EVALUATION OF THE HEALTH ASPECTS OF WHEAT GLUTEN, CORN GLUTEN, AND ZEIN AS FOOD INGREDIENTS

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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 wheat gluten, corn gluten, and zein as food ingredients. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (Rogers, 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 22810-11814) that opportunity would be provided for any interested persons 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 wheat gluten, corn gluten, and zein 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 prem­marketing 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 wheat gluten, corn gluten, and zein 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 this substance 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

Wheat gluten

The protein of wheat flour consists of about 10-15% water- or salt-soluble albumins and globulins; the remainder is difficultly soluble or insoluble gliadin and glutenin (Cluskey et al., 1961; Orth and Bushuk, 1972; Pence et al., 1954). The latter two protein components comprise the major proportion of wheat gluten. When flour is hydrated and mechanically worked, gluten forms as a cohesive, elastic mass which can be separated from starch and other flour components. Although many processes have been described for the separation of gluten, the Martin and the batter processes appear to be the principal ones used in the commercial production of gluten (Fellers, 1973).

In the Martin process, a dough is formed in a continuous mixer by the addition of water to flour in a ratio of 0.4-0.6 to 1 (Fellers, 1973). The dough is washed with water in a continuous kneader to remove starch and solubles, leaving a mass of wet gum gluten. In drying gluten care must be taken to retain the elastic properties that are important in breadmaking. Dry gluten in which the elastic properties are retained is known as vital gluten. Adding some dry gluten to the wet gum gluten allows the mixture to be broken into small particles; these are fed into a flash drier where the moisture is removed at relatively low temperatures. The elastic properties of gluten are also retained if it is spray-dried from approximately 10% dispersions in acetic acid, or in ammonium hydroxide at pH 9-10. Gluten to be used for purposes other than breadmaking, e.g., for preparation of protein hydrolyzates, may be dried at higher temperatures or under other conditions which destroy the elastic properties.

In the batter process, sufficient water is added to flour to form an elastic but free-flowing batter (Anderson, 1967). The batter is broken up mechanically in the presence of additional water to yield curds of gluten. The curds are recovered by screening, further washed to remove starch and solubles, and dried as described for the Martin process.

About 80% of flour protein is recovered as gluten from first and second clear flours, flours relatively high in ash commonly used for gluten production (Anderson, 1967). On a moisture-free basis, commercial gluten contains 70-85% protein, about 10% lipids, 3-5% starch, and 0-2% other polysaccharides (Simmonds and Orth, 1973). The product is essentially free of albumins and globulins.

Gluten contains about equal proportions of gliadin (soluble in 70% ethanol) and glutenin (insoluble in 70% ethanol) (Fellers and Mecham, 1974; Simmonds and Orth, 1973). The gliadins
can be divided into four groups (α, β, γ, and ω) based on mobility in starch gel electrophoresis (Patey, 1974). By combinations of ion-exchange chromatography and electrophoresis, as many as three α, four β, three γ, and six to eight ω components have been identified, each variety of flour having a characteristic set of components. The same variety of wheat grown in different parts of the world will produce identical proteins although not necessarily in the same proportions (Patey, 1974). The molecular weights of the α, β, and γ gliadins (each about 30% of the total gliadin mixture) are between 32,000 and 44,000. The ω gliadins have molecular weights about twice those of the other gliadins and are devoid of sulfur-containing amino acids (Patey, 1974). Glutenin appears to be more polydisperse; ultracentrifugal analysis indicated it to be a complex mixture of proteins ranging in molecular weight from about 25,000 to 2-3x10⁶ (Jones et al., 1961; Nielsen et al., 1962).

The amino acid composition reported for gluten separated from a hard wheat flour is given in Table 1 (Woychick et al., 1961). Most glutamic acid reported in gluten is present as glutamine, as indicated by the large amount of ammonia liberated in the hydrolysis of gluten.

Table 1. Amino Acid Composition of Wheat and Corn Glutens

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Wheat gluten* (%)</th>
<th>Corn gluten† (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>2.6</td>
<td>8.4</td>
</tr>
<tr>
<td>Arginine</td>
<td>2.6</td>
<td>3.1</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>3.2</td>
<td>5.9</td>
</tr>
<tr>
<td>Cystine</td>
<td>2.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>40.8</td>
<td>22.3</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.4</td>
<td>2.0</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Leucine</td>
<td>7.4</td>
<td>16.3</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.0</td>
<td>3.1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>5.4</td>
<td>6.1</td>
</tr>
<tr>
<td>Proline</td>
<td>15.0</td>
<td>8.9</td>
</tr>
<tr>
<td>Serine</td>
<td>5.7</td>
<td>5.0</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.7</td>
<td>3.2</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>1.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>4.2</td>
<td>4.7</td>
</tr>
<tr>
<td>Valine</td>
<td>4.5</td>
<td>4.4</td>
</tr>
<tr>
<td>Ammonia</td>
<td>5.6</td>
<td>---</td>
</tr>
</tbody>
</table>

* Calculated from analyses of Woychick et al. (1961).
† Reiners et al. (1973a).
Ponté et al. (1967) compared the composition of the fraction extracted from gluten with benzene-ethanol (50:50, v:v) with the corresponding fraction extracted from the parent flour. The flour contained 13.9% protein and 2.1% lipid compared with 78.8 and 9.5%, respectively, for gluten. Distribution of the principal components of the lipid fractions of flour and gluten was similar as determined by thin layer chromatography (Table 2).

Table 2. Lipid Distribution in Flour and Gluten (Ponté et al., 1967)

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Flour, %</th>
<th>Gluten, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polar lipids*</td>
<td>46</td>
<td>51</td>
</tr>
<tr>
<td>Diglycerides</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>37</td>
<td>36</td>
</tr>
<tr>
<td>Nonpolar lipids</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Phosphatides, glycolipids, monoglycerides.

Gallus and Jennings (1971) detected a number of low-molecular weight components separated by dialysis of a phenol-acetic acid solution of gluten against 10% acetic acid solution. Substances detected included amino acids, peptides, polysaccharides, lipids, reducing sugars, neoinositol, glycerol, inorganic phosphate, phenolic compounds, aromatic and heterocyclic compounds, and pigments.

The principal use of wheat gluten is as vital gluten in bakery products (Fellers and Mecham, 1974). It may be added to white bread formulas to improve dough-handling properties, crumb texture, and loaf volume, and to hamburger and hotdog buns to increase hinge strength. Crumb strength of breads that carry fruit, nuts, or other non-bakery substances is often increased by the addition of gluten. It is also used as a protein supplement and binder in such products as high-protein breakfast cereals, lunch meats, and meat and poultry rolls; as a texturizing protein and meat substitute in meat-like products; as a strengthening agent in pretzels, crackers, and macaroni; and as a protein source for the preparation of hydrolyzed vegetable protein.

The FDA considers gluten to be GRAS both as an ingredient in foods and as a source of hydrolyzed plant protein (Henry, 1978).
Corn gluten

Corn endosperm contains four classes of proteins which can be separated in sequential extraction as follows: albumins soluble in water, globulins in saline, zein in 70% ethanol, and glutelin in dilute alkali. These components make up about 4, 4, 47, and 39%, respectively, of corn endosperm protein (Paulis and Wall, 1969; Paulis et al., 1969; Reiners et al., 1973b). Zein is similar to the gliadin component of wheat gluten in that both are prolamine (Pomes, 1971). In the wet milling of corn for starch, zein and glutelin are recovered as components of the gluten fraction. Before it is milled, the corn is steeped in 0.1-0.2% SO₂ for 30-50 h (Watson, 1967). Fermentation of soluble sugars by lactobacilli yields lactic acid which reduces the pH to 3-4. Under these conditions, SO₂ cleaves some of the disulfide bonds in zein and glutelin. This disrupts the protein matrix surrounding the starch granules, and permits more complete starch separation in the milling operation. Separated starch and gluten are washed with water used in the steeping operation, extracting residual water-soluble proteins.

About 35% of the protein in corn is recovered in the gluten fraction (generally designated commercially as corn gluten meal) which has a protein content of 60-70% (Watson, 1967; Watson and Yahl, 1967). The protein consists of about 1% globulins, 68% zein, and 27% glutelin. Average protein content (N x 6.25) of 33 samples of corn gluten meal produced in three plants of one company was 61.9% (Reiners et al., 1973a). Other components reported were moisture, 10%; fat, 5.6%; fiber, 1.2%; and ash, 1.8%. Average amino acid composition of these samples is given in Table 1. Proximate analysis reported by Feldberg (1965) for a product identified as corn gluten and proposed for food use was moisture, 5-8%; protein, 70-73%; carbohydrate, 5-15%; crude fiber, 2-3%; ash, 1-2%; oil (ether extraction), 3-4%. Also present were the carotenoid pigments of the yellow corn used as the starting material.

The use of corn gluten as a food ingredient was approved by FDA in 1954 (Kneeland, 1954), and thus appears to be prior-sanctioned.

Little, if any, corn gluten appears to be used as such in food (Bell, 1980). It is reported to serve as a protein source for the production of hydrolyzed vegetable protein used as a flavoring agent (SCOGS, 1978a). Feldberg (1965) reported that corn gluten meal improved the color and textural properties of specialty breads and cakes when up to 3% of the flour content was added to baking formulations (about 2% in total product). However, the Select Committee has no information that corn gluten is currently used for this purpose.
Zein

Zein is produced commercially by extracting corn gluten at 60°C with 88% aqueous isopropyl alcohol containing 0.25% sodium hydroxide (Reiners et al., 1973b). The clarified extract is cooled to about −15°C causing the zein to precipitate. The zein is separated and dried on a vacuum drum dryer. This product contains about 3−4% oil and up to 500 mg/kg xanthophylls. A purer product, containing 1−2% oil and less xanthophylls, is prepared by redissolving and reprecipitating the zein. Before 1967, zein was prepared by a two-solvent process. Corn gluten was extracted with hot, aqueous 86% isopropyl alcohol, treated with sodium hydroxide, cooled, brought to pH 5.6 with acid, and filtered. An equal volume of hexane was added to the neutralized solution, forming two layers. The lower layer, containing zein, was pumped into chilled water precipitating the zein, which was then filtered and dried.

Boundy et al. (1967) showed that the steeping of corn in sulfur dioxide solution before wet milling reduced the disulfide sulfur content of its zein. They compared the disulfide content of unmodified zein extracted from cornmeal with 70% aqueous ethanol with that of commercial zeins (Table 3). The commercial zeins were extracted from corn gluten by the two-solvent process, with (G-200) and without (HV-9) the alkali treatment (Table 3). Both commercial gluten samples had much lower cystine content (determined as disulfide) than did unmodified zein. Neither commercial nor unmodified zeins had free sulfhydryl groups; however, the cysteine content of HV-9 determined as cysteic acid after performic acid oxidation was similar to that of unmodified zein, suggesting that cysteine in HV-9 was present as the S-sulfo derivative formed by reaction with sulfur dioxide. The investigators pointed out that the sulfur content of HV-9 not accounted for by methionine and cysteine was probably present in the S-sulfo cysteine. Amino acid analyses of the three zein samples after performic acid oxidation are given in Table 4. Lysine and tryptophan contents, not listed in Table 4, were reported by Paulis et al. (1969) to be 1 and 0 mmol/16g N for a sample of unmodified zein.

Boundy et al. (1967) suggested that the lower sulfur content of sample G-200 (Table 3) than that of the other zein samples resulted from the desulfurizing action of alkali on cystine and its derivatives, forming dehydroalanine as a predominant product. Dehydroalanine can react with cysteine to form lanthionine, or with lysine to form lysinoalanine. The authors considered the former the most likely product because of the low content of lysine in zein. No analyses for either compound were reported. If it is assumed, however, that the difference in total sulfur between the unmodified and the alkali-treated zein (0.07%) represented sulfur lost in the formation of dehydroalanine, and that all dehydroalanine formed reacted to form lanthionine and/or lysinoalanine, then the content of the latter compounds in the alkali-treated zein would be approximately 0.4%.
Table 3. Distribution of Sulfur in Different Zeins (Boundy et al., 1967)

<table>
<thead>
<tr>
<th>Composition</th>
<th>Unmodified</th>
<th>Commercial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% wt</td>
<td>% wt</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>14.3</td>
<td>15.8</td>
</tr>
<tr>
<td>Sulphydryl</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Disulfide sulfur</td>
<td>0.24</td>
<td>0.09</td>
</tr>
<tr>
<td>Cysteic acid sulfur</td>
<td>0.25</td>
<td>0.24</td>
</tr>
<tr>
<td>Methionine sulfur</td>
<td>0.37</td>
<td>0.36</td>
</tr>
<tr>
<td>Cystine and methionine sulfur</td>
<td>0.62</td>
<td>0.54</td>
</tr>
<tr>
<td>Total sulfur</td>
<td>0.59</td>
<td>0.66</td>
</tr>
<tr>
<td>Unaccounted-for sulfur</td>
<td>--</td>
<td>0.12</td>
</tr>
</tbody>
</table>

* Alkali-treated

Table 4. Amino Acid Composition of Performic Acid-Oxidized Zeins (Boundy et al., 1967)

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Unmodified</th>
<th>HV-9</th>
<th>G-200</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol/16g N</td>
<td>mmol/16g N</td>
<td>mmol/16g N</td>
</tr>
<tr>
<td>Cysteic acid</td>
<td>8.8</td>
<td>7.9</td>
<td>2.0</td>
</tr>
<tr>
<td>Methionine</td>
<td>13.2</td>
<td>9.4</td>
<td>14.9</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>42.2</td>
<td>45.7</td>
<td>46.9</td>
</tr>
<tr>
<td>Threonine</td>
<td>27.0</td>
<td>27.3</td>
<td>25.4</td>
</tr>
<tr>
<td>Serine</td>
<td>65.9</td>
<td>60.1</td>
<td>54.6</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>176.0</td>
<td>181.5</td>
<td>177.1</td>
</tr>
<tr>
<td>Proline</td>
<td>95.5</td>
<td>83.4</td>
<td>94.2</td>
</tr>
<tr>
<td>Glycine</td>
<td>18.5</td>
<td>16.6</td>
<td>17.5</td>
</tr>
<tr>
<td>Alanine</td>
<td>119.6</td>
<td>125.4</td>
<td>120.5</td>
</tr>
<tr>
<td>Valine</td>
<td>27.4</td>
<td>34.4</td>
<td>33.6</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>24.0</td>
<td>33.6</td>
<td>30.4</td>
</tr>
<tr>
<td>Leucine</td>
<td>156.9</td>
<td>170.6</td>
<td>169.9</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>44.7</td>
<td>47.1</td>
<td>54.0</td>
</tr>
<tr>
<td>Arginine</td>
<td>9.2</td>
<td>8.9</td>
<td>8.3</td>
</tr>
<tr>
<td>Ammonia</td>
<td>258.9</td>
<td>249.1</td>
<td>258.0</td>
</tr>
</tbody>
</table>
Electrophoresis of unmodified laboratory-prepared zein showed two major bands, two minor bands, and immobile material that remained at the origin of the starch gel plates (Boundy et al., 1967; Turner et al., 1965). Sulfite-reduced zein also migrated as four bands in starch gel electrophoresis but at different rates; no material remained at the origin. Commercial alkali-treated zein showed two major components and a diffuse trailing band. The molecular weight of unmodified zein as determined by ultracentrifugal analysis was 44,000; after reduction and alkylation, the molecular weight was approximately 21,000 (Turner et al., 1965).

Zein is considered GRAS as a food ingredient when used in accordance with good manufacturing practice (Spiher, 1960; Wulfsberg, 1960).
III. CONSUMER EXPOSURE DATA

Neither wheat gluten nor corn gluten was included in surveys conducted by the National Research Council on the levels of addition and poundage of GRAS food ingredients used by the food industry (Subcommittee on Review of the GRAS List--Phase II, 1972; Committee on GRAS List Survey--Phase III, 1979). A recent study conducted by Mittleider et al. (1978) indicates that 96 million lb (43.5 million kg) of wheat gluten were marketed in the United States in 1977. This included domestic production of 43 million lb (19.5 million kg) and imports of 53 million lb (24 million kg). Information on the relative proportions used for direct food use and for production of protein hydrolyzates was not available to the Select Committee. If one assumes that all was consumed as such in food, per capita daily consumption would have been 0.6 g. This per capita disappearance may be compared with the quantity of gluten consumed as a component of products made from wheat flour. Annual per capita consumption of wheat flour in 1977 was 116 lb (U.S. Department of Agriculture, 1980). If the average protein content is 12% and if 80% of the protein is gluten, then the daily per capita consumption of gluten from this source would be 14 g, more than 20 times that of gluten added to food.

As noted in the Background section, the principal use of corn gluten in food is as a protein source for the production of hydrolyzed vegetable protein. No information was available to the Select Committee on the quantities so used.

The 1977 NRC survey of industry (Committee on GRAS List Survey--Phase III, 1979) on the use of food additives reported that 3000 lb (1360 kg) of zein were used in processed food products in 1976. This corresponds to a per capita daily disappearance of 20 μg. The principal use in foods appears to be as a rinse-resistant coating on enriched rice where it is applied at a level of 0.6%.

Zein is also used in the pharmaceutical industry in the manufacture of tablets as a sealant, granulating agent, coating polymer, and a sustained-release coating material (Minehan, 1980). In foods, it is used in the seal coating of confectionery pieces, nuts, grains, and other food items, where a moisture barrier or glaze is desired.
IV. BIOLOGICAL STUDIES

Absorption and metabolism

Rats (Radke et al., 1969) and pigs (Babcock and Markley, 1967) demonstrated better utilization of nitrogen from a mixture of casein and lactalbumin than from wheat gluten when each protein was adjusted to the FAO amino acid pattern (estimated human requirement for essential amino acids, mg/g dietary nitrogen) by addition of crystalline amino acids. The diets were fed to rats at 1.2 and 1.6% nitrogen and to pigs at 2% nitrogen. Cook et al. (1971) demonstrated that the ratio of increase in nitrogen content of carcass to nitrogen intake was greater when wheat gluten, adjusted to the FAO pattern, was fed to weanling CFN (Carworth Farms) rats at 2.4% nitrogen in the diet than when it was fed at 1.2%, or at several levels greater than 2.4%. When wheat gluten and casein-lactalbumin, each adjusted with amino acids to the FAO pattern, were fed for 8 wk at a level of 2.4% nitrogen, nitrogen utilization was similar to that obtained with an amino acid mixture in the FAO pattern.

Hepburn et al. (1966) reported high availability for rats of methionine, threonine, tryptophan, valine, phenylalanine, leucine, and isoleucine in wheat gluten. Gluten was added to amino acid basal diets limiting in the respective amino acid under test to provide that amino acid in incremental amounts, and bioavailability was judged by the increase in carcass nitrogen per unit increment.

Ten adult male white rats were fed a diet with casein as the protein source; two or three metabolic balance studies of 5-7 d duration were carried out with each rat (Ribeiro et al., 1957). The rats were then fed the same diet except with 2% gluten replacing an equivalent quantity of casein. Rats fed the gluten-containing diet consumed significantly less food and excreted more fat than did rats fed the gluten-free diet.

Acute toxicity

No reports of acute toxicity studies with wheat gluten, corn gluten or zein have come to the attention of the Select Committee.

Short-term studies

A number of studies have been reported in which animals have been fed wheat gluten, corn gluten, or zein as the sole source of protein (Rogers, 1978). Because these proteins are known to be deficient in certain essential amino acids, the poor
performance of the animals is considered to have little relevance to evaluation of the proteins as food ingredients in human diets. Feeding studies in which animals have been given wheat gluten plus appropriate amino acid supplementation have already been mentioned.

Human subjects fed wheat gluten. Three Agricultural Experiment Stations collaborated in comparing the effects on university students of consuming low-protein diets (0.4 or 0.5 g/kg body weight): an amino acid mixture in the FAO pattern; casein-lactalbumin adjusted to the FAO pattern with the addition of amino acids; or wheat gluten adjusted to the FAO pattern (Morse et al., 1969). In each study either casein-lactalbumin or wheat gluten (each adjusted with amino acids) was compared with the amino acid mixture in a balanced, cross-over design; each nitrogen source was fed for 10 d. The investigation suggested that wheat gluten adjusted to the FAO pattern by amino acid supplementation was somewhat less effective in promoting nitrogen retention than was an amino acid-supplemented casein-lactalbumin mixture or a mixture of amino acids in the FAO pattern.

Gormican et al. (1967a,b) studied two healthy men in a metabolic ward for 5 mo while they received 51 g/d of gluten in the form of cereal products and desserts. The diet provided approximately 19% of energy from protein and 40% from fat (114-160 g/d); energy was sufficient to maintain body weight. A fat tolerance test was carried out monthly. Fat excretion in all instances was less than 3% of intake. Although some changes in serum concentrations of lipids were observed over the period of study, it is impossible to determine whether such changes were related to the gluten content of the diet without control data.

Levine et al. (1966) carried out 34 studies with 26 subjects—13 healthy, young, male volunteers and 13 convalescent patients with normal jejunal mucosa and no evidence of malabsorption. A nutritionally complete diet was fed in a metabolic ward with 4-7 menus of equivalent nutrient composition rotated on a daily basis and consumed completely. After a 2-wk control period, all diets were supplemented for 8 wk with wheat gluten (100 or 150 g/d), gliadin, powdered dry egg white, or glucose, followed by a 2-wk recovery period. The 26 subjects were assigned to 6 groups based on clinical diagnosis (normal or convalescent) and type of supplement. No significant differences related to the type of supplement were demonstrated in the following parameters: fecal weight or nitrogen content; urinary excretion of 5-hydroxyindoleacetic acid; plasma or serum concentrations of non-esterified fatty acids, total protein, albumin, total glycerides, lipid phosphorus, or calcium; blood concentration of glucose; glucose tolerance test, prothrombin time, hematocrit, leukocyte and differential blood counts, and roentgenographic studies of the gastrointestinal tract. Serial intestinal mucosal biopsies demonstrated no abnormality. Among the healthy volunteers there was no significant difference
in fecal excretion of fat between those receiving the gluten supplement and those receiving the egg white supplement. In the convalescent patients given the gluten supplement, fecal excretion of fat increased significantly from a mean value of 3.5 g/d during the control period to 7.4 g/d. The increase in fat excretion (from 2.9-4.7 g/d) by convalescent patients fed the egg white supplement was not significant. Serially performed gel-diffusion tests failed to demonstrate humoral antibodies in normal subjects. The investigators concluded that dietary supplementation with as much as 150 g of gluten daily did not induce acute tissue response or circulating antibodies to gluten, suggesting that an intact intestinal mucosa prevents sensitization to dietary proteins.

No studies have been located in which human subjects have been fed corn gluten or zein supplemented with appropriate amino acids or a nutritionally adequate diet containing a specified quantity of corn gluten or zein.

Long-term studies

No reports of long-term animal or human studies were available on the feeding of wheat gluten, corn gluten, or zein.

Special studies

Celiac disease. Celiac disease, also termed celiac sprue, gluten-induced enteropathy, and nontropical sprue, is a disorder in which consumption of gluten from wheat, rye, or perhaps oats and barley, is associated with atrophy of the intestinal villi and malabsorption of dietary components. There are no reliable figures for incidence of celiac disease in North America, but there is a general impression that it is less common in North America than in Western Europe (Dennis, 1978) and perhaps less in the United States than in Canada (Roy et al., 1975). Incidence in England, Wales, and Scotland appears to be in the range of 1 in 2000-8000 (Black, 1964) although in various regions of Ireland, Austria, and Switzerland an incidence greater than 1 in 1000 has been reported (Dennis, 1978). It is rare among blacks and persons of Jewish and Mediterranean ancestry (Spiro, 1977). Commonly, the patient with celiac disease has a close relative with the disorder. The histocompatibility antigen, HLA-8, is four times as common in patients with celiac disease than in the normal population, a phenomenon suggesting that celiac disease may be the result of an abnormal "immune response gene" which leads either to production of anti-gluten antibody or an abnormal binding of gluten to the epithelial cells (Spiro, 1977).

Much work has been done in attempting to identify the component of wheat responsible for provoking the manifestations of celiac disease in susceptible individuals (Kasarda, 1975). The
provocative factor is known to be present in gliadin and is not destroyed by digestion with pepsin and trypsin. Complete hydrolysis of gliadin or partial hydrolysis with 1 M HCl for 45 min at 100°C renders it harmless. Several studies with enzymatic digests suggest that the provocative factor is probably a peptide with a molecular weight of 3000 or less. Intact α-gliadin also produces characteristic toxic effects when instilled directly into the jejunum of celiac patients and in tissue cultures from celiac patients, suggesting that portions of α-gliadin molecules contain sequences of amino acids responsible for the toxic effect.

In most instances the elimination of gluten from the diet of a patient with celiac disease produces a feeling of well-being within a few days, although restoration of the normal villous structure of the intestinal mucosa may require months (Spiro, 1977).

Improvement in the intestinal lesions of some patients with dermatitis herpetiformis after removal of gluten from the diet may be explained by the coexistence of celiac disease in these patients (Weinstein et al., 1971).

Neither corn gluten nor zein has been implicated as a factor in the pathogenesis or aggravation of celiac disease. In fact, corn flour and cornmeal are prominent components of substitutes for wheat flour used in management of patients with celiac disease (Ohlson, 1972).

Mental disorders. Dohan (1978) has reviewed circumstantial evidence in support of the hypothesis that one or more components of cereal grains (and possibly other foods) are the major environmental factors that provoke schizophrenia in those with hereditary susceptibility. Celiac disease appears to be more common in the medical histories of patients with schizophrenia than in the general population (Dohan, 1970; Graff and Handford, 1961). A highly significant positive correlation was reported between the number of first admissions for schizophrenia and per capita consumption of wheat and rye during World War II (Dohan, 1966). Other epidemiologic evidence suggests that societies consuming only small amounts of wheat and rye demonstrate a lower incidence of schizophrenia than do societies with large consumptions (Dohan, 1966).

Two studies were interpreted as demonstrating more rapid improvement in patients with schizophrenia when they consumed a grain-free, milk-free diet than when they consumed a high-cereal diet with milk (Dohan et al., 1969; Dohan and Grasberger, 1973). This difference in rate of improvement was not noted in patients receiving the grain-free, milk-free diet to which wheat gluten was added. Other investigators reported that in patients with schizophrenia managed with a grain-free, milk-free diet and neuroleptic
medications, interruption in improvement was noted during the course of a "blind" challenge with wheat gluten (Singh and Kay, 1976a). The conclusion of these investigators has been questioned (Anonymous, 1976; Levy and Weinreb, 1976; Smith, 1976), and a rebuttal has been offered (Singh and Kay, 1976b). All parties appear to be in agreement that further controlled trials are needed.

Two sets of experiments may be relevant to consideration of the possible adverse effects of wheat gluten in patients with mental disorders (Freed et al., 1978; Zioudrou et al., 1979). Freed et al. (1978) reported that gastric intubation with wheat gluten 20 min before oral administration of tritiated haloperidol (a neuroleptic agent used in treatment of schizophrenia) resulted in less radioactivity in plasma and in brain of mice than was observed after a similar pretreatment with soy flour. The relevance of this study to the apparent adverse effect of high-cereal diets in the management of schizophrenia was questioned on several grounds, including the high doses of wheat gluten given to the mice (2 g/kg), the administration of only a single oral dose, and the failure to determine the area under the curve (Dohan, 1979; Singh, 1979).

More recently, Zioudrou et al. (1979) have demonstrated that certain peptides derived from pepsin hydrolyzates of wheat gluten demonstrate naloxone-reversible inhibition of (1) adenylate cyclase in homogenates of neuroblastoma X-glioma hybrid cells and (2) electrically stimulated contractions of the mouse vas deferens. Because of the morphine-like effects of these substances of exogenous origin, they have been termed exorphins. These exorphins were demonstrated to compete with morphine for binding to opiate receptors in rat brain homogenates.

S-sulfocysteine. As mentioned in the Background section, the steeping of corn in sulfur dioxide solution before wet milling appears to result in the formation of the S-sulfo derivative of cysteine in the proteins of the gluten component. Rat liver enzymes have been shown to catalyze the conversion of S-sulfocysteine to thiosulfate, pyruvic acid, and ammonia (Sörbo, 1958), and, in the presence of α-ketoglutarate (or oxaloacetate), to glutamate (or aspartate), pyruvate, and thiosulfate (Coletta et al., 1961). Increased urinary excretion of thiosulfate was observed in rats fed S-sulfocysteine (De Marco et al., 1960). The health aspects of thiosulfate as a food ingredient have been evaluated by the Select Committee (SCOGS, 1975); no information indicating potential adverse effects has come to the attention of the Select Committee since that evaluation. Olney et al. (1975) have reported that lesions develop in the retinas and arcuate nuclei after subcutaneous administration of free S-sulfocysteine to 5-day-old rats in doses of 80-800 mg/kg body wt, but not 8 mg/kg. Similar lesions also developed in 10-day-old mice after subcutaneous administration of 120 mg/kg of free L-cysteine (Olney et al., 1971). No feeding
studies of sulfurous acid-treated protein in which the experimental animals were examined for neurotoxic effects have come to the attention of the Select Committee; however, no evidence has been presented that ingestion of food proteins containing the amino acids cysteine, glutamic acid, and aspartic acid residues, all demonstrated to cause brain lesions if administered in free form by gavage to rodents, have similar toxic effects (SCOGS, 1978a,b; 1980a,b).

Other studies. No studies of carcinogenesis, mutagenesis, reproductive performance, teratogenesis, or fetotoxicity of wheat gluten, corn gluten, or zein have come to the attention of the Select Committee.
V. OPINION

The principal use of wheat gluten is as vital gluten in bakery products although it is also used as a protein supplement, binder, texturizing agent, or strengthening agent in other food products. The per capita intake of wheat gluten added to foods is estimated to be less than 0.6 g/d whereas about 14 g/d are consumed from wheat flour. The principal and perhaps only use of corn gluten in food is as a protein source for production of hydrolyzed vegetable protein. No information is available to the Select Committee on the quantities so used. The principal use of zein in foods appears to be as a rinse-resistant coating on enriched rice. The per capita disappearance is about 20 μg/d.

Commercial wheat gluten, dry basis, contains about 70-85% protein, 10% lipids, 3-5% starch, and 0-2% other polysaccharides. An analysis of corn gluten proposed for use as a food ingredient was protein, 70-73%; carbohydrate, 5-15%; crude fiber, 2-3%; ash, 1-2%; oil, 3-4%; and moisture, 3-4%. Zein is the alcohol-soluble component of corn gluten comprising about 70% of the protein of gluten. Amino acid analyses and animal feeding studies indicate that these protein products are deficient in certain essential amino acids and support poor growth when used as the sole source of dietary protein. However, these deficiencies are of little consequence in view of the relatively small contribution of these products to the per capita intake of protein from all sources.

Studies of human adults have suggested that wheat gluten supplemented with amino acids to conform to the FAO pattern promoted somewhat less retention of nitrogen than did a casein-lactalbumin-amino acid mixture adjusted to the FAO pattern. Metabolic studies of adults fed gluten daily as part of the diet in the form of bakery goods demonstrated normal absorption of fat. A study of normal and convalescent patients fed similar diets supplemented with 100-150 g/d of wheat gluten revealed no adverse effects. Similar studies with corn gluten or zein do not appear to have been carried out.

Patients with celiac disease, an uncommon disorder in North America, are intolerant to gluten of wheat and rye but not to corn gluten or zein. Some authors have suggested that consumption of gluten may be a factor in pathogenesis of schizophrenia but further study will be necessary before placing much weight on such a relationship.

The steeping of corn gluten in sulfur dioxide solution before wet milling appears to result in the formation of S-sulfo-cysteine as a component of the proteins of corn gluten. The available evidence indicates that the metabolic products of S-sulfo-cysteine are also metabolites of common dietary constituents.
Subcutaneous administration of free S-sulfocysteine to young rats has been demonstrated to cause neuronal lesions. Similar lesions resulted from subcutaneous injection or gavage feeding of free L-cysteine. The Select Committee is aware of no evidence that either L-cysteine or its S-sulfo derivative cause adverse effects when ingested in protein-bound form.

Because the amount of wheat gluten, corn gluten, and zein added to foods is small in relation to the amount consumed as natural components of wheat and corn, the Select Committee in this instance did not place much toxicological significance on the absence of studies of carcinogenesis, mutagenesis, reproductive performance, teratogenesis, or fetotoxicity.

The Select Committee has weighed the available evidence and concludes that:

There is no evidence in the available information on wheat gluten, corn gluten, or zein that demonstrates, or suggests reasonable grounds to suspect, a hazard when they are used as food ingredients in the manner now practiced or that might reasonably be expected in the future.
VI. REFERENCES CITED


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