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Attempts to Establish the Safety Margin for Neurotoxicity of Monosodium Glutamate

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For every chemical, with practically no exceptions, it is possible to find a route of administration and a dose that will induce a toxic effect in some animal species. Once this toxic effect has been found, the problem is to establish its relevance to man. Usually, a safety ratio is calculated by dividing the dose that induces a toxic effect in a given animal species by the dose utilized in man; the larger the ratio, the safer the chemical. More recently, however, there has been a trend to use not the dose, but the plasma, or tissue level, of the chemical under study as a more reliable parameter in making calculations (1,3). It is, in fact, known that different animal species dispose of chemicals in a different manners, thus making the plasma or tissue concentrations a better parameter than the dose for extrapolating biological activity across animal species.

This is the approach utilized here in an attempt to interpret the relevance for man of some interesting findings concerning the neurotoxicity of an ubiquitous amino acid, glutamic acid (Glu).

Necrotic lesions of the hypothalamus (arcuate nucleus) have been found as a result of the administration of monosodium glutamate (MSG) to newborn animals (12,14–17). The mouse appears to be the most sensitive species, followed by rats and guinea pigs (Heywood and Worden, *this volume*). At variance with initial observations (17; Olney, *this volume*) in a variety of experimental conditions, monkeys are apparently insensitive to this toxic effect of MSG (11,21,24; Reynolds et al., *this volume*).

BASAL PLASMA AND BRAIN LEVELS IN DIFFERENT ANIMAL SPECIES

Preliminary investigations indicated that the basal plasma concentration of Glu did not substantially change in rats as a result of overnight fasting, so no reference will be made to the nutritional conditions of control animals. Similarly, no significant differences were found between the plasma or brain Glu levels in male and female rats. Furthermore, no difference was found between venous (jugular vein) and arterial (carotid artery) concentrations of Glu, either in the basal condition or after a load of Glu in rats. The ratio of plasma to red cells also showed no change in arterial and venous blood following administration of Glu.

TABLE 1. *Glu in plasma and brain of several animal species at different ages*

Animal species	Age (days)	Plasma (μmoles/ml ± SE)	Brain (μmoles/g ± SE)
Mouse	1	0.15 ± 0.01	—
Mouse	7	0.15 ± 0.01	4.42 ± 0.18[a]
Mouse	Adults	0.17 ± 0.01	8.21 ± 0.21
Rat	1	0.25 ± 0.02	3.21 ± 0.14[a]
Rat	7	0.20 ± 0.01	4.83 ± 0.14[a]
Rat	15	0.17 ± 0.01	6.50 ± 0.06[a]
Rat	Adults	0.15 ± 0.02	8.05 ± 0.25
Guinea pig	1	0.19 ± 0.01	8.01 ± 0.19
Guinea pig	7	0.17 ± 0.01	8.04 ± 0.15
Guinea pig	15	0.23 ± 0.01	9.15 ± 0.41
Guinea pig	Adults	0.20 ± 0.01	7.56 ± 0.15
Rabbit	Adults	0.12 ± 0.01	10.65 ± 0.40
Dog (beagle)	Adults	0.050 ± 0.003(81)	5.80 ± 0.02
Monkey (rhesus)	Adults	0.13 ± 0.01(22)	9.14 ± 1.97
Human	Premature newborns	0.50 ± 0.07(21)	—
Human	Adults	0.06 ± 0.003(109)	—

Glu was assayed by an enzymatic method according to Bernt and Bergmeyer (2). Results represent the average of at least five determinations unless otherwise indicated in parentheses.
[a] $p < 0.01$ compared to values in adults of the same species (Student's t- and Dunnett tests).

Table 1 indicates that the plasma Glu levels in mice, rats, guinea pigs, rabbits, and rhesus monkeys are not markedly different. In contrast, dog and man show Glu plasma values that are about 50% lower than the other species considered. Furthermore, they are not influenced by growth, the levels in newborns and adults being similar in mice, rats, and guinea pigs, with the possible exception of the high plasma Glu levels of newborn rats the first day of life. Conversely, human premature newborns show much higher Glu plasma levels than adults. The brain levels of Glu are comparable in adult animals with the exception of dogs, which have the lowest level among the species investigated.

In mice and rats, brain Glu was low at birth and increased with age, whereas in guinea pigs, brain levels were similar at and after birth. These results are in agreement with the finding that guinea pigs are born with a more mature brain than mice and rats (6). It should be recalled that human newborns are also considered relatively more mature in their brain myelinization than rats and mice.

KINETICS OF Glu IN NEWBORNS AND ADULTS

Administration by gavage of a standard dose of MSG (1 g/kg, 10% w/v) results in a marked increase of plasma Glu in all the animal species studied (Table 2). However, the extent of the increase is different. Peak plasma Glu levels are about 12 times higher than the basal concentration in mice, 13 in rats, 11 in guinea pigs, 2 in rabbits, 35 in dogs, and 4 in monkeys. The area under the curve (AUC) is also different, being lowest in rabbits and highest in guinea pigs; the plasma half-life

TABLE 2. *Kinetic parameters for Glu in different animal species after administration by gavage of 1 g/kg of MSG (10% w/v)*

Animal species	Age (days)	Plasma peak (μmoles/ml ± SE)	Plasma AUC (μmoles/ml × min)	Plasma $T_{1/2}$ (min)
Mouse	7	2.10 ± 0.06	375	111
Mouse	90	2.08 ± 0.02	309	98
Rat	7	2.89 ± 0.11	1,391	237
Rat	90	1.91 ± 0.09	288	88
Guinea pig	7	1.91 ± 0.02	321	101
Guinea pig	90	2.28 ± 0.03	487	173
Rabbit	Adults	0.29 ± 0.14	13	53
Dog (beagle)	Adults	1.78 ± 0.31[a]	143	49
Monkey (rhesus)	Adults	0.58 ± 0.33[a]	53	99

Results represent the average of at least five animals.
[a] Vomiting occurred.

TABLE 3. *Kinetic parameters of plasma Glu in 7-day-old mice after administration by gavage of different doses of MSG (10% w/v)*

Dose (g/kg)	Plasma peak (μmoles/ml ± SE)	Plasma AUC (μmoles/ml × min)	Plasma $T_{1/2}$ (min)
0.25	0.72 ± 0.04	103	79
0.50	1.07 ± 0.02	200	71
1.00	2.10 ± 0.05	375	111

Results represent the average of 5 animals.

$T_{1/2}$ ranges from 49 min in dogs to 173 min in guinea pigs. There are also, as summarized in Table 2, quantitative age-dependent differences. For rats, and to a lesser extent for mice too, plasma Glu levels expressed as AUC are higher for newborns than adults, whereas for guinea pigs the opposite holds true. This confirms the importance of maturity at birth as a factor in the ability to dispose of Glu. Table 3 shows that the plasma peak level, the plasma AUC, but not the $T_{1/2}$, increase linearly for newborn mice as a function of the MSG dose given by tube.

The concentration at which MSG is given is also an important factor in the kinetics of Glu. At equal doses the MSG concentration determines the volume in which it is given and hence the time required to administer the dose orally to newborn animals. Table 4 confirms that the plasma peak rises for newborn mice and rats as the concentration at which MSG is given increases. When the same dose of MSG (1 g/kg) is given to newborn rats by tube, the plasma AUC is five times higher when the concentration is increased from 2 to 10%. Similarly in mice, given an oral dose of 0.5 g/kg at concentrations from 2 to 20%, the AUC increases by a factor of about 2.5. Similar results were obtained in adult animals. However, the concentration at which MSG is given becomes less important when the dose is increased (7).

TABLE 4. *Kinetic parameters of plasma Glu in 7-day-old mice and rats after administration by gavage of MSG at different concentrations*

Animal species	Dose (g/kg)	Concentration (% w/v)	Plasma peak (μmoles/ml ± SE)	Plasma AUC (μmoles/ml × min)	Plasma $T_{1/2}$ (min)
Mouse	0.5	2	0.82 ± 0.03	126	88
Mouse	0.5	5	0.86 ± 0.04	147	117
Mouse	0.5	10	1.08 ± 0.02	200	71
Mouse	0.5	20	1.43 ± 0.02	318	106
Rat	1.0	2	0.97 ± 0.05	275	183
Rat	1.0	5	2.26 ± 0.36	1,085	237
Rat	1.0	10	2.89 ± 0.11	1,391	241

Different times were required for administering MSG by gavage at different concentrations. Results represent the average of five animals.

TABLE 5. *Plasma peak, AUC, and $T_{1/2}$ of MSG given to adult mice either by gavage or mixed with the diet*

MSG (g/kg)	Percent	Plasma peak (μmoles/ml ± SE)	Plasma AUC (μmoles/ml × min)	Plasma $T_{1/2}$ (min)
1.00	10[a]	2.08 ± 0.02	309	98
1.00	Meal[b]	0.27 ± 0.01	4.8	—

Results represent the average of five animals.
[a] By gavage (1 min).
[b] Mixed with food (30 min).

PLASMA LEVELS OF Glu WHEN MSG IS GIVEN WITH FOOD

Usually, Glu is not given to humans by tube feeding, but is added in various amounts to food eaten in a given period of time. It therefore seems reasonable to investigate differences in plasma Glu levels in laboratory animals when the substance is given by tube feeding or mixed with food.

To this end, adult mice were trained to eat their daily food intake during 1 hr in the morning. Glu (as MSG) was added to a commercial diet in such a way as to give 1 g/kg body weight during an eating period of 30 min; mice were then killed at various times after the meal for determination of plasma Glu and for calculation of the kinetic parameters (Table 5).

It is interesting to note that 1 g/kg of MSG given by tube feeding induces a high peak level of plasma Glu and a large AUC, whereas when given with the meal results in only very slight increases in plasma Glu. Hence, when MSG is given with a meal, it is much more slowly absorbed and/or much more rapidly disposed than when given by gavage. The reasons for this difference are not yet clear, but may include decreased absorption of MSG due to competition with other amino acids,

TABLE 6. *Relation between plasma Glu peak and brain concentrations in different species (newborn and adult animals)*

Animal species	Age (days)	Oral dose MSG (g/kg)	Glu factor of increase	
			Plasma	Brain
Mouse	3	0.5 (5%)	8	NS
	7	1 (10%)	13	NS
	90	1 (10%)	12	NS
Rat	3	1 (10%)	14	NS
	7	1 (10%)	18	NS
	7	2 (5%)	12	NS
	7	2 (20%)	19.6	1.6[a]
	90	1 (10%)	10	NS
Guinea pig	7	2 (20%)	12	NS
	90	1 (10%)	12	NS

NS, not significant.
[a] $p < 0.05$.

increased metabolism in the intestine due to the slower rate of absorption, interaction with other nutrients, and others.

LEVELS OF Glu IN BRAIN

Throughout the variety of conditions described above, the levels of brain Glu remained unchanged in both mice and rats, adults and newborns, even when plasma Glu levels were up to 15 times the basal concentrations. Rat brain Glu concentration significantly increased, by about 60%, only when the oral dose of MSG was raised to 2 g/kg (20% w/v) and peak plasma Glu exceeded the basal level by about 19 times (Table 6).

The fact that the level of whole brain Glu was not changed even by doses of MSG that induce some brain damage (15) does not exclude the possibility, as shown by Perez and Olney (19), that Glu may increase in a small brain area such as the arcuate nucleus of the hypothalamus, where the amino acid has been seen to produce toxic effects (necrosis). However, recent data (8) indicate that in adult rats Glu does not substantially increase in the retina, another tissue where, nevertheless, it causes damage (4).

In relation to these data, it was of interest to study whether Glu was capable of accumulating in discrete brain areas, particularly the nucleus arcuatus. Experiments were carried out in rats. The nucleus arcuatus was obtained by the punching technique of Palkovitz et al. (18). The thalamus lateralis was selected as a control area. The results obtained are reported in Table 7 for adult rats and in Table 8 for newborn rats. The levels of Glu are expressed in nmoles/mg protein. Doses of MSG that increase plasma levels by about 11 times in both adult and newborn rats do not affect the level of Glu in the arcuate nucleus or in the lateral thalamus (L. Airoldi et

TABLE 7. *Relation between Glu levels in plasma and in the nucleus arcuatus and lateral thalamus in adult male rats*

Min. after treatment	Plasma Glu (μmoles/ml ± SE)	Glu (nmoles/mg protein ± SE)	
		N. arcuatus	L. thalamus
0	0.21 ± 0.02	132 ± 5	110 ± 4
15	1.56 ± 0.21	136 ± 5	113 ± 9
30	2.30 ± 0.11	136 ± 7	118 ± 2
60	1.63 ± 0.08	125 ± 6	117 ± 5
180	1.43 ± 0.09	131 ± 9	109 ± 2
360	0.62 ± 0.08	125 ± 6	112 ± 6

MSG was given by gavage at the dose of 4 g/kg (20% w/v). Glu was measured according to the method of Young et al. (25). Results represent the average of five animals.

TABLE 8. *Relation between Glu levels in plasma and in the nucleus arcuatus and lateral thalamus in 4-day-old rats*

Min after treatment	Plasma Glu (μmoles/ml ± SE)	Glu (nmoles/mg protein ± SE)	
		N. arcuatus	L. thalamus
0	0.23 ± 0.02	114 ± 10	124 ± 14
60	2.76 ± 0.37	115 ± 3	126 ± 23
180	0.86 ± 0.28	123 ± 10	128 ± 4

MSG was given by gavage at the dose of 2 g/kg (20% w/v). Glu was measured according to the method of Young and Lowry (25). Results represent the average of five animals.

al., *unpublished*). The reason for the discrepancy between these results in rats and the data obtained by Perez et al. (20) in mice is at present unknown. However, it seems that the explanation given for the selective neurotoxic effect of MSG in mice cannot be generalized to all animal species.

SIGNIFICANCE OF KINETIC DATA FOR THE NEUROTOXICITY OF Glu

The kinetic data previously summarized might help us interpret the incidence of hypothalamic lesions observed in newborn and adult animals (12,14). Table 9 summarizes some of the data reported in the literature or in this book concerning the incidence of hypothalamic lesions in various animal species, both newborns and adults. The newborn mouse is the species most sensitive to the neurotoxic effect of MSG given by gavage, followed by rats and guinea pigs. For monkeys there are contradictory reports, but most of the authors could not find the lesion. Dogs are also apparently insensitive (Heywood and Worden, *this volume*).

The effect of chemicals always depends on two factors: the concentrations present at the site of action and the sensitivity of the target organ. If we try to correlate the

TABLE 9. *Correlation between the incidence of hypothalamic lesions and Glu plasma AUC after administration of MSG by stomach tube to several animal species*

Dose (g/kg)	Incidence of hypothalamic lesions (and AUC)	
	Newborns	Adults
0.5	0 (—)[a] Rat	
	0 (200) Mouse	
		0 (288) Rat
1.0	0 (813) Rat	0 (309) Mouse
	0 (321) Guinea pig	0 (487) Guinea pig
	100 (375) Mouse	0 (143) Dog
		0 (53) Rhesus monkey
2.0	60 (—)[a] Rat	30 (—)[a] Rat
	20 (516) Guinea pig	0 (179) Dog
		0 (86) Rhesus monkey
4.0	100 (—)[a] Rat	60 (494) Rat
	80 (—)[a] Guinea pig	0 (187) Dog
		0 (150) Rhesus monkey

[a] Data not available.

exposure to MSG, expressed as plasma AUC (Table 9) with hypothalamic lesions, we note that no lesions are observed unless the AUC is more than 200 μmoles/ml $\times$ min. However, this value may not be enough to induce a lesion, as shown for instance in newborn rats and guinea pigs. It should be stressed that this conclusion is tentative, since so little data is available and most of the reports used for this correlation were from different laboratories.

The discussion can be more elaborate concerning mice, since this species is the most sensitive to the neurotoxicity of MSG and has therefore been widely tested. Table 10 summarizes the percentage of mice with hypothalamic lesions according to several authors who used various doses and concentrations of MSG. The toxicological data compared with the plasma AUC obtained under similar conditions show that the percentage of lesions is related more to the plasma AUC than to the dose of MSG administered. For instance, the same dose of MSG (0.5 g/kg) can give 50% lesions if used at a 20% concentration or no lesions when employed at 2, 5, or 10%. The total exposure to MSG, therefore, expressed in this study by the AUC value, may be a better index of toxicity than the administered dose. Within the limits of the present results, it looks as though plasma exposure to MSG over about 200 μmoles/ml $\times$ min (or about 33.330 μg/ml $\times$ min) may result in hypothalamic damage.

PLASMA Glu LEVELS IN HUMAN PREMATURE NEWBORNS

Plasma Glu levels measured in 21 premature newborns (average weight 2,008 $\pm$ 17 g, age 19 $\pm$ 3 days) in blood obtained from the umbilical vein were considerably higher than in human adults (0.50 $\pm$ 0.07 μmoles/ml $\pm$ SE as opposed to 0.06

TABLE 10. *Correlation between hypothalamic lesions and plasma AUC in newborn mice after administration of MSG at various doses and concentrations*

Oral dose (g/kg)	Concentration (% w/v)	Hypothalamic lesions (%)	Plasma AUC (μmoles/ml × min)
0.25	10	0[a]	103
0.50	2	0[b]	126
0.50	5	0[b]	147
0.50	10	0[c]	200
0.50	20	50[a]	318
0.75	20	75[a]	—
1.00	2	50[b]	—
1.00	5	80[b,d]	—
1.00	10	100[b]	375
1.00	20	100[a]	—

[a] From Olney, ref. 13.
[b] R. Heywood et al., *personal communication.*
[c] From Takasaki, ref. 23.
[d] From Lemkey-Johnston and Reynolds, ref. 9.

± 0.003 μmoles/ml ± SE). There was no correlation between Glu plasma level and weight ($r^2 = 0.25$) or age ($r^2 = 0.30$).

Human milk is known to contain relatively high levels of free Glu (22). By calculating the amount of milk given with a meal, it is possible to work out that premature newborns receive between 1 and 11 mg/kg (2.76 ± 0.51) of Glu in a single intake.

In blood samples taken for medical reasons from premature newborns, it was possible to measure the levels of Glu at various times after a meal. The results obtained show that Glu plasma levels were 0.54 ± 0.09 μmoles/ml (± SE) when measured between 5 and 90 min after the milk meal. This value is not statistically different from the basal value. These studies indicate that premature newborns can metabolize Glu after a milk meal so that plasma Glu levels are kept within the range of normal values (B. Assael et al., *unpublished*).

PLASMA Glu LEVELS IN MAN

As previously mentioned, the levels of plasma Glu in man are somewhat lower than in the rodents examined in this study. A total of 109 volunteers (49 females and 60 males, 26.04 ± 0.53 years of age and 62.03 ± 0.99 kg body weight) received a dose of 60 mg/kg of MSG with a bouillon (2% solution), which is a large dose for any food enriched with MSG. The subjects were fasted for 5 hr and were then required to drink the bouillon in 3 min, after which they received no further food for another 5 hr. Blood samples were withdrawn before MSG and 15, 30, 60, and 120 min after MSG.

The average peak level was reached 30 min after MSG administration (0.194 ± 0.09 μmoles/ml). The apparent plasma $T½$ was 68 ± 4.7 min, while the AUC was

TABLE 11. *AUC of plasma Glu after oral doses of MSG in man*

No. subjects	Dose MSG		AUC
	mg/kg	Percent	(μmoles/min × min)
7	30	2	1.69
109	60	2	5.56
6	120	4	8.00
5	60	Tomato juice	2.89
5	—	Meal	0.22

different ($p < 0.05$) for females (4.74 ± 0.53 μmoles/ml × min) and males (6.23 ± 0.49 μmoles/ml × min). The distribution of basal Glu levels and of the AUC was normal (0.06 and 0.08 by Kolmogorov-Smiznov's test); there was no correlation between basal Glu and AUCs (linear regression) or between basal plasma levels or AUCs and age, weight, or cigarette, coffee, or tea consumption (multiple regression analysis). The AUCs of subjects with postprandial symptoms (headache, gastric acidity, etc.) were not statistically different from subjects free of symptoms. Previous studies performed according to a double-blind design by Morselli et al. (10) did not show any specific effect of MSG in terms of side effects (26).

Table 11 summarizes the plasma AUC in adults after the administration of 30, 60, and 120 mg/kg of MSG. Plasma AUCs are clearly proportional to the dose given. Table 11 also gives the plasma AUC values obtained after adding 60 mg/kg of MSG to tomato juice at the concentration of 2%. MSG added to tomato juice results in lower Glu plasma levels than when it is given with a bouillon. Therefore, in man, as in mice, MSG taken with nutrients gives considerably lower plasma Glu levels.

RELEVANCE OF Glu NEUROTOXICITY FOR MAN

The data from the literature summarized above (see also Table 9) indicate that the newborn mouse appears to be the most sensitive animal species to the neurotoxicity of MSG. In the absence of better criteria, it seems safest to take the kinetic data of newborn mice as a basis for extrapolating safety ratios from animals to man.

As reported for mice in Table 10, an ED_{50} of MSG (dose producing hypothalamic lesions in 50% of the subjects; i.e., 500 mg/kg by gavage in solution at 20%) results in a plasma AUC of 318 μmoles of Glu/ml × min. If this kinetic parameter is compared with the AUC obtained in man after different doses of MSG, it is possible to obtain the safety ratios reported in Table 12. A ratio of 188 is calculated for human ingestion of a single dose 14 to 15 times the U.S. average daily intake of MSG (5). This figure becomes 57 when a dose of 60 mg/kg is utilized. It should be remembered that this is the largest single dose still palatable, at least according to Western taste preferences. Even at unpalatable doses (120 mg/kg of MSG), unlikely to be ingested under normal conditions, the safety ratio is still 40.

TABLE 12. *Safety ratios for MSG calculated assuming that the sensitivity of man to the neurotoxic effect of MSG is similar to that of newborn mice*

Animal species	Oral dose (mg/kg)	AUC (μmoles/ml × min)	Safety ratio according to	
			Dose	AUC
Mouse	500	318	—	—
Man	30[a]	1.69	16.6	188
	60	5.56	8.3	57
	120[b]	8.00	1.6	40
	60[c]	2.89	8.3	110
	Meal	0.22	—	1,445

[a] This is 14–15 times the average daily intake of MSG in U.S.
[b] Unpalatable.
[c] Dissolved in tomato juice.

It should, however, be clearly stated that these calculations have only theoretical value because they have no direct bearing on the utilization of MSG as a food additive. In fact, when 60 mg/kg of MSG are given in tomato juice instead of in aqueous solution, the safety ratio increases to 110.

Furthermore, when MSG was given to sensitive animal species with a meal even at very high doses and for prolonged periods of time brain lesions or any other toxic effect were not reported (Takasaki, *this volume*), reinforcing the view that gavage of aqueous solutions is a route of administration that gives rise to high Glu plasma levels not achievable by regular feeding.

The data utilized for establishing safety ratios were obtained in adult humans. It is, however, believed that these conclusions can also be applied to infants in view of the fact that newborn prematures can metabolize free Glu at the dose of 2.7 ± 0.51 mg/kg (see above) and that within the range of doses at which MSG can be consumed, Filer et al. (*this volume*) have not observed any difference between infants and adults in absorption and/or disposal of MSG.

In conclusion, therefore, although Olney's findings are extremely interesting in terms of utilizing MSG as a toxicological tool, it is fortunate that they were obtained under a number of experimental conditions (animal species, age, maturity, route of administration, dose, and concentration of MSG) that all tend to induce high, long-lasting plasma Glu levels not likely to occur in man, infant, or adult, even under extreme conditions of MSG intake.

REFERENCES

1. Baker, S. B. de C., and Foulkes, D. M. (1973): Blood concentrations of compounds in animal toxicity tests. In: *Biological Effects of Drugs in Relation to Their Plasma Concentrations*, edited by D. S. Davies and B. N. C. Prichard, pp. 41–50. Macmillan, London.
2. Bernt, E., and Bergmeyer, H. U. (1974): UV assay with glutamate dehydrogenase and NAD. In: *Methods of Enzymatic Analysis, Vol. 4*, edited by H. U. Bergmeyer, pp. 1704–1708. Academic Press, New York.
3. Bizzi, A., Veneroni, E., Salmona, M., and Garattini, S. (1977): Kinetics of monosodium glutamate in relation to its neurotoxicity. *Toxicol. Lett.*, 1:123–130.

4. Cohen, A. I. (1967): An electron microscopic study of the modification by monosodium glutamate of the retinas of normal and "rodless' mice. *Am. J. Anat.*, 120:319–326.
5. Committee on GRAS List Survey—Phase III (1976): *Application of a probabilistic method in estimating daily intakes of certain GRAS substances*. Food and Nutrition Board, Division of Biological Sciences, Assembly of Life Sciences, National Research Council, National Academy of Sciences, Washington, D.C. (*In preparation*).
6. Folch-Pi, J. (1955): Composition of the brain in relation to maturation. In: *Biochemistry of the Developing Nervous System*, edited by H. Waelsch, pp. 121–136. Academic Press, New York.
7. James, R. W., Heywood, R., Worden, A. N., Garattini, S., and Salmona, M. (1978): The oral administration of MSG at varying concentrations to male mice. *Toxicol. Lett.*, 1:195–199.
8. Liebschutz, J., Airoldi, L., Brownstein, M. J., Chinn, N. G., and Wurtman, R. J. (1977): Regional distribution of endogenous and parental glutamate, aspartate and glutamine in rat brain. *Biochem. Pharmacol.*, 26:443–446.
9. Lemkey-Johnston, N., and Reynolds, W. A. (1972): Incidence and extent of brain lesions in mice following ingestion of monosodium glutamate (MSG). *Anat. Rec.*, 172:354–359.
10. Morselli, P. L., and Garattini, S. (1970): Monosodium glutamate and the Chinese restaurant syndrome. *Nature*, 227:611–612.
11. Newman, A. J., Heywood, R., Palmer, A. K., Barry, D. H., Edwards, F. P., and Worden, A. N. (1973): The administration of monosodium L-glutamate to neonatal and pregnant rhesus monkeys. *Toxicology*, 1:197–204.
12. Olney, J. W. (1969): Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. *Science*, 164:719–721.
13. Olney, J. W. (1969): Glutamate-induced retinal degeneration in neonatal mice. Electron microscopy of the acutely evolving lesion. *J. Neuropathol. Exp. Neurol.*, 28:455–474.
14. Olney, J. W. (1971): Glutamate-induced neuronal necrosis in the infant mouse hypothalamus. An electron microscopic study. *J. Neuropathol. Exp. Neurol.*, 30:75–90.
15. Olney, J. W., and Ho, O.-L. (1970): Brain damage in infant mice following oral intake of glutamate, aspartate or cysteine. *Nature*, 227:609–611.
16. Olney, J. W., Ho, O.-L., and Rhee, V. (1971): Cytotoxic effects of acidic and sulphur containing amino acids on the infant mouse central nervous system. *Exp. Brain Res.*, 14:61–76.
17. Olney, J. W., Sharpe, L. G., and Feigin, R. D. (1972): Glutamate induced brain damage in infant primates. *J. Neuropathol. Exp. Neurol.*, 31:464–488.
18. Palkovits, M., Brownstein, M., Saavedra, J. M., and Axelrod, J. (1974): Norepinephrine and dopamine content of hypothalamic nuclei of the rat. *Brain Res.*, 77:137–149.
19. Perez, V. J., and Olney, J. W. (1972): Accumulation of glutamic acid in the arcuate nucleus of the hypothalamus of the infant mouse following subcutaneous administration of monosodium glutamate. *J. Neurochem.*, 19:1777–1782.
20. Perez, V. J., Olney, J. W., and Robin, S. J. (1973): Glutamate accumulation in infant mouse hypothalamus: Influence of temperature. *Brain Res.*, 59:181–189.
21. Reynolds, W. A., Lemkey-Johnston, N., Filer, L. J. Jr., and Pitkin, R. M. (1971): Monosodium glutamate: Absence of hypothalamic lesions after ingestion by newborn primates. *Science*, 172:1342–1344.
22. Stegink, L. D., Filer, L. J. Jr., and Baker, G. L. (1972): Monosodium glutamate: Effect on plasma and breast milk amino acid levels in lactating women. *Proc. Soc. Exp. Biol. Med.*, 140:836–841.
23. Takasaki, Y. (1978): Studies on brain lesion by administration of monosodium L-glutamate to mice. I. Brain lesions in infant mice caused by administration of monosodium L-glutamate. *Toxicology*, 9:293–305.
24. Wenn, C., Kenneth, C. H., and Gershoff, S. N. (1973): Effects of dietary supplementation of monosodium glutamate on infant monkeys, weanling rats and suckling mice. *Am. J. Clin. Nutr.* 26:803–813.
25. Young, R. L., and Lowry, O. H. (1966): Quantitative methods for measuring the histochemical distribution of alanine, glutamate and glutamine in brain. *J. Neurochem.*, 13:785–793.
26. Zanda, G., Franciosi, P., Tognoni, G., Rizzo, M., Standen, S. M., Morselli, P. L., and Garattini, S. (1973): A double blind study on the effects of monosodium glutamate in man. *Biomedicine*, 19:202–204.