both areas, the *Selaginella* appeared to be largely free of the diurnal and nocturnal predators so frequently encountered on other plants in the tropics.

The paucity of insect species that feed on *Selaginella*, coupled with the abundance of the plants, implies disadvantages attached to the switch from feeding on monocots to feeding on lycopsids which has occurred in *E. westwoodi*. All previous records of the larvae of Papilionidae, Pieridae, Nymphalidae, and Libytheidae are from seed plants (*1*). A few species of the remaining butterfly family, Lycaenidae, are predaceous, or feed on lichens or fungi. There are no records of butterfly larvae feeding on bryophytes, sphenopsids, or ferns and *Euptychia westwoodi* is the first record from the lycopsids. One might suspect that *Selaginella*, a relatively succulent plant, possesses rather potent biochemical defenses. Recent evidence (*3*) suggests that ferns, which are shunned by butterfly larvae and suffer little from insect attack in general, are defended biochemically.

Although at least some species of *Euptychia* are quite palatable to birds (*4*), there may well be variation among species in the degree of palatability. Several species of *Euptychia* are involved in interesting and little understood mimetic complexes, often involving species of Lycaenidae. *Euptychia westwoodi* itself appears to be in a complex with *E. hesione* and *Mesosemia molina* (Lycaenidae, Riodinidae).

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Monosodium Glutamate: Absence of Hypothalamic Lesions after Ingestion by Newborn Primates

Abstract. *After receiving monosodium glutamate by stomach tube, the brains of infant macaques were perfused for examination by light and electron microscopy. No morphological differences were observed in the hypothalamic regions of treated and control monkeys. However, inadequately fixed tissue had the same appearance as that of the previously reported brain lesion in a newborn monkey.*

The nervous system in certain neonatal species appears to be highly susceptible to injury after the injection or oral administration of large doses of monosodium glutamate (MSG). Acute neuronal necrosis has been reported to occur in the retina of neonatal mice (*1, 2*) with concomitant biochemical (*3*) and electrophysiological (*4*) alterations. It has been reported that the subcutaneous injection of MSG (0.5 to 4.0 g per kilogram of body weight) in the newborn mouse is followed by a lesion in the arcuate nucleus (*5*). Further, newborn mice receiving daily subcutaneous injections of MSG over a 10-day period developed into obese adults with delayed skeletal maturation. Female mice so treated were reported to be sterile (*5*). Because of its serious implications for the human infant whose diet may contain MSG, the most disquieting report has been that of a hypothalamic lesion in the periventricular-arcuate nucleus 3 hours after the injection of MSG into a single newborn rhesus monkey (*6*).

We administered MSG by nasogastric tube to 16 infant monkeys and then examined the hypothalamic region by both light and electron microscopy. Distilled water was administered in an identical manner to five infant monkeys (controls).

Nineteen animals were obtained from dated conceptions delivered in the Primate Breeding Facility of the University of Illinois at the Medical Center. The other two were purchased from a commercial supplier. In two instances, infants were delivered at term by cesarean section. The two species, *Macaca mulatta* and *Macaca irus*, are similar, although *M. irus* is a smaller animal, which is reflected in its lower weight at birth. The conditions of the experiment are shown in Table 1. Infants were fasted for 4 hours before being given, by stomach tube, a 50 percent solution of MSG in distilled water. Control animals received only distilled water by stomach tube. The doses given (1, 2, and 4 g/kg) included levels both higher and lower than that (2.7 g/kg) of Olney and Sharpe (*6*). We chose to administer MSG by stomach tube rather than by injection because its use by man is primarily as a food additive. Each infant was maintained in an incubator with handling and cuddling at intervals for a 6-hour period. No unusual behavior was exhibited by the infants.

At the end of the 6-hour period, the infant monkey was sedated with Sernylan (1 mg/kg), and an endotracheal tube was inserted, either through the oral pharynx or a tracheostomy incision. Halothane was administered by positive pressure (2 percent), and a thoracoabdominal incision was made. The abdominal aorta was clamped, and a 5 percent solution of sodium nitrite was injected into the left ventricle. A cannula was threaded through the left ventricle into the aorta and Tyrode solution containing gum acacia was perfused for 2 minutes. This was followed immediately by a perfusate of 19 percent glutaraldehyde (25 ml). The third perfusate consisted of 2 percent glutaraldehyde with acrolein and gum acacia in phosphate buffer. Solutions were prepared according to Schultz (*7*).

To diminish rapid cerebral vasoconstriction at the time of perfusion, six monkeys were chilled to 26°C (rectal temperature) by placing them on ice bags for approximately 1 hour prior to perfusion. In these instances gum acacia was not added to the perfusing solutions.

At the end of the perfusion, the head was removed and stored over-

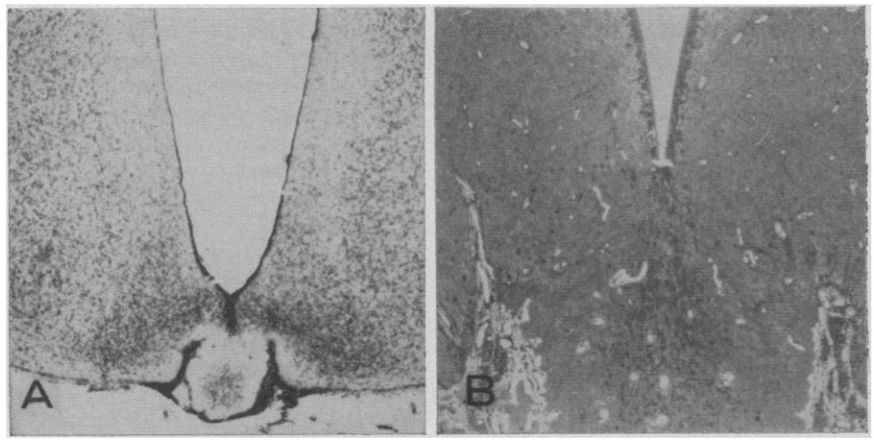

Fig. 1. (A) Paraffin section through arcuate nucleus–median eminence region from neonate monkey which had received 4 g of MSG per kilogram of body weight (× 90). (B) Epon section of ventral hypothalamus cutting through infundibular stalk from another monkey receiving MSG (4 g/kg). Sections such as these were indistinguishable from comparable regions of control monkeys (× 90).

night in the final perfusate. The following day, two or three sample blocks (1 mm thick slices) were cut through the arcuate–median eminence area with a Sorvall TC-2 tissue sectioner. These slices were post-fixed in osmium, dehydrated, and embedded in Epon. Sections (1 μm thick) were cut and stained with methylene blue–azur II and examined with the light microscope. Ultrathin sections of each block were then examined with an RCA EMU 3H microscope.

For light microscopic examinations, monkeys were perfused with physiologic saline followed by 10 percent formalin containing gum acacia. Serial paraffin sections of the hypothalamus extending from the preoptic area to the mammillary bodies were cut at 15 μm and stained with cresyl violet.

The hypothalamic area was prepared for light microscopic examinations in eight infant macaques and for electron microscopic examinations in 13. The hypothalamus was first studied by light microscopy in order to evaluate the presence or absence of a lesion induced by MSG, because light microscopy has been shown by Olney to be adequate for detecting retinal (*2*) and hypothalamic (*5*) lesions. Also, at the light microscopic level, we have been able to confirm hypothalamic lesions in the mouse (*8*) characterized by swollen cell somas and pyknotic nuclei. Close examination of serial paraffin sections of the hypothalamic areas derived from infant monkeys that were treated with MSG revealed no differences between these sections and comparable serial sections obtained from control animals (Fig. 1A). Similarly, examination of 1 μm Epon sections prepared from 16 sample blocks of the arcuate–median eminence area revealed no significant differences between control and treated animals (Fig. 1B). Dendrites and cell bodies of neurons were normal in appearance at all dosage levels.

Ultrathin sections of the arcuate nucleus confirmed our light microscopic observations in that no significant differences were found in the appearance of the cell somas and their nuclei between normal infant monkeys and those treated with MSG (Fig. 2, A and B). However, electron microscopy did reveal certain details not readily apparent in paraffin and Epon sections. Badly to marginally fixed areas became readily observable and in these regions were seen swollen dendrites lacking internal cytoplasmic contents (Fig. 3) as well as neuronal perikarya exhibiting a spectrum of "degenerative" changes. As is often true of inadequately perfused brain, we found that poor fixation characterized one brain region while immediately adjacent areas presented a well-fixed appearance.

No significant morphological difference at the light or ultrastructural level could be detected in the periventricular-arcuate area between neonatal monkeys which served as controls and those ingesting MSG. In small areas

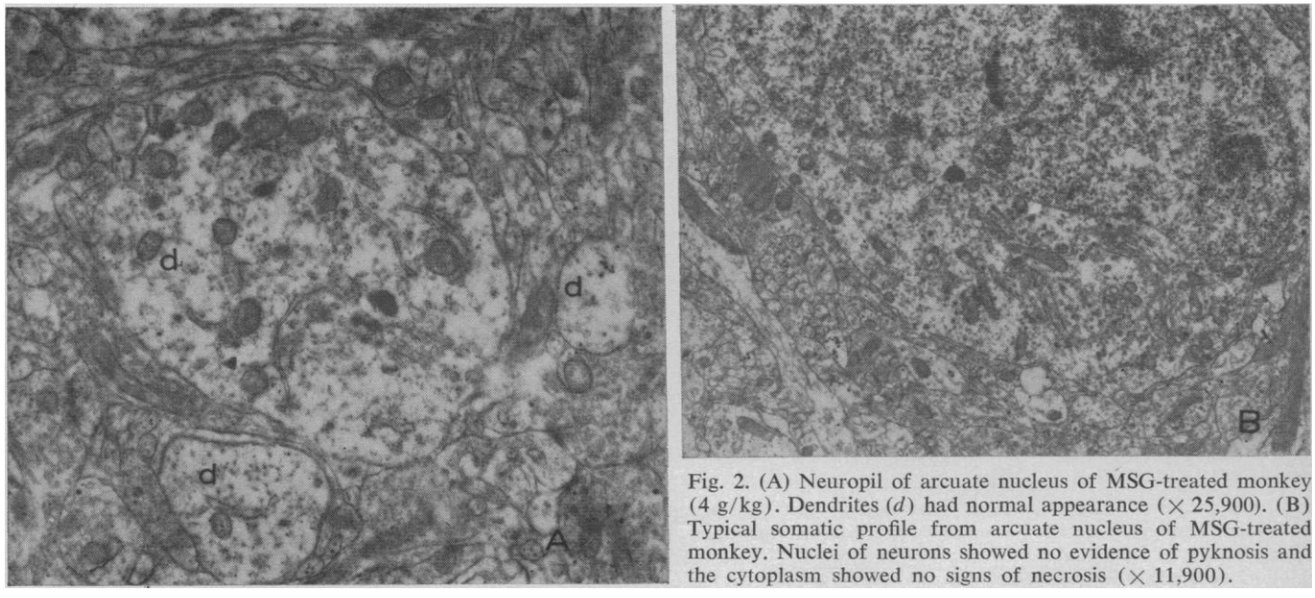

Fig. 2. (A) Neuropil of arcuate nucleus of MSG-treated monkey (4 g/kg). Dendrites (*d*) had normal appearance (× 25,900). (B) Typical somatic profile from arcuate nucleus of MSG-treated monkey. Nuclei of neurons showed no evidence of pyknosis and the cytoplasm showed no signs of necrosis (× 11,900).

Table 1. Species, age, body weight, and administered dose of MSG.

Species	Age (days)	Weight (g)	MSG dose (g/kg)
Electron microscopic examination			
M. irus	9	310	Water
M. irus	21	360	Water
M. irus	36	335	Water
M. mulatta	0.02	550	1
M. mulatta	2	450	1
M. mulatta	4	430	1
M. irus	7	445	2
M. mulatta	8	390	2
M. irus	3	320	3
M. irus	2	345	4
M. irus	3	340	4
M. irus	6	300	4
M. irus	8	300	4
Light microscopic examination			
M. irus	15	310	Water
M. irus	51	410	Water
M. irus	1	510	2
M. irus	4	375	2
M. irus	6	320	2
M. mulatta	8	240	2
M. irus	8	340	4
M. mulatta	14	480	4

of the periventricular-arcuate region in both normal and treated infants, poorly fixed tissue appeared similar at the ultrastructural level to that described in a newborn monkey after MSG administration (*6*). The obstacles to obtaining consistently superior fixation, especially in a large animal, do create difficulties in interpreting neuronal pathology. For these reasons, it appears imperative that such studies be performed on a number of animals, that wide-scale sampling of both experimental and control blocks be undertaken, and that the electron microscopic appearance of controls be examined with great care.

According to Olney (*2*, *5*), the lesion induced by MSG in the rodent is characterized by swollen dendrites and cell bodies accompanied by mitochondrial transformations. Degeneration leading to neuronal necrosis was followed by completion of phagocytosis 48 hours after injection. Arees and Mayer (*9*) have described necrotic areas in the arcuate nucleus of the mouse resulting from degenerating microglia a few hours after MSG treatment. We, too, have been able to confirm reproducibly a lesion in the arcuate nucleus of the newborn mouse and in other brain areas in response to the ingestion of MSG (*8*).

With respect to both functional and morphological indices of maturation, the central nervous system of the newborn primate and the newborn rodent are hardly comparable. It remains to be determined whether it is glutamate or one of its metabolites that is responsible for the damage observed in the newborn mouse. Conceivably, slight species differences in the metabolism of glutamate may make the rodent mouse more susceptible to neuronal damage than the primate. The final parameter that may vary with developmental age or on a species basis is that of route of access to the area susceptible to injury. The blood-brain barrier, the cerebrospinal fluid, and the integrity of the ependyma lining the ventricles all deserve closer scrutiny in this respect.

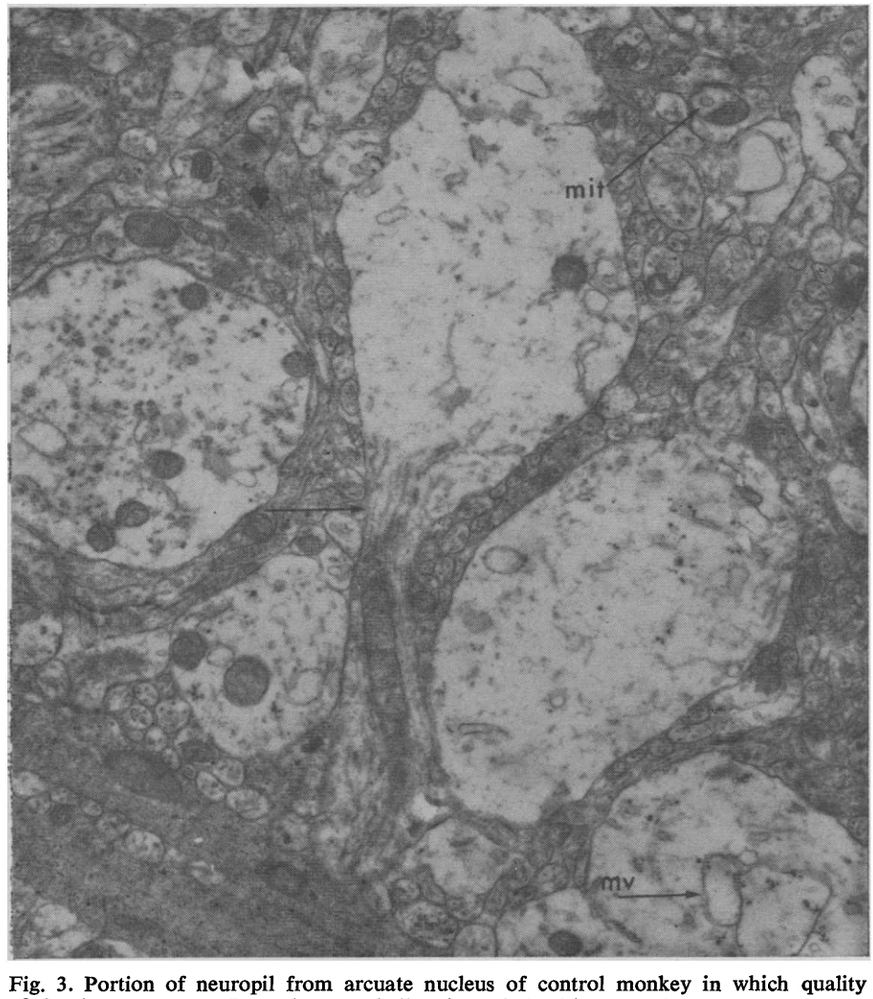

Fig. 3. Portion of neuropil from arcuate nucleus of control monkey in which quality of fixation was poor. In such areas ballooning of dendrites, as shown at arrow, was relatively frequent. Also, membrane vesiculation (*mv*), mitochondrial swelling (*mit*), and vacuolization of organelles in neuronal profiles was often found. Epon sections, 1 μm, which preceded ultrathin sections, showed a "Swiss-cheese effect" because of dilation of badly preserved dendritic processes ($\times$ 17,000).

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