NATURE AND EXTENT OF BRAIN LESIONS IN MICE RELATED TO INGESTION OF MONOSODIUM GLUTAMATE

A Light and Electron Microscope Study

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ABSTRACT

The incidence of arcuate neuron damage following 1 to 4 mg/gm oral doses of monosodium glutamate (MSG) to neonatal mice was studied. The first morphological manifestation of the lesion involved edematous astrocytic glia and pathological changes in neurons close to the median eminence within 15–20 minutes. The sequence of the lesion involved at least three phases: 1) neuronal edema and death, 2) multiple phagocytic cells, viz. a type of "dense" cell and an electron "lucid" cell (astrocyte), and 3) proliferation of astrocytic processes. It was noted that at 4 mg doses, the lesions occurred in other areas such as tectum, habenular nuclei, subfornical organ, dorsolateral surface of the thalamus, dentate-hippocampal gyri, cerebral cortex, and in the lower medulla, the nuclei gracilis and cuneatus and area postrema. In all these structures, the lesion was initiated superficially and radiated inward, suggesting an inflow of the deleterious agent from the cerebrospinal fluid. Comparison of our neuropathological findings with those of others, emphasized the critical aspects of species variation, developmental age, route of administration, time of examination of brain material after dosage and thoroughness of sampling methods.

INTRODUCTION

The potential toxicity of food additives for the neonate has generated great interest in recent years, especially with regard to the flavor enhancer monosodium glutamate (MSG). The early reports of Olney (21–23) regarding the susceptibility of the mouse retina and the arcuate nucleus of the hypothalamus to neuronal damage after subcutaneous administration of large doses of MSG were subsequently contradicted by a series of investigations employing animals of various species and ages, and utilizing different routes of administration (2, 27). The observation that the arcuate lesion affected primarily neurons was opposed by Arees and Mayer (4) who maintained that the target of damage was the glial cell. Confirmation of neuronal damage has been supplied by Abraham et al (1), who found that under certain conditions, mouse arcuate neurons were damaged following MSG administration.

Our studies are an examination of the incidence of this lesion in mouse brain as well as an attempt to elucidate some of the underlying factors contributing
to these lesions. We have identified the successive elements of the arcuate nuclear region involved in the lesion at selected time intervals following dosage. Most importantly, the spread of damaged regions in relation to various intervals after administration and to different dosage levels has been ascertained. Finally, attention has been given to whether the phenomenon of MSG-induced central nervous system lesions is restricted to the neonatal period or whether such lesions can be induced in adult animals as well. The results reported here suggest that the study of the susceptibility of brain to exogenous toxic influences for neonatal animals of various species be expanded in scope.

METHODS

1. Animal Care. Three strains of Swiss Albino mice were employed in this study: A/Jax (Jackson Laboratories, Bar Harbor, Maine); SCH:ARS, HA/ICR (Abrams Breeders, Chicago, Illinois); and an A/Jax-ICR hybrid mouse. Unless otherwise indicated, MSG was administered by stomach tube to neonatal mice 7 to 10 days of age. After administration of the designated dose of MSG, the mice were isolated from their dams but kept warm under a heat lamp until the end of the treatment interval (usually 3 to 4 hours). For studies involving an interval of 12 or more hours, infant mice were permitted to suckle.

2. Acute Studies of MSG. To study the histologic and temporal aspects of the brain lesion, neonatal mice received a single dose of MSG administered as a 20 percent solution via stomach tube at dosages of 1, 2 or 4 mg/gm of body weight. Neonatal mice given the largest dose (4 mg/gm) became markedly quiet and ceased motion. Some animals exhibited slight tremors following administration of the amino acid. Death rate was highest for those mice receiving the largest dose (table 1). In this study MSG was most toxic for the A/Jax-ICR hybrid (table 1). These data were analyzed by means of a four-way contingency table and differences between inbred and hybrid strains were statistically significant at both the 2 and 4 mg/gm dosages.

3. Chronic Studies of MSG. For these studies infant hybrid mice received 22 mg/gm body weight of a 20 percent solution of MSG by subcutaneous injection from the second through the eleventh day of life. Littermates in equal numbers were given saline injections. All injections were given between 10:00 and 12:00 a.m. Growth and reproduction studies performed on these animals are reported elsewhere (31).

4. Preparation of Brain for Electron Microscopy. All mice were anesthetized with Nembutal®. The chest was opened and the whole animal perfused via a sharp cannula inserted into the left ventricle. The perfusate which consisted of 2 percent glutaraldehyde and 2 percent paraformaldehyde in phosphate buffer (pH 7.3) was allowed to flow for 10 minutes. By this time 15 to 20 mls of perfusate had passed through the animal. Heads were removed, immersed in perfusate and refrigerated overnight before removal of the brain. Tissue for study was obtained by making 2 parasagittal cuts, approximately 1mm on either side.

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side of the longitudinal fissure of the cerebrum. Next, a cut close to the midline was made. The resulting two slices of tissue were trimmed anteriorly at the preoptic-anterior com-
miszure level and posteriorly through the superior colliculus-midbrain level. These slices
were post-fixed in 1 percent phosphate buffered osmium and processed through a graded
series of acetones into propylene oxide and finally epon.
Sampling of epon embedded brain was done initially in the coronal plane at 2 levels
through the arcuate nucleus. However, it became apparent that sampling in the parasagittal
plane close to the midline was a more efficient method of visualizing the arcuate nucleus in
its entire anterior-posterior extent. This became the method of choice and most control and
-treated mice were sampled in this plane. If initial epon sections were midventricle, further
sections were cut laterally at several levels.
For study by electron microscopy, the large tissue slices were trimmed to include the
arcuate nucleus, peripheral neuropil and median eminentia. Ultrathin sections (1 μm) were
stained with uranyl acetate and lead citrate and examined with an RCA EMU 3H electron
microscope.
5. Preparation of Brain for Light Microscopy. Brain fixation was achieved by immersion
of fresh whole brain in 10 percent formalin. The specimens were routinely dehydrated in an
alcohol series, placed in one percent celloidin in methyl benzoate for two days, then processed
in benzene to paraffin. Serial sections were cut at 10 μ and stained with Cresyl Echt-Violet
solution.
Sampling of paraffin embedded brains consisted of examination of serial sections of brains
from four 9-day-old hybrid mice treated with 4 mg/gm MSG for 3 hours. Sections were
cut in the parasagittal plane from olfactory bulb to cervical spinal cord. Three 7-day-old
animals given MSG at 1 mg/gm body weight were similarly studied. For optimal interpreta-
tion of the structures involved in lesions occurring in the lower medulla, the brains of 9
animals ranging from 8 to 10 days of age were serially sectioned in the coronal plane from
the level of the inferior olives to the level of the cervical spinal cord. Five male mice which
had been chronically treated with MSG as neonates were killed at 10 months of age and
serial sections through the entire brain were made, 3 in the coronal plane and 2 in the
sagittal plane. To serve as controls the brains of ten sham-injected animals ranging in age
from 8 days to 3 months were serially sectioned in both coronal and sagittal planes.

RESULTS

1. Time Course for the Arcuate Nuclear Lesion in Neonatal Mice Following a
Single Oral Dose of MSG

In order to gain more information as to the nature of the lesion that follows
the ingestion of large doses of MSG by the mouse, it was of prime importance
to confirm the nature of the cellular components involved in the lesion and the
order of their involvement. Were the damaged structures primarily ependymal
cells, capillary cells, glial cells or neurons? In what sequence, if any, did these
cells manifest pathological change? To answer these questions a series of
animals were given MSG (4 mg/gm) in a 20 percent solution via stomach tube
and killed at 10, 15, 20 and 30 minute intervals for short-term morphological
studies and at 1, 1.5, 3, 6, 12, 24, 36 and 48 hours for long-term morphological
studies. No central nervous system (CNS) lesion was observable by light
microscopy in mouse brains obtained 10 to 15 minutes following administration
of MSG. With the electron microscope, however, there was some evidence that a
small number of astrocytic somata showed early signs of edema at 15 minutes
while ependymal cells and neurons appeared normal. However, 20 minutes after
the ingestion of MSG, lesions were easily observable by either light or electron microscopic methods. With the light microscope, swollen spaces in the arcuate neuropil were the first evidence of abnormality. Electron microscopic observations again revealed that one of the first elements to appear abnormal was the astrocytic glial cell. The somas and astrocytic processes in the central area of the lesion, but not the margins, appeared markedly swollen. Some large dendrites showed early evidences of abnormality, namely, the appearance of large vacuoles, and expansion of agranular reticular profiles. By 20 minutes, neuron somata exhibited abnormalities such as dispersal of ribosomes, swelling of the agranular reticulum and occasionally mitochondrial swelling. The affected areas were those closest to the median eminence. Thirty minutes after ingestion the lesion could be easily detected in plastic sections (fig. 1) since the damaged area was filled with large dilated processes, and some neurons appeared swollen with vacuolated cytoplasm. Ultrastructural changes were mainly an expansion of phenomena observed at 20 minutes. Great numbers of vastly dilated glial and dendritic profiles filled the fields examined by electron microscopy. It was not always possible to distinguish between the two cell processes since massively dilated and vacuolated dendrites could only be identified if they possessed synaptic contacts. Moreover, in neonatal material, synapses, which are just forming, are not found in great numbers. That dendritic profiles were indeed involved is shown in figure 2 in which two synapses are found on

![Image](http://jnen.oxfordjournals.org/) Fig. 1. Pansagittal epon section through the arcuate nucleus of an 8-day-old mouse indicating the degree of damage to the arcuate nuclear area thirty minutes after oral administration of MSG. The most obvious feature is dilated spaces in the neuropil which represent edematous astrocytic and dendritic processes. Close inspection shows neuronal somas are swollen as well (arrows). Methylene Blue—Azure II. × 180.
Fig. 2. Electron microscopic field of arcuate nucleus from an 8-day-old mouse shown in figure 1. An astrocytic cell (A) and its processes are markedly edematous and many swollen profiles are seen in the neuropil. That at least some of these profiles are dendritic (D) is demonstrated by the presence of synapses on two of the profiles (arrows). An apparently normal neuron (N) lies to the right of the astrocyte. × 11,750.
extensively dilated dendrites. Thirty minutes after the administration of MSG, a large number of neurons are drastically affected. The cytoplasm becomes dense and the disarray of the agranular reticulum and dispersal of ribosomes throughout is marked. At this time many nuclei show clumping of chromatin along the periphery (fig. 3). In some neuronal profiles exhibiting advanced organelle disarray, large membrane-line "vacuoles" appear in the cytoplasm. In other profiles, cytoplasmic vacuolation appears without presence of internal membrane. For the first three hours after MSG administration the predominant picture is that of astrocytic and neuronal edema, vacuolation of neurons and their processes and nuclear pyknosis. All other tissue elements appear normal.

Between three and six hours after administration of MSG the second stage of the lesion, that of phagocytosis, begins. At six hours, observations by light microscopy (fig. 4a) indicate that the number of pyknotic nuclei has greatly increased. Numerous basophilic cells occur within the lesioned area and at its periphery. Electron microscopic observations indicate that the basophilic cells are phagocytic in nature and would probably best correspond to current descriptions of "microglia". Within these cells are found whorled, lamellated bodies and clumps of cytoplasmic debris (fig. 5). Typically, extremely pyknotic cells and dilated dendrites were found to be surrounded by the processes of these dark, phagocytic cells. At twelve hours the situation is essentially the same, although the frequency of lamellated membranous whorls occurring in the microglial cells may be increased, as if these represented later stages in the breakdown of ingested elements.

At twenty-four hours, the appearance of the lesion by light microscopy has changed markedly. Very few edematous processes are found within the lesion which now appears to be filled with phagocytes and nuclei in advanced stages of pyknosis. Random microscopic fields observed in corresponding ultrathin sections reveal a reduced number of dilated dendritic processes and necrotic cells. Swollen profiles of dendritic processes along with considerable numbers of "normal" appearing neurons persisted. The most striking change on the ultrastructural level was the proliferation of astrocytic processes (fig. 6a) which can be easily distinguished from those of the dark microglial cells. Phagocytic inclusions were numerous in both the somas and processes of astrocytic glial cells. At thirty-six and forty-eight hours, the number of neurons was drastically diminished (fig. 4b) and large numbers of phagocytes were present in each section. From electron microscopic observations astrocytes and microglia seemed to be present in equal numbers, however astrocytic processes predominated. Both cell types showed continued evidence of vigorous phagocytic activity. At this time microglial processes contained many large dense bodies and vacuoles (fig. 6b).

2. Time Course and Spread of Lesions in Neonatal Brain Following Dosing

From observations confined to diencephalic-midbrain levels (epon sections) in all experimental situations the first areas to sustain lesions were the arcuate and/or the preoptic region of the hypothalamus. These changes were observed
Fig. 3. Electron micrograph from same animal as figure 1 and 2 showing one of the drastically affected neurons. The agranular reticulum (AGR) is vastly expanded, the ribosomes are dispersed throughout the cytoplasm, the nuclear chromatin is clumped toward the periphery of the nuclear membrane. A synapse (arrow) on the projecting dendrite unequivocably identifies this profile as a neuron. $\times$ 11,975.
Fig. 4a. Parasagittal epon section through the arcuate nucleus of an 8-day-old animal representing degree of damage six hours after oral administration of MSG. There is a dramatic change in the number of damaged neurons which by now exhibit pyknotic nuclei and markedly swollen somata. Scattered throughout the neuropil are dark basophilic elements (arrows) which are probably microglia. Methylene Blue—Azure II. × 172.

Fig. 4b. Parasagittal epon section through the arcuate nucleus of a 10-day-old mouse representing the appearance of the lesioned area 48 hours after oral administration of MSG. Basophilic cells (macrophages) persist, and some neurons (arrows) persist although in vastly depleted numbers. Stain as above. × 200.
Fig. 5. Electron micrograph of major phagocytic cell from the same animal as seen in figure 4a. Totally engulfed within this phagocyte, tentatively identified as a microglial cell, are the remnants of a degenerated arcuate neuron (N), including its pyknotic nucleus (PN). The cytoplasm of some, but not all, degenerating arcuate neurons becomes totally dense as well as vacuolated as seen in this profile. Note that microglial processes (MP) at the same time surround two degenerating profiles (D). X 11,750.
Fig. 6a. Electron micrograph of astrocyte at 24 hours. At this time, astrocytes actively participate in the phagocytic process and continue to do so for at least as long as the 48-hour sampling period. Note that both the somata and astrocytic processes are filled with debris (*) and not merely “dense bodies”. × 8,172.

Fig. 6b. Electron micrograph of microglial cell at 48 hours. While some microglial cells still contain entire portions of degenerating neurons, most profiles show large numbers of vacuoles (v) and multivariegated dense bodies (db). × 8,172.
15 to 60 minutes after dosing. In some animals lesions could be detected also in the habenular nucleus and the most superficial layers of the tectum. By two hours, the lesion had spread inward from the involved outer surface of the brain. This change occurred in the tectum and habenular and areuate nuclear regions. In this time interval the outer aspects of the dorsolateral thalamus and the subfornical organ were also damaged (fig. 7). Lesions in the tectal area were of considerable interest since the tectum is separated from cerebrospinal fluid by pial-glial membranes rather than ependymal cells. In 2 to 3 hours the teetral lesion resembles that of the areuate nucleus in that the neuropil is filled with edematous processes and vacuolated neurons, some of which contained

Fig. 7. Parasagittal epon section of 7-day neonatal mouse (ICR) given 4 mg/gm MSG orally and sacrificed 2 hours subsequently. It can be seen that the lesion has spread to many areas other than the "areuate region", viz. the preoptic region (PO), the subfornical organ (SFO), the thalamus (TH), the habenular nucleus (HAB) and the tectum (TECT). Methylene Blue—Azure II. × 40.
pyknotic nuclei. By 24 hours the lesion in the tectum presented an appearance never observed in the arcuate nucleus, i.e. the development of large cystic spaces almost devoid of neuronal or glial cells (Fig. 8b). These observations suggest that lesions in the inferior and superior colliculi are more substantive and potentially more capable of producing functional disabilities than lesions in the arcuate regions. Lesions in the subfornical organ observed at 3 hours were very similar to those of the arcuate nucleus (Fig. 8a). Most of the neurons appeared highly vacuolated with pyknotic nuclei. With exception of the tectum, the progress of lesions observed in other brain areas tended to resemble those of the arcuate nucleus.

3. Incidence of Lesioned Areas in Serial Sectioned Mouse Brain

Observations made on sections embedded in epon could not be extended to include sagittal sections of whole brain. Thus a series of whole mouse brains inclusive of the olfactory bulb to the level of the cervical spinal cord were embedded in paraffin and sectioned in the sagittal plane.

The evaluation of lesions in Nissl-stained paraffin sections involved slightly different criteria than those used for plastic sections. Careful use of control sections was necessary. In paraffin sections, areas with lesions showed "bloating" or edema plus the presence of abundant pyknotic nuclei. Vacuolation of cell somas and dilation of processes in the neuropil readily discernible in ultrathin sections could not be discretely observed in thicker paraffin sections. The appearance of arcuate lesions in epon sections (Fig. 1, 4a–b) can be contrasted with the same seen in paraffin in figure 9.

Examination of serial paraffin sections confirmed the presence of lesions in the diencephalic-midbrain area and permitted observation of additional areas of involvement. Figure 10a shows lesions involving the rostral and superior surfaces of the colliculi. Large areas surrounding the thalamic portion of the third ventricle also are involved. Serial sections demonstrated massive damage to dentate-hippocampal gyri surfaces abutting the ventricle, while the entire superficial dorsolateral surfaces of the thalamus and hippocampal-parahippocampal cortex were also extensively affected (Fig. 10b). Figure 10d depicts the relationship of the hippocampal-cortical lesions to the lateral ventricles. Lesions could be found in the olfactory bulb (Fig. 10c) but were confined to regions abutting the small finger of ventricle protruding into the core of the olfactory bulb. A large expansive lesion was observed in lower medullary levels (Fig. 11a). In sagittal sections, the lesion could be traced from the spinal canal to lateral levels. Coronal sections showed that the superficial layers of the nuclei gracilis and cuneatus were edematous, containing cells with pyknotic nuclei. The area postrema varied in degree of damage from animal to animal. In some instances the damage was characterized by numerous pyknotic nuclei while in other cases by numerous edematous cells. Another region of brain frequently showing superficial lesions was the molecular layer of cerebral cortex. This part of the brain, like tectum, is separated from cerebrospinal fluid by pial-glial
Fig. 8a. Coronal epon section of a lesion as it appears in the subfornical organ 3 hours subsequent to MSG dosage. There is marked similarity to the appearance of the damaged arcuate nucleus, viz. edematous processes in the neuropil, swollen neuronal somata with pyknotic nuclei, and infiltration of basophilic elements (macrophages) (arrows). Methylene Blue—Azure II. × 228.

Fig. 8b. Development of a lesion in the lictum of the mouse 24 hours subsequent to oral dosage. Large cystic spaces developed in this structure—a phenomenon not observed in other damaged regions of brain. Stain as above. × 59.
membrane rather than by ependymal cells. Lesions here might be small focal areas as shown in figure 11b or massive ones involving almost an entire lobe.

4. Incidence of Brain Lesion and Mouse Strain

During the course of this study strain differences in response to MSG were suggested. At the lowest dose studied the arcuate and sometimes the preoptic area were involved in the lesion formation in all mouse strains. In the hybrids small lesions were observed in the area postrema and dentate gyrus as well. At 2 mg/gm body weight in inbred strains the lesion was confined to the arcuate nucleus although in the A/Jax strain the subfornical organ was sometimes involved. In hybrid mice at this dosage the area of lesion included the habenular nucleus and tectal areas. It is possible that lesions occurred in brain areas other than the diencephalon and midbrain at the 1 and 2 mg doses both in inbred and hybrid animals, since serial sections of entire brains were prepared only for hybrids at 1 and 4 mg/gm body weight doses. Thus, for inbred strains, only diencephalon and midbrain areas were sampled. In these preparations the thalamus always contained a lesion in the ICR mice but not in the A/Jax mice. Three points seem to emerge from our observations: 1) One of, if not the most vulnerable area to neuronal damage appears to be the arcuate region. 2) At a given dose of MSG, hybrid strains sustain lesions in more brain areas than do the inbred strains. 3) With large dosages of MSG, brain regions other than the arcuate area are susceptible to the development of substantial lesions.
Fig. 10. Sagittal paraffin sections showing extent of damaged areas in brain other than hypothalamus after a 4 mg/gm dose of MSG. Brains were processed 3 hours after dosage. These figures and subsequent figures of paraffin sections received the same experimental treatment. All Cresyl Violet.

a. The external layers of both superior (SUP) and inferior (INF) colliculi are damaged as are midbrain levels surrounding the cerebral aqueduct (CA). $\times 35$.

b. Portions of dentate gyrus (DG) abutting the ventricles as well as parahippocampal gyrus (PHG) show damage. Serial sections show that large amounts of the dorsolateral thalamus (TH) facing the third ventricle are similarly damaged. $\times 64$.

c. Olfactory bulb lesions (arrow) are confined to small areas surrounding the extension of the ventricle. $\times 64$.

d. Some large lesions were observed to occur in brain regions bordering the lateral ventricles (arrows). In this instance, the external surfaces of the hippocampal lobe (H) and cerebral cortex (C) are involved. $\times 56$. 

Fig. 11a. The lower medulla frequently manifested an expansive lesion which could be traced from spinal canal to the most lateral surface. Sagittal section showing appearance and extent of the lesion which in this case is well limited. It was observed that the lesion in other animals sometimes involved only the more superficial layers of cells bordering the fourth ventricle. Cresyl Violet. × 85.

Fig. 11b. Sagittal paraffin section of cerebral cortex from MSG-treated mouse. Focal lesion of cerebral cortex. Such lesions were found frequently in mouse brain serial sections. Stain as above. × 85.
Although the numbers were too small for statistical validity, it was interesting to note that the 8 animals 10 or more days old (hybrids and inbreds) given a 1 mg/gm body weight dose, failed to sustain lesions. The lesion rate for 6- to 8-day-old animals given this dose was 80 percent. Three of four 10-day mice treated with 2 mg/gm body weight dose sustained a lesion; the lesion rate mice mice 7 to 10 days old given this dose was 88 percent. All animals given MSG at 4 mg/gm body weight exhibited a lesion. Thus, there is an indication that slightly older animals (at the lowest dosage employed) may be less susceptible to MSG.

5. The Effect of Monosodium Glutamate on Adult Mice

Almost all work completed thus far has focused on the toxicity of monosodium glutamate for the neonate. The two reports (4, 22) suggesting that arcuate lesions can be induced in adult mice at the grossly high dosages of 5 to 10 mg/gm body weight have been largely ignored and unconfirmed. Thus, we decided to administer MSG to a series of adult inbred mice at the following doses: 2, 4, 5 and 7 mg/gm body weight. Sampling was limited to epon sections of the diencephalic-midbrain levels. In every animal an arcuate nuclear lesion developed within the 3 to 5 hour dosage period (Fig. 12) though it was never as acute or expansive in appearance as the lesion observed in the neonate. Furthermore, the lesion was not observed in other nearby brain regions, such as preoptic nucleus, habenular nucleus or tectum.

Fig. 12. Sagittal epon section through hypothalamus of an adult mouse treated with 2 mg/gm MSG intraperitoneally and sacrificed within 3 hours after dosage.

Low power view showing less extensive lesion in adult which may be contrasted to the expansive lesion found in neonates. Methylene Blue—Azure II. × 85.
DISCUSSION

1. Pathological Changes Involving Neurons and Glia in the Arcuate Nucleus

Although there have been general descriptions of the progress of the lesion in the retina (21) and in the arcuate nucleus (23) the phagocytic elements involved in the process have not been identified.

At the electron microscope level our observations confirm the involvement of at least two distinct types of glial cells in the evolution of the lesion. One of these is a “dense” cell, which can be correlated with the large densely staining basophilic cell observed by light microscopy. The second is a cell with electron lucid cytoplasm. While identification of the latter cell type as astrocyte is not difficult, easy identification of the “dense” cell is more controversial. A detailed analysis of this phagocytic process will be reported elsewhere (16).

Pathological changes in the arcuate nucleus were found to occur in three distinct phases: 1) neuronal degeneration and astrocytic edema, 2) phagocytosis first by dense cells that might be microglia or a neuronal type similar to that of Vaughn and Peters (39), and 3) many hours later phagocytosis by astrocytes which simultaneously proliferated to fill the spaces left by degenerating neurons and their processes.

2. The Incidence of Damage to Other Areas of Mouse Brain

The CNS lesion induced by MSG was not confined to the arcuate nucleus but spread with time to other areas of the brain. In every instance, the damaged area occurred in a region of the CNS which was in close proximity to circulating cerebrospinal fluid and damage was initiated in that portion of a structure bordering CSF. At high doses, the lesion was found to be non-specific for distinct nuclear groups, spreading instead as a broadly diffuse or radiating band. In no instance was a lesion seen to begin within the geometric center of a given structure and radiate outward in a circular fashion, nor could the lesion ever be correlated with the pattern of distribution of blood vessels.

With time, the lesion radiates steadily inward from those areas adjacent to the CSF. Observations of lesions in the inferior and superior colliculi best illustrate this. Lesions first appear on the outer surface but with time they are observed to envelop deeper and deeper collicular layers (Fig. 7).

The same pattern occurs in the arcuate nucleus where thirty to 60 minutes following a 4 mg/gm body weight dose of MSG, the lesion is more or less contained in the nuclear area bordering the infundibular recess. After 2 to 5 hours the lesion has spread dorsally to encompass portions of the ventromedial nucleus (fig. 7).

These observations suggest that one of the access routes to central neurons, if not the route of entry of the deleterious agent is via cerebrospinal fluid. We cannot rule out entry from some blood vessels since in the well-documented lesions occurring in the retina (17, 21) direct access to CSF is lacking. Nor can the possibility that the deleterious agent enters from capillary systems in which the blood brain barrier is modified be ruled out as a possible contributory
access route. The presence of fenestrated capillaries in the median eminence—neurohypophysis (34), adjacent to the arcuate nucleus, as well as in the subfornical organ and area postrema (41) might be involved in facilitating entry. It was disconcerting to note, however, that cells of the subfornical organ and area postrema in many instances did not sustain a lesion, while arcuate cells were consistently found damaged.

3. Nature of the Toxic agent and Arcuate Neuron Susceptibility

The observation that the arcuate nuclear region is particularly susceptible to neuronal damage following ingestion of MSG raises two questions of prime importance. First, following administration of MSG either by the subcutaneous route or the oral route, what is the final molecular moiety which enters the brain substance and causes the death of neurons, and second, why are cells in the arcuate nuclear region so susceptible to this agent?

Most workers have found that the oral or parenteral administration of glutamic acid has no effect on total brain glutamate levels in the adult rat (9, 12, 19, 20, 29, 30, 33). In one report of 24 hour old neonatal mice receiving MSG by injection, there was no change in brain glutamate levels at 30 minutes and a small increase after 3 hours (38). But in another report involving 24 hour old rats, brain glutamate levels were found to increase 30 minutes after injection of MSG (10). Rats fed a diet containing up to 20 percent for 16 weeks experienced no alteration in brain glutamate levels (29), but mice fed 1.25 gm/kg of MSG for 8 weeks were reported to have doubled their brain content of glutamic acid (13).

From microchemical assay for glutamic acid of tissue samples obtained by microdissection of the arcuate nuclear region, Perez and Olney (28) reported increases in glutamic acid for this region of mouse brain following subcutaneous administration of MSG. They found the glutamate level doubled in 15 minutes, which is about the time we observed the first evidence of a lesion. Their study infers that a localized increase in glutamic acid accompanies the development of the lesion found in the arcuate area after subcutaneous dosage of MSG. However, proof that glutamic acid is the actual substance entering the arcuate nuclear area (rather than being secondarily induced) and still further, that glutamate is the final molecule reacting with neuronal and/or glial membranes is unanswered. Just what substance does enter the mouse brain after oral administration of MSG?

A more recent method employed to resolve the question of what molecules enter the brain following MSG administration has involved the use of radioisotopes. Lajtha et al (15) showed no net transfer of $^{14}$C-glutamic acid into whole brain in adult mice and rats although small amounts of labeled glutamate quickly reached the brain suggesting that there is rapid exchange between blood and brain glutamic acid pools. Studies in our laboratories have shown that neonatal or adult mice injected with $^{14}$C-L-glutamate rapidly convert large quantities into glucose, acetoacetate, a-ketoglutarate and lactate, with
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Glucose and acetooacetate predominating (5, 32). Contrasting, glucose and lactate predominate after administration of 14C-glutamate to the neonatal pig or monkey, and little if any acetooacetate and a-ketoglutarate are noted (36, 37). Interestingly, acetooacetate is the major ninhydrin-negative metabolite found in whole mouse brain extracts following glutamate injection (5).

The majority of published studies support the notion that glutamic acid enters the brain in small amounts representing exchange between glutamate in plasma and brain tissue. High circulating levels of glutamic acid are seemingly not followed by aceretion of brain glutamate.

Glutamic acid and its metabolite, alpha-ketoglutaric acid, are putative neurotransmitter substances (8, 14). The concentration of glutamine plus glutamic acid within mammalian brain far exceeds that of any other amino acid (18). Further, glutamic acid is one of the amino acids whose increase in concentration during rapid brain growth provides an index of brain maturation (3, 11). The fact that high peripheral levels of glutamic acid are not reflected in brain levels of the substance strongly suggest that most glutamate found in the brain is synthesized there. Local brain regulation of glutamic acid levels and relative impermeability of the blood-brain barrier to glutamic acid are probably mandates for the role of a neurotransmitter substance. Some evidence points toward a metabolite of glutamic acid, perhaps acetooacetate, as being the deleterious agent responsible for the lesion rather than glutamic acid itself.

The second question of why the neurons in the arcuate region appear to be initially the most susceptible to damage is even more difficult to answer. It would seem that a combination of factors may be at work: a) variations in cell metabolism and/or b) local concentration gradients of the toxic substance be it glutamic acid or a metabolite. It has been observed that those cells in close proximity to cerebrospinal fluid are the cells which are damaged (retinal ganglion cells being the only exception), but that not all brain regions next to cerebrospinal fluid are equally susceptible. Perhaps neonatal arcuate neurons are least able to survive large local increments of glutamic acid or its metabolites when these molecules rapidly pernecate the neuropil. It is possible that arcuate nuclear susceptibility is related to the fact that ependymal cells in the arcuate nuclear region are different from those in other parts of the CNS.

Brawer (6) has observed that arcuate tanyeytes possess zoulae adherentes instead of maculae ocellundentes, meaning that the extracellular space between adjacent ependymal cells may extend uninterrupted into the substance of the arcuate nucleus. However, a molecule as small as glutamic acid should have free passage from cerebrospinal fluid into brain extracellular space at any ependymal site. Perhaps we should reemphasize here the proximity of arcuate neurons to fenestrated capillaries. Ready passage of many substances, including glutamic acid, might be expected through fenestrated blood vessels such as occur in the external layers of the median eminence and the neurohypophysis and this factor might contribute to a faster rate of influx here than in other areas. It should be repeated that arcuate lesions initiate in regions closest to the
median eminence. The fact that neurons of the subfornical organ and area postrema are similarly affected might point to this route, although these cells do not seem as vulnerable as arcuate neurons.

4. **MSG Toxicity in Neonates vs. Adult Mice**

The problem of whether MSG toxicity is limited to the neonate and if so, to what extent, deserves consideration. A major finding in this study was the observation that the adult mouse brain arcuate nucleus sustains a readily detectable lesion after a dosage as low as 2 mg/gm body weight (the lowest dosage employed). While neurons were damaged unequivocally in the adult, the number of involved cells and areas of damage at this dose were markedly less than those of any of the neonates given an equivalent oral dose. These observations are supported by the fact that at 1 mg/gm body weight older neonates (10 to 12 days of age) sometimes fail to sustain an observable lesion. It appears that susceptibility of neurons to damage does change with maturation, but the threshold of susceptibility is not an absolute one at large doses.

With regard to MSG toxicity, it must be remembered that doses in the 1 to 4 mg/gm body weight range are exceedingly high. At levels of 1 mg/gm body weight a lesion is not always found. Thus, the MSG lesion in the mouse is a phenomenon of "unphysiologic" or very high doses. In our laboratory systematic sampling studies at lower dose levels (0.1 to 0.5 mg/gm body weight) are underway. The potential value of MSG as a selective agent for inducing chemical lesions in arcuate neurons is still to be exploited.

5. **Interpretation of Neuropathological Results Following MSG Administration**

Many of the various investigators studying the neuropathologic changes following the administration of monosodium glutamate (1, 2, 4, 7, 22–25, 27) have overlooked or underemphasized one extremely important variable, viz., the route of administration. Many of these studies have involved subcutaneous routes (2, 4, 21–23, 25, 28). This method while having certain experimental advantages, ignores relevance to nutrition. The interpretation of lesions following subcutaneous or intraperitoneal versus oral administration have often failed to take into account differences in brain susceptibility derived from route of administration. Abraham et al (1) did find oral administration of MSG less profound in its effects upon the arcuate nucleus in mice than subcutaneous dosing. Investigators employing procedures involving oral dosing must consider such variables as maturation of the intestinal mucosa and rate of intestinal absorption. There is some evidence that the nutritive state of man or animal influences glutamic acid absorption by the intestinal mucosa (35, 36, 40). Studies involving all routes of administration should consider factors affecting liver metabolism of glutamic acid such as maturation of enzyme systems. All of these factors influence the rate of passage of glutamate into the circulation, and ultimately the amount of glutamic acid or its metabolic by-products available to enter the brain.
Another major consideration in evaluating and comparing reports dealing with MSG-induced lesions is tissue sampling methods. The extent of sampling and levels of brain examined have usually been very sketchily described. It would seem to us that the method of choice for sampling of any given region of brain is by serial sections of plastic embedded material. This method permits observation of the most minute lesions, since these sections readily show edematous neurons and dendrites, even at early stages. However, this method is extremely tedious for even small regions of the brain and of doubtful feasibility for surveying damage throughout the entire brain of a large series of experimental animals. It is also difficult to apply to whole brain whose dimensions exceed that of neonatal mouse. An alternate method of sampling is serial paraffin sections throughout entire neonatal brains. Experience in our laboratory has shown that with frequent referral to control material, paraffin sections are adequate for showing large to medium-sized lesions. Serial paraffin sections can quickly and easily demonstrate the full extent of well-developed lesions, and of course permit easy sampling of an entire brain. However, we do think caution must be exerted when paraffin sections are used for “all or none” detection of brain lesions.

A “microlesion” consisting of but 2 to 3 cells in a single plane of section and observable only in serial 1 μ plastic sections has been reported (26). This “microlesion” was found only in the subependymal portion of the infundibular nucleus of neonatal monkeys receiving large oral doses of MSG, in contrast to the massive lesions sustained in the lateral hypothalamic extensions of rodent arcuate nucleus. This interesting finding, if confirmed, raises the further consideration of the clinical significance of 50 to 90 lesioned cells. Numbers of lesioned cells approaching this order of magnitude must be compared with the ongoing rate of cell death due to excessive neuronal proliferation characteristic of developing brains. However, if such microlesions should give evidence of clinical significance, revisions would be necessary in sampling methods in order to detect the presence of brain lesions in circumscribed brain areas.

REFERENCES


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