Monosodium Glutamate: Feeding of Large Amounts in Man and Gerbils

Abstract. *Oral administration of large amounts of glutamic acid to adult humans and animals in a formula diet appeared to cause no clinical pathological changes. The only biochemically demonstrable effect was a decrease in serum cholesterol and associated beta lipoproteins.*

Interest in the toxicity of monosodium glutamate (MSG) as a food additive has increased greatly in the past year because of the association of this compound with both the Chinese-restaurant syndrome in humans (*1*) and a lesion of the hypothalamus in newborn mice and monkeys (*2*, *3*). The burning sensation and, most probably, the headache in the Chinese-restaurant syndrome appear to be of vascular origin and represent a chemical idiosyncrasy (*1*). A discrete brain lesion is unlikely in this syndrome since the symptoms are transient and since sensitive individuals need only avoid exceeding their tolerance threshold in order to prevent a recurrence. No permanent neurological lesions have been observed. On the other hand, the brain lesions induced in newborn mice and monkeys by parenteral or oral administration of glutamate are characterized by a degeneration of dendritic processes and cell bodies of hypothalamic neurons (*3*, *4*). Associated with these lesions is a complex syndrome of metabolic, nutritional, and reproductive abnormalities visible in newborn mice for a 9-month period. These discoveries have given rise to concern about the safety of sodium glutamate when added to foods ingested by pregnant women and by infants. We report the harmless effects of large amounts of monosodium glutamate given to both animals and man by mouth. This project had its origin in a study of the hypocholesterolemic effects of glutamic acid when it was fed as a sole source of nonessential nitrogen in amino acid formula (AAF) diets (*5*). Subsequently, related studies were carried out in gerbils (*6*), in which a similar hypocholesterolemic effect was noted.

Table 1. Effects of glutamate feeding in human subjects. Δ-Cholesterol represents the difference in concentrations of plasma cholesterol from those in subjects on the control diet (AAF). None of the subjects showed any clinical effects.

Subject	Time (days)	Dose (g/day)	Δ-Cholesterol (mg/100 ml)
J.B.	34	147	−27
R.S.	28	88	−82
R.M.	27	147	−16
J.M.	21	137	−46
P.B.	21	137	−40
P.K.	21	147	−55
J.A.*	14	25	−16
	14	50	− 7
	21	100	+ 3
S.S.†	22	131	− 5
R.M.	21	133	−23
M.E.†	21	126	−20
M.E.	21	68	−41
	21	137	−42
G.R.	28	137	−29
W.B.	42	137	−75
D.M.	21	147	−40

* Monosodium glutamate was added to a house diet. † Monosodium glutamate was added to a low protein (25 g) diet.

A chemically defined diet [amino acid formula with glutamate (AAFG)] supplying all of the essential amino acids in optimum amounts, 16.0 g of total nitrogen including 137 g of glutamic acid as the sole source of nonessential nitrogen, and calories to maintain body weight has been fed to 11 adult male subjects for periods ranging from 14 to 42 days. Lesser amounts were fed to three other subjects. All subjects tolerated the regimen well, showed no changes in neurologic or hepatic function, and demonstrated a reduction in serum cholesterol and β-lipoproteins of 25 to 40 percent of control levels. None developed the Chinese-restaurant syndrome. Substitution of glycine plus ammonium ion for glutamate (AAF) abolished the hypocholesterolemic effect. Supplements of glutamate of as much as 100 g/day to an ordinary diet were ineffective in lowering the serum lipids but showed no toxic manifestations. Table 1 shows the effects of feeding from 25 to 147 g of MSG per day to adult human subjects. No changes in weight, irritability, appetite, or mentation were noted. The average fall in plasma cholesterol was 42 mg (± 5.8 standard error of the mean) per 100 ml.

When these same diets (*6*) were fed to gerbils, with animals fed a 30 percent casein diet and a chow diet as controls, it was observed that AAFG was less effective in maintaining body weight than the casein diet but more effective than AAF. Nevertheless, casein, AAF, and AAFG did not differ in their effects upon hair coat and general neurological status. When AAFG was replaced by food control diet (FCD), changes in body weight, hair appearance, and serum cholesterol concentration were significantly, if not completely, reversed within 1 week. No increase or decrease in neurological activity was noted during the feeding of AAFG. More specifically, there was no evidence of alteration of visual acuity or change in frequency of convulsion. A summary of these results is shown in Table 2. Animals on the glutamate diet were more tranquil than those on casein or chow diets. The oral doses employed in the study of gerbils (30 g per kilogram of body weight per day) should approximate the effect of the parenteral doses

Table 2. Effects of various diets on mature male Mongolian gerbils. Observations on test diets were recorded at the end of 1 week. Weight is reported as the mean ± standard error (S.E.) of the mean. Food control diet (FCD) was Purina laboratory chow for small animals; 20 of the gerbils received FCD with 1 percent (by weight) of cholesterol added. See text for casein diet; 26 of the gerbils on this diet received 30 percent casein with 1 percent (by weight) of cholesterol added. (Δ-Cholesterol represents the difference in concentration of cholesterol from that in animals on the preceding diet ± S.E. of the difference.) Amino acid formula (AAF) was fed to 56 gerbils, 28 of which received AAF with 1 percent (by weight) of cholesterol added. For animals fed amino acid formula with glutamate (AAFG), the statistical evaluation is limited to the AAF-AAFG interaction ($P < .005$). (Δ-Cholesterol represents the difference in concentration of cholesterol from that in animals on the preceding diet ± S.E. of the difference.) Twenty-eight of these gerbils received AAFG with 1 percent (by weight) of cholesterol added.

Diet	Animals (No.)	Weight (g)	Δ-Cholesterol (mg/100 ml)	Observations
FCD	110	72 ± 1.0		Hair is smooth; occasional convulsions during handling
Casein (30%)	49	72 ± 1.1	−8 ± 7.8	Hair loss; increased number of convulsions; increased mortality rate
AAF control	56	60 ± 1.2		Hair ruffled with occasional hair loss; rare hyperactivity, ataxia without additional convulsions
AAFG experimental	56	65 ± 1.0	−53 ± 14.0	As above

employed by others in animal studies [Lucas and Newhouse (*7*), Potts *et al.* (*8*), Freedman and Potts (*9*), Olney (*2*), and Olney and Sharpe (*3*)], who administered MSG in the range of 2 to 10 g per kilogram of body weight. In their latest report, Olney and Sharpe (*4*) reported effects of 1 g/kg, by feeding tube, in newborn mice.

Mayer-Gross and Walker (*10*) demonstrated a transient toxic effect (vomiting) after intravenous administration of 20 g of sodium glutamate to normal adults, while this dose produced a favorable metabolic effect (arousal) in comatose, hypoglycemic patients. To our knowledge, then, no permanent neurotoxic effect for glutamate has been definitely reported for adult humans.

Rapid utilization of MSG would prevent the accumulation of free amino acid in the plasma and would reduce the danger of damage to susceptible tissue. Oral MSG is well utilized by human subjects, as indicated by no significant alterations in concentrations of plasma glutamic acid plus glutamine 12 hours after thrice daily feeding of glutamate formulas. The subjects also were at constant body weight and in nitrogen balance (*11*).

Clearly, glutamate produced metabolic effects consisting of decrease in serum cholesterol, phospholipids, and β-lipoproteins without alterations of body weight, nitrogen balance, liver function, or neurological activity (*12, 13*).

The question of MSG toxicity appears, therefore, to center around the permeability of the blood-brain barrier to glutamic acid. How much of the fed or injected glutamate does actually reach the brain? It is obvious that significant amounts reach the brain in newborn animals and possibly infants. Himwich *et al.* (*14*) have reported ready access of glutamate to the brain at birth but exclusion of it with establishment of a blood-brain barrier by the 10th day. In view of this finding, it is likely that even with parenteral administration of large doses of MSG only small amounts of it would reach the adult brain. In newborn mice and monkeys prior to the establishment of this barrier, the brain could be susceptible to the toxic effects of MSG described by Olney (*2, 3*).

We conclude from our studies that very high oral doses of glutamate (147 g/day) are tolerated, with no neurological changes, by adult gerbils and humans. The problem of determining the age of transition from susceptibility to tolerance for MSG in human subjects remains open.

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Echinoid Skeleton: Absence of a Collagenous Matrix

Abstract. *Lack of hydroxyproline and proline in the calcified distal spines and Aristotle's lantern of the echinoderm* Strongylocentrotus *indicated the absence of a collagenous matrix. The fact that the small amount of collagen present in the base of the spines and in the test with sutures was removed by bacterial collagenase indicates that this collagen was not calcified.*

The structural role of a collagenous matrix in the calcified tissues of vertebrates is clearly established (*1*). However, evidence for the existence of a collagenous matrix in the calcified tissues of invertebrates consists of the amino acid composition and the appearance in the electron microscope (*2*) of the calcareous plates and spines of the sea urchins *Strongylocentrotus droebachiensis* (Müller) and *Lytechinus variegatus* (Lamarck) after treatment with pronase. Two phyla (Porifera and Mollusca) have been shown not to have significant quantities of collagen (*2*). This conclusion was reached because hydroxyproline (an amino acid unique to collagen and elastin) was absent or was a minor component of the amino acids present in the skeletal tissues.

The existence of a collagenous matrix needs to be considered in the light of other types of evidence. During the embryological development of the echinoid skeleton (*3*), a large intracellular crystal of calcite, the initial spicule, is first formed. Subsequent skeletal development occurs extracellularly by crystal growth on the initial spicules. Histological studies (*4, 5*) of the decalcified or untreated skeleton of echinoderms have shown the presence of uncalcified collagenous fibers only at the sutural areas between the calcareous plates and at the basal attachment of the spines to the skeleton. Observations of the intact and fragmented trabeculae from spine or plate with the scanning electron microscope (*5–7*) have led to the suggestion (*6*) that no organic phase was present within the trabeculae. In addition, x-ray diffraction studies (*7, 8*) of the mineral phase indicate that the spines and plates behave as single crystals (*9*) of calcite.

We now demonstrate that collagen or proteins containing proline are a minor component of the skeleton of the echinoid *Strongylocentrotus droebachiensis* and that the collagen that is present represents soft connective tissue.

Dried specimens of *S. droebachiensis* were divided into the distal spine, base of the spine, test with sutures, and Aristotle's lantern (excluding teeth). After the dry weights were obtained, each sample was hydrolyzed by being autoclaved for 5 hours at 120°C in 6*N* HCl. Samples of the hydrolyzate containing 160 to 274 mg of sample were chromatographed (*10*) for hydroxyproline and proline. These imino acids were analyzed by methods previously described (*10*).

The data obtained from the four echinoid tissues are presented in Table 1. An analysis of dog bone, a typical calcified collagen, is included for comparison. Chromatograms of the hydrolyzates showed traces (1 to 8 μg) of hydroxyproline in Aristotle's lantern and distal spine. The small amount (174 to 248 μg) of hydroxyproline present in the test (the "shell") or in the base of the spine suggested that