LIFE SCIENCES RESEARCH OFFICE

REVIEW OF THE RECENT LITERATURE OF THE HEALTH ASPECTS OF RIBOFLAVIN AND RIBOFLAVIN 5'-PHOSPHATE AS FOOD INGREDIENTS

1977

Review of the recent literature of the **Health Aspects of Riboflavin & Riboflavin 5'-Phosphate** as food ingredients
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LIFE SCIENCES RESEARCH OFFICE
FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY
9650 Rockville Pike
Bethesda, Maryland 20014

FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB) provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in work in specific areas of biology and medicine.

This technical report was prepared for the LSRO Select Committee on GRAS Substances (SCOGS) as a part of their review of the health aspects of using these food ingredients as stipulated in the Food, Drug, and Cosmetic Act for Generally Recognized as Safe substances. Dr. Michael J. Wade prepared the report based on a comprehensive search and evaluative assessment of the current literature in accordance with the provisions of contract no. FDA 223-75-2004. Acknowledgment is made of the assistance of the LSRO staff who provided much of the background information.

Kenneth D. Fisher, Ph. D.
Director
Life Sciences Research Office

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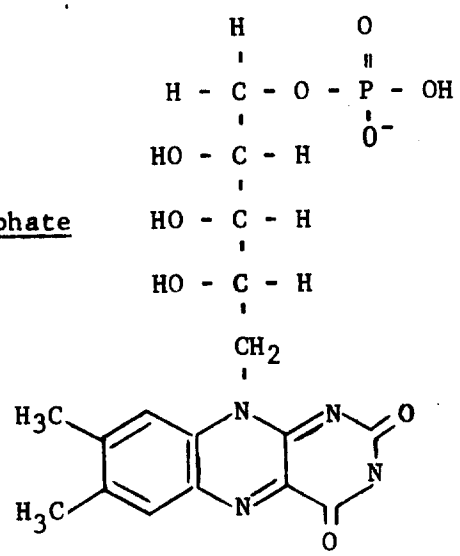
INTRODUCTION

This report concerns the health aspects of using riboflavin and riboflavin 5' phosphate as food ingredients. It reviews the world scientific literature from 1972 through 1976.

To assure completeness and currency as of the date of this report, information has been obtained by searches 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; and by the combined knowledge and experience of members of the LSRO staff. This report supplements and updates information contained in a scientific literature review (monograph) prepared for FDA by Informatics, Inc.[1] which summarizes the world's scientific literature up to 1974.

Riboflavin (21 CFR 182.5695) and riboflavin 5'-phosphate (21 CFR 182.5697) are listed as GRAS substances under the Federal Regulations[2] for use as dietary supplements. The structure of riboflavin 5' phosphate is given below. Riboflavin has the identical structure minus the phosphate group esterified to the ribitol moiety.

<u>Riboflavin 5' phosphate</u>



[1] The document is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.

[2] Office of the Federal Register, General Services Administration. 1977. Food and Drug Administration: rules and regulations. Food for human consumption: reorganization and republication. Fed. Regist. 42:14301-14669.

I. ABSORPTION AND METABOLISM

Riboflavin absorption and metabolism were recently reviewed by Matthews (1974). In man, riboflavin and riboflavin 5'-phosphate taken orally are apparently absorbed in the small intestine by a carrier, and there is a linear relationship between oral dose and urinary riboflavin excretion up to some maximum dose at which the carrier is saturated. The maximum amount of riboflavin that can be absorbed by man at any one time may be as low as 10-12 mg (Matthews, 1974).

Riboflavin 5'-phosphate given orally appears in the blood as riboflavin. However, riboflavin is phosphorylated in the intestinal mucosa during absorption (Marks, 1975); and dephosphorylation may take place in the liver since riboflavin 5'-phosphate is not found in the peripheral blood (Matthews, 1974).

Axelson and Gibaldi (1972) studied the absorption and excretion of riboflavin in the rat. Male Sprague-Dawley rats were assigned to either a standard laboratory diet or a special riboflavin-deficient diet, and some animals were randomly chosen for bile duct ligation. Animals maintained on the standard diet showed a much higher level of background urinary riboflavin excretion than those on the riboflavin-deficient diet. One mg doses (5.5 mg per kg) of riboflavin 5'-phosphate were administered to the animals by intraperitoneal injection or by gavage. When the riboflavin 5'-phosphate was administered by gavage, about 4 percent of the dose was recovered from the urine excreted within 72 hours by animals fed the riboflavin-deficient diet; this value was increased to about 6 percent in animals with ligated bile ducts. About 10 percent of riboflavin 5'-phosphate administered by gavage was recovered within 72 hours from the urine of nonligated rats given the standard diet. (The urinary excretion was not reported for rats on the standard diet with ligated bile ducts.) When the compound was given by injection, from 40 to 70 percent of the dose was recovered from the urine. When 0.2 mg of riboflavin 5'-phosphate (1.1 mg per kg) was injected into the animals, a lower percentage of the injected dose was recovered in the urine than when 1 mg was injected.

Leclerc (1974) studied the effect of different feeding levels of riboflavin on the tissue concentrations of riboflavin in lactating rats and their offspring. A group of female Wistar rats was mated and then divided into three dietary groups differing only in the amount of B vitamins in the diet. Group A received the NRC recommended levels of thiamine (4 mg per kg), riboflavin (4 mg per kg), and vitamin B_6 (1 mg per kg). Group B received 4 mg per kg of each of the three vitamins and group C received 40 mg per kg of each of the three vitamins. Only females bearing

eight or more young were used and three hours after delivery each litter was reduced to eight pups. After 21 days of lactation, the mothers and all of their litters were sacrificed along with control (nonlactating) females There were no differences among the three dietary groups of lactating mothers with regard to weight gain, liver weight and total body weight. Similar results were seen in the nonlactating rats. The offspring of the rats in the three dietary groups were similar with respect to weight, liver weight and total litter weight. The reproductive performance of each group was similar as measured by number of rats mated, number becoming pregnant, size of litters and number of offspring surviving. No differences in liver or carcass levels of riboflavin were seen between the different dietary groups, or between pregnant and nonpregnant females. Similarly, no differences were noted between the offspring of the three dietary groups with respect to liver or carcass levels of riboflavin. Leclerc (1974) concluded that saturation of the tissues with riboflavin occurs in both lactating and nonlactating rats at a dietary level of 4 mg per kg per day.

Riboflavin 5'-phosphate and flavin adenine dinucleotide (FAD) appear to be the physiologically active forms of the vitamin in most tissues, serving as cofactors in a large number of enzymatic reactions. Sanpitak and Chayutimonkul (1974) monitored the activity of the FAD-dependent enzyme erythrocyte glutathione reductase as a measure of riboflavin nutriture in Thai women taking oral contraceptives. Forty-two women taking oral contraceptives showed a significant decrease of about 18 percent in the activity of glutathione reductase as compared to 31 control women of similar age and social status not taking the contraceptives. When FAD was included in the assay mixture there was a large increase in the activity of enzyme samples from women taking oral contraceptives and a moderate increase in the enzyme activity of samples from control women, so that both groups had about equal glutathione reductase activity with in vitro FAD addition to the assay mixture. These workers suggested that oral contraceptives may interfere with riboflavin absorption or its conversion to FAD.

II. ACUTE TOXICITY

In mice, subcutaneous injection of 60 or 120 mg per kg riboflavin caused increased neuromuscular sensitivity and death in about 30 minutes; mild hypothermia was the only ill effect noted in mice injected with a 30 mg per kg dose of riboflavin (Beidz-Bielawski, 1973).

III. SENSITIVITY

Neumann (1971) described the case of a 50-year-old woman who developed severe pruritic dermatitis which covered her body and limbs but not her face. The eruptions cleared up 48 hours after the woman ceased taking over-the-counter vitamin B complex capsules. Neumann (1971) found that the dermatitis could also be brought about if the patient ate bread enriched with thiamine, riboflavin and niacin. However, the author did not report any investigations on which of these three substances provoked the dermatitis.

Allergic dermatitis was also seen in a 22-year-old woman hospitalized for the fourth time with an exacerbation of microbial eczema (Soloshenko and Brailovskii, 1975). She had a previous history of allergic disorders including drug allergies. The patient was injected with 1 ml of a 1 percent solution of riboflavin (0.17 mg per kg) for treatment of the eczema. Within 30 minutes she exhibited chills, edema of the face and hands, itching and intense hyperemia of the skin on the torso. The leukocyte agglomeration test showed 3.1 percent agglomeration in the controls and 32.7 percent with riboflavin. Upon cessation of riboflavin treatment, the symptoms subsided.

IV. SPECIAL STUDIES

A recent review has cited studies in mice and rats that suggest riboflavin deficiency slows the growth of spontaneous and transplanted cancers (Anonymous, 1974).

Williams et al., (1970) found that in the rat the metabolism of the carcinogen 4-dimethylaminoazobenzene by gut bacteria was affected by the amount of riboflavin in the diet. One group of eight male Fisher rats was maintained on a low riboflavin diet, 2 ppm (0.2 mg per kg body weight per day). Two other groups of four animals each, received supplemental riboflavin to 20 or 200 ppm in the diet (2 or 20 mg riboflavin per kg body weight per day). After four or six weeks of feeding, the rats were sacrificed and the azo-reductase activities of the liver and the cecal contents were measured in the presence and absence of riboflavin. [The enzyme azo-reductase catalyzes the reductive cleavage of dimethylaminoazobenzene into two monophenylamine moieties. (Mueller and Miller, 1949)] The amount of azo-reductase activity per mg of protein was higher in the cecal contents than in the liver. This was true for all three feeding groups whether enzyme activities were measured in the presence or absence of riboflavin. Assayed in the absence of riboflavin, the cecal levels of azo-reductase were 1.5 times higher in the 200 ppm riboflavin feeding group as compared to

the 2 ppm feeding group. When measured in the presence of riboflavin, no increase in cecal azo-reductase activity was seen with increasing dietary riboflavin. In the liver, there was a marked increase in reductase activity in the 20 ppm riboflavin feeding group as compared to unsupplemented controls. The 200 ppm riboflavin feeding group also showed a similar but smaller increase in azo-reductase activity. These relative changes in liver reductase activity took place whether or not riboflavin was added in vitro to the assay mixture, but the changes were more marked when riboflavin was not added to the assay mixture.

In the presence of molecular oxygen, riboflavin and riboflavin 5'-phosphate are able to act as sensitizers for the photooxidation by visible light of susceptible molecules including proteins and nucleic acids (Speck et al., 1975; Plimmer and Klingebiel, 1971; and Spikes and Glad, 1964).

Newberne, et al., (1974) found that exposure to riboflavin and light enhanced the toxicity of aflatoxin in the rat. Weanling male Sprague-Dawley rats were fed either normal diets or diets marginal in lipotropes for two weeks. The animals were then dosed intragastrically with 28 mg per kg riboflavin and 7 mg per kg aflatoxin. Fifty other animals were dosed with the same amount of riboflavin and with 70 mg per kg monocrotaline. The doses of aflatoxin and monocrotaline represent LD_{50} values. Some of these animals were further treated by irradiation with a light source having a spectral distribution close to that of sunlight. For rats on the control diet, the cumulative mortality after two weeks was 45 percent for animals dosed with aflatoxin alone, 57.4 percent for aflatoxin plus light, 50 percent for aflatoxin and riboflavin, and 75 percent for aflatoxin, light and riboflavin. Each of the above values was based on test groups of 40 rats each. Qualitatively similar results, but with lower mortality, were seen in rats undergoing the same treatments but maintained on a low lipotrope diet. In the case of animals dosed with monocrotaline, riboflavin appeared to exert a protective effect.

Illumination of calf thymus DNA in vitro with high output blue lamps in the presence of 1.33×10^{-4} M riboflavin caused changes in the physical properties of the DNA (Speck et al., 1975). Changes were also seen in the DNA of HeLa cells irradiated in the tissue culture in the presence of riboflavin. These changes included a decreased ultraviolet absorption peak and a shift in the wavelength of the absorption maxima. There was also an increase in the buoyant density and decreases in the sedimentation coefficient and temperature of the helix coil transition. These changes were interpreted by the authors as resulting from the selective destruction of guanine residues in the DNA. No changes were seen in the DNA during control experiments performed in the absence of either riboflavin or light.

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