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Morphology of the Fetal Monkey Hypothalamus After *In Utero* Exposure to Monosodium Glutamate

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Exhaustive studies by light and electron microscopy on the hypothalamus of the infant monkey have yielded no evidence of damage following monosodium glutamate (MSG) administration (1,2,12,20,25,28). Some 59 infant primates received oral loads of MSG ranging from 0.25 to 4 mg/g and developed no indication of neuronal damage in the hypothalamus (Table 1). These studies, by four independent laboratories, contrast with those of Olney and colleagues (15), who reported neuronal death in the subinfundibular region of three infant monkeys who received

TABLE 1. *Infant monkeys receiving MSG*

Investigators	Number	Route	Dose (g/kg)	Neuroanatomical findings
No neuropathologic findings				
Abraham et al. (1975)	3	Oral	1–4	Normal
	2	s.c.[a]	4	Normal
	2	Dietary	0.25–1	Normal
Wen et al. (1973)	8	Dietary	5–20%	Normal
Newman et al. (1973 and *unpublished*)	26	Oral	2–4	Normal
Reynolds et al. (1971 and *unpublished*)	18	Oral	1–4	Normal
Total	59			
Neuropathologic findings				
Olney et al. (1972)	3	s.c.[a]	2.7–4	Spreading lesions
	3	Oral	1–4	Small focal lesions

[a] Subcutaneous.

TABLE 2. *Incidence of arcuate lesions in neonatal mice after MSG ingestion*

Dose[a] (mg/g)	Number examined	Number with lesions	Incidence (%)
4	24	24	100
2	23	20	87
1	40	25	62
0.5	40	9	22
0.25	6	0	0

[a] 20% solution for 4, 2, and 1; 5% for 0.5; and 2.5% for 0.25 dose for nonsuckling animals.

oral dosages of MSG ranging from 1 to 4 mg/g. There have been many attempts to reconcile these differences in findings. The preponderance of infant primates used in the studies involving no observed lesions (Table 1) had been born in primate facilities under optimum environmental conditions. The infants utilized by Olney and colleagues were purchased from a supplier. Three of these infants were used for injection studies involving MSG and NaCl, one was premature, and two were apparently dehydrated due to difficulties in nursing. This contrast in the condition of the experimental primate infants, along with the sheer weight of numbers of infants studied in different laboratories, leads to the conclusion that Olney's claims of the susceptibility of the neonatal primate hypothalamus to high levels of MSG cannot be confirmed by others.

It is now well accepted that the rodent brain does incur damage in response to high loads of MSG (1,3,9,14). The neonatal mouse sustains hypothalamic lesions (Table 2) upon ingesting 0.5 mg/g of MSG about 22% of the time (19); with increasing age, higher intakes of MSG are necessary for damage to occur. This finding has caused the speculation that because of the immaturity of the rodent brain at birth, it is exquisitely sensitive to high levels of MSG. In contrast, the neonatal brain of the monkey and human is relatively mature and therefore less susceptible to damage by MSG and, for that matter, other chemicals in high doses, such as sucrose and NaCl, than is the neonatal rodent (8). Whether the maturational state of the brain or whether a basic species difference is responsible for the lack of susceptibility of primate brain to MSG could be resolved in part by administering the substance *in utero* directly to primate fetuses and searching for alterations in their developing brains.

MATERIALS AND METHODS

We have shown that MSG does not cross the hemochorial placenta (24) except following extreme elevation of maternal plasma glutamate. Thus, it was necessary to inject MSG directly into the umbilical vasculature or into the fetus in order to create elevated MSG levels in the fetal circulation. The experiments are summarized

TABLE 3. *Macaque fetuses receiving MSG* in utero[a]

Animal number	Species	Gestational age (days)	Weight (g)	Length[b] (cm)	Dose (mg/g)	Interval (hr)	Comment
GG	*M. irus*	38	6	CH = 3.0	16.6	6	IP injection
JJ	*M. arctoides*	66	11.2	CH = 7.0	3.6	4.25	Heart not beating at birth
KK	*M. arctoides*	85	47	CR = 7.5 CH = 10.5	6.8	2.25	
II	*M. arctoides*	89	60	CR = 10.0 CH = 14.5	0.4	6	Fetus alive at birth
I	*M. mulatta*	100	69	CR = 12.0 CH = 15.2	5.8	2	Ecchymosed
EE	*M. irus*	142	275	CR = 16.0 CH = 24.0	1.8	6.75	Fetus alive at birth
DD	*M. arctoides*	142	340	CR = 15.3 CH = 24.3	2.4	5.5	IP injection, fetus alive at birth

[a] All MSG administration was by injection into the umbilical vein unless otherwise indicated.
[b] CR = crown to rump; CH = crown to heel.

in Table 3. Six of the pregnant primates were obtained from the University of Illinois Primate Facility Colony; in these instances, the dates of conception are known and the length of gestation can be pinpointed. For the one pregnant animal (I) obtained from a commercial source, the age of the fetus was estimated from its weight and length, using growth tables. Of necessity, the required dose was estimated, hopefully to achieve a dosage of 4 mg/g. However, as Table 3 indicates, the end result is variable as to the actual dose achieved because of the impossibility of accurately predicting weight *in utero*. All but one of the dosages turned out to be above the minimal 1 mg/g level required to obtain a lesion in the rodent with reliability (19). It should be noted that experiments I and JJ were the last two performed, and the dosage achieved in them was closer to the desired level because we had learned better how to predict weight *in utero* from the preceding experiments.

Under Sernylan (Parke-Davis Co.) and halothane anesthesia, abdominal laparotomy was performed. The gravid uterus was then exposed and transilluminated so as to determine the location of the bipartite macaque placenta. An incision was then made into the uterus avoiding the placenta. The umbilical cord was carefully located and a short segment of it exteriorized, taking care not to interfere with umbilical blood flow. The estimated dose, in a 20% solution (w/v), was then injected toward the fetus into the umbilical vein and the needle hole was sutured. For the smallest fetus, it was necessary to inject intraperitoneally, since the umbilical cord was too small to exteriorize.

We then chose to perform an intraperitoneal injection of MSG for fetus DD so

that circulating levels of glutamate could be compared following this route of administration with those after injection into the umbilical vein. The uterus was replaced into the abdomen and the incision closed temporarily with clamps. At the end of the experimental interval, the fetus was delivered by cesarean section.

In two experiments (EE and DD), a tracer dose of 10 μCi ^{14}C-MSG was added to the loading dose of MSG. At the termination of these two experiments, 6.75 and 5.5 hr after dosing with MSG, respectively, fetal plasma, urine, CSF, and amniotic fluid samples were obtained for amino acid analysis. Maternal plasma samples were obtained at intervals throughout the experiment from an indwelling catheter placed in the saphenous vein. These samples were subjected to simultaneous measurement of radioactivity and amino acid composition (23) in order to see if glutamate moved from fetal to maternal circulation. All fluid samples were deproteinized immediately with sulfosalicyclic acid and stored at $-40°$ C. Amino acid analyses were performed on a Technicon NC-1 analyzer using the Efron buffer system (23). Control values were obtained from earlier studies of fetal (24) and neonatal (25) glutamate metabolism in the monkey or from samples obtained from normal macaque fetuses delivered for other research projects.

The fetal head was removed and the cranium carefully dissected away with Ronjour forceps. Each brain was immersed immediately in 10% buffered formalin. After fixation, the brains were dehydrated in an alcohol series, placed in 1% celloidin in methyl benzoate for 2 days, then processed in benzene to paraffin. Serial sections of the entire brain from the smaller animals and of the hypothalamic regions of the larger animals (over 200 g) were cut at 10 μm and then stained with cresyl echt violet solution.

RESULTS

The only fetus that exhibited any abnormality upon delivery was I, which was ecchymosed, although otherwise normal in appearance. It was derived from a pregnant macaque that had been purchased and arrived in Chicago from New York the day before the experiment. *In utero* deaths in pregnant macaques subjected to the stresses of shipping are common, and it is possible that this fetus was traumatized before the experiment. Fetuses II, EE, and DD were vigorous upon delivery and exhibited spontaneous movement, gasping, respiratory activity, and normal heart rates. Fetuses GG, JJ, and KK were too small to exhibit these signs of vigor; GG and KK possessed beating hearts, whereas the heart of JJ was not beating and was refractile to stimulation.

Maternal plasma glutamate levels increased slightly at 30 min and 1 hr (Exp. DD, 6.5 to 11.1 μmoles/dl; Exp. EE, 2.8 to 24.9 μmoles/dl) and in both instances had returned to base-line levels by 2.5 hr. The radioactivity in maternal plasma was primarily incorporated in glucose and lactate (Fig. 1). Small amounts of ^{14}C-labeled glutamate were observed at 15 min in Exp. EE, but had disappeared by 2 hr; this phenomenon was even more transient and less extensive in Exp. DD. Significant

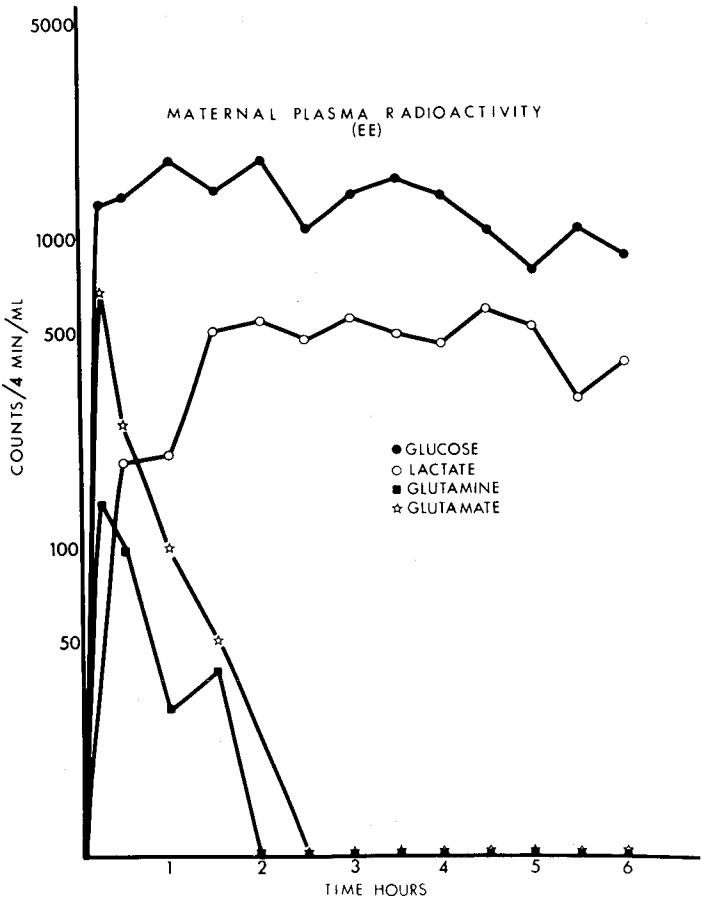

FIG. 1. Most of the counts/min in maternal plasma were found in glucose and lactate. A small, transient increase in labeled glutamate occurred at 15 min and had disappeared by 2 hr. Only when circulating fetal levels of glutamate are exceedingly high does the substance breach the placental barrier.

levels of glutamate radioactivity were still present in cord blood at the end of the experimental period (Exp. DD, 800 counts/4 min; Exp. EE, 900 counts/4 min).

Negligible amounts of radioactivity were associated with glutamate in CSF in both experiments. Similarly, total glutamate levels were not elevated significantly above control values (1.8 ± 1.1 μmoles/dl) in the CSF of fetuses DD and EE).

That both fetuses sustained extensive elevations in circulating glutamate levels is corroborated by the plasma amino acid values encountered at the end of the experiments, 5.5 and 6.5 hr after administration of the dose (Table 4). Glutamate levels were still two to three times those of control animals. Amniotic fluid

TABLE 4. *Amino acid levels in fetal plasma and in amniotic fluid*

	Aspartate	Glutamine	Glutamate	Proline	Alanine
Plasma					
Control fetus (5)	0.5 ± 0.5	57 ± 18	6 ± 3	18 ± 7	33 ± 10
DD[a]	1.56	63	20.2	24.1	29.1
EE[b]	0.59	148	12.9	11.1	54
Amniotic fluid					
Control pregnancy (5)	0.2 ± 0.2	18.8 ± 4.7	2.4 ± 1.0	9.1 ± 2.0	11.6 ± 2.4
DD[a]	0.9	14.8	22.9	8.8	20.1
EE[b]	0.5	14.8	32.8	8.8	19.0

All values in μmoles/dl.
[a] 5.5 hr after i.p. injection.
[b] 6.8 hr after i.v. injection.

glutamate levels were also markedly elevated (Table 4), probably as a marker of a prior extensive rise in fetal blood levels, clearance to urine, and then micturition into the amniotic fluid. Fetal urine correspondingly contained enormous concentrations of glutamate (Table 5). Most of the radioactivity in fetal urine was associated with glutamate (Table 6).

All brain sections were studied by light microscopy, by which it is possible to

TABLE 5. *Amino acid in fetal urine*

Amino acid	Control fetus (3)	DD	EE
Glutamate	6.8 ± 4.1	2,650	3,550
Aspartate	2 ± 1	44	35
Glutamine	60 ± 25	62	71
Glycine	75 ± 30	62	70
Histidine	15 ± 9	13	14
Threonine	29 ± 15	35	27

All values in μmoles/dl.

TABLE 6. *Percent distribution of total radioactivity in fetal urine*

Amino acid	DD	EE
Glutamate	86	91.7
α-Ketoglutarate	4.2	1.3
Aspartate	3.8	2.8
Glucose	2.8	2.0
Lactate	1.0	8.0
Alanine	0.9	0.7
Urea	0.5	0.3
Acetoacetate	0.3	0.05

observe lesions resulting from the ingestion of high levels of MSG in neonatal rodents (9,13).

Ventral hypothalamic morphology was embryonic in fetus GG and quite immature in fetuses JJ, KK, II, and I. The hypothalamic nuclei of GG (Fig. 2) were not yet differentiated. The hypothalamus at this stage is still an evagination of the thalamus, and the optic primordium was still visible in the diencephalon (Fig. 2). The brain of JJ was still very immature, but the median eminence, anterior pituitary, and infundibular nucleus area could be identified. Although fetuses KK, II, and I were more mature (Fig. 3), the median eminence lacked the differentiation it undergoes in later development, probably under the influence of thyroid hormone. As a result of the rapid rate of cell division in the ependymal layer, mantle and marginal layers were packed with cells. However, the cells were still quite undiffer-

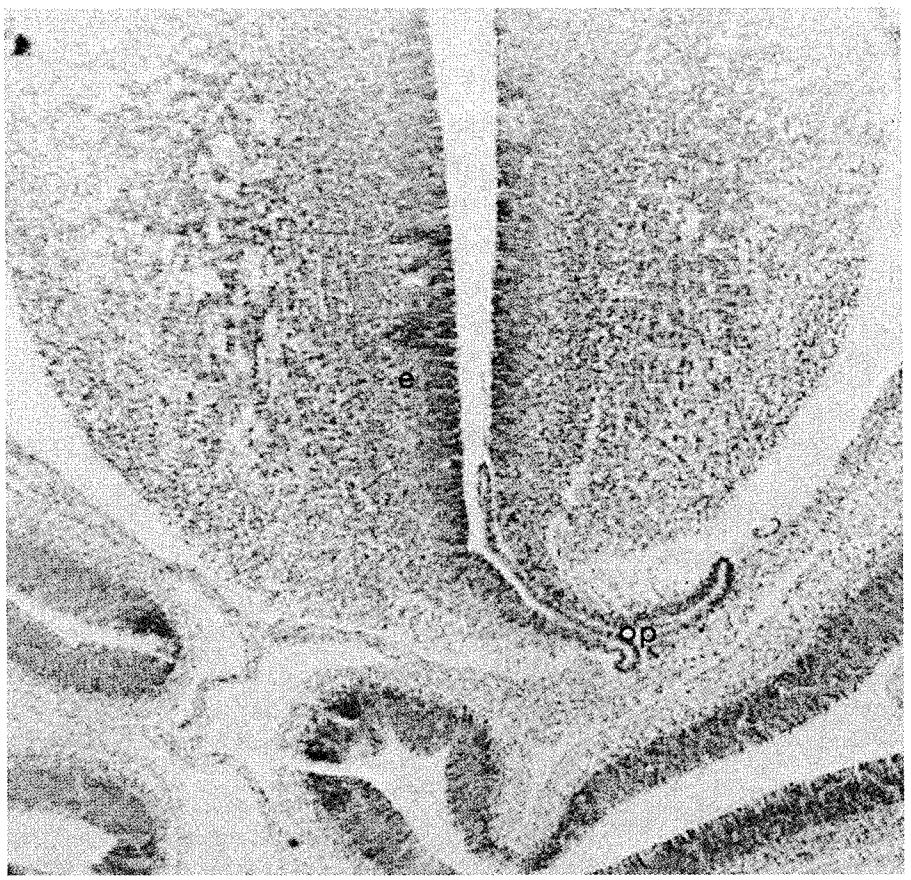

FIG. 2. Section through ventral diencephalic area of fetus GG (38 days gestation). Note the optic primordium *(op)* evaginating from the third ventricle. Cell division in the ependymal *(e)* layers is still occurring at a rapid rate. ×208.

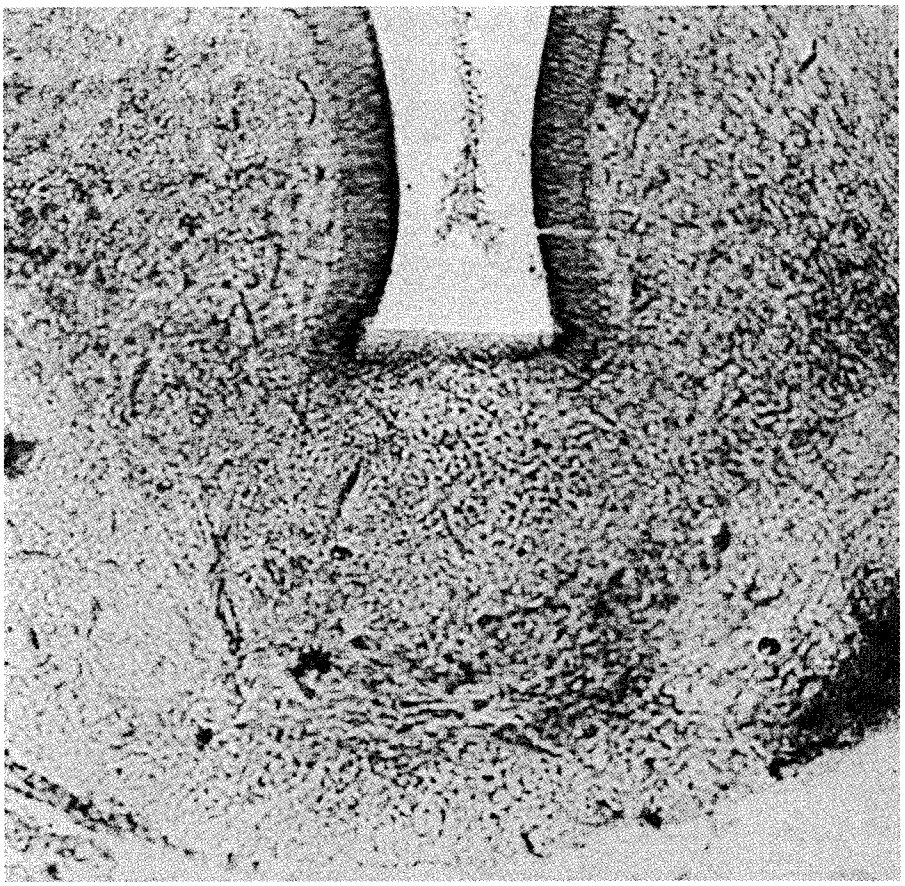

FIG. 3. Section through ventral hypothalamus of fetus KK (85 days). This brain is still immature, but the median eminence is now distinct at the base of the third ventricle. Numerous, undifferentiated cells populate this region. ×524.

entiated, with little cytoplasm, and it was impossible to discern future neurons from glia at this stage (Fig. 4). The cells within the hypothalamic areas of these four fetuses were normal in appearance. There was no evidence of pyknotic nuclei, tissue edema, or cell loss in the subinfundibular region, undifferentiated though it was.

The brains of fetuses DD and EE were more differentiated than those of the younger ones. However, they did not exhibit the degree of myelination, neuronal differentiation, and maturation of the median eminence seen in the neonatal monkey brain (1,2,12,15,19,20). Thus, considerable hypothalamic differentiation was yet to occur in the remaining three to four weeks of gestation. Gestation in the rhesus monkey is 168 ± 7 days (27).

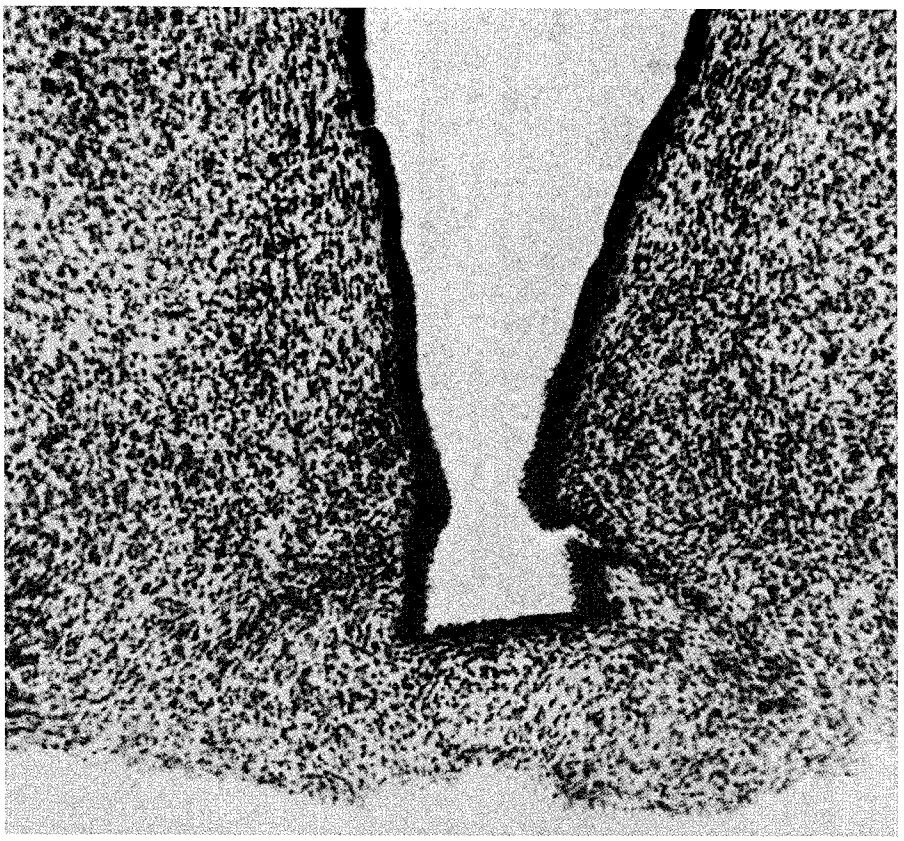

FIG. 4. Section through ventral hypothalamus of fetus II (89 days gestation). The median eminence is not present in its entirety because of mechanical damage. Note the dense population of immature cells, whose differentiative fates cannot yet be determined. ×416.

No neuronal abnormalities were seen in the ventral hypothalamic area of fetus DD or EE (Fig. 5, p. 226). Excessive numbers of cells whose accruing cytoplasm suggests they have a future as neurons are present in this area (Fig. 5). No evidence of cell death or vacuolated cytoplasm, which are characteristics of MSG damage in the neonatal mouse, could be found.

DISCUSSION

A few other studies have involved the fetal effects of MSG. Murakami and Inouye (11) observed hypothalamic damage in the neutromedial and arcuate nuclei of mouse fetuses whose dams received 5 mg/g injections of MSG late in gestation. This is an enormous, acute load, and probably wreaked havoc with the placental barrier. In contrast, when Takasaki (26) fed mice with diets containing up to 15%

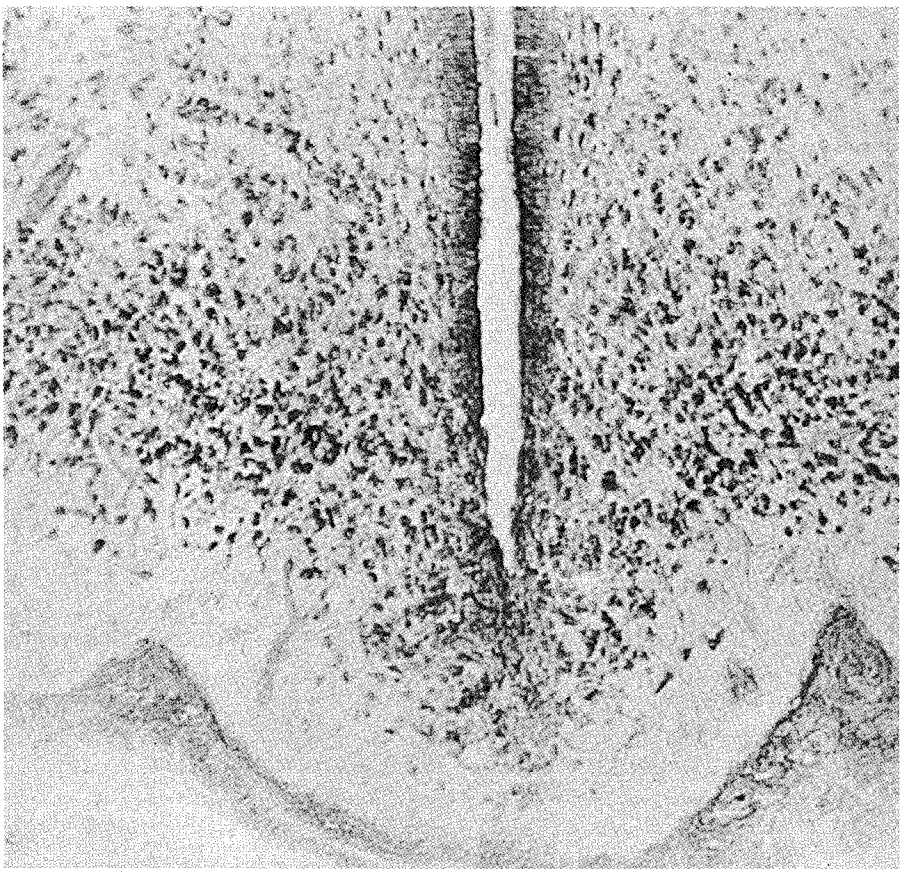

FIG. 5. Section through ventral hypothalamus of fetus DD (112 days gestation). Infundibular area contains cells whose increasing cytoplasm indicates that they are neurons in the process of becoming arranged in the infundibular nucleus. ×524.

MSG or with 5% aqueous solutions of MSG *ad libitum* for up to 4 days during pregnancy, no necrosis of neurons in the fetal brains occurred. Olney (16) double-injected a very large dose of glutamate into a single pregnant monkey and reported a lesion in the fetus.

In contrast Newman et al. (12) administered MSG at a daily level of 4 mg/g in drinking water to six pregnant monkeys during the last third of gestation; the hypothalamic areas of the newborns of these animals exhibited no abnormalities. These latter findings are in line with the fact that glutamate, even injected in large loads, does not cross the neurochorial placenta of the primate (23).

Either intravenous or intraperitoneal administration served to elevate glutamate levels markedly in the fetuses. The early backcrossing of a small amount of glutamate into the maternal circulation (Fig. 1) was similar to that seen previously

when 5 mg/g MSG was infused into a fetus, elevating maternal glutamate levels slightly (24). In this instance, fetal levels rose to 400 times base line or 2,000 μmoles/dl. The administration of lesser amounts of glutamate (1.5 or 2.4 mg/g) to the fetus was insufficient to breach the fetomaternal placental barrier. Thus, since glutamate did backcross slightly into the maternal circulation, it would appear that all of these fetuses experienced major elevations in circulating glutamate levels following the injections. These levels would far surpass those achieved by oral administration, where the time required for intestinal absorption attenuates and lowers the peak blood levels of glutamate.

Another phenomenon may have contributed markedly to constantly elevated fetal blood levels of MSG, at least in the more mature fetuses. The monkey fetus forms urine at the rate of about 5 mg/kg/hr (4). In both amniotic fluid (Table 4) and fetal urine (Table 5) the glutamate levels were exceedingly high at the termination of the experiments. The fetuses old enough to drink were probably "recycling" glutamate contained in amniotic fluid at a rate roughly equivalent to 3 ml/kg/hr, extrapolating from the human rate (18).

Even though the fetuses DD and EE were 4 weeks younger than the newborns studied previously (25), their blood-brain barrier impermeability to glutamate was already effective. Neither animal had any increase in CSF glutamate or glutamate labeling with radioisotope. The failure of glutamate to enter CSF in even the fetal monkey may be a critical component of a species difference between the rodent and the primate. We have hypothesized that glutamate may gain access to certain brain regions via CSF, since damage appears to be restricted to areas adjacent to CSF in the mouse (9,19). Many studies have confirmed that there is little if any net transfer of glutamate into CSF in several adult mammals, including mouse (21), rat (10), and dog (7). Thus, lack of a totally effective blood-brain barrier integrity in the young rodent could be a major reason for the susceptibility of its brain to damage by glutamate.

The one embryonic and seven fetal brains studied all exhibited striking cell differentiation and turnover in comparison to that seen in the neonatal primate brain (1,2,12,15,20,28). During the embryonic and early fetal period, the extensive rate of cell division, primarily in the ependymal region, results in a dense cellular population of the neuropil. A minority of this cell population will differentiate into glial and neuronal elements. Most of these cells, however, are destined to die, probably because they fail to make functional connections to the periphery or to adjacent cells (6). Submammalian species have been the most closely studied in this respect; in the frog and chick, less than 50% of the cells persist in given areas after differentiation has occurred (17).

The damage incurred by the neonatal monkey brain following ingestion of MSG is described by Olney (15) as numbering but 2 to 3 neurons per section and totaling 50 to 90 in the rostral subventricular portions of the infundibular nucleus. The lateral portions of the hypothalamus, primarily damaged in rodents (1,14) were not affected in the primate. Paraffin preparations, suitable for discerning lesions in the mouse brain (9,13), would not possess the resolution necessary to discern

only 2 to 3 moribund cells per section; plastic sections are required for the study of individual neurons. However, since cell death in the CNS is a normal part of the differentiative process, an entirely different approach would be required to resolve the possibility of "microlesions" involving but a few cells per section. Such analyses require morphometric techniques for painstaking analysis of the actual numbers of viable and nonviable cells present in a given area. It is certainly possible that the CNS experiences microlesions in response to anoxia, drugs, alterations in pH, and other stresses. At present, the search for microlesions awaits the modification of the appropriate statistical and morphometric techniques. It will be a meticulous and difficult task for the primate brain because of the expense and the variability between animals. Further, because of the vasculature and water content of the brain, only fetuses weighing more than 200 g can be perfused adequately so as to prepare epon sections for study.

Even following the extraordinary measure of injecting MSG into the fetal monkey, no damage to the hypothalamus was observed. These seven fetuses, added to 59 neonatal monkeys (Table 1) represent a major search for susceptibility of the primate brain to MSG. Even in the embryonic and fetal period, no hypothalamic damage was found.

For studies of substances involved in human diets, the nonhuman primate is the model of choice. Phocomelia cannot be induced in the fetal period in the rodent by thalidomide; it is readily induced in the fetal primate following maternal administration (5). Insulin is highly teratogenic in the rodent but not in the human (29). The primate brain is not affected by even large dosages of MSG. Differences in glutamate metabolism between the rodent and primate (22), greater integrity of the blood-brain barrier, or specific neuronal susceptibility—all or in concert—could be responsible. Perhaps it is now more appropriate to regard damage to the rodent brain from the ingestion of MSG as an unusual and interesting phenomena, restricted to the order Rodentia.

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