EVALUATION OF THE HEALTH ASPECTS OF COLLAGEN
AS A FOOD INGREDIENT

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
EVALUATION OF THE HEALTH ASPECTS OF COLLAGEN
AS A FOOD INGREDIENT

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

Life Sciences Research Office
Federation of American Societies
for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014
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
## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>II. Background information</td>
<td>2</td>
</tr>
<tr>
<td>III. Consumer exposure data</td>
<td>8</td>
</tr>
<tr>
<td>IV. Biological studies</td>
<td>9</td>
</tr>
<tr>
<td>V. Opinion</td>
<td>13</td>
</tr>
<tr>
<td>VI. References cited</td>
<td>15</td>
</tr>
<tr>
<td>VII. Scientists contributing to this report</td>
<td>22</td>
</tr>
</tbody>
</table>
I. INTRODUCTION

This report concerns the health aspects of using collagen as a food ingredient. 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 November 7, 1980 (45 FR 74056) 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 collagen as a food ingredient. The Select Committee received no request for such 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 collagen 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

Collagen is the most abundant of all proteins of higher vertebrates, comprising more than one-third of body protein. Skin, tendons, ligaments, bone, and cornea are particularly rich in collagen. Its amino acid composition is distinctive, generally containing about 35% glycine, 11% alanine, 12% proline, and 9% hydroxyproline (Lehninger, 1975). Hydroxylysine and only a small amount of tyrosine are present, while tryptophan and cystine are absent (Montgomery et al., 1977).

Collagen fibrils are arranged in various structures in relation to biologic function; e.g., fibrils of tendons are arranged in parallel bundles which provide strength but resist stretching, whereas fibrils in cowhide form an interlacing network laid down in sheets (Lehninger, 1975).

The collagen molecule (tropocollagen) consists of three similar polypeptide chains, termed $\alpha$-chains, wound around each other to form a three-strand rope (Lehninger, 1975; Miller, 1976). Each polypeptide chain is a left-handed helix with approximately three residues per turn. The molecule is about 15 Å in diameter and 2800 Å in length. Terminal amino acid sequences of $\alpha$-chains do not have the triple-chain helix structure and appear to be the site of interchain crosslinks in certain types of collagen and contain major antigenic determinants in others (Piez, 1976).

Collagen fibrils are made up of tropocollagen units assembled in a highly ordered array as evidenced by the regularly banded patterns in electron micrographs of fibrils (Stimler and Tanzer, 1977). The three-dimensional structure of the fibrils is stabilized by intermolecular crosslinks. Some of these crosslinks, however, are relatively unstable to non-physiologic conditions. Fibrils are about 50-1000 Å in diameter and may be up to 2 mm in length as found in dehydrated cowhide (Karmas, 1974). Bundles of collagen fibrils constitute fibers in hide that may be 20 μm or more in diameter.

When collagen fibers are treated with dilute acid, they dissociate into fibrils and go into solution (Harrington, 1958; Noda and Wyckoff, 1951). The fibrils can be precipitated by changes in pH or salt concentration. Electron microscopic examination of fibrils precipitated from 1% sodium chloride show the cross-striated pattern characteristic of the native fibrils. Fibrils precipitated with other salts may have different structural patterns. Electron micrographs of regenerated collagen sausage casings reveal the presence of fibrils having a cross-striated structure (Lieberman, 1964). If collagen fibers are heated in water above a specific temperature (about 67°C for calfskin collagen) (Gustavsson, 1960), they undergo a sharp contraction to less than one-third their length. In this process, many of the physical
properties of the native fiber are lost, including the characteristic x-ray diffraction pattern and resistance to trypsin digestion. This thermal shrinkage temperature is associated with the transformation of collagen to gelatin in which the helical conformation of polypeptide chains in the tropocollagen molecules is destroyed and converted to a randomly coiled configuration. Polypeptide chain hydrolysis, in addition to conformational change, occurs in the production of commercial gelatin (SCOGS, 1975f).

Food-grade native collagen is prepared from hides of animals slaughtered for human consumption. Bovine tendon has been reported in the patent literature (Lieberman, 1967) as a source of collagen for the manufacture of sausage casing, but appears not to be a current source (Devro, Inc., 1977). Food-grade collagen must come from inspected slaughter, and identity with acceptable carcasses must be established (Whitmore et al., 1970).

Animal hides consist of three principal layers: the epidermis, or outer layer, containing hair follicles; the corium comprising the bulk of the hide and consisting mainly of bundles of collagen fibers; and the flesh or connective tissue layer comprising the inner layer of the hide (O'Flaherty and Stubbings, 1967). Fresh hide contains about 30-35% proteins, about 2% lipids, 0.5% carbohydrates, and 1% mineral salts and other substances such as pigments. The solids in fresh hides are made up of 90-95% proteins, both fibrous and nonfibrous. Of the fibrous proteins, collagen accounts for over 85% of the corium; elastin is present in small quantities. The nonfibrous proteins are albumins, globulins, and mucins, mucoids, or glycoproteins (Nayudamma, 1974). In the production of collagen, the corium is separated from the other two layers by mechanical means, usually after chemical pretreatment. Processing chemicals are subsequently neutralized and removed by washing, resulting in a product that is principally collagen (Karmas, 1974).

Two principal processes for producing a purified corium layer are described in the patent literature as a first step in the production of reconstituted collagen sausage casing (Karmas, 1974). This purified corium is identified as food-grade collagen (Devro, Inc., 1977). In one process, hides fresh from the slaughter house are washed, and then soaked for several days in a dilute aqueous solution of a weak acid, such as acetic, lactic, formic, or citric (e.g., Fagan and Lieberman, 1965). After the acid treatment, the epidermal and flesh layers are removed mechanically and the resultant layer of corium is neutralized by treatment with sodium bicarbonate or ammonia solution, followed by washing to remove residual salts.

In a second process, a corium layer is separated mechanically from fresh or salt-cured hides which have been treated with lime to assist in the removal of hair. Adjuncts in the lime solution may include sodium sulfhydrate and dimethylamine sulfate. The product, identified commercially as "limed splits," is washed,
then treated with an acid to neutralize the excess lime and permit its removal by washing (e.g., Cohly, 1968; Talty and Cohly, 1968).

Whitmore et al. (1970) described another process for the preparation of food-grade collagen from limed splits. After washing, the splits (corium layer) were treated with ammonium chloride and a mixture of lactic and sulfuric acids and then washed, resulting in a collagen product with pH between 4.5 and 6.8. Composition of collagen prepared by this method, dried by lyophilization and ground, was 76.2% collagen (calculated from nitrogen concentration determined by Kjeldahl analysis), 10.8% moisture, 9.8% fat, 0.7% fiber, and 0.4% ash.

Specifications for food-grade native collagen do not appear in Food Chemicals Codex (National Research Council, 1972). Proposed specifications for food-grade collagen require that solids comprise 25-35%, and that on a dry-weight basis the material be not less than 70% collagen and not more than 20% fat (Devro, Inc., 1977).

In the manufacture of regenerated collagen sausage casings, purified corium, such as that described above, is treated with solutions of weak organic acids, e.g., acetic, lactic, citric, or dilute mineral acid (Karmas, 1974), or carbon dioxide under pressure (Froehlich, 1979) to disperse the collagen fibers and produce a homogeneous solution of swollen collagen fibrils. The collagen fibril solution is extruded through an annular orifice into a coagulating medium to produce a tubular body of regenerated collagen, which may be subsequently treated with tanning agents, plasticizers, and other substances that become part of the finished casing. The coagulating medium described in several patents is a concentrated solution of ammonium sulfate (Karmas, 1974). Tannings agents described include alum [NH₄Al(SO₄)₂·24H₂O], ferric salts (sulfate, nitrate, ammonium sulfate, halides), ammonium hydroxide, glutaraldehyde, glutaraldehyde-acid phosphate, and basic aluminum citrate complex. Tanning agents are applied by passing the casing through a solution of the compounds, after which the casing is washed. The washing steps employed in the preparation of casings are stated to remove essentially all of the reagents used in liming the hides and in tanning the extruded casing, except for calcium (less than about 0.5%) and aluminum (about 2%) which are bound to the collagen (Talty, 1969). Analysis of casings of domestic manufacture tanned with glutaraldehyde revealed no detectable free glutaraldehyde using a method sensitive to 0.1 ppm (Froehlich, 1980). Bound glutaraldehyde was 0.18%. No glutaraldehyde was liberated by heating casings for 30 min at 120°C in water or 0.5% acetic acid using a method sensitive to 16 ppm (Nehring, 1978).

Crosslinks are formed by reaction of glutaraldehyde with the ε-amino groups of lysine in neighboring collagen molecules (Bowes and Cater, 1968). The crosslinks are stable to the action of dilute acids and bases (Harlan and Fearheller, 1977). Bowes and Cater (1968) showed that the aldehyde-amino bond was retained
during hydrolysis of glutaraldehyde-treated collagens in 6N HCl. They also presented evidence indicating that most of the ε-amino groups that reacted were involved in glutaraldehyde crosslinks.

Hardy et al. (1976) proposed that at least part of the crosslinks have a 1,3,4,5-tetrasubstituted pyridinium structure analogous to desmosine, which forms the crosslinks in elastin as it occurs in tendons, arterial walls, and alveoli. Their proposal was based on the similarity in properties of reaction products of glutaraldehyde with model amino acids to those of compounds isolated from hydrolyzates of glutaraldehyde crosslinked collagen.

Gaseous ammonia is also described as a coagulation medium (Fagan, 1970). The collagen tube is inflated with air as it is extruded and ammonia is passed into the interior of the tube. This neutralizes the acid in the extrusion solution, as well as the collagen fibrils, and increases the tensile strength of the casing, thus eliminating the need for tanning. The casing may be washed to remove salts, or dried directly, producing a casing containing residual ammonium salts of the acid used to disperse the collagen.

Plasticizing or softening agents mentioned in patents include glycerin, sorbitol, dextrin, sucrose, glucose, polyoxyalkylene glycol, fatty monoglyceride, and acetylated fatty monoglyceride (Karmas, 1974). Carboxymethyl cellulose, added to processing baths and particularly to the plasticizer bath, is reported to increase the strength of the casing. Reducing sugars (glucose, fructose, mannose, xylose, and galactose) applied to the casing in dilute solution (0.005-0.2%) increase its wet strength. Other additives described in patents include sodium alginate, added to the collagen solution prior to extrusion; egg white, incorporated as a component of the plasticizing bath; antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propylgallate in their commercial formulations, and edible oils—mineral, vegetable, or animal—that may be applied at various stages in the production of the casing.

The Select Committee does not have information on the extent of usage by industry of the various substances mentioned in patents as useful in the preparation of collagen and sausage casings. However, many have GRAS status as food ingredients. Calcium hydroxide (lime) used in the liming of hides prior to separation of collagen is considered GRAS (Office of the Federal Register, 1980 [21 CFR 182.1205]; SCOGS, 1975a). GRAS acids mentioned for use in the isolation of collagen and its dissolution prior to regeneration include the following organic and mineral acids: acetic (CFR 192.1005; SCOGS, 1977a), citric (CFR 182.1033; SCOGS, 1977b), lactic (CFR 182.1061; SCOGS, 1978a), hydrochloric (CFR 182.1057; SCOGS, 1979a), and sulfuric (CFR 184.1095; SCOGS, 1975b). Sodium bicarbonate used in the neutralization of acids is also GRAS (CFR 182.1736; SCOGS, 1975c). Substances identified as coagulating or hardening agents that are considered GRAS include ammonium sulfate.

An analysis of one company's regenerated collagen sausage casing (Froehlich, 1979) is as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>56%</td>
</tr>
<tr>
<td>Glycerin</td>
<td>18%</td>
</tr>
<tr>
<td>Fat</td>
<td>1%</td>
</tr>
<tr>
<td>Oil</td>
<td>2%</td>
</tr>
<tr>
<td>Salts (NH₄⁺, Na⁺, Al³⁺⁺)</td>
<td>5.5%</td>
</tr>
<tr>
<td>Salts and bicarbonates</td>
<td></td>
</tr>
<tr>
<td>Nitrite</td>
<td>2-3 ppm</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>0.22 ppm</td>
</tr>
<tr>
<td>Water</td>
<td>balance</td>
</tr>
</tbody>
</table>

This analysis suggests that the major constituents of the casing other than collagen, i.e., glycerin and salts (ammonium, sodium, and aluminum sulfate and bicarbonates) were GRAS substances. The source of the fat (1%) and oil (2%) was not specified, but proposed specifications for food-grade collagen permit 20% fat, dry basis (Devro, Inc., 1977).

Alkaline treatment of proteins including ribonuclease (Bohak, 1964), soy protein (Woodard et al., 1975), casein (Creamer and Matheson, 1977), and various proteinaceous foods (Sternberg et al., 1975), particularly at high pH and/or elevated temperature, has been shown to form lysinoalanine, a compound that has produced renal lesions in rats (Woodard et al., 1975; SCOGS, 1979b). However, no lysinoalanine was detected in collagen prepared from hides treated with lime (Froehlich, 1979). Temperature during the liming treatment did not exceed 50°C, and the pH was no greater than 7.2.
A GRAS letter was issued in 1960 for the use of regenerated collagen casings in sausage products (Cassidy, 1960). At the outset of this evaluation, the Select Committee was requested to review all current food uses and preparations of collagen, especially regenerated collagen used in sausage casings and their preparation (Miles, 1979). Gelatin, a hydrolysis product of collagen, has been evaluated in another report of the Select Committee (SCOGS, 1975f). Although sausage casings appear to be the only current food use for native collagen, it has been suggested that the binding and texturizing properties of collagen may make it suitable for other uses in food. According to Happich (1975), collagen offers the potential of varying the character, viscosity, consistency, mouth feel, tenderness, and juiciness (succulence) of extended meat products. However, the Select Committee has no knowledge as to whether such uses are now current or are contemplated by industry.
The National Research Council's (NRC) survey of industry on the use of GRAS food ingredients in 1977 reported that collagen (Aviten®) was used at a level of 0.69% in meat and meat products (Committee on GRAS List Survey--Phase III, 1978). Presumably this use is as sausage casing. The NRC survey of industry conducted on 1970 usage reported that 5,948 lb of sausage casings from regenerated collagen were used that year (Subcommittee on Review of GRAS List--Phase II, 1972). However, this poundage was based on reports of three or fewer food processors. A casing manufacturer has estimated that about 360 million lb (162 million kg) of sausage were packed in regenerated collagen casings in 1978, somewhat less than the quantity packed in animal casings (Sellers, 1979). Assuming the casing is about 1% of the weight of the sausage, about 3-4 million lb (1.4-1.8 million kg) of casings which contain about 1.8-2.7 million lb (0.8-1.2 million kg) of collagen were used. This represents a per capita daily intake of about 10-15 mg. Such an estimate is likely to be somewhat in excess of consumption values because production figures do not take into account inevitable losses that occur between production and consumption.

The quantity of collagen contributed by regenerated sausage casings may be compared with the collagen content of the sausage meat. Based on Sellers' (1979) figures, the collagen content of casing is about 0.6% of the weight of the sausage. This is in general agreement with the estimate of Froehlich (1979), who reported that the casing size most used for processed meat is 0.42% of the meat and with the value, 0.69%, reported in the NRC survey (Committee on GRAS List Survey--Phase III, 1978). Wiley et al., (1979) determined the collagen content of 21 different sausage raw materials, including nine from beef and ten from pork. Collagen content was calculated from the hydroxyproline content and would also include any elastin present. The collagen content of beef meats ranged from 0.87-2.53% with a mean value of 1.7%. Values for pork sausage meats ranged from 0.76-2.43%, also with a mean value of 1.7%. Assuming that this mean value is representative of the blends used in various sausages, about three times as much native collagen is contributed by the sausage meat as regenerated collagen by the casing.
IV. BIOLOGICAL STUDIES

The literature provides few biological studies on collagen. The studies that have been reported concern digestibility, protein efficiency ratio, and subacute toxicity. The Select Committee is not aware of reports of acute or chronic toxicity, reproductive effects, or carcinogenicity.

Digestibility

Whitmore et al. (1970) reported that dried collagen appeared to be completely digestible by rats and demonstrated an energy value 86% that of casein. No details were given, but a subsequent publication from the same laboratory (Whitmore et al., 1975) reported in detail the digestibility of hide collagen from fresh limed cattlehide splits that had been delimed, neutralized, and washed, then cut while frozen to obtain hide particles which passed a 10 mm screen. These in turn were freeze-dried and ground to pass through a 2 mm screen. Digestibility and caloric availability were studied in 4-wk-old Sprague-Dawley rats, 5 per group. To 5 g of a basal, calorically restricted diet providing a near-maintenance amount of protein, 2 g of casein or collagen was added and fed for 7 d. The apparent digestibility of collagen preparations containing 0, 3, or 10% fat was 90% in each instance.

Sizer (1949) reported that small pieces of beef tendon collagen were 70% dissolved when suspended in a 0.5% pepsin/1% HCl solution for 21 h at 38°C. Collagen was more rapidly dissolved in gels prepared by treating tendon powder or very thin slices of tendon with acetic acid at pH 2.5. Ninety percent of the collagen in a gel containing 1.5% collagen and 0.1% pepsin was dissolved after standing overnight at 37°C. Collagen filaments formed by extruding collagen gels into a dehydrating bath digested twice as fast as the original tendon fibers. Heating the filaments at 130°C increased their tensile strength and also their resistance to digestion by pepsin or trypsin. Regenerated collagen casing also receives heat treatment after extrusion. Klajn et al. (1975) reported digestibilities of regenerated collagen casings from five manufacturers, including products from U.S. companies. Values ranged from 94-97%; but details of the methodology were not given.

Booth (1969) reported that glutaraldehyde-treated collagen, 4.6% glutaraldehyde content (Whitmore, 1969), was only 31% digested by rats. Fecal output of five Sprague-Dawley rats fed a basal diet (5 g) supplemented with treated collagen (1 g) for 9 d was compared with that of a similar group fed the basal diet. Digestibility of a sample of the untreated collagen was 100%.
Protein efficiency ratio

Protein efficiency ratio (PER) of cattlehide collagen mixed with lean beef, whey protein concentrate, or soy protein concentrate has been reported by Happich (1975). PER values corrected to that of casein at 2.5 were 2.8 for 100% lean beef protein, 2.7 for 100% whey protein concentrate, and 2.2 for 100% soy protein concentrate. Lean beef to collagen ratios of 90:10, 50:50, and 10:90 gave PER values of 2.5, 1.7, and 0, respectively. Lean beef, collagen, whey protein concentrate ratios of 90:5:5, 50:25:25, and 25:25:50 gave PER values of 2.5, 2.4, and 2.6, respectively. Lean beef, collagen, soy protein concentrate ratios of 50:25:25 and 25:25:50 gave PER values of 2.2 and 2.1, respectively. It was concluded that collagen as an added component in meat products or meat analogues in limited quantities can result in products with a satisfactory PER.

Short-term feeding studies

In a 90-d study, Booth (1970) fed groups of eight Sprague-Dawley female rats diets containing 15% (about 10 g/kg body wt) gelatin, collagen, or glutaraldehyde-treated collagen (4.6% bound glutaraldehyde). No significant differences were observed in body weight gains among the groups. The kidney:body wt ratio was significantly greater (P<0.05) for the animals fed collagen than for animals fed the control diet, but was less than the ratio for the group fed gelatin. Organ:body wt ratios for the spleen and heart were significantly lower (P<0.05) for the animals fed treated collagen as compared with those fed the basal diet, but not as compared with those receiving untreated collagen. Urinalyses were within normal limits as were hematology values for the group fed treated collagen. Blood-clotting time tended to be less for animals fed glutaraldehyde-treated collagen as compared with those fed the basal diet but the values were not significantly different (P>0.10). Urine output also tended to be lower in rats fed the treated collagen (14 ml/108 h) as compared to the output (19 ml/108 h) of the animals given the basal diet. Water losses in the feces were not reported. No significant lesions were detected in any tissues of the rats fed the treated or untreated collagen diets.

Whitmore et al. (1975) fed cattlehide collagen particles (prepared as previously described) or casein to weanling Sprague-Dawley rats, five of each sex in each feeding group. Collagen or casein was mixed with a commercial rat diet to provide 20% of diet weight and fed for 90 d. Hemoglobin concentrations in rats fed casein or collagen were greater than in those fed the basal diet. The rats fed collagen appeared to be more sensitive to ether anesthesia than controls and their blood clotted more rapidly. No significant lesions in the collagen-fed rats were noted in abdominal organs, thorax, or brain. The only significant difference from controls was a greater kidney weight in the rats fed the collagen supplement, a finding the authors tentatively attributed to
the greater nitrogen concentration of collagen than of casein. However, further study of this point was suggested.

These findings for collagen (Booth, 1970; Whitmore et al., 1975) are similar to those reported for rats fed gelatin in high concentrations in the diet (SCOGS, 1975f). It should be noted that collagen, after treatment with heat in a moist environment as in cooking, will be quite similar not only chemically, but physically, to gelatin.

In a study of the molecular configuration of compounds causing central–peripheral distal axonopathy, glutaraldehyde was included in order to observe the effects of a compound capable of crosslinking proteins (Spencer et al., 1978). Groups of three young (200 g) Sprague-Dawley rats received drinking water containing 0.1, 0.25, or 0.5% (about 140, 350, or 700 mg/kg body wt) glutaraldehyde for 11 wk. Body wt gains and clinical signs were normal in the treated animals. No pathological changes were observed in the central or peripheral nervous systems.

Morgareidge (1967) reported results of 90-d rat and dog feeding studies with a coacervate of gelatin and gum arabic (1:1 mixture) crosslinked with glutaraldehyde. The coacervate contained 4.5% bound and 10–14 ppm free glutaraldehyde. It was fed to groups of weanling albino rats, 22 of each sex, at dietary levels adjusted biweekly to provide 0.5, 1.75, or 5 g coacervate/kg body weight. The control diet contained 10% coacervate which had not been treated with glutaraldehyde. Hemoglobin, hematocrit, total and differential leukocyte counts, blood urea nitrogen, glucose, and serum glutamic pyruvic transaminase as well as urinary pH, albumin, glucose, and occult blood determined at 6 and 12 wk revealed no significant differences among groups. Microscopic examination of 15 organs and tissues of 10 male and 10 female rats from the control group and the group receiving 5 g/kg coacervate indicated no histopathological effect of the test substance.

In the dog study (Morgareidge, 1967) 5- to 7-mo-old beagles, six per group balanced as to sex and weight, were fed diets containing 10% glutaraldehyde-crosslinked coacervate (1:1 gelatin–gum arabic) or an equal amount of untreated coacervate. Hemoglobin, hematocrit, total and differential leucocyte counts, prothrombin time, blood urea nitrogen, blood glucose, serum glutamic pyruvic transaminase, serum alkaline phosphatase, urinary specific gravity, occult blood, albumin, glucose, ketones, and pH determined at 6 and 12 wk were within normal limits. Gross examination of organs at sacrifice after the thirteenth week showed cecal redness in two dogs in the test group and one dog in the control group. The investigators attributed this to handling since no histopathological changes were found. Microscopic examination of 25 organs and tissues revealed no findings in the treated animals attributable to ingestion of the test substance.
Regenerated collagen casing tanned with glutaraldehyde did not demonstrate mutagenic activity in assays employing *Salmonella typhimurium* TA-1535, -1537, -1538, -98, and -100 with and without rat liver microsomal activation (Litton Bionetics, Inc., 1977). The test material (½ inch squares) was placed on the surface of agar plates, and cells were spread over the surface of the plate covering the test material.

Glutaraldehyde did not exhibit mutagenic activity in assays with *S. typhimurium* TA-100 when tested at the highest nontoxic level with and without rat liver microsomal activation (Lieberman, 1980). However, Haworth (1980) and Senti (1981) reported weak mutagenic activity when glutaraldehyde was tested with *S. typhimurium* TA-100 with and without rat or hamster liver microsomal activation. The samples of glutaraldehyde assayed came from different suppliers.
V. OPINION

Food-grade collagen is currently prepared from bovine hides by separating and purifying the corium layer, which consists mainly of bundles of collagen fibers. The only present food use of this native collagen is in the production of sausage casings. For this purpose, the purified corium layer of hides is dissolved in dilute acid and precipitated in tubular form under conditions that regenerate the fibrillar structure of the native collagen. Various plasticizing, softening, and strengthening agents may be included in the casing to obtain desired physical properties. Such regenerated collagen sausage casings are considered GRAS.

Regenerated casing is estimated to account for only about 0.7% of sausage weight. Intake of collagen from sausage casings is likely to be considerably less (less than one-fourth of total) than that contributed by the remainder of the sausage and certainly a minor fraction of total intake of collagen. Collagen makes up a significant percentage of the weight of meats, poultry, and fish as they are consumed and the likelihood of adverse effects from such consumption seems remote. Collagen consumed in the form of sausage casings appears to be digestible. There is little reason to suspect that the regeneration process results in a product that is more likely to produce adverse effects than native collagen. However, specifications for food-grade collagen, native or regenerated, have not been established.

Several substances are described in the patent literature as useful in the preparation of sausage casings from collagen. Most are GRAS substances and their use in sausage casings would add relatively little to the amount of these substances now consumed in other GRAS applications. Their content in casings would be only a fraction of the amount of collagen in the casing and total use of the latter is estimated to be 10-15 mg/capita/d. Glutaraldehyde, preferred for use as an agent to strengthen casings, is not included among the compounds listed as GRAS. However, no significant adverse effects were observed in short-term feeding tests with rats and dogs fed collagen or gelatin diets that provided several orders of magnitude more free and/or bound glutaraldehyde/kg body wt than would be estimated for humans consuming glutaraldehyde-treated sausage casings. Chemical evidence indicates that the binding of glutaraldehyde to collagen is stable under the acid and alkaline conditions of the digestive system. This is supported by markedly decreased digestibility of collagen containing higher levels of bound glutaraldehyde indicating that portions of collagen molecules containing bound glutaraldehyde are not absorbed, but are eliminated in the feces. Reports on the mutagenic activity of free glutaraldehyde are equivocal. One investigator reported no activity, another weak activity. Tests on the mutagenic activity of enzymic digests of glutaraldehyde-treated collagen appear not to have been conducted, but should be undertaken in due course.
Although the addition of collagen to foods in forms other than sausage casings is not now practiced, it has been suggested that native collagen has functional properties that may be useful in other processed foods. Native collagen is digestible by rats and presumably by humans. After heat treatment, as in cooking, it seems likely that native collagen will be similar not only chemically but physically to gelatin. Although feeding native collagen, or gelatin, to rats in high concentration has been reported to result in kidney enlargement, it seems most unlikely that a hazard to the public would result from consumption of native collagen as a food ingredient.

In light of the foregoing, and on the assumption that specifications will be established for food-grade regenerated collagen and food-grade native collagen, the Select Committee concludes that:

There is no evidence in the available information on regenerated collagen that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used in sausage casings in the manner now practiced or that might reasonably be expected in the future.

There is no evidence in the available information on native collagen that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public should it be used as a food ingredient.
VI. REFERENCES CITED


- 15 -


Senti, F.R. 1981. Federation of American Societies for Experimental Biology, Bethesda, MD. Memorandum, dated February 20, concerning information (about mutagenicity assay on glutaraldehyde) supplied by S. Haworth, EG&G Mason Research Institute, Rockville, MD.


VII. SCIENTISTS CONTRIBUTING TO THIS REPORT

1. Members of the Select Committee on GRAS Substances:

Joseph F. Borzelleca, Ph.D., Professor of Pharmacology, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Virginia.

Harry G. Day, Sc.D., Professor Emeritus of Chemistry, Indiana University, Bloomington, Indiana.

Samuel J. Fomon, M.D., Professor of Pediatrics, College of Medicine, University of Iowa, Iowa City, Iowa.

Bert N. La Du, Jr., M.D., Ph.D., Professor and Chairman, Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan.

John R. McCoy, V.M.D., Professor of Comparative Pathology, New Jersey College of Medicine and Dentistry, Rutgers Medical School, New Brunswick, New Jersey.

Gabriel L. Plaa, Ph.D., Professor and Chairman, Department of Pharmacology, University of Montreal Faculty of Medicine, Montreal, Canada.

Michael B. Shimkin, M.D., Professor Emeritus of Community Medicine and Oncology, School of Medicine, University of California, San Diego, La Jolla, California.

Ralph G.H. Siu, Ph.D., Consultant, Washington, D.C.

Marian E. Swendseid, Ph.D., Professor of Nutrition, School of Public Health, University of California, Los Angeles, California.

John L. Wood, Ph.D., Professor Emeritus, Department of Biochemistry, University of Tennessee Center for Health Sciences, Memphis, Tennessee.

George W. Irving, Jr., Ph.D., Chairman, Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Maryland.
2. LSRO staff:

Kenneth D. Fisher, Ph.D., Director
Frederic R. Senti, Ph.D., Associate Director
Richard G. Allison, Ph.D., Staff Scientist
Sue Ann Anderson, Ph.D., Staff Scientist
Herman I. Chinn, Ph.D., Senior Staff Scientist
John M. Talbot, M.D., Senior Medical Consultant

Report submitted by:

June 8, 1981
Date

George W. Irving, Jr., Chairman
Select Committee on GRAS Substances