

Fig. 1. Enlargements of active stalactite tips. (A) Drop on stalactite tip (diameter 11 mm). (B), (C), and (D) Enlargements of (A) ( $\times 6$ ). Striped areas indicate calcium carbonate.

tites, and other limestone structures. With a special microscope, mounted horizontally on an adjustable bracket, sliding along a vertical steel bar, stalactites were observed *in situ*. As in telescopes, the eye piece on the horizontal microscope was attached at a right angle by means of a prism, making it possible to observe even in tight spots. A flashlight was mounted near the microscope to illuminate the stalactites.

Along the limestone surface of all active stalactites, short very thin hyphae extended perpendicularly (Fig. 1D). Some were branched and had conidiphores. On dissolving the limestone with dilute acetic acid, the mycelium was found only in the surface layer of the stalactites, and only sparingly. Some hyphae extended into the surface of the water drop hanging from the stalactite.

Whereas some of the growth of the stalactite surface was obviously by extension of the existing surface crystals downward into the drop surface, a large number of smaller and larger crystals and crystal aggregates were dispersed in the drop surface, having no continuity with the crystals of the stalactite (Fig. 1, B and C). Yet in some way the crystals were attached: When individual stalactites were observed over a 24-hour period, the unattached crystals retained the same relative position (1 to 20  $\mu$  distance) to the stalactite tube. In a number of cases hyphae were seen to connect these crystals to the body of the stalactite, and in other cases strings

of crystals formed in a long arc, along hyphae which connected at both ends with the stalactite (Fig. 1A). Thus the hyphae function both as crystallization nuclei and as attachment, without which individual crystals would be eliminated by the falling drop. In one case a cotton fiber was attached to the tip of a stalactite, and all along its length, as far as it was coiled up and immersed in the drop, a row of crystals covered the fiber. From it, secondary strings of crystals emerged, apparently along hyphae.

It is conceivable that the presence of these hyphae allows the orderly

growth of stalactites, which, if entirely dependent upon accretionary growth of preexisting crystals, do not grow straight down, as found in the case of helictites.

Cultures were made on nutrient agar of the microorganisms living in and on active and inactive stalactites. Cultures from the terminal drop and the active rim of the stalactite gave, in five out of six cases, pure cultures of *Cephalosporium lamellaecola* F. E. Smith, a slow-growing fungus with very fine white mycelium; in one case bacterial colonies developed in addition to the fungus. In another test, 42 out of 46 cultures from stalactite tips produced *Cephalosporium*. Of the 100 microorganisms developing on helictites, not a single one was *Cephalosporium*. In the active stalactites investigated in Lehman Caves the same fungus was associated with them, and no other microorganisms, and certainly no bacteria, can be involved in their growth. The nutrition of these fungi must depend on organic materials that are leached from soil and move down with the calcium bicarbonate solution seeping into the cave. All calcium carbonate deposits of known biological origin in oceans (corals, algae, and molluscs) are attributed to the activity of an enzyme, carbonic anhydrase, produced by these organisms.

F. W. WENT

Desert Research Institute,  
University of Nevada System,  
Reno 89507

#### Note

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## Brain Lesions in an Infant Rhesus Monkey Treated with Monosodium Glutamate

**Abstract.** In an infant rhesus monkey brain damage resulted from subcutaneously administered monosodium glutamate. Although a relatively high dose of monosodium glutamate was used, the infant was asymptomatic for a 3-hour observation period during which time hypothalamic neurons were undergoing a process of acute cell death. With the electron microscope it was observed that dendrites and cell bodies of neurons are the tissue components primarily affected in brain damage induced by monosodium glutamate.

Susceptibility of the developing central nervous system to damage from subcutaneously administered monosodium glutamate (MSG) has been observed in every species of experimental animal tested thus far—mice (1, 2), rats (2, 3), and rabbits (4). In mice,

which have been studied more extensively for MSG-induced brain damage than other species, the lowest effective dose for the baby animal (0.5 g/kg) was approximately one-tenth that for the adult (5 g/kg) (2). Additional studies are needed to clarify mecha-

nisms underlying the MSG effect and to elucidate the basis of enhanced vulnerability on the part of the immature nervous system. In the meantime, the question arises whether glutamate could have an occult etiologic involvement in any of the unexplained brain damage syndromes occurring in the course of human ontogenesis and whether the widespread practice of feeding glutamate-enriched diets to human infants is a wise one (5). The feasibility of studying these questions in the primate is suggested by our evidence that the infant rhesus monkey (*Macaca mulata*) is susceptible to glutamate-induced brain damage.

Our report is based on only one test subject because we were unable to obtain additional baby monkeys at this time. However, the pattern of neuronal necrosis induced in the hypothalamus of experimental animals by MSG is highly selective for certain cell types and has a very distinctive appearance. Furthermore, as a frame of reference, we have extensive light and electron microscopic data pertaining to the evolution of this type of lesion in the retina (6) and the hypothalamus (7) of numerous rats and mice. The fact that the margins of the MSG lesion are sharply demarcated was helpful for evaluating fixation variables with the

electron microscope because normal, well-fixed cells of every kind typical for a given region could be examined, just beyond the margin of the lesion, for comparison with degenerating cells within the damaged area.

We separated an infant rhesus monkey from its mother 8 hours after birth; the infant was an alert, healthy-appearing male with an active cry and appropriate spontaneous motor activity. However, it weighed only 260 g and measured 16.5 cm from crown to rump, so that, judged by size, it would probably be classified as a premature infant (8). Glutavene, a commercially available preparation of MSG in 25 percent aqueous solution, was injected subcutaneously in a volume of 2.8 ml, the total dose being 0.7 g or 2.7 g per kilogram of body weight. The treated infant was then held and cared for in a maternal manner (but not provided with food) for a 3-hour observation period; during this time there were no manifestations of a central nervous system disturbance. Three hours after treatment the infant was given 1 mg of Sernylan (Parke, Davis) intramuscularly, which provided excellent anesthesia characterized by a deep sleep with loss of responsiveness to painful stimuli but with retention of full rhythmic respirations. Thoracotomy was then performed so that a cannula could be clamped into the ascending aorta,

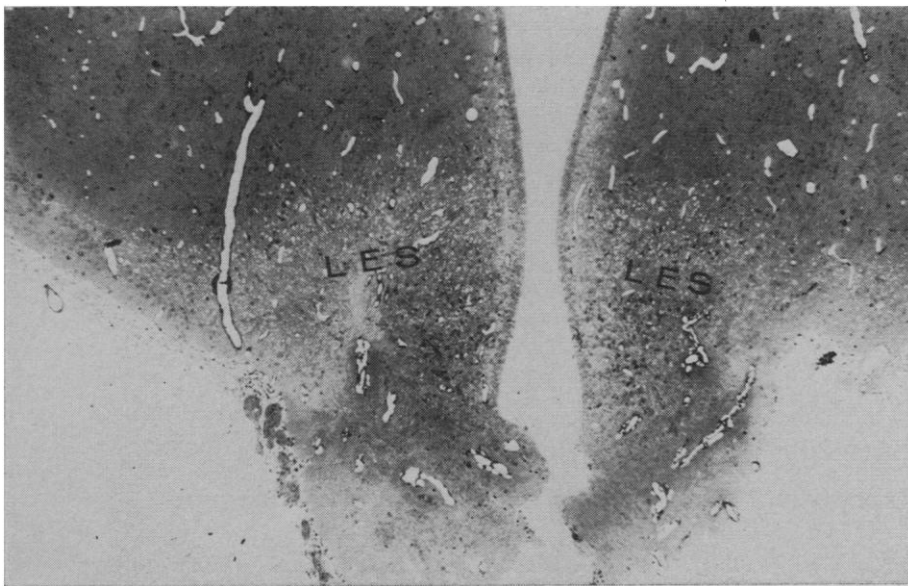


Fig. 1 (top). Cross section of the ventral hypothalamus cutting through the infundibular stalk. The lesion (LES) affects the periventricular-arcuate regions bilaterally, giving these areas a rarefied appearance. A "Swiss cheese effect" is created by the dilatation of dendritic processes. The larger holes and open spaces are dilated blood vessels resulting from perfusion fixation ( $\times 50$ ).



Fig. 2 (bottom left). An electron micrograph showing a massively dilated dendritic process (*d*) in synaptic contact with a normal-appearing axon terminal (*a*). The internal content of the dendrite consists primarily of diffusely distributed particulate debris. The axon is not swollen and contains numerous synaptic vesicles and normal-appearing mitochondria ( $\times 10,300$ ).

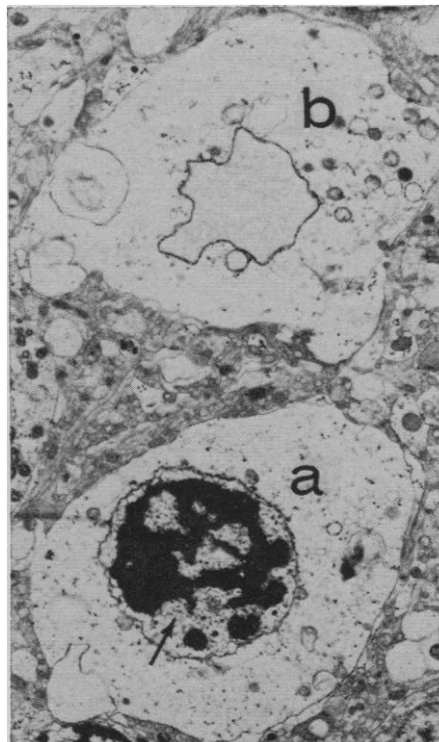


Fig. 3 (bottom right). An electron micrograph of two degenerating neurons (*a* and *b*) illustrating alteration of nuclear chromatin pattern (arrow, *a*) and disintegration of cytoplasmic components. The membrane system comprising the endoplasmic reticulum has degenerated beyond recognition and mitochondria have either ruptured or become completely spherical ( $\times 6000$ ).

and perfusion of the brain was begun within 30 seconds. The perfusate consisted of 3 percent glutaraldehyde in 0.1M cacodylate buffer and 0.02 percent  $\text{CaCl}_2$ . After 20 minutes of perfusion, the brain, pituitary gland, eyes, and optic nerves were removed and placed in jars containing the perfusion fluid. Areas of special interest were dissected out from these tissues which were then fixed further in osmium tetroxide for 2 hours, dehydrated in graded ethanols, and embedded in Araldite after an intermediate stage in toluene. Sections 1  $\mu\text{m}$  thick were cut with glass knives (0.95 cm) and stained for light microscopy (9). Sites of lesion formation identified by light microscopy were examined with the electron microscope in ultrathin sections prepared from the same block.

A lesion affecting the periventricular-arcuate region of the hypothalamus and essentially identical in light microscopic appearance to the form of pathology seen in mouse brain after MSG treatment (2, 7) was readily apparent (Fig. 1). Electron microscopic examination established that the cellular constituents primarily affected were dendrites and cell bodies of neurons. Many synaptic complexes could be found in which the postsynaptic (dendritic) component was massively dilated (Fig. 2). These processes were either empty or contained degenerating organelles and diffusely distributed particulate debris. The presynaptic component (axonal) of such complexes was usually unaffected, as were axon bundles passing through the region of injury. Many neuronal cell bodies were swollen with intracellular edema and, in some, the cytoplasmic organelles appeared to have undergone a lytic process, while nuclei showed marked alterations in chromatin pattern (Fig. 3). A mild intracellular edema of the ependyma was evident, but this was not accompanied by degenerative changes in either nuclei or intracellular organelles, and no alterations were noted in the appearance of junctional complexes between ependymal cells. No structural alterations were detected in glial or vascular components to suggest involvement of these elements in the pathological process.

The lack of symptoms in this primate infant during the time when a small percentage of its brain cells were being destroyed is evidence of a subtle process of brain damage in the developmental period, which could easily go unrecognized were it to occur in the

human infant under routine circumstances. However, a high dose of MSG was used to produce brain damage in this neonatal monkey, and it was administered by the subcutaneous rather than oral route. Thus, while we have demonstrated susceptibility of a primate species to the mechanism of the glutamate effect, it remains to be seen whether this mechanism can be triggered by any set of naturally occurring circumstances. Presumably, an elevated blood concentration of glutamic acid is an important prerequisite to lesion formation.

In attempting to evaluate the risk of glutamic acid blood concentrations rising high enough to produce brain damage in the human infant, it is important to recognize that the oral dose of MSG is but one among several potential determinants of glutamic acid concentrations in the blood. Other factors, such as circadian periodicity (10), viral infection (11), immaturity of enzyme systems, rapid absorption from an empty gastrointestinal tract, and individual variations in metabolic capabilities could act in concert with a high glutamate diet to produce much

higher concentrations of glutamic acid in an infant's blood than might be expected were such factors overlooked.

JOHN W. OLNEY

LAWRENCE G. SHARPE

Department of Psychiatry,  
Washington University School of  
Medicine, St. Louis, Missouri 63110

#### References and Notes

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5. Monosodium glutamate is the sodium salt of glutamic acid, an amino acid found as a protein constituent in the normal diet. It is also added as a flavoring agent to a variety of commercially prepared foods, including nearly all brands of baby food.
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## Luteinizing Hormone-Releasing Activity in Hypophysial Stalk Blood and Elevation by Dopamine

**Abstract.** Pituitary halves incubated in pituitary stalk plasma release more luteinizing hormone than their opposite halves incubated in plasma from peripheral blood. Glands incubated in stalk plasma from dopamine-treated rats release more luteinizing hormone than glands incubated in stalk plasma from untreated controls. Luteinizing hormone-releasing activity in stalk plasma may be due to the luteinizing hormone-releasing factor, and the secretion of luteinizing hormone-releasing factor may be controlled by a dopaminergic mechanism.

Adrenergic and cholinergic mechanisms are thought to be involved in the regulation of gonadotropin release from the anterior pituitary (1). For example, monoamine oxidase (2) and cholinesterase (3) activities and the monoamine content (4) of the hypothalamus vary during the estrous cycle and at other times when there are changes in the production of ovarian or testicular steroids, as during pregnancy and after castration. It has been shown by means of a histochemical fluorescence technique (5) that monoamines are present in high concentrations in several regions of the mammalian nervous system and that adrenergic nerve terminals are especially dense in the hypothalamus near the median eminence

(6). Recent results indicate that dopamine stimulates the release of gonadotropins from pituitaries incubated with hypothalamic tissue in vitro (7).

We observed that in rats the concentration of luteinizing hormone (LH) in systemic blood increases after the injection of dopamine into the third ventricle of the brain (8). When the anterior pituitary was perfused directly with dopamine by means of a microcannula inserted into a pituitary stalk portal vessel (9), so as not to involve the hypothalamus, LH release was unaffected (8). We now report that dopamine increases LH release by stimulating the secretion of luteinizing hormone-releasing factor (LRF).

Hypophysial stalk blood was col-