

**ASPARTAME ADMINISTRATION TO THE INFANT MONKEY:
HYPOTHALAMIC MORPHOLOGY AND BLOOD AMINO ACID
LEVELS**

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FINAL REPORT

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INTRODUCTION

The administration of large amounts of the neurotransmitter amino acid, monosodium glutamate (MSG) to neonatal mice results in retinal (1) and brain lesions (2-4). At high dosages, numerous brain areas are affected including the tectum, habenular nuclei, subfornical organ, dorsal lateral surface of the thalamus, dentate-hippocampal gyri, cerebral cortex as well as the nuclei gracilis and cuneatus, and area postrema of the lower medulla (4, 5). The lesion is characterized by neuronal death resulting in pycnotic nuclei, dilated dendrites, and ultimately, phagocytic processes involving glia (4). Neonatal mice are sensitive to glutamic acid at dosages as low as 0.5 mg/gm where about 22% of infant mice sustain damage restricted primarily to the arcuate area; at 0.25 mg/gm of MSG, no lesions are observed (4, 6).

Another acidic amino acid, aspartate, is also capable of eliciting retinal and hypothalamic damage in the fetal (6) and neonatal (6-8) mouse at high dosages (1 mg/gm). Glutamate and aspartate appear to be additive in effect because when mice receive an oral dose of a mixture of MSG (0.5 mg/gm) and sodium aspartate (0.5 gm/kg) hypothalamic damage characteristic of animals treated with either substance at 1 mg/gm is noted (6). In addition, aspartic acid is one of three amino acids implicated in brain damage in infant mice following the administration of commercially available casein and fibrin hydrolysates used for parenteral alimentation (9).

Of late, overwhelming evidence has indicated that susceptibility to lesions following the administration of glutamate seems to be a phenomenon of the immature rodent brain. Only Olney and colleagues have reported hypothalamic damage in neonatal primates following MSG

intake (10, 11). No other laboratories, in spite of extensive and painstaking attempts (3, 8, 12-15) have found any indication of hypothalamic damage resulting from MSG administration to infant primates. In fact, even the direct injection of MSG in utero to fetal monkeys does not result in hypothalamic damage (16). All told, some 59 infant monkeys have been treated with MSG and found to sustain no evidence of hypothalamic damage (16) in contrast to the studies of Olney (11) involving 3 animals receiving subcutaneous injections and 3 animals receiving oral loads.

Aspartame (APM) is a potent sweetener, some 200 times as sweet as sugar, composed of phenylalanine and aspartic acid. Because of concern for developing brain, it was decided to search for any possible hypothalamic effects of administering acute, massive loads of APM in the neonatal period. In addition, blood samples were taken at intervals following dosing in order to determine amino acid metabolism following abuse loads, and to be sure that the infants did indeed experience exposure to high blood levels of aspartame.

Materials and Methods

The three species of macques used for these experiments were Macaca mulatta, fascicularis, and arctoides, all born in the Primate Facility at the University of Illinois at the Medical Center from timed pregnancies. Each experimental infant was isolated early on the morning of experimentation in a humidity and temperature-controlled incubator. After a 3-4 hour fast, each infant was lightly tranquilized with Sernylan (phencyclidine hydrochloride) and placed on a warming pad to maintain body temperature at 37°C for the duration of the experiment. In order to obtain blood samples, a polyvinyl catheter was placed into the umbilical vein in infants of 4 days of age or less. For older infants, a polyvinyl catheter was inserted into the saphenous vein. Each infant monkey was then dosed with the appropriate compound by stomach tube. Aspartame has relatively low solubility in water, and so this solution was in effect prepared as a slurry. For the experiments involving APM alone (Table 2) a 10% solution was prepared and the appropriate dosage computed on a kilogram weight basis. For experiments involving 2 gm/kg of APM plus 1 gm/kg of MSG, a 30% solution was prepared immediately prior to dosing. Before dosing an infant monkey, a stomach tube was introduced into the stomach and fluid was aspirated to make sure the tubing was in the stomach rather than the respiratory tree. After dosing, each infant was observed constantly over the following sampling period so that vomiting, respiratory difficulties, cyanosis, or other difficulties could be observed. If an infant regurgitated, the infant was observed until the spell of vomiting was over, and then redosed with the estimated amount regurgitated.

At the end of four hours, the infant was intubated and anesthetized with Halothane. Under sterile conditions, the chest cavity was then opened and 1 cc of a 5% sodium nitrate solution was injected into the ventricle to encourage vasodilation. Next, a small incision was made into the left ventricle and a trocar inserted into the aorta. The right atrium was then cut for drainage and a normal saline solution permitted to flow into the body under gravity pressure. When the saline flow back through the atrium was clear, indicating that most of the blood had been drained from the brain, perfusion of a 2% glutaraldehyde solution in 0.1M phosphate buffer (pH 7.3) was initiated. At this point, an attempt was always made to clamp the abdominal aorta so that the anterior portion of the body would receive the greatest amount of the perfusate.

At the end of the perfusion period, the head was removed and stored overnight in the glutaraldehyde perfusate. The next day, two or three samples (1 mm slices) including the arcuate-median eminence area were cut grossly with a razor blade. Subsequently, the slices were post-fixed in osmium, dehydrated and embedded in Durcupan ACM. In order to scrutinize the entire hypothalamic region, plastic sections were cut at 1 micron on an LKB III with a glass knife, stained with methylene blue and azure II, and studied by light microscopy.

Thin sections were cut on a Sorvall MT-2 with a diamond knife at 700 angstroms, stained with uranyl acetate and lead citrate, and examined with a RCA EMU3H electron microscope.

A control series of infant monkeys received either no treatment or water by a stomach tube (Table 1). Eight infant monkeys received aspartame at a level of 2 gm/kg (Table 2). Because of concern about the possible additive effects of aspartame and MSG in the diet, a series of six infant monkeys were given an acute load of 2 gm/kg of APM plus 1 gm/kg of MSG (Table 3).

During the experimental period, blood samples were obtained at 0, 20, 40, 60, 90, 120, 180 and 240 minutes. Each sample was immediately centrifuged to remove erythrocytes and the plasma was prepared for amino acid analysis as described by Stegink et al. (17). Amino acid analyses were carried out on automated amino acid analyzers (Beckman 121 M, Beckman Instruments, Palo Alto, California).

RESULTS

Infant monkeys A-14 and A-6 regurgitated small amounts after dosing and required redosing. Infant A-12 exhibited agitated movement after dosing. It was dosed on an empty stomach as evidenced by failure to aspirate stomach contents prior to dosing. Infant A-7 was also agitated and along with A-6, had cyanotic episodes. Infant A-9 stopped breathing an hour after dosing but responded to intubation and oxygen administration with a resumption of respiration. These responses are to be expected following the loading of a fasted animal to the full capacity of the stomach with concentrated acidic amino acid solutions.

It is technically difficult to perfuse a brain as large as that of the infant monkey with glutaraldehyde so as to achieve uniformly excellent fixation throughout and therefore allow study of plastic sections at the electron microscope level. Therefore, it is important to have a good series of control animals so that careful reference can be made to discern between real morphological abnormalities and perfusion artifacts. For this reason, the entire hypothalamus was serially sectioned in all of the animals listed in Table 1-3, which is an enormously painstaking and time-consuming process. Close examination of the one micron sections prepared from the animals receiving 2 gm/kg of APM revealed no significant differences in the appearance of the neurons, processes, or glia between normal infant monkeys and those treated with APM (Fig. 1 A and B). Similarly, those animals receiving 2 gm/kg APM plus 1 gm/kg MSG exhibited no abnormalities in the various cytological constituents of their hypothalami (Fig. 2 A and B).

When sectioned for electron microscopy, greater cellular details became apparent which were not readily seen in the thicker 1 micron Durcupan ACM sections. Well-fixed areas containing normal neurons would be closely adjacent to areas where the fixatives had not entered as thoroughly and here could be seen swollen processes or other evidence of poor fixation (Fig. 3A). We repeatedly noted a large cell containing large, dense inclusion bodies (Fig. 3B) which has not been reported before for the central hypothalamic area and which we had not observed over the years in many studies involving the infant mouse hypothalamus. It is interesting to speculate on the nature of this cell which contains large, dense granules (Fig. 3B) and which resembles osmoreceptors of the superior colliculus or a mast cell. Its existence in other brain regions and whether it persists into the brain of the juvenile and the adult primate are unknown.

Considerable variation in the plasma amino acid absorption curves for phenylalanine, tyrosine, glutamate and aspartate was noted after either aspartame or aspartame plus monosodium glutamate administration. This undoubtedly reflects the fact that aspartame must be administered as a slurry to achieve a dose level of 2 gm/kg body weight. Plasma aspartate and phenylalanine curves in human subjects show a much greater variability after slurry administration than solution administration (18).

Individual plasma aspartate and glutamate levels in four of the animals administered aspartame are shown in Table 4.

Aspartame administration significantly increased plasma aspartate levels above baseline values (0.69 ± 0.43 umoles/dl) with mean (\pm S.D.)

peak levels of 23 ± 33 umoles/dl noted at 60 minutes. Individual peak values for aspartate at any given time ranged from 21 to 84 umoles/dl. Plasma glutamate increased from baseline levels of 9.38 ± 1.71 umoles/dl to mean (\pm S.D.) peak levels of 31 ± 14 umoles/dl at 120 minutes, presumably reflecting conversion of aspartate to glutamate. Individual peak values for glutamate at any given time ranged from 32 to 44 umoles/dl.

Individual plasma phenylalanine and tyrosine levels in animals administered aspartame are shown in Table 5. Plasma phenylalanine increased from baseline values of 5.93 ± 2.81 umoles/dl to mean peak values of 95 ± 59 umoles/dl 90 minutes after aspartame loading. Individual peak values ranged from 36.6 to 215 umoles/dl. Plasma tyrosine increased from baseline values of 6.69 ± 2.62 umoles/dl to mean (\pm S.D.) peak values of 35.5 ± 6.70 umoles/dl 4 hours after aspartame ingestion. The increase in plasma tyrosine undoubtedly reflects conversion of phenylalanine to tyrosine.

Individual plasma glutame and aspartate levels in 5 of the animals administered aspartame plus MSG are also shown in Table 4. Plasma aspartate levels increased significantly over baseline (1.36 ± 0.97 umoles/dl) reaching mean (\pm S.D.) peak values of 49 ± 30 umoles/dl 120 minutes after ingestion (Table 1). Individual peak values ranged from 20 to 94 umoles/dl. Plasma glutamate levels were also significantly elevated overbaseline levels (8.01 ± 1.42 umoles/dl), reaching a mean (\pm S.D.) peak level of 168 ± 88 umoles/dl 120 minutes after ingestion of the test load. Individual peak levels ranged from 121 to 280 umoles/dl in these animals.

Individual plasma phenylalanine and tyrosine levels in animals administered aspartame plus MSG are shown in Table 5. Plasma phenylalanine and tyrosine levels are similar to those noted in animals

administered aspartame alone. Plasma phenylalanine levels increased from baseline values of 6.76 ± 2.11 umoles/dl to mean peak values of 120 ± 127 umoles/dl at 120 minutes after aspartame loading. Individual peak values ranged from 45 to 380 umoles/dl. Plasma tyrosine levels also increased over baseline values (6.59 ± 1.11 umoles/dl), reaching a mean peak level of 27.9 ± 9.76 umoles/dl 4 hours after administration.

The plasma glutamate levels observed in infant monkeys administered glutamate plus aspartame were similar to those noted in infant monkeys administered an equivalent dose of MSG (1 gm/kg body weight) without added aspartame (19).

DISCUSSION

The plasma aspartate, glutamate and phenylalanine data (Tables 4 and 5) clearly demonstrate the administration and absorption of the test compounds. Thus, the failure to observe neuronal necrosis does not reflect a failure to elevate plasma levels. This issue has previously been raised by Olney et al. (11, 20) as an explanation when four other research groups (3, 8, 12-16) were unable to reproduce their data which had suggested that administration of glutamate to neonatal primates resulted in hypothalamic neuronal necrosis. We have previously demonstrated elevated plasma glutamate levels in infant monkeys studied morphologically after glutamate administration (19), also discrediting those claims. The plasma glutamate and aspartate levels found in neonatal monkeys administered 1 gm/kg MSG with 2 gm/kg aspartame are similar to those noted in neonatal monkeys given MSG alone at 1 gm/kg body weight (19).

The data in Table 4 and 5 show a marked variability in the absorption and metabolism curves for plasma aspartate, phenylalanine and glutamate. The variability is consistent. Thus, both plasma aspartate and phenylalanine levels show a rapid early peak in monkey A-5, with peak values much higher than the mean peak values noted for all animals. Similarly, levels of both show a much slower increase in monkey A-14, with peak values being lower than expected from mean peak values noted in all animals.

This variation likely reflects the administration of aspartame in slurry form. Administration in slurry form probably results in

considerable differences in gastric emptying among the animals. We have previously compared the effects of aspartame administration in either solution or slurry form in human subjects (18). These studies showed large variations in plasma aspartate and phenylalanine levels after slurry administration, contrasting with low variability noted after aspartame administration in solution. However, mean peak values for plasma phenylalanine and tyrosine were similar with both forms of administration.

The plasma aspartate and phenylalanine absorption curves in human subjects administered aspartame in slurry form showed the same variation in shape and peak values noted in the monkeys studied here. Thus, human subjects showing what appears to be rapid gastric emptying had a rapid peak in both plasma aspartate and phenylalanine levels, with peak values considerably above the mean peak values noted in all subjects. Similarly, subjects showing apparent delayed gastric emptying had a lower, broader absorption curve for both amino acids, with peak values below the mean peak values noted for all subjects studied.

The data from the present study are consistent with our previous study indicating that the monkey metabolizes amino acids more rapidly than man. The mean peak, or individual peak, plasma phenylalanine levels in monkeys administered aspartame were lower than might be expected from human studies with aspartame. Human studies using aspartame doses ranging from 34 mg/kg to 200 mg/kg body weight demonstrated a linear relationship between peak plasma phenylalanine levels and aspartame dose (21). Extrapolating these data to an aspartame dose of 2 gm/kg body weight predicted a higher peak plasma phenylalanine level than those observed. Similarly, plasma glutamate levels noted

those observed. Similarly, plasma glutamate levels noted after administration of MSG with aspartame were lower than might have been predicted from studies in man. Peak plasma glutamate levels in man show a positive dose-response curve to increasing loads of MSG (60 to 240 mg/kg body weight) (22). Extrapolation of these data to an MSG load of 1 gm/kg predicted a higher plasma glutamate level than levels noted in the animals studied.

To evaluate this question further, we recently compared plasma glutamate levels in mice, monkey and man administered equivalent doses of MSG either in water or as part of a meal (23). These data demonstrate that mice and monkeys metabolize glutamate more rapidly than man, and have lower plasma glutamate levels after ingestion of doses of MSG. Both the glutamate and phenylalanine data are consistent with the known protein requirements of monkey and man. The monkey has a much higher dietary requirement than man (24), suggestive of a more rapid catabolism of ingested amino acids. Thus, it is reasonable to expect lower plasma levels after administration of an equivalent load.

The administration of APM or APM plus MSG in this study was designed to be as akin to an abuse situation as possible in that the additives were administered as a slurry in water by stomach tube into a fasted infant. Even so, this massive load of APM or APM plus MSG did not create abnormalities in hypothalamic morphology. Since 8 infant monkeys received a 2 gm/kg of APM alone, and 6 received APM plus MSG, another 14 carefully studied infant monkey brains after the administration of large dosages of acidic amino acids can be added to the 47 infants receiving massive acute oral loads of MSG in the neonatal period whose brains exhibited no evidence of abnormal hypothalamic morphology. In

scientific endeavor, it is necessary to have an overwhelming number of studies involving negative findings to dispel a single investigator with positive findings as was true of Olney's earlier claim of damage to 3 infant monkey hypothalami following acute oral loads of MSG (11). Olney also dosed three infant monkeys subcutaneously; Abraham et al. (15) dosed two infants subcutaneously, two infants by dietary administration, and Wen et al. (14) dosed eight infants by dietary administration. Only Olney claimed to find any hypothalami abnormalities in infant monkeys receiving MSG administration by any route. The preponderance of scientific data indicating that the neonatal primate does not sustain hypothalamic damage even when having ingested massive loads of acidic amino acids would indicate that there is no longer a need to attempt to explain scientifically the difference between the findings of Olney and all of the other investigators who have been involved in similar studies. It should be noted, however, that the infant monkeys obtained by Olney were not raised in a primate facility. One of the infants was premature and two experienced dehydration. The damage reported by Olney (11) occurring in the three neonatal monkey brains following ingestion of MSG numbered by 2-3 neurons per section, and totaled only 50-90 cells in the rostral subventricular portion of the infundibular nucleus in contrast to the rodent where lateral portions of the hypothalamus sustained the most obvious characteristic damage, involving dozens of neurons per section (4, 5). Cell death in the developing central nervous system is a requisite and normal part of the differentiative process. During embryonic and fetal periods, the ependymal cells lining the ventricles of the brain divide rapidly and create a dense cellular population of the neuropil. Only a minority of this cell population will persist as glia and neuronal

elements. Many neurons or even the majority of the cell populations are destined to die probably because of a failure to connect either to peripheral neurons or to adjacent cells. This well-known developmental process of the central nervous system leads to the question of whether Olney's observations were upon isolated areas of normal neuronal cell death, perhaps somehow exaggerated by the dehydrated state of the infant monkeys.

The infant Old World primate brain is an excellent model of the human infant brain, both with respect to morphology and developmental events. Thorough study, section by section, through the infant hypothalamus has laid aside the concern about damage to this area resulting even from inordinately high loads of APM. Similarly, the additive administration of two acidic amino acids, APM and MSG, to the infant primate created no abnormalities in the vulnerable infant primate hypothalamus. It should be noted that this is strong testimony to the safety of these substances resulting from extensive neuroanatomical studies to which only a few food additives have been subjected.

SUMMARY

Infant monkeys received 2 gm/kg of aspartame (APM) or 2 gm/kg APM plus 1 gm/kg monosodium glutamate (MSG) by gastric tube. Control infant monkeys received nothing or water. Blood samples were obtained at intervals over the ensuing four hours and analyzed for amino acid levels. At termination, each infant was perfused with glutaraldehyde and the hypothalamus embedded in plastic followed by the preparations of serial sections at 1 micron.

Hypothalamic morphology was normal in all 8 infant brains exposed to 2 gm/kg APM as well as in the 6 infant brains experiencing the 2 gm/kg APM plus 1 gm/kg MSG loads. No signs of pycnotic nuclei, neuronal degeneration or dendritic swelling were noted. Localized areas of poor perfusion exhibited abnormal morphology in both experimental and control brains. Elevated plasma levels of aspartate, glutamate and phenylalanine indicated the test compounds had been administered and absorbed. Variable rates of absorption were evident, probably due to the necessity of administering APM as a slurry, due to its low solubility. The infant monkeys metabolized the amino acids somewhat more rapidly than does man.

It is concluded that APM given alone or with MSG, in large acute doses, does not result in hypothalamic damage in the newborn monkey.

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Table 1. Control Infant Monkeys Receiving Nothing
or Water by Gastric Tube

| Age (days) | Sacrifice date | Code | Species | Sex | Weight (gm) | Dose |
|---------------|-------------------|---------|---------------------|-----|----------------|-----------------------|
| 6 | 9/10/70 | 1008-MY | <u>M. fasc.</u> | F | 335 | nothing |
| 8 | 4/11/74 | 2564 | <u>M. mulatta</u> | M | 450 | nothing |
| 9 | 7/13/70 | 953-MO | <u>M. fasc.</u> | M | 310 | 5 ml H ₂ O |
| 15 | 8/18/70 | 978-MV | <u>M. fasc.</u> | M | 310 | nothing. |
| 16 | 6/11/73 | 2189 | <u>M. mulatta</u> | F | 590 | 5 ml H ₂ O |
| 18 | 6/4/74 | 2594 | <u>M. fasc.</u> | M | 350 | 2 ml H ₂ O |
| 19 | 2/6/74 | 761 | <u>M. fasc.</u> | F | 370 | 3 ml H ₂ O |
| 21 | 8/6/70 | 958-MS | <u>M. fasc.</u> | M | 360 | nothing |
| 30 | 7/11/75 | 3007 | <u>M. arctoides</u> | M | 520 | nothing |
| 51 | 8/19/70 | 950-MW | <u>M. fasc.</u> | M | 410 | nothing |
| 120 | 6/11/73 | 1951 | <u>M. arctoides</u> | F | 820 | 5 ml H ₂ O |

Table 2. Infant Monkeys Receiving APM
by Gastric Tube

| Age (days) | Sacrifice date | Code | Species | Sex | Weight (gm) | Dose per kg |
|---------------|-------------------|-------------|---------------------|-----|----------------|----------------|
| 1 | 11/4/74 | (A-4) 2747 | <u>M. arctoides</u> | F | 430 | 2 gm |
| 1-1/2 | 11/5/74 | (A-5) 2758 | <u>M. mulatta</u> | F | 370 | 2 gm |
| 2 | 1/24/75 | (A-13) 2839 | <u>M. arctoides</u> | M | 430 | 2 gm |
| 3 | 1/31/75 | (A-14) 2858 | <u>M. mulatta</u> | F | 410 | 2 gm |
| 3 | 10/18/74 | (A-2) 2746 | <u>M. arctoides</u> | F | 470 | 2 gm |
| 5 | 1/15/75 | (A-12) 2836 | <u>M. arctoides</u> | F | 500 | 2 gm |
| 8 | 10/22/74 | (A-3) 2742 | <u>M. fasc.</u> | F | 280 | 2 gm |
| 22 | 10/15/74 | (A-1) 2740 | <u>M. mulatta</u> | M | 530 | 2 gm |

Table 3: Infant Monkeys Receiving
APM Plus MSG by Gastric Tube

| Age (days) | Sacrifice date | Code | Species | Sex | Weight (gm) | Dose per kg |
|---------------|-------------------|--------------|---------------------|-----|----------------|----------------------|
| 1 | 1/8/75 | (A-11) 2835 | <u>M. mulatta</u> | F | 422 | 2 gm APM 1 gm MSG |
| 1 | 12/11/74 | (A-7) 2796 | <u>M. arctoides</u> | M | 460 | 2 gm APM 1 gm MSG |
| 1 | 12/4/74 | (A-6) 2823 | <u>M. mulatta</u> | M | 480 | 2 gm APM 1 gm MSG |
| 2 | 1/3/75 | (A-9) 2832 | <u>M. mulatta</u> | F | 370 | 2 gm APM 1 gm MSG |
| 2 | 1/2/75 | (A-8) 2822 | <u>M. arctoides</u> | F | 380 | 2 gm APM 1 gm MSG |
| 3 | 1/3/75 | (A-10) 2831) | <u>M. mulatta</u> | M | 380 | 2 gm APM 1 gm MSG |

Table 4: Plasma glutamate and aspartate levels in neonatal monkeys administered Aspartame or Aspartame Plus Monosodium L-Glutamate

| Amino Acid | Monkey | Compound | Time After Administration Of Dose (minutes) | | | | | | | |
|------------------|--------|-----------|---|------|------|-------|-------|-------|-------|-------|
| | | | 0 | 20 | 40 | 60 | 90 | 120 | 180 | 240 |
| <u>Aspartate</u> | A-5 | APM | 0.28 | 14.1 | 84.2 | 72.3 | 36.3 | 25.4 | 12.5 | 4.20 |
| | A-12 | APM | 1.30 | 1.22 | 1.81 | 10.2 | 24.2 | 14.1 | 4.11 | 2.71 |
| | A-13 | APM | 0.70 | 2.10 | 3.88 | 9.10 | 18.2 | 25.2 | 11.2 | 12.4 |
| | A-14 | APM | 0.51 | 0.45 | 0.65 | 0.41 | 0.31 | 4.4 | 12.0 | 21.1 |
| | Mean | | 0.69 | 4.46 | 22.6 | 23.0 | 19.8 | 17.3 | 9.95 | 10.1 |
| | S.D. | | 0.43 | 6.45 | 41.2 | 33.2 | 15.0 | 10.1 | 3.93 | 8.47 |
| <u>Aspartate</u> | A-6 | APM + MSG | 1.61 | 3.22 | 7.11 | 16.4 | 52.2 | 92.4 | 24.2 | 9.11 |
| | A-7 | APM + MSG | 2.50 | 4.01 | 6.20 | 14.2 | 32.2 | 28.1 | 8.80 | 8.00 |
| | A-8 | APM + MSG | 2.00 | 8.11 | 10.4 | 14.2 | 20.1 | 16.8 | 18.2 | 18.4 |
| | A-10 | APM + MSG | 0.40 | 2.81 | 35.4 | 58.2 | 48.2 | 44.2 | 40.1 | --- |
| | A-11 | APM + MSG | 0.30 | 11.2 | 46.2 | 54.2 | 40.1 | 65.2 | 43.2 | 33.0 |
| | Mean | | 1.36 | 5.87 | 25.3 | 31.4 | 38.6 | 49.3 | 26.9 | 17.1 |
| <u>Glutamate</u> | A-5 | APM | 8.41 | 16.2 | 29.3 | 31.4 | 32.4 | 23.8 | 15.2 | 11.7 |
| | A-12 | APM | 11.6 | 19.2 | 19.9 | 27.4 | 26.3 | 42.3 | 43.0 | 20.0 |
| | A-13 | APM | 9.81 | 18.3 | 23.4 | 27.4 | 37.4 | 43.5 | 40.2 | 44.3 |
| | A-14 | APM | 7.71 | 9.43 | 8.61 | 7.24 | 6.61 | 16.3 | 22.4 | 33.1 |
| | Mean | | 9.38 | 15.8 | 20.3 | 23.4 | 25.7 | 31.5 | 30.2 | 27.3 |
| | S.D. | | 1.71 | 4.41 | 8.70 | 10.9 | 13.5 | 13.6 | 13.5 | 14.4 |
| <u>Glutamate</u> | A-6 | APM + MSG | 8.11 | 16.2 | 24.3 | 48.2 | 170 | 200 | 79.2 | 43.0 |
| | A-7 | APM + MSG | 7.4 | 10.2 | 20.4 | 51.4 | 121 | 60.4 | 54.3 | 40.3 |
| | A-8 | APM + MSG | 10.0 | 29.4 | 48.2 | 49.3 | 80.1 | 100 | 190 | 180 |
| | A-10 | APM + MSG | 6.12 | 23.4 | 32.4 | 172 | 180 | 200 | 125 | --- |
| | A-11 | APM + MSG | 8.42 | 50.2 | 152 | 247 | 197 | 280 | 240 | 188 |
| | Mean | | 8.01 | 25.9 | 55.5 | 113.6 | 149.6 | 168.1 | 137.7 | 112.8 |
| | S.D. | | 1.42 | 15.4 | 55.0 | 91.5 | 48.1 | 87.7 | 77.0 | 82.3 |

Table 5: Plasma phenylalanine and tyrosine levels in neonatal monkeys administered aspartame or aspartame plus monosodium L-glutamate.

| Amino Acid | Monkey | Compound | Time After Administration Of Dose (Minutes) | | | | | | | |
|---------------|--------|-----------|---|------|------|------|-------|-------|-------|------|
| | | | 0 | 20 | 40 | 60 | 90 | 120 | 180 | 240 |
| Phenylalanine | A-5 | APM | 10.1 | 215 | 196 | 150 | 140 | 99.1 | 86.1 | 71.1 |
| | A-12 | APM | 4.51 | 19.2 | 52.1 | 100 | 109 | 102 | 48.2 | 41.1 |
| | A-13 | APM | 4.00 | 29.1 | 74.2 | 94.8 | 121 | 118 | 72 | 61.2 |
| | A-14 | APM | 5.11 | 6.00 | 5.24 | 5.94 | 7.99 | 18.2 | 30.4 | 36.6 |
| | MEAN | | 5.93 | 67.3 | 81.9 | 87.7 | 94.5 | 84.3 | 59.2 | 52.5 |
| | S.D. | | 2.81 | 98.9 | 81.3 | 59.9 | 59.1 | 44.9 | 24.8 | 16.4 |
| Phenylalanine | A-6 | APM + MSG | 5.21 | 27.3 | 52.4 | 75.4 | 98.2 | 121 | 70.4 | 56.2 |
| | A-7 | APM + MSG | 10.2 | 17.4 | 18.2 | 31.3 | 43.8 | 44.6 | 20.1 | 15.2 |
| | A-8 | APM + MSG | 5.00 | 120 | 160 | 110 | 77.2 | 40.3 | 40.3 | 30.2 |
| | A-10 | APM + MSG | 7.22 | 15.4 | 22.8 | 133 | 240 | 340 | 380 | --- |
| | A-11 | APM + MSG | 6.21 | 64.2 | 52.4 | 54.2 | 42.5 | 54.3 | 54.3 | 40.8 |
| | Mean | | 6.76 | 48.9 | 61.2 | 80.8 | 100.3 | 120.0 | 113.0 | 35.6 |
| Tyrosine | A-5 | APM | 5.11 | 15.2 | 20.3 | 24.3 | 31.4 | 36.4 | 35.2 | 31.2 |
| | A-12 | APM | 10.1 | 16.2 | 21.4 | 22.3 | 34.2 | 37.4 | 40.2 | 41.2 |
| | A-13 | APM | 7.33 | 16.3 | 31.4 | 38.4 | 47.2 | 52.8 | 46.2 | 41.3 |
| | A-14 | APM | 4.22 | 5.00 | 5.22 | 5.31 | 5.44 | 8.99 | 14.3 | 28.4 |
| | Mean | | 6.69 | 13.2 | 19.6 | 22.6 | 29.6 | 33.9 | 34.0 | 35.5 |
| | S.D. | | 2.62 | 5.47 | 10.8 | 13.6 | 17.5 | 18.2 | 13.9 | 6.70 |
| Tyrosine | A-6 | APM + MSG | 6.22 | 11.2 | 12.4 | 11.3 | 16.2 | 19.3 | 28.4 | 35.3 |
| | A-7 | APM + MSG | 7.11 | 13.2 | 12.4 | 14.8 | 17.2 | 21.4 | 15.3 | 14.8 |
| | A-8 | APM + MSG | 8.22 | 17.4 | 24.5 | 28.3 | 31.3 | 32.3 | 36.4 | 35.4 |
| | A-10 | APM + MSG | 6.11 | 11.2 | 12.3 | 11.4 | 14.3 | 26.3 | 28.1 | --- |
| | A-11 | APM + MSG | 5.31 | 9.22 | 14.3 | 16.3 | 17.4 | 20.4 | 24.3 | 26.0 |
| | Mean | | 6.59 | 12.4 | 15.2 | 16.4 | 19.3 | 23.9 | 26.5 | 27.9 |
| | S.D. | | 1.11 | 3.10 | 5.27 | 6.98 | 6.83 | 5.38 | 7.65 | 9.76 |

Figure 1.

Light micrographs:

- A. Section through rostral aspect of hypothalamus of infant monkey A-5 receiving 2 gm/kg APM by stomach tube. Neurophil is normal in appearance. (X100)**
- B. Enlarged section through subventricular region from infant monkey A-5 receiving 2 gm/kg APM. Neurons appear normal. (X250)**

A



B

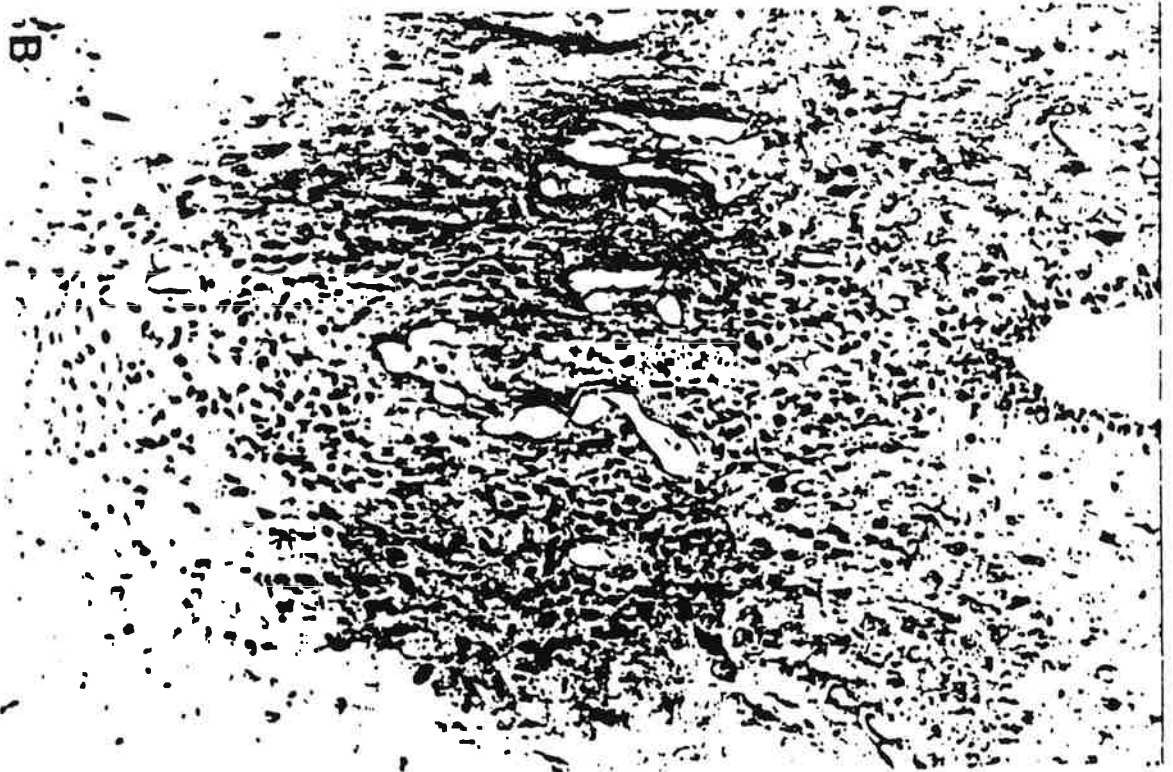
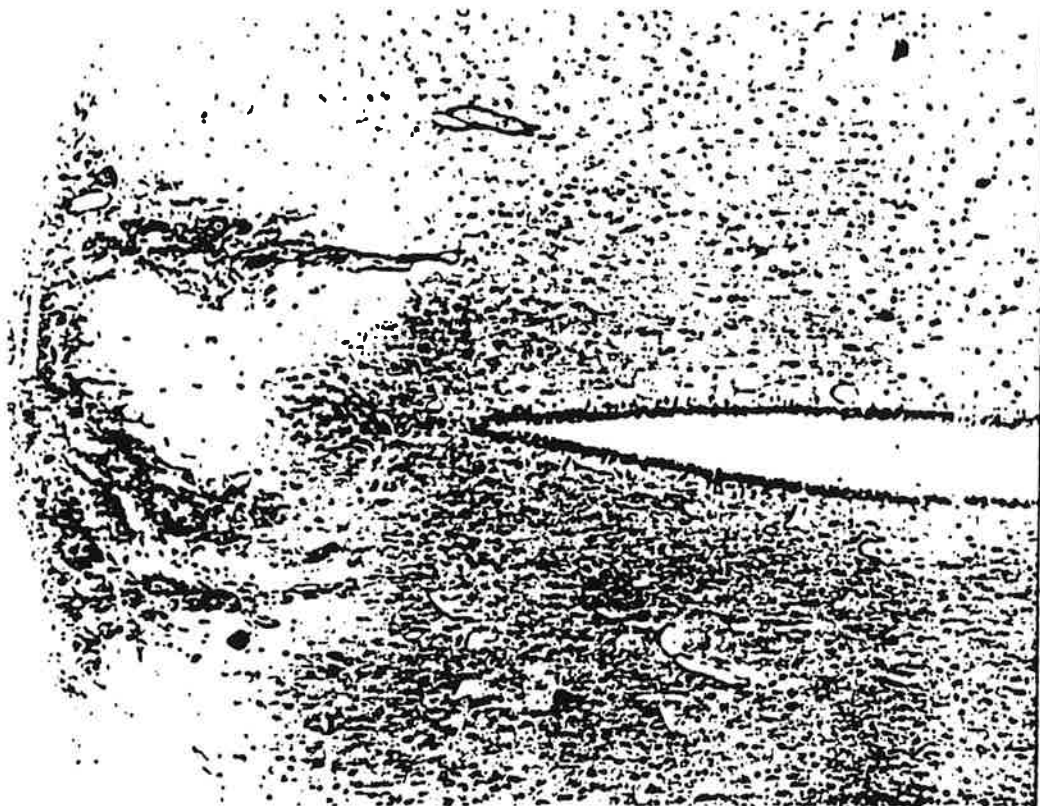


Figure 2.

Light micrographs:

- A. Section through infundibular area of hypothalamus of infant monkey A-6 receiving 2 gm/kg APM plus 1 gm/kg MSG by stomach tube. (X100)**
- B. Enlarged section through subventricular region from infant monkey A-6 receiving 2 gm/kg APM plus 1 gm/kg MSG. Neurons appear normal. (X250)**

A



B

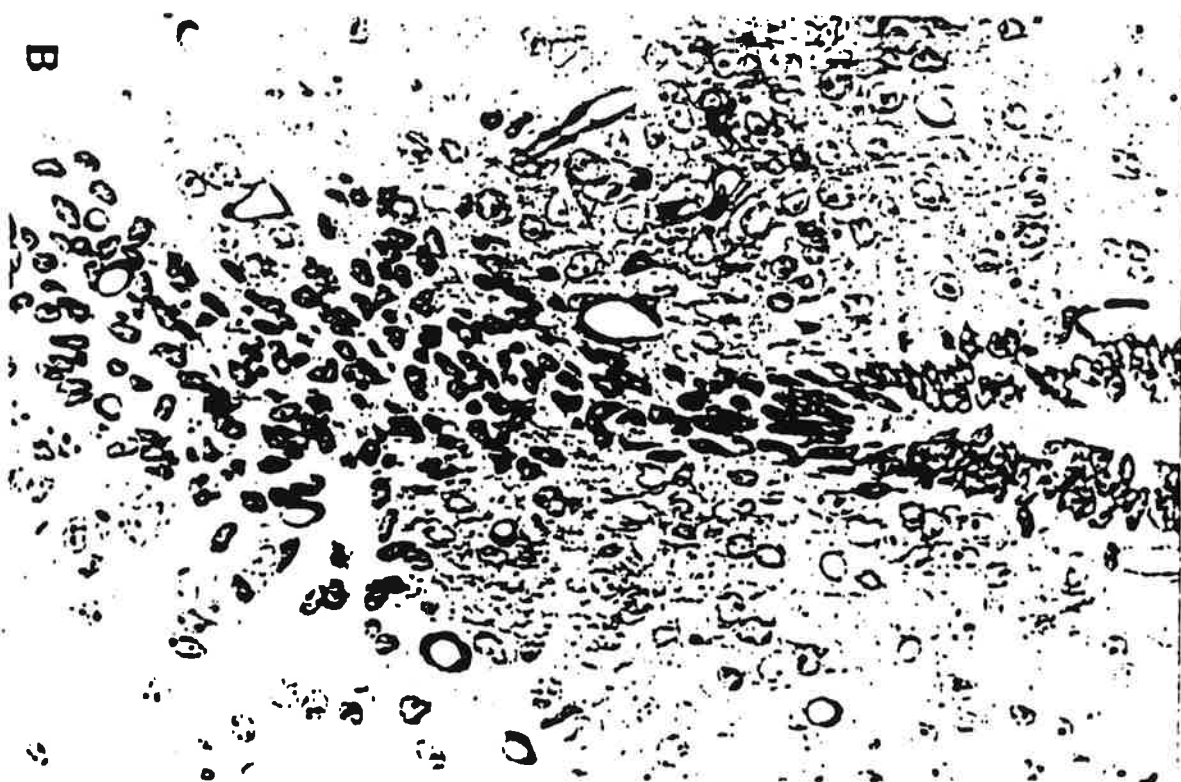


Figure 3.

- A. Electron microscopic view of area from subventricular aspect of hypothalamus of infant monkey A-14 receiving 2 gm/kg APM. The cytoplasm and nucleus (N) appear normal, in an area of good fixation. Immediately adjacent is an area of poor fixation (arrow) characterized by smaller dendrites.
(X8600)
- B. Electron microscopic view of area from subventricular portion of hypothalamus from infant monkey A-8 receiving 2 gm/kg APM and 1 gm/kg MSG. The nucleus of a cell is seen in a background of dense neuropile with many small dark granules. The nucleus is surrounded by intracytoplasmic dark granules somewhat reminiscent of osmoreceptor cells in the central grey in the superior colliculus or a mast cell.
(X5600)

