EVALUATION OF THE HEALTH ASPECTS OF CERTAIN
GLUTAMATES AS FOOD INGREDIENTS
Supplemental Review and Evaluation

1980

Prepared for
Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D.C.

Contract No. FDA 223-75-2004
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Life Sciences Research Office
Federation of American Societies
for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014
NOTICE

This report is one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior-sanctioned food substances as food ingredients, being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-75-2004 with the Food and Drug Administration (FDA), U.S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VI.

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

The Federation and the FDA have agreed that when additional information and data become available subsequent to completion of an evaluation report, the FDA may request review and evaluation of the supplemental information and data by the Select Committee. Based upon the evaluation of all available data, the Select Committee will prepare a supplemental report. The supplemental reports are also approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee. Upon completion of these review procedures, the supplemental reports are approved and transmitted to FDA by the Executive Director of FASEB.
While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

Kenneth D. Fisher, Ph.D., Director
Life Sciences Research Office, FASEB
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<td>30</td>
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</table>
I. INTRODUCTION

The Select Committee, in its 1978 report on the evaluation of the health aspects of certain glutamates as food ingredients, reached guarded conclusions as to the safety of the various salts (1).

The conclusions were based in part upon controversial experimental results which indicated neuronal damage in common laboratory animal species, particularly in neonates, following parenteral or oral administration of high doses of monosodium glutamate in aqueous solution. Other reported deleterious effects on growth, reproduction, and behavior as well as the development of obesity were clearly associated with parenteral administration, or oral forced feeding, of very large amounts of the tested agent. Oral intakes of the glutamates as components of a diet at various levels gave no indications of toxicity. Reports of the condition known as "Chinese Restaurant Syndrome," were also of concern to the Select Committee. Since the frequency of occurrence of this syndrome in the population and its differential diagnosis were based upon controversial and largely anecdotal data, the Committee could not reach a conclusion as to the public health significance of the syndrome.

Evidence existed for the presence of protective mechanisms regulating glutamate transport and metabolism at various sites in the intestine, liver, retina, and brain. Precise evidence was not available in regard to glutamate absorption in the presence of other dietary components, or to toxic thresholds and pharmacokinetics of the glutamates. Sufficient long-term studies on glutamate effects had not been reported. The Select Committee could not conclude that the glutamates were safe for unrestricted use as food ingredients under the circumstances. It made known the uncertainties and the need for additional data in the opinion section of its 1978 evaluation report (1).

Since the issuance of the Select Committee's evaluation report on glutamates (1), additional information has been forthcoming. This has included the proceedings of a symposium on glutamic acid held in Milan, Italy, on May 29-31, 1978. Following this symposium, the Food and Drug Administration (FDA) requested that the Select Committee update its evaluation of the health aspects of glutamates as food ingredients (2). This request was announced in the Federal Register (44:7232-7233, 1979). The announcement also listed additional data and information on the safety of glutamates that were available to the public, invited public comment on the data, and requested that any new safety information not previously considered in the review of the GRAS status of glutamates, be submitted to FDA and the Select Committee.
The Select Committee has reviewed the information presented in the published proceedings of the Milan Symposium (3), in the publications and letters listed in the Federal Register announcement, in submissions to the Select Committee following the announcement, and in publications that have appeared in the recent scientific literature. This information is reviewed in this supplemental report; together with the information in the Select Committee's previous report on glutamates, it constitutes the basis of a revised opinion.
II. ADDITIONAL BIOLOGICAL STUDIES

Absorption and metabolism

Glutamic acid together with its amide, glutamine, approximates 20 percent of food protein. Substantial amounts of this amino acid are present in free form in body cells. However, plasma concentrations of glutamate are relatively small, and are closely regulated by metabolic processes in both intestine and liver. As indicated by Munro (4) in a recent review, the quantitative aspects of glutamate regulation at the subcellular level have not been established.

The pronounced effect of dietary constituents on plasma glutamate levels following monosodium glutamate (MSG) ingestion has been indicated in several well-designed studies. The quantity of administered MSG which caused an increase in plasma glutamate levels was dependent on whether MSG was ingested with or without food. Administered in varying amounts of water with total quantity of MSG fixed, plasma glutamate levels increased with glutamate concentration and were higher in fasted than in fed animals.

The ingestion of 34 mg MSG per kg body weight by six adults with a hamburger-milk shake meal (providing 1 g protein and 1 g carbohydrate per kg body weight) resulted in no increase in plasma glutamate level above that observed when the meal was consumed without added MSG (5). Ingestion of the same quantity of MSG in water resulted in an increase in the plasma glutamate level of about 10 \( \mu \text{mol per dl} \) over the basal level of about 4 \( \mu \text{mol per dl} \). Ingestion by eight adults of 150 mg MSG per kg in water solution (3.6 percent MSG) providing 1.1 g partially hydrolyzed starch (Polycose\textsuperscript{®}) per kg body weight resulted in a peak plasma glutamate level of 7.18 ± 3.48 \( \mu \text{mol per dl} \), compared to a basal level of 3.46 ± 1.95 \( \mu \text{mol per dl} \). Ingestion of the MSG solution without added Polycose\textsuperscript{®} produced a maximum plasma glutamate level of 59.4 ± 46.5 \( \mu \text{mol per dl} \) (6).

Marrs et al. (7) studied six human adults fasted overnight and administered 60 mg MSG per kg body weight in 200 ml water during a 5-min period. They observed a mean peak plasma glutamate level of 15.5 ± 2.6 \( \mu \text{mol per dl} \). The basal mean plasma glutamate level was 4.3 ± 0.3 \( \mu \text{mol per dl} \). Plasma alanine levels did not increase. Plasma glutamate levels did not increase significantly in six fasted adults after ingestion of a water solution containing 440 mg per kg of a pancreatic hydrolyzate of casein providing 60 mg glutamate per kg. Alanine levels increased, presumably due to absorption of alanine contained in the hydrolyzate as well as that resulting from transamination.

Bizzi et al. (8) reported that area under the curve (AUC) of plasma glutamate concentration versus time, increased 2.5-fold
for human adults given 60 mg MSG per kg when the concentration in water solution was increased from 2 to 8 percent.

Airoldi et al. (9) reported the effect on AUC of concentration of MSG administered to rodents in water solution. A dose of 1 g per kg body weight given to newborn rats led to a five-fold increase in AUC when concentration in the administered solution was increased from 2 to 10 percent. In mice given an oral dose of 0.5 g per kg, AUC increased 2.5-fold when concentration was increased from 2 to 20 percent.

Table I summarizes the effects of ingestion of food with MSG on plasma glutamate levels and AUC in human adults, and in infant, weanling, and adult mice.

In studies of the kinetics of plasma glutamic acid, Airoldi et al. (9) reported half-life values (T\textsubscript{1/2}) for glutamate in plasma after administration of MSG to various animal species. The T\textsubscript{1/2} values after intubation of 1 g per kg in 10 percent aqueous solution were: 7-day-old mice, 111 min; 7-day-old rats, 237 min; 90-day-old mice, 98 min; adult monkey, 99 min. For adult humans administered 60 mg MSG per kg in bouillon (2 percent solution), the T\textsubscript{1/2} was 68 min.

Baker et al. (12) estimated that a breast-fed 3.5 kg infant ingests an average quantity of free glutamate equivalent to 46 mg MSG per kg body weight per day (range 30-60 mg per kg) in addition to the glutamate present in milk protein. There is now substantial evidence that metabolic factors regulating plasma glutamate levels are well developed early in infancy, even in premature infants. Plasma glutamate levels obtained 5 to 90 min after feeding human milk to 21 premature human newborns (up to 11 mg MSG per kg in a single intake) were not statistically different from the basal values (9). Healthy term infants, 28 to 33 days of age, showed no statistically significant differences in serum glutamate or aspartate when fed human milk (65 mg glutamate per kg) or when fed a casein hydrolyzate (Nutramigen\textsuperscript{®}) (80 mg glutamate per kg) in which about half of the total amino acids was present as free acids (13). Serum glutamate concentrations, 2 h postprandially, were 12.3 ± 3.1 and 10.1 ± 2.1 μmol per dl, respectively.

In additional studies, eight low-birth-weight infants, 11 to 35 days of age, weighing 1.3 to 1.8 kg at birth, were fed commercial casein hydrolyzate (modified Pregestimil\textsuperscript{®}) or cow milk (13). Two-hour postprandial plasma glutamate levels (μmol per dl) were 11.0 ± 2.9 (casein hydrolyzate), 12.4 ± 3.1 (cow milk, SMA\textsuperscript{®}), 13.5 ± 5.8 (cow milk, Enfamil\textsuperscript{®}) and 10.3 ± 3.3 (cow milk formula). Twenty-four 1-year-old infants and six adults were each fed quantities of custard providing 1 g protein per kg body weight. Two hours after ingestion, plasma glutamate concentrations for both age groups increased by the same percentage (90 percent). Postprandial levels
<table>
<thead>
<tr>
<th>Number of subjects</th>
<th>Dose mg/Kg</th>
<th>Vehicle</th>
<th>Concentration %</th>
<th>Plasma glutamate level, ( \mu \text{mol/dl} )</th>
<th>AUC* ( \mu \text{mol/mlxmin} )</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Adult humans</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>hamburger-milk shake</td>
<td>8.8 ± 5.0</td>
<td></td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>None added</td>
<td>hamburger-milk shake</td>
<td>7.1 ± 3.9</td>
<td></td>
<td>-</td>
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<td>6</td>
<td>150</td>
<td>water</td>
<td>3.6</td>
<td>72 ± 36</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>150</td>
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<td>49 ± 7</td>
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<tr>
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<td>150</td>
<td>Sustagen</td>
<td>3.6</td>
<td>10.5 ± 2.7</td>
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<tr>
<td>8</td>
<td>None added</td>
<td>Sustagen</td>
<td>-</td>
<td>7.6 ± 1.6</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>150</td>
<td>water</td>
<td>3.6</td>
<td>59 ± 46</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>150</td>
<td>Polysse®</td>
<td>**</td>
<td>7 ± 3.5</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>120</td>
<td>bouillon</td>
<td>2</td>
<td>-</td>
<td>8.00</td>
<td>9</td>
</tr>
<tr>
<td>109</td>
<td>60</td>
<td>bouillon</td>
<td>2</td>
<td>19.4 ± 9</td>
<td>5.56</td>
<td>9</td>
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<td>60</td>
<td>tomato juice</td>
<td>2</td>
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<td>2.89</td>
<td>9</td>
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<td>30</td>
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<td>-</td>
<td>1.69</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>None added</td>
<td>meal</td>
<td>-</td>
<td>-</td>
<td>0.22</td>
<td>9</td>
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<td>8</td>
<td>1000</td>
<td>water</td>
<td>10</td>
<td>314 ± 66</td>
<td>10,11</td>
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<tr>
<td>8</td>
<td>1000</td>
<td>infant formula</td>
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<td>126 ± 26</td>
<td>10,11</td>
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<td>8</td>
<td>None added</td>
<td>infant formula</td>
<td>-</td>
<td>47 ± 12</td>
<td>10,11</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1000</td>
<td>water</td>
<td>10</td>
<td>219 ± 31</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1000</td>
<td>basal diet</td>
<td>10</td>
<td>45 ± 8</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>None added</td>
<td>basal diet</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3000</td>
<td>synthetic diet†</td>
<td>22.2</td>
<td>162 ± 46</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>None added</td>
<td>synthetic diet†</td>
<td>-</td>
<td>17 ± 6</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td><strong>Adult mice</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1000</td>
<td>water</td>
<td>10</td>
<td>344 ± 37</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1000</td>
<td>clear soup</td>
<td>10</td>
<td>192 ± 12</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1000</td>
<td>basal diet++</td>
<td>10</td>
<td>43 ± 6</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>None added</td>
<td>basal diet++</td>
<td>10</td>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1000</td>
<td>water</td>
<td>10</td>
<td>208 ± 20</td>
<td>309</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>1000</td>
<td>basal diet++</td>
<td>10</td>
<td>27 ± 1</td>
<td>4.8</td>
<td>9</td>
</tr>
</tbody>
</table>

*AUC = Area under curve of plasma glutamate concentration plotted vs. time after administration of MSG.

**Polysse® is partially hydrolyzed cornstarch. The quantity administered with MSG provided 1.1 glucose equivalent per kg body weight; mean fasting plasma glutamate level was 4.1 ± 1.7 \( \mu \text{mol per dl} \).

†Synthetic diet, a paste administered by gavage, consisted of casein, sucrose, dextrin, corn oil, vitamins, and minerals. No hypothalamic lesions were observed after administration of the diet containing MSG [11].

‡Food eaten in 15 to 30 min.
(11 μmol per dl) in the 1-year-old infants were similar to those in formula-fed, low-birth-weight infants and in formula-fed and breast-fed term infants.

The regulation of plasma glutamate levels is of interest since elevations in plasma glutamate or AUC have been associated with the development of hypothalamic lesions in experimental animals of some species, although an exact correlation has not been established.

Perez and Olney (14) reported a fourfold elevation of glutamate in the arcuate nucleus and contiguous median eminence in 4-day-old mice following subcutaneous administration of a 2 g per kg dose of MSG. However, analyses of brain tissues of rats showed that oral doses of MSG (2 g per kg, infants 4 or 7 days of age; 4 g per kg, adults) which increased plasma levels by 11-fold over basal levels (230 μmol per dl or greater) in both infants and adults did not affect the level of glutamic acid in the arcuate nucleus or the lateral thalamus (9,15). The glutamate level in the whole infant rat brain increased by about 60 percent only when peak plasma levels exceeded the basal level by 19 times. Whole brain levels in infant and adult mice and guinea pigs were not increased by oral doses (1 g per kg in 10 percent aqueous solution) that increased plasma levels 12-fold. Airolodi and Garattini (16) found no increase in glutamate concentration in the arcuate nucleus or the lateral thalamus of 3-day-old guinea pigs administered 2 g MSG per kg by gavage, or in adults intubated with 4 g per kg. Thus, increases in glutamate concentration in the arcuate nucleus of rats and guinea pigs were not detected at doses and plasma glutamate concentrations associated with tissue damage.

Some clarification of these results has been provided by investigation of hepatic glutamate uptake and by direct studies of glutamate uptake and efflux from the brain (17). The Michaelis-Menton constants for transport of glutamate across liver cell membrane are \( K_m \) (half-saturation constant) = 2.7 mM, and \( V_{max} = 1.3 \) μmol per min per g. As normal portal plasma levels of glutamate are < 0.3 mM, the liver cell membrane is not saturated by physiological doses of glutamate. Pardridge (17) reported that oral doses of 1–4 g MSG per kg in the mouse result in portal amino acid levels that approach the maximum capacity of the liver to take up glutamate, and concluded that the major factor protecting brain cells from high levels of plasma glutamate is the blood–brain barrier (BBB). The potency of the BBB and the blood–retina barrier (BRB) are due to a unique capillary structure. The brain capillary endothelial cells are fused together by tight junctions which convert the brain capillary wall into an epithelial barrier. The BBB segregates the cerebral and systemic extracellular fluids, and is effectively a plasma membrane (with regional specializations) for the entire brain. The rate at which metabolic substrates penetrate the BBB is a function of the kinetic characteristics \( (K_m, V_{max}, K_d) \) of the specific carrier systems which transport the
respective substrates. A specific carrier for glutamate and aspar-
tate has been documented (\(K_m\) and \(V_{max}\) values for glutamate trans-
port are approximately 0.04 mM and 0.4 nmol per min per g, respec-
tively) (17). This carrier is virtually saturated by physiologic
plasma levels of glutamate (0.15 mM); therefore, brain glutamate
does not rise or fall in parallel with changes in plasma levels,
as is the case with the neutral or basic amino acids. Indeed, the
 glutamate carrier is an active efflux system which transports glu-
tamate from brain interstitium to blood against a concentration
gradient.

In addition to the saturable route of glutamate influx
into the brain, there is also a nonsaturable route which trans-
ports about 0.3 nmol per min per g at a plasma level of 0.15 mM
and as much as 80 nmol per min per g when the plasma level is
raised to 40 mM by parenteral administration of 2 g per kg gluta-
mate (17). The active efflux carrier system appears to have the
capacity to keep the glutamate level constant in regions of the
brain protected by the BBB. Certain regions of the brain lack a
BBB and take up glutamate under conditions of very high plasma
levels (14). These regions are the circumventricular organs (CVO)
which include the median eminence (contiguous to the arcuate
nucleus, hypothalamus), and the organum vasculosum of the lamina
terminalis (contiguous to the pre-optic area, hypothalamus).
Radial diffusion of glutamate from the extracellular space of the
CVO to the arcuate nucleus, or retrograde axoplasmic flow of gluta-
mate from nerve endings in the CVO, may account for the vulnerabili-
ity of the arcuate nucleus to glutamate toxicity.

Partridge (17) has stated that the BBB is anatomically
intact in the newborn; the brain endothelial tight junctions are
formed in the first trimester of human fetal life. The BBB imper-
meability to proteins is complete by the 15th day of fetal life in
the rat. However, specific carrier systems may not have developed
to maximum transport capacity. Development of the active efflux
system for organic acids is markedly species dependent; this sys-
tem is p-dipropylsulfamyl benzoic acid sensitive (lower \(K_m\) value
for efflux) in mice and rats but insensitive in rabbits and rhesus
monkeys.

Toxicity studies

Recent studies on the incidence of hypothalamic lesions
in infant, weanling, and adult mice administered MSG in aqueous
solution subcutaneously or by gavage are summarized in Table II.
Also shown are associated peak plasma glutamate levels and AUCs.

Takasaki (18) (Table II) observed that peak plasma glutama-
te levels reached after administration of the lowest effective
dose for inducing hypothalamic lesions in mice were 104 ± 18, 385
± 32, and 631 ± 30 μmol per dl for infants (10-day-old), weanlings

- 7 -
TABLE II
Neurotoxic MSG Doses in Mice and Associated Peak Plasma Glutamate Levels and AUCs

<table>
<thead>
<tr>
<th>Dose g/kg</th>
<th>Concentration of solution %</th>
<th>Route</th>
<th>Peak plasma concentration, μmol/dl</th>
<th>AUC b</th>
<th>Incidence hypothalamic lesions, %</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td></td>
<td></td>
<td>Infants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>10</td>
<td>p.o.</td>
<td>62 ± 6</td>
<td>-</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>0.7 a</td>
<td>10</td>
<td>p.o.</td>
<td>104 ± 18</td>
<td>-</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>0.8</td>
<td>10</td>
<td>p.o.</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>18</td>
</tr>
<tr>
<td>0.5 d</td>
<td>10</td>
<td>p.o.</td>
<td>-</td>
<td>200 d</td>
<td>52</td>
<td>9,19,22</td>
</tr>
<tr>
<td>0.5</td>
<td>5</td>
<td>p.o.</td>
<td>88 e</td>
<td>-</td>
<td>22</td>
<td>20,21</td>
</tr>
<tr>
<td>1.0</td>
<td>10</td>
<td>p.o.</td>
<td>282 e</td>
<td>375 f</td>
<td>62</td>
<td>9,20,21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weanlings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.7 a</td>
<td>4</td>
<td>s.c.</td>
<td>385 ± 32</td>
<td>-</td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td>1.0</td>
<td>4</td>
<td>s.c.</td>
<td>760 ± 52</td>
<td>-</td>
<td>75</td>
<td>18</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>p.o.</td>
<td>-</td>
<td>-</td>
<td>&gt;50</td>
<td>18</td>
</tr>
<tr>
<td>3 g</td>
<td>22.8</td>
<td>p.o.</td>
<td>162 ± 46</td>
<td>-</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>4</td>
<td>s.c.</td>
<td>539 ± 25</td>
<td>-</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>1.2 a</td>
<td>4</td>
<td>s.c.</td>
<td>631 ± 30</td>
<td>-</td>
<td>50</td>
<td>18</td>
</tr>
<tr>
<td>1.5</td>
<td>4</td>
<td>s.c.</td>
<td>841 ± 105</td>
<td>-</td>
<td>50</td>
<td>18</td>
</tr>
</tbody>
</table>

a MSG administered in water solution except in case of 3 g per kg dose to weanling mice.

b AUC = Area under curve of plasma glutamate concentration plotted vs. time after administration of MSG.

c Dose identified by Takasaki (18) as Least Effective Dose.

d AUC determined by Airoldi et al. (9) under conditions shown by Olney and Ho (19) to result in incidence of hypothalamic lesions given in column 6. Dose and AUC identified by Airoldi et al. (9) as ED50 and AUC50, respectively. AUC is revised value reported by Garattini (22).

e Peak plasma level determined by Stegink et al. (21) under conditions reported by Reynolds et al (20) to result in incidence of hypothalamic lesions given in column 6.

f AUC reported by Airoldi et al. (9) after administration of 1 g MSG per kg in 10 percent solution.

† A synthetic diet containing casein, sucrose, dextrin, corn oil, vitamins, and minerals plus added MSG. Administered by gavage in the form of a paste.
(23-day-old), and adults (3-month-old), respectively. Corresponding doses (g per kg body weight) were 0.7 p.o., 0.7 s.c., and 1.2 s.c., respectively.

James et al. (23) found no damage in the hypothalamus after per os administration of 1.5 g MSG per kg to 43-day-old mice, which resulted in a mean peak plasma glutamate of 290 µmol per dl.

Stegink et al. (21) estimated from observations of Reynolds et al. (20) that 88 µmol per dl was the lowest effective plasma glutamate level for inducing hypothalamic lesions in 9- to 10-day-old mice. Their evidence indicated that in neonatal mice administered 250 mg MSG per kg orally, neuronal necrosis did not occur, but lesions were observed in 22 percent of mice given 500 mg per kg orally. The values (Table II) for least effective toxic oral doses in mice are in fair agreement with those reported by Olney (24): 500 mg per kg for 10-day-old mice, 1000 mg per kg for 21-day-old mice and 2000 mg per kg for 60-day-old mice.

Airoldi et al. (9) reported that the threshold AUC was greater than 200 but less than 375 µmol per dl x min for 7-day-old mice. AUC after oral doses of 0.25, 0.50, and 1.0 g MSG per kg body weight (10 percent w/v in water) were 103, 200, and 375 µmol per ml x min, respectively. Takasaki (18) observed no lesions after per os administration of 0.5 g per kg, whereas Olney and Ho (19) reported lesions in 52 percent of the mice at this dose. No lesions occurred in adult mice at an AUC of 309 µmol per ml x min (9). Heywood and Worden (25) found that the incidence of hypothalamic lesions was 60 percent in 3-day-old rats administered 2 g MSG per kg in 20 percent solution; the incidence in 2- to 3-day-old guinea pigs was 20 percent after similar dosage. No lesions in adult beagles were observed after per os administration of 2 g MSG per kg in 10 percent solution.

Takasaki (26) administered by gavage to 10-day-old mice 2 g MSG per kg body weight in admixture with 1.93 g per kg body weight of glucose, fructose, galactose, or lactose, or 1.83 g per kg sucrose. Controls were given 2 g MSG per kg as a 10 percent solution. In a pilot experiment, mice were administered 2 or 4 g MSG per kg, or 2 g MSG per kg in combination with 0.63 g NaCl per kg. The dosage of NaCl as administered was equiosmolar to 2 g MSG per kg, twice the osmolality of the monosaccharides and four times that of the disaccharides. Mice were sacrificed 8 h after treatment, brains were fixed, embedded in acrylate and 6 µm sections cut. A section 120 µm anterior to the base of the hypophyseal stalk was taken as a representative section in each animal, and the number of necrotic neurons in the arcuate nucleus was counted under the light microscope. These results, showing the protective effects of sugars, are given in Table III.
TABLE III

Effect of Simultaneous Administration of Sugars with a Neurotoxic Dose of MSG on the Number of Necrotic Neurons in a Representative Section of the Arcuate Nucleus of Infant Mice (26)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice examined</th>
<th>No. of necrotic neurons ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>8</td>
<td>1 ± 0.4</td>
</tr>
<tr>
<td>MSG 4 g per kg</td>
<td>8</td>
<td>462 ± 18</td>
</tr>
<tr>
<td>MSG 2 g per kg</td>
<td>6</td>
<td>195 ± 18</td>
</tr>
<tr>
<td>MSG 2 g per kg + NaCl 0.63 g per kg</td>
<td>9</td>
<td>209 ± 8</td>
</tr>
<tr>
<td><strong>Experiment II</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSG 2 g per kg + glucose</td>
<td>7</td>
<td>263 ± 15</td>
</tr>
<tr>
<td>+ fructose</td>
<td>6</td>
<td>138 ± 9</td>
</tr>
<tr>
<td>+ galactose</td>
<td>6</td>
<td>142 ± 16</td>
</tr>
<tr>
<td>+ sucrose</td>
<td>7</td>
<td>155 ± 24</td>
</tr>
<tr>
<td>+ lactose</td>
<td>6</td>
<td>158 ± 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>133 ± 19</td>
</tr>
</tbody>
</table>
Protective effects of leucine and arginine were demonstrated by Takasaki (27) in another study using the same technique as employed with saccharides (26). Simultaneous intubation of 0.2 g leucine or 2.28 g arginine hydrochloride per kg body weight with 2 g MSG per kg reduced the number of lesions observed with MSG alone by 35 and 44 percent, respectively. Preinjection of 0.02 unit of insulin 2 or 4 h before intubation of MSG reduced the number of lesions by 40 and 60 percent, respectively. The author suggested that elevation of plasma insulin level following the ingestion of saccharides or amino acids may have an important role in reducing plasma glutamate levels and subsequent neuronal damage.

Reynolds et al. (20) found no evidence of hypothalamic lesions in fetal monkeys after in utero injection of 20 percent MSG solutions into the umbilical vein of macaque fetuses of gestational age 66, 85, 89, 100, and 142 days, at levels of 3.6, 6.8, 0.4, 5.8, and 1.8 g per kg body weight, respectively. The first three fetuses were Macaca arctoides, the 4th and 5th, M. mulatta and M. irus, respectively. A tracer dose of $^{14}$C-labeled MSG was added to MSG solution injected into the 5th fetus. Two additional fetuses received MSG by intraperitoneal injection: an M. irus fetus, 16.6 g per kg dosage at 38 days gestational age; and an M. arctoides fetus, 2.4 g per kg dosage at 142 days gestational age. The dose administered to the latter fetus was $^{14}$C-labeled MSG. Fetuses were delivered by cesarean section 2 to 6.75 h after injection of MSG. A control fetus at 130 days of gestation was delivered by hysterotomy. Negligible radioactivity associated with glutamate was found in cerebrospinal fluid (CSF) taken at delivery from the two fetuses administered MSG intraperitoneally or injected intravenously. Total glutamate levels in the CSF of these fetuses were not elevated above control values ($1.8 \pm 1.1 \mu\text{mol per dl}$). (CSF levels were measured since brain damage after MSG administration appears to be restricted to areas adjacent to CSF.) Glutamate levels in plasma were two to three times those of control values indicating sustained elevations (blood samples were taken 6.75 and 5.5 h after injection) in circulating glutamate levels. Levels in fetal urine of these two fetuses were 380 and 500 times those of control animals; levels in amniotic fluid were also elevated, indicating clearance to urine and passage to the amniotic fluid.

The cells within the hypothalamic areas of the fetuses of 66, 85, 89, and 100 days gestational age were immature but normal in appearance (20). There was no evidence of pyknotic nuclei, tissue edema, or cell loss in the subinfundibular region. The investigators noted that ventral hypothalamic morphology in the 38-day fetus was embryonic and did not mention any effects of MSG administration. Although the brains of the 142-day fetuses were more differentiated than the younger ones, they did not exhibit the degree of myelination, neuronal differentiation, and maturation of the median eminence seen in the neonatal monkey brain (gestation period in the rhesus monkey is 168 ± 7 days).
No neuronal abnormalities were seen in the ventral hypothalamic area of the fetus. No evidence was found of cell death or vacuolated cytoplasm which are characteristic of MSG damage in the neonatal mouse.

The investigators concluded that difference in susceptibility of the monkey and mouse may result from (a) differences in glutamate metabolism, (b) greater integrity of the blood-brain barrier, or (c) specific neuronal susceptibility of the mouse, or (d) a combination of these factors.

A summary of results of various investigators studying neuropathologic effects of MSG administration to infant monkeys has been compiled by Reynolds et al. (20) and is included as Table IV.

Additional studies of effects of high levels of dietary glutamate in several species of animals have now been completed. No pathological changes in the hypothalamus were observed in pregnant, lactating, or weanling mice fed a commercial basal diet containing 5, 10, or 15 percent MSG providing maximum intakes of 14, 43, and 42 g MSG per kg body weight per day to the respective groups (33). Diets were fed 1-4 days and animals were sacrificed 2-3 h after completion of feeding. Similar results were reported by Heywood et al. (34) who fed weanling mice diets containing 10 percent MSG or drinking water containing 5 percent MSG (45.5 and 20.9 g MSG per kg body weight, respectively) for 4 days.

In a three-generation study involving 1,826 mice, MSG was fed at 1 and 4 percent levels (about 1.8 and 7.2 g per kg body weight mean intake for females) during pregnancy only, during pregnancy and lactation, and subsequently postweaning (35). MSG intake of dams increased during lactation to 25.1 g per kg maximum on the 4 percent diet; immediately postweaning, MSG intake was about 13 g per kg in pups fed this diet. Fertility, gestation, viability, and lactation indices were similar for all groups in all generations. Differences in body weights between treatment groups of the same generation were not statistically significant (weights were reported up to 32 weeks of age). Heavier animals were identified as coming from small-sized litters, and as heavier at weaning. No incidence of brain lesion or other pathological change was observed in pups of the F₃.1.1 generation examined within 90 min after birth, and at 3, 14, and 21 days of age.

In a two-generation feeding trial, Yonetani et al. (36) administered MSG at 2 and 4 percent dietary levels (4 and 8 g per kg body weight) with IVCS and Swiss albino mice. Males and females of the P generation were mated 2 weeks after MSG diets were initiated and were sacrificed 100 days postdelivery. The F₁ generation was sib-mated at 90 days and the remaining mice were killed at 130 days of age. Growth, food consumption, estrous cycle, date of sexual maturation, organ weight, litter size, body weight of neonates, and histopathology of major organs including brain and retina, did not differ significantly compared with controls.
<table>
<thead>
<tr>
<th>Investigators</th>
<th>Number</th>
<th>Route</th>
<th>Dose</th>
<th>Neuroanatomical Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. No Neuropathologic Findings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abraham et al. (28)</td>
<td>3</td>
<td>Oral</td>
<td>1-4 g/kg</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>s.c.</td>
<td>4 g/kg</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Dietary</td>
<td>0.25-1 g/kg</td>
<td>Normal</td>
</tr>
<tr>
<td>Wen et al. (29)</td>
<td>8</td>
<td>Dietary</td>
<td>5-20%</td>
<td>Normal</td>
</tr>
<tr>
<td>Newman et al. (30)</td>
<td>26</td>
<td>Oral</td>
<td>2-4 g/kg</td>
<td>Normal</td>
</tr>
<tr>
<td>Reynolds et al. (31) and unpublished</td>
<td>18</td>
<td>Oral</td>
<td>1-4 g/kg</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B. Neuropathologic Findings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olney et al. (32)</td>
<td>3</td>
<td>s.c.</td>
<td>2.7-4 g/kg</td>
<td>Spreading lesions</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Oral</td>
<td>1-4 g/kg</td>
<td>Small focal lesions</td>
</tr>
</tbody>
</table>
MSG fed to beagle dogs at dietary levels (2.5, 5.0, or 10.0 percent) providing about 0.6, 1.3, or 2.5 g per kg body weight for two years caused no adverse effects on weight-gain, general behavior, electrocardiogram (ECG), ophthalmological findings, hematology, blood chemistry, organ weights, or mortality (37).

Wen et al. (29) fed one cynomolgus and one bushbaby monkey a diet containing 9.1 percent MSG (7.6 g per kg body weight) from 1 to 12 months of age. A control monkey of each species was fed the basal diet during the same period. Growth and development were periodically assessed. Final weight gains, electroretinogram (ERG), electroencephalogram (EEG) and plasma amino acids patterns were similar to controls. Daily observation revealed no behavioral abnormalities. No evidence of gross obesity developed. Wen et al. (29) also injected a 3-week-old cynomolgus monkey with 2.7 g per kg MSG intramuscularly. Plasma glutamate rose to 360 mol per dl. This monkey was observed for 30 months with no apparent sequela developing from the MSG injection.

In especially relevant studies, Takasaki et al. (38), Iwata et al. (39), and Matuszawa et al. (40) reported long-term effects on Wistar rats administered subneurotoxic doses of MSG as neonates. These researchers found that the least effective doses of MSG for induction of hypothalamic lesions in 2-day-old rats injected s.c., or administered p.o. to 10-day-old rats, were 0.4 and 1.4 g per kg body weight, respectively. There were 12 rats per group. Daily s.c. injection (from 2 to 11 days of age) of subneurotoxic doses (0.2 g per kg) in neonates, or daily forced intubation (from 10 to 19 days of age) of 0.5 g per kg in the infant rat did not affect body weight, body length, tail length, or Lee's index ($\sqrt[3]{\text{body weight/body length}}$) at 16 and 36 to 40 weeks of age. No sign of obesity or stunted growth was observed in weanling rats fed ad libitum diets containing 5 percent MSG (8 g per kg) for 10 days (from 20 to 29 days of age) after intubation as described above.

Positive controls administered s.c. 4 g MSG per kg body weight daily as neonates (2 to 11 days of age) or infants (10 to 19 days of age) showed significant reductions in body weight, body length, tail length, and increases in Lee's index (38). Effects were considerably less in rats treated in the infant stage.

Male rats examined at 5 and 12 months and females at 3 and 12 months, both given subneurotoxic doses of MSG subcutaneous or per os as described above, showed no changes in weight of the anterior pituitary, gonads, seminal vesicles, uterus, adrenals, or thyroid as compared to control animals (40). No abnormalities were observed in females in the onset of vaginal opening and estrous cycle. Serum levels and pituitary contents of luteinizing hormone
(LH) and follicle stimulating hormone (FSH) measured in the morning and evening on the day of proestrus in 3-month-old rats did not differ from those of controls. No differences in serum levels and pituitary contents of LH and FSH were found in treated adult and control males.

The following tests were applied to rats given subneurotoxic doses of MSG as neonates or infants as described above (39): spontaneous motor activities of 1-, 3-, and 9-month-old male rats measured by ANIMEX® in which activity was measured in pairs of rats under illumination schedule 12L:12D; center latency and ambulation scores of 1-, 3-, and 7-month-old male rats exposed to an open field arena for 3 min; neurological and muscle strength of male and female rats at 1, 3, and 7 months as determined by the rotating rod and inclined plane tests; learning ability of male rats at 3 months of age by the Lashley III maze test, and at 6 months by a fixed-ratio food reinforcement schedule learning test; learning and swimming abilities of 6-month-old female rats as measured by the water-filled multiple T-maze; corneal and pinna reflexes; and observations of self mutilation. No differences were observed in the treated rats in any of these tests as compared with control rats; differences were observed in some of the tests with positive controls administered 4 g MSG per kg subcutaneously as neonates, but slight or no differences when administered as infants.

Vorhees et al. (41) included MSG among other substances in a preliminary analysis of a developmental test battery for neurobehavioral toxicity. Sprague-Dawley rats whose parents had been fed diets containing 1.7, 3.4, or 5.1 percent MSG (2.6, 4.7, and 8.2 g per kg body weight), beginning two weeks prior to mating and continuing through gestation and lactation, were used. Pups received the same diets from weaning until 90 days of age. Each treatment group consisted of 18 litters of F₁ animals. Two males and two females of each litter were selected for preweaning testing; two additional animals of each sex per litter were included in tests applied postweaning. No difference in body weights were observed in F₁ rats of treated parents at 1, 7, 14, or 21 days of age as compared with controls. Mean age at which incisors erupted in litters from treated animals tended to be lower than that of controls but did not show dose dependence (10.5, 10.0, and 10.1 days at 1.7, 3.4, and 5.1 percent dietary MSG versus 10.8 days for controls; standard error was 0.2 for all groups). There were no differences in mean age at eye opening or pinna detachment.

No significant effects were observed on righting development, cliff avoidance, auditory startle development, motility, or visual placing development. At 6 days of age, infant rats from dams fed 5.1 percent MSG had significantly lower performance scores in swimming direction tests than controls, but not at 8 days. On day 12, but not at other ages, infants from dams fed 1.7 percent MSG had significantly higher performance scores than controls.
In postweaning tests, no significant differences were observed between treated and control rats in rotord or activity wheel performance, appetitive position description, or ambulation in open field tests (41). Rearing frequency was significantly lower in the latter tests in rats from the 5.1 percent MSG treatment groups but the response was not dose dependent. In the active avoidance task, males receiving the highest dietary levels of MSG required more time to reach a 90 percent avoidance rate (2.6 ± 0.4, 2.3 ± 0.4, and 4.6 ± 0.9 days, respectively, for the 1.7, 3.4, and 5.1 percent MSG dietary groups versus 2.9 ± 0.5 days for control group), but then lost the response more rapidly than controls (2.2 ± 0.4, 3.0 ± 0.6, and 1.9 ± 0.4 days, respectively, for the three dietary groups versus 2.6 ± 0.4 days for the control group). In the passive avoidance test, only rats from the 5.1 percent MSG group showed an increase in retention latency. Neurohistological examination at 90 days of age showed a significant reduction in neuronal cell count in rats fed diets containing 1.7 percent MSG but no differences compared with controls in animals fed 3.4 or 5.1 percent MSG. The investigators concluded that MSG produced statistically significant behavioral deviations in some tests at high doses, but that the significance of these was minor from a safety standpoint.

In studies on the retinal barrier, Olney (42) reported retinal damage in Swiss mice administered 1 g MSG per kg body weight subcutaneously between the 9th and 10th postnatal days. He stated that after the 10th to 11th days it was difficult, even with lethal doses of MSG, to produce a significant lesion in the retina. Takasaki (18) determined the threshold value for appearance of changes in the arcuate nucleus to be 0.4 g per kg in ICR strain mice 10 days old when administered 10 percent aqueous solutions of MSG intraperitoneally, whereas the threshold for retinal change was 2 g per kg.

In a two-generation study, mice fed diets providing 8 g MSG per kg body weight demonstrated no histopathologic changes in the retinas of the F1 generation sacrificed at 100 days of age (36).

In a report by Owen et al. (43) weanling Charles River CD rats were fed diets containing 1, 2, or 4 percent MSG (up to about 13 g per kg as weanlings) for 104 weeks. Each animal was examined ophthalmoscopically every 13 weeks. No adverse effects were reported.

Studies on human responses to MSG administration and the "Chinese Restaurant Syndrome (CRS)"

Kenney and Tidball (44,45) reported three studies on the responses of human subjects to MSG ingestion. In the first study, 32 percent of 77 subjects given 5 g MSG in 150 ml tomato juice reported one or more sensations of warmth or burning, stiffness or tightness, weakness in the limbs, or tingling. When 2 g MSG in
150 ml tomato juice were administered, symptoms were no more frequent than after ingestion of salted tomato juice.

In a second study with 51 volunteers, Kenney (45) identified 11 subjects as reactors to 5 g MSG taken orally in 150 ml tomato juice and 11 as nonreactors. On subsequent ingestion of 150 ml tomato juice containing 1-5 g MSG or 0.7 g sodium chloride in randomized sequence over a 9-day period, the reactors responded to 3, 4, and 5 g MSG with increasingly intense sensations as MSG concentration was increased. Nonreactors did not distinguish MSG from placebo.

In the third study, MSG was administered to 57 volunteers as a component of a soft-drink mix unfamiliar to the subjects (45). Tightness, pressure, tingling, weakness, warmth, and burning were reported only after ingestion of beverage (150 ml) containing MSG (6 g) and reports were confined to 16 subjects. In a double-blind trial of 12 of the reactors with 3 g MSG per 150 ml beverage, three subjects reported characteristic but less intense sensations. Two of these three subjects reported mild, brief (2 min), transient sensations, and the third experienced nothing after ingestion of 150 ml beverage containing 1.5 g MSG. Further tests with reactor subjects after ingestion of 6 g MSG (3 percent concentration) in double-blind administration, showed no changes in heart rate, electrocardiographic pattern, muscular electrical activity (in areas where sensations of tightness, pressure, or fatigue were reported), blood pressure, or skin temperature in temporal association with symptom experience. Kenney (45) proposed that sensations experienced following ingestion of MSG are referred from the upper alimentary tract for which high concentrations of MSG may be a specific or non-specific irritant.

In another study with 60 subjects, Kenney (46) administered 200 ml of reconstituted frozen orange juice, a spiced tomato juice, black coffee, chocolate-flavored milk, and a 2 percent solution of MSG in a soft-drink vehicle. Test materials were given in randomized order on nonsequential days to fasting subjects. All materials provoked some post-ingestional symptoms, and symptoms of burning, tightness, or pain in the chest, neck, face, or arms or numbness were reported in response to coffee (6 subjects), spiced tomato juice (6 subjects), and MSG solution (2 subjects). Kenney (46) concluded that MSG is not unique in provoking CRS symptoms.

Based on the frequency of symptoms characteristic of MSG ingestion reported by 15 reactor subjects after ingestion of MSG in solutions of various concentrations on three different occasions and the percentage of reactors in his test population, Kenney (47) estimated that about 4 percent of the population might react occasionally, but less than 2 percent regularly, to MSG ingestion at 1 percent concentration in a vehicle comparable to a soft-drink mix.
Stegink et al. (48) investigated the incidence of reactions in adults given 150 mg MSG per kg body weight (3.6 percent concentration) in tomato juice or water, or in tomato juice or water with 1.1 g per kg body weight added Polycose® (partially hydrolyzed starch). Tomato juice with added NaCl was given as a control. In their first study, five of eight subjects given MSG in tomato juice reported some reaction, whereas none was reported when tomato juice containing NaCl was ingested. In a second study, six of eight subjects ingesting MSG in water solution reported a reaction, but only one reacted when the MSG solution containing Polycose® was ingested. The reactor was classified as acutely sensitive, since she responded to 25 mg MSG per kg body weight dissolved in soup. As in the first study, there was no correlation of plasma glutamate level with reaction.

In a third study, 16 medical students were given MSG in tomato juice, or in tomato juice with added Polycose®, or were given tomato juice with NaCl in double-blind fashion (48). Eleven of 16 reported a reaction to MSG in tomato juice, one to tomato juice with added NaCl, and none to MSG in tomato juice with added Polycose®. Mean maximum plasma glutamate levels in eight subjects after ingestion of 150 mg MSG per kg in water solution with and without added Polycose® were 7.2 ± 3.5 and 59.4 ± 46.5 μmol per dl, respectively. Fasting level was about 4 μmol per dl.

Stegink et al. (48) compared levels of the major metabolites of glutamate (aspartate, alanine, glucose, and lactate) and histidine and choline esterase in the plasma of reactors and non-reactors ingesting 150 mg MSG per kg body weight in water or tomato juice. No significant differences were found. These investigators suggested that the response to MSG might be caused by an allergic reaction to an impurity in MSG, by a differing metabolism of glutamate leading to the production of the active metabolite, or by intestinal cells with receptors for MSG that release a second messenger to the blood, resulting in the characteristic reactions.

Kerr et al. (49,50) described a survey conducted by Market Research Corporation of America (commissioned by Ajinomoto, USA) on incidence of symptoms considered characteristic of CRS. In this survey, the same questionnaire sequence was administered as had been used by Kerr et al. (51) in surveying students of Harvard summer school, faculty, students, and staff of Harvard School of Public Health, and employees of the Children's Hospital Medical Center of Boston. In the first questionnaire in the Harvard survey, 3 to 7 percent reported symptoms which could possibly represent characteristic symptoms of CRS. When asked in a second questionnaire whether they had experienced CRS, 31 percent responded affirmatively. Analysis of responses to the first questionnaire in the Market Research Corporation survey identified no instance of "definite CRS" or "probable CRS" but 1.8 percent of respondents were considered to represent cases of "possible CRS."
In response to the second Market Research Corporation questionnaire, 74 respondents, or 2.3 percent of the population, stated they had experienced CRS. Of these 74, however, only six (0.19 percent of the total population) had been classified as "possible CRS" on the basis of their symptoms. Characteristic CRS symptoms were also reported with many ethnic foods such as Italian, Mexican-Spanish, and American, and with food classes such as spices and beverages.

Of the 3,222 respondents to the Market Research Corporation survey, 43 percent indicated one or more unpleasant or adverse reactions to food consumption (50). Heartburn (25 percent), diarrhea (12 percent), abdominal cramps (11 percent), and unusual thirst (10 percent) were the most frequently reported adverse reactions to the 65 classes of foods surveyed. The greatest number of respondents (18 percent) reported adverse reactions to spices. Characteristic CRS symptoms were reported from 3 to 5 percent of respondents who indicated unpleasant symptoms after ingestion of each food class.
III. RECAPITULATION

The Select Committee is appreciative of the new information received since the publication of its report on the health aspects of glutamates. It recognizes that the monograph entitled "Glutamic Acid: Advances in Biochemistry and Physiology," is a record of the proceedings of a conference on the subject held a month prior to the issuance of the Select Committee's report in 1978. Enlightening new studies of short duration were included in the proceedings of the Milan conference. Some of the investigators have kindly shared more recent data with the Committee. Among the latter are additional data indicating that glutamates, in water solution administered by methods and in concentrations favoring overloading of physiological barriers, are capable of inducing neuropathological lesions in neonatal rodents. However, at more mature ages, there are metabolic reactions which limit the neurological effects. With the exception of one investigator, neurologic lesions have not been reported in neonatal subhuman primates administered glutamate at doses comparable on a body weight basis to those given to rodents. No evidence of hypothalamic lesions was found in fetal monkeys after the in utero injection of glutamate.

Additional short- and long-term studies, in which MSG was administered in the diet to several animal species, have now been conducted. There were no reports of adverse effects. When pregnant, lactating, and weanling mice were fed up to 15 percent MSG in a commercial diet for 1-4 days and sacrificed, no hypothalamic lesions were found. Three-generation studies in mice were similarly free of toxic effects at up to 25.1 g MSG per kg body weight in dams and 13 g per kg in pups fed the diet postweaning; parameters measured included fertility, gestation, viability, lactation indexes and body weight. No statistically significant differences were observed in histopathological changes in brain tissues of the $F_{1,1}$ and $F_{3,1,1}$ generations when the latter were examined at birth and at 3, 14, and 21 days. Small but statistically significant deviations from controls were reported in some of the neurobehavioral tests applied pre- and postweaning to $F_1$ litters of rats fed diets containing 5.1 percent MSG (4.0 g per kg during gestation; 8.2 g per kg during lactation) beginning two weeks prior to mating, but none occurred in the progeny of rats fed 1.7 or 3.4 percent MSG in their diet. These tests require validation; caution must be exercised in their interpretation.

Observations at 5 and 12 months in male rats and at 3 and 12 months in female rats given oral subneurotoxic doses of MSG during the immediate postnatal period demonstrated no changes in endocrine organ weights, sexual maturation, estrous cycles, or in pituitary and serum levels of luteinizing hormone. Neurophysiological tests elicited similar results in 1-, 3-, 7-, and 9-month-old rats, both controls and treated. These observations have diminished the concern of the Select Committee that infants might be
exposed to doses of glutamates that would induce "silent" lesions with manifestations appearing later in life as behavioral effects or endocrine dysfunction.

In the initial report, the Select Committee expressed a need for information concerning protective mechanisms against glutamate toxicity. The blood-brain barrier has been studied by investigators using mice, rats, guinea pigs, rabbits, and subhuman primates. Increased resistance to the passage of glutamate may start as early as the first trimester of gestation in some species and is usually well developed by infancy. In terms of threshold values, only those for mice are available.

Liver glutamate uptake is saturated by toxic oral doses of 1-4 g per kg in the mouse, and this makes the blood-brain (and blood-retinal) barrier the chief protector against neurotoxic effects of high intakes of glutamates. The presence of an active efflux system for glutamates further enhances protection of the brain through transport of glutamate from brain to blood against a concentration gradient. Even this barrier may be breached, however, when very large doses of glutamate are administered. Susceptibility to breaching varies among species. In subhuman primates, the blood-brain barrier permits only negligible amounts of MSG to enter the cerebrospinal fluid of fetuses injected intraperitoneally or intravenously, while plasma glutamate levels were two to three times and urine values up to 500 times those of control animals as measured 5.5-6.5 h after administration. The differences in susceptibility to glutamate treatment among the species have been attributed to variations in glutamate metabolism, greater integrity of the blood-brain barrier, specific neuronal susceptibility in the mouse, or a combination of these factors. Although neurotoxicity in all rodent species has been associated with elevated plasma glutamate levels, elevated levels in the arcuate nucleus after neurotoxic doses have been observed only in the mouse, and not in the rat or guinea pig.

The blood-retinal barrier is apparently less susceptible to breaching than the blood-brain barrier, since higher doses are necessary to induce neuronal damage in the retina. Age appears to increase the resistance to neuronal damage. Studies in mice receiving 8 g MSG per kg in their diet indicated no retinal damage as determined by histological examination. In rats fed 1, 2, or 4 percent MSG for 104 weeks, no adverse effects were noted by ophthalmoscopic examinations, but there were no histological reports.

In its initial report, the Committee also called for information on the effect of other dietary components on the rate of glutamate absorption in view of the fact that, in use, glutamates would be ingested with other foodstuffs. There is now convincing evidence that when MSG is added to foods such as bouillons, tomato juice, infant formulas, and various basal diets, plasma glutamate levels are less than those found when water is used as a vehicle.
Partially hydrolyzed starches and amino acids also serve to reduce plasma glutamate concentration. These observations may explain why, when large amounts of glutamates are added to the diets of experimental animals, the neurotoxic signs fail to appear. In one experiment, the ingestion of mono- or disaccharides, along with a toxic dose of MSG to mice, reduced the incidence of hypothalamic lesions by 50 percent. Ingestion of the amino acids, leucine, and arginine gave similar reductions.

The initial report of the Select Committee stated, "The mature animal has mechanisms which protect against the intestinal absorption of excessive amounts of glutamic acid...The degree of protection by these barriers is not accurately known, nor is their time of appearance in the developing infant." It has been shown that there are no statistically significant differences in serum glutamate levels in premature, low-birth-weight, or 3.5 kg infants when fed human milk or in healthy, full-term infants 28 to 33 days of age, and low-birth-weight babies fed commercial infant formulas based on casein hydrolyzate or cow milk. In addition, plasma glutamate levels increased equally in one-year-old infants and adults fed quantities of custard providing 1 g protein per kg body weight. Thus, no significant differences have been observed in the capability of premature, low-birth-weight, and full-term babies to metabolize free glutamate from casein hydrolyzates or cow milk. Capabilities to metabolize glutamate appear to be similar in year-old infants and in adults.

"Chinese Restaurant Syndrome" (CRS) is a condition of unknown cause which has been associated with ingestion of large amounts of glutamate, but the signs and symptoms also occur after ingesting other food substances. Recently published information regarding the frequency of occurrence of CRS in the general population has demonstrated the elusive qualities of the syndrome and a wide range of severity. However, the purview of the Select Committee extends only to the effects of glutamates as they are added to various processed foods sold to the public.

In current practice, the highest reported level of glutamate added to a processed soup is one percent. This level is calculated to provide an intake of 2 g MSG in a 200 ml portion, or about 30 mg per kg. This amount may be sufficient to induce a reaction in an "acute responder", as demonstrated by the one case in which a person reacted acutely to servings of soup containing MSG at a level delivering 25 mg MSG per kg body weight. Another study was the source of data predicting that about 4 percent of the population would react occasionally, and less than 2 percent regularly, to a food (soft drink) processed to contain 1 percent MSG. According to the 1970 NAS/NRC survey, highest weighted mean levels of usual addition of MSG were 0.46 percent in milk products, 0.24 percent in soups, and 0.29 percent in pastas and rice dishes; highest maximum
level reported used in one soup was one percent MSG. The frequency of responders to MSG in the population is less than the frequency of responders or hypersensitive persons who exhibit reactions to common foods. The cause or causes of reaction to MSG remain unknown, although postulations include buccal or esophageal receptor stimulation, allergy to impurities, differing metabolic reactions inducing a metabolite producer of CRS, and special cells in the intestinal epithelium with receptors for MSG that release a second "messenger" to the blood, resulting in CRS.
IV. OPINION

The Select Committee notes that the new information on long-term oral administration of monosodium glutamate (MSG) in the diet to various animal species has revealed no adverse effects, while data showing neuropathological lesions in neonatal animals resulting from subcutaneous or forced oral dosing of MSG has thus far been confirmed only in rodents. The administration of protein or carbohydrate products concurrently with MSG has been shown to lower plasma glutamate levels, and in two recent studies, to reduce the incidence of hypothalamic lesions in mice. In pregnant monkeys, glutamate does not appear to readily cross the placental barrier. From comparisons of plasma glutamate levels, healthy term and premature infants have already developed the capability to metabolize glutamates.

Ingestion of MSG solutions has been demonstrated to cause transient clinical symptoms resembling those of "Chinese Restaurant Syndrome" and there is evidence that some individuals may respond to relatively small doses. Similar symptoms can be evoked by certain other food substances. The use of MSG in restaurant- and/or home-prepared foods does not fall within the purview of the Select Committee since it is limited in its considerations to the use of glutamates as ingredients of processed foods. According to industry sources, MSG is not added to infant and junior foods. Because, however, a proportion of the consuming public may be sensitive acute responders, even though the unpleasant symptoms are transient, the Select Committee believes there should be some constraint placed on the addition of MSG to processed foods.

In light of the foregoing studies on the biological effects of monosodium glutamate and the information considered in its previous report on glutamates, the Select Committee concludes that:

There is no evidence in the available information on L-glutamic acid, L-glutamic acid hydrochloride, monosodium L-glutamate, monoammonium L-glutamate, and monopotassium L-glutamate that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current and in the manner now practiced. However, it is not possible to determine, without additional data, whether a significant increase in consumption would constitute a dietary hazard.
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