EVALUATION OF THE HEALTH ASPECTS OF THIAMIN HYDROCHLORIDE AND THIAMIN MONONITRATE AS FOOD INGREDIENTS

1978

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
for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014
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.

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 thiamin* (thiamine) hydrochloride and thiamin mononitrate as food ingredients. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (2), 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 13, 1978 (43 FR 25487-25489) 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 thiamin hydrochloride and thiamin mononitrate as food ingredients. The Select Committee received no requests for such a hearing on thiamin hydrochloride and thiamin mononitrate.

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 (3) [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 (3) recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

*This spelling has been recommended by the International Union of Nutrition Sciences and by the American Institute of Nutrition (1).

**The document (PB-241 951/3) 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 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 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 Committee, there are insufficient data upon which to base a conclusion. The Committee is aware that its 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 thiamin hydrochloride and thiamin mononitrate and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of these substances under the Federal Food, Drug, and Cosmetic Act.
II. BACKGROUND INFORMATION

Thiamin, vitamin B₁, is widely distributed in plant and animal tissues, especially in yeast, rice husks, cereal grains, green leaves, roots, tubers, eggs, milk and liver (4). Practically all of the marketed thiamin is now produced synthetically, usually in the form of the hydrochloride or the mononitrate:

Thiamin hydrochloride

\[
\begin{align*}
\text{CH}_3&\text{N}^+\text{NH}_3 \\
\text{CH}_2\text{CH}_2\text{OH} \\
\text{CH}_3&\text{N} \text{S} \\
\end{align*}
\]

Thiamin mononitrate

\[
\begin{align*}
\text{CH}_3&\text{N}^+\text{NH}_2 \\
\text{CH}_2\text{CH}_2\text{OH} \\
\text{CH}_3&\text{N} \text{S} \\
\end{align*}
\]

Thiamin hydrochloride crystallizes as colorless monoclinic plates in rosette-like crystals. It has a characteristic odor and a slightly bitter taste. It melts at 248°C with some decomposition. When exposed to air, the anhydrous product rapidly absorbs about 4 percent of water, forming a hydrate. It is very soluble in water, but virtually insoluble in ether and other fat solvents. In its dry form and in acid solution, thiamin hydrochloride is quite stable, but it is rapidly destroyed in solutions above pH 5.5 (4, 5).

Thiamin mononitrate is only moderately soluble in water and is practically nonhygroscopic. It is more stable than the hydrochloride and is preferred by the food industry for the enrichment of flour mixes (6).

The Food Chemicals Codex (5), the U.S. Pharmacopeia (7) and the U.S. Dispensatory (8) specify that both vitamin forms must contain not less than 98 nor more than 102 percent of the respective compounds calculated on the dry basis. The following additional specifications are stipulated by the Food Chemicals Codex and the U.S. Pharmacopeia: The nitrate shall lose not more than 1 percent and the hydrochloride not more than 5 percent weight on drying. After complete ignition of either compound, the residue must not exceed 0.2 percent of the original weight. The nitrate shall not contain more than 600 ppm of chloride nor the hydrochloride show the presence of nitrate. The pH of a 1 in 100 solution of thiamin hydrochloride must be between 2.7 and 3.4 and of a 1 in 50 solution of thiamin mononitrate, between 6.0 and 7.5.
Thiamin hydrochloride [21 CFR 182.5857] and thiamin mononitrate [21 CFR 182.5878] are classified by the Food and Drug Administration as nutritive/dietary supplements with GRAS status (3). When used to enrich various foods such as bakery, cereal and pasta products, limits are specified and generally vary from 1.8 mg per pound (4 mg per kg) for bread [21 CFR 136.115] to 4.0 to 5.0 mg per pound (8.8 to 11 mg per kg) for macaroni and noodle products [21 CFR 139.115; 139.122; 139.155]. Thiamin forms are also incorporated in tablets and capsules, singly or in combination with other dietary supplements, for over-the-counter sale. The Physicians' Desk Reference (9) lists over 100 preparations of this type sold in the United States.
III. CONSUMER EXPOSURE DATA

In keeping with their vitamin character, the thiamin salts are normally added to food in very small amounts. Table I is based on information supplied to the National Research Council subcommittee (10) by manufacturers who reported adding thiamin hydrochloride or thiamin mononitrate to at least one food in a given category.

The level of addition shown in Table I does not indicate that any particular product contains that amount of thiamin, nor does inclusion of a food category within this table mean that it is present in a majority of the foods in that category. Actually many of the foods within the listed food categories may contain no added thiamin or much lower levels than indicated in the table. The data for several categories in Table I probably represent an overestimation of the actual average levels of added thiamin.

Nevertheless, in the absence of more reliable data, the NRC subcommittee has utilized these values in an attempt to calculate the possible average daily intake of added vitamin in several age groups. For this purpose, the subcommittee has used the values shown in Table I, together with data on the mean frequency with which foods in each category are consumed (provided by the Market Research Corporation of America) and the mean portion size of these various foods as determined by the Department of Agriculture. The resulting calculations yield the following combined possible average daily intakes of added thiamin hydrochloride and thiamin mononitrate for the different age groups: 0 to 5 months, 2.0 mg; 6 to 11 months, 10.5 mg; 12 to 23 months, 15.7 mg; and over 2 years of age, 29.5 mg. These estimates almost certainly grossly overstate the average intake of added thiamin.

An alternative approach for estimating the intake of added GRAS substances is based on the total amount added annually to food by the manufacturers. Surveys by the NRC and by the Flavor and Extract Manufacturers' Association (FEMA) revealed that a total of 37,200 kg of thiamin hydrochloride and 60,000 kg of thiamin mononitrate had been added to foods in 1970 by industrial respondents to the survey. The National Research Council estimated that the reported amounts represented only 60 percent of the actual industrial use. Corrected to 100 percent, the per capita daily intake of added thiamin hydrochloride was calculated to be no more than 0.8 mg and that of thiamin mononitrate 1.3 mg, for a total maximum daily intake of 2.1 mg. This amount probably represents a more reasonable estimate of the average daily intake of added thiamin than that calculated by the previously described technique.

A comparison of 1970 and 1960 usage of thiamin hydrochloride and thiamin mononitrate for respondents reporting data for both years indicates only a 1.2-fold increase in usage during the 10-year period.
<table>
<thead>
<tr>
<th>Food category</th>
<th>Thiamin Hydrochloride</th>
<th>Thiamin Mononitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weighted mean</td>
<td>Weighted mean</td>
</tr>
<tr>
<td></td>
<td>mg per kg</td>
<td>mg per kg</td>
</tr>
<tr>
<td>Baked goods, baking mixes</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Grain products such as pastas or rice dishes</td>
<td>6*</td>
<td>4</td>
</tr>
<tr>
<td>Milk products</td>
<td>33</td>
<td>8</td>
</tr>
<tr>
<td>Frozen dairy desserts, mixes</td>
<td>50*</td>
<td></td>
</tr>
<tr>
<td>Processed fruits, juices and drinks</td>
<td>3</td>
<td>3*</td>
</tr>
<tr>
<td>Meat products</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Poultry products</td>
<td>490*</td>
<td></td>
</tr>
<tr>
<td>Fish products</td>
<td>490*</td>
<td></td>
</tr>
<tr>
<td>Condiments, relishes, salt substitutes</td>
<td>48*</td>
<td></td>
</tr>
<tr>
<td>Soft candy</td>
<td>50*</td>
<td></td>
</tr>
<tr>
<td>Sweet sauces, toppings, syrups</td>
<td>7*</td>
<td>10*</td>
</tr>
<tr>
<td>Gelatins, puddings, fillings</td>
<td>2*</td>
<td>2*</td>
</tr>
<tr>
<td>Snack foods</td>
<td>3*</td>
<td>1*</td>
</tr>
<tr>
<td>Beverages, nonalcoholic</td>
<td>2*</td>
<td></td>
</tr>
<tr>
<td>Beverages, alcoholic</td>
<td>2*</td>
<td></td>
</tr>
<tr>
<td>Nuts, nut products</td>
<td>24*</td>
<td></td>
</tr>
<tr>
<td>Gravies, sauces</td>
<td>12*</td>
<td></td>
</tr>
<tr>
<td>Dairy products analogs</td>
<td></td>
<td>20*</td>
</tr>
<tr>
<td>Hard candy</td>
<td>10*</td>
<td></td>
</tr>
<tr>
<td>Chewing gum</td>
<td>24*</td>
<td></td>
</tr>
<tr>
<td>Baby baked goods</td>
<td></td>
<td>10*</td>
</tr>
<tr>
<td>Baby cereals</td>
<td>40*</td>
<td>1*</td>
</tr>
<tr>
<td>Baby formulas</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Baby combination dinners</td>
<td>3*</td>
<td></td>
</tr>
</tbody>
</table>

*Reports from fewer than 4 food processors.

Blanks in the table mean that the substance is not added to the foods indicated. Level of addition of thiamin hydrochloride, and thiamin mononitrate is the weighted mean of the levels reported by manufacturers as their usual addition to one or more products in a food category. For discussion of weighted mean see text, also Section X and Exhibit 50 of reference 10.
The intake of thiamin includes not only the amounts added to food products, but also the quantity found naturally in foods and that provided from vitamin supplements. This potential intake may be reduced by deterioration or destruction during food storage and processing. Cooking may cause considerable loss of thiamin. One-third to one-half of the thiamin in beef and pork may be lost by roasting. Similar losses occur during the cooking of vegetables. Storage of frozen meats for several months results in a 20 to 40 percent loss. Losses during canning depend upon the acidity of the food and the duration and temperature of processing. Retention of thiamin in canned fruit juice is high, but low in canned meats and vegetables (11). Sulfite (3000 ppm) added to an average meal containing approximately 0.7 mg thiamin destroyed 15 percent within 15 minutes and virtually all within 18 hours (12). However, the FDA does not allow sulfiting agents in meats or in food recognized as a significant source of thiamin [21 CFR 182.3798].

Estimates of thiamin requirements are based on the following: the level producing or alleviating deficiency symptoms; the level of excretion of thiamin or its metabolites; and the transketolase activity of the red blood cells, which is reduced before the appearance of any clinical signs of deficiency (13). The Food and Nutrition Board of the National Academy of Sciences has recommended an allowance of 0.5 mg thiamin daily per 1000 kcal for persons of all ages with certain exceptions (14). Because older persons may utilize thiamin less efficiently than younger adults, the Board recommended an intake of 1 mg per day even for those consuming less than 2000 kcal daily. The recommended level of 0.5 mg per 1000 kcal was considered adequate for lactating women, since their daily intake would increase because of the markedly higher caloric requirements during this period. Similarly, 0.5 mg per 1000 kcal was believed sufficient during the first 6 months of pregnancy, but an increase to 0.6 mg per 1000 kcal was recommended for the last trimester. These recommendations compare closely with those formulated by other nutritional advisory bodies (Table II) (15).

A nationwide survey in the spring of 1965 showed that the average diets for most sex, age and income groups approached (90 to 100 percent) or exceeded the RDA for thiamin. Only women between 15 and 17 and those over 65 years had diets containing less than 90 percent of the RDA (16).

More recent attempts to assess the nutritional status of the U.S. population were conducted by the National Center for Health Statistics during 1971-1974. The dietary intakes of more than 20,000 persons were analyzed according to their age, sex, race and income (17). The mean intake of thiamin was 1.28 mg per day. The mean intake for thiamin per 1000 kcal for all persons surveyed was 0.64 mg. The RDA of 0.5 mg per 1000 kcal was exceeded by every age, sex, race and income group. The lowest intake of any of these groups was 0.54 mg per 1000 kcal for white women, aged 15 to 17 in families with incomes below the poverty level.
<table>
<thead>
<tr>
<th>Subjects</th>
<th>Food and Nutrition Board, NAS</th>
<th>WHO/FAO</th>
<th>Committee for Revision of Dietary Standards, Canada</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>0.3 - 0.5</td>
<td>0.3</td>
<td>0.3 - 0.5</td>
</tr>
<tr>
<td>Children</td>
<td>0.7 - 1.2</td>
<td>0.7 - 1.0</td>
<td>0.7 - 1.1</td>
</tr>
<tr>
<td>Teenagers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.4 - 1.5</td>
<td>1.2</td>
<td>1.2 - 1.4</td>
</tr>
<tr>
<td>Female</td>
<td>1.1 - 1.2</td>
<td>0.9 - 1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.2 - 1.5</td>
<td>1.2</td>
<td>1.4 - 1.6</td>
</tr>
<tr>
<td>Female</td>
<td>1.0 - 1.1</td>
<td>0.9</td>
<td>1.0 - 1.1</td>
</tr>
<tr>
<td>Pregnant Women</td>
<td>+0.3</td>
<td>+0.1\textsuperscript{b}</td>
<td>+0.2</td>
</tr>
<tr>
<td>Lactating Women</td>
<td>+0.3</td>
<td>+0.2</td>
<td>+0.4</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Adapted from (15)
\textsuperscript{b}For latter half of pregnancy.
Absorption, distribution, and metabolism

Rindi and Ventura (18) in their review of the literature, concluded that thiamin is absorbed by a dual mechanism. At low, physiological concentrations, intestinal absorption in many animal species occurs as an active transport mechanism requiring sodium ion and adenosine triphosphate. Upon entering the intestinal mucosal cells, thiamin is phosphorylated to thiamin pyrophosphate (19) which is then partially dephosphorylated on the serosal side (20). When the concentration in the intestinal lumen is high, thiamin is absorbed by passive diffusion (18).

Using the everted sac technique, Schaller and Hölter (21) found the absorption of thiamin in the rat to decrease significantly from the proximal to distal segments. Absorption was more complete with low (1.5 μM per liter) than with high (30 μM per liter) concentrations of thiamin. These data support the concept of an active transport of thiamin which may be reduced or prevented when the transport receptors are saturated by high levels of thiamin in the intestine.

The concept that thiamin absorption is dependent upon saturable receptor sites is further supported by observations on human subjects (22). The percentage of thiamin excreted in the urine has been reported to vary inversely with the oral dose. Approximately 50 percent of a 1 mg oral dose, 36 percent of a 5 mg dose and 24 percent of a 20 mg dose were excreted in 72 hours. In malnourished alcoholic patients with fatty livers or cirrhosis, absorption was about one-third that of control subjects.

Thiamin is rapidly absorbed, as evidenced by a rise in the thiamin level of blood within 6 minutes after oral administration of 15 mg to normal human subjects (23). The thiamin pyrophosphate concentration rose concomitantly and continued to increase for 15 to 30 minutes, reaching and maintaining an elevated level longer than free thiamin.

Williams and Bissell (23) concluded from their study and a review of the literature, that most of the thiamin is phosphorylated before it is stored, with the liver and kidney being particularly active in this process. This phosphorylation is extremely rapid as is the removal of the thiamin pyrophosphate from the blood. When 15 mg thiamin were injected intravenously into normal subjects, the thiamin pyrophosphate level in the blood increased sharply and immediately. Within 6 minutes, the thiamin level had returned to normal.
The total amount of thiamin in the body has been estimated to be about 25 mg (24). The storage can be increased somewhat by repeated large doses, but there is a limit, not much above normal levels, beyond which no appreciable increase in stores results. The greatest concentrations of thiamin are found in heart, kidney, liver and brain with lesser, but significant, amounts in the spleen, lung, adrenals and muscle (11).

Thiamin pyrophosphate, also termed diphosphothiamin and cocarboxylase in the older literature, is necessary for normal carbohydrate metabolism. It serves as the coenzyme for two classes of enzyme-catalyzed reactions in which aldehyde groups are removed or transferred: (1) the decarboxylation of alpha keto acids and (2) the formation or degradation of alpha ketols (25). In the absence of thiamin pyrophosphate, pyruvate is not metabolized normally and the steep rise of blood pyruvate is a typical consequence of thiamin deficiency (26).

The fate of thiamin in the body has been investigated by a number of investigators using $^{14}$C- or $^{35}$S-labeled preparations. A large number of thiamin metabolites have been detected in the urine of animals given the vitamin by mouth. A minimum of 22 different catabolic products were reported from the pyrimidine moiety alone in the urine of rats and rabbits when $^{14}$C-pyrimidine-labeled thiamin was fed (27). In addition, at least a dozen unidentified degradation products of $^{14}$C-thiazole-labeled thiamin products have been reported by Iacono and colleagues (28, 29). Only a few of these have been identified, most notably 4-methyl-5-beta-hydroxymethylthiazole (HT) (30), which would be generated by hydrolytic cleavage between the methylene bridge and the thiazole ring of thiamin.

Labeled thiamin has also been administered to human subjects. Baker et al. (31) gave $^{14}$C-thiazole-labeled thiamin by mouth to a normal male subject and measured the radioactivity excreted in the urine, feces and breath. Approximately 32 percent was excreted in the urine within 10 days and 3.8 percent in the feces within 3 days. There was no detectable radioactivity in the expired air. The half-life of the vitamin was estimated to be 18 days. Similar results were obtained by Ariaey-Nejad and colleagues (32) in healthy young men who ingested thiamin labeled either in the thiazole or in the pyrimidine moiety. Again, no $^{14}$CO$_2$ was detected in the respiratory air and less than 5 percent of the dose could be recovered in a 5-day fecal sample. The half-time excretion varied from 9.5 to 18.5 days and seemed related to the dietary thiamin intake.

-10-
Acute toxicity

The acute toxic effects of thiamin salts have been determined in several species and by various routes of administration. Mice have proved to be the most sensitive species and monkeys the most resistant of the animals tested. The minimum lethal doses (mg per kg) of thiamin hydrochloride by intravenous injection are: mice, 125 (33); guinea pigs, 180 (34); rats, 250 (33); rabbits, 300 (33); and dogs, 350 (33). By subcutaneous administration the lethal dose is sixfold, and by mouth, fortyfold, that of intravenous injection (33). In monkeys, intravenous injection of 200 mg per kg elicited no toxic signs and 600 mg per kg were required to produce the first toxic manifestations (35). Death is due to depression of the respiratory center. With artificial respiration, dogs were able to tolerate blood levels of up to 120 mg thiamin per 100 ml blood, whereas 7 to 10 mg per 100 ml were otherwise lethal (36). The signs of thiamin poisoning are restlessness, labored respiration, vasodilation, cyanosis, muscular twitching, clonic convulsions and death by respiratory paralysis. No significant difference has been detected in the toxicity between thiamin hydrochloride and mononitrate (37). Oral LD₅₀'s are available only for mice and rats (Table III).

TABLE III

<table>
<thead>
<tr>
<th>Animal</th>
<th>&quot;Thiamin&quot;*</th>
<th>Thiamin Hydrochloride</th>
<th>Thiamin Mononitrate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>2450</td>
<td>3000</td>
<td>7000</td>
<td>38, 40, 44</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>&gt;5000</td>
<td></td>
<td>39, 41</td>
</tr>
<tr>
<td></td>
<td>6000</td>
<td></td>
<td>7000</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>8224</td>
<td></td>
<td></td>
<td>43</td>
</tr>
<tr>
<td>Rat</td>
<td>9500</td>
<td></td>
<td></td>
<td>39</td>
</tr>
</tbody>
</table>

*Salt form not stated

Short-term studies

Administration for several weeks of relatively massive oral doses of thiamin to animals causes few deleterious effects. Rats were given 500 to 1500 mg per kg (several thousand times their normal requirements) by stomach tube, 5 days weekly for 6 weeks. No apparent ill effects were noted in the general conditions of the animals. The organs were normal as were the urinary sediments and the various hematological and liver function indices. The higher dose depressed slightly the growth of both sexes although female growth was depressed more at the lower dose (41).
Brown (45) supplemented the basal diet of male, weanling rats for 2 to 6 weeks with 12 to 10,000 µg thiamin per g of diet (about 1.2 to 1000 mg per kg body weight per day) or up to several thousand times the daily requirement. No effect was detected upon the growth of the animals or upon the fat, nitrogen or moisture content of their livers. The thiamin level increased in all tissues examined except the adrenals, with the greatest concentrations found in the heart, kidney, liver and testes.

Kato (44) also studied the effect on growth of large supplements of thiamin to the diet of young rats. He fed 45-day-old male rats for 4 weeks a basal diet supplemented with 1, 10 or 50 mg thiamin hydrochloride per kg body weight. The weight gain of the rats receiving additional thiamin surpassed that of the controls during the first 2 weeks but returned to control levels by the end of the experiment. The biosynthesis of protein-bound lipoic acid in the liver mitochondria showed a similar pattern; an original increase was followed by a return to control levels within 4 weeks.

A somewhat longer experiment with older rats also failed to demonstrate any significant differences between control animals and those on fortified diets (46). Ninety-day-old female rats were fed ad libitum for 20 weeks an adequate control ration providing 4.5 mg thiamin per kg per day or a ration containing approximately 50 times this amount. The test group had normal hemoglobin, erythrocyte and leukocyte counts, a higher granulocyte count and a lower basal metabolism and weight gain than the controls. However, when all rats were restricted to an intake of 6 g of food daily, supplying about 2 and 100 mg thiamin per kg per day to the control and treated animals respectively, no difference could be detected in any of these measurements between the two groups.

Oral doses far in excess of physiological requirements have been administered to man in attempts to effect clinical improvements in various disorders. Cooper (47) in treating subacute necrotizing encephalopathy (Leigh's Disease) gave children 1 to 4 g of thiamin daily for as long as a year or more with no discernible ill effects. Lonsdale and Price (48) similarly treated four cases of different forms of encephalopathy with massive daily doses of thiamin. Three patients received 600 mg daily for 5, 6 and approximately 36 months while a fourth was given 2 g daily for 9 months. Kunstmann (49) treated 20 patients with chronic nephritis with daily doses of 150 mg thiamin alone or in combination with vitamin E. Improvement was claimed after total doses of 7.5 to 22.5 g (50 to 150 days). One of the patients received this treatment for 2 years with no reported side effects. A 12-year-old boy who developed symptoms typical of beri-beri received daily doses of 250 mg thiamin intramuscularly for 3 months followed by 250 to 500 mg daily by mouth for 23 months (50). Jones (51) states that severe cases of beri-beri heart disease require 300 mg and milder cases 30 to 90 mg thiamin hydrochloride daily. Vorhaus (52) could detect no untoward effects in "polyneuritis"
patients receiving as much as 270 mg daily for an unspecified period. Ruiz-Maldonado and Tamayo (53) gave 200 to 300 mg thiamin hydrochloride by mouth daily to children with papular urticaria for 1 to 6 months. An overall improvement of 88 percent was claimed with no reported side effects. Roch and Sciclounoff (54) gave daily subcutaneous or intramuscular injections of up to 50 mg thiamin to more than 200 patients with a variety of clinical conditions. The treatment was generally 2 to 3 weeks, but in some cases was "much longer." No untoward effects were reported.

The only report known to the Select Committee of human toxicity following oral administration of thiamin is that of Mills in 1941 (55). Toxicity was reported in a 47-year-old woman who had been taking 10 g of thiamin hydrochloride daily for 2 1/2 weeks. The signs and symptoms resembled those of overdosage with thyroid extract: headache, increased irritability, insomnia, rapid pulse, weakness and trembling. Within 2 days after discontinuance of the thiamin ingestion, the symptoms disappeared. After one week of rest, the patient resumed vitamin ingestion, taking 5 mg of thiamin hydrochloride daily. At this intake level for 4 1/2 weeks, a recurrence of the same toxic manifestations was observed. Mills also refers to "other patients with thiamin toxicity" observed in Panama on daily doses of 20 to 40 mg. He describes only one other case, in which a young woman, receiving an average of 17 mg daily, developed symptoms similar to those of thyroid hyperactivity, with fine and coarse muscle tremor, rapid pulse and nervous hyperirritability. This report is puzzling in view of the numerous cases in which much larger amounts of thiamin hydrochloride have been given without toxicity.

Reactions to thiamin have been recorded in some 200 cases in the world literature (56), nearly all following parenteral administration of 5 to 100 mg. Five cases of sudden death following intravenous or intramuscular injection of thiamin have been reported (57). Most of the patients in whom reactions were observed had previously tolerated parenteral injections of equal amounts of thiamin without untoward effects. A large percentage of the reactions occurred after four or more injections. One death occurred after sensitization to oral thiamin hydrochloride. A 55-year-old woman had taken 100 mg thiamin hydrochloride daily for 15 days. Two months later she ingested a 100 mg tablet and developed a pruritic rash, dyspnea and choking sensation. Treatment relieved the symptoms temporarily, but the patient died the following day (58).

Three cases of occupational dermatitis have been reported among women filling ampules with thiamin or vitamin solutions (59, 60). All showed positive patch tests for thiamin. In one case relapse occurred after ingestion of 200 mg thiamin and later after intracutaneous injection of 10 mg (60).
A 25-year-old man under treatment for suspected encephalitis and head injuries was treated with various drugs including thiamin. A pruritic rash which developed was demonstrated to be a thiamin allergy. Subsequent ingestion of thiamin caused prompt recurrence of the rash (61).

**Reproductive performance; teratogenesis**

Various studies have been conducted in animals to determine the effect on mother and offspring of large amounts of thiamin in the diet.

Mice were maintained on a commercial dog chow supplemented with 250 mg thiamin per kg of diet (62). The growing mice consumed an average of 625 to 750 μg of thiamin daily (about 50 to 75 mg per kg body weight). During lactation the thiamin intake of the mothers was approximately 2 mg per day (about 100 mg per kg body weight). Observations during three generations on this diet revealed no abnormalities of fertility or lactation.

A three-generation study was also conducted with rats with comparable findings (42). Male and female weanling rats were fed a stock diet supplemented with 300 mg per kg of thiamin hydrochloride or mononitrate. This represented a daily intake of approximately 30 mg thiamin per kg body weight or several hundred times the rats' requirements. Rats consumed this diet from weaning to 6 months of age. Growth, reproductive performance and necropsy findings were indistinguishable from those of control animals.

Groups of 12 female weanling rats were fed complete diets containing 75 mg per kg diet of thiamin hydrochloride, pyridoxine hydrochloride or both for 12 weeks (63). The estimated daily intake was 7.5 mg per kg body weight of the respective vitamins. The animals were mated to stock males and the mothers killed after the young were weaned. The high thiamin, pyridoxine or combination diets had no effect on weight gain or reproductive performance. The levels of solids, total lipids, riboflavin, pyridoxine, pantothenate and vitamin B₁₂ in the livers of the dams after parturition and lactation did not differ from those of controls. The liver thiamin concentrations in both mothers and offspring were markedly increased.

Shumacher et al. (64) fed five female rats 100 mg thiamin per kg diet (about 10 mg per kg per day) for 2 weeks, at which time they were mated. The rats were maintained on this diet throughout gestation and lactation. The average birth weight, number of young per litter, average weight of the offspring at weaning and vitamin requirements of the young did not differ significantly from the controls.
Telford et al. (65) studied the resorption rate in pregnant rats receiving by mouth 10 g thiamin hydrochloride per kg body weight or several hundredfold the normal requirement. Twenty-two days after positive mating, the rats were killed and the young delivered by caesarian section. Of 126 untreated rats, 40.8 percent had one or more resorptions, with 10.5 percent of all implantations terminating in resorption. Among 14 thiamin-treated animals, the comparable values were 69.2 and 9.4 percent, respectively. Similar results were seen with a variety of other substances including glucose and ascorbic acid. The investigators concluded that the resorption rate after thiamin did not differ significantly from the control series.

The lack of toxicity of such excesses of thiamin on reproductive performance suggested by these studies is at variance with earlier experiments of Sure (66) and Perla (67). Sure (66) in 1939 fed female rats on a stock diet containing an estimated 100 to 110 μg thiamin per day's ration, supplemented with daily oral doses of 10 to 400 μg thiamin. Sterility was observed in some of the second generation rats receiving 100 μg thiamin supplementation. A decrease in lactation efficiency occurred in the third generation when the diet contained an additional 200 μg thiamin daily for a total intake of about 1.5 mg per kg body weight. Rats showed no ill effects for two generations on 400 μg thiamin supplement daily by demonstrated a failure of lactation and a high infant mortality in the third generation when the added thiamin was increased to 800 μg (about 4.5 mg per kg daily).

Perla (67) also observed interference with lactation in second generation rats receiving about 150 μg (about 1.5 mg per kg) thiamin daily. Mollitor (39) claimed similar findings in experiments extending over four generations with "large" but unspecified doses. In subsequent studies, Perla and Sandberg (68) attributed this toxicity to inadequate dietary manganese. A daily intake of 2 mg manganese as manganese chloride completely neutralized during three successive generations the lactation impairment resulting from 400 μg (about 2 mg per kg) thiamin per day. Unna (57) suggests that the apparent toxicity of thiamin in such studies was more likely the result of dietary inadequacies than of thiamin excess.

Taylor et al. (69) increased the amount of thiamin in white Leghorn eggs 10 to 20 percent by injecting thiamin, dissolved in egg white, directly into the eggs before incubation. The 11- to 13-day chick embryos showed a modest increase in hemoglobin and relative brain size and a decrease in the relative size of the feet. The thiamin had no effect on the size of the heart or liver or upon the total embryo weight. Injection of thiamin dissolved in "toxic" media (olive oil, distilled water, saline, etc.) doubled or tripled the number of defective embryos. Defective brain and eye developments were the most common abnormalities.
The injection of 1 to 18 mg of thiamin hydrochloride, in a gum arabic emulsion, into the yolk sac of eggs produced no changes in the embryonic heart (70). Relatively minor and reversible changes were observed in the kidneys. Sekiya (71) injected 100 μg thiamin hydrochloride into eggs on the fifth day of incubation. No malformations were detected upon examination of the embryos after 6 to 18 days of incubation.

Carcinogenesis

Male mice were implanted with Crocker sarcoma 180 and daily paratumor injections of 0.5 or 1.5 mg thiamin begun 6 days later. The lower dose was reported to accelerate tumor growth from the 11th day after implantation, while the larger dose retarded growth (72).

Roe (73) gave "101" strain mice 0.2 percent thiamin in their drinking water, together with semiweekly subcutaneous injections of 2.5 mg thiamin. Skin papillomas were induced with dimethylbenzantracene or benzo[a]pyrene. The thiamin administration exerted no discernible effect.

Di Grazia (74) reported that 0.2 mg thiamin administered thrice weekly (orally?) to rats hastened the appearance of papillomas induced by the simultaneous topical application of benzo[a]pyrene. Scott (75), on the other hand, claimed that feeding 0.5 mg thiamin hydrochloride per week prevented the formation of tumors in rats treated with dimethylbenzantracene topically.

Special studies

Unna (57) has summarized the pharmacological effects of thiamin when administered parenterally in massive doses. Upon rapid intravenous injection of 5 to 50 mg per kg to cats and dogs, the respiratory center is depressed together with a transient and dose-related fall in blood pressure. At high concentrations, thiamin inhibits the contraction of the isolated intestine and the rise in blood pressure normally induced by nicotine. In very large doses, thiamin inhibits cholinesterase, depresses ganglionic action and neuromuscular transmission and may produce bronchoconstriction.

Liver homogenates of male and female rats receiving 2.0 mg (about 10 mg per kg) thiamin daily metabolized aniline, zoxazolamine and aminopyrine at significantly lower rates than did homogenates from thiamin-deficient rats (76). Similarly, heptachlor metabolism was decreased in male rats receiving a high thiamin diet (77). Grosse and Wade (76) attribute the reduced metabolism to the observed reduction in the activity of hepatic microsomal cytochrome b₅, cytochrome P-450 and nicotinamide adenine dinucleotide phosphate-cytochrome c reductase activity.
Thiamin exhibits an antithyroid effect both in vitro and in vivo similar in some aspects to that of propyl thiouracil (78). The presence of 0.001M thiamin reduced the uptake of $^{131}$I by calf thyroid slices. A high thiamin diet (about 100 mg per kg body weight daily) inhibited the formation of organic radiiodine compounds in rats. There was no enlargement of the thyroid.

Thiamin hydrochloride did not exhibit mutagenic activity in microbial assay, either with or without metabolic activation, using Saccharomyces cerevisiae strain D4 and Salmonella typhimurium strains TA-1535, -1537, -1538, -98, and -100 in both plate and suspension tests (79).
V. OPINION

Thiamin* (thiamine) salts have been administered to man for months in daily doses up to 1 g or more without reported adverse effects, except for the development of sensitivity in rare cases. This dosage is several hundred times the estimated intake of thiamin hydrochloride and thiamin mononitrate added to foods. Most cases of sensitivity were induced by previous topical or parenteral exposure to thiamin.

Similarly, mice and rats fed daily for three generations with several hundred times their normal requirements of thiamin showed no adverse effects.

Absorption of orally administered thiamin is regulated by a transport mechanism which offers an effective protection against overdosage. Excess thiamin in the tissues is rapidly excreted in the urine.

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

There is no evidence in the available information on thiamin hydrochloride or thiamin mononitrate that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used at levels that are now current or that might reasonably be expected in the future.

*This spelling has been recommended by the International Union of Nutrition Sciences and by the American Institute of Nutrition (1).
VI. REFERENCES CITED


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