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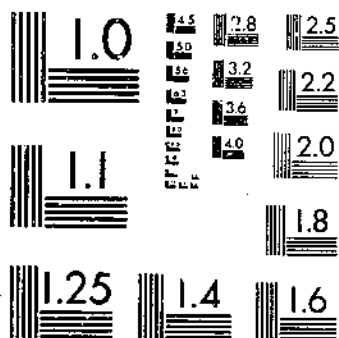
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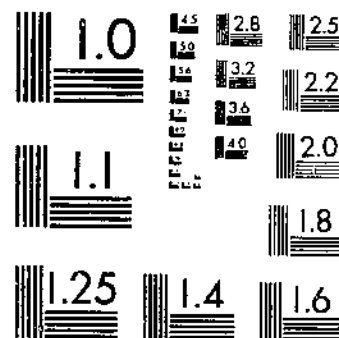
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THE VITAMIN B CONTENT OF FOODS IN TERMS OF CRYSTALLINE THIAMIN
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MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A



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NATIONAL BUREAU OF STANDARDS-1963-A

UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

THE VITAMIN B₁ CONTENT OF FOODS IN TERMS OF CRYSTALLINE THIAMIN¹

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INTRODUCTION

The vitamin B complex may be separated into vitamin B₁ (the anti-neuritic or anti-beriberi vitamin) and the vitamin B₂ complex. In the vitamin B₂ complex, riboflavin, nicotinic acid, vitamin B₆, and pantothenic acid are clearly differentiated, both with regard to chemical properties and to specific physiological functions. Recent researches indicate that other factors of the vitamin B complex will be clearly differentiated in the near future.

The letter with its subscript numeral in the term vitamin B₁ signifies that this vitamin is one belonging to the group of B vitamins and at the same time emphasizes that it is only one of the vitamins into which the B complex has been resolved.

The complete chemical structure of vitamin B₁ is now known. Unlike any of the other known vitamins it contains sulfur. Because of this unique characteristic, the name thiamin has been proposed as a substitute for the term vitamin B₁. The American Society of Biological Chemists, the American Institute of Nutrition, and the Committee on Nomenclature of the American Chemical Society have tentatively approved the name thiamin. The council on pharmacy and chemistry of the American Medical Association has adopted this new name also with the proviso that if the International Committee

¹ Submitted for publication May 4, 1939.

REVIEW OF RECENT LITERATURE

The compilation of vitamin content of foods published in 1937 by Daniel and Munsell (8) includes values for vitamin B determined previous to the differentiation of vitamin B₁, values estimated for vitamin B₁ on the basis of standard growth rates, and vitamin B₁ values determined by direct comparison of some specific physiological effect of the food tested with that of the 1934 international standard for vitamin B₁. These tables are fairly complete for the vitamin B₁ values of foods reported previous to January 1936.

Williams and Spies (28) have recently summarized the vitamin B₁ content of a number of different foods and have discussed the probable relationships of the various vitamin B₁ units which have been used in the past to express vitamin B₁ activity.

Fixsen and Roscoe (10) released a compilation of the vitamin content of foods in 1938. All of the vitamin B₁ values therein were obtained from reports of experiments in which direct comparison was made of the physiological effect of the food tested with that of the 1934 international standard adsorption product. These authors point out that their compilation of the vitamin B₁ content of foods is to be considered as preliminary and that many of the values probably need to be rechecked.

Baker and Wright (1, 2) have reported their analyses of a rather extensive list of food items in terms of International Units of vitamin B₁. These workers used the bradycardia test method proposed by Birch and Harris (3) and the 1934 international standard adsorption product as reference standard of vitamin B₁. According to Birch and Harris and according to Baker and Wright the vitamin B₁ values of foods as determined by the bradycardia method are in good agreement with values determined by the rat-growth method.

Investigators in many laboratories have contributed values for the vitamin B₁ content of a variety of different food items. Mickelsen, Waisman, and Elvehjem (22) have reported an experimental study of the distribution of vitamin B₁ (thiamin) in meat and meat products, the values being given in terms of micrograms of vitamin B₁ and in terms of International Units of vitamin B₁. These workers have also presented data showing that during the roasting, broiling, or stewing of meats the destruction of vitamin B₁ is on the order of 50 percent.

Copping and Roscoe (7) have determined the International Unit values for vitamin B₁ in several samples of yeast and in different types of wheat flours and breads. They found that the proportions of yeast used in bread-making were too small to contribute significantly to the vitamin B₁ value of the bread and that there was no appreciable destruction of vitamin B₁ as a result of the baking process.

Harris (11) and Leong and Harris (16) made a special investigation of the relative vitamin B₁ values of brown and white breads used in England. A given weight of whole-wheat or brown bread carried seven to eight times as many International Units of vitamin B₁ as white bread made of unbleached flour.

Morgan, Hunt, and Squier (23) have reported the assays of prunes, whole wheat, and wheat germ in terms of International Units of vitamin B₁.

In general, the vitamin B₁ values of foods as reported in this bulletin agree fairly well with those reported by Baker and Wright and with such other values as have been determined by biological assay in which the international standard has been used as a reference value.

METHODS USED BY DIFFERENT WORKERS FOR THE DETERMINATION OF VITAMIN B₁ VALUES

The methods for the determination of vitamin B₁ are based upon certain specific properties or physiological effects induced by vitamin B₁, namely:

1. Chemical reactions of vitamin B₁.
2. The stimulation of growth or of the fermentation process of some specific micro-organism.
3. The cure of a polyneuritic condition (head retraction) in pigeons.
4. The prevention of polyneuritis in chicks.
5. The cure of polyneuritic convulsions in rats.
6. Relief of bradycardia induced in rats as a result of vitamin B₁ deficiency.
7. Weight gains in young standardized rats.

Chemical methods for the estimation of vitamin B₁ values depend upon some specific interaction of chemical reagents with vitamin B₁ to produce some measurable effect, such as the development of color. The color reaction proposed by Prebluda and McCollum (24) utilizes the intensity of red coloration resulting from a coupling reaction between diazotized p-amino acetanilide or p-amino acetophenone and vitamin B₁ as a measure of vitamin B₁ values. This reaction apparently has a high degree of specificity for the 4-methyl-5- β -hydroxy-thiazole portion of the thiamin molecule. However, thiamin in the form of vitamin B₁ pyrophosphate must first be hydrolyzed before the pigment compound can be extracted from an aqueous phase into a nonaqueous phase (for example, xylene) suitable for quantitative colorimetric measurement.

Melnick and Field (19, 20, 21) recently made rather intensive studies of the chemical method proposed by Prebluda and McCollum. As a result means have been developed for concentrating the vitamin from extracts of biological materials, for separating the vitamin from the impurities which inhibit the coupling reaction, for addition of reagents to increase the sensitivity of the method, and for conversion of the phosphorylated vitamin B₁ into free thiamin. These refinements in the method have been used successfully for yeast, rice polishings, and wheat germ. The results are in good agreement with the biological assays reported for these materials. However, it would seem that this method required further study of its application to a variety of foods as compared with biological assays on the same samples before it could be regarded as a reliable substitute for the latter.

The intensity of the blue fluorescent light of thiochrome produced by the oxidation of vitamin B₁ with ferricyanide in alkaline solution was proposed by Jansen (18) and later modified by other investigators as a means of measuring vitamin B₁ values. According to Pyke (25) analyses of wheat-embryo samples indicated that the thiochrome method yielded results from 42 to 73 percent lower than the biological assay method (bradycardia method) for vitamin B₁ in the same samples. Thiochrome is an intermediate compound in the oxidation of thiamin and the blue fluorescent color is rather unstable.

The method is apparently not entirely specific for thiamin and does not include the thiamin in the form of vitamin B₁ pyrophosphate. Hennessy and Cerecedo (12) have recently suggested improvements for the thiochrome method for determining vitamin B₁ activity, including removal of interfering materials by the use of a base-exchanging zeolite as an adsorbent for vitamin B₁, the use of an enzyme preparation from beef kidney for conversion of thiamin phosphoric esters to free thiamin, and the use of a more sensitive fluorometer. This method has not yet been applied to any large number or variety of foodstuffs.

Methods based on the stimulation of growth or of the fermentation process of micro-organisms by vitamin B₁ involve very complex relationships of nutrients and also require quantitative extraction of the vitamin B₁ from foods, neither of which difficulties has been satisfactorily surmounted to date.

Irregularities in the production and cure of head retraction in pigeons are quite generally recognized. Occasionally, cures of a polyneuritic condition in pigeons have been attributed to food components other than thiamin. In view of the uncertainty as to specificity and interpretation of this method, it was considered unsuitable for the assays to be undertaken here.

Kline, Keenan, Elvehjem, and Hart (14) introduced the use of young chicks in the quantitative determination of thiamin. This method was subjected to further tests and summarized by Elvehjem (9). These investigators used a reinforced, heated grain ration supplemented with different levels of the food under assay. The lowest level of the food which is capable of protecting all the chicks from polyneuritis was determined. This level of food is presumably equivalent in thiamin value to the level of pure thiamin that accomplishes the same result. Although this method appears to have definite possibilities, since the vitamin B₁ assays undertaken in this laboratory were made for the specific purpose of making it possible to evaluate the vitamin B₁ values of human dietaries, it would seem more appropriate to use some species of mammal as experimental animals rather than to use chicks.

The so-called polyneuritic curative methods for rats are modifications or refinements of the method originally proposed by Smith (26). The polyneuritic curative method has been found reasonably satisfactory by many workers and especially valuable in assaying concentrates of vitamin B₁. However, many of our common foods contain only very small proportions of vitamin B₁, and it is impossible to get a polyneuritic animal to eat sufficiently large quantities of such foods to render the curative method generally applicable.

Rats maintained on the usual vitamin B₁-deficient rations are subject to a marked fall in heart rate as the state of vitamin B₁ deficiency progresses toward an obvious polyneuritic condition. The bradycardia or heart rate method proposed by Birch and Harris (3) is based on the measurement of the interval of relief of this condition following the administration of a single dose of vitamin B₁ or of a vitamin B₁-carrying material and the subsequent decline of the heart rate to approximately the rate at the time of dosing. The so-called bradycardia method as described by Birch and Harris and as applied subsequently to a rather extensive list of food items by Baker and Wright

(1, 2) appears to be reliable and adapted to the assay of foods both rich and poor in vitamin B₁ value. This method, however, does involve the availability of a suitable electrocardiograph and a considerable amount of experience and skill in operating it satisfactorily, neither of which conditions prevailed in this laboratory.

The rat-growth methods as proposed by Chase and Sherman (4) or by Chick and Roscoe (5) with or without modifications are based on the average gains in weight induced in suitably standardized young rats receiving a vitamin B₁-deficient ration supplemented by daily known quantities of foodstuffs.

The rat-growth methods have been criticized because the criterion of measurement is a nonspecific effect unless the vitamin B₁-deficient diet contains optimal quantities of all other nutrients except vitamin B₁. Experience in this laboratory has shown that the average weekly gains of groups of rats receiving different levels of crystalline thiamin over a 4-week test period produces a curve of response practically parallel to that of comparable groups of rats receiving different levels of foodstuffs that are good or rich sources of vitamin B₁. The differences in growth response of different strains of rats are of no particular concern provided the growth response is judged in relation to strict litter-mate comparisons between rats receiving supplements of vitamin B₁ and the foodstuff under test. Birch and Harris (3) determined the vitamin B₁ values for three different products (a dried yeast sample, a yeast concentrate, and a sample of wheat germ) by the bradycardia, rat-curative, and rat-growth methods. The respective international vitamin B₁ values of the three products were essentially the same as judged by any one of these three methods.

With the choice of method most applicable to the task of assaying a wide variety of common food items reduced to a choice between the bradycardia method and a rat-growth method, the latter was chosen for reasons of expedience and convenience.

MATERIALS AND METHOD USED IN THIS STUDY

PREPARATION OF SPECIAL INGREDIENTS USED IN EXPERIMENTAL VITAMIN B₁-DEFICIENT RATION

VITAMIN B₁-FREE CASEIN

To 4 liters of 60-percent (by weight) ethyl alcohol in an 8-liter wide-mouthed bottle, 800 gm. of dry acid-precipitated casein were added and the mixture agitated with a mechanical shaker for at least 30 minutes. The contents of the bottle were then transferred to a muslin bag and the alcohol pressed out in a tincture press. The filter cake was broken up in the bag and washed with 2 liters of 60-percent ethyl alcohol. The casein was transferred from the cloth bag back to the 8-liter bottle and the process (shaking, pressing, and washing the filter cake) repeated. The filter cake was broken up and washed with 1 liter of 95-percent (by volume) ethyl alcohol to facilitate drying. The extracted casein was then spread on a suitable tray and dried in the air. The dried casein was sieved and stored in a clean, dry, stoppered jar.

SALT MIXTURE

(Modified Osborne and Mendel Salt Mixture)

The following solution was prepared in a beaker with the use of heat:

| | | |
|---------------------------|-------------|-------|
| Iron citrate..... | grams..... | 29.5 |
| Water..... | liters..... | 1.5 |
| Citric acid crystals..... | grams..... | 444.0 |

The following were added to the above solution:

| | | |
|---|------------------|----|
| KI (0.5 gm. in 250 ml.)..... | milliliters..... | 40 |
| MnSO ₄ ·4H ₂ O (2.9 gm. in 250 ml.)..... | do..... | 40 |
| NaF (6.2 gm. in 250 ml.)..... | do..... | 40 |
| K ₂ Al ₂ (SO ₄) ₄ (0.61 gm. in 250 ml.)..... | | |

or

| | | |
|---|------------|-------|
| K ₂ Al ₂ (SO ₄) ₄ ·24H ₂ O (1.13 gm. in 250 ml.)..... | do..... | 40 |
| Phosphoric acid (85 percent, sirupy)..... | do..... | 284 |
| Hydrochloric acid (36 percent)..... | do..... | 517 |
| Sulfuric acid (98 percent)..... | do..... | 21 |
| CuSO ₄ ·5H ₂ O..... | grams..... | 0.425 |

The following salts were mixed and added slowly (stirring) to the above solution:

| | | |
|--------------------------|------------|-----|
| Calcium carbonate..... | grams..... | 539 |
| Sodium carbonate..... | do..... | 137 |
| Potassium carbonate..... | do..... | 565 |
| Magnesium carbonate..... | do..... | 91 |

The resulting salt mixture was dried and ground as per instructions given for drying and grinding the autoclaved yeast.

These ingredients in quantities specified make about 1,700 gm. of dry salt mixture.

AUTOCLAVED YEAST

Sufficient tap water was added to wet the desired quantity of baker's yeast and the mixture worked into a thick paste, free of lumps. The yeast paste was spread in smooth layers not deeper than 1 inch on flat agate pans or flat pyrex baking dishes having a depth of about 3 inches. These pans or dishes with yeast were placed in the autoclave and heated to 15 pounds steam pressure (120° C.) for 6 hours.

When the autoclaving of the yeast was finished, the pans or dishes were placed in the drying oven (about 50° C.) and stirred each day until the yeast became hardened. Then the lumps were broken up and the drying continued until the yeast could be ground in a mill. The dry, finely ground, autoclaved yeast was kept in the refrigerator. The yeast may be kept under these conditions for a period of about 1 month.

SULFITE-TREATED YEAST

Five hundred grams of dried baker's yeast were mixed with 4 liters of 0.1-percent sodium sulfite solution and placed in a wide-mouthed 8-liter bottle. Sulfur dioxide gas was passed into the suspension until it reached pH 4.0±0.1 as measured with a glass electrode. The mixture was then allowed to stand in the dark at room temperature for 5 days. At the end of this period the suspension was poured over 500 gm. of cornstarch, which was spread in a thin layer in a shallow pan, and dried at a temperature of about 50° C. The dried

mixture was finely ground and was then ready for use in the preparation of the vitamin B₁-free basal diet.

SELECTION AND MANAGEMENT OF ANIMALS USED IN THIS STUDY

Young albino rats from 21 to 28 days of age and varying from about 35 to 45 gm. in body weight were kept until they reached stationary weight on a vitamin B₁-deficient diet of the following composition:

| Vitamin B ₁ -deficient ration: | | Grams |
|---|-------|-------|
| Extracted casein..... | ----- | 180 |
| Agar..... | ----- | 20 |
| Cod-liver oil..... | ----- | 40 |
| Cottonseed oil..... | ----- | 60 |
| Autoclaved bakers' yeast ¹ | ----- | 150 |
| Salt mixture..... | ----- | 40 |
| Cornstarch..... | ----- | 510 |
| Total..... | ----- | 1,000 |

¹ In assaying the vitamin B₁ values of rye and of the lean portion of loin lamb chops, strictly parallel assays were made using 15 percent of sulfite-treated bakers' yeast in place of the same proportion of autoclaved yeast. This method of treating yeast preliminary to its incorporation into vitamin B₁-deficient rations was first suggested by Kline, Tolle, and Nelson (15). The details of preparation of the sulfite-treated yeast will be found on p. 7.

The animals were fed the vitamin B₁-deficient diet ad libitum and had access to water at all times. The animals were weighed at the end of 1 week after they were placed on this diet and again at the end of 10 days and on each day thereafter until they reached stationary weight.

The young rats very regularly reached the stage of stationary weight in 12 to 18 days after having been placed on the vitamin B₁-deficient diet. At this time each rat was housed in a separate small cage with a raised screen bottom. One rat from each litter (the so-called negative control) was continued on the vitamin B₁-deficient diet without supplement of any kind. The remaining rats of each litter were given supplements of thiamin chloride or supplements of the food item to be assayed for vitamin B₁ value. The thiamin chloride and the food items under test were fed six times weekly. On Saturdays and Mondays these supplements were increased 50 percent in order to compensate for the lack of supplement on Sundays. In practically every case six assay rats (three males and three females) were used on each level of test food. Two separate strains of albino rats were used in conducting these assays.

During the experimental period of 4 weeks' duration the assay rats were weighed once each week.

METHOD OF CALCULATING VITAMIN B₁ VALUES

The average weekly weight increases were calculated for each rat that had received a given supplement level of the test food and for each of the litter-mate control rats that had received 1.5 or 3 micrograms of the reference standard of crystalline thiamin chloride. The average weekly weight increments for each pair of litter mates was plotted against the respective level of test food administered as a vitamin B₁ supplement. A straight-line relationship was assumed for the average weekly weight increments intermediate between those induced in the paired litter mates that had received the two different levels of supplement of test food. The quantity of test food, which

presumably would have resulted in an average weekly weight increase equal to that induced in the litter-mate control that had received 1.5 or 3 micrograms of the crystalline thiamin, was then determined by inspection.

The average quantity of a given test food equivalent to 1.5 or 3 micrograms of thiamin, as judged by the response of six litter-mate pairs, was used in the final calculation.

In most cases the average weekly weight increment of the rats that received supplements of thiamin chloride was intermediate between those of their litter-mate pairs, which had received two different levels of the food item under test. In a few cases the quantity of test food equivalent to 1.5 or 3 micrograms of thiamin necessitated some measure of extrapolation. It is not feasible to present the experimental data for each set of paired litter mates together with the litter-mate control, but the average weight increments of all the rats on each supplement of vitamin B₁ are shown in table 1.

The evaluation of the vitamin B₁ or thiamin value per 100 gm. of each food was made according to the equation $X = \frac{3 \text{ or } 1.5 \text{ micrograms}}{A} \cdot 100$

wherein X = micrograms of vitamin B₁ or thiamin per 100 gm. of food under test, and A = grams of test food that presumably would induce an average gain in weight equivalent to that induced by the 3 or 1.5 micrograms of thiamin. These values are given in column 3 of table 1.

The vitamin B₁ potencies of each food item in terms of International Units are given in column 2 of table 1. The value of 3 micrograms of crystalline thiamin chloride has recently been adopted as the new international standard for vitamin B₁ by the permanent commission on biological standardisation of the Health Organisation of the League of Nations (18). The vitamin B₁ values in terms of International Units, are, therefore, one-third of the corresponding numerical values in terms of micrograms of thiamin.

DESCRIPTION AND PREPARATION OF FOOD SAMPLES

All of the assays included in table 1 are experimental values determined in the nutrition laboratories of the Bureau. The assays were made during the interim of June 1938 to May 1939.

The specimens of food selected for assay were bought on the open markets of Washington, D. C., and doubtless were grown in many different regions and under widely different cultural conditions. Only the edible portions, prepared as for cooking or table use, were subject to analysis for thiamin values. The assay of each of the fresh fruits and vegetables was made, insofar as possible, during the period coincident with the season of its respective greatest abundance. In view of the perishable nature of many of the foodstuffs fresh specimens were purchased two or three times weekly during the course of the assays. Each assay of the fresh fruits and vegetables, fresh meats, and dairy products, therefore, necessitated the use of 10 or more different samples of these items.

The detailed descriptions of the specimens of food assayed and the method of preparing the samples of these to be fed as vitamin B₁ supplements are as follows:

Apples.—Stayman Winesap variety, bought during October and November. For sampling the fruit was thinly pared and cored.

Asparagus.—A thin-stalk variety, fairly green in color, bought during March and April. The butt ends were removed before sampling, and the entire stalks were chopped fine and well mixed.

Avocado.—Ripe fruit with green outer rind purchased in August. The seed and rind of the fruits were discarded.

Bananas.—Fully ripe fruit, bought during August and September. The fruit was peeled before sampling.

Beans.—Snap beans, green fleshy pods, bought during September and October. In preparation for sampling the ends and strings if any were removed and the whole pods chopped fine and mixed.

Yellow wax beans, succulent pods, bought during July and August. These were sampled in the same manner as the green snap beans.

Fresh lima beans, immature seeds, light green in color, bought during August and September. Just previous to the preparation of the sample, the limas were shelled and chopped fine.

Dried lima beans and dried navy beans were ground fine before sampling and mixed with small portions of the vitamin B₁-deficient diet for feeding.

Beef.—Fresh lean muscle from the top-round cut.

Beets.—Pared roots 1½ to 3 inches in diameter, bought in August and September.

Blackberries.—Large, juicy berries, bought during June and July.

Blueberries.—Large, firm, ripe berries purchased during June and July.

Broccoli.—Green flowers, stalks, and some small, dark-green leaves, bought during December and January, and finely chopped before sampling.

Brussels sprouts.—Medium-size heads with green outer leaves and bleached inner leaves, bought during March and April. Entire heads were finely chopped and mixed.

Cabbage.—Summer cabbage with green outer leaves and bleached inner leaves, bought during August and September. The entire head was used with the exception of the hard center core, which was discarded. The leaves were chopped fine and mixed well.

Carrots.—Young roots, bought in March and April. The outer skin was removed by scraping and whole roots were finely chopped and thoroughly mixed before sampling.

Cauliflower.—Finely chopped flowers and flower stalks of solid white heads, bought in November and December.

Celery.—Finely chopped, crisp bleached stalks. All leaves were excluded from the samples.

Cheese.—A yellow American Cheddar made in Wisconsin.

Cherries.—Ripe red "Bing" cherries purchased during June and July. Samples of entire fruit exclusive of the pits were used for assay.

Chicken.—Dark and light flesh of fryers were assayed separately. Dark meat was taken from the leg and thigh; the light meat from the breast. The skin in both cases was discarded.

Cocoa.—Powdered cocoa, bought in packaged form, was mixed with small portions of the vitamin B₁-deficient ration.

Collards.—Fresh green leaves and edible part of the stem were chopped together and well mixed for sampling. Purchased during July and August.

Corn, sweet.—Country Gentleman and Golden Bantam varieties in milk stage of ripeness, bought during July, August, and September. Tips and pulp of the kernels were cut from the cob immediately before sampling.

Corn flakes.—A packaged breakfast cereal.

Corn meal.—White and yellow commercial products, prepared by identical milling processes. A small amount of corn bran was removed in the milling process in each case.

Cowpeas or black-eyed peas.—Dried mature seeds, finely ground before sampling and mixed with small portions of the vitamin B₁-deficient ration for feeding.

Dates.—Hayany variety supplied by the Bureau of Plant Industry from the United States Date Station at Indio, Calif. The dates were processed for 2 to 3 days at 130° F. then stored for about 6 months at 23° to 34° F. The entire edible portion (skin and pulp) was included in the material assayed.

Eggs.—Bought on the open market during December and January. The yolks were separated from the whites with special care to leave as little of the whites as possible on the surface of the yolks. The yolks were then very thoroughly mixed in a definite proportion with the vitamin B₁-deficient diet. A new sample was prepared each week and kept stored in the refrigerator at about 10° C.

The raw egg whites were cut with scissors in order to obtain a more homogeneous sample and then mixed in a definite proportion with the vitamin B₁-

deficient diet. This mixture was also prepared once a week and kept in the refrigerator at 10° C.

Endive.—Bleached heads purchased during May and June. The entire heads were finely chopped and well mixed for sampling.

Fish.—Fresh halibut and fresh-water trout, bought once a week during the period of assay, and canned red salmon. The juice of the canned salmon was discarded. The fish was kept in a refrigerator at a temperature a few degrees below 0° C.

Flour.—A white "straight-run" soft-wheat flour milled for the purpose by the Bureau of Plant Industry, a commercial packaged white patent flour, and a white flour said by the producer to contain the germ portion of the wheat grain. All samples were incorporated in small portions of the vitamin B₁-deficient diet in place of equal weights of starch.

Grapefruit.—Fully ripened, heavy juiced fruit, bought during November and December. The white membranes between the sections of the fruit were removed.

Kale.—Dark-green, crisp, whole leaves, bought during October and November. The leaves were finely chopped and thoroughly mixed before sampling.

Lamb.—Lean portion of loin chops.

Lettuce.—Outer green leaves and inner bleached leaves of head lettuce, bought in November and December. The leaves were finely chopped and well mixed before the samples were taken.

Liver.—Fresh beef livers.

Milk.—Commercial condensed, evaporated, dry skim, and dry whole milk, bought on local markets. The fluid skim milk and fluid whole milk were bought from a large local dairy during June, July, and August. The skim milk had a very low fat content on the order of 0.02 percent.

Molasses.—A tinned commercial product, dark brown in color and of a type widely used in cooking. The supplements of molasses were mixed with the vitamin B₁-deficient ration for feeding.

Muskmelon.—Fruit with a bright orange-colored flesh and a gray and green rind. The seeds were discarded and the flesh was removed to within about one-quarter inch of the rind and finely chopped before sampling.

Mustard greens.—Medium-green leaves, bought during November and December. Preparation was the same as for kale.

Oats.—Packaged commercial "quick-cooking" oatmeal and rolled oats.

Okra.—Fresh specimens, bought during December and January. The entire pods and seeds were finely chopped.

Onions.—Mature, white-fleshed, winter globe type, bought during November and December. The outer thin brown skins were discarded.

Oranges.—Fully ripened juicy fruits grown in Florida and bought during December and January. The samples included the pulp, with the membrane between sections removed.

Peaches.—White-fleshed, fully ripe fruits, bought in June and July. For the assay samples skins and pits were discarded.

Peanuts.—Virginia-type peanuts supplied by the Bureau of Plant Industry. The germ, thin red skins, and cotyledons plus germ were assayed separately. The red skins were ground and mixed with the vitamin B₁-deficient diet in order to make them palatable.

Spanish peanuts from a single sample bought from the local market, assayed raw and roasted, red skins removed. The roasting was done by heating raw peanuts in a single layer at 190° C. for 20 minutes.

Pears.—Fruit bought in October and November was pared and cored before sampling.

Peas.—Green peas, bought during July and August, in the pod, and freshly shelled just before weighing the samples.

Pineapples.—Fully ripened fruit bought during November and December. The samples represent the flesh without parings or core.

Plums.—A blue damson variety bought during October and November. The stems and pits were removed before sampling.

Pork.—Lean flesh of fresh loin chops and the lean portion of smoked raw ham.

Potatoes.—New potatoes of the Irish Cobbler variety bought in August and September; mature potatoes of the same variety bought in January and February. The new potatoes were scraped and the mature ones were pared before sampling.

Prunes.—Average size domestic dried prunes of good quality. Both skins and pulp were included in the samples.

Raspberries.—Fresh juicy black and red berries bought in June and July.

Rhubarb.—Early spring rhubarb bought in March and April. The entire stems were finely chopped and well mixed before sampling.

Rutabagas.—Light yellow roots bought in December and January, pared and chopped before sampling.

Rye.—Whole grains ground in the laboratory.

Sauerkraut.—"Bulk" sauerkraut bought in January and February.

Soybeans.—Fresh green beans obtained from the Arlington Experiment Farm three times each week during August and September. Several varieties were included. The beans were shelled and the whole seeds finely chopped for sampling.

The whole dried soybeans were finely ground before feeding.

Spinach.—Fresh, dark-green leaves and the more tender portions of the leaf-stalk, finely chopped and well mixed for sampling, from spinach bought during June and July.

Squash.—Hubbard squash bought during November and December. The flesh was finely chopped after removing the rind and about one-quarter inch of flesh adjoined to the rind.

Small white cymblings purchased during May and June. The seeds and flesh were chopped together after removing the rind.

Strawberries.—Fresh, firm, fully ripened berries bought during the last week of March and through the first 3 weeks of April. The whole berries were finely chopped before sampling.

Sweetpotatoes.—Nancy Hall variety bought in October and November, pared and finely chopped before sampling.

Tomatoes.—Firm red specimens bought during August and September. The skins were removed for the assay samples.

Turnip greens.—Leaves, medium dark green in color, bought during October and November. The entire leaves and a small part of the stems were finely chopped and well mixed before sampling.

Turnips.—Globe-shaped, white-fleshed turnips with purple-pigmented skins on the upper part, bought during November and December. The roots were pared and finely chopped before sampling.

Walnuts.—Large firm meats of Persian (English) walnuts bought without shells.

Wheat, shredded.—A packaged breakfast cereal.

Wheat, whole.—Soft winter, hard winter, and hard spring varieties ground in the laboratory and fed as separate supplements. The entire grains were included.

EXPERIMENTAL RESULTS

The vitamin B_1 values of the perishable food items (table 1) were determined on fresh samples purchased two or three times a week over the 4-week period of assay. These values, therefore, represent a composite assay involving 10 to 12 specimens. The vitamin B_1 values were determined on uncooked samples unless otherwise indicated in column 1 of the table.

GROWTH RATES OF VITAMIN B_1 ASSAY RATS

Two different strains of albino rats, referred to as stock A and stock B, were used for the vitamin B_1 assays reported in table 1. About 15 percent of the experimental animals were from stock A and about 85 percent from stock B.

The average weekly weight increment of the 18 males and of the 18 females from stock A, which received 3 micrograms of thiamin per day, were 6.3 ± 4.0 and 6.3 ± 2.3 gm., respectively. The average weekly weight increment of the 107 males and of the 112 females from stock B, which received 3 micrograms of thiamin per day, were 12.9 ± 4.1 and 10.6 ± 3.1 gm., respectively. This wide difference in average growth response between: say rats of the two strains receiving identical supplements of thiamin indicates clearly the unreliability of absolute growth rates as criteria of the vitamin B_1 values of the supplements fed as sources of vitamin B_1 .

TABLE 1.—The vitamin B₁ (thiamin) assay values of foods

(Estimated precision, 15 to 20 percent)

| Food | Vitamin B ₁ (thiamin) content per 100 grams (edible portion) | | Summary of experimental data | | | |
|--|--|------|------------------------------|--------------------------------------|--------------------------|--------------------------------------|
| | | | Crystalline thiamin | | Food supplement | |
| | | | Amount fed per day | Weight incre- ment per week | Amount fed per day | Weight incre- ment per week |
| Apples (<i>Malus sylvestris</i>): | I. U. | μgm | μgm | Grams | Grams | Grams |
| Stayman Winesap..... | <8 | <25 | 3 | 11.3 | 4 | 1.3 |
| Asparagus (<i>Asparagus officinalis</i>)..... | 59 | 177 | 3 | 12.0 | 2 | 15.3 |
| Avocado (<i>Persea</i> spp.)..... | | | | | 4 | 20.6 |
| | 34 | 102 | 2 | 12.9 | 3 | 13.3 |
| | | | | | 5 | 10.4 |
| Bananas (<i>Musa sapientum</i>)..... | 18 | 54 | 2 | 13.5 | 3 | 5.3 |
| | | | | | 5 | 13.0 |
| Beans (<i>Phaseolus</i> spp.): | | | | | | |
| Green, snap..... | 24 | 72 | 3 | 11.0 | 2 | 2.3 |
| | | | | | 4 | 3.5 |
| Lima, fresh..... | 114 | 342 | 3 | 8.9 | 1 | 9.7 |
| | | | | | 2 | 17.0 |
| Lima, dried..... | 170 | 510 | 3 | 13.6 | .5 | 11.8 |
| | | | | | 1 | 17.0 |
| Navy, dried..... | 128 | 384 | 3 | 10.2 | 1.67 | 8.9 |
| | | | | | 1 | 12.5 |
| Yellow, wax..... | 29 | 87 | 3 | 11.8 | 2 | 5.7 |
| | | | | | 4 | 13.5 |
| Beef: | | | | | | |
| Lean muscle..... | 38 | 114 | 3 | 13.2 | 2 | 12.4 |
| | | | | | 3 | 13.6 |
| Beets (<i>Beta vulgaris</i>)..... | ca17 | ca50 | 3 | 10.9 | 2 | 1.9 |
| | | | | | 4 | 4.5 |
| Blackberries (<i>Rubus</i> spp.)..... | <8 | <25 | 3 | 10.0 | 4 | 2.1 |
| Blueberries (<i>Vaccinium</i> spp.)..... | 15 | 45 | 3 | 12.1 | 3 | 3.9 |
| | | | | | 5 | 8.4 |
| Broccoli (<i>Brassica oleracea botrytis</i>)..... | 33 | 99 | 3 | 11.5 | 1 | 2.8 |
| | | | | | 3 | 11.6 |
| Brussels sprouts (<i>Brassica oleracea gemmifera</i>)..... | 67 | 171 | 3 | 10.2 | 2 | 11.1 |
| | | | | | 4 | 18.9 |
| Cabbage (<i>Brassica</i> spp.): | | | | | | |
| Fresh..... | 27 | 81 | 3 | 8.6 | 2 | 1.9 |
| | | | | | 4 | 9.9 |
| Fermented (sauerkraut)..... | <10 | <30 | 3 | 11.9 | 4 | 3.9 |
| Carrots (<i>Daucus carota</i>)..... | 24 | 72 | 3 | 12.4 | 2 | 3.1 |
| | | | | | 4 | 11.4 |
| Cauliflower (<i>Brassica oleracea botrytis</i>)..... | 56 | 168 | 3 | 10.9 | 3 | 13.6 |
| | | | | | 5 | 18.3 |
| Celery (<i>Apium graveolens</i>)..... | 12 | 36 | 1.5 | 7.7 | 3 | 4.5 |
| | | | | | 5 | 9.8 |
| Cheese: | | | | | | |
| American Cheddar..... | 14 | 42 | 1.5 | 8.1 | 1 | -1.1 |
| | | | | | 3 | 6.6 |
| Cherries (<i>Prunus</i> spp.): | | | | | | |
| Bing..... | 17 | 51 | 3 | 10.0 | 3 | 6.6 |
| | | | | | 5 | 8.8 |
| Chicken: | | | | | | |
| Dark meat..... | 37 | 111 | 3 | 14.3 | 2 | 9.0 |
| | | | | | 3 | 10.9 |
| Light meat..... | 26 | 78 | 3 | 13.7 | 2 | 4.9 |
| | | | | | 3 | 9.6 |
| Cocoa..... | ca25 | ca75 | 3 | 13.9 | 2 | 7.2 |
| Collards (<i>Brassica oleracea acephala</i>)..... | 67 | 201 | 3 | 13.1 | 1.5 | 14.0 |
| | | | | | 3 | 20.3 |
| Corn (<i>Zea mays</i>): | | | | | | |
| Sweet, Country Gentleman..... | 40 | 120 | 3 | 10.3 | 2 | 8.4 |
| | | | | | 4 | 17.2 |
| Sweet, Golden Bantam..... | 50 | 150 | 3 | 11.7 | 2 | 11.7 |
| | | | | | 4 | 21.3 |
| Corn flakes..... | (9) | (9) | 3 | 13.4 | 3 | 1.6 |
| Corn meal: | | | | | | |
| White..... | 101 | 303 | 3 | 12.3 | .8 | 15.2 |
| | | | 3 | 9.3 | 2 | 22.8 |
| | | | | | 4 | 24.4 |
| Yellow..... | 78 | 234 | 3 | 11.6 | .8 | 8.7 |
| | | | | | 1.5 | 13.1 |
| Cowpeas (<i>Vigna sinensis</i>): | | | | | | |
| Dried..... | 312 | 936 | 3 | 10.4 | .5 | 16.6 |
| | | | | | 1 | 15.9 |

*Trace.

TABLE 1.—The vitamin B₁ (thiamin) assay values of foods—Continued

| Food | Summary of experimental data | | | | | |
|--|--|-------|--------------------------|--------------------------------------|--------------------------|--------------------------------------|
| | Vitamin B ₁ (thiamin) content per 100 grams (edible portion) | | Crystalline thiamin | | Food supplement | |
| | | | Amount fed per day | Weight incre- ment per week | Amount fed per day | Weight incre- ment per week |
| Dates (<i>Phoenix dactylifera</i>): | I. U. | μgm | μgm | Grams | Grams | Grams |
| Hayany..... | 24 | 72 | 3 | 13.2 | 1.5 | 7.2 |
| | | | | | 3 | 9.5 |
| Eggs: | (1) | (1) | 3 | 12.3 | 4 | .8 |
| Whites..... | 118 | 354 | 3 | 13.3 | 1.75 | 12.7 |
| Yolks..... | | | | | 1.5 | 17.4 |
| Endive (<i>Cichorium endivia</i>)..... | 33 | 99 | 3 | 11.5 | 3 | 11.7 |
| | | | | | 0 | 17.9 |
| Fish: | | | | | | |
| Halibut..... | 28 | 84 | 3 | 12.7 | 1.5 | 7.5 |
| | | | | | 3 | 12.1 |
| Salmon, red, canned..... | (1) | (1) | 3 | 10.1 | 2 | -2.0 |
| | | | | | 4 | -1.6 |
| Trout, fresh-water..... | 29 | 87 | 3 | 11.7 | 1.5 | 5.9 |
| | | | | | 3 | 10.6 |
| Flour: | | | | | | |
| White, straight milled..... | 29 | 87 | 3 | 14.4 | 1 | 6.3 |
| | | | 1.5 | 8.3 | 3 | 12.4 |
| White, patent..... | ca17 | ca50 | 1.5 | 9.2 | 2 | 9.0 |
| | | | | | 3 | 7.8 |
| White, "plus germ"..... | 43 | 129 | 3 | 14.8 | 1 | 6.2 |
| | | | | | 3 | 12.3 |
| Grapefruit (<i>Citrus grandis</i>)..... | 24 | 72 | 3 | 10.0 | 4 | 9.7 |
| | | | | | 6 | 12.2 |
| Ham: | | | | | | |
| Smoked..... | 476 | 1,428 | 3 | 11.9 | .25 | 12.0 |
| | | | | | 4 | 15.1 |
| Kale (<i>Brassica oleracea acephala</i>)..... | 63 | 189 | 3 | 8.7 | 1 | 6.5 |
| | | | | | 3 | 14.2 |
| Lamb: | | | | | | |
| Chop, lean portion..... | 79 | 237 | 3 | 14.6 | 1 | 12.6 |
| | | | | | 2 | 23.5 |
| Chop, lean portion ¹ | 86 | 258 | 3 | 17.3 | 1 | 16.8 |
| | | | | | 2 | 23.8 |
| Lettuce (<i>Lactuca sativa</i>): | | | | | | |
| Head..... | 29 | 87 | 3 | 12.8 | 3 | 11.6 |
| | | | | | 5 | 16.5 |
| Liver: | | | | | | |
| Beef..... | 89 | 267 | 3 | 14.1 | .5 | 5.9 |
| | | | | | 1 | 12.1 |
| Milk: | | | | | | |
| Condensed..... | 24 | 72 | 3 | 11.0 | 3 | 7.8 |
| | | | | | 5 | 13.2 |
| Evaporated..... | 17 | 51 | 3 | 10.3 | 5 | 9.0 |
| | | | | | 2 | 14.7 |
| Fluid, skim..... | 15 | 42 | 3 | 9.1 | 3 | .7 |
| | | | | | 6 | 5.5 |
| Fluid, whole..... | 16 | 48 | 3 | 9.4 | 3 | 4.3 |
| | | | | | 6 | 9.1 |
| Powder, skim..... | 125 | 375 | 3 | 8.8 | .6 | 7.4 |
| | | | | | 1 | 10.3 |
| Powder, whole..... | 105 | 315 | 3 | 13.0 | .8 | 6.9 |
| | | | | | 1 | 13.9 |
| Molasses: | | | | | | |
| Brown..... | (1) | (1) | 3 | 13.6 | 3 | -.5 |
| Muskmelon (<i>Cucumis melo</i>)..... | 19 | 57 | 3 | 13.1 | 4 | 12.3 |
| | | | | | 6 | 13.9 |
| Mustard greens (<i>Sinapis</i>)..... | 46 | 138 | 3 | 11.2 | 1 | 8.1 |
| | | | | | 3 | 14.7 |
| Oatmeal: | | | | | | |
| "Quick-cooking"..... | 270 | 810 | 3 | 8.9 | .3 | 7.1 |
| | | | | | .6 | 15.5 |
| Oats (<i>Avena sativa</i>): | | | | | | |
| Rolls..... | 242 | 726 | 3 | 9.2 | .2 | 2.4 |
| | | | | | .4 | 7.8 |
| Okra (<i>Hibiscus esculentus</i>)..... | 42 | 126 | 3 | 13.2 | 2 | 12.1 |
| | | | | | 3 | 14.9 |

¹ Trace.² This assay was made with sulfite-treated yeast instead of autoclaved yeast in the vitamin B₁-deficient diet.³ Milliliters.

TABLE 1.—The vitamin B₁ (thiamin) assay values of foods—Continued

| Food | Summary of experimental data | | | | | |
|---|--|------------|--------------------------|--------------------------------------|--------------------------|--------------------------------------|
| | Vitamin B ₁ (thiamin) content per 100 grams (edible portion) | | Crystalline thiamin | | Food supplement | |
| | | | Amount fed per day | Weight incre- ment per week | Amount fed per day | Weight incre- ment per week |
| Onions (<i>Allium cepa</i>): | I. U. >10 | μgm >30 | μgm 3 | Grams 12.2 | Grams 2 | Grams 3.4 |
| Winter, globe | | | | | 4 | 4.7 |
| Oranges (<i>Citrus</i> spp.): | | | | | | |
| Florida | 28 | 78 | 3 | 10.6 | 3 | 8.1 |
| | | | | | 5 | 14.7 |
| Peas (<i>Pisum</i> spp.): | | | | | | |
| Fresh, green | 130 | 360 | 3 | 12.6 | 1 | 16.3 |
| | | | | | 3 | 24.6 |
| Peach (<i>Amigdalus persica</i>): | | | | | | |
| White fleshed | <8 | <25 | 3 | 9.8 | 4 | -1.1 |
| Peanut (<i>Arachis hypogaea</i>): | | | | | | |
| Whole, Spanish | 350 | 1,050 | 3 | 14.1 | .3 | 15.2 |
| | | | | | .6 | 22.3 |
| Whole-roasted, Spanish | 78 | 234 | 3 | 14.1 | 1.0 | 4.2 |
| | | | | | 1.5 | 9.1 |
| Whole, Virginia type | 250 | 750 | 3 | 7.9 | 1.5 | 8.8 |
| | | | | | .1 | 19.4 |
| Germ, Virginia type | 204 | 882 | 3 | 8.8 | .5 | 1.0 |
| | | | | | .5 | 13.4 |
| Red skins, Virginia type | 2,632 | 7,896 | 8 | 8.0 | .03 | 5.0 |
| | | | | | .1 | 20.1 |
| Pear (<i>Pyrus</i> spp.) | <8 | <25 | 1.5 | 4.7 | 3 | .4 |
| Pineapple (<i>Ananas sativus</i>) | 30 | 90 | 3 | 13.6 | 3 | 13.9 |
| | | | | | 5 | 18.9 |
| Plum (<i>Prunus domestica</i>): | | | | | | |
| Blue damson | 18 | 48 | 1.5 | 6.5 | 3 | 5.0 |
| | | | | | 5 | 10.9 |
| Pork: | | | | | | |
| Chop, lean portion | 455 | 1,365 | 3 | 10.8 | .2 | 11.3 |
| | | | | | .4 | 16.0 |
| Ham, smoked | 470 | 1,410 | 3 | 11.9 | .25 | 13.0 |
| | | | | | .4 | 10.1 |
| Potatoes (<i>Solanum tuberosum</i>): | | | | | | |
| New, Irish Cobbler | 62 | 186 | 3 | 9.1 | 2 | 11.9 |
| | | | | | 4 | 18.7 |
| Mature, Irish Cobbler | 49 | 147 | 3 | 14.3 | 1 | 7.5 |
| | | | | | 2 | 13.8 |
| Prunes (<i>Prunus domestica</i>): | | | | | | |
| Dried | 60 | 180 | 3 | 12.4 | .8 | 6.9 |
| | | | | | 1.5 | 11.4 |
| Raspberries: | | | | | | |
| Black (<i>Rubus occidentalis</i>) | <8 | <25 | 3 | 6.8 | 4 | 3.0 |
| Red (<i>Rubus strigosus</i>) | <8 | <25 | 3 | 10.0 | 4 | 1.1 |
| Rhubarb (<i>Rheum raphanifolium</i>) | <8 | <25 | 3 | 11.6 | 4 | .7 |
| Rutabagas (<i>Brassica campestris</i>) | 25 | 75 | 3 | 11.2 | 2 | 1.1 |
| | | | | | 4 | 11.2 |
| Rye (<i>Secale cereale</i>): | | | | | | |
| Whole grain | 156 | 468 | 3 | 13.4 | .75 | 14.6 |
| | | | | | 1 | 18.4 |
| Whole grain ^a | 131 | 393 | 3 | 15.1 | .75 | 13.1 |
| | | | | | 1 | 18.6 |
| Sauerkraut | <10 | <30 | 3 | 11.9 | 4 | 3.9 |
| Soybeans (<i>Glycine max</i>): | | | | | | |
| Green | 159 | 472 | 3 | 18.6 | .4 | 14.5 |
| | | | | | .8 | 21.5 |
| Dried | 485 | 1,455 | 3 | 12.1 | .15 | 9.0 |
| | | | | | .3 | 18.0 |
| Spinach (<i>Spinacia oleracea</i>) | 35 | 105 | 3 | 9 | 2.6 | 8.0 |
| | | | | | 3.5 | 10.3 |
| Squash (<i>Cucurbita maxima</i>): | | | | | | |
| Hubbard | 16 | 48 | 1.5 | 7.3 | 2 | 5.4 |
| | | | | | 4 | 8.6 |
| Cymding | 14 | 42 | 3 | 12.0 | 3 | 3.5 |
| | | | | | 6 | 8.7 |
| | | | | | 4 | 1.9 |
| Strawberries (<i>Fragaria</i> spp.) | <8 | <25 | 3 | 10.8 | | |
| Sweetpotatoes (<i>Ipomoea batatas</i>): | | | | | | |
| Nancy Hall | 31 | 93 | 3 | 11.6 | 8 | 11.3 |
| | | | | | 5 | 14.2 |

^a This assay was made with sulfate-treated yeast instead of autoclaved yeast in the vitamin B₁ deficient diet.

TABLE 1.—The vitamin B₁ (thiamin) assay values of foods—Continued

| Food | Vitamin B ₁ (thiamin) content per 100 grams (edible portion) | Summary of experimental data | | | |
|--|--|------------------------------|--------------------------------|--------------------------|--------------------------------|
| | | Crystalline thiamin | | Food supplement | |
| | | Amount fed per day | Weight increase per week | Amount fed per day | Weight increase per week |
| Tomatoes (<i>Lycopersicon esculentum</i>): | I. C. | μgm | μgm | Grams | Grams |
| Red..... | 26 | 78 | 3 | 9.8 | 2 |
| Turnips: | | | | | 4 |
| Greens..... | 46 | 138 | 3 | 5.9 | 2 |
| White fleshed (<i>Brassica rapa</i>)..... | 20 | 60 | 3 | 13.1 | 2 |
| Walnut meats (<i>Juglans regia</i>): | | | | | 4 |
| Persian (English)..... | 114 | 342 | 3 | 14.1 | .5 |
| Wheat (<i>Triticum aestivum</i>): | | | | | 1.0 |
| Shredded..... | 73 | 219 | 3 | 8.9 | .8 |
| Whole, hard spring..... | 175 | 525 | 3 | 11.5 | 1.5 |
| Whole, hard winter..... | 159 | 477 | 3 | 13.1 | .2 |
| Whole, soft winter..... | 118 | 354 | 3 | 11.0 | .6 |
| | | | | | 1.0 |

One rat from each litter (the so-called negative control) was continued on the vitamin B₁-deficient diet without a supplement of any kind. All of the negative control rats from stock A lost weight steadily and 76 percent were dead before the end of the assay period. Of the 156 negative control rats from stock B, 77 percent lost weight or died during the assay period and 23 percent showed small gains in weight despite having reached an apparent stage of stationary weight before the assay period was begun.

Large variations in growth rate were observed in the groups of vitamin B₁ assay rats whose vitamin B₁ supplements supported an average growth rate of less than 5 gm. per week. Maximum regularity in the growth rates of the assay rats was observed when the vitamin B₁ supplements supported an average weekly growth within the limits of 6 and 20 gm.

In the comparison of the use of sulfite-treated yeast and autoclaved yeast in the basal diet of rats used in parallel assays of rye and of lamb, values were obtained that were comparable within the limits of error of the method of assay. According to the assays of these two products, therefore, it would seem that the choice between sulfite-treated yeast and autoclaved yeast could be decided on the basis of convenience to the experimenter.

STATISTICAL ANALYSIS OF RESULTS

In the assay of the "straight-milled" wheat flour two sets of litter-mate reference rats from stock B were included, one set receiving 1.5 micrograms and the other set 3 micrograms of thiamin per rat per day. Each set consisted of three males and three females. The average weekly weight-increment of those receiving 1.5 micrograms of thiamin per day was 8.3 ± 4.9 gm. and of those receiving 3 micrograms of thiamin, 14.4 ± 5.8 gm.

The vitamin B₁ content of the flour as interpolated from these two sets of reference rats differed by less than 10 percent, a difference

within the limits of the reproducibility of the method. The mean difference between litter-mate pairs of rats receiving these two levels of crystalline thiamin was 6.2 gm. with a standard deviation of 2.3 gm.

In testing for the significance of this difference in average growth between pairs the value of t from the equation, $t = \frac{\bar{X}\sqrt{N}}{S.D.}$ was 5.4,

indicating a very high degree of probability that the difference was significant. Assuming a standard deviation of the same magnitude, the chances are 20:1 that a mean difference of 2.9 gm. would be a significant difference ($t=2.57$) in average weekly weight increment induced by two levels of thiamin as a vitamin B₁ supplement.

It would appear, therefore, that the rat-growth method for assay of vitamin B₁ as described here is sensitive to about 0.75 microgram of this vitamin when daily supplements are fed at levels that will induce average growth rates within the limits of 8 and 14 gm. per week.

Similar calculations based on the average growth rates of assay rats on two levels of a food containing appreciable quantities of vitamin B₁ also indicate that an average difference in weekly gain in weight of 3 to 5 gm. between carefully matched pairs is probably significant.

The reproducibility of the vitamin B₁-assay method together with the differences in assay values of 12 foods as calculated from 4-week and 3-week experimental assay periods, respectively, are shown in table 2. From these results it may be concluded that there is little, if anything, to be gained by prolonging the vitamin B₁-assay period beyond 3 weeks.

TABLE 2.—Data showing the reproducibility of the vitamin B₁-assay method and differences in potencies of 12 food items, calculated on the basis of 4-week and 3-week assay periods

| Food | Quantity of food equivalent to 3 micrograms of crystalline thiamin | | Difference in assay value |
|-------------------------------------|--|---------------------------------|---------------------------|
| | On basis of 4-week assay period | On basis of 3-week assay period | |
| | Grams ¹ | Grams ¹ | Percent |
| Pork, fresh, lean..... | 0.22±0.09 | 0.22±0.12 | 0 |
| Cowpeas, dried..... | .32±.06 | .32±.05 | 0 |
| Oatmeal, "quick cooking"..... | .37±.06 | .33±.10 | +11 |
| Beans, dried lima..... | .59±.14 | .75±.17 | -27 |
| Egg yolk..... | .85±.23 | .77±.25 | +10 |
| Wheat, shredded..... | 1.37±.85 | 1.45±1.14 | -8 |
| Potatoes..... | 1.62±.56 | 1.34±.17 | +17 |
| Prunes, dried..... | 1.67±.23 | 2.04±.82 | -22 |
| Mustard greens..... | 2.19±.89 | 2.36±.82 | -8 |
| Wheat flour, "straight milled"..... | 3.48±.88 | 3.52±1.10 | -1 |
| Turnips, white..... | 4.05±1.91 | 4.56±.56 | +9 |
| Milk, fresh, whole..... | 6.20±2.60 | 5.46±2.10 | +12 |

¹ Figures following the symbol ± refer to standard deviation, not standard error.

The average coefficient of variation ($100 \times S. D. / \text{mean}$) of the vitamin B₁ (thiamin) assay values for the 12 foods listed in table 2 was 33 percent. The average precision or coefficient of variation of the mean ($100 \times S. D_m / \text{mean}$) of the assay values for these same 12 food items was 13 percent (only one of the coefficients included in this average

¹ This equation is frequently referred to as Student's t test of the significance of the mean of a small sample. The symbol \bar{X} represents the mean differences between pairs; N , the number of pairs and $S. D.$ the standard deviation of a single measurement.

was greater than 17 percent). It is probably safe to assume therefore that the precision of the assay values of most of the foods included in table 1 is of the order of 15 to 20 percent.

FOODS CLASSIFIED AS SOURCES OF VITAMIN B₁

Although vitamin B₁ is widely distributed in common foodstuffs, certain classes of foods are outstanding with respect to their vitamin B₁ values. The most important natural food sources of vitamin B₁ for human dietaries are whole-grain cereals, legumes, nuts, and lean pork, both fresh and cured. Although, according to Mickelsen, Waisman, and Elvehjem (22) and to unpublished work done at the Bureau of Home Economics, as much as 50 to 60 percent of the vitamin B₁ value in lean pork is ordinarily destroyed as a result of cooking processes, the cooked product is still an excellent source of this vitamin.

Fruits, in general, are not important sources of vitamin B₁. For the most part vegetables other than legumes are distributed between fair and unimportant sources of vitamin B₁. The following classification of foods is somewhat arbitrary and is intended to furnish only a general concept of the distribution of vitamin B₁ in foods. Foods listed in the class of fair or poor, such as potatoes and milk, may furnish appreciable amounts of the daily needs for vitamin B₁ if consumed in liberal quantities.

EXCELLENT (150 INTERNATIONAL UNITS OR MORE PER 100 GM.)

| | | |
|--------------------------|--------------------------|-----------------------------------|
| Beans, lima, dried | Peanut germ | Pork, ham, smoked (lean portion) |
| Cowpeas, dried | Peanut skins | |
| Oatmeal, "quick cooking" | Peanuts, whole, raw | Soybeans, fresh, green, and dried |
| Oats, rolled | Pork chop (lean portion) | |

GOOD (100 TO 150 INTERNATIONAL UNITS PER 100 GM.)

| | |
|--------------------|----------------------------|
| Beans, lima, green | Milk powder, whole |
| Beans, navy, dried | Peas, green |
| Corn meal, white | Rye, whole |
| Egg yolk | Walnuts, Persian (English) |
| Milk powder, skim | Wheat, whole |

FAIR (30 TO 100 INTERNATIONAL UNITS PER 100 GM.)

| | | |
|--------------------|-------------------|-----------------|
| Asparagus | Corn, sweet | Pineapple |
| Beef, lean muscle | Kale | Potatoes |
| Broccoli | Lamb, lean muscle | Prunes, dried |
| Brussels sprouts | Liver | Spinach |
| Cauliflower | Mustard greens | Sweetpotatoes |
| Chicken, dark meat | Okra | Turnip greens |
| Corn meal, yellow | Peanuts, roasted | Wheat, shredded |

POOR (LESS THAN 30 INTERNATIONAL UNITS PER 100 GM.)

| | | |
|---------------------|--------------------------------|--------------------|
| Apples | Fish, halibut | Onions |
| Bananas | Fish, salmon, red, canned | Oranges |
| Beans, green, snap | Fish, trout, fresh-water | Peaches |
| Beans, yellow, wax | Flour, wheat "straight milled" | Pears |
| Beets | Flour, patent | Plums |
| Blackberries | Grapefruit | Raspberries, black |
| Cabbage | Lettuce, head | Raspberries, red |
| Carrots | Milk, condensed | Rhubarb |
| Celery | Milk, evaporated | Rutabagas |
| Cheese | Milk, skim | Sauerkraut |
| Chicken, white meat | Milk, whole | Squash |
| Cocoa | Molasses | Strawberries |
| Corn flakes | Muskmelon | Tomatoes, red |
| Egg white | | Turnips |

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