THE THIAMIN CONTENT OF CERTAIN FOODS CONTAINING ADDED WHEAT GERM

by

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INTRODUCTION

The deficiency of vitamin B_1 or thiamin in the average American diet is becoming more and more a matter of concern among nutrition workers. The people of the United States, for the most part, are living on diets which afford only a small margin of safety in their thiamin content. This safety margin, although sufficient to prevent beriberi in its true form, does not prevent the subclinical symptoms due to partial vitamin B_1 deficiency or to its prolonged inadequacy. These conditions are rarely recognized, yet they are widespread and important.

The preference shown for highly refined foodstuffs has decreased the intake of thiamin more than 50 percent in the past century. Since the discovery that wheat germ is one of the richest natural sources of this vitamin, there has been an increasing trend toward its incorporation into the diet. Therefore, it seemed desirable to determine, by biological assay, whether the addition of wheat germ to certain foods increases their thiamin content sufficiently to justify advocating its use as a means of increasing the level of vitamin B_1 in the dietary.

REVIEW OF LITERATURE

The vitamin theory, as it is known today, was first conceived and developed from studies made on the disease called beriberi, which for many years had ravaged the population of the eastern Asiatic countries. The first evidence that the disease was in any way related to the diet was presented by Takaki (51), a medical officer in the Japanese Navy. He almost completely eradicated beriberi from the Navy by the substitution of a moderate amount of meat, barley and vegetables for a portion of the rice which had previously constituted a substantial fraction of the food.

In 1897, Eijkman (13), appointed by the Dutch Government to study the cause and seek a cure for the disease, produced in fowls a paralytic condition, now known as polyneuritis, which he recognized as similar to beriberi in man. Although he brought about this condition by feeding an exclusive diet of polished rice the thought that there might be something lacking in the diet which would prevent the disease did not occur to Eijkman. He believed beriberi to be caused by a toxin which was counteracted by some substance present in rice polishings. According to Williams and Spies (57), the work of Eijkman was extended by Grijn who, in 1901, demonstrated that the paralytic condition in fowls was due to some substance which was present in the whole rice grain but absent from the kernel. This discovery marked the beginning of vitamin history which has made such rapid progress during the part three decades.

In 1911 Funk (18) publicly announced that he had isolated a substance from rice polishings which was active in curing polyneuritis in pigeons. He called this substance "vitamine" because he thought it was an amine which was essential for life. McCollum (33), in 1913 while analyzing the dietary requirements of the rat, found that there were some factors necessary for the growth of the rat, which, as yet, were unidentified. He proposed the name "water-soluble B" for one of these factors and indicated that, on the basis of similarity of distribution in foodstuffs, this substance might be identical with Funk's "vitamine". This factor, isolated by Funk and described by McCollum, was thought to be a single substance which Drummond later called vitamin B. In 1920 Emmet and Luros (15) suggested the dual nature of the vitamin and six years later Smith and Hendrick (47) proved that the substance contained at least two essential factors. Jansen and Donath (21) in 1927 announced the isolation of the anti-beri-beri vitamin and prepared it in pure crystalline form.

Williams with his associate, Cline, (56), after elucidating the chemical structure, in 1936 accomplished the synthesis of the compound. The synthesis of vitamin B_1 was a great step in the advancement of the science of nutrition, one which opened the way for controlled and extensive research. McCollum's "water-soluble B" is now separated into vitamin B_1 and the vitamin B_2 complex which includes riboflavin, nicotinic acid, pyridoxine, and pantothenic acid, all of which are clearly differentiated as to chemical properties and specific physiological functions.

Vitamin B_1 is a derivative of pyrimidine. It contains the thiazole nucleus, which is otherwise unknown in nature. It is the only one of the known vitamins which contains sulfur. It was this characteristic that caused the name thiamin to be adopted as a substitute for the term vitamin B_1. The empirical formula for the vitamin is $C_{12}H_{18}ON_4SCl_2$. It is a white crystalline substance, water soluble, and has a moderately bitter, slightly salty taste.

Being the thermolabile part of the B complex, thiamin is destroyed by heat, but, according to Williams (54), only in alkaline or neutral solution. He believed that the heat merely aggravated the splitting tendencies. Kinnersley, O'Brien and Peters (26) have shown that hot alkalies split off the sulfur as hydrogen sulfide. Besides its sensitivity to alkalinity, thiamin is also destroyed readily by sulfite. Morgan (35) found a significant loss in the vitamin B_1 content of dried fruits as a result of treatment with sulfur dioxide in the process of preparation. That the vitamin is not sensitive to atmospheric oxidation was noted by Williams and Spies (57). In solution it is oxidized to thiochrome by various reagents. Kinnersley, O'Brien, and Peters (27) have shown that blue fluorescent oxidation-products of pure thiamin are produced by the action of permanganate and manganese oxides at pH values more acid than pH 6. Lipmann (31) reduced thiamin by treating it with hydrosulphite. He was of the opinion that the mode of reaction with hydrosulphite may be indicative of the function of thiamin in the living cell. The vitamin was completely lost when Malcolm (32) treated it with formaldehyde.

Williams (54) stated that the reactions of thiamin are important for three reasons:

> They may result in the loss of the natural supply by improper methods of distributing and preparing foods; they may serve as a basis for the devising of adequate chemical methods of assaying foods, concentrates, and body secretions; they are of interest in the interpretation of the mode of functioning of the substance in living tissues.

Due to the rapid advances in the study of the physiological role of thiamin it has become necessary to develop means whereby quantitative estimations may be made. A number of chemical as well as biological tests have recently been proposed to make possible the determination of the thiamin content of various biological materials.

Jendrassik (22) observed that solutions containing thiamin reduced ferric ferricyanide to form a blue colored solution which he thought owed its color to the presence of ferric ferrocyanide. It was found on further investigation that the color was given by the ortho and polyphenols. Barger et al. (2) oxidized thiamin with alkaline ferricyanide to form a pale, yellow-blue fluorescent compound known as thiochrome, which shows an intense blue fluorescence in neutral or alkaline solutions. Jansen (20) proposed using the intensity of the blue fluorescent light of thiochrome as a means of quantitative estimation after it had been extracted with isobutyl alcohol.

Kinnersley and Peters (25) observed that, by adding diazotized sulfanilic acid to a solution containing thiamin in the presence of formaldehyde, a red color is produced. Prebluda and McCollum (40) obtained a red pigment from thiamin and alkaline diazotized p-aminoacetophenone, which was stable and insoluble in water. Villera and Leal (52) observed that ammonium molybdate in sulfuric acid solution gave with vitamin B_1 an intense blue color. They used this method for the estimation of vitamin B_1, the color intensity being determined photometrically.

In an attempt to determine quantitatively the thiamin content of various organic materials by biological assays the problem has been approached from many different angles. Probably the first method of assay was that of Kinnersley and Peters (24). They used the pigeon curative test based on the alleviation of acute head retraction and other polyneuritic symptoms after the administration of the test material in graded doses to pigeons made severely neuritic by restriction to a vitamin B_1 free diet. Young chicks were used by Kline and Keenan (28) in a quantitative determination of the vitamin. The lowest level of test sample which was capable of protecting chicks from polyneuritis was determined. This amount of food was regarded as equivalent in thiamin value to the amount of pure thiamin which would accomplish the same results.

Chase and Sherman (6) estimated the amount of vitamin B_1 in foods by determining the smallest amount of the assay material which, when added to the basal diet, would maintain growth in young rats at a rate of 3 gm. per week during a period of 4 to 8 weeks. Chick and Roscoe (7) placed rats on a thiamin free diet at time of weaning. When growth ceased the material to be tested was added to the diet in graded doses for different groups of animals and the amount which restored growth at the rate of 10 to 14 gm. per week was determined.

Smith (46) devised a curative test. With this method rats were placed on a basal diet until a polyneuritic state was reached, then the minimum dose which would effect a cure was administered. Kline, Tolle, and Nelson (29) modified the technique used by Smith. They changed the basal diet making it more complete except for vitamin B_1. They also made a change in the method of interpreting the response of the animal to a test dose. That rats on diets deficient in vitamin B_1 had low heart rates was found by Birch and Harris (4). Rapid cures resulted on administration of vitamin B_1, the increase in heart rate being proportional to the amount of thiamin given.

Schultz and Atkins (43) described a fermentation test for vitamin B_1 based on the "powerful action exerted by the vitamin on the rate of alcoholic fermentation." The method involved measurements of the rate of gas evolution and, with a suitable sugar-salt buffer mixture, as little as one microgram of the vitamin could be detected. The same workers (44) found that the growth of Staphylococcus aureus is proportional to the amount of vitamin B_1 in the culture media and proposed this as a basis for a method of measuring the vitamin.

An accurate chemical procedure for the determination of thiamin would be more rapid and more convenient than biological tests. However, none of the proposed chemical methods have been perfected to a degree that the results compare satisfactorily with the results obtained from the biological assays.

Thiamin, unlike most of the other vitamins which are essential for the well being of a given organ or group of cells, has a more generalized function in the body. Being associated with the metabolism of carbohydrate a deficiency of thiamin causes many disorders because it impairs a metabolic process which is essential to all the cells in general. The role of thiamin in carbohydrate metabolism is not definitely established at the present time. In a review of the functions of the vitamin B complex in nutrition, Elvehjem (14) stated that, on entering the body, thiamin is changed to cocarboxylase which functions as a coenzyme for the metabolism of pyruvic acid. In other words, the process of carbohydrate metabolism which normally proceeds from glucose to lactic acid and pyruvic acid and thence to carbon dioxide and water is, in the absence of cocarboxylase, arrested at the pyruvic acid stage, resulting in an accumulation of toxic substances in the body.

Changes in the nervous system occur in experimental animals deprived of thiamin causing an inability to maintain equilibrium followed by paralysis and finally death. Whether these symptoms are due to degeneration of nerve tissue or to an accumulation of metabolic products, particularly pyruvic acid, is not known. Williams and Spies (57) found degenerative changes in the peripheral nerves, the sciatic nerves being particularly affected in animals on thiamin-deficient diets.

Birch and Harris (4) found bradycardia so common among animals deprived of the vitamin that they are now using that symptom as a method of assay. Cowgill (8) observed that vitamin B_1 has no specific influence on the normal heart; only in the deficient organism does administration result in a demonstrable effect.

Thiamin has another important role in the maintenance of appetite. Cowgill (8) has concluded that the appearance of anorexia is, in most cases, the first definite sign that a state of deficiency exists. He also stated that the urge to eat can be restored either by introducing thiamin directly into the blood stream or by giving it orally.

Williams and Spies (57) found that besides the profound effect of thiamin on the appetite it also promotes the efficient utilization of food thus exerting an influence on the promotion of growth.

Fein, Jolliffe, and Ralli (17) studied a group of diabetic patients, nine of whom had symmetrical peripheral neuropathy characteristic of the peripheral neuropathy found in subjects having proved vitamin B_1 deficiency. Treatment by daily administration of 10 mg. of thiamin hydrochloride by mouth, without otherwise changing the regimen, resulted in the cure of eight of the patients and improvement in the condition of the ninth.

Williams et al. (53) recently published the results of an experiment on human subjects on diets low in thiamin. The deficiency did not produce beriberi as there was no evidence of edema, cardiac dilatation, or neuritic pain. However, a mild tenderness of the muscles of the calves, paresthesia of the feet and legs, and diminution of tendon reflexes were observed. The early stages of the condition induced by the restricted intake of the vitamin closely resembled neurasthenia, the later stage simulated anorexia nervosa. The authors suggest that states of thiamin deficiency such as these exist in our part of the country where few cases of beriberi are encountered and should be looked for in cases diagnosed as neurasthenia. Favorable responses to treatment with thiamin have been reported in a wide diversity of disordered conditions. Williams and Spies (57) said,

> In vain have we endeavored to find the specific effect of a deficiency of the substance and yet it is credited with almost panacean properties. The reason is that carbohydrate metabolism cannot go forward in any living cell without thiamin.

Although widely distributed in common foodstuffs, thiamin is present in greater abundance in certain classes of foods. The whole grain cereals, dried legumes, nuts, and lean pork are the most important natural sources. Mickelsen, Waisman, and Elvehjem (34) found that although as much as 50 percent of the thiamin content of pork is destroyed during the cooking process, it is still an excellent source of the vitamin. They stated that, when used to the extent of 7 percent of the diet, meat may supply one-third of the daily requirement for this vitamin. Cowgill (11) found oatmeal to be the richest of the cereals in its thiamin content. The mean value was 300 International Units per 100 gm.

Williams and Spies (57) ranked cereals first in importance as a source of thiamin in the human diet. The high starch and low water content together with the fact that they may be purchased at a low cost makes them one of the most popular sources of calories. These authors classified foods, with regard to thiamin content, as follows:

1. Rich in B_1 - seeds, pork, milk, internal organs.
2. Poor in B_1 - highly milled grains, white bread, macaroni, and sugar.
3. Indifferent foods - fruits and vegetables.
4. Fats - which do not require B_1 for metabolism.

Booher and Hartzler (5) divided food sources of thiamin into four groups:

1. Excellent - containing 150 I. U. per 100 gm.- oatmeal, dried lima beans, peanut germ, lean pork, soybeans.
2. Good - containing 100 to 150 I. U. per 100 gm.- cornmeal, egg yolk, milk powder, rye, walnuts, whole wheat.
3. Fair - containing 30 to 100 I. U. per 100 gm.- lamb, liver, beef, potatoes, asparagus, and broccoli.
4. Poor - containing less than 30 I. U. per 100 gm.- fish, milk, fruits, and vegetables.

The amount of thiamin contained in the various foods has usually been reported on the basis of that food in the raw state. The vitamin may be greatly affected by the method of preparation. Aughey and Daniels (1) found no loss in boiling carrots, navy beans, or rolled oats. They observed a 20 percent loss in baked and boiled potatoes; 59 percent of the vitamin content was lost when beans were boiled with sodium bicarbonate, and only 18 percent when boiled without sodium bicarbonate. Peas lost 9 percent by destruction and 11 percent by solution. Pork lost 15 percent when braised; 43 percent when roasted. Munsell (38) noted little loss of thiamin in foods during the process of canning. The only loss was due to preparation before the foods were put into cans. She also observed that the vitamin is preserved in frozen foods but it is more easily extracted in cooking than from fresh foods. Parker (39) found no significant loss of the vitamin in bread during the baking process. Baking powder biscuits, on the contrary, suffered a considerable loss. This is due to the alkalinity of the baking powder which tends to accelerate the loss of thiamin at baking temperatures.

Since the human body is unable to store any significant amount of thiamin it is necessary, in order to maintain optimum nutrition, that the diet regularly supply an adequate amount of this substance. For the normal adult the thiamin requirement is related to the caloric intake, particularly to the calories obtained from carbohydrates. Cowgill (9) stated the minimum daily requirement of an adult as approximately 300 to 350

International Units which is equivalent to about 1 mg. of pure thiamin. This amount is equal to 10 International Units per 100 Calories daily. The requirement increases with an increase of energy expended. Preschool children need as much as 20 to 25 International Units per 100 Calories. Williams (55) placed the minimum requirement for adults as 0.6 mg. or 200 International Units per day for a 2500-Calorie intake. Becker (3) found the minimum requirement for adults to be 400 to 500 International Units. He saw no reason for increasing the requirement for children above that level. Elvehjem (14) observed that animals on high fat diets required less thiamin than those on high carbohydrate rations. He said that the normal adult, on the high carbohydrate diet which is so prevalent, requires 1 to 2 mg. of the vitamin daily. An editorial in the Journal of the American Medical Association (12) stated that, while soldiers were kept in good condition on 300 International Units of thiamin daily, an increase in alertness was observed and the capacity for physical work was almost doubled when the intake was increased to 600 International Units per day.

Rose (41) observed that the requirement for children increased with the rate of growth and the amount of energy expended. She stated that the minimum requirement for children is somewhere between 13 to 46 International Units per 100 Calories. Morgan and Berry (36) found a marked influence on the growth of children when the thiamin content of the diet was increased above the ordinary requirement for children.

Special studies of the thiamin requirement of rats during pregnancy and lactation have been reported by Evans and Burr (16) and by Sure (50), who observed that a more generous supply is necessary for proper lactation than is required for ordinary conditions. Williams and Spies (57) stated that the nursing mother should receive at least 5 mg. of thiamin daily and the infant should receive 0.5 mg. per day. He also stated that the intake of persons suffering from fever, gastro-intestinal disorders, hyperthyroidism, and other conditions should be distinctly above the amount recommended for the individuals in normal conditions.

Although the American diet is one of the best in the world it is still far from being optimal for maximum health. The most prevalent deficiency seems to be a lack of vitamins and minerals. Scott and Herman (45) observed that the diets of farmers in the southern states were extremely inadequate in thiamin. Although they produce chickens, eggs, milk, and various kinds of vegetables, they sell these and buy polished rice as their staple cereal. The occurrence of beriberi is not uncommon among this particular group. Muller (37) found that the average diet falls far short of the established minimum requirement for vitamin B_1. He suggested that the excessive use of sugar and meat should be discouraged and more potatoes, legumes, and fruits eaten in the place of white flour and pastries. After studying the American diet Jolliffe (23) concluded that "a 55 percent fraction of the calories of the American diet of 1840 containing a minimum of 600 I. U. of thiamin has been replaced in a contemporaneous diet by a like fraction containing only about 50 I. U." Rubenstone and Meranze (42) also studied the American dietary in regard to thiamin content and found that the intake of the normal adult rarely exceeds the minimum of 300 International Units per day and often falls below the necessary level. They found inadequacy of the vitamin in the diet especially frequent in association with poverty, improper dietary habits, and organic diseases. The same workers made an analysis of hospital diets and observed that none of these exceeded the minimum requirements; most totaled two-thirds of the minimum, and a few were as low as one-third of the minimum. Patients on reduction diets, diabetics, and those suffering from cardiac disturbances were receiving diets very inadequate in their thiamin content. Williams et al. (53) observed that routine hospital diets contain approximately 0.6 to 0.8 mg. of the vitamin and special diets often contain even less. They are of the opinion that, when the nature of the patient's disease is such as to cause the doctor to reduce certain kinds of food in the diet, supplements should be prescribed in order that all essentials are supplied in the required amounts.

Although much has been written regarding the low thiamin content of the average American diet, little has been accomplished in the way of a practical solution of the problem. The American Medical Association Council on Foods (11) recently approved of the fortification of cereals, both the ready-to-eat variety and those eaten after cooking. The cereal product having the highest thiamin value is oatmeal which contains about 300 International Units per 100 gm. of dry material. The addition of thiamin to cereals up to this level would be in agreement with the Council's policy. Rose (41) found the foods which form a major source of energy in the diet lacking in thiamin. She has stated that a change from refined to whole grain cereals would increase the thiamin intake 100 percent. The same results might be accomplished by adding one-half ounce of wheat germ to refined cereals. The English Government, recognizing the improvement in soldiers fed a diet high in thiamin, has passed a law demanding the fortification of all bread flour. This will raise the vitamin intake 180 International Units from this source when the diet includes 100 gm. of bread. Parker (39) suggested that a similar law be passed in our country whereby millers would be obliged to bring the thiamin content of patent flour up to the level of whole wheat. Each ounce of whole wheat has a potential value of 45 International Units of this vitamin and each ounce of average patent flour a value of about 5

International Units. For restoration, about 40 International Units per ounce or 125,000 International Units per barrel will be necessary. This latter amount of vitamin B_1 is slightly more than one-third of a gram of pure thiamin crystals.

The literature contains little concerning the enrichment of the diet with thiamin. However, in the light of the growing knowledge of the importance of this factor and the definite benefits resulting from an increased intake in a variety of conditions, every effort should be made to enrich the diet in a practical way so that at least the minimum of 300 International Units is assured everyone including those on low cost dietaries.

PROCEDURE

The food samples tested were cooked cream of wheat (unfortified) and meat loaf made according to standard recipes, and the same products supplemented with wheat germ.

Cream of wheat was prepared according to the following formula:

Cream of wheat	120 gm.
Salt	10 gm.
Water	616 gm.

This was prepared by adding the cereal and salt to the boiling water and cooking for 15 minutes in a double boiler. The supplemented cereal was made in the same way, with 50 gm. of the cereal replaced by an equal weight of wheat germ.

The meat loaf was prepared as follows:

Ground beef	250 gm.
Ground pork	125 gm.
Egg	50 gm.
Cracker crumbs	50 gm.
Tomato juice	60 gm.
Salt	10 gm.

The ingredients were mixed lightly, formed into a loaf and baked for one hour at 350° F. The supplemented meat loaf was made according to the same recipe, with the cracker crumbs replaced with an equal weight of wheat germ.

Biological assays of the thiamin content of these food samples were carried out according to the rat curative method of Nelson, Kline, and Tolle (29). The basal diet of the experimental animals was adequate in all factors now known to be required by the rat with the exception of vitamin B_1. It contained the following ingredients:

	percent
Sucrose	61.25
Purified casein	18.00
Osborne and Mendel salt mixture	4.00
Autoclaved yeast	4.00
Cod liver oil	2.00
Autoclaved peanuts	10.00
Purified liver extract	0.75

Sucrose was chosen as the carbohydrate because Guerrant, Dutcher, and Brown (19) found that difficulties due to refection and coprophagy were minimized when sucrose was used. The casein was purified by extracting with 60 percent alcohol twice and filtering, then washing it with 95 percent alcohol to absorb the moisture. The purified casein was then dried at 50° C. and finely ground. The yeast was prepared by making a paste of 100 gm. of yeast and 125 cc. of 0.1 N solution of NaOH and autoclaving for six hours under 15 pounds of pressure. This product was then dried and finely ground. The autoclaving destroyed the thiamin but the thermostable part of the B complex was unaffected, thus retaining riboflavin, nicotinic acid, pyridoxin, and pantothenic acid. Raw shelled peanuts were ground finely and autoclaved for six hours, dried and ground with the sucrose before the other constituents of the diet were added. The peanuts served as a source of vitamin B_4 which is necessary for the prevention of a specific nutritional paralysis in rats. The liver extract was purified by reprecipitation. It was first dissolved in water; ethyl alcohol and ether were added, and the material was filtered. The residue was redissolved in water, reprecipitated and again filtered. After a third precipitation the final residue was dissolved in water and dried on a small amount of casein. This preparation served as a source of factor W, essential for the growth of rats.

Litters of young rats 12 to 13 days old were placed with their mothers on wire screens in suitable cages and fed the depletion diet. When the young rats had attained a weight of 40 to 50 gm. they were placed in individual cages with elevated wire screen bottoms to prevent access to excreta. The animals were weighed weekly until they began to show a decline in weight. Then they were weighed daily and observed for symptoms of polyneuritis which occurred 25 to 50 days after weaning.

Polyneuritis is a condition in which the animal suffers from extreme contraction of the musculature. There are three stages of this disease generally recognized, viz., the slight, the acute, and the severe. In the case of the slight polyneuritis, the animal is rotated by the tail and displays, upon being dropped to the table, a slight paralysis and an inability to regain his equilibrium. However, recovery follows after a few seconds. In the second or acute stage the animal displays signs of convulsions on being rotated. Recovery does not follow for several seconds. In the severe stage the animal is extremely convulsive and is unable for a long period of time to control his movements. Animals in the second stage are preferred for the test.

Ninety six rats in the acute stage of polyneuritis were standardized by injecting into the hind leg muscles 0.1 cc. of a standard solution containing 6 micrograms or 2 International Units of thiamin. This amount of the vitamin brought about a complete cure in the animals. They gained from 4 to 8 gm. during the period of recovery and remained cured from 6 to 15 days.

Upon the occurrence of a second attack of the disease a sample of the food to be tested was fed. That amount of the sample which effected the same curative response as 6 micrograms of pure thiamin was assumed to contain 2 International Units of thiamin. Cream of wheat without added germ was fed as a cooked cereal (in amounts ranging from 6.0 to 10.0 gm.), a dry cereal (9.0 and 10.0 gm.) and as a concentrated alcoholic extract (equivalent to 10 gm. of dry cereal) to 12 rats. Thirty four samples of cream of wheat with added germ were fed to 21 rats at levels ranging from 2.0 to 6.0 gm. Twenty five samples of meat loaf were fed to 21 rats at levels ranging from 1.75 to 3.0 gm. Thirty three samples of meat loaf containing added wheat germ were fed to 27 rats in amounts varying from 1.0 to 3.0 gm.

RESULTS

The cream of wheat without added wheat germ fed as a cooked cereal, a dry cereal, and a concentrated alcoholic extract failed to relieve the polyneuritic symptoms of thiamin deficient rats and the animals succumbed to the disease. Hence, it was concluded that this product contained too small an amount of thiamin to be measured by this method.

The rats receiving 2 and 2.5 gm. of cream of wheat with added germ were not cured of the polyneuritis but when levels of more than 3 gm. were fed the symptoms disappeared within 24 hours. Those animals receiving 3 gm. remained in good health for only a few days. Those receiving 3.5 gm. exhibited a curative response equal to that produced by 6 micrograms or 2 International Units of the thiamin standard, as shown in Table 1.

Table 1. Curative response of thiamin-deficient rats to 6 μg. of thiamin and to 3.5 gm. of cream of wheat.

Animal number	Curative response to 6 μg. of thiamin	Curative response to 3.5 gm. cream of wheat with added germ
	days	
631	15	16
633	10	7
634	10	14
637	12	9
643	7	13
645	12	10
648	10	9
654	14	10
Average	11.2	11.0

Amounts of less than 2.5 gm. of meat loaf containing cracker crumbs failed to restore the animals to normal health, but when amounts ranging from 2.5 to 3.0 gm. were fed there was complete recovery and the animals showed gains in weight, during the recovery period, varying from 5 to 10 gm. Those receiving 2.5 gm. exhibited a curative response equal to that produced by 6 micrograms of the thiamin standard, as shown in Table 2.

Table 2. Curative response of thiamin-deficient rats to 6 μg. of thiamin and to 2.5 gm. of meat loaf containing cracker crumbs.

Animal number	Curative response to 6 μg. of thiamin	Curative response to 2.5 gm. of meat loaf containing cracker crumbs
	days	
881	11	13
885	13	12
886	11	11
980	11	11
912	12	10
978	8	8
979	6	6
999	9	10
Average	10.1	10.1

All the animals receiving 1 gm. and more of meat loaf containing wheat germ recovered from the polyneuritic symptoms and showed gains in weight from 5 to 8 gm. Those fed 1 gm. showed a curative response equal to that produced by 6 micrograms of the thiamin standard, as shown in Table 3.

Table 3. Curative response of thiamin-deficient rats to 6 μg. of thiamin and to 1.0 gm. of meat loaf containing wheat germ.

Animal number	Curative response to 6 μg. of thiamin	Curative response to 1.0 gm. of meat loaf containing wheat germ
	days	
876	15	14
878	16	15
879	11	12
881	11	12
886	11	11
981	7	6
996	10	11
1001	8	9
Average	11.1	11.2

Based on the experimental findings, Table 4 shows the thiamin content of the food samples tested, with and without added wheat germ.

Table 4. Thiamin content of cream of wheat and meat loaf, unsupplemented and with wheat germ.

Test sample	μg. of thiamin per gm. of food without wheat germ	μg. of thiamin per gm. of food with wheat germ	Difference in thiamin (μg. per gm.)
Cream of wheat	0.0	1.7	1.7
Meat loaf	2.4	6.0	3.6

It is to be concluded that the addition of wheat germ to such foods as cereals and meats increases the thiamin content considerably. In order that these figures may be practical for general purposes it is necessary to express them in terms of household measures. A 100-gram serving, or one-half cup, of cereal made according to the previously stated formula with added wheat germ supplies 170 micrograms or 57 International Units of vitamin B_1 which is approximately one-seventh of the estimated day's requirements. A 100-gram serving of meat loaf prepared by the recipe previously mentioned with wheat germ added to replace the cracker crumbs would furnish 600 micrograms or 200 International Units of thiamin which is one-half the estimated daily requirement for this vitamin. Supplementing such foods as cereal and meat loaf with wheat germ also adds a pleasing flavor to the finished product. The experimental animals consumed the reinforced samples more quickly as if they preferred them to the unsupplemented ones.

DISCUSSION OF RESULTS

The most commonly used methods for the quantitative determination of the vitamin B_1 content in foods are the bradycardia, the rat growth, and the rat curative technics.

The bradycardia or heart rate method, while it appears to be reasonably reliable, involves the use of the electrocardiograph and experience and skill in operating it. Since this is not always available the bradycardia method has proved impractical for general use.

The rat growth method is considered by Munsell (38) to give the most accurate results because the rats used in this method are healthy and well able to utilize the thiamin in contrast with the depleted rats used in the determination by the curative method. However, growth is not a response to a specific stimulus but involves the consideration of a number of factors. If the vitamin B_1 deficient diet did not contain optimal quantities of all other nutrients the results obtained would not be a true picture of a thiamin deficiency but might be due in part to the lack of some other factor which is necessary to promote growth. If this other factor were present in the test sample, the increase in growth would not result from thiamin alone. Hence, until all the nutritional requirements of the rat can be supplied with certainty in a synthetic diet, weight gain cannot be considered a sufficiently specific criterion for the assay of thiamin.

The curative method has been criticized on the grounds that since most of our common foods contain only a small proportion of vitamin B_1 it is difficult to get polyneuritic animals to eat enough of such foods to render the method applicable. However, Williams and Spies (57) found the rat curative the most trustworthy of all methods because it is the most specific. The rat curative technic is the official testing method specified in the United States Pharmacopeia and is believed to be the most accurate. This method has the advantages of speed and convenience over the growth test. Animals may be kept several weeks on the verge of polyneuritis by careful adjustment of the thiamin intake and then only three or four days are required to develop polyneuritis when they are wanted for the test. Rats may be used repeatedly for successive assays by using this method.

After considering these three methods the latter was chosen for this problem because it is the most specific. To cure polyneuritis in an animal with a definite amount of assay material gives an accurate means of computing the thiamin content of that particular food.

The problem was not underway very long, however, until it was discovered that this method has its difficulties. It has been shown by Guerrant et al. (19) that vitamin B_1 may be formed in the cecum probably as the result of bacterial action on the food residue there. The vitamin B_1 thus synthesized is not absorbed from the lower intestinal tract, but the rat may obtain benefit from it by the simple expedient of consuming his feces. The screen bottom cages are used to prevent coprophagy but during the course of this experiment the animals developed a severe dysentery with the result that the loose evacuations remained on the wire screens and were consumed by the animal. After the rat discovered that his condition could be improved in this manner it was impossible to prevent his obtaining the excreta. A metal harness has been designed to fit on the body of the animal to prevent access to the feces. An attempt was made to use the harnesses but they were so uncomfortable that the animals were not normal. They refused to eat and were not in condition for the test, so had to be discarded. An entirely new group was used for the continuation of the problem.

In spite of the difficulties encountered the results obtained in this experiment prove that the use of wheat germ in the diets of our people would help to solve, to a great extent, the problem of the widespread thiamin deficiency so prevalent at the present time.

Wheat germ is one of the richest natural sources of thiamin. While it makes up only 2.5 percent of the wheat berry it contains 13.6 percent of the vitamin content of that grain. The Ralston Research Laboratories[1], in a recent study on the vitamin content of wheat, have shown that the endosperm, which makes up about 70 percent of the wheat berry, contains only 75 to 166 International Units of thiamin per pound. The bran contains about 1400 International Units per pound or nearly 12 times as much as the endosperm. Middlings average roughly 2200 International Units per pound or 18 times as much as the starchy particles. The average of 18 samples of fresh wheat germ showed 3700 International Units per pound or 38 times as much as the endosperm.

Plate I shows the distribution of the vitamin in the grain of wheat.

Modern methods of milling, due to the demand for refined cereals, use the endosperm and discard those parts of the berry which are rich sources of thiamin. Cowgill (10) stated that, prior to the present method of milling, flour represented 81.4 percent of the original wheat kernel and contained an average of 1.65 International Units of thiamin per gram. Modern wheat flour represents only 72.5 percent of the kernel and contains an average of 0.15 International Units of thiamin per gram. In other words, modern milling gives a

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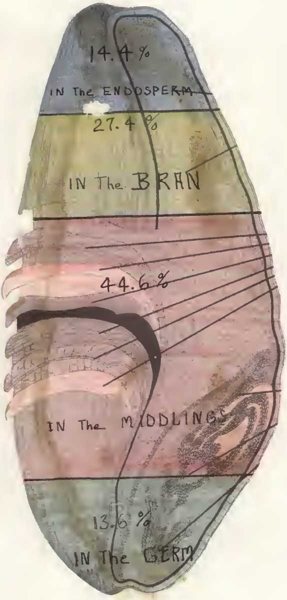
1 Personal communication from Ralston Research Laboratories, St. Louis, Missouri.

EXPLANATION OF PLATE I

The distribution of thiamin in the wheat grain.

(By courtesy of the Ralston Research Laboratories)

PLATE I


14.4 %
IN The ENDOSPERM
27.4 %
IN The BRAN
44.6 %
IN The MIDDLINGS
13.6 %
IN The GERM

product containing about one eleventh of the amount of the vitamin found in the old stone ground flour. This change would not be of much practical importance if the wheat products formed only a small part of the dietary but when they furnish 25 percent of our total calories, as recent surveys show, the decrease in the vitamin content becomes a matter of great concern.

The use of wheat germ as a means of increasing the vitamin content of the diet has received much attention recently, particularly in regions where wheat is grown in abundance. In many instances, however, the housewife is at a loss as to just how the germ may be used. There are many ways of including wheat germ in the diet. One of the most practical is its addition to cereals. Since it makes up part of the grain it is only reasonable to expect a satisfactory product when it is restored to its original place. The addition of wheat germ to a cereal of the bland type, such as cream of wheat, not only increases the thiamin content but definitely improves the flavor. The reason the cereal manufacturers do not add the germ is that, because of its high fat content, it is subject to rancidity. Incorporating wheat germ in meat loaf to replace cracker crumbs produced a very palatable product.

An attempt to increase the thiamin content of muffins and refrigerator rolls by the addition of wheat germ proved unsuccessful. The volume of the finished product was indirectly proportional to the amount of germ used; as the amount of germ was increased the volume of the product decreased. There is a substance in the germ which has a deleterious effect on flour. Sullivan et al. (49) made a study of the germ to determine the nature of this substance. They thought it was the lipoid content that was responsible but the baking quality was not improved when the fat was removed by extraction with ether. Sometime later Sullivan and Howe (48) reported that glutathione had been extracted in pure form from the germ and that it was the presence of this substance that was responsible for the degrading action of the germ on the baking quality of flour. The glutathione may be extracted with water but the vitamin, being water soluble, would be removed at the same time.

It may be concluded that, while it is not practical to use wheat germ as a substitute for flour in bakery goods, there are a number of ways in which it may be used to increase both the vitamin content and the palatability of foods which form an important part of the American dietary.

SUMMARY

1. The purpose of this study was to determine, by biological assay, whether the addition of wheat germ to certain foods increases their thiamin content sufficiently to justify its use in the ordinary household as a means of increasing the level of vitamin B_1 in the dietary.

2. The rat curative method was used for the quantitative determination of the vitamin B_1 content of cream of wheat and meat loaf made according to standard recipes, and the same products with wheat germ added.

3. The cream of wheat without wheat germ contained insignificant amounts of vitamin B_1. A 100-gm. serving of the cream of wheat with wheat germ at the level of this study provides 170 micrograms or 57 International Units of thiamin, which is approximately one-seventh of the estimated daily requirement. A 100-gm. portion of the meat loaf with wheat germ at the level of this study supplies 600 micrograms or 200 International Units of thiamin (2½ times that of the meat loaf without wheat germ), which is one-half the daily requirement.

4. The extensive use of wheat germ in the ordinary household would help in a great measure to overcome the thiamin deficiency so widespread at the present time.

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