EVALUATION OF THE HEALTH ASPECTS OF RENNET AS A FOOD INGREDIENT

1977

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D.C.

Contract No. FDA 223-75-2004
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Life Sciences Research Office
Federation of American Societies
tor Experimental Biology
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NOTICE

This report is one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior sanctioned food substances as food ingredients, being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-75-2004 with the Food and Drug Administration (FDA), U.S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

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

This report concerns the health aspects of using rennet as a food ingredient. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (1), which summarizes the world's scientific literature from 1920 through 1973.* To assure completeness and currency as of the date of this report, this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, an announcement was made in the Federal Register of June 7, 1977 (42 FR 29105-29107) 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 rennet as a food ingredient. The Select Committee received no requests for such a hearing on rennet.

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

*The document (PB-228 541/9) is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.
The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety, the Select Committee, in accordance with FDA's guidelines, is relying primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. While the Select Committee realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited, it recognizes that there can be instances where, in the judgment of the Select Committee, there are insufficient data upon which to base a conclusion. The Select Committee, aware that biological testing is dynamic, bases its conclusions on information now available; it cannot anticipate the results of experiments not yet conducted or those of tests that may be reconducted, using new technologies. These conclusions will need to be reviewed as new or better information becomes available.

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

II. BACKGROUND INFORMATION

Rennet (rennin) is included on the list of GRAS substances (2) as a multiple purpose GRAS food substance [21 CFR 182.1685]. Rennet is an extract containing the enzyme rennin that is used principally to clot milk in cheese making; historically, it has been prepared from the abomasum of young calves and until recently has been the major clotting agent used in the commercial manufacture of cheese. A similar preparation sometimes called bovine rennet comes from the abomasum of older animals. An increased demand for cheese in the past decade and a decrease in the number of calves slaughtered led to the development of rennet substitutes (3, 4). Porcine pepsin is currently used as an extender for rennet. Microbial proteases have been developed and are widely used as milk-clotting agents for cheese-making (3, 5). Fungal and bacterial enzymes produced by pure culture fermentations of specified microorganisms are approved as regulated food additives [21 CFR 173.150] in the production of standardized cheeses [21 CFR Part 133] (2). These microbial products are sometimes referred to as microbial rennets and milk-clotting enzymes from plant sources as plant rennets (3, 6). However, the present report will be limited to the consideration of calf-stomach extracts, the product to which the term "rennet" has been historically applied.
In the preparation of rennet extracts, the vells (calf abomasa) are opened at slaughter, washed and packed in salt. For processing, the vells are cleaned and dried, shredded, mixed with an inert filler and extracted with sodium chloride solutions. The crude extract is activated by acidifying to pH 5.0. It may be clarified with alumina before filtering (3, 7).

Rennet extract is preserved as a high salt (14 to 20 percent) solution which contains preservatives such as propylene glycol (5 percent), sodium propionate (2 percent), and sodium benzoate (0.1 percent) or methyl and propyl paraben (0.1 percent) (3, 7, 8). Nitrogen content of one commercial rennet extract was reported as 5 mg per ml which, as protein, would represent a concentration of about 3 percent (3). Specific activity of rennin in the extract was 20 rennet units (R. U.) per mg N whereas that of the crystalline enzyme rennin separated from the extract was 401 R. U. per mg N indicating that the concentration of active rennin in the extract was about 0.15 percent.

Rennet powders are prepared from precipitates obtained by acidifying activated extracts or by saturating with sodium chloride or both. Gelatin is often added to assist in precipitation of rennet.

Although rennet has been used for centuries in cheese-making, commercial production began in the United States in the late 1800's (7).

Rennin, the active principle of rennet, is a protease which occurs in the mucosa as a zymogen, prorennin. Rennin attacks one specific peptide bond in kappa-casein of milk to cleave it to para-kappa-casein and a macro-peptide. The stabilizing action of kappa-casein on the caseinate micelles in milk is destroyed by rennin; a precipitate of calcium paracaseinate forms resulting in the milk clot. Several mechanisms describing the details of the process have been proposed (3).

Food Chemicals Codex (9) does not list rennet but specifications for the identity and purity of enzyme preparations for food processing appear in the WHO Food Additive Series, No. 2. General specifications for purity of the enzyme preparations are given which include limits on heavy metal content and pathogenic microorganisms (6).

III. CONSUMER EXPOSURE DATA

A National Research Council (NRC) subcommittee surveyed manufacturers in 1970 concerning the level of addition of GRAS substances to foods and estimated the possible average daily intake of these substances for several age groups (10). Based on information supplied by those manufacturers who reported adding a GRAS substance to at least one food in a category, weighted means were calculated for the usual and maximal
addition of the substances to foods in a category. Weighted means of the usual level of addition of rennet in four categories are given in Table I. The NRC subcommittee also estimated possible average daily intakes of rennet for four age groups (Table II) from Market Research Corporation of America data on the mean frequency of eating food by food category, U.S. Department of Agriculture data on the mean portion size of foods in these categories and the assumption that all food products within a category contained the substance at the level shown in Table I. This estimate of daily intake for the 2 to 65+ yr age group, 2.7 mg, may be questioned because the level of addition given for cheese (Table I) appears to be the amount added to milk in the cheese-making process (7, 8) and not the concentration in the cheese produced. If all extract is retained in the cheese as the NRC survey appears to have assumed, then the level of rennet extract in cheese would be about nine times greater since the average yield of cheese is about 11 percent as shown by comparing statistics on U.S. cheese production with the quantity of milk used for that purpose (13).

A per capita consumption of rennet of 2.8 mg can be calculated from the quantity 213,000 kg*, of rennet estimated to have been used in food based on reports from the food manufacturers who responded in the NRC survey. No data were available to the Select Committee on domestic production but a domestic usage of 213,000 kg appears inconsistent with U.S. import data (14) which show that 768,731 pounds (349,000 kg) of rennet were imported in 1970. However, these figures cannot be compared directly since the import figure does not represent rennet extract alone but also includes vells and rennin powder (15). A more reliable estimate of the quantity of rennet extract used in U.S. cheese production can be made from the quantity of cheese produced and the level of addition of rennet extract used in producing cheese. In 1970, 2,201,433,000 pounds of cheese were produced (13) which at a level of addition (Table I) of 0.021 percent of rennet extract to the milk (approximately 0.2 percent in the cheese, assuming a yield of 100 pounds of cheese per 1000 pounds milk) would require 4,600,000 pounds of extract. According to Davis (5), 80 percent of U.S. cheese in 1970 was made with a 50:50 rennet-pepsin mixture, and the remainder mainly with rennet extract. On this basis, about 2.8 million pounds of rennet extract were used in cheese-making in 1970, a quantity about six times greater than that estimated from food manufacturers' reports in the NRC survey. If it is assumed that the same level of addition of rennet applies to the 160 million pounds of cheese imported in 1970 (16), an additional 200,000 pounds of rennet extract were used in making cheese.

*This figure was calculated from the quantities used in foods that were reported to the National Research Council subcommittee, assuming that the survey data represented about 60 percent of actual usage as suggested by the NRC subcommittee (10).
TABLE I

Level of Addition of Rennet to Foods by Food Category (10)

<table>
<thead>
<tr>
<th>Food category</th>
<th>Rennet Weighted mean percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk, milk products</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cheese</td>
<td>0.021</td>
</tr>
<tr>
<td>Frozen dairy desserts, mixes</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gelatins, puddings, fillings</td>
<td>0.003</td>
</tr>
</tbody>
</table>

TABLE II

Possible Average Daily Intake of Added Rennet by Age Groups (10)

<table>
<thead>
<tr>
<th>Age group</th>
<th>Total intake mg</th>
<th>Intake* mg/kg body weight</th>
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<tbody>
<tr>
<td>0-5 mo</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>6-11 mo</td>
<td>1.1</td>
<td>0.14</td>
</tr>
<tr>
<td>12-23 mo</td>
<td>2.2</td>
<td>0.20</td>
</tr>
<tr>
<td>2-65+ yr</td>
<td>2.7</td>
<td>0.05</td>
</tr>
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*Calculations based on the average weight of 60 kg for an adult (11) and the following estimated weights of infants by age groups: 0-5 mo, 5 kg; 6-11 mo, 8 kg; 12-23 mo, 11 kg (12).
consumed in the U.S. in that year. Total per capita usage of rennet extract in the manufacture of cheese in 1970, then, would be about 18 mg as compared to 2.7 mg given in Table II for the 2 to 65+ yr age group.

Microbial proteases have replaced rennet to a considerable extent over the past five years and it is estimated that, in 1974, about 60 percent of all milk coagulants used in the cheese industry were microbial proteases; 25 percent were rennet and porcine pepsin blends and 15 percent were rennet extracts. About 75 percent of the rennet extract was bovine pepsin rather than calf rennet (17). On this basis 6 mg of rennet extract were used per capita in the production of the 3.017 billion pounds of cheese consumed in 1975 (18).

The extent to which the components of rennet extract appear in cheese depends on their distribution between the curd and the whey which are produced in approximately a 10:90 ratio in the cheese-making operation. Holmes and Ernstrom (19) found that 30 percent of the rennin activity is retained in the fresh cut curd. Activity is lost in subsequent operations and only 6 percent of the original activity is present after overnight pressing of the curd. Assuming 30 percent of the rennet components are retained in the curd, 6 mg per capita addition of rennet extract to milk in the cheese-making operation would result in the following per capita intake of rennet components in cheese: 0.06 mg total protein, 0.003 mg rennin, 0.1 mg propylene glycol, 0.04 mg sodium propionate, 0.3 mg NaCl and 0.002 mg sodium benzoate or 0.002 mg total methyl and propyl parabens. The Select Committee has evaluated the health aspects of sodium benzoate (20), methyl and propyl parabens (21), and propylene glycol (22) and reported no evidence of hazard at the foregoing levels of daily intake. Sodium propionate will be evaluated in a future report on propionates.

Presence of rennin in cheese ranging in age from three weeks to 1.5 years was reported by Elliot and Emmons (23) who developed a passive indirect hemagglutination test for calf rennin.

IV. BIOLOGICAL STUDIES

Very few studies on the biological properties of rennet were available to the Select Committee.

Effect of rennin on the digestion of milk was studied in five patients by determining the ratio of soluble to insoluble nitrogen at several intervals after gastric intubation of 200 ml milk containing 10 g of dextrose and 1 g of commercial rennin (junket). Three to five such experiments were conducted on each patient; on alternate days rennin was omitted from the milk to
provide the control. Samples taken over a period of two hours showed rennin had no effect on rate or extent of the gastric digestion of milk. No adverse effects of rennin were reported (24).

Driver (25) investigated the effect of several proteolytic enzymes, including rennin, on the production of ulcers in the intestines of dogs. Intestinal loops were exposed to 0.1 percent solutions of rennin (Armour rennet, N.F.) and pepsin in 0.1 N HCl under a pressure of 90 cm of water. Perforation occurred in 114 minutes in the rennin solution and 82 minutes in the pepsin solution, whereas in HCl alone perforation resulted after 174 minutes. No information was given on the purity of the rennin preparation with respect to content of pepsin, a common rennet component.

Favorable results were observed in 130 hospitalized infants suffering from dystrophy, atrophy, loss of appetite, pylonospasm and certain forms of milk dyspepsia when fed sweetened cow's milk curdled with a rennin preparation called "pegnin." Improvements noted were weight gain and tolerance of the diet. The infants received the curdled milk for periods varying from a few days up to six weeks (26).

Rennet powder was tested for teratogenicity in the developing chick embryo. It was administered in water via the air cell and the yolk at preincubation (10 to 200 mg per kg of egg) and at 96 hours of incubation (5 to 100 mg per kg of egg). Air cell treatment at 0 hour showed no toxicity above background; at 96 hours, the LD₅₀ was 85 mg per kg of egg. Scattered abnormalities were observed for all four conditions of test but in no instances were these significantly higher than or different from those observed in solvent-treated or untreated control eggs. The author concluded that rennet displayed no teratogenicity under the test conditions employed (27). It should be noted that 1 g of the rennet powder tested contained three and a third to five times the rennin activity of 1 ml of rennet extract used in cheese making (28).

No short or long-term animal toxicity studies on rennet were available to the Select Committee nor were there any reports relating to carcinogenicity or mutagenicity of rennet preparations.

V. OPINION

Rennet is an extract containing the milk-clotting enzyme, rennin, that is obtained from edible tissues of animals commonly used for food. It has been used for centuries in the production of cheese. Consumption of cheese, the major source of rennet in the diet, adds to the diet an
insignificant amount of rennet protein (0.06 mg of which 0.003 mg is rennin) and very small amounts (0.1 mg or less per person per day) of preservatives such as sodium benzoate or methyl and propyl parabens, levels at which the Select Committee found no evidence of hazard in previous evaluations. Although rennin is a protease, it is unlikely to exert significant proteolytic activity on the mucosa of the alimentary tract since it is ingested at very low concentrations in the presence of large amounts of substrate and would be rapidly inactivated by digestion. No adverse effects have been reported in infants fed milk coagulated with rennin preparations. Teratogenicity tests on rennet by the chick embryo method have given negative results.

In view of the foregoing, the Select Committee concludes that:

There is no evidence in the available information on rennet that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when used at levels that are now current and in the manner now practiced, or those that might reasonably be expected in the future.
VI. REFERENCES CITED


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