Glutamic Acid: Advances in Biochemistry and Physiology, edited by L. J. Filer, Jr., et al. Raven Press, New York © 1979.

In Utero and Dietary Administration of Monosodium L-Glutamate to Mice: Reproductive Performance and Development in a Multigeneration Study

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Safety evaluation of a food additive or ingredient must necessarily involve a progression from the acquisition of a thorough knowledge of its nonbiological aspects to a basic framework of appropriate biological tests that permits the judicious interpretation of the data. In the context of short- and long-term exposures of the test system, pharmacokinetics, biotransformation, special tests such as those for reproductive function, teratology, and neurotoxicity, and other miscellaneous tests to establish synergism or antagonism, all fall in the category of appropriate biological or toxicological procedures. Certain basic considerations and assumptions underlying these test procedures may be emphasized:

1. All substances can elicit a response in an appropriate biological test system.
2. Such a response is a function of the dose administered and the duration of the exposure.
3. The response may be modified by other factors such as sex, age, nutritional and health status, diet and strain of the animal, and interaction of the test material with other substances to which the animal may be exposed before, during, or after administration.
4. The appropriateness of the test procedures employed is critical. Tests should fit the material. The same experimental design may not be suitable for all, or any other, test substance.

Where a subchronic or prolonged toxicity test is planned, it therefore needs to be recalled that it is truly "a test of (the) measurable harmful effect of the substance on the biological system, occurring as a consequence of administering repeated doses, usually by dosing the system (animal) on a daily basis for 90–120 days, or longer."

Safety-in-Use of Monosodium L-Glutamate (MSG)

The absolute safety of any substance can only be questionably proved to one's own satisfaction. One may therefore appreciate that the literature on the safety-in-

use of the ubiquitous glutamates as food-flavoring ingredients, although abundant and clear, continues to cause concern. Why? A brief recapitulation of certain pertinent though contrasting published findings in this context can be useful.

The repeated subcutaneous administration of MSG to newborn mice resulted in severe damage to the inner layers of the retina (5,12). Olney (16), under the same conditions of administration, discovered discrete brain lesions, mainly in the preoptic and arcuate nuclei of the hypothalamus, together with scattered neurons within the median eminence. Olney and Ho (17) further found that the arcuate nuclei were damaged when mice that were 10 to 12 days old were given oral doses of 3 mg MSG/g body weight. Equivalent amounts of NaCl had no effect. The early brain damage in MSG-treated rodents is considered to account, at least in part, for many of the endocrine disturbances observed in later life, such as adult obesity without accompanying hyperphagia (2,3,13,15,16), skeletal stunting (2,16), and reproductive dysfunction and sterility in both sexes (16,22). Djazayery and Miller (6) and Djazayery et al. (7) injected 5 mg MSG/g body weight intraperitoneally to weanling female mice, but with only a moderate success in inducing obesity.

Although administration of MSG to newborn rodents by either s.c. or i.p. injection almost always resulted in brain lesions, by and large, the dietary administration of MSG at even very high doses was not found to result in any of these symptoms, including the endocrine disturbances (3,10,20,26,27,30). Ebert (8) reported that in chronic toxicity trials with rats and mice fed over a 2-year period with two levels of MSG incorporated in diet, there were no abnormalities in body weight gain, food intake, hematology, or histopathology. Owen et al. (20) fed weanling rats diets containing added MSG at 1, 2, or 4% w/w for 2 years and found no adverse effects on body weight gain, economy of food consumption, hematology, blood chemistry, organ weights, or mortality by comparison with control rats receiving the basal diet. Besides focal mineralization at the renal corticomedullary junction occurring with equal frequency in all groups, including the controls, they observed no other histological changes of any significance. It is our experience that the renal change itself is probably due to a mineral (Ca/P) imbalance in the diets.

Different routes of administration of MSG have, therefore, much different effects on even the same test animal species. This is an important point.

Route of Administration

Since MSG is a food-flavoring ingredient, what would be the ideal approach of assessing its safety-in-use, or even potential toxicity in the experimental animal?

The most uncertain aspect of safety evaluation, whatever the primary concept underlying the experimentation—be it nutritional, pharmacological, or physiological—is the relevance of animal data to humans. Indeed, in the case of MSG it would be reasonable to argue that the oral route of administration, especially when admixed with the diet of the animal, represents the only true logical approach to an investigation of its long-term safety, or its cumulative effect on the animal. The present investigations have centered on the assessment of the effects of "*in utero*

and preweaning exposures and postweaning dietary administration of MSG to mice on their growth, food and MSG intake, reproductive performance, and brain morphology."

Principal Questions

Why mice? The species differences noticed by a number of workers may be broadly attributed to differences in (a) the effect of the animal on the substance and (b) the effect of the substance on the animal. For the dietary evaluation of the long-term, or subchronic toxicity of MSG, it might be agreed that the mouse appears to be the most sensitive species. The trials involved three generations of mice and attempted to answer such principal questions concerning the safety of MSG as whether it would bring about any of the following:

Reduced reproductive capacity.
Malformation or stunted development of the newborn.
Brain lesions, early or later in life.
Abnormal or excessive growth and food intake during postnatal life.
Adult obesity, with or without accompanying hyperphagia.
Reduced fertility.
Any other pathological change.

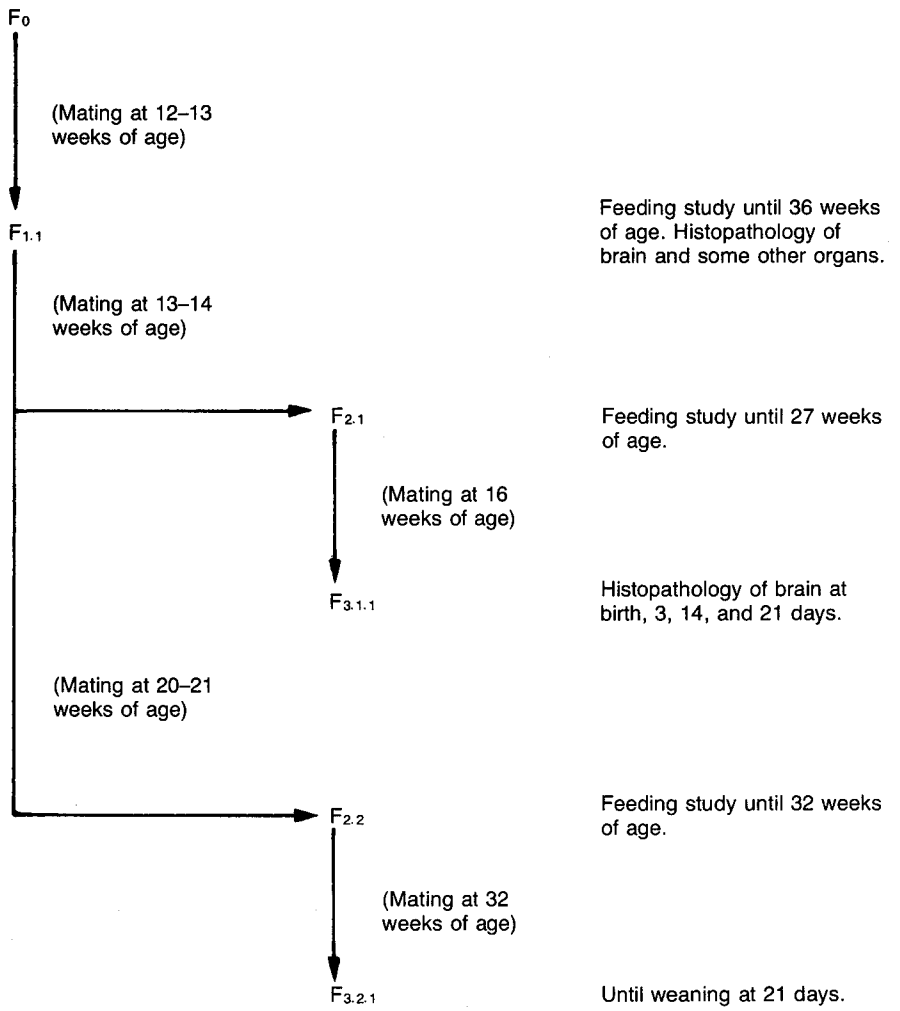
DESIGN OF THE MULTIGENERATION STUDY

The organization of the multigeneration trials is set out schematically in Table 1, and the dietary treatments, starting with those for the animals of the F_0 generation, in Table 2.

Among the variety of possible toxic effects that can interfere with the functions of organs and tissues are those occurring during reproduction, i.e., fertility, parturition, and lactation. It is feasible to study these aspects during a period shorter than the animal's lifetime. Information on reproductive performance is essential to an evaluation of a potential hazard, since this complex physiological state is highly susceptible to specific deleterious effects. The fact that previous experimental evidence has shown the increased susceptibility of the newborn, as well as of the embryo and of the fetus to MSG compared to the older animal, made it desirable to investigate the influence of both the *in utero* and dietary administration of this substance on mouse reproduction. The composition of the basal diet is set out in Table 3. MSG was incorporated into this basal diet at two levels: 1 and 4% w/w.

Mice have an acute sense of hearing, and the audiogenic seizures that are provoked by intense noises in certain strains lead to interference with breeding performance (31). To ensure freedom from all stress, including that due to transportation, the F_0 generation of the SPF-derived, CD-1, COBS strain of mice on receipt from Charles-River Farms, France, postweaning, were maintained behind a barrier, as were the future generations. Animals for the feeding trials were housed singly, with

TABLE 1. *Organization of multigeneration protocols for the safety evaluation of MSG in mice*



all receiving food and water supply freely. The mating condition was three females to one male.

Besides the free-feeding trials with the $F_{1.1}$, $F_{2.1}$, and $F_{2.2}$ generations, reproduction studies in the $F_{1.1}$, $F_{2.1}$, $F_{2.2}$, $F_{3.1.1}$, and $F_{3.2.1}$ generations, and food and MSG intake in a voluntary manner, histopathological evaluation of brain tissue was carried out for animals of the $F_{1.1}$ generation and in the newborn 3-, 14-, and 21-day-old mice of both sexes of the $F_{3.1.1}$ generation (Table 4).

TABLE 2. *Dietary treatment: schematic representation*

Generation	Experimental groups						
	A	B	C	D	E	G	H
F_0							
On arrival at 3–4 weeks of age until mating at 12 weeks	Basal, reference diet without added MSG						
During gestation, 12–15 weeks of age	Basal	1% MSG	4% MSG	1% MSG	4% MSG	1% MSG	4% MSG
During lactation, for 3 weeks	Basal	1% MSG	4% MSG	Basal	Basal	1% MSG	4% MSG
F_1							
Postweaning, to 36 weeks of age	Basal	1% MSG	4% MSG	Basal	Basal	Basal	Basal
During gestation and lactation	Basal	1% MSG	4% MSG	—	—	—	—
F_2							
Postweaning, to 27 weeks—$F_{2.1}$ to 32 weeks—$F_{2.2}$	Basal	1% MSG	4% MSG	—	—	—	—
During gestation and lactation	Basal	1% MSG	4% MSG	—	—	—	—
F_3							
To dams until pups were weaned	Basal	1% MSG	4% MSG	—	—	—	—

TABLE 3. *Composition of basal diet*

Gross energy (on dry matter):	4.63	kcal/g	
Protein:[a]	230	g/kg	
Total lipids:	50	g/kg	
Nonnutritive cellulose:	40	g/kg	
Total carbohydrates:	500	g/kg	
Composite vitamin-mineral mixture:	60	g/kg	
Moisture (maximum):	120	g/kg	

Mineral components (mg/kg):		Vitamins:		
Phosphorus (P):	7,800	Vitamin A:	16,800	IU/kg
Calcium (Ca):	8,400	Vitamin D_3:	4,000	IU/kg
Potassium (K):	7,500	Thiamine:	8	mg/kg
Sodium (Na):	3,400	Riboflavin:	13	mg/kg
Magnesium (Mg):	1,700	Pantothenic acid:	27	mg/kg
Manganese (Mn):	67	Pyridoxine:	4	mg/kg
Iron (Fe):	280	Niacin:	88	mg/kg
Copper (Cu):	30	Menadione:	6	mg/kg
Zinc (Zn):	64	Vitamin E:	47	mg/kg
Cobalt (Co):	2	Folic acid:	1	mg/kg
Iodide from marine algae		Biotin:	0.1	mg/kg
		Vitamin B_{12}	0.04	mg/kg
		Choline:	2,100	mg/kg

Note: MSG was added at 1 or 4% w/w to this basal diet, ensuring that all diets were finally isocaloric and isonitrogenous.

[a] Protein (% N × 6.25) as defatted soybean meal, food yeast, fish meal, and milk whey solids.

TABLE 4. *Numbers of mice used in trials—all generations*

	Control		1% MSG		4% MSG	
Generation	Male	Female	Male	Female	Male	Female
F_0	33	99	17	51	17	51
$F_{1.1}$	370	357	123	133	136	116
$F_{2.1}$	229	219	84	93	91	85
$F_{2.2}$	122	114	59	67	66	63
$F_{3.1.1}$	110	107	58	59	53	57
$F_{3.2.1}$	35	31	27	31	38	27
Total/sex	899	927	368	434	401	399
Total	*1,826*		*802*		*800*	

Note: Numbers of mice in treatment groups D, E, G, and H in the F_0 and $F_{1.1}$ generations are not shown here.

GROWTH AND BODY WEIGHT DISTRIBUTION FREQUENCY

The growth data set out in Figs. 1, 2, and 3 for both sexes of the $F_{1.1}$, $F_{2.1}$, and $F_{2.2}$ generations, respectively, do not need further elaboration. In all the trials, growth curves for the MSG-treatment groups were similar to those for the controls. There were no abnormal developments or abnormal rates of growth in either sex,

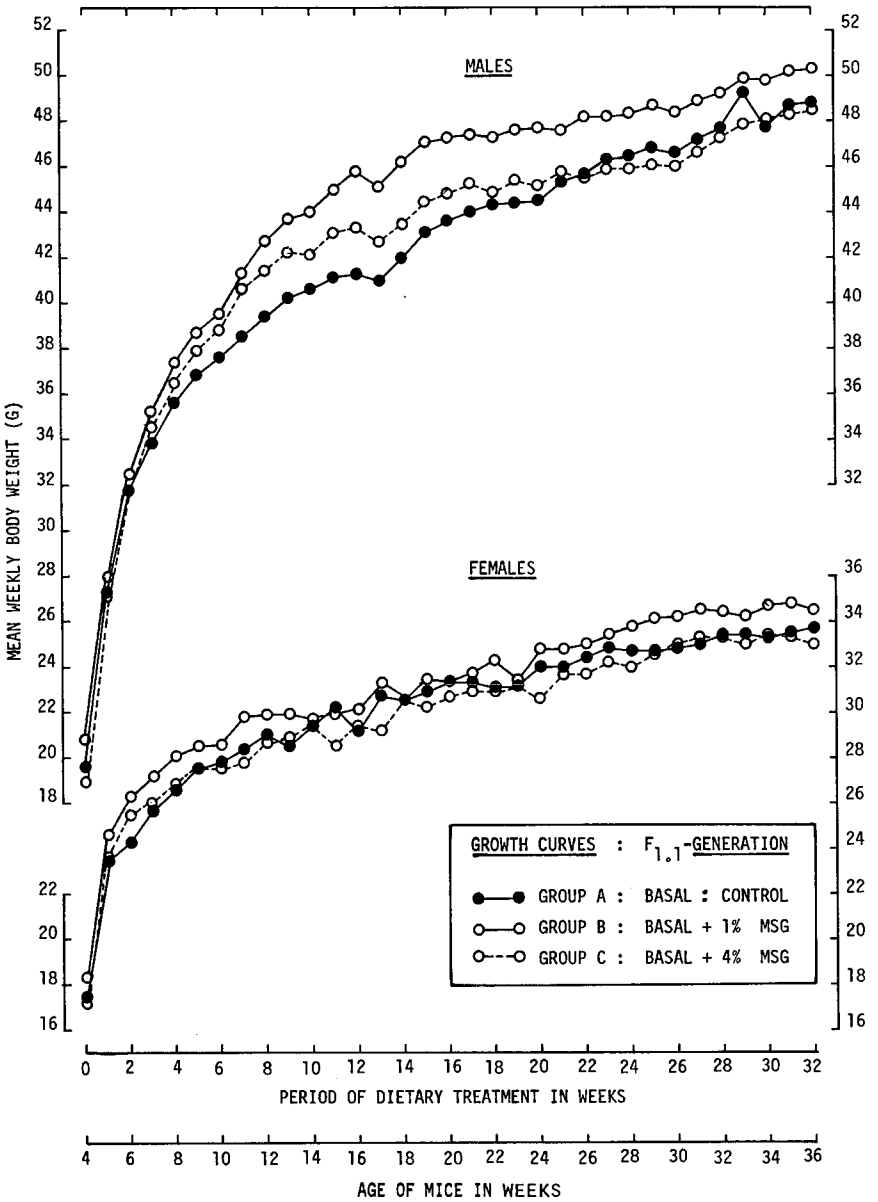


FIG. 1. Growth curves for the $F_{1.1}$ generation.

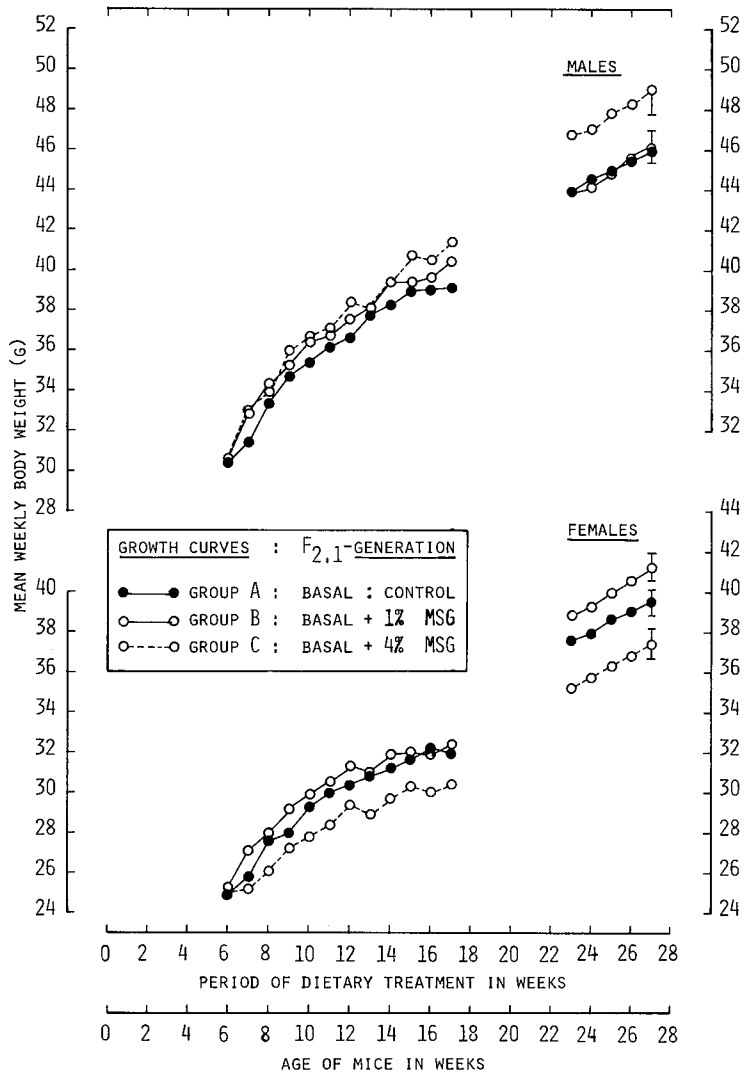


FIG. 2. Growth curves for the $F_{2.1}$ generation. Animals were not weighed between weeks 18 and 23. Terminal values indicated ± SEM.

nor in any of the generations or litters. None of the animals were found to feel fatty or predisposed to obesity.

Females of both the MSG and the control groups in the $F_{2.1}$ and $F_{2.2}$ generations were slightly heavier than those of the parent generation, although the differences in body weight between the treatment groups of the same generation were not statisti-

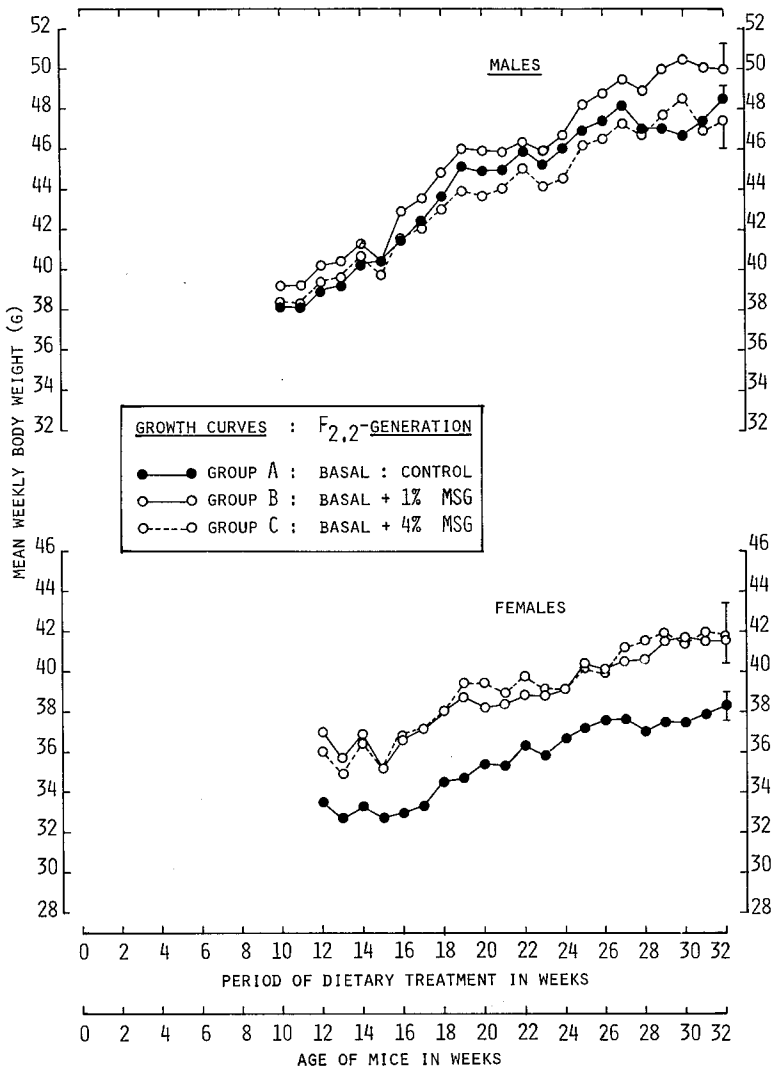


FIG. 3. Growth curves for the $F_{2.2}$ generation. Animals were weighed beginning week 12. Terminal values indicated ± SEM.

cally significant. This is explained as due to the small number of heavier animals in these subsequent generations at the start of trials, chiefly because they were derived from small-sized litters.

As descriptions of the frequency distribution of a series of observations, the most important values are usually the mean and standard deviation. With a normal distribution, only 1 in 20 observations will differ from the mean by more than twice

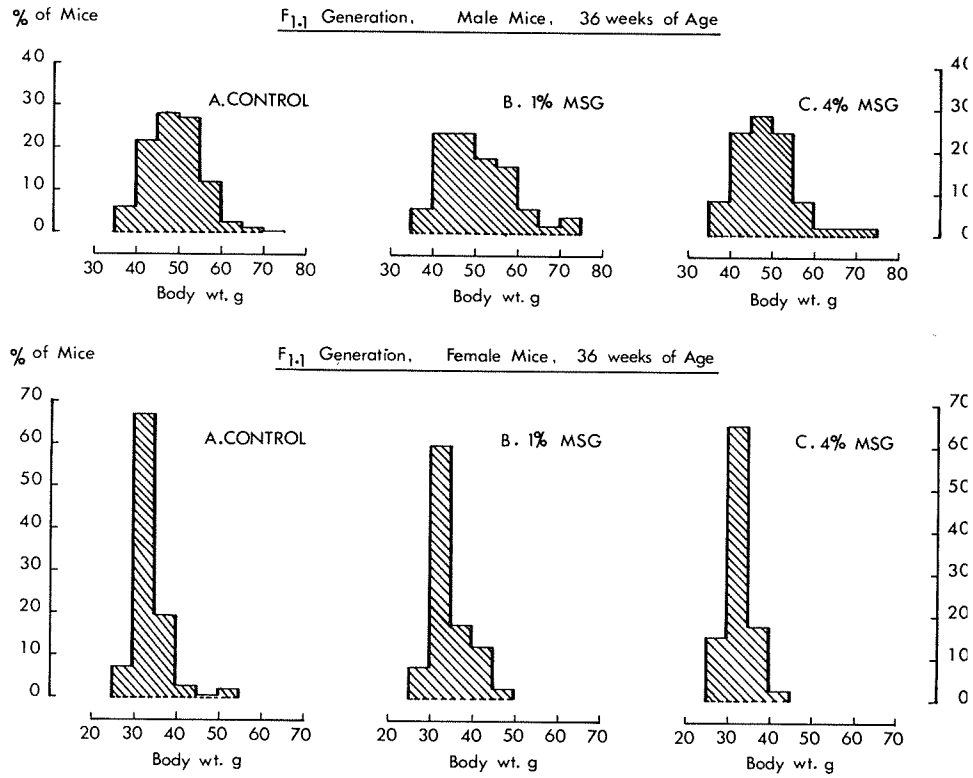


FIG. 4. Body weight distribution frequency for the $F_{1.1}$ generation. Histograms represent terminal values at age 36 weeks.

the standard deviation (±), and only some 3 in 1,000 will differ from the mean by more than three times the standard deviation (±).

Body weight distribution frequencies of both sexes of the $F_{1.1}$, $F_{2.1}$, and $F_{2.2}$ generations are represented as histograms in Figs. 4, 5, and 6, respectively. The outliers in each case were again traced to small-sized litters. Otherwise, the histograms permit an eye-fit evaluation of the similarity in the frequency distribution between the controls and the MSG groups.

FOOD INTAKE

Weekly food intake, measured throughout the 32 weeks of trial in the $F_{1.1}$ generation (Fig. 7) showed that, by and large, mice of both sexes of all three groups ingested similarly. Regular fluctuations in food intake occurred in both sexes, although a small but definite increase in food intake in females of all treatment groups was also registered with the progress of the trial, visibly so until the mice

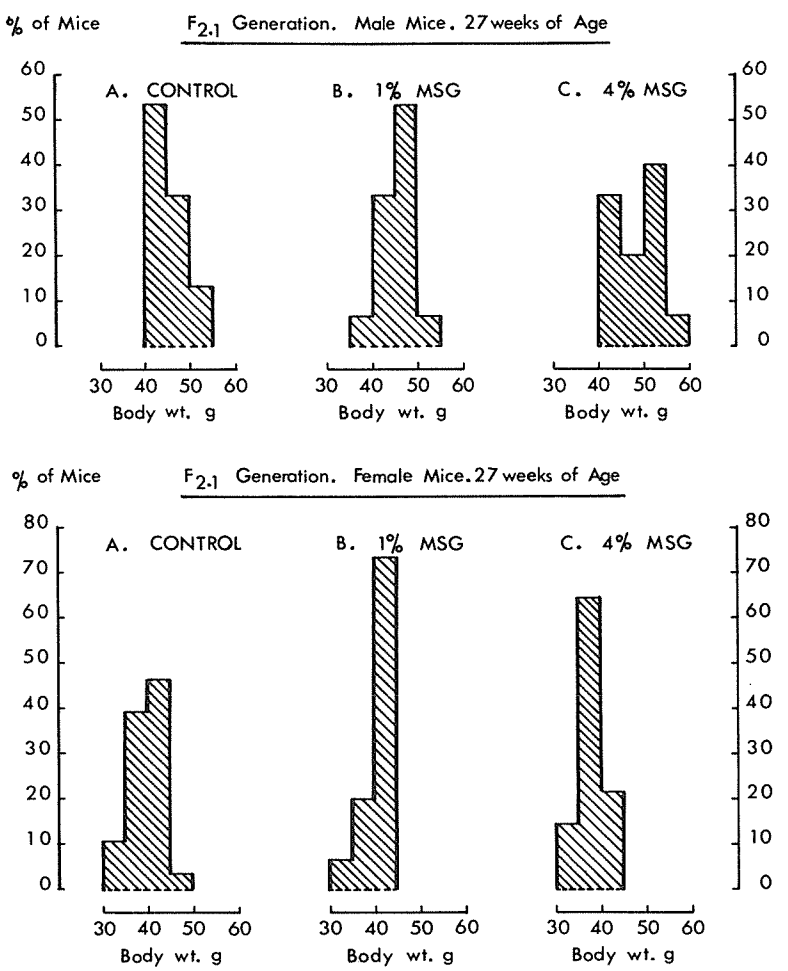


FIG. 5. Body weight distribution frequency for the $F_{2.1}$ generation. Histograms represent terminal values at age 27 weeks.

were 26 weeks of age. A similar pattern of fluctuation was also noted for females of the $F_{2.1}$ generation (Fig. 8), although there was no hyperphagia, nor any significant differences between control and the MSG-treated groups. In this generation, however, food intake was not measured throughout the duration of the trial.

How then explain the progressive, though small, increase with age, as well as the occasional, but definite, decrease in food intake of female mice? A number of investigators have observed that the food intake and energy expenditure of the female cycling rodent varies with the stage of the ovulatory cycle, which is itself distinguished by a waxing and waning of the plasma estradiol concentrations, with the maximum levels reached at proestrus and estrus, and the nadir at diestrus and

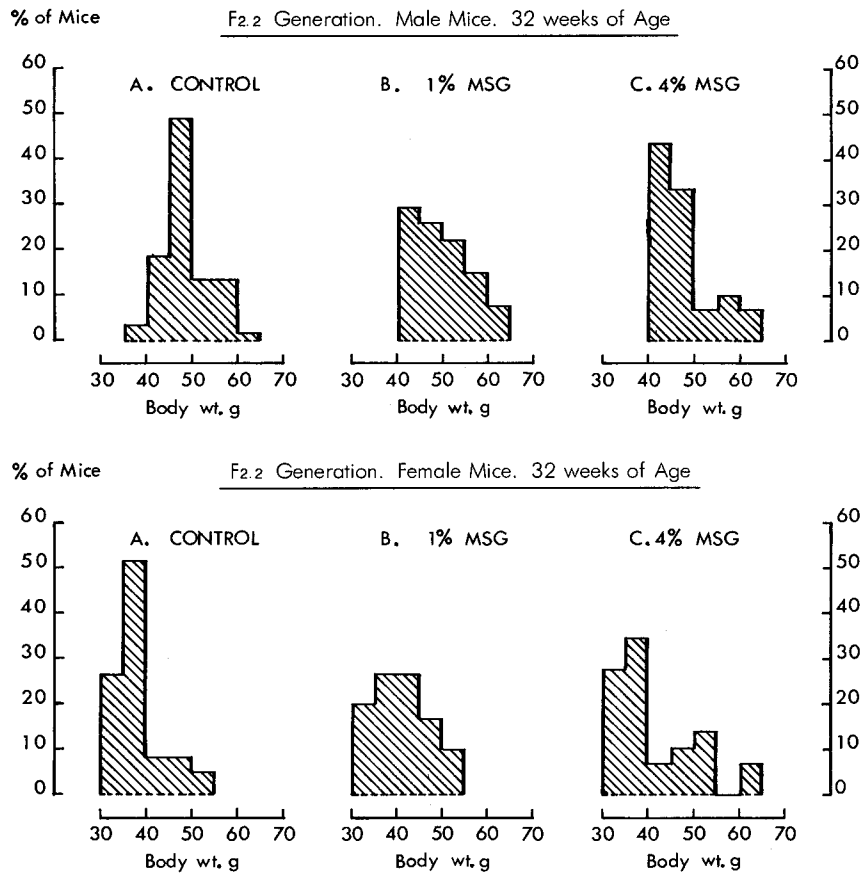


FIG. 6. Body weight distribution frequency for the $F_{2.2}$ generation. Histograms represent terminal values at age 32 weeks.

metestrus (28,29). The food intake of the cycling rodent varies inversely with the plasma estradiol concentration, diminishing at estrus and increasing at diestrus. Any attempt to explain appetite and food intake behavior, and the influence of hormones on these, would be out of place here, though it would seem the logical explanation for the present findings.

MSG INTAKE

Safety evaluation is currently founded on the concept of the "maximum no-effect dose." All approaches are designed to determine the largest daily intake over extended periods that will not produce the injurious effects characteristic of the test substance when given in larger, i.e., toxic amounts. Just as important, these approaches attempt to exclude the possibility that these "subtoxic" amounts will produce some hitherto unsuspected reaction.

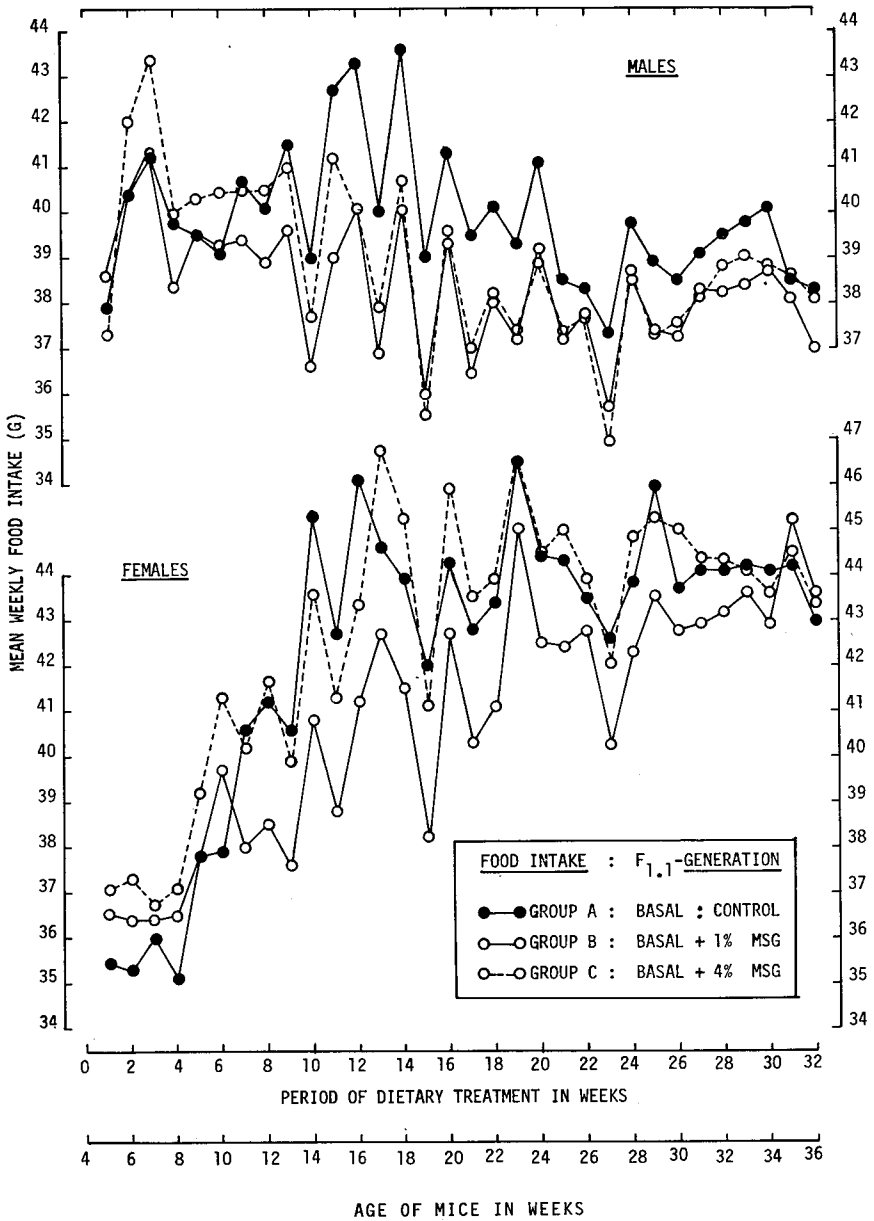


FIG. 7. Food intake curves for the $F_{1.1}$ generation.

The two supplementary levels of MSG were chosen based on a knowledge of the usual food intake of the mouse, i.e., between 5 and 6 g/day, so as to ensure a safe level, and a fairly high level of MSG intake. Now, how much MSG did the animals ingest?

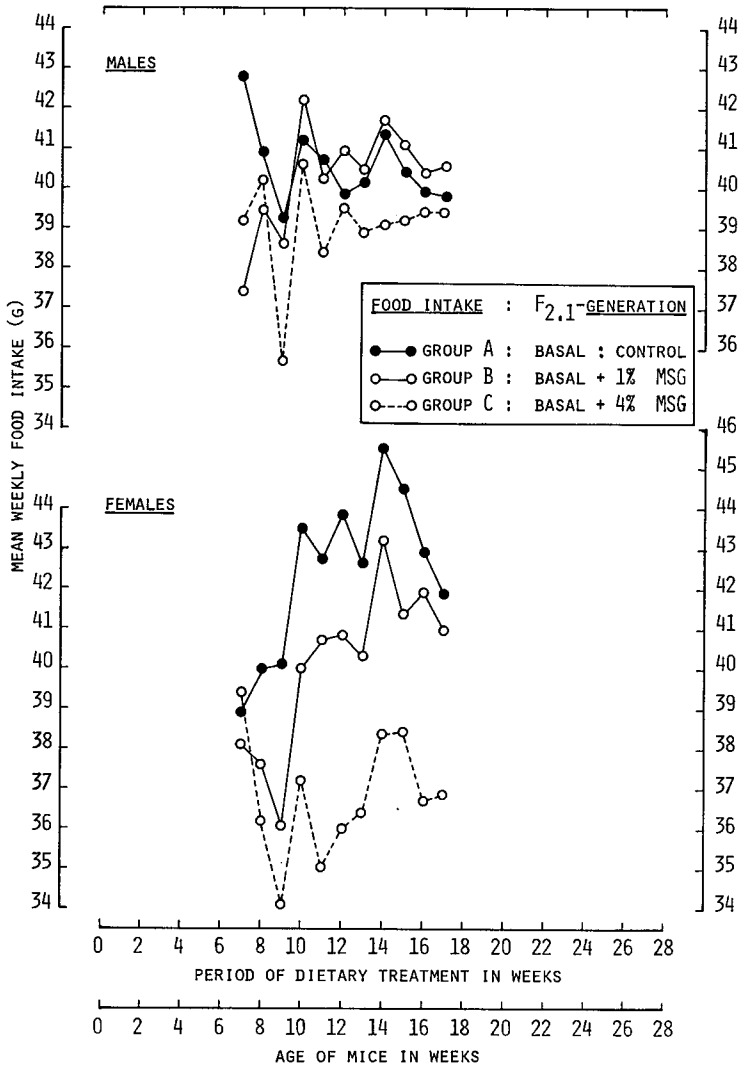


FIG. 8. Food intake curves for the $F_{2.1}$ generation.

MSG Intake During Free Feeding and Lactation

The median intake of MSG as g/kg body weight/day (Table 5) amounted to 1.5 and 6.0 g for males on the 1 and 4% MSG diets, respectively; for the females they were 1.8 and 7.2 g, respectively, due to their lower body weight but similar food intake. The food intake of the adult male, despite the fluctuations noticed, could be considered to be fairly constant over prolonged periods, but that of the dam increases progressively during lactation, averaging 18 to 20 g/day for the 3-week

TABLE 5. *Median MSG intake from diets in growth trials—all generations*

Variable	Males		Females	
	1% MSG	4% MSG	1% MSG	4% MSG
Median body weight (g)	40	40	33	33
Median food intake (g/day)	6.0	6.0	6.0	6.0
Median MSG intake (g/kg/day)	1.5	6.0	1.8	7.2

period. Therefore, the intake of MSG, too, increased during lactation (Table 6). Thus, on the 4% MSG diet, the average intake during the last week of lactation was as high as 25 g/kg body weight/day; yet no adverse effect on the young was observed. Takasaki (26) has reported that ingestion of a 30% w/w MSG diet in a single meal by the lactating dam had no deleterious effect on the dam or on the young. This and other similar observations show that high levels of dietary MSG do not impose any stress or toxic overload on the suckling young, nor cause any abnormal pathology.

When injected subcutaneously or by intragastric means, MSG is absorbed rapidly, leading to elevations of plasma glutamate, whereas dietary ingestion, even in very high amounts, does not lead to such high elevations.

MSG Intake During the Early Postweaning Period

When normally weaned at the age of 21 days, the mouse weighs around 12 g, but its rate of growth during the next 14 days is approximately 1 g/day. The food intake of the mouse during this period is indeed almost near the adult level of intake, which ensures meeting the energy requirements of rapid growth. For a further 4 weeks or so, the rate of weight gain continues at a relatively rapid pace, but with little further increase in food intake. Under these circumstances, the newly weaned mouse would ingest slightly more than 50% of the amount of dietary MSG consumed by the lactating dam during the last week of lactation. The average MSG intake by the newly weaned mouse over a 90-day period is set out graphically in Fig. 9. Thus, on the 4% MSG diet, immediately postweaning, the mouse would ingest around 13 g MSG/kg body weight/day, as against the 25 g/kg body weight/day by the dam.

None of these mice, male or female, developed hyperphagia, hyperactivity, or obesity. Similar observations have been reported by Wen et al. (30) in a study of mice that were injected subcutaneously with varying amounts of MSG from day 6 through 10, and involving a follow-up of the survivors over a 1-year period.

REPRODUCTION PERFORMANCE

A typical reproduction study includes measurement of the following parameters:

Fertility index: the proportion of matings that are successful.
Gestation index: the proportion of pregnancies that result in live litters.

TABLE 6. *Mean body weight, and food and MSG intake of dams during lactation weeks 1 to 3: $F_{1.1}$, $F_{2.1}$, and $F_{2.2}$ generations*

Generation	Body weight (g)			Food intake (g/day)			MSG intake (g/kg/day)		
	1	2	3	1	2	3	1	2	3
$F_{1.1}$									
A. Control	35	35	35	13.0	18.1	21.9	—	—	—
B. 1% MSG	36	36	36	12.9	18.9	23.9	3.6	5.3	6.6
C. 4% MSG	36	36	36	13.6	18.2	22.4	15.1	20.2	24.9
$F_{2.1}$									
A. Control	36	36	36	13.6	19.2	21.7	—	—	—
B. 1% MSG	37	37	37	13.7	17.3	21.7	3.7	4.7	5.9
C. 4% MSG	35	35	35	14.1	19.2	23.1	16.1	21.9	26.4
$F_{2.2}$									
A. Control	42	42	42	14.4	20.0	23.5	—	—	—
B. 1% MSG	44	44	44	16.1	21.6	25.6	3.7	4.9	5.8
C. 4% MSG	44	44	44	16.0	21.5	26.5	14.5	19.5	24.1

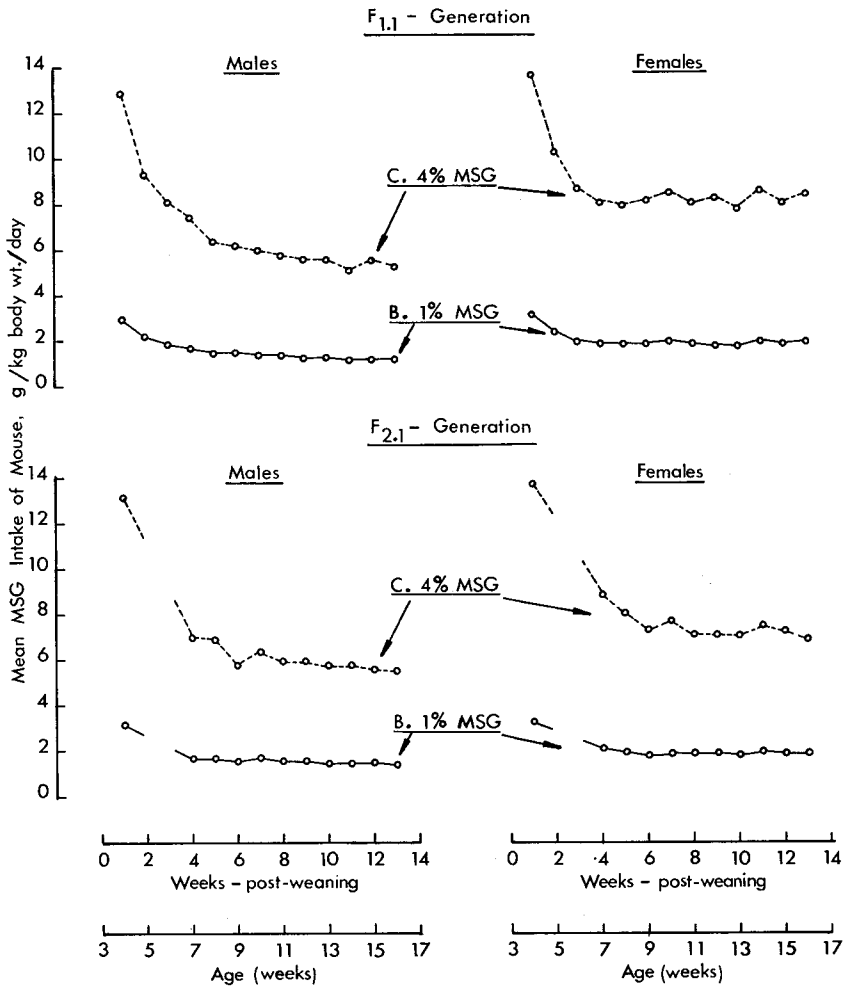


FIG. 9. MSG intake. Values for the $F_{1.1}$ generation are given from postweaning through 90 days of trial. Values for the $F_{2.1}$ generation were measured during 1 week postweaning, and then again from week 7 through 90 days of trial.

Viability index: the proportion of pups born that are alive at 4 days of age.
Lactation index: the proportion of pups alive at 4 days that survive until weaning.

A high viability index usually predisposes a high lactation index, implying a high percentage of weaned young.

The various reproductive parameters for the $F_{1.1}$, $F_{2.1}$, and $F_{2.2}$ generations are set out in Table 7. In all three generations, fertility index, as well as the other parameters, was high (90 to 100%) and identical in all treatment groups. Over 95% of the newborn that were alive on day 4 were weaned by the dam in all groups.

TABLE 7. *Reproductive data in the multigeneration trials: $F_{1.1}$, $F_{2.1}$, and $F_{2.2}$ generations*

Generation	Fertility index (%)	Gestation index (%)	Viability index (%)	Lactation index (%)
$F_{1.1}$				
A. Control	77/99 (78)	77/77 (100)	769/814 (95)	727/769 (95)
B. 1% MSG	28/34 (82)	28/28 (100)	272/283 (96)	256/272 (94)
C. 4% MSG	28/34 (82)	28/28 (100)	254/281 (90)	252/254 (99)
$F_{2.1}$				
A. Control	50/60 (83)	50/50 (100)	462/496 (93)	448/462 (97)
B. 1% MSG	20/20 (100)	20/20 (100)	187/220 (85)	177/187 (95)
C. 4% MSG	18/20 (90)	18/18 (100)	180/192 (94)	176/180 (98)
$F_{2.2}$				
A. Control	26/30 (87)	26/26 (100)	239/250 (96)	236/239 (99)
B. 1% MSG	12/15 (80)	12/12 (100)	133/137 (97)	126/133 (95)
C. 4% MSG	13/15 (87)	13/13 (100)	140/149 (94)	129/140 (92)

There was no adverse influence on any of the reproductive parameters that was attributable to MSG ingestion. In all these cases, the first mating was initiated (Table 1) when the animals were young, i.e., soon after attaining maturity, in accordance with good breeding practice.

Data for the $F_{3.1.1}$ and $F_{3.2.1}$ generations are set out separately for comparison in Table 8. The sharp differences in the reproductive characteristics exhibited by the $F_{3.2.1}$ generation in contrast to the still high fertility of the $F_{3.1.1}$ generation is striking. Evidently, fertility is at its peak soon after maturation. Current breeding practice goes so far as to recommend breeding mice from around 60 days of age, keeping with the view that the breeder life of the animal could be exploited to maximum. In mice of the $F_{3.2.1}$ generation, for which mating was at 32 weeks of age, fertility dropped below 50%, irrespective of dietary treatment. Again, in this case there was no discernible adverse MSG effect.

Semprini et al. (24) observed no reduction in fertility when consecutive litters were raised on diets containing 1 and 2% w/w MSG. On the other hand, supporting the work of Olney (16), Pizzi et al. (21,22) have reported that MSG administered subcutaneously to newborn mice from day 2 to 11 resulted in a sequence of events that manifested in adulthood as marked reproductive dysfunction in both sexes, with treated females having fewer pregnancies and smaller litters, and treated males showing reduced fertility. In contrast, Adamo and Ratner (1) did not observe any pronounced disturbances in the reproductive function of rats that had been sub-

TABLE 8. *Reproductive data in the multigeneration trials: $F_{3.1.1}$, and $F_{3.2.1}$ generations*

Generation	Fertility index (%)	Gestation index (%)	Viability index (%)	Lactation index (%)
$F_{3.1.1}$				
A. Control	22/30 (73)	22/22 (100)	—	—
B. 1% MSG	12/15 (80)	12/12 (100)	—	—
C. 4% MSG	11/15 (73)	11/11 (100)	—	—
$F_{3.2.1}$				
A. Control	9/20 (45)	9/9 (100)	81/85 (95)	66/81 (81)
B. 1% MSG	8/15 (53)	8/8 (100)	77/79 (97)	58/77 (75)
C. 4% MSG	7/15 (47)	7/7 (100)	65/70 (93)	65/65 (100)

Note: The $F_{3.1.1}$ generation was obtained by mating $F_{2.1}$ at 16 weeks of age. This generation of mice was almost entirely taken up for brain histopathology. The $F_{3.2.1}$ generation was obtained by mating $F_{2.2}$ at 32 weeks of age.

cutaneously treated with MSG when 3 to 4 days old, when later evaluated in adult life. Matsuyama et al. (13), however, observed that newborn mice treated subcutaneously with MSG became obese in adult life, but showed no remarkable changes in their reproductive system or in the sexual cycles of the female, although further generations were not raised for additional evidence. These varying observations lead one to infer that in addition to the importance of the route of administration for a desired effect, one needs to take note of species specificity and age of the animal in making evaluations of MSG. Nevertheless, one point seems clear. Dietary administration of MSG to the gestating or lactating dam, or the newly weaned mouse did not result in reproductive dysfunction, or any other associated disorders (24,26,27).

LITTER SIZE

A variety of factors may adversely affect litter size in a reproduction study. A strain possessing a measure of genetic uniformity and a uniform environment are necessary for producing animals of uniform quality and characteristics. One of the commonest causes of lack of uniformity in the environment, especially in the early environment, is variation in litter size and preweaning influences. In the several hundreds of litters in the present trials, variations in litter size were minimum, although there were some litters as low as 4 and as high as 18 in the population taken as a whole. Nonetheless, these were equally to be seen in the control groups and in the MSG treatment groups.

Individual weights of pups vary inversely with litter size, both at birth and at

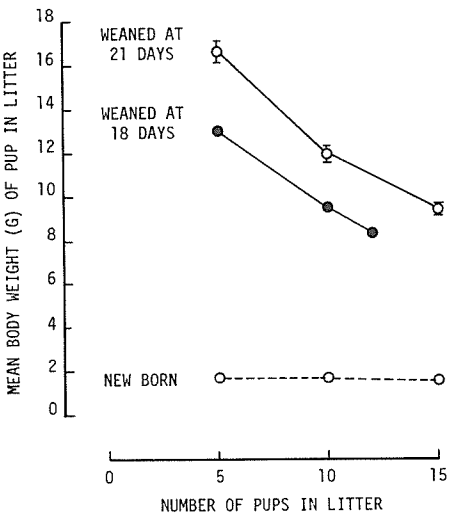


FIG. 10. Graphical representation of litter size as influencing birth and weaning weight of mouse pup. Values for pups weaned at 18 days of age. (From Festing, ref. 9; and Lane-Petter and Lane-Petter, ref. 11.)

weaning. Festing (9) has reported that in inbred mice the average weaning weight goes down by 0.13 ± 0.03 g for every extra pup in the litter. A much greater variation has been recorded for random-bred mice (11). The data in Fig. 10 show the poor performance of large litters compared to optimally sized litters, i.e., around 10 pups to a litter. Especially when early weaning is practiced, there is a compelling reason to ensure uniformly sized litters, for otherwise a pup poorly reared might be expected to continue poorly, postweaning.

With mice treated in early life with MSG subcutaneously, Pizzi et al. (22) observed fewer pregnancies and smaller litters. All through our multigeneration trials, conducted under careful housing and husbandry conditions, the litter size was uniformly about 10 in all groups including the controls, with a mean birth weight of between 1.6 and 1.7 g, and a mean weaning weight of 12 g. Details are presented in Table 9.

HISTOPATHOLOGICAL EXAMINATION OF BRAIN TISSUE

A large number of randomly selected animals of both sexes from the $F_{1.1}$, and the $F_{3.1.1}$ generations were employed for histopathological examinations of brain. The mice of the latter generation were examined at birth (within 90 min after birth), and at 3, 14, and 21 days of age.

The neuronal densities, especially in the arcuate and other nuclei of the hypothalamus, in the basal ganglia, in the hippocampus formation, and the thalamus, as well as in the cortex, of the different treatment groups and the control were compared. Special attention was directed to possible presence of any of the following:

TABLE 9. *Mean litter size and mean body weight at birth and at weaning: $F_{1.1}$, $F_{2.1}$, $F_{2.2}$, and $F_{3.2.1}$ generations*

Generation	No. live pups/litter (± SEM)[a]		Body weight (g ± SEM)[a]	
	At birth	At weaning	At birth	At weaning
$F_{1.1}$				
A. Control	10.32 ± 0.36	9.82 ± 0.35	1.65 ± 0.02	12.02 ± 0.32
B. 1% MSG	9.86 ± 0.70	9.85 ± 0.65	1.63 ± 0.03	12.77 ± 0.55
C. 4% MSG	9.57 ± 0.60	9.69 ± 0.54[b]	1.63 ± 0.04	12.00 ± 0.59
$F_{2.1}$				
A. Control	9.60 ± 0.46	9.14 ± 0.45	1.68 ± 0.03	11.76 ± 0.43
B. 1% MSG	10.25 ± 0.62	9.32 ± 0.68	1.55 ± 0.05	12.67 ± 0.78
C. 4% MSG	10.50 ± 0.38	9.78 ± 0.49	1.62 ± 0.03	11.74 ± 0.58
$F_{2.2}$				
A. Control	9.50 ± 0.60	9.83 ± 0.57[b]	1.72 ± 0.03	12.50 ± 0.39
B. 1% MSG	11.33 ± 0.50	11.45 ± 0.25[b]	1.71 ± 0.05	12.03 ± 0.31
C. 4% MSG	11.38 ± 1.00	11.73 ± 1.03[b]	1.72 ± 0.03	11.80 ± 0.77
$F_{3.2.1}$				
A. Control	9.60 ± 0.41	9.41 ± 0.39	1.71 ± 0.03	13.06 ± 0.32
B. 1% MSG	9.80 ± 0.33	9.58 ± 0.32	1.69 ± 0.03	12.68 ± 0.27
C. 4% MSG	10.00 ± 0.34	9.33 ± 0.45	1.70 ± 0.04	12.88 ± 0.46

[a] Represents mean of litter means.
[b] Higher values compared to at birth would indicate a litter in this group did not survive until weaning.

Ganglial cell degeneration and necrosis
Phagocytosis of decaying ganglial cells
Decreased density in ganglial cells, especially of the hypothalamic nuclei
Disturbed bilateral symmetry of ganglial cell pattern
Glial proliferation, altered glial cells, or evidence of edema and myelin changes.

In addition, the incidence of occurrence of technical artifacts that were observed was compared between the treatment groups, and with the controls. Histopathology, which involved the evaluation by light microscopy of thousands of brain sections from hundreds of mice of different age groups for the possible presence of any of the changes listed above, clearly showed that none of them were present in any of the MSG groups.

The main conclusion from this exercise is, indeed, that the dietary administration of MSG over prolonged periods, including exposure *in utero*, does not cause or provoke the typical brain lesions attributed to the administration of glutamate by different routes (4,14,16,17,25). Other investigators have also underscored the fact that the dietary oral administration of MSG does not cause the brain lesions and other specific changes associated with neuronal damage (18,19,23).

We may conclude that these trials, involving thousands of mice over three generations, have clearly shown the tolerance of the mouse, the most sensitive of

the laboratory animal species for this work, to prolonged ingestion of MSG at elevated dietary levels.

SUMMARY

The present study involved the subchronic dietary administration of 1 and 4% w/w MSG admixed with a basal diet; its aim was to investigate the possible adverse cumulative effect(s) of such a diet. The treatment crossover design employed permitted the evaluation of the effects of dietary ingestion of MSG by pregnant dams during gestation only, during gestation through lactation, and subsequently during postweaning.

Median, voluntary food intake was 6 g/day for both sexes, with median body weights of 40 and 33 g for males and females, respectively. MSG intakes under these conditions were 1,500 and 1,800 mg/kg/day and 6,000 and 7,200 mg/kg/day on the 1 and 4% diets for males and females, respectively. The food intake of dams increased considerably in all groups during lactation, with the intakes of dams on the 4% diet rising to 25,100 mg MSG/kg/day. Nevertheless, the pre- and postweaning performance of the young were unaffected.

Reproduction characteristics—fertility, gestation, viability, and lactation indices—were comparable in all groups and in all generations. There was no evidence of male or female sterility attributable to MSG. There was no incidence of hyperphagia or obesity throughout the trial.

No incidence of brain lesions, nor any other pathological change, was encountered in any of the animals of any treatment group. Overall, the dietary administration of MSG was without any untoward incidence, reinforcing the safety-in-use of MSG.

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