EVALUATION OF THE HEALTH ASPECTS OF PROTEIN
HYDROLYZATES 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
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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 V.

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 the individual members of its constituent societies.

[Signature]
Kenneth D. Fisher, Ph.D., Director
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I. INTRODUCTION

The 1978 report of the Select Committee on GRAS Substances, entitled "Evaluation of the Health Aspects of Protein Hydrolyzates as Food Ingredients," assessed the safety of their use as flavoring agents in commercially processed foods (1). Following the presentation of new information on protein hydrolyzates and monosodium glutamate (MSG), a principal component of protein hydrolyzates, at the Milan Symposium on Glutamic Acid, May 29-31, 1978 (2), the Food and Drug Administration (FDA) requested that the Select Committee on GRAS Substances update its review of the health aspects of protein hydrolyzates as flavoring ingredients, and of casein hydrolyzate as a nutrient (3). The FDA indicated that this review need not include use of protein hydrolyzates in special diets for rapid weight reduction since this subject is under review by the Protein Diet Task Force of the United States Public Health Service (3), or include the use of protein hydrolyzates for parenteral administration (4).

FDA announced its request to the Select Committee in the Federal Register (44:7232-7233, 1979). The announcement also listed the public availability of additional data and information on the safety of protein hydrolyzates and glutamates, invited public comment on the data, and requested submission to FDA and to the Select Committee of any new safety information not previously considered in the review of the GRAS status of glutamates and protein hydrolyzates.

The Select Committee has reviewed the information presented in the proceedings of the Milan Symposium (2), 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.

The statement that follows is the Select Committee's revised analysis and evaluation of protein hydrolyzates as flavoring agents in foods and enzymatic casein hydrolyzates as nutrients in foods, including infant formulas.
II. DISCUSSION

Six adverse effects reported in the literature had particularly raised reservations on the part of the Select Committee regarding the use of protein hydrolyzates in their original report (1). These were:

I. Hypothalamic lesions induced by approximately 1 g MSG per kg body weight administered subcutaneously or orally to mice, rats, guinea pigs, and monkeys (5-9).

II. Retinal lesions induced within a few hours after subcutaneous injection of 2.2-5.4 g MSG per kg in 2- to 16-day-old mice (10).

III. Abnormalities in growth, reproductive functions, behavior, and other characteristics in adult rodents which had been injected subcutaneously with high doses of glutamate during the neonatal or infant stage (5).

IV. Hypothalamic lesions induced by oral or parenteral administration of 1 g sodium aspartate per kg body weight in infant mice (7).

V. Endocrine disturbances with obesity, skeletal stunting, and decreased weight of adenohypophyses, ovaries, and testes induced by subcutaneous administration of multiple doses of 2.3 g sodium aspartate per kg body weight to neonatal mice (11).

VI. Hypothalamic lesions induced in 10-day-old mice by the subcutaneous administration of 1-5 g of enzymatic casein hydrolyzate per kg body weight (12).

Adverse effect I (hypothalamic lesions produced by MSG)

Commercial protein hydrolyzates contain approximately 5 to 20 percent glutamic acid. However, there are no official specifications for commercial hydrolyzates, and the glutamic acid concentration may be much higher (1). As a result, safety considerations on the use of protein hydrolyzates are necessarily affected by those of glutamic acid. A more detailed treatment of the latter compound is provided in a separate report entitled, "Evaluation of
Aspects of Certain Glutamates as Food Ingredients, Supplemental Review and Evaluation," dated 1980 (13). This supplemental report covers the cogent points related to protein hydrolyzates.

The original account of neuronal damage in the hypothalamus induced by glutamate has been confirmed by independent investigators for neonatal mice (14,15), rats (16-18), hamsters (19,20), and guinea pigs (21), but not for monkeys (22-26).

The smallest effective oral dose of MSG for hypothalamic lesions in infant mice was found by one researcher to be 0.7 g MSG per kg body weight (27,28). No lesions were observed at 0.5 g per kg. Two other groups recorded 22 and 52 percent incidences, respectively, at 0.5 g per kg (24,7). The smallest effective subcutaneous doses for weanling and adult mice were determined as 0.7 g per kg and 1.2 g per kg, respectively (27,28). The smallest effective subcutaneous dose was determined to be 0.4 g per kg for 2-day-old rats, and 1.4 g per kg for 10-day-old rats (28).

Adverse effects were not noted under conditions of ad libitum feeding of diets containing added MSG or soy protein hydrolyzate. When mice and rats were fed a diet containing 2 percent MSG ad libitum during pregnancy and lactation, histological examination of the offspring at 0, 15, and 30 days showed no hypothalamic abnormalities (29). No necrosis of neurons was seen in the hypothalamus of young rats fed diets containing 6 percent (about 6 g per kg body weight) glutamate ad libitum for 80 days (30). Dietary administration of glutamate, with mean intakes as high as 7.2 g per kg per day, to mice up to 32 weeks, including in utero, did not give rise to hypothalamic lesions or significant differences over the controls in pathology or hyperplasia (31). MSG intake of dams increased during lactation to 25.1 g per kg per day maximum immediately postweaning, and was as high as 13 g per kg in pups. No hypothalamic lesions were detected when glutamate was administered ad libitum in the diet or drinking water at levels of 45.5 g per kg per day or 20.9 g per kg per day, respectively, to weanling mice (32).

No adverse effects were reported in weanling rats fed; for 13 weeks, a laboratory diet to which 5 percent of a commercial soy protein hydrolyzate (acid hydrolyzed) was added (33). Intake of protein hydrolyzate ranged from about 5 g per kg body weight initially to 1.6 g per kg at 13 weeks. Pathologic studies included microscopic examination of brain tissues. The dicarboxylic acid content reported for commercial soy protein hydrolyzates is greater than that for commercial casein hydrolyzates (1).

Plasma glutamate levels are affected by many exogenous and endogenous factors. Their association with neurotoxicity has been reported in many investigations. Forced feeding of 5 g L-glutamic acid per kg body weight to fasted rats significantly increased the glutamic acid in portal plasma (34). However, glutamate levels in
portal and systemic plasma of fasted rats were no higher after intubation of 5 g casein hydrolyzate per kg body weight than those observed after intubation of an equal quantity of casein (35).

The plasma glutamate levels in six fasted adults did not increase significantly after ingestion of water solutions providing 440 mg of a pancreatic hydrolyzate of casein and 60 mg glutamate per kg body weight (36). This suggested that the tolerance for a given dose of glutamate in a mixture of amino acids in a hydrolyzate was higher than that for an equal quantity of glutamate administered in relatively pure solutions that were used in most of the neonatal animal tests. Based on a long series of studies on the plasma levels of glutamate and aspartate after their administration to human infants in various enteral and parenteral feeding mixtures, the conclusion was reached that these amino acids were effectively metabolized, whether given as such, as peptides, or as proteins (37). No overloading occurred in the serum aminograms of glutamate and aspartate of 28- to 33-day-old healthy term infants, or in 11- to 35-day-old low-birth-weight infants, despite the high content of free glutamate in enzymatic casein hydrolyzate (Nutramigen® or modified Pregestimil®) fed enterally (38,39). Six sick infants were infused with an enzymatically digested casein solution (Amigen®) or an enzymatically digested beef fibrin solution (Aminosol®), which provided 0.2 g glutamate per kg and 0.05 g aspartate per kg. Their plasma glutamate and aspartate levels stayed within normal postprandial limits of enterally fed infants (40). Filer et al. (37) have cited such data as strong support for the safety of formulas containing protein hydrolyzates for human infant feeding, since no significant increase was observed in plasma glutamate or aspartate after enteral or parenteral feeding.

While data on plasma amino acid levels are of considerable value, the Select Committee does not believe they alone dissolve the challenge posed by the data on hypothalamic lesions. Even if the correlation between the two were quantitatively as well as qualitatively perfect, the former would at best constitute an indirect indication, rather than a direct measure, of toxicity.

Considerable assurance on the safety of protein hydrolyzates is derived from the experimental evidence that when glutamate was administered ad libitum at relatively high levels in the diets of rodents, it did not elicit adverse effects (26,29-32). The relation of these data to conditions of human ingestion is weakened somewhat, since the enteral feeding of casein hydrolyzate to infants cannot be regarded as exactly ad libitum. However, O'hara and Takasaki (41) reported no neuronal lesions in weanling mice administered by gavage 3 g MSG per kg body weight as a component of a synthetic diet in the form of a paste. This mode of administration would be more comparable to that for human infants under home and clinical feeding conditions.
Adverse effect II (retinal lesions induced by MSG)

Confirmation of retinal lesions induced by subcutaneous injections of large doses of MSG has been realized for neonatal mice (42-44), rats (45-47), and rabbits (48). Retinal damage in mice appears to be less sensitive to glutamate than do changes in the arcuate nucleus (27). Two g MSG per kg body weight were required to produce retinal damage in rats when introduced intra-peritoneally, as compared to 0.4 g per kg to produce hypothalamic damage. Olney (43) reported retinal damage in mice injected subcutaneously with 1 g MSG per kg body weight between the 9th and 10th postnatal days, but no damage when even larger doses were administered on subsequent days. Yonetani et al. (49) reported that no abnormalities were observed in the microscopic examination of retinas of F₁ generation mice fed diets providing 8 g MSG per kg body weight for 130 days after weaning. A similar diet had been fed to the parent generation.

No additional significant and relevant disclosures have come to the attention of the Select Committee. It is not possible to pinpoint the highest no-adverse-effect dose for retinal impairment. Nor does the Select Committee know of any comparable work that might have been done on protein hydrolyzates, so that safety extrapolations might be made with high confidence. Nevertheless, in assessing the implications of these findings to the real-life situation, the results of the enteral feeding of MSG as an ingredient of a diet appear to be most significant.

Adverse effect III (abnormalities in growth induced by MSG)

Confirmations of growth abnormalities with glutamate in relatively pure solutions have been announced by independent laboratories (28,50-55).

Speculations have been voiced that less than frankly neurotoxic doses in early development might result in functional abnormalities which manifest themselves, at maturity, in the form of behavioral abnormalities or endocrine dysfunction (56). Twelve-month-old rats given subneurotoxic doses of glutamate during the neonatal and infant stage showed no deterioration in activity, learning ability, corneal and pineal reflexes, or self-mutilation (57-59). Some differences were noted in positive control neonates given subcutaneous doses of 4 g MSG per kg body weight, but few differences were noted in those administered the same doses as infants. Delayed aberrations were not recorded even when significant amounts of glutamate were added to the diet, instead of being administered in relatively pure solutions. No obesity or alterations in reproductive patterns resulted from diets providing as high as 13 g MSG per kg body weight per day to mice as weanlings, and 25 g per kg during lactation (31). No adverse results regarding fertility, lactation, or viability were engendered by glutamate fed at 1 or 4 percent (up to 13 g per kg to weanlings) dietary levels through three generations of mice (31).
In extensive feeding studies, balanced mixtures of amino acids supplying total nitrogen requirements have demonstrated their capacity to support maintenance, growth, and reproduction in rats over several generations (60-62). Enzymatic casein digest was used for three decades as a source of nitrogen in the feeding of infants, and there were no clinical reports of delayed abnormalities (63). Donnell et al. (64) observed 15 children with galactosemia for periods up to 12 yr (mean 5 yr, 9 mo); 12 were given a casein hydrolyzate based formula (Nutramigen®) during infancy. Growth patterns compared favorably in both height and weight with those of normal children. The data indicated that early diagnosis and initiation of galactose-free diets resulted in most satisfactory mental development. In a review of galactose metabolism and galactosemia, Isselbacher (65) stated that maintenance of patients on formulas such as Nutramigen® was compatible with normal growth and development. None of the patients had mental retardation, cataract formation, liver disease, or other symptoms. A male 6-day-old infant received parenteral hyperalimentation (fibrin hydrolyzate) for 153 days after undergoing a massive small bowel resection (66). From the 60th postoperative day, he also received by mouth, formulas based on casein hydrolyzates or amino acid mixtures. At 2 years of age, the child's growth was retarded, but there was no evidence of gross mental retardation.

Formulas based on casein hydrolyzates also have been used successfully in the treatment of food allergies (67), and in the management of children with kwashiorkor, marasmus (68), and acute gastrointestinal disturbance (69). The same general absence of significant adverse effects appears to hold for parenteral experience with casein hydrolyzates in the hands of specialists trained in their use (70-72).

**Adverse effect IV (hypothalamic lesions induced by aspartate)**

Aspartic acid makes up about 3 to 10 percent of the weight of commercial protein hydrolyzates, as compared to about 5 to 20 percent for glutamic acid (1). Experimental confirmation of claims (7) of hypothalamic lesions resulting from oral intubation of aspartate has been reported by Okaniwa et al. (73). These investigators established the minimum lesion-producing dose (10 percent incidence in treated animals) of potassium aspartate in 1-week-old rats as 0.55 g per kg body weight, and in 3-week-old rats as 2.4 g per kg. No lesions were observed in 1-week-old rats after administration of three 0.50 g per kg doses in a 24 hour period, indicating this to be a maximum no-adverse-effect dosage. However, definitive studies on potential synergism between glutamate and aspartate have not been made to the best of our knowledge.

There are reasonable grounds to expect that the absorption and cellular uptake of aspartate parallels that of glutamate (74). If so, this means that the above observed effects on hypothalamic lesions would be attenuated when aspartate is fed in
food mixtures. This expectation is supported by experiments on protein hydrolyzates and on the methyl ester of aspartyl-phenylalanine (75,76).

Therefore, reported adverse effect IV may be considered essentially resolved. At the same time, it would be prudent to test this reasoned judgement with controlled experimentation, as has been done in the case of glutamic acid.

Adverse effect V (endocrine dysfunction induced by aspartate)

Observations of endocrine dysfunction induced by subcutaneous administration of multiple doses of sodium aspartate to neonatal mice (11) also have been made by another group of researchers (53); no other relevant publication has come to the attention of the Select Committee. However, the doses and conditions of the original experiment were such that high mortality was incurred among the animals. This would suggest that the other adverse effects observed should not be considered extrapolatable to the effect of aspartate as a constituent of casein hydrolyzate under conditions of normal ingestion.

Adverse effect VI (hypothalamic lesions induced by casein hydrolyzates)

Another laboratory published findings of some damage to the arcuate nucleus of 10-day-old mice after subcutaneous administration of 5 g per kg casein hydrolyzate, but none when distributed as five 1 g per kg doses over 8 hours (77). No other directly relevant observations have been reported. However, the Select Committee does not feel that the response to ingestion of protein hydrolyzates can be predicted from the results of subcutaneous administration experiments in view of the intervention of the intestines and the liver in absorption and metabolism of the component amino acids and peptides after ingestion. Subcutaneously injected casein hydrolyzates bypass the enteral-hepatic system and enter the circulation via lymphatic and/or vascular routes. The experimental conditions do not, therefore, represent real-life conditions of assimilation. Questions concerning the neurotoxic effects of the dicarboxylic amino acid components of casein hydrolyzates as they may be expressed under dietary and clinical conditions of expected use are discussed in the preceding sections.
III. OPINION

The average level of consumption of protein hydrolyzates for flavoring purposes is less than 3 mg per kg per day. Protein hydrolyzates are not used for flavoring purposes in commercially processed baby foods which formerly may have contained about 2 percent by weight. The Select Committee was unable to locate reports of experimentally demonstrable adverse effects of high concentrations of glutamate in dietary mixtures.

In light of the above, and assuming that appropriate product specifications are adopted, the Select Committee concludes that:

There is no evidence in the available information on acid hydrolyzed proteins, enzymatically hydrolyzed proteins, yeast autolysates, and soy sauces, that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used as flavoring agents at levels that are now current or that might reasonably be expected in the future.

The situation is different regarding the use of enzymatic casein hydrolyzates as nutrients. These hydrolyzates are consumed or administered in much higher doses, frequently as the sole source of dietary protein in products that are used as special dietary foods.

Decades of clinical experience have revealed no reports of untoward effects when casein hydrolyzates are administered orally in combination with other nutrients such as glucose. Adverse effects of the dicarboxylic amino acid components have been reported only in rodents under unusual conditions of administration (e.g., gavage or subcutaneous injection) and are not considered relevant to the use of casein hydrolyzates by humans.

The Select Committee therefore concludes that:

There is no evidence in the available information on enzymatically hydrolyzed casein that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used as a nutrient in special dietary foods at levels that are now current or that might reasonably be expected in the future.
IV. REFERENCES CITED


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