EVALUATION OF THE HEALTH ASPECTS OF CERTAIN
GLUTAMATES AS FOOD INGREDIENTS

1978

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 professional 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 VII.

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.

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.

[Signature]
Kenneth D. Fisher, Ph.D., Director
Life Sciences Research Office
FASEB


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I. INTRODUCTION

This report concerns the health aspects of using certain glutamates as food ingredients. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (1), which summarizes the world's scientific literature from 1920 through 1973. 

To assure completeness and currency as of the date of this report this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, announcement was made in the Federal Register on April 1, 1977 (42 FR 17526-17529) that opportunity would be 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 health aspects of using certain glutamates as food ingredients. Seventeen requests were received. The Select Committee held a hearing on July 25-26, 1977. Those who requested opportunity to present data, information, and views are identified at the end of this report. The material presented at the hearing has been considered by the Select Committee in reaching its final conclusions.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premarketing clearance that is required for food additives. It is stated in the Act and in the Code of Federal Regulations (2) [21 CFR 170.3 and 170.30] that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. These sections of the Code also indicate that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. FDA (2) recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

*The document (PB-229 856/0) is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.
The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety, the Committee, in accordance with FDA's guidelines, is relying primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. While the Committee realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited, it recognizes that there can be instances where, in the judgment of the Committee, there are insufficient data upon which to base a conclusion. The Committee is aware that its conclusions will need to be reviewed as new or better information becomes available.

In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on certain glutamates and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of these substances under the Federal Food, Drug, and Cosmetic Act.
II. BACKGROUND INFORMATION

L-Glutamic acid, or L-2-aminopentanedioic acid, is a naturally occurring amino acid of plant and animal proteins.

The average glutamic acid content of food proteins is 20 percent. Expressed as glutamic acid per 100 g of the edible portions, medium fat beef contains about 2.65 g of glutamic acid; whole liquid cow milk, 0.82 g; lean pork, 2.16 g; haddock, 2.32 g; peas, 5.58 g; soybeans, 7.01 g; corn-meal, 1.62 g; and whole grain wheat flour, 4.16 g (3). In addition, free glutamic acid is present in many vegetables, fish, and meats in small amounts (0.005 to 0.23 g per 100 g) and as high as 2 g per 100 g in some varieties of cheese (4, 5). Recent estimates of free amino acids in the milk of various species indicated that the free glutamic acid in human milk is about 0.22 g per 100 ml (6).

Glutamic acid and its salts are prepared commercially principally by fermentation of glucose and, to a lesser extent, by isolation from hydrolyzates of wheat, corn, soybean, and sugar beet protein (7, 8). Glutamic acid and the hydrochloride as well as the mono-sodium, -potassium, and -ammonium salts of L-glutamic acid share similar physical properties: they are nearly odorless, white, free-flowing crystalline powders, and except for glutamic acid and glutamic acid hydrochloride, are freely soluble in water. Glutamic acid is slightly soluble and glutamic acid hydrochloride moderately soluble in water (9). The Food Chemicals Codex (9) gives the following specifications for the food grades of these substances: all must assay not less than 99 percent of the compound named and may not contain more than 3 ppm arsenic, 10 ppm lead, and 20 ppm heavy metals (as lead). Chloride is limited to 0.2 percent in monosodium glutamate and glutamic acid and to 0.1 percent in monopotassium glutamate.

Monosodium glutamate (MSG) is listed in the Federal Regulations (2) as an example of a common food ingredient that is considered GRAS (21 CFR 182.1). In other sections of the Regulations, monoammonium glutamate (21 CFR 182.1500), monopotassium glutamate (21 CFR 182.1516), glutamic acid (21 CFR 182.1045), and glutamic acid hydrochloride (21 CFR 182.1047) are listed as multiple purpose GRAS food substances. Glutamic acid, its hydrochloride, and the monosodium, monopotassium, and mono-ammonium salts are all used in foods as flavor enhancers; the monoammonium and monopotassium salts are used primarily as components of salt substitutes. Hydrolyzates of animal or vegetable proteins which contain glutamic acid or its salts and which are considered by FDA as GRAS in the context of 21 CFR 182.1 are also used by the food industry for some of these purposes. Protein hydrolyzates are not considered here but are evaluated in a forthcoming report of the Select Committee (10).
Monosodium glutamate has been used for its seasoning properties in the Orient for several centuries, principally as a naturally-occurring component of the hydrolyzates of soybeans and certain seaweeds (7, 8). It became available commercially in the United States in the early 1940's and its use by the food industry and in home and restaurant cooking has become well established. According to a comprehensive survey of the food industry conducted by a subcommittee of the National Research Council (NRC) (11), substantial increases in consumption of glutamic acid and monoammonium and monosodium glutamates occurred in the United States between 1960 and 1970 (Table I).

The report of the NRC subcommittee (11) has also provided information on the usual use levels of glutamic acid and its ammonium, potassium, and sodium salts in various categories of foods. The subcommittee surveyed manufacturers by questionnaire concerning the addition of glutamic acid or the three salts to their processed products. Based on the information supplied by those manufacturers who reported making such additions to at least one product in a food category, a weighted mean was calculated for the usual percentage addition to foods in that category. The weighted means for the percentage glutamic acid and the three salts added to each food category are given in Table II.

The Select Committee has been informed that no glutamic acid or glutamate salts are being added to commercially prepared infant and junior foods (12).
<table>
<thead>
<tr>
<th>Substance</th>
<th>Relative amounts used&lt;sup&gt;a&lt;/sup&gt; 1970/1960&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Total used &lt;sup&gt;b&lt;/sup&gt; (1970) kg</th>
<th>Per capita daily intake&lt;sup&gt;c&lt;/sup&gt; mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Glutamic acid</td>
<td>125</td>
<td>4,400</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Monoammonium L-glutamate</td>
<td>10</td>
<td>14,000</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Monopotassium L-glutamate</td>
<td>---&lt;sup&gt;d&lt;/sup&gt;</td>
<td>65,000</td>
<td>1</td>
</tr>
<tr>
<td>Monosodium L-glutamate</td>
<td>---&lt;sup&gt;d&lt;/sup&gt;</td>
<td>15,300,000&lt;sup&gt;e,f&lt;/sup&gt;</td>
<td>204&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>L-Glutamic acid hydrochloride</td>
<td>1</td>
<td>30,000</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based only on the reports from those respondents to the National Research Council (NRC) survey who submitted information for both 1960 and 1970.

<sup>b</sup> Recalculated to 100 percent from survey data that the NRC subcommittee estimated to represent about 60 percent of the actual usage.

<sup>c</sup> Based on a U.S. population of 205 million.

<sup>d</sup> Insufficient data to make an estimate.

<sup>e</sup> This is a corrected value. The correction is included in a 1976 report of an NRC Committee (13).

<sup>f</sup> Official statistics show that U.S. production and imports of monosodium glutamate in 1970 were 37.2 million and 10.4 million pounds, respectively, or a total of about 48 million pounds (22 million kg). On this basis, per capita daily intake in 1970 would be about 294 mg (14, 15). The corresponding figures for 1973 were 24.4 million kg and 318 mg per capita daily.
<table>
<thead>
<tr>
<th>Food category</th>
<th>Monosodium L-glutamate</th>
<th>L-Glutamic acid</th>
<th>Monoammonium L-glutamate</th>
<th>Monopotassium L-glutamate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weighted mean percent^a</td>
<td>Weighted mean percent</td>
<td>Weighted mean percent</td>
<td>Weighted mean percent</td>
</tr>
<tr>
<td>Baked goods, baking mixes</td>
<td>0.07</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain products, such as pastas or rice dishes</td>
<td>0.29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fats and oils</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk products</td>
<td>0.46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td>0.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frozen dairy desserts, mixes</td>
<td></td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Processed fruits, juices, and drinks</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat products</td>
<td>0.13</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poultry products</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg products</td>
<td>0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish products</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Processed vegetables, juices</td>
<td>0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condiments, relishes, salt substitutes</td>
<td>0.17</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft candy</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatins, puddings, fillings</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soups, soup mixes</td>
<td>0.24</td>
<td></td>
<td>0.42^o</td>
<td></td>
</tr>
<tr>
<td>Snack foods</td>
<td>0.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beverages, nonalcoholic</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beverages, alcoholic</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Nuts, nut products</td>
<td>0.39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reconstituted vegetable proteins</td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gravies, sauces</td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seasonings and flavors</td>
<td>70.8</td>
<td>0.27</td>
<td>1.07</td>
<td></td>
</tr>
</tbody>
</table>

^a Blanks in the table mean that the substance is not added to the foods indicated. For discussion of weighted mean see text, and Section X and Exhibit 50 of reference 11.

^b Figures in this column are corrected figures, taken from a 1976 report of the NRC committee (13).

^c Reference 11 includes an incorrect figure for the percentage of monoammonium glutamate in soups and soup mixes. The figure as corrected by the NRC subcommittee and as communicated to the Select Committee by Durward Dodgen, NRC, on December 20, 1974, is given in this table.
III. CONSUMER EXPOSURE DATA

Since the NRC subcommittee (11) did not request food consumption data in their survey, they derived estimates of possible average daily intakes (Table III) by utilizing Market Research Corporation data on mean frequency of eating foods by category, USDA data on mean portion size, and by assuming that all food products within a food category contain glutamic acid or the three salts at the levels shown in Table II. The Select Committee has converted these figures to intakes in mg per kg of body weight for each of the age groups. Because of factors detailed in the NRC subcommittee report, they believe that their estimated average intakes (Table III) are likely to be higher than would be the intakes achieved through consumption of a diet consisting totally of processed foods to which glutamic acid or one of the three salts has been added at maximum levels. It should also be recognized, as the NRC subcommittee has pointed out, that its calculations of intakes of GRAS substances are overstated in most cases, often by considerable margins.

New estimates that appear to be more typical of actual intakes by age groups of the glutamate added to foods in the greatest total amount, monosodium glutamate, have been developed in 1976 by another NRC committee (13). The new method of estimation employed the existing data on usage levels obtained in the 1971-72 GRAS survey (11) as well as the same basic information on food consumption (frequency of eating and portion size). However, it applied a probabilistic factor based on whether or not any particular eating of a food product within a major food category would contain monosodium glutamate. The revised estimates are given in Table IV.

This tabulation reveals that actual mean consumption of monosodium glutamate is of the order of 2 mg per kg per day or less for all but the 12 to 23 month age group which consumes at about three times this rate. Consumption of the 99.9th percentile ranges from 25 to 64 mg per kg daily with children in the 6 to 23 month age group consuming at the highest level. The figures in Table IV correlate well with the estimated per capita daily intake of 204 mg monosodium glutamate (Table I).

New estimates of intakes of glutamic acid, monoammonium glutamate, and monopotassium glutamate have not been made. However, the estimates in Table III, which are known to be considerably overstated, still reveal that added glutamic acid constitutes little, relative to monosodium glutamate, of the daily glutamate load. Likewise, monopotassium glutamate, which is added only to seasonings and flavors, and monoammonium glutamate, which is added only to soups and soup mixes, are unlikely to make a significant contribution, relative to that made by monosodium glutamate, to total daily


<table>
<thead>
<tr>
<th>Substance</th>
<th>0-5 months mg</th>
<th>mg/kg&lt;sup&gt;a&lt;/sup&gt;</th>
<th>6-11 months mg</th>
<th>mg/kg&lt;sup&gt;a&lt;/sup&gt;</th>
<th>12-23 months mg</th>
<th>mg/kg&lt;sup&gt;a&lt;/sup&gt;</th>
<th>2-65+ years mg</th>
<th>mg/kg&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Glutamic acid</td>
<td>1</td>
<td>&lt;1</td>
<td>5</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>27</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Monoammonium L-glutamate&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
<td>&lt;1</td>
<td>97</td>
<td>12</td>
<td>144</td>
<td>13</td>
<td>131</td>
<td>2</td>
</tr>
<tr>
<td>Monopotassium L-glutamate</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Monosodium L-glutamate&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43</td>
<td>9</td>
<td>584</td>
<td>73</td>
<td>799</td>
<td>73</td>
<td>1,035</td>
<td>17</td>
</tr>
</tbody>
</table>

<sup>a</sup>Calculations of intakes in mg per kg were based on an average weight of 60 kg for an adult (16) and the following estimated weights of infants by age groups: 0-5 mo, 5 kg; 6-11 mo, 8 kg; and 12-23 mo, 11 kg (17).

<sup>b</sup>Reference 11 includes incorrect figures for the possible daily intakes of monoammonium glutamate and monosodium glutamate. The figures as corrected by an NRC committee (13), and as communicated to the Select Committee by Durward Dodgen, NRC, are given in this table.

<sup>c</sup>Asterisks (***<sup>c</sup>) in the table mean that there were insufficient data on which to base an estimate.
**TABLE IV**

Revised Estimates of the Daily Intake of Added Monosodium Glutamate by Age Group\(^a\) (13)

<table>
<thead>
<tr>
<th>Age group</th>
<th>Mean intake mg</th>
<th>Mean intake mg/kg(^b)</th>
<th>90th Percentile mg</th>
<th>90th Percentile mg/kg(^b)</th>
<th>99th Percentile mg</th>
<th>99th Percentile mg/kg(^b)</th>
<th>99.9 Percentile mg</th>
<th>99.9 Percentile mg/kg(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5 mo</td>
<td>2.2</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>91</td>
<td>11</td>
<td>183</td>
<td>25</td>
</tr>
<tr>
<td>6-11 mo</td>
<td>19</td>
<td>1.9</td>
<td>19</td>
<td>1.9</td>
<td>295</td>
<td>36</td>
<td>430</td>
<td>46</td>
</tr>
<tr>
<td>12-23 mo</td>
<td>75</td>
<td>6.8</td>
<td>329</td>
<td>30</td>
<td>449</td>
<td>43</td>
<td>659</td>
<td>61</td>
</tr>
<tr>
<td>2-65+ yr</td>
<td>99</td>
<td>2.1</td>
<td>500</td>
<td>8</td>
<td>680</td>
<td>24</td>
<td>1,080</td>
<td>38</td>
</tr>
</tbody>
</table>

\(^a\) Because of the very small sample size and the low percentage of eaters in the younger age groups, the 90th, 99th, and 99.9th percentile values shown above are only approximations and should be interpreted accordingly.

\(^b\) Based on actual weights of consumer in the sampled population.
glutamate consumption, but again children between the ages of 6 and 23 months appear to be consuming at the highest level.

It is to be noted that the intake of naturally occurring glutamic acid from foods is relatively large. For example, data presented by Fomon (18) suggest that protein intake of a 3-week-old infant might be 2.7 g per kg (180 ml per kg per day of infant formula providing 1.5 g of cow milk protein per 100 ml). Because glutamic acid accounts for nearly 20 percent of the amino acids of cow milk, the intake of glutamic acid from this source would be approximately 500 mg per kg per day. However, as has been recently noted by Gaull and Rassin (6) free glutamic acid represents only 2 to 3 mg of this total. While it may be argued that introduction of glutamic acid as an integral part of food protein differs from the introduction of free glutamic acid or its salts, it is noteworthy that this same infant receiving human milk, would be consuming free glutamic acid at the level of 30 to 40 mg per kg body weight per day (6, 19). An adult receiving 1 g of protein per kg daily of which 20 percent was glutamic acid, would receive 200 mg of glutamic acid per kg body weight daily.

The Joint FAO/WHO Expert Committee on Food Additives (20) has established an acceptable daily human intake of up to 120 mg per kg of body weight for L-glutamic acid and its monosodium, monopotassium, mono-ammonium, and calcium salts, calculated as glutamic acid. The Joint Committee, in the light of findings concerning the susceptibility of neonates to glutamates, concluded that it would not be prudent to apply this acceptable daily intake to infants under 12 weeks of age, even though they regarded the reported findings as controversial.
IV. BIOLOGICAL STUDIES

Absorption

Various studies suggest that glutamic acid is transaminated prior to absorption from the small intestine. Neame and Wiseman (21, 22) showed that in rabbits an average of 49 percent of the dose of glutamic acid solution (120 to 200 mg per kg of body weight) introduced into the small intestine, bypassing the stomach, disappeared from the lumen in 30 minutes. The disappearance, measured similarly, averaged 92 percent in cats and 74 percent in dogs. At this level, a small increase in blood glutamate concentration occurred. On the other hand, at this level of intraintestinal administration, significant increases in blood alanine were observed. With larger intraintestinal doses (200 to 1,000 mg per kg) in dogs, most of the glutamic acid was absorbed as such and alanine production was relatively low. In man, variable results were obtained in measuring glutamic acid content of venous blood after oral administration of glutamic acid (100 mg per kg), making it difficult to estimate the dose level at which glutamate itself is absorbed (23). Himwich (24) showed that great variations in absorption rate occurred in man depending on whether glutamic acid, the hydrochloride, or the sodium salt, was fed; the salt was absorbed most readily. An oral dose of 250 mg per kg of the salt resulted in a plasma glutamate level of 35.3 mg percent within an hour; plasma concentration after similar doses of the free acid or the hydrochloride did not exceed 15 mg percent. These studies suggest that the minimum oral dose of glutamic acid for intestinal absorption as glutamic acid in the adult human is approximately 100 mg per kg body weight. Garattini and Bizzi and their colleagues (25, 26) have shown that a single oral dose of 30 mg per kg body weight of MSG to fasted human volunteers induces a measurable increase in plasma glutamate. It appears that at moderate oral intakes of glutamic acid and its salts, most is transaminated and absorbed as alanine and α-ketoglutaric acid. After larger doses, this mechanism is apparently overloaded, permitting the absorption of glutamic acid itself rather than as products of transamination.

Some evidence suggests that the capacity of gut transamination of glutamic acid develops postnatally in several species and cannot be prematurely induced by early feeding of high levels of glutamates. Cutler (27) using $^{14}C$-labeled monosodium L-glutamate showed a dose-related rise in labeled serum glutamic acid when 1-day-old rats were dosed with monosodium glutamate in a rat's milk formula. By 5 days of age, the rise did not occur and no label could be found in serum glutamate. A simultaneous increase in intestinal transaminase activity was found. Stegink et al. (28) reported a rapid labeling of plasma glutamate as well as other amino acids, urea, and nonamino acid metabolites in the neonatal pig following administration of $^{14}C$-labeled monosodium glutamate by stomach tube. Wen and Gershoff (29) showed an increasing activity of intestinal transaminases with age in neonatal rats.
Because glutamic acid is metabolized largely via aspartate or alanine aminotransferases, which are vitamin B₆-dependent enzymes, it is understandable that Wen and Gershoff (29) also demonstrated in rats, that removal of intravenously administered glutamate from the blood is much slower in vitamin B₆ deficiency than with an adequate intake of the vitamin. Predictably in their studies on the development of the activity of these enzymes in the intestinal mucosa of suckling rats and in weanling rats fed diets deficient or adequate in vitamin B₆, the activity of both aminotransferases was low in suckling rats and increased when they were fed the vitamin B₆-supplemented diet. The activities decreased when the vitamin B₆-deficient diet was fed. This is in accord with the response of these enzymes to dietary vitamin B₆ in other tissues of the rat. Thus, it is possible that the vitamin B₆ status of the treated animal may influence the results of transamination experiments.

The intestinal absorption of glutamate is retarded by the presence of other nutrients. For example, in adult mice, plasma peak glutamate levels were more than 7.5 times and plasma area under the curve values 64 times higher when monosodium glutamate was given by intragastric tube than when fed mixed with meals (25).

**Metabolism and excretion**

Glutamic acid is concentrated in the brain (30, 31) where it is the only amino acid oxidized (32). Based on experiments on rats and mice by Schwerin et al. (33) and others, Weil-Malherbe (30) noted that the rate of transfer of glutamic acid across the blood-brain barrier is very slow, and Bazzano et al. (34) referred to a protective effect of the blood-brain barrier against monosodium glutamate in gerbils. Although no firm biochemical evidence for a blood-brain barrier against glutamate exists, the mature brain appears to be protected from high levels of this amino acid by a mechanism that maintains a steady state of brain glutamate levels. There is a free exchange between plasma and brain glutamate with no net increase in brain glutamate levels (31, 35). In addition, all glutamate and glutamate product pools are rapidly labeled when glutamate is administered intravenously. Although it has been suggested that in the infant this protective mechanism is not fully functional, little evidence is available to support this idea. However, the glutamic acid present in the cerebrum of 17-day-old rats is more than twice the levels at 1 day of age. In the rat, the period between 10 and 20 days of age covers the onset of cortical function, myelination, maturation of neurons, and development of blood-brain barrier functions (31).

Garattini and his colleagues (25, 26) have shown in studies with mice, rats, and guinea pigs that neurotoxicity is related more to plasma levels of glutamate, expressed as plasma area under the curve (AUC) than to the dose administered. The plasma AUC and peak plasma levels are sensitive to several other variables in addition to the dose. These include the concentration as well as the rate and route of administration of the glutamate.
However, data on the threshold amounts of circulating glutamate necessary for brain uptake, that is, to overcome the blood-brain barrier, have not been reported. These investigators have shown also that the dose of monosodium glutamate by gastric intubation to newborn mice that results in an increase in plasma glutamate levels is less than 250 mg per kg body weight. In the neonatal rat, Cutler (27) reported that all glutamic acid is absorbed unchanged. Garattini et al. (25, 26) estimated that, within the limits of their experimental method, plasma area under the curve of monosodium glutamate over about 150 μmoles per ml x min (or 25,000 μg per ml x min) may result in hypothalamic damage. These results indicate that in order for a sufficient amount of glutamate to reach the brain and produce lesions, the blood level of glutamate must exceed a threshold level for a minimal time period.

In rats and rabbits the main pathways of glutamate catabolism have been identified (36-39). The most important of these is the conversion of glutamate to succinate via α-ketoglutarate. This conversion process, in turn, apparently increases the need for pyruvate which results in increasing mobilization of liver glycogen when large amounts of glutamate are fed. There is no reason to assume that once absorbed, amino acids are metabolized differently depending on their source.

The question of transplacental transfer of amino acids is in dispute. In his studies with rhesus monkeys, Kerr (40) concluded that the placenta maintains a higher blood level of glutamine and glutamic acid in the fetus than in the mother and that, if the mother has an elevated serum level of free amino acids, the placenta will magnify this abnormality in the fetal circulation. Murakami and Inouye (41) found cellular necrosis in the ventromedial and arcuate nuclei of the hypothalamus in mouse fetuses examined 3 and 6 hours after treatment of pregnant mice late in gestation (day 17 or 18) with single 5 mg per g subcutaneous doses of monosodium glutamate; no lesions in the ventromedial nucleus were observed in fetuses examined 24 hours after treatment. On the other hand, Stegink et al. (42) using 14C-glutamic acid infused intravenously into rhesus monkeys at a rate of 20 mg per minute for a total dose of 1 g (160 to 220 mg per kg body weight), found no significant transfer of glutamic acid through the primate placenta and concluded that this organ is virtually impermeable to glutamic acid even when maternal plasma glutamate levels increased twentyfold over baseline values. In other experiments, pregnant rhesus monkeys were given monosodium glutamate in their drinking water at a level to provide 4 g per kg body weight per day during the last third of gestation. No evidence of neurotoxicity was found in the brains of the offspring, which were sacrificed 4 hours after birth (43).

A similar dispute exists over the source of glutamic acid in milk. Prosky and O'Dell (44) reported that the stomach contents of 5-day-old rats suckled on mothers fed 10 percent monosodium glutamate (estimated
to be about 5 g per kg body weight per day) in the diet had 20 percent more free glutamate in their stomachs than did the controls. In contrast, Stegink et al. (19) could not show any significant change in glutamic acid (as glutamate) or alanine in the milk of lactating women when monosodium glutamate was orally administered in amounts of 100 mg per kg of body weight.

There was a hypocholesterolemic effect when glutamate was fed to gerbils, 30 g per kg for 1 week (34); chicks, about 3 g per kg for 2 weeks (45); and man, up to 147 g per day for 3 to 6 weeks (34, 46, 47); while no effect was noted in rabbits fed 0.7 g per kg for 2 weeks; and rats fed 1.3 g per kg for 2 weeks showed higher serum cholesterol levels (45). The mechanism of this effect is not well understood but is apparently specific for glutamic acid.

Acute toxicity

Acute toxicity data on monosodium and monoammonium glutamate in mice, rats, and chickens are summarized in Table V. In the rat, the oral LD₅₀ of monosodium glutamate was estimated to vary between 14.5 and 18.9 g per kg of body weight, while the parenteral LD₅₀ was 3.6 g per kg of body weight, suggesting a protective action of the gastrointestinal tract.

Short-term studies

Neurotoxicity. Since 1957, a series of reports has appeared suggesting that the parenteral administration or forced feeding of glutamates results in lesions of the central nervous system. In the first of these, Lucas and Newhouse (50) reported injecting monosodium glutamate subcutaneously into adult mice in single doses of 4 to 8 g per kg of body weight, and into neonatal mice, 2 to 16 days of age, in single doses of 2.2 to 5.4 g per kg of body weight. Retinal lesions were found within a few hours after the injections, involving necrosis of ganglion cells, inner fiber layer, and some of the bipolar cells; in very young animals, damage to the inner layers was more extensive. These findings were later confirmed by Potts et al. (51). Other workers showed that the critical period in mice for the development of retinal effects from parenteral doses up to 4 g of monosodium glutamate per kg of body weight, was the first 10 postnatal days (52) and that a single subcutaneous injection of monosodium glutamate, given on the ninth to tenth postnatal day, could produce that lesion (53).

In neonatal rats, subcutaneous or intraperitoneal injections of monosodium glutamate (about 2 to 5 g per kg of body weight) also resulted in retinal lesions (54, 55). The biochemical lesion associated with this pathology was correlated with the repression of glutaminase-I activity in the inner retina (54). A blood-retina barrier was also shown to develop by the twelfth postnatal day in rats, explaining the reduction in retinal damage observed after this time (56). Adult rabbit retinal degeneration has also been shown
### TABLE V

#### Acute Toxicity

<table>
<thead>
<tr>
<th>Substance</th>
<th>Animal</th>
<th>Route</th>
<th>Dosage, g/kg body wt</th>
<th>Measurement</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monosodium L-glutamate</td>
<td>mouse</td>
<td>oral</td>
<td>19.20 (16.13-22.84)</td>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>48</td>
</tr>
<tr>
<td>Monosodium L-glutamate</td>
<td>rat</td>
<td>oral</td>
<td>16.60 (14.50-18.90)</td>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>48</td>
</tr>
<tr>
<td>Monosodium L-glutamate</td>
<td>rat</td>
<td>i.p.</td>
<td>3.60</td>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>37</td>
</tr>
<tr>
<td>Monoammonium L-glutamate</td>
<td>rat</td>
<td>i.p.</td>
<td>1.00</td>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>37</td>
</tr>
<tr>
<td>Monosodium L-glutamate</td>
<td>chick</td>
<td>s.c.</td>
<td>3.00-4.00</td>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>49</td>
</tr>
<tr>
<td>Monosodium L-glutamate</td>
<td>chick</td>
<td>s.c.</td>
<td>5.00</td>
<td>LD&lt;sub&gt;100&lt;/sub&gt;</td>
<td>49</td>
</tr>
</tbody>
</table>
to follow intravenous or intraperitoneal injections of monosodium glutamate at doses greater than 250 mg per kg body weight, given daily for 16 days (57). Studies by Reif-Lehrer (58) show rapid and appreciable damage to rodent retinas in culture at monosodium glutamate concentrations of the order of those reported in some normal vertebrate sera.

The mechanism of this action of glutamate can be explained by its effect on the ion permeability of the dendritic and somatic plasma membranes. Curtis et al. (59) were the first to demonstrate that glutamate iontophoretically applied to neurons in the central nervous system causes their depolarization and firing by an increase in ion (especially Na) permeability of the plasma membrane. A marked increase in Na permeability of the neuronal plasma interferes with the double Donnan equilibrium which maintains the normal ion distribution across the plasma membrane and results in an inflow of extracellular NaCl, for osmotic reasons accompanied by water, into the affected cells. This causes a swelling of dendrites and somas and a loss of extracellular material (60). The increase in Na permeability causes depolarization of the neurons, as during excitation, and results in an exchange of intracellular K for extracellular Na. Ames (61) and Ames et al. (62) showed that the application of a 5 mM L- or D-glutamate solution to the isolated rabbit retina caused a marked gain of the tissue levels of Na, Ca and Cl and a loss of K. It can be surmised that these changes in the ion composition of the retinal tissue are responsible for the swelling, opaqueness and irreversible loss of function observed.

Spreading depression (a decreased rate of spread of an electric impulse in the cortex from a stimulated area, as measured by the electrocorticogram), which can be elicited in the retina, is initiated by a release of glutamate from the extracellular compartment where it is present in high concentration (10 mM) into the extracellular space where it causes the enhanced ion permeability of plasma membranes described above (60, 63). Indeed Kow and Van Harreveld (64) showed that during spreading depression in the isolated chicken retina the tissue gained Na and water and suffered a severe loss of K. The recovery of the tissue from these ionic changes was found to be extraordinarily slow. After repeated spreading depressions the isolated retina shows the same changes as after glutamate application, although to a lesser degree.

Changes in other areas of the central nervous system have been reported following parenteral administration of monosodium glutamate. In 1969, Olney (65) reported acute lesions in the preoptic and arcuate nuclei of the hypothalamus of newborn mice following single subcutaneous injections of monosodium glutamate at doses as low as 500 mg per kg of body weight. These lesions occurred soon after treatment and were characterized by intracellular edema and neuronal necrosis. In a longer experiment, Olney reported that subcutaneous injections in mice for 10 days after birth with
daily doses of monosodium glutamate ranging from 2,200 to 4,400 mg per kg of body weight resulted in a variety of changes including the brain lesions reported previously, reduction in body size, greater postweaning weight gain, adiposity, female sterility, and smaller pituitary glands. The central nervous system lesions, greater postweaning weight gain, and adiposity were later confirmed by Matsuyama (66) following the subcutaneous administration of monosodium glutamate to infant mice in single or multiple injections of 2,000 to 4,000 mg per kg of body weight. Similar hypothalamic lesions were reported by O'ney and Ho (67) following administration by gavage of monosodium glutamate to 10- to 12-day-old mice. In these experiments, doses as low as 500 mg per kg of body weight produced lesions in more than half of the animals; no lesions were found in animals receiving 250 mg per kg. Sodium chloride, monosodium glutarate, and several monocarboxylic amino acids at a dose of 3,000 mg per kg had no effect, but 3,000 mg per kg of cysteine or 1,000 mg per kg of sodium aspartate produced lesions in all animals. The specific hypothalamic lesions attributed to glutamate have been reported to occur in fetal mouse brains after transplacental exposure from a single 5,000 mg per kg subcutaneous dose to the dam on day 17 or 18 of pregnancy (41), and after parenteral or oral administration in a variety of species including mice (68-70), rats (69-71), and rhesus monkeys (72). In the study with monkeys, oral doses of monosodium glutamate as low as 1,000 mg per kg of body weight produced small focal lesions in the infundibular nucleus. Recently Nemeroff et al. (73) reported that exposure of neonatal rats to large intraperitoneal doses (4 g per kg) of MSG resulted in disappearance of perikarya of the arcuate nucleus dopamine neurons, with no effect on catecholamine neurons in surrounding or distal brain areas.

Other investigators have found hypothalamic lesions in adult mice given monosodium glutamate parenterally in single doses of 2,000 to 10,000 mg per kg of body weight (65, 74). Recently, Lemkey-Johnston and Reynolds (75) reported arcuate neuron damage in adult mice given monosodium glutamate in intraperitoneal doses of 2,000, 4,000, 5,000, and 7,000 mg per kg body weight. While neurons were damaged unequivocally in the adult, the number of involved cells and areas of damage were less than those in neonates. At levels of 1,000 mg per kg in adults and in older neonates (10 to 12 days of age), lesions were not always found.

Reynolds, Butler, and Lemkey-Johnston (76) intubated 6- to 12-day-old mice with an aqueous solution of monosodium glutamate in single doses of 0.25, 0.5, 1.0, 2.0, or 4.0 g per kg body weight, and infant macaques (30 minutes to 14 days old) with single doses of 1.0, 2.0, and 4.0 g per kg. Brain tissues were taken for microscopic examination 3 to 6 hours after dosing. Hypothalamic lesions were encountered at dose levels equal to or exceeding 0.5 g per kg in mice but hypothalamic morphology remained normal in neonatal macaques after doses as high as 4.0 g per kg. In parallel experiments with intubated aqueous slurries of aspartame (aspartyphenylalanine), hypothalamic lesions that were much smaller than those found with
monosodium glutamate were encountered in neonatal mice at dose levels equal to or exceeding 1.0 g per kg, and no morphological changes were observed in neonatal macaques after doses as high as 2.0 g per kg. The authors suggest that the blood-brain barrier and liver metabolism are two potential morphologic and physiologic parameters that may cause susceptibility of neonatal mouse brains to large loads of the dicarboxylic amino acids while rendering the neonatal primate resistant (76).

In contrast to these reports of neurological changes as a result of neonatal treatment with glutamic acid or monosodium glutamate, other studies do not confirm such findings. Oser et al. (77) gave single oral or subcutaneous doses of monosodium glutamate or potassium glutamate, 1,000 mg per kg of body weight, to 3-day-old mice, rats and beagles and found no specific effects associated with the treatment. Animals were sacrificed and brains and eyes examined 24 hours after treatment. Similarly, Abraham et al. (78) were not able to find significant neuronal changes after oral treatment of infant mice with 1,000 mg monosodium glutamate per kg of body weight. In neonatal rats, Adamo and Ratner (79) were unable to find neuronal lesions following single subcutaneous injections of monosodium glutamate in doses of 4,000 mg per kg of body weight. No neuronal changes were observed in three studies with infant monkeys (78, 80, 81) in which doses ranging from 1,000 to 4,000 mg per kg of body weight were given orally even though high blood glutamate levels were achieved (81). Wen et al. (82) were unable to find the hypothalamic lesions reported by others in infant mice fed 2,000 to 9,000 mg of monosodium glutamate per kg of body weight, nor were they able to confirm such effects when monosodium glutamate was added to the diets of weanling rats (20 to 40 percent monosodium glutamate), suckling mice (2,000 to 9,000 mg per kg of body weight per day), and infant monkeys (3,800 to 13,800 mg per kg of body weight per day). These workers attributed their inability to find these lesions to the fact that they did not inject or force-feed the test material, but offered it as a supplement to a normal diet. Newman et al. (43) were not able to produce these lesions in neonatal monkeys with single oral doses of 2,000 to 4,000 mg per kg of body weight of monosodium glutamate. Hazleton Laboratories, Inc. (83) found no evidence of neuronal lesions in brain sections of 10 pups from rabbit does that had received up to 0.825 percent (about 250 mg per kg body weight) monosodium glutamate in the diet. Experiments on infant rhesus monkeys 5 to 50 days of age intubated with 2 g monosodium glutamate per kg body weight and examined 4 hours after dosing, revealed no hypothalamic changes associated with the glutamate administered (84). Similarly negative results were obtained in 80-day-old monkeys receiving 4 g monosodium glutamate per kg body weight.

There are common pathologic states in which the blood-brain protective barriers (BBB) may be compromised. Although the anatomic location of the BBB is uncertain (being in either the capillary walls, the neuroglia, or both),
data from animal experiments suggest that vascular disease, acute hypertension, or brain edema may affect the barriers (85-87). There is experimental evidence that damage to the brain, for instance, by ischemia secondary to atherosclerosis, emboli, or thrombi, or from cold injury, affects the BBB, permitting diffusion of the relatively large blood proteins into the brain (85,86). Acute hypertension in dogs, induced by clamping the thoracic aorta or by injection of metaraminol, resulted in extravasation of Evans blue-albumin into cerebral and cerebellar tissues (87). Although hypoxia itself will damage brain tissue, the damage may be increased by the entrance of glutamate into the tissue. It would seem prudent to restrict the intake of glutamate by patients in whom such pathologic states are present. In addition, inflammation of the eye and surgical procedures such as ocular paracentesis are known to increase the permeability of the blood-ocular barrier (88), and vitamin B₆ deficiency is known to decrease the rate of removal of circulating glutamate in experimental animals (29). The relationship of this experimental finding to a probable need for supplemental vitamin B₆ in women taking oral contraceptives needs study (89).

**Long-term studies**

Administration of monosodium glutamate in the diet in long-term studies in mice, rats and dogs at levels between 1 and 10 percent of the diet (between 1 and 4 percent in the majority of the studies) appears to have no adverse effects including such parameters as growth and development, reproduction, brain morphology, organ weights and organ morphology (90-94). However, in a two-generation study, Semprini et al. (95) observed that mice fed MSG at levels of 1 or 2 percent in the diet showed higher average weights at weaning compared with controls. Examples of estimated average MSG intakes per animal per day were 84 and 209 mg, respectively, for the 1 and 2 percent MSG diets during days 1 to 15 and 150 and 344 mg during days 16 to 30.

On the other hand, various endocrine disturbances have been reported in adult animals following parenteral treatment with monosodium glutamate in infancy. After subcutaneous administration to neonatal rats of doses ranging from 2, 200 to 4, 200 mg per kg body weight for 10 days and autopsy after 40 days, the animals showed decreased body weight, testicular and ovarian atrophy, reduction in adrenal and thyroid weights and decreased levels of growth hormone and luteinizing hormone in the anterior pituitary gland (96, 97). In similar studies rats became obese as adults following subcutaneous monosodium glutamate for the first 10 days of life (98). These animals demonstrated increased epididymal fat pad weight and cell size and a reduction in adipose cell numbers. In addition, the larger cells of the treated animals were less responsive to the lipolytic effect of epinephrine and more responsive to the antilipolytic effect of insulin. The authors suggested that the obese state produced by glutamate administration results from an increase in adipose cell lipid content which is maintained, in part, by altered lipolytic responsiveness to epinephrine and insulin.
Similar endocrinologic effects were noted in a study reported by Nemeroff et al. (73) in which rats given single or a series of five intraperitoneal injections of MSG (4 g per kg) during the first 10 days of life showed, as adults, significantly smaller pituitaries, adrenals, ovaries and testes than controls. In a more recent report, Nemeroff and his colleagues (99) noted that rats receiving five intraperitoneal injections of MSG (4 g per kg) during the first 10 days of life showed, as adults, growth, endocrinologic and behavioral abnormalities including tail automutilation in a large proportion of test animals. There were marked stunting and obesity, and the females had smaller ovaries, uteri, and pituitaries than controls. Another group of neonatal rats that received single intraperitoneal injections of MSG (4 g per kg) had few of the "characteristic symptoms associated with neonatal MSG treatment."

Newborn male and female mice given subcutaneous doses of monosodium glutamate gradually increased from 2,200 to 4,200 mg per kg body weight from day 2 to day 11 showed reproductive dysfunction in adulthood. Females had fewer pregnancies and smaller litters; males showed reduced fertility (100). Nikoletas (101) reported that eight consecutive daily subcutaneous doses (increasing from 2.5 to 4.2 mg per g body weight) of monosodium glutamate to neonatal male and female rats had no effect on activity levels but resulted in skeletal stunting and obesity during growth and development and disturbances of the reproductive system of the females.

Although the effect on body weight in adult life of parenteral administration of monosodium glutamate to neonatal animals is clear, the situation with oral administration is not. On the whole, if there is an effect of orally administered glutamate on weight gain, it is small, and seems to affect a limited number of animals in the population. This raises the question of whether there may be some animals that are more susceptible to the effects of orally administered glutamate on weight gain.

Human responses

Two types of responses have been described following human ingestion of monosodium glutamate. In the first, symptoms normally identified as allergic symptoms, are experienced by individuals sensitive to the raw materials from which the glutamate is prepared, e.g. beets, corn or wheat (102). The second is the so-called "Chinese restaurant syndrome" (CRS), first reported in 1968 (103-105). Apparently this syndrome appears in sensitive individuals within 5 to 35 minutes after consuming, on an empty stomach, foods containing on the order of several grams of added monosodium glutamate. In order of frequency of appearance, the signs and symptoms include: a sensation of tightness in the back of the neck; a feeling of pressure behind the eyes; frontal or temporal headache; drowsiness, facial flushing, sweating, nausea, a feeling of pressure on the side of the face, thirst, pressure, and burning sensations in the chest; and abdominal pain. These
Manifestations are transient, usually lasting for less than 1 hour; however, headache may occasionally persist for as long as 5 hours.

Susceptibility to CRS has been reported to be greater in women than in men (106). No correlation between the effects and blood levels of glutamate could be found (107). While the effects are described as uncomfortable, inconvenient, and sometimes alarming, there is no evidence of significant untoward consequences. Susceptible individuals may avoid the syndrome by not eating the offending foods or by eating other foods before taking items containing added monosodium glutamate. Although several attempts have been made to elucidate the mechanism of the syndrome, they have been unsuccessful.

Reif-Lehrer (108) has indicated that 25 percent of those exposed to Chinese restaurant food report adverse reactions, presumably to its monosodium glutamate content. In contrast to this, Kerr et al. (109), using a more restricted definition of CRS in terms of specific signs and symptoms, reported an incidence of between 3 and 7 percent of 530 subjects surveyed. Using the same restricted definition as Kerr et al., Abrams and Kadushin (110) found only 1.8 percent of 3,222 questionnaire respondents had experienced signs and symptoms that were compatible with a diagnosis of possible CRS.

During the 5-year period, 1970-1974, 139 outbreaks involving 1,012 cases of foodborne illnesses of chemical etiology were reported to the Center for Disease Control (111). Ten (7 percent) of the total outbreaks including 41 individual cases (4.1 percent of the total cases) were reported to the Center as monosodium glutamate intoxication (CRS). The prevalence of CRS cannot be estimated from these data because reporting of CRS by physicians or patients is optional. The data suggest, but do not establish, that the reported illnesses were caused by ingestion of foods containing added monosodium glutamate (111).

However, a number of studies do not support the conclusion that oral monosodium glutamate loading causes CRS. Several experiments by different groups of investigators using human volunteers from the general, unselected population and double blind techniques led to the conclusion that there were no significant differences in the incidence of symptoms between control and treated groups who ingested 3 to 4.4 g of monosodium glutamate (8,107,112-114). The variability of responses to monosodium glutamate has made it impossible to determine the proportion of the population susceptible to this syndrome or to confirm its etiology.

Behavioral studies

A review of the literature up to 1964 on the effects of orally administered glutamic acid on maze learning in rats noted that while several
investigators had reported improved maze learning performances when rats were fed glutamic acid supplements, others were unable to confirm such findings (115). In 1969, Wincze and Vogel (116) reported that in young rats, moderate levels of glutamic acid in the diet (200 mg per day or approximately 1.3 g per kg body weight per day) accelerated learning of simple perceptual-motor tasks (bar pressing) but that high levels (400 mg per day or approximately 2.6 g per kg body weight per day) caused overactivity and "behavioral disorganization." These investigators chose a simple perceptual-motor task (bar pressing) instead of a perceptual-restructuring task (maze learning) on the theory that any behavioral influence of glutamic acid is probably mediated through its adrenergic effect. Pinto-Scognamiglio et al. (48) found that a very high dose (10 g per kg) of monosodium glutamate orally in rats was effective in depressing avoidance acquisition and performance but that tolerance appeared rapidly with repeated treatment.

Pradhan and Lynch (117) gave daily doses of 1.25 to 5 g of monosodium glutamate per kg body weight by stomach tube to rats between the fifth and tenth postnatal days. Significant decreases in spontaneous motor activity and maze learning discrimination were observed in tests conducted during the ensuing 12 weeks. The deficits were more pronounced in animals that were pretreated at the 5 g per kg level. The authors concluded that a behavioral deficit develops in the adult following treatment of newborn rats with high doses of monosodium glutamate. Feeding 10 percent monosodium glutamate (about 10 g per kg per day) in the diet of male weanling rats resulted in learning deficits in avoidance lever pressing behavior while monopotassium glutamate produced an enhancement of avoidance learning (118). Weanling albino rats, given dry laboratory chow ad libitum for 16 weeks supplemented with monosodium glutamate at the 1, 5, 10, and 20 percent levels (estimated to be 1, 5, 10, and 20 g per kg of body weight, respectively), displayed increased irritability and decreased concentrations of brain γ-aminobutyric acid. The irritability was similar to that observed in vitamin B₆ deficiency in which decreased production of γ-aminobutyric acid has been demonstrated (119).

In the period beginning about 30 years ago, there was wide interest in the apparent ability of orally administered glutamic acid to improve "intelligence" in human mental retardates. In numerous clinical trials, many of which were uncontrolled, doses as high as 40 g of glutamate were given daily for periods of several months. While careful toxicological studies were not made, reports of side effects after sustained, high levels of orally administered glutamic acid in human patients (30 to 40 g per day) included occasional instances of gastric disturbance, insomnia, hyperactivity, and impulsiveness during intelligence testing. Reviews of these studies in subsequent years concluded that specific effects of supplemental glutamic acid upon human intelligence had not been convincingly demonstrated and that the more carefully designed studies tended to be negative (120,121).
No recent studies of the influence of glutamic acid on mental retardation have been reported. While it has not been demonstrated that glutamic acid is of value therapeutically in mental deficiency, it is appropriate to note that a sample of 25 reports of clinical studies reveals that of the 529 subjects evaluated, doses of glutamate ranging from 3 to 40 g per day, in some cases administered for as long as 2 years, did not elicit obvious or significant signs of acute or chronic toxicity.

Special studies

In preliminary experiments with rabbits, Tugrul (122) found a variety of untoward effects in the dams and fetal malformations associated with oral administration of glutamic acid hydrochloride to the parents at 25 mg per kg of body weight for 30 days. No confirming information or expansion of this study has been reported. However, other teratologic studies with monosodium glutamate and glutamic acid hydrochloride in rabbits (doses from 25 mg to 2.5 g per kg body weight) yielded negative results (123-125). Semprini et al. (126) fed monosodium glutamate to rats in amounts up to 2 percent of the diet (estimated to vary with age of animals from 1 to 2 g per kg per day) for two generations without evidence of teratogenesis, and Prosky and O'Dell (44) found no effect in two generation studies in rats fed 10 percent monosodium glutamate in the diet (estimated to vary with age of animals from 5 to 10 g per kg per day). In the teratologic evaluation of orally intubated monosodium glutamate at doses up to 1140 mg per kg daily in mice (day 6 through day 15 of gestation), up to 845 mg per kg daily in rats (day 6 through day 15 of gestation), and up to 640 mg per kg daily in rabbits (day 6 through day 18 of gestation), no discernible effects on nidation or on maternal or fetal survival were observed. The number of abnormalities seen in soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls (127). In identical tests, similarly negative results were obtained with monopotassium glutamate at doses up to 520 mg per kg in mice and up to 450 mg per kg in rats (128).

Following injection of 0.05 ml of a 547 mg per ml saline solution of L-glutamic acid hydrochloride (about 27 mg) into the yolks of white Leghorn chicken eggs, Landauer (129) found a 3 percent greater incidence of rumplessness in the developing chicks compared with controls. Verrett (130-132) found no significant increase in abnormalities in the chick embryo test at levels up to 200 mg of monosodium glutamate, monopotassium glutamate or monoammonium glutamate per kg. A low level of serious anomalies was found in chick embryos after yolk or air cell administration of monosodium glutamate; doses varied up to 3.0 g per kg (133). At present, there is no reliable way to extrapolate to mammals these apparent effects of glutamates in the chick embryo toxicity test.
There was no evidence of mutagenic effects of monosodium glutamate in either the dominant lethal assay with mice (single oral dose in amounts up to 5.4 g per kg body weight (134) or the host-mediated assay in rats (5.7 g per kg daily for 14 days) (135). The following substances at concentrations indicated did not exhibit mutagenicity in a series of in vitro microbial assays using revertant strains of Salmonella typhimurium and Saccharomyces cerevisiae with and without activation by mouse, rat, and monkey liver homogenates: monoammonium glutamate at up to 0.58 percent, monopotassium glutamate at up to 3.0 percent, L-glutamic acid at up to 5.0 percent, and L-glutamic acid hydrochloride at up to 0.025 percent in the culture medium (136-139). Feeding monosodium glutamate for 2 years to C57 black mice at dietary levels of 1 percent and 4 percent (about 3 to 10 g per kg per day) and to Sprague-Dawley rats at 0.1 percent and 0.4 percent (about 200 to 400 mg per kg per day) produced no evidence of carcinogenicity (140,141). Weisburger et al. (142) reported that approximately 50 percent of rats fed 2.5 percent acetamide in the diet for 12 months developed malignant liver tumors. Addition of 5.6 percent arginine glutamate to the test diet resulted in nearly complete inhibition of the carcinogenicity.
V. OPINION

Glutamic acid comprises about one-fifth of the amino acids in proteins which are regularly consumed as food; thus, the average adult consumes about 15 g of glutamic acid daily from dietary proteins, and a 3-week-old infant, about 1.5 g. It is also present in the free form in many vegetables, fish, and meats in concentrations from 0.005 to 0.23 percent and as high as 2 percent in some cheese. The estimated daily average adult consumption of glutamic acid in food protein may be compared with the per capita human consumption of food additive monosodium glutamate (MSG), which for 1970 was estimated to be about 200 mg per day. Estimated adult consumption per person per day of added glutamic acid, glutamic acid hydrochloride, and monoammonium glutamate, is less than 1 mg each, and of monopotassium glutamate is not more than 1 mg. As far as the Select Committee has been able to ascertain, none of these compounds is currently being added to infant formulas and/or commercially prepared strained and junior baby foods.

Oral LD₅₀ values for monosodium glutamate in experimental animals are high; an average of 19.2 g per kg body weight in mice and 16.6 g per kg in rats. However, lethal doses have not been reported for such species as subhuman primates. Children who were being treated with orally administered glutamic acid for mental retardation tolerated daily doses as high as 40 g for periods of 6 months or longer without evidence of significant side effects.

Neurotoxicity has been reported following large doses of glutamic acid and its salts, especially in neonatal animals. Monosodium glutamate and L-glutamic acid had approximately equal neurotoxic effects in mice. For mature animals, the safety of these substances appears relatively clear. The mature animal has mechanisms which protect against the intestinal absorption of excessive amounts of glutamic acid and limit its concentration in the brain. While it is apparently possible to overload these mechanisms, this evidently occurs only at very high levels of intake, as in an experiment. The degree of protection from excess glutamate by these barriers is not accurately known, nor is their time of appearance in the developing infant. The presence of other foods in the diet appears to restrict and modulate the absorption of this amino acid.

Several investigators have reported retinal and hypothalamic neuronal damage in neonatal animals following single, large, parenteral or "oral" doses of monosodium glutamate. The functional significance of the hypothalamic lesions is not clear. In addition, there are several reports of similar neurotoxic effects in the brains of adult animals given monosodium glutamate parenterally or "orally." It should be noted that the oral doses referred to in the animal studies involved forced feeding via stomach tube.
Despite the fact that the brain damage reported in neonatal mice, rats, and monkeys by some research groups could not be confirmed by other groups, the Select Committee concludes that the morphologic changes are real and are reproducible. However, their significance is undetermined. Based on evidence from laboratory animal studies, some investigators have postulated that the hypothalamic neuronal damage which could be suffered by an infant exposed to a toxic dose of monosodium glutamate would be a "silent lesion" whose manifestations would probably not appear until later life in the form of behavioral effects or endocrine dysfunctions.

There is some evidence indicating that glutamate is not significantly concentrated in the milk of lactating women. A recent report indicates that the primate placenta is impermeable to glutamate except when massive doses (greater than twentyfold above normal plasma levels) are given; thus, monosodium glutamate ingested in the amounts anticipated in the United States (Table IV) will probably not cross the normally functioning placenta.

Behavioral effects of glutamic acid and monosodium glutamate, both adverse and beneficial, have been reported in laboratory animals, but the accumulated evidence remains equivocal as does the evidence for any beneficial effect in human cases of mental retardation.

When one considers the ubiquity of glutamic acid in food protein, the long history of monosodium glutamate as a flavor enhancer, the estimated average adult intake of about 200 mg per day of monosodium glutamate as a food additive, and the physiological protective mechanisms (except in neonatal experimental animals) against excess absorption of all but massive doses of glutamate, it might be logical to conclude that glutamate as currently used as a food ingredient is without biological hazard in adults. However, in view of its neurotoxicity, albeit in doses that are very large, and in view of the fact that there are several important contradictions in the literature, such a conclusion should be guarded. Clarification is needed concerning problems of minimal levels for intestinal absorption, amounts of ingested MSG required for uptake in the central nervous system, and the effect of other dietary components on the rate of absorption.

The minimal concentration of glutamic acid required to cause brain lesions in rodents has not been precisely determined, but it appears that glutamate blood levels above a threshold value must be exceeded for a minimal period of time to produce them. These pharmacokinetic requirements are suggested by the experimental data currently available. However, there may be significant differences among species in thresholds, properties of the blood-brain barriers, and changes during development which would modify the extrapolation of pharmacokinetic conclusions derived from experiments in rodents to other species, including man. Therefore, answers to questions of possible risk to the public from ingestion of glutamates as
added food ingredients require more definitive studies of their pharmacokinetics and toxic thresholds as well as the influence of admixture with other food components.

There is some evidence to show that the "Chinese restaurant syndrome" may be caused by monosodium glutamate when used as a food ingredient. While this phenomenon appears to involve an unknown percentage of people who eat glutamate-seasoned food and apparently causes no permanent damage, it is stated to be a very disturbing or temporarily incapacitating experience. Rough estimates of the prevalence of CRS vary from 1.8 to 25 percent of the population. Available data suggest that the nature, causes, definition and, therefore, the prevalence of CRS are not clearly understood. In order to determine whether CRS is significant to the public health, such information should be obtained.

Questions concerning protective mechanisms against glutamate toxicity such as intestinal, hepatic, retinal, and blood-brain barriers need answers. Studies should include the development of and changes in these mechanisms in different species, including man, thresholds, and the effects of abnormal states. Long-term studies that concentrate on retinal damage in addition to the reported damage in the hypothalamus should be conducted.

The Select Committee has weighed the foregoing information on the biological effects of glutamic acid, its hydrochloride, and its food additive salts and concludes that:

There is no evidence in the available information on L-glutamic acid, L-glutamic acid hydrochloride, monoammonium L-glutamate, and monopotassium L-glutamate that demonstrates or suggests reasonable grounds to suspect, a hazard to individuals beyond infancy when used at the 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.

While no evidence in the available information on monosodium L-glutamate demonstrates a hazard to individuals beyond infancy when it is used at the levels that are now current and in the manner now practiced, uncertainties exist requiring that additional studies should be conducted.

The evidence is insufficient to determine that the adverse effects reported are not deleterious to infants should L-glutamic acid, L-glutamic acid
hydrochloride, monoammonium L-glutamate, monopotassium L-glutamate, or monosodium L-glutamate be added to commercially prepared infant or junior foods. Current information indicates that glutamic acid and its salts are not now being added to these foods.
VI. REFERENCES CITED


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