EVALUATION OF THE HEALTH ASPECTS
OF PEPTONES AS FOOD INGREDIENTS

1981

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

Contract No. FDA 223-78-2100
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Life Sciences Research Office
Federation of American Societies for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20814
NOTICE

This report, one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior-sanctioned food substances as food ingredients, is being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-78-2100 with the Food and Drug Administration (FDA), U.S. Department of Health and Human Services. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshaling 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 Dockets Management Branch, 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.

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 peptones as food ingredients. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (Dailey, 1978), which summarizes the world's scientific literature from 1920 through 1978. To ensure 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, an announcement was made in the Federal Register of April 21, 1981 (46 FR 22810-22814) 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 peptones as food ingredients. The Select Committee received no request for a hearing.

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 (Office of the Federal Register, 1980) [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 recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

The Select Committee on GRAS Substances of LSRO reviewed and evaluated the available information on peptones in full recognition of the foregoing provisions. In reaching its conclusions on safety, the Committee, in accordance with FDA's guidelines, relied primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. This report is intended for the use of FDA in determining the future status of these substances under the Federal Food, Drug, and Cosmetic Act. The Committee anticipates that its conclusions will be reviewed as new information becomes available.
II. BACKGROUND INFORMATION

Peptones were defined by Gortner (1929) as "The partial hydrolytic decomposition products of proteins. They are soluble in water, non-coagulable by heat and not precipitated by saturating the solutions with ammonium sulfate." Proteoses were differentiated from peptones by their precipitability with ammonium sulfate. The failure to precipitate with ammonium sulfate places a rough upper limit of a few thousand on the molecular weight of the polypeptides present in peptones, while the condition that the protein is only partially hydrolyzed implies that most of the protein is retained in polypeptide form rather than as free amino acids. The Select Committee considered a group of products identified as protein hydrolyzates in an earlier report (SCOGS, 1978a). These were defined by the statement: "Protein hydrolyzates represent a group of acid or enzymatically treated protein sources designed to provide a mixture of amino acids or amino acids and small peptides." The implication of this definition is complete or nearly complete hydrolysis as contrasted with partial hydrolysis in the case of peptones. Compositional data on the protein hydrolyzates (SCOGS, 1978a) indicated a relatively high degree of hydrolysis.

The United States Pharmacopeia (1975) describes meat peptone as a powder, soluble in water, containing 14.2–15.5% nitrogen corresponding to not less than 89% protein. No precipitate should form when a solution (1:20) is heated to boiling, and no more than a slight flocculent precipitate should result when 5 ml of solution (1 in 10) is mixed with 20 ml of zinc sulfate solution (50 g in 35 ml water).

Several letters have been issued by the FDA confirming the GRAS status of peptones. Cassidy (1961) approved beef peptone as GRAS for use in food. The request for approval described beef peptone as a product prepared by digesting acidified beef tissues (minced beef lungs) with pepsin (sliced hog stomach linings) at 122°F for 16 h, then separating and evaporating the supernatant to dryness (Lazo-Wasem, 1961). The product was described as containing 13% nitrogen, 4–8% ash, and 6% moisture.

Wulfsberg (1960) approved a request (Shapiro, 1960) for GRAS status of a peptone prepared from defatted fatty tissues obtained from the steam rendering of lard. This peptone was prepared by heating at 285°F for 3 h, separating the aqueous layer, acidifying with phosphoric acid to prevent bacterial growth during storage for 20 h, then boiling and adding slaked lime to precipitate phosphate as calcium phosphate. The solution was then evaporated to a paste and vacuum-dried. He stated that the peptone was used to improve the quality of the foam in beer. No compositional data were provided. However, data are available for a peptone currently manufactured by this procedure: nitrogen 16.29%, ash
3-4%, moisture 5-6% (Bard, 1980). The protein in fatty tissue is
considered to be mainly collagen; hence the protein content of peptone
powder would be about 90.4% (16.29 x 5.55) protein. Bard
(1980) stated that this peptone was used in industrial fermentation
media and as a protein supplement in animal feed preparations.

Peptones produced by the hydrolysis of food-grade gelatin
with papain were approved as GRAS (Cassidy, 1959). The proposed
use of these peptones was as an additive to beer at 125 ppm concen-
tration (Chase, 1959). Chemical analyses of the products were not
provided.

Several companies manufacture products marketed as, or
recognized as, peptones (Allen, 1980; Bard, 1980; Kullick, 1980;
Meyerson, 1980; Senti, 1980b). Principal intended use for these
products is as a nutrient in microbiological culture media,
although one product, a soy peptone, has found use as a foam sta-
bilizer in fermented beverages (Meyerson, 1980). Other products
are stated to have pharmaceutical, as well as microbiological,
applications. One company notes that its raw materials are all
food-grade or pharmaceutical-grade materials collected under U.S.
Department of Agriculture guidelines, and are handled and pro-
cessed according to FDA-approved good manufacturing practice
(Allen, 1980).

Some information on the methods of preparation, protein
source, amino acid composition, and extent of hydrolysis was pro-
vided in the manufacturers' technical specifications for their
products (Allen, 1980; Bard, 1980; Kullick, 1980; Meyerson, 1980;
Senti, 1980b). Of some 30 products identified as peptones, about
40% were derived from casein, 25% from animal tissues, 15% from
soy protein or meal, 10% from gelatin, and the remainder from
mixtures of these proteins. About 80% of the peptones were pre-
pared by hydrolysis with enzymes, and 10% with acid; the remainder
were identified as beef extracts (2 products) whose processing pro-
cedures were not specified, or as heat-hydrolyzed defatted fatty
tissue (1 product). The enzymes specified included trypsin, pep-
sin, pancreatin, and papain; however, for most products the enzyme
used was not identified.

A measure of the extent of hydrolysis in preparation of
the peptones is given by the ratio of free amino acid nitrogen to
total nitrogen. For 22 peptones prepared by enzymatic hydrolysis,
the distribution of values for this ratio was:

<table>
<thead>
<tr>
<th>No. of peptones</th>
<th>Free amino N/total N</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.40 - 0.49</td>
</tr>
<tr>
<td>10</td>
<td>0.30 - 0.39</td>
</tr>
<tr>
<td>1</td>
<td>0.20 - 0.29</td>
</tr>
<tr>
<td>5</td>
<td>0.10 - 0.19</td>
</tr>
<tr>
<td>2</td>
<td>0.01 - 0.09</td>
</tr>
</tbody>
</table>
A ratio, 0.76, was given for one of three products prepared by acid hydrolysis, indicating a considerably greater proportion of free amino acids and small peptides in this product than in those listed above.

For comparison, the ratio of free amino acids to total amino acids in commercial protein hydrolyzates used as flavoring agents ranged from 0.76-0.95 for nine products prepared by acid hydrolysis, and varied from 0.46-1.0 for six autolyzed yeast extracts (SCOCS, 1978a).

In addition to one product identified as a soy peptone, other enzyme-modified proteins not designated as peptones but having properties of peptones, as defined by Gortner (1929), are used in foods. These products are identified as soy albumens (Meyerson, 1980; Smith and Circle, 1972) or as enzyme-modified whipping proteins (Gunther, 1979; Meyerson, 1980). The latter appellation indicates the primary functional property of the product, i.e., whipping or foaming. Smith and Circle (1972) noted that the soy albumens are soluble in both acid and alkaline aqueous solutions, can be pasteurized without coagulation, and resemble peptones more nearly than albumens.

Gunther (1979) stated that enzyme-modified whipping proteins may be manufactured by treatment of oil-free soy flakes or flour, soy protein isolate or concentrate, or wheat gluten with pepsin, papain, ficin, trypsin, or bacterial proteases. Soy protein isolate and soy flakes appear to be the protein sources most frequently used, and pepsin the commonly used enzyme (Meyerson, 1980; Senti, 1980a). Free amino acid content of the products is no more than 2 or 3%; most of the protein is present as polypeptides 5,000-30,000 in molecular weight (Senti, 1980a).

The recommended usage and level of addition in foods of products sold as soy albumen, soy peptone, and whipping agent are given in Table 1 (Meyerson, 1980).
Table 1. Recommended Usage and Level of Addition of Peptone Products in Foods (Meyerson, 1980)

<table>
<thead>
<tr>
<th>Peptone product</th>
<th>Food</th>
<th>Function</th>
<th>Level of addition, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy albumen</td>
<td>Aerated confections</td>
<td>Whipping agent</td>
<td>0.25-1.00</td>
</tr>
<tr>
<td></td>
<td>Baked goods</td>
<td>Whipping agent &amp; texture modifiers</td>
<td>0.25-0.50</td>
</tr>
<tr>
<td>Soy peptone</td>
<td>Fermented beverages</td>
<td>Foam stabilizer</td>
<td>0.10-0.75</td>
</tr>
<tr>
<td>Whipping agent</td>
<td>Aerated confections</td>
<td>Whipping agent</td>
<td>0.25-0.75</td>
</tr>
<tr>
<td></td>
<td>Prepared mixes</td>
<td>Whipping agent</td>
<td>1.00-2.50</td>
</tr>
<tr>
<td></td>
<td>Non-alcoholic beverages</td>
<td>Foaming agent</td>
<td>0.25-0.50</td>
</tr>
<tr>
<td></td>
<td>Frozen desserts</td>
<td>Whipping agent &amp; stabilizer</td>
<td>0.20-0.30*</td>
</tr>
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</table>

*FDA approved use up to 0.60% in standardized and non-standardized frozen desserts; the limit approved in California is 0.50% in frozen yogurt products.
III. CONSUMER EXPOSURE DATA

The 1970 survey of the food industry conducted by the National Research Council (NRC) (Subcommittee on Review of the GRAS List--Phase II, 1972) reported 43,593 lb (19,800 kg) of peptones used as a processing aid in alcoholic beverages (presumably in their GRAS use as a foam stabilizer in beer). Weighted mean level of addition was 28 ppm. Based on the poundage data, the calculated daily per capita consumption in 1970 was 0.27 mg. Use as aerating and whipping agents was not reported in the 1970 survey. Peptones were not included among the substances reported in the survey of the food industry conducted by NRC in 1977 (Committee on GRAS List Survey--Phase III, 1979). However, the principal manufacturer of peptone products used in foods (Table 1) estimates that per capita consumption in this usage is less than 5 mg daily. It will be noted that Table 1 includes uses as aerating and whipping agents in addition to foam stabilization, explaining the larger per capita consumption reported in 1980.
IV. BIOLOGICAL STUDIES

The term peptone has now been abandoned by protein chemists in favor of precise definitions of the peptide chain length. However, the original designation of peptone would appear to be operationally useful in referring to a mixture of soluble peptides of varying chain lengths which contain minimal amounts of free amino acids. These "peptones," or oligopeptides, representing partially digested proteins, would normally be present in the gastrointestinal tract following ingestion of protein-containing foods.

Absorption and metabolism

Until quite recently, proteins were thought to be hydrolyzed in the intestinal lumen to free amino acids which were then absorbed by the mucosal cell. Van Slyke and Meyer (1913) showed a large increase of α-amino nitrogen in portal blood following protein ingestion in dogs. Dent and Schilling (1949) fed casein or hydrolyzed casein to dogs and found large increases in plasma amino acid content measured directly by paper chromatography.

However, more recent experiments have shown that peptides may not be completely hydrolyzed before or during absorption. Newey and Smyth (1959) and Peters and MacMahon (1970) found that the dipeptide glycyldglycine (Gly-Gly) introduced by intubation into the intestines of dogs and rats was present in plasma in addition to large amounts of glycine. Adibi (1971) demonstrated that Gly-Gly appeared in plasma of man during intestinal perfusion with the dipeptide.

Subsequent investigations during the past decade have greatly altered the perception of protein digestion and absorption. There is now substantial evidence that oligopeptides are absorbed intact from the mammalian intestinal lumen into the mucosal cell by specific transport mechanisms similar to those observed in bacteria (Matthews, 1975; Matthews and Adibi, 1976). The relative proportion of amino acids transported from the intestinal lumen in free form or as oligopeptides remains a controversial question at this time.

The studies conducted during the last 20-30 yr give ample evidence that the initial stages of protein digestion are extremely rapid. Within 1 h following a protein meal, the intestinal lumen contains a large amount of peptone-like material that is soluble in protein precipitants, is dialyzable, and consists of a mixture of peptides of about 5000 mean molecular weight (Nixon and Mawer, 1970).
In many instances, peptides appear to be transported from the intestinal lumen more rapidly than amino acids. Crampton et al. (1971) compared the rates of absorption of pancreatic hydrolyzates of several proteins with mixtures of free amino acids. The hydrolyzates prepared by digestion of protein in vitro with crude pancreatic enzymes consisted of two-thirds oligopeptides and one-third free amino acids. The mean chain length of the peptides was 2-3 amino acid residues for casein, serum albumin, and lactalbumin, and six residues for lysozyme. Absorption was measured by disappearance of α-amino nitrogen from tied rat jejunal loops in vivo. In all instances, absorption of pancreatic hydrolyzates was substantially greater than that of corresponding amino acid mixtures. After 10 min, the absorption of serum albumin and lactalbumin hydrolyzates was approximately twice that of the amino acid mixture.

Silk et al. (1973) conducted similar studies in humans by perfusing through jejunal loops a pancreatic hydrolyzate of casein and an amino acid mixture simulating the amino acid composition of casein. Total absorption of α-amino nitrogen was greater from the pancreatic hydrolyzate than from the amino acid mixture.

It has also been reported that a given quantity of amino acid could be absorbed more rapidly from the intestinal lumen when administered as a small peptide rather than in free form (Adibi and Phillips, 1968; Craft et al., 1968). Studies have been conducted with di- and tripeptides of glycine and with mixed peptides of glycine and leucine. Furthermore, when glycine is given together with Gly-Gly, the amount of glycine absorbed is the same as when it is given alone. Thus, there appear to be specific peptide transport sites that are independent of those for free amino acids.

The independence of peptide and amino acid uptake is also shown in the congenital intestinal amino acid transport defects of Hartnup disease and cystinuria. Patients with these diseases do not suffer from amino acid deficiencies because amino acids are absorbed in peptide form. In Hartnup disease (Asatoor et al., 1970), it was found that phenylalanine could not be absorbed from the intestinal lumen, but phenylalanyl-phenylalanine was; analogous findings were obtained in cystinuria with lysine and lysyl-lysine (Hellier et al., 1972).

Adibi and Morse (1977) concluded from perfusion studies with human jejunum that the largest homologous glycine oligopeptide which can be taken up by the intestinal mucosa is the tripeptide. The results of Smithson and Gray (1977) indicated that the tetrapeptide Gly-Leu-Gly-Gly was hydrolyzed on the cell surface of rat jejunum to its constituent amino acids and C-terminal dipeptide; these products were subsequently transported into the intestinal cells.
Since, as already indicated, protein products in portal blood are chiefly amino acids, peptides must be hydrolyzed within the mucosal cell. In the guinea pig, about 10% of dipeptidase activity was found in the brush border fraction and about 90% in the cytosol, whereas 20-75% of oligopeptidase activity was detected in the former fraction (Peters, 1972). Oligopeptidases that hydrolyze peptides with six amino acid residues are present in the brush border fraction.

There is evidence that some peptides can be absorbed directly into the portal blood. As mentioned earlier, Gly-Gly has been detected in blood plasma when infused in the intestine (Adibi, 1971). After gelatin feeding, peptides of hydroxyproline appear in peripheral plasma (Hueckel and Rogers, 1970). Both carnosine and anserine are absorbed by man from diets containing meat, and are excreted in the urine (Block et al., 1965). Thyrotropin-releasing factor, a tripeptide, and luteinizing hormone releasing-factor, a decapeptide, are active when given orally to rodents (Amoss et al., 1972 Vale et al., 1970).

Zioudrou et al. (1979) separated a peptide fraction from a pepsin hydrolyzate of wheat gluten that had opioid activity as demonstrated by naloxone-reversible inhibition of adenylate cyclase in homogenates of neuroblastoma X-glioma, NG 108-15, hybrid cells, naloxone-reversible inhibition of electrically stimulated contractions of the mouse vas deferens, and displacement of $[^3]H$ dihydromorphine in rat brain homogenates. A peptide fraction with similar, but less potent, properties in these bioassays was separated from a pepsin hydrolyzate of $\alpha$-casein. These fractions were not inactivated by treatment with trypsin; the casein peptides were completely inactivated by treatment with pronase while the gluten peptide fraction lost 70% of its activity by this treatment. Both fractions were partially inactivated by treatment with chymotrypsin, subtilisin, or thermolysin.

A second fraction separated from the pepsin hydrolyzate of wheat gluten stimulated both NG 108-15 adenylate cyclase activity and electrically induced contractions of the mouse vas deferens (Zioudrou et al., 1979). Adenylate cyclase stimulatory activity also was observed for pepsin hydrolyzates of proteins of cereal grains: zein (maize), hordein (barley), avenin (oats), and secalin (rye). Pepsin hydrolyzates of soy $\alpha$-protein, cytochrome, and $\alpha$-lactalbumin inhibited adenylate cyclase activity; this activity was not reversed by naloxone. Pepsin hydrolyzates of edestin, ovalbumin, and bovine $\alpha$-globulin were inactive in the adenylate cyclase bioassay. On the basis of these in vitro tests, Zioudrou, et al., (1979) speculated that biologically active peptides may be formed in the normal digestion of food proteins, absorbed in the blood, and reach opiate receptors in the central nervous system, triggering their functions. They also suggested that the stimulation of the adenylate cyclase of intestinal mucosa cells by peptides in gluten hydrolyzates would lead to diarrhea and could thereby play a role in celiac disease.
According to Matthews (1975), lipid-soluble peptides of any size, water-soluble peptides small enough to pass through aqueous pores, and peptides which fulfill the requirements for carrier transport by the intestine can be absorbed if they are relatively resistant to protease digestion. In peripheral blood plasma and in extracellular and intracellular fluids, it may be considered that there is a very small "pool" of peptides derived from breakdown of tissue proteins, estimated to account for no more than 1% of the total free amino acid pool (Matthews and Payne, 1975). Some of the peptides present in urine have been demonstrated to be of dietary origin (Hueckel and Rogers, 1970).

After absorption, the amino acids derived from protein hydrolyzates are transported to the liver and metabolized similarly to amino acids derived from other sources. 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 (Birnbaum et al., 1957; Greenstein et al., 1957; Schultze, 1957).

Acute toxicity

An LD$_{50}$ of 26.0 ± 1.6 g/kg body weight with death occurring within 4 h was reported for Wistar rats fed by intubation a casein hydrolyzate prepared by treatment of casein with pancreatin (Boyd et al., 1967). The extent of hydrolysis was not specified.

Short-term studies

Good growth has been reported in albino rats fed soybean and ground nut protein hydrolyzed by papain (Mital and Mathur, 1969). The ratio of amino nitrogen to total nitrogen in these hydrolyzates was 0.22. Normal growth and serum biochemical values were reported for 14 female infants fed a formula based on an enzymatically hydrolyzed casein in which the ratio of free to total amino acids was about 0.65 (Pomon and Filer, 1974). This formula also has been used successfully to meet the nitrogen requirements of patients with galactosemia (Donnell et al., 1961; Isselbacher, 1959).

No treatment-related changes in serum biochemical values or histopathology in any organ was reported in a 6-mo rat feeding study with fermented soy sauce solids (Ohshita et al., 1977). The protein source was a 1:1 mixture of soy grits and wheat. Proteolytic enzymes were produced by Aspergillus sojae or A. oryzae, and the ratio of amino nitrogen to total nitrogen was about 0.5. Groups of 30 STD-Wistar rats of each sex, 80-100 g initial body weight, were given diets containing 0.4, 1, 2, 5, or 10% soy sauce solids (about 0.3 to 7 g/kg body weight), or the basal diet containing equivalent levels of sodium chloride.
Long-term studies

An 18-mo mouse feeding study was conducted with fermented soy sauce solids prepared as described in the preceding paragraph (Ohshita et al., 1977). Groups of 40 weanling ICR-JCL mice of each sex were given diets containing 0, 0.11, 1.1, or 11.0% soy sauce solids, or the basal diet containing equivalent amounts of sodium chloride. No differences were observed in body weight gain, mortality, organ:body weight ratios, or histopathology of the treated animals as compared with the controls receiving sodium chloride. No evidence of carcinogenicity was observed.

Special studies

Hemmings et al. (1977) reported that as much as 40% of the radioactivity of \(^{131}\text{I}\)- or \(^{125}\text{I}\)-labeled \(\alpha\)-gliadin, ferritin, or IgG administered by stomach tube to adult Wistar rats was present in carcass tissues as tungstic acid precipitable material. They stated, but did not present supporting experimental data, that the antigenic content of the tissues precipitated by immune serum agreed fairly closely with the isotopic measure of proteinaceous material. In ultracentrifugal supernatants of brain tissue macerates of adult rats fed bovine IgG, Hemmings (1978) found that 57% of the radioactivity present was precipitable with immune serum. The whole brain content of protein-bound radioactivity (tungstic acid precipitable) was \(10^{-4}\%\) of the administered dose. Hemmings speculated that dietary protein is only partially degraded in the gut to small peptides and amino acids, and that a larger part is transmitted into the lymph and plasma as large peptides which enter tissue cells throughout the body where they are further degraded and digestion completed. It should be noted that these studies did not include control animals that were administered labeled iodinated tyrosine. However, Jones (1977) reported that moniodotyrosine was not significantly incorporated into carcass protein when fed to rats.

Dogs given peptones intravenously developed profound hypotension with sometimes fatal results (Csaba et al., 1963; Dragstedt and Mead, 1937; Dragstedt et al., 1938; Holmes et al., 1941; Mota et al., 1956; Pieroni and Levine, 1969). Other effects of peptone shock included the release of heparin and histamine with increased vascular permeability and splanchnic congestion. The peptone composition was not stated in most reports. However, Dragstedt et al. (1938) showed that a preparation having 42% of its nitrogen present in free amino acids produced a much more severe reaction than a preparation having 73% nitrogen in this form. Enzymatic hydrolyzates of casein, in which one-third of the amino acids were in peptide form with a mean chain length of three or four amino acid residues, gave no adverse reaction when given parenterally to dogs (Frazier et al., 1959). In man, formulas containing casein and
fibrin hydrolyzates have been infused intravenously during total parenteral nutrition without significant adverse effect (Filler et al., 1975). Enzymatic hydrolyzates used for this purpose were estimated to contain 55-70% of their nitrogen as free amino nitrogen (Ghadimi, 1975).

Olney et al. (1973) reported lesions in the hypothalamus of 10-d-old Webster Swiss mice given enzymatic casein hydrolyzates (nearly 50% free amino acids) subcutaneously at doses ranging from 1-5 g/kg body weight. The lesions were attributed to the content of free glutamic, aspartic, and cysteic acids, which other studies had shown to have brain-damaging properties. No lesions were observed after subcutaneous injection of amino acid mixtures not containing these acidic amino acids. Guzman et al. (1975) also observed damage to the arcuate nucleus of 10-d-old mice after subcutaneous injection of 5 g/kg of enzymatic casein hydrolyzate (62% free amino acids), but not when the same total dose was administered in five smaller doses (1 g/kg) over a period of 8 h.

Many feeding studies have been conducted with the sodium salt of glutamic acid, the major acidic amino acid present in protein hydrolyzates. No hypothalamic lesions were observed when rodents were fed monosodium glutamate ad libitum in the diet at levels up to 45.5 g/kg/d (Anantharaman, 1979; Heywood et al., 1977; Huang et al., 1976; Semprini et al., 1974). Based on studies of plasma levels of glutamate and aspartate after their administration to human infants in various enteral and parenteral feeding mixtures, (Filler et al. 1979) concluded that these amino acids were effectively metabolized, whether given as such, as peptides, or as proteins. These and other studies on the biological properties of monosodium glutamate, potassium aspartate, and protein hydrolyzates were reviewed by the Select Committee in previous reports (SCOGS, 1978a,b; 1980a,b). The Committee concluded that protein hydrolyzates used for flavoring purposes and enzymatic casein hydrolyzates used in special dietary foods posed no health hazards to the public.

One possible adverse effect of a substance that could be classified as a peptone has appeared. It has been widely confirmed that the manifestations of celiac disease, a syndrome occurring in both children and adults, is caused in some way by the normal protein dietary constituent, gliadin, which is present in wheat flours (Ezeoke et al., 1974; Falchuk et al., 1974; Hekkens et al., 1970; Hekkens et al., 1974; Kendall et al., 1972). The factor producing the symptoms is known to be present after digestion of gliadin by pepsin and trypsin, but toxicity is lost after complete hydrolysis or partial hydrolysis with 1 M HCl at 100°C for 45 min (Kasarda, 1975). It appears likely to be a polypeptide with a molecular weight less than 3000 that may represent a partial digestion product of gliadin. Its mechanism of action is uncertain. Experimental evidence does not support the hypothesis that the celiac
enterocyte lacks an enzyme concerned with the digestion of gluten (Kasarda, 1975). In any case, it appears that wheat gluten is not currently in use as a protein source for preparation of peptones.

Evaluation of the mutagenic activity of an acid hydrolyzed soy protein preparation (FDA 71-85) in a series of in vitro microbial assays demonstrated no increase in reversion frequency with Salmonella typhimurium strains TA-1535, -1537, and -1538 with and without metabolic activation (Litton Bionetics, Inc., 1974). Ratio of free amino nitrogen to total nitrogen in the hydrolyzate was not given. Teratologic evaluation of the same protein hydrolyzate revealed no effect on fetal development or on maternal and fetal survival in CD-1 mice fed up to 1.6 g/kg body weight and Wistar rats fed up to 1.4 g/kg body weight for 10 d beginning on day 6 of gestation (Morgareidge, 1973).
V. OPINION

The term "peptone" was the original designation given by protein chemists to partial hydrolytic decomposition products of proteins that are water soluble, non-coagulable by heat, and not precipitated in solutions saturated with ammonium sulfate. The definition and differentiation from protein products of other degrees of hydrolysis imply that peptones were considered to be a mixture of soluble peptides of varying chain lengths that contained minimal amounts of free amino acids. Another group of products identified as "protein hydrolyzates," also approved as GRAS food ingredients, was considered by the Select Committee in a previous report. Data supplied by manufacturers of peptones and protein hydrolyzates indicate that products marketed as peptones generally have a lower degree of hydrolysis than those marketed as protein hydrolyzates, although there is some overlap in the ranges encompassed by the two sets of products.

Peptones are prepared from casein, animal tissue, soy protein isolate, concentrate or meal, gelatin or defatted fatty tissue (mostly collagen) by hydrolysis catalyzed by trypsin, pepsin, pancreatin, papain, acid or heat. Most products identified as peptones are sold for use as nutrients in microbiological culture media, and the only food use reported for peptones in the 1970 NRC survey of the food processing industry was as a foam stabilizer in beer. Per capita daily usage in this application was 0.27 mg. However, other enzymatic protein hydrolyzates that would be included in the above definition of a peptone are used as food ingredients. These uses include that of whipping, aerating, or foaming agents in cakes, candies, confections, toppings, frozen desserts, and non-alcoholic beverages. Extent of current usage of peptones in food is estimated to be less than 5 mg/capita daily.

There is ample evidence that a distribution of oligopeptides and amino acids similar to that found in peptones may be formed in the gastrointestinal tract during the normal digestive process following the ingestion of protein. It is now known that oligopeptides, particularly di- and tripeptides, are directly absorbed into the intestinal mucosal cell and, in some instances, a small amount of peptide may enter the portal blood directly. As an example, peptides containing hydroxyproline are present in plasma and in urine following the ingestion of gelatin. Reports that pepsin-resistant peptides derived from food proteins may have opioid activity as indicated by in vitro techniques were not considered significant in the absence of in vivo evidence. The Select Committee has no evidence of toxic symptoms in healthy individuals associated with any peptide produced from food proteins. In patients with celiac disease, symptoms are produced by the ingestion of wheat gluten or peptic digests of gluten. However, no peptone products appear to be produced commercially from wheat gluten.
Because natural acid and enzymatic hydrolytic products of digestion are "peptones" and because commercial peptones prepared by mammalian digestive enzymes, papain, or heat should react similarly toxicologically, it is likely that they would not be associated with any toxicity if food-grade protein sources and good manufacturing practices were used in their preparation.

In light of the above considerations, the Select Committee concludes that:

There is no evidence in the available information on peptones that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used as food ingredients at levels that are now current or that might reasonably be expected in the future.
VI. REFERENCES CITED


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