DAILY THIAMINE EXCRETIONS AND THEIR RELATIONSHIP TO CREATININE EXCRETIONS IN FOUR ADULTS ON CONTROLLED DIETS

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INTRODUCTION

This study of urinary thiamine and creatinine excretions of four adult women on controlled diet formed a part of a larger study made in the Nutrition Research Laboratory, School of Home Economics, Oregon State College. The purpose of the whole investigation was to study the effect of two different levels of thiamine intake on the nutritional status of four apparently normal adult women on constant diet. The nutritional status was evaluated by the following daily determinations: concentration of thiamine in whole blood, serum total and free riboflavin, blood hematocrit, and total daily urinary excretion of riboflavin, thiamine and creatinine.

The determination of daily urinary thiamine excretion is used in many laboratories in assessing the thiamine nutrition of man since the picture it gives is fairly reliable. It has been noticed, however, that the excreted amount of thiamine varies between different individuals on similar intakes. Also, in a given individual, there may be marked variation in thiamine excretions from time to time. Some causes for these variations have been detected by several investigators, but more experimental work is needed to find the complete explanation for them.

To determine the total daily excretion of thiamine, a 24-hour urine collection of the subject is needed. Since this is a time

consuming procedure, it would be desirable to find some shorter way to estimate the value of the total daily thiamine excretion. Adamson and co-workers (1, p.25) indicated that the excretion of thiamine is related to the excretion of creatinine, that it may be calculated in mcg. per gm. of creatinine, and that this ratio is fairly constant from voiding to voiding in a given individual. This would mean that to assess the thiamine nutrition of an individual, only one voiding of urine would need to be collected and analyzed for thiamine and creatinine. The amount of thiamine in mcg. per gm. of creatinine in this voiding would then indicate the thiamine nutrition of the individual. No further information concerning this method of thiamine estimation is given in the literature.

The purpose of this study was to add more data to previous studies on daily thiamine excretions of subjects on controlled intake and to study individual variations in the relationship of thiamine excretions to creatinine excretions of these subjects on the controlled diet. In addition a very brief study was conducted to assess the validity of estimating the total daily thiamine excretion by the thiamine per gm. of creatinine ratio in single voidings.

REVIEW OF LITERATURE

METHODS OF ASSESSING THE LEVEL OF THIAMINE IN NUTRITION

The difference between radiant health and the presence of symptoms of real thiamine deficiency can be easily detected. During the state of suboptimal health when no clinical evidence of thiamine deficiency is seen, objective tests to determine the level of thiamine nutrition are needed. The measurements of the thiamine content of the tissues, feces, blood, and urine by biochemical tests have all been used.

Carleen, et al. (6, pp.47-49) introduced a method for measuring the thiamine content of tissues, but it has not been widely used. Concerning the thiamine content of feces, Cowgill (10, pp.805-812) stated that the thiamine in feces, except during diarrhea, is found in the bodies of the bacteria present in the intestine and does not originate from the ingested food. Since thiamine is water soluble, it will be absorbed from the food and the excess excreted in urine. When assaying both urine and feces in a balance study with children in 1936, Knott (34, pp.597-611) found that the thiamine content of feces did not increase proportionately to increases in dietary thiamine intake. As these and several other studies indicate, the thiamine content of feces does not have any direct relationship to the thiamine nutrition of the individual and, hence, cannot be used in assessing the thiamine level.

The thiamine content of blood, however, is found to vary with

the intake of thiamine. Chemical methods have been used to determine the thiamine level of the blood and a microbiological test has been proposed (20, pp.372-381). The fact that comparatively large amounts of blood are needed for these methods, however, makes their frequent use difficult. A micromethod for determining thiamine in blood has been reported by Burch, but so far no method has been published.

During thiamine deficiency, an excess of pyruvate and other metabolites is accumulated in the tissues. Lu (35, pp.249-254) proposed a method to determine the blood pyruvate concentration as an indication of thiamine nutrition. However, the blood pyruvate concentration was found to vary also for reasons other than thiamine intake. Urine also may be analyzed for pyruvic acid. A disadvantage of this procedure, as well as for the measurement of blood pyruvate, is that in mild cases of deficiency little pyruvic acid can be detected.

METHODS OF DETERMINING THIAMINE NUTRITION BY URINARY TESTS

The excretion of thiamine in urine is the most widely used indication of thiamine nutrition of the individual. The presence of thiamine in human urine was first demonstrated by Muckenfuss in 1918 (42, p.595), and this finding was subsequently confirmed by van der Walle in 1922. Investigations of the factors influencing the urinary excretion of thiamine were necessary before significance could be attached to the values obtained. Melnick and Field (40, pp.97-107) reported in 1939 that thiamine in urine is in the free

form which makes the quantitative chemical measurement fairly simple. The excretion of thiamine of a given individual has been found in many laboratories to be roughly proportional to the intake of the vitamin under normal conditions. (24, pp.886-894, 63, pp.638-642, 30, pp.198-211, 39, pp.139-151) Many different ways of measuring thiamine nutrition by means of urinary analysis have been suggested and used in different laboratories.

One-Hour Fasting Sample

The amount of thiamine in a one-hour fasting specimen has been considered by a number of investigators as an indication of thiamine nutrition. The critical level in a deficiency case was stated to be zero by Holt and Najjar in 1942 (29, pp.329-330). By Johnson it was said to be .6 mcg. (31, p.128), and by Papageorge and Lewis (48, p.301), 4 mcg. These investigators do not agree whether or not the fasting-hour test gives any reliable indication of the thiamine nutrition of the subject.

Four-Hour Load Tests

There is even more disagreement in regard to the interpretation of load tests. It is agreed that in people on controlled intakes, the excretion following the administration of a thiamine test dose decreases as the general intake level of thiamine is reduced. According to Melnick's findings (42, pp.593-610), in 1939, most of a parenteral test dose of thiamine is recovered in the urine during the first 4 hours after the administration. Najjar and Holt (31, p.

128) have found that the thiamine excretion after the intravenous administration of 1 mg. of the vitamin drops to less than 50 mcg. in 4 hours when symptoms of deficiency are apparent. Williams and Mason (60, pp.71-97), making the same type of a study in 1943, stated that an excretion of less than 100 mcg. of a 1 mg. test dose indicates deficiency. Johnson (31, p.128) used an oral test dose of 5 mg. and concluded that a 4-hour output of less than 20 mcg. was subnormal. Goldsmith and Sarett found that 7 persons on normal diets excreted about 150 mcg. in 4 hours after the same oral test dose. Oldham, Davis and Roberts (47, p.163) concluded that load test excretions gave a better idea of tissue stores than did the output of one-hour fasting or even 24-hour urine collections. Robinson, Melnick and Field (50, p.399) and Wang and Harris (57, p.1356) also suggested the detection of thiamine deficiency by means of oral and parenteral test doses. In 1939, Melnick, Field and Robinson (42, pp.593-610) stated that variations in test dose recovery values of different individuals are not related to differences in urine volume, in metabolic rate, or vitamin to calorie, or thiamine to non-fat calorie ratio in the diet. "The response of the subject to the oral test dose of extra thiamine is governed by the nutritional status of the individual and is independent of the adequacy of the diet consumed at the time of the test," (42, p.608). The percentage of the available vitamin which is excreted is a function of how great an excess is present. Melnick and Field (41, pp.131-138) in 1942 studied the effects of an intramuscular test dose the amount of which depended

on the surface area of the subject's body, being 350 mcg. per square meter of the body area. A 4-hour collection was taken after the administration of thiamine. They suggested that 6% or less of the test dose excreted indicated deficiency, 9% being a safety borderline. They also considered that the subjects which excreted less than 7% of a 5 mg. oral test dose in 4 hours were deficient. In the same study, 4-hour fasting urinary determinations were done, the fasting starting 12 hours after the last meal. It was concluded that 10 mcg. or less of thiamine in a 4-hour fasting specimen of urine indicates deficiency. The minimal normal level should be 15 mcg.

Expression in Terms of Percentage

Some investigators state the amount of excreted thiamine in terms of percentage of the intake. Jolliffe and others (30, pp.198-211) in 1939 showed that their subjects excreted thiamine in the range of 7-25% of intake during the entire study. Changes in intake caused corresponding changes in output. During the high thiamine period, the subjects excreted 13-25% of the intake. During the following deficiency period, the excretion dropped to 7-23% of the intake. After the depletion period, a recovery period followed, and the excretion during that time was 11.7-21% of the intake. In 1942, Melnick (39, pp.139-151) studied a group of adult men and women on controlled thiamine intakes. The men were given 1 mg. of thiamine daily, and they excreted a mean of 200 mcg. a day. The women on a 700 mcg. intake excreted 90 mcg. daily. On this level the response of both men and women to the test dose was 13%. It

may be assumed that adequate storage had been obtained on both intake levels. Gifft and Hauck (18, pp.635-645), in 1946, had their subjects in the stage of saturation before starting the experiment. The experimental diet contained 600 mcg. thiamine per 1000 calories. The excretion of thiamine was 9-17% of the intake. A 5 mg. oral test dose was given to the subjects, and the response to it in a 24-hour collection was 15-22% of the dose. The response of the subjects to a 1 mg. intramuscular test dose was 15-24% at the beginning of the study and 8-21% at the end of it. In 1942, Melnick and Field (41, pp.131-138) observed that 23 normal adults with good stores of thiamine excreted 7-30% of a test dose, and 14 normal adults with poor stores excreted 1-7% of the same test dose. Six other adults studied by Melnick (39, pp.139-151) excreted 13-20.8% of a test dose of 10 mg. daily given for one week.

Twenty-four-Hour Urine Collections

The most commonly used method to determine the thiamine nutrition of a person is the determination of thiamine excretion in a 24-hour urine collection. Melnick, Field and Robinson (42, pp.593-610), in 1939, stated that the volume of urine in 24 hours appeared to be an insignificant factor governing the urinary output of thiamine. Daum, et al. (13, p.1049), in 1948, had a group of young women on 4 different levels of thiamine intake. The subjects receiving 140 mcg. thiamine daily excreted about 20 mcg. They developed deficiency symptoms. Another group received 200 mcg. thiamine daily. Their excretion diminished progressively for 19 weeks. On

625 mcg. daily thiamine intake level, the excreted amount diminished for 3 months, then there was a slight increase during the next two months. The thiamine excretion of subjects ingesting 1340 mcg. a day increased noticeably. The group on the lowest level was later transferred to the highest intake, but their thiamine excretion did not increase until the third month of the diet. In 1942, Mason and Williams (36, pp.247-255) had 15 female subjects on controlled diets. Their lowest thiamine intake level was 150 mcg. daily. The output on that level was 11-26 mcg. In their report, they stated that if the intake of thiamine was 1000 mcg. and the output 100±10 mcg. and the recovery after a 1 mg. test dose 20±2%, the nutrition with respect to thiamine was sufficient for minimal needs. They also indicated that 400 mcg. per 1000 calories was the minimal daily requirement for the 5 adult women in their experiment. The thiamine excretion levels of individuals on varied intake levels was also studied by Mickelsen and co-workers (44, pp.254-258) in 1946. Before starting the study the subjects were given thiamine supplements to reach the saturation level. On a 1 mg. level of daily intake, the output was 32-91 mcg. and on a 2 mg. level of intake, it was 86-310 mcg. daily. No apparent explanation was found for the relationship between the individual and his level of thiamine excretion. There was no correlation between the body weight and the amount of thiamine excreted, nor did the fecal excretion or physical activity seem to influence the thiamine output of the individual.

As already indicated in the study of Mason and Williams, a test

dose is often given to the subjects after they have been on constant thiamine intake over a certain period of time. The 24-hour recovery after the test dose is used as an indication of thiamine nutrition of the person.

ESTIMATES OF NORMAL THIAMINE NEEDS

As early as 1920 it was realized that thiamine was a necessary factor in normal nutrition. The search for facts about human requirements had begun, although the pure vitamin had not yet been isolated. The first attempt to express the thiamine requirement in the form of a mathematical formula was made by Cowgill in 1934 (9, pp.73-78). It was based on calorie intake and body weight. His formula was:

K (constant) x wt. in pounds x calorie intake,

with the K value for man as developed after isolation of pure thiamine being .00213 (14, p.104). This formula does not provide any safety factor. Applied to a 150 lb. man who consumes 2400 calories daily, it calls for 767 mcg. thiamine daily. After that several investigators studied the daily thiamine requirements of human subjects with variable results. In 1938, Harris (23, pp.67-69) recommended thiamine intakes of 300-600 I.U. daily which equals approximately 0.9-1.8 mg. According to Wilbur's estimation in 1939 (59, pp.246-253) the daily thiamine requirement of man was about 500 I.U. which equals 1.5 mg. He also stated that the requirement varies with body weight and total metabolism. The next year

Williams and co-workers (61, pp.785-799) reported that 950 mcg. of thiamine daily prevented deficiency symptoms, but 2 mg. resulted in the best performance as measured in terms of physical work done by normal women. In contradiction, Keys and Henschel (32, pp.259-269) stated that when large supplements of thiamine were added to diets containing 430 mcg. thiamine per 1000 calories, no improvement in ability to do physical work could be detected in adult subjects. The subjects of Elson and others (15, 569-577) ingested 651 mcg. thiamine daily for 120 days. Their minimum requirements seemed to be met. The authors, however, suggested that this amount might prove inadequate if consumed over a longer period of time. To determine the thiamine needs of normal adults, Melnick (39, pp.139-151) made several studies. According to his results 350 mcg. per 1000 calories is an adult requirement, but he recommended a safety guide of 500 mcg. per 1000 calories daily. The recommendation of Williams, Mason, and Wilder (60, 71-97) in 1943 was a little higher. Their subjects were kept on a thiamine intake of 450 mcg. per 1000 calories daily and slight deficiency symptoms developed. Therefore, in the opinion of these investigators, 600 mcg. per 1000 calories is none too high an allowance. After a study of different thiamine intake levels of adults, Hathaway and Strom (25, pp.1-8) stated that 0.84-1.0 mg. thiamine per day appears to be sufficient for a normal adult. The recommended daily allowance by the Food and Nutrition Board of the National Research Council was based on these and several other studies. The National Research Council recommended daily

allowance of 500 mcg. per 1000 calories which was suggested in 1948 (16, p.16) was used as a basis in this study.

CREATININE EXCRETION

Under normal conditions, the daily excretion of creatinine by an adult ranges from 1.0-1.8 gm. per day. The exact amount varies somewhat with the diet because the creatinine contained by the diet is excreted in the urine. It was shown by Folin and Myers (56, pp.696-699) that the daily excretion of creatinine on a meat-free diet is constant in a given individual. Often the excreted amount of creatinine is expressed as a creatinine coefficient which means the 24-hour excretion in milligrams per kilogram of body weight. The normal range of the creatinine coefficient is 19-30 (26, p.842). Maw (37, pp.482-486), in 1947, reported that the daily excretion of creatinine may vary up to 300 mg. from day to day, and that there is a significant correlation between the level of creatinine excretion and the day of the week, presumably due to the influence of a weekly routine common to all subjects. The next year Friedemann and others (17, pp.117-136) studied the effect on the creatinine coefficient of diets restricted in protein and the vitamin B-complex. The diet was so low in those nutrients that deficiency symptoms appeared which were reflected in metabolic changes associated with the formation and excretion of creatinine. It was concluded that the creatinine excretion paralleled the rise and fall of body weight. The creatinine coefficients of the subjects decreased gradually on the

deficiency diet. During the second period, thiamine was supplemented to the diet, but the coefficients did not increase. Friedemann states that the administration of thiamine to 2 subjects did not increase their creatinine coefficients in 2 months. The recovery was very slow even when other restricted nutrients were supplemented.

In their report on a medical survey of nutrition in Newfoundland in 1945, Adamson and his associates (1, pp.25-26) express the urinary thiamine values as mcg. per gm. of creatinine in a given voiding. The normal values for urinary thiamine excretion according to their report range between 150-300 mcg. per gm. of creatinine. It is assumed by them that an excretion of less than 50 mcg. thiamine per gm. of creatinine represents an inadequate thiamine intake and that well-nourished individuals excrete more than 150 mcg. thiamine per gm. of creatinine. It was also stated in their report that the amount of urinary thiamine per gm. of creatinine may be considered roughly as the amount of the vitamin excreted per day by a small adult.

The same way of measuring thiamine nutrition was used by Burch and co-workers (5, pp.9-30) when surveying the nutritional status of the population in Bataan, Philippines, in 1950. The literature does not yield any further information about this method of estimating the thiamine nutrition of man.

EXPERIMENTAL PROCEDURE

This study was started January 19, 1951. The previous day served as a preliminary day in which the determinations were made, but the diet was not controlled. The study lasted for 30 days, February 17 being the last, and was divided into two periods of 15 days each. During the first period, the subjects received 628 mcg. thiamine daily, furnished by the diet, plus an additional amount of 400 mcg. of thiamine hydrochloride given orally in water solution before breakfast. This way the total ingested thiamine daily during the first period was 1028 mcg. per 2000 calories which approximately follows the recommended daily allowance of the National Research Council, 1000 mcg. per 2000 calories (16, p.16). During the second period, no thiamine supplement was given to the subjects. Hence, the thiamine intake was the 628 mcg. furnished by the daily diet.

EXPERIMENTAL DIET

The subjects were maintained on a constant daily diet which provided approximately 2000 calories. The foods were selected to meet the National Research Council recommended daily allowances for all nutrients except thiamine. The calorie, thiamine, riboflavin, protein, fat, and carbohydrate values of the foods contained in the diet are given in Table 1. These food values were calculated from the food composition tables by Watt and Merrill, U.S. Department of Agriculture (58, pp.10-147) except for thiamine and riboflavin values

TABLE 1.

AMOUNTS AND NUTRITIVE VALUES OF FOODS OF THE DAILY DIET

Food	Amount gm.	Calories	Thiamine mcg.	Riboflavin* mcg.	Protein gm.	Fat gm.	Carbohydrate gm.
Milk, evaporated	100	139	75	373	7.0	7.9	9.9
Carrots, canned	100	30	18	22	.5	.4	6.1
Beef, round	100	177	77	27	19.5	11.0	--
Wheat germ	6	24	129	20	1.5	.6	3.1
Pears, canned	100	75	10	12	4.2	.1	18.4
Peaches, canned	100	75	11	18	4.4	.1	18.2
Green beans, canned	100	22	45	67	1.0	.1	4.2
Orange juice	166	80	127	68	1.0	.3	18.4
Cream of wheat	30	108	19	--	3.3	.2	23.2
Egg, E.P.	54	96	51	264	7.8	7.0	.4
Cheese, American Cheddar	30	120	14	181	7.5	9.7	.6
Biscuits	110	372	28	15	9.0	11.8	57.4
Cookies	96	422	24	101	5.8	12.2	52.0
Butter	30	220	--	9	.2	24.4	--
Sugar	20	80	--	--	--	--	20.0
Total		2040	628	1177**	72.7	85.8	231.9

* Riboflavin values were determined by Mei-ling Wu.

** The riboflavin intake was less than the National Research Council's recommended daily allowance of 1500 mcg.

which were determined in this laboratory. The individually determined values for the thiamine content of the foods are given in Table 5.

DESCRIPTION OF SUBJECTS

Four apparently normal adult women served as subjects of this study. One of them was a faculty member, three others were graduate students. Their activities during the study were quite similar. All of them were working in the laboratory doing chemical determinations. MLW, HAL, and CAS were in good health throughout the entire period. RBD had some nausea on 3 individual days. Any deviations which seemed to have affected the results of the determinations are indicated in the table of daily values and were considered in the statistical treatment of data, Table 6. A description of the subjects in terms of age, height, weight, and weight variations during the study is given in Table 2.

TABLE 2.

AGE, HEIGHT, AND WEIGHT OF THE SUBJECTS

Subject	Age	Height in.	Mean Weight lb.	Mean Weight kg.	Weight Range lb.	Weight Variation lb.
MLW	35	65.5	122.0	55.5	121.0 - 123.5	2.5
HAL	31	68.0	140.3	63.8	138.5 - 142.0	3.5
CAS	44	68.5	152.6	68.5	151.0 - 154.0	3.0
RBD	29	62.0	104.6	47.5	102.5 - 106.0	3.5

URINE COLLECTION AND PRESERVATION

The collection of urine was started the first day of the experiment after the first voiding before breakfast was discarded. The first voiding of the next day was always included in the previous day's collection. Immediately after each voiding the volume of the urine was measured and recorded. A preservative, glacial acetic acid, in the amount of 2% of the voiding was added. The pool of preserved voidings was stored in a brown bottle under refrigeration. When it was completed, the total volume was again measured. In calculations for the different analyses, the total volume, the calculated sum of individual voidings, was used unless there was an obvious error in measurement detected by the total measuring.

ANALYTICAL PROCEDURE

To determine the thiamine content of the foods and urine, the thiochrome method was used. This method was first proposed by Jansen in 1936, and later modified by Karrer and Kubli (7, pp.380-384). It was further modified by Hennessy and Cerecedo in 1939 (28, pp.179-183) who introduced the use of synthetic zeolite, called Decalso, for adsorbing the thiamine and thereby separating it from interfering substances. They also were the first ones to employ a sensitive photoelectric instrument to measure fluorescence. The thiochrome method is based on 1) oxidation of thiamine to thiochrome by potassium ferricyanide in an alkaline medium, 2) extraction of thiochrome with isobutanol which has been redistilled to free it

from fluorescent material, and 3) estimation of the intensity of the violet-blue fluorescence in ultra-violet light. Mickelsen, Condiff and Keys (45, pp.361-370), in 1947, suggested an additional adjustment of pH before the extraction step to minimize interference of other fluorescent substances often present in the samples. The procedure as followed in this study includes all these modifications and has been previously used in this laboratory with satisfactory results (unpublished data).

Reagents and Equipment Used

During the procedure for determination of thiamine in the foods and urine, the following reagents and equipment were used.

Standard Solutions

1. Quinine Sulfate Stock Solution A: 0.0108 gm. of U.S.P. quinine sulfate was dissolved in 0.1 N H_2SO_4 and diluted to 1 liter with the same solvent. It was stored in a brown, glass-stoppered bottle under refrigeration.
2. Quinine Sulfate Working Standard Solution: 5 ml. of the stock solution A was pipetted to a 200 ml. volumetric flask and diluted to volume with 0.1 N H_2SO_4. The solution was stored as explained above, and it was used to set the fluorometer reading at 70 before reading of unknown.
3. Thiamine Stock Solution A: 25.0 mg. of Merck's crystalline thiamine hydrochloride, kept dry over concentrated H_2SO_4, was dissolved with 20% ethanol of pH 4 and made up to 500 ml. in a volumetric flask.

4. 20% Ethanol of pH 4: 120 ml. of 95% ethyl alcohol was added to 450 ml. distilled water. The pH of the solution was measured with a Beckman pH meter and adjusted to pH 4 by adding 0.1 N HCl drop by drop.

5. Thiamine Intermediate Solution: 5 ml. of thiamine stock solution A was diluted to 50 ml. with buffered water pH 4.5. One ml. of this solution was equivalent to 5 mcg. of thiamine hydrochloride.

6. Thiamine Working Solution: 10 ml. of thiamine intermediate solution was pipetted into a 250 ml. volumetric flask and diluted to volume with buffered water, pH 4.5. Thus, 1 ml. of this solution was equivalent to 0.2 mcg. of thiamine hydrochloride.

Other Reagents

7. Buffers

   a. pH 4.5: 55 ml. glacial acetic acid and 66.6 gm. anhydrous sodium acetate were made up to 1 liter with distilled water. The pH was measured with Beckman pH meter and adjusted if necessary.

   b. pH 5.65 to 6.68: Anhydrous sodium acetate was dissolved in full strength glacial acetic acid to the point of saturation.

8. Buffered water, pH 4.5: Distilled water was adjusted to pH 4.5 little by little by adding the buffer pH 4.5 to it and testing the pH with Beckman pH meter. The approximate concentration of the solution was 5:100.

9. 1.0 N HCl: 83 ml. of concentrated C.P. HCl was diluted to 1 liter with distilled water.

10. 0.1 N HCl: 10 ml. of concentrated HCl was diluted to 1200 ml. with distilled water.

11. 0.1 N H_2SO_4: 5 ml. of concentrated H_2SO_4 was diluted to 1800 ml. with distilled water.

12. Silver Nitrate Solution: 25 ml. of 5% silver nitrate solution in distilled water and 25 ml. of concentrated HNO_3 were mixed together. The solution was used to test whether Decalso was washed free from chlorides.

13. 2% Acetic Acid: 40 ml. of glacial acetic acid was diluted to 2 liters with distilled water.

14. 1% Potassium Ferricyanide: 1.0 gm. of crystalline potassium ferricyanide was dissolved in 100 ml. of distilled water. It was stored in a dark bottle.

15. Anhydrous Sodium Sulfate: Preparation of Mallinckrodt Chemical Works.

16. Isobutanol: Isobutyl alcohol was redistilled before using. The portion only which distilled between temperatures of 106-108°C. was used as a reagent. The fluorescence of the distillate was tested to be the same or less than that of distilled water.

17. 15% Sodium Hydroxide Solution: 2.5 liters of distilled water were boiled for 10 minutes to get rid of CO_2 and cooled to room temperature. 300 gm. of NaOH pellets were disolved in 1.5 liters of this water which was then cooled again to room temperature. The solution was diluted to 2000 ml. with the same CO_2-free water. 20 gm. of C.P. $Ba(OH)_2$ was added to this

solution, stirred well, and allowed to stand in a dark place until the next day. The clear liquid was then decanted and the residue discarded.

18. 25% KCL in 0.1 N HCL: 800 ml. of 0.1 N HCl was heated to boiling. 250 gm. of C.P. KCl were dissolved in it, and the solution was cooled to room temperature. It was made up to 1 liter with 0.1 N HCl.

19. Acid Solution: 50% concentrated HCl and 50% concentrated H_3PO_4 for adjusting the pH of the sample before adding isobutanol.

20. Enzyme Solutions for Food Analyses: 5% takadiastase solution was made up by weighing 2.5 gm. takadiastase which was dissolved in 50 ml. of buffer, pH 4.5.

    5% papain was prepared the same way using papain.

    Both enzyme solutions were prepared fresh just before using them.

21. Decalso, Synthetic Zeolite. Activation: About 1 kg. of Decalso, 50-80 mesh, obtained from Permutit Co., was soaked in 2 liters of hot 2% acetic acid and stirred well several times. The liquid with floating debris was decanted. The Decalso was soaked again with three successive portions of hot acetic acid. When the last amount of acid was poured off, the Decalso was drained as dry as possible. Then it was soaked for 20 minutes in boiling 25% KCl and stirred well. The KCl was decanted. Two washings with hot 2% acetic acid followed and then at least three washings with hot distilled water. The last washing water was tested with silver nitrate solution to determine whether it was free

from chlorides and also with litmus paper as to whether neutral. If either test was positive, Decalso was washed more with hot distilled water. Finally it was drained well and spread on trays to dry in air.

Equipment

Beckman pH Meter, laboratory model G, National Technical Laboratories.

Coleman Electronic Photofluorometer, model 12.

International Centrifuge, size I, type SB, series no. W9601.

Base Exchange Tubes, called Decalso columns. When prepared for use, a small plug of glass wool was first placed into the bottom opening. The tube was filled with distilled water. Decalso which had been soaked in distilled water previously was introduced with a spoon into the funnel part of the tube and allowed to settle down by gravity while the column was slowly rotated. Air bubbles in the tube were avoided. Decalso was added until the level of it was 2-3 mm. above the opening to the funnel. Water was allowed to drain just before introducing the sample into the tube.

Reaction Tubes. Glass-stoppered, conical Maizel-Gerson reaction vessels were used. Wooden racks were prepared to support the vessels.

Matched Cuvettes were used for fluorometer readings.

Incubator Oven for food samples was maintained at the temperature of 37°C.

Syringe Pipettes

20 ml. syringe, B-D Yale, was used for isobutanol

Hypo-syringe, Chieftain, 5 ml., was used for NaOH

Hypodermic tuberculin syringe, B-D Yale

Syringe Pipette Holders were of suitable size for each pipette.

Determination of Thiamine Standard Curve

To calculate the thiamine values of unknown samples, a thiamine standard curve was determined by using known amounts of thiamine hydrochloride. Different volumes of the thiamine working solution, containing 0.2 mcg. thiamine hydrochloride per ml., were pipetted into 25 ml. glass-stoppered graduated cylinders and diluted to volume with buffered water, pH 4.5. The following amounts of thiamine were used: 0.0; 0.2; 0.4; 0.6; 0.8; 1.0; 1.2; 1.4; 1.6; 1.8; 2.0; 2.4; 2.8; 3.2; 3.6; and 4.0 mcg. Each 25 ml. sample was run through a Decalso column. Three washings, 10 ml. each, of buffered water, pH 4.5, followed to rinse the graduated cylinder and the sides of the column. Activated Decalso during this process adsorbs thiamine from the solution. After the last washing was through, the washings were discarded and a new 25 ml. graduated cylinder was placed under the column for collecting the thiamine which is eluted with hot 25% KCl solution. Enough KCl was added to make the eluate exactly up to volume.

Two 10 ml. portions of the eluate were pipetted into the reaction vessels. Into each aliquot, 3 drops of 1% potassium ferricyanide were added followed by 3 ml. of 15% NaOH solution. The pH of the

aliquot was measured and adjusted to a range between pH 8.5-9.8 with an acid solution containing equal amounts of concentrated HCl and concentrated H_3PO_4. 13 ml. of redistilled isobutanol was then added and the vessels were shaken vigorously for 1.5 minutes. They were centrifuged for 2 minutes. The bottom aqueous layer was removed by suction with a capillary tube. 2-3 gm. of anhydrous Na_2SO_4 were added to the extract to remove the last traces of water. The vessels were shaken to mix and centrifuged for 1.5 minutes. The clear extract was poured into dry fluorometer cuvettes and the fluorescence was measured in a Coleman Electronic Photofluorometer set at 70 with the quinine sulfate reference solution. A blank reading, blank containing no thiamine but all the reagents, was subtracted from each sample reading to correct for any preformed fluorescence present in the reagents. The data collected in at least three trials of each amount of thiamine are shown in Table 3. They were treated statistically by the method of least squares suggested by Coward (8, pp.20-21) to obtain a theoretical curve. The statistical data are summarized in Table 4. The calculated curve was plotted (Figure 1.) and used for thiamine calculations in food and urine samples.

Determination of Thiamine in Foods

Lohmann and Schuster (7, pp.382) have shown that a great part of the naturally occurring thiamine may be present in foods in the form of its pyrophosphoric ester known as cocarboxylase. This is

TABLE 3.

SUMMARY OF RESULTS IN DETERMINATION OF
THIAMINE STANDARD CURVE
[Each number is the mean value of two corrected readings (reading - blank)]

Mcg. thiamine in aliquot	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	2.0	2.4	2.8	3.2	3.6
Corrected fluorometer readings	4.75	9.5	13.0	18.5	24.5	25.5	32.0	36.0	47.5	44.75	59.25	68.0	78.25	88.75
	5.0	10.5	16.5	19.25	22.0	33.0	33.0	37.5	38.75	45.5	57.5	63.5	78.5	82.0
	5.0	9.0	13.75	17.0	24.0	31.25	35.0	37.5	35.75	44.25	61.75	69.0	77.25	81.0
			14.0		21.0	25.5			44.25		55.5	57.5	77.0	79.5
						26.5					57.25	56.0		80.5
												61.5		
												72.5		
												59.0		
												80.0		
												81.0		
												78.5		
												68.0		
Average	4.92	9.67	14.31	18.25	22.88	28.35	33.33	37.00	41.56	44.83	58.25	67.92	77.75	82.35

TABLE 4.

DETERMINATION OF THEORETICAL THIAMINE STANDARD CURVE BY METHOD OF LEAST SQUARES

Concentration of thiamine,	Observed readings	Deviation from mean	Product of	Squares of deviation from mean	Corrected Y
X	Y	$X - \overline{X}$	$Y(X - \overline{X})$	$(X - \overline{X})^2$	$\overline{Y} - B(X-\overline{X})$
0.2	4.92	-1.443	- 7.0996	2.0822	4.57
0.4	9.67	-1.243	- 12.0198	1.5450	9.31
0.6	14.31	-1.043	- 14.9253	1.0878	14.04
0.8	18.25	-0.843	- 15.3848	0.7106	18.78
1.0	22.88	-0.643	- 14.7118	0.4134	23.51
1.2	28.35	-0.443	- 12.5591	0.1962	28.25
1.4	33.33	-0.243	- 8.0992	0.0590	32.99
1.6	37.00	-0.043	- 1.5910	0.0018	37.72
1.8	41.56	+0.157	+ 6.5249	0.0246	42.46
2.0	44.83	+0.357	+ 16.0043	0.1274	47.19
2.4	58.25	+0.757	+ 44.0953	0.5730	56.67
2.8	67.92	+1.157	+ 78.5834	1.3386	66.14
3.2	77.75	+1.557	+121.0568	2.4242	75.61
3.6	82.35	+1.957	+161.1590	3.8298	85.08

The value of corrected Y, the theoretical reading, is obtained by the equation $Y = \overline{Y} - B(X - \overline{X})$

$\overline{X}$ = mean value of X

Y = corrected fluorometer reading (reading - blank)

$\overline{Y}$ = mean value of Y

B = slope of the curve, obtained by equation $\frac{\Sigma Y(X - \overline{X})}{\Sigma (X - \overline{X})^2}$

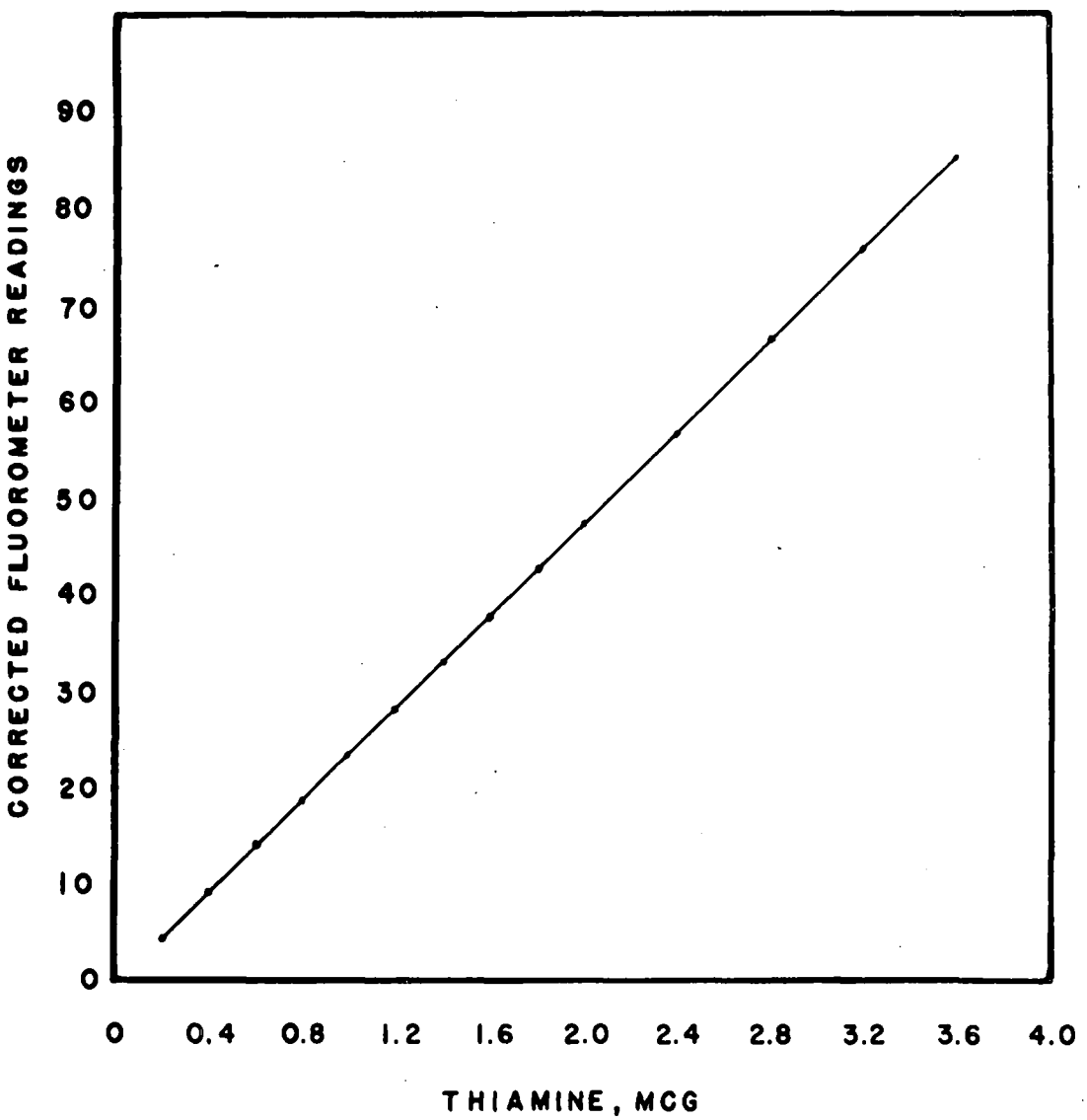
$\overline{X} = 1.643$

$\overline{Y} = 38.67$

$B = \frac{341.0331}{14.4143} = 23.68$

FIGURE 1.

THEORETICAL THIAMINE STANDARD CURVE



converted to the pyrophosphoric ester of thiochrome by potassium ferricyanide, but Kinnersley and Peters (33, p.697) have shown that this compound is not extractable with isobutanol and must be broken down to obtain correct thiamine values in analysis. Therefore, the cocarboxylase has to be hydrolyzed prior to oxidation with potassium ferricyanide. Enzymatic action for hydrolysis has been used by several investigators. Hennessy and Cereceda (28, p.179-183) used a liver preparation. Tauber and Lohmann and Schuster (20, p.93) tried starch-splitting enzymes like mylase, clarase, takadiastase. Conner and Straub made an extensive study to determine the effectiveness of these different enzymes. They came to the conclusion that 5% takadiastase or 5% clarase are the most effective ones, and similar in their action in hydrolysis of starches. Halliday and Deuel, in 1941, (21, p.555) proposed a mixture of 5% papain and 5% takadiastase to free thiamine from its protein complex. To determine the thiamine values of the foods used in the experimental diet of this study, the following procedure was used.

Four samples of each kind of food were weighed out--the size of the sample depending on the estimated thiamine content of the food. The sample was mixed in a Waring blendor with 75 ml. of 2% acetic acid for 2 minutes; 25 ml. of acid were added rinsing the lid and sides of the blendor and mixing was continued for one more minute. The total volume of the mixture was then measured, one half of it was poured into an Erlenmeyer flask, and the other half was discarded. 5 ml. of 5% papain and 5 ml. of 5% takadiastase were used to rinse

the sides of the measuring cylinder and then added to the sample. The sample was incubated at 37°C. over night, at least 14 hours. After this digestion period the mixture was brought to boil to inactivate the enzymes. It was cooled under running water and centrifuged for 10 minutes. The supernatant liquid was filtered through a sharkskin filter paper. 5 to 25 ml. aliquots of the filtrate were used acoording to the estimated thiamine content. The pH of the aliquot was adjusted to 4.5 and the volume was made up to 25 ml. with buffered water, pH 4.5. The procedure after that was the same as in the determining of the thiamine standard curve, described above. One sample of each kind of food was used for a blank determination to detect any other fluorescent material present in the food. The blank was run as the other samples except that the oxidation with potassium ferricyanide was omitted. The blank reading was then subtracted from the readings of the other samples to obtain the value of fluorescence caused by thiamine in the sample. To the fourth sample of each food, a certain amount of thiamine hydroohloride was added for a recovery test. The values obtained in food determinations are shown in Table 5.

Determination of Thiamine in Urine

To determine the thiamine content of preserved urine, a 100 ml. sample of each collection was measured. The pH of the preserved urine was determined and adjusted to pH 4.5 with saturated sodium acetate buffer. The volume of the added buffer was measured carefully and added to the volume of the 100 ml. sample for later

TABLE 5.

DETERMINATION OF THIAMINE IN FOODS OF THE DAILY DIET

Food	Amount gm.	Weight of sample gm.	Thiamine in sample mcg.	Average thiamine in sample mcg.	Thiamine per 100 gm. food mcg.	Thiamine in daily amount mcg.
Milk, evaporated*	100	2	1.47			
			1.51			
			1.51	1.50	75	75
Carrots, canned	100	30	5.68			
			5.40			
			5.40	5.49	18	18
Beef, round	100	10	9.92			
			6.40			
			6.26			
			8.16			
			7.31			
			8.16	7.70	77	77
Wheat germ	6	1	23.89			
			22.08			
			19.11			
			22.54			
			20.30			
			22.06			
			20.53	21.50	150	129

* The enzyme solutions used for determining thiamine in milk were made up in distilled water instead of buffer.

TABLE 5.

DETERMINATION OF THIAMINE IN FOODS OF THE DAILY DIET
(Continued)

Food	Amount gm.	Weight of sample gm.	Thiamine in sample mcg.	Average thiamine in sample mcg.	Thiamine per 100 gm. food mcg.	Thiamine in daily amount mcg.
Pears, canned	100	100	9.51			
			9.98			
			9.51			
			12.42			
			10.58	10.40	10	10
Peaches, canned	100	50	5.39			
			5.39			
			5.99			
			5.67	5.61	11	11
Green beans, canned	100	20	8.31			
			8.82			
			9.52			
			10.08			
			8.82	9.11	45	45
Orange juice	166	10	8.52			
			8.04			
			7.08			
			8.04			
			7.44			
			7.44			
			7.44	7.71	77	127

TABLE 5.

DETERMINATION OF THIAMINE IN FOODS OF THE DAILY DIET
(Continued)

Food	Amount gm.	Weight of sample gm.	Thiamine in sample mcg.	Average thiamine in sample mcg.	Thiamine per 100 gm. food mcg.	Thiamine in daily amount mcg.
Cream of wheat	30	8.5	5.51 5.13 5.13	5.26	59	19
Egg, E.P.*	54	10	9.79 9.16 9.69	9.55	955	51
Cheese, American Cheddar	30	5	2.12 2.55 2.46	2.38	48	14
Biscuits	110	20	5.33 4.87 5.21	5.14	26	28
Cookies	96	10	2.30 2.68 2.55	2.51	25	24

* The average weight of the edible portion of the eggs was used in calculations.

thiamine calculation. Aliquots of 2 to 15 ml. in volume were taken of the buffered urine, the volume of the aliquot depending on the expected thiamine content of the urine. The aliquot was made up to 25 ml. volume with buffered water, pH 4.5. Three aliquots of each sample were taken and to one of them a known amount of thiamine hydrochloride was added for a recovery test. The rest of the procedure was essentially the same as in the determination of the thiamine standard curve, differing only in the blank determination. When the two 10 ml. portions of the 25 ml. KCl eluate were pipetted into 2 reaction vessels, one of them was used as a blank by omitting the addition of potassium ferricyanide. This blank reading was subtracted from the aliquot reading. For the recovery, both portions were oxidized and the average of the blank readings of both other aliquots was subtracted from the readings of the recovery aliquots.

Determination of Creatinine in Urine

The creatinine content of preserved urine was determined with Folin's method (26, p.839) which is based upon the property of creatinine to yield a definite color reaction in the presence of picric acid in an alkaline medium.[1]

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1. The creatinine determinations were made by Betty E. Hawthorne.

RESULTS AND DISCUSSION

The daily data of thiamine and creatinine excretion values are summarized in Table 6. and in Figure 2. In the statistical treatment of data, for the first period of the study the values of the last 10 days were used. The first 5 days were considered as an adjustment period. For the second period when the thiamine intake was lowered, the first 4 days served as an adjustment period, and only the values of following 10 days were used in the statistical treatment of the data. On the last day of the study a 5 mg. oral test dose of thiamine hydrochloride was given to each subject.

TWENTY-FOUR-HOUR EXCRETIONS OF THIAMINE

As shown in Table 6., the mean values of the daily thiamine excretions of the four subjects ranged from 100.5 to 275.6 mcg. for the first period and from 35.6 to 78.5 mcg. for the last period of the study. The variation in day-to-day values of each subject is more noticeable on the higher intake level. It is especially great for the subject RBD who either had big enough stores of thiamine because she had been taking brewers' yeast previous to the study or because her requirement was smaller than that of the others due to her smaller body size, indicated in Table 2. and also indicated by her creatinine excretion value, Table 6. The first reason may be closer to the truth since her thiamine excretion was constantly diminishing throughout the first period. Mickelsen, Caster, and Keys (44, pp.254-258) showed that variations in thiamine

TABLE 6.

DAILY EXCRETIONS OF THIAMINE, CREATININE, AND THIAMINE PER GRAM OF CREATININE FOR FOUR SUBJECTS ON CONTROLLED DIET

Day of Study		Thiamine Intake	MLN			HAL			CAS			RBD		
			Thiamine	Creatinine	Thiamine/gm. Creatinine	Thiamine	Creatinine	Thiamine/gm. Creatinine	Thiamine	Creatinine	Thiamine/gm. Creatinine	Thiamine	Creatinine	Thiamine/gm. Creatinine
		mcg.	mcg.	gm.	mcg.	mcg.	gm.	mcg.	mcg.	gm.	mcg.	mcg.	gm.	mcg.
Preliminary			331.5	1.10	301.4	64.1	1.18	54.3	238.4	1.51	157.9	643.0	1.08	595.4
1		1028	442.2	1.05	421.1	142.1	1.21	117.4	250.1	1.55	161.4	549.8[4]	1.07[4]	513.8[4]
2			323.7	1.13	286.5	99.5	1.24	80.2	178.3	1.54	115.8	359.4	1.06	339.1
3			319.4	1.03	310.1	119.1	1.28	93.0	177.8	1.54	115.5	363.9	1.13	322.0
4			312.7	1.01	309.6	117.1	1.28	91.5	159.7	1.50	106.5	347.4	1.01	344.0
5			253.0	1.11	227.9	108.7	1.25	87.0	*	*	*	385.2	1.10	350.2
6			304.7	1.12	272.1	*	*	*	181.3	1.52	119.3	332.4	1.10	302.2
7	First period		260.7	1.12	232.8	96.2	1.20	80.2	114.8	1.42	80.8	275.7	1.11	248.4
8			311.5	1.12	278.1	94.9	1.26	75.3	140.8	1.45	97.1	328.2	1.09	301.1
9			235.8	1.12	210.5	92.8	1.23	75.4	123.0	1.46	84.2	287.5[5]	1.10[5]	261.4[5]
10			271.0	1.13	239.8	84.0	1.21	69.4	117.6	1.45	81.1	302.4	1.09	277.4
11			298.5	1.14	261.8	111.6	1.28	87.2	134.9	1.47	91.8	303.0	1.08	280.6
12			283.0	1.21	233.9	106.9	1.28	83.5	140.5	1.53	91.8	287.9	1.16	248.2
13			201.4	1.15	175.1	102.8	1.26	81.6	106.3	1.53	69.5	230.2	1.18	195.1
14			258.0	1.16	222.4	112.1	1.25	89.7	126.0	1.51	83.4	201.2	1.09	184.6
15			255.4	1.18	216.4	103.1	1.29	79.9	139.8	1.50	93.2	207.9	1.04	199.9
16		628	135.3	1.15	117.7	67.0	1.27	52.8	84.2	1.48	56.9	176.2	1.24	142.1
17			117.3	1.17	100.3	54.8	1.29	42.5	83.5	1.51	55.3	150.1[6]	1.08[6]	139.0[6]
18			114.6	1.13	101.4	49.4	1.29	38.3	66.6	1.57	42.4	97.7	1.08	90.5
19			100.1	1.10	91.0	51.9	1.27	40.9	54.9	1.59	34.5	109.7	1.12	97.9
20			88.5	1.10	80.5	47.8	1.27	37.6	64.0	1.57	40.8	87.4	1.08	80.9
21			85.5	1.07	79.9	55.1	1.28	43.0	69.3	1.56	44.4	83.6	1.08	77.4
22	Second period		83.4	1.12	74.5	43.9	1.27	34.6	55.3	1.56	35.4	68.0	1.10	61.8
23			72.8	1.13	64.4	40.1	1.27	31.6	55.0	1.56	35.3	*	*	*
24			73.0	1.09	67.0	32.1	1.35	23.8	45.8	1.55	29.5	78.1	1.11	70.4
25			71.0	1.14	62.3	30.9	1.32	23.4	48.0	1.62	29.6	78.2	1.12	69.8
26			81.2	1.13	71.9	24.5	1.26	19.4	39.2	1.58	24.8	66.3[7]	1.08[7]	61.4[7]
27			77.0	1.09	70.6	27.5	1.31	21.0	36.8	1.54	23.9	53.0	1.07	49.5
28			79.7	1.13	70.5	28.3	1.35	21.0	39.5	1.56	25.3	80.8	1.10	73.5
29			72.6	1.07	67.9	25.3	1.28	19.8	32.2	1.53	21.0	48.3	1.04	46.4
30	5 mg. test dose		194.7	1.11	175.4	130.8	1.32	99.1	141.5	1.48	95.6	375.7	1.06	354.4
First period														
Mean			268.0	1.15	234.3	100.5	1.25	80.2	132.5	1.48	89.2	275.6	1.10	249.9
S.D. of mean[1]			±10.6	±0.0105		±3.1	±0.012		±6.6	±0.0105		±14.9	±0.0105	
Second period														
Mean			78.5	1.12	71.0	35.6	1.30	27.5	48.5	1.56	31.0	71.5	1.09	65.7
S.D. of mean[1]			±1.9	±0.0105		±3.3	±0.0105		±3.9	±0.0105		±4.6	±0.008	
S.D. of diff. between means[2]			±10.8			±4.5			±7.6			±15.5		
Diff. between means			189.5			64.5			84.0			204.1		
			Significant[3]			Significant[3]			Significant[3]			Significant[3]		

* Value missing because of some loss of the urine.

TABLE 6. (Continued)

1) The standard deviation of the mean in each case has been calculated by using the formula: $S.D.m = \pm\sqrt{\frac{\Sigma\ d^2}{n(n-1)}}$ where S.D.m = the standard deviation of the mean and d = the deviation from the actual mean.

2) The standard deviation of the difference between two means has been calculated by using the formula: $\sigma Dm_1 - m_2 = \pm\sqrt{S.D.m_1^2 + S.D.m_2^2}$ in which $Dm_1 - m_2$ = difference between first and second mean.

3) For the purposes of this study, a difference between the two means which is two times the standard deviation of the difference between means is considered significant.

4) Subject vomited after having ingested the thiamine supplement and part of her breakfast.

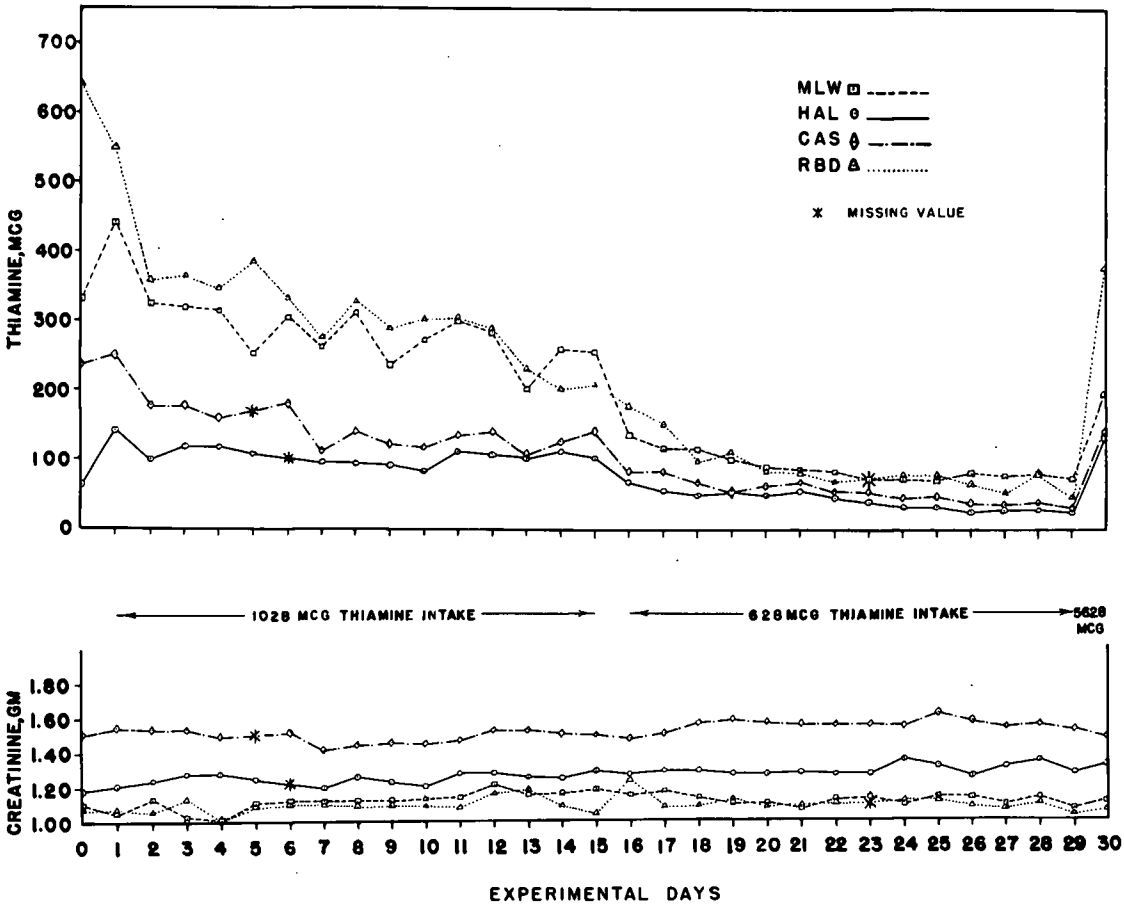
5) Subject forgot to eat 10 cookies and ate them the next morning.

6) Subject was nauseated and had diarrhea, vomited lunch and skipped dinner.

7) Subject did not eat her cookies.

FIGURE 2.

DAILY URINARY EXCRETIONS OF THIAMINE AND CREATININE
OF FOUR ADULTS ON CONTROLLED DIET



excretions of individuals are considerable on intake levels of 0.6 to 2.0 mg. of thiamine daily. The same authors (43, p.415) stated that variability in data became more marked when thiamine intake was increased. Concerning individual variation in excretion of thiamine, Daum, Tuttle, and Wilson (12, pp.398-403) reported that "on any constant daily level of thiamine intake an individual showed day-to-day variation in urinary excretion of thiamine, the more so as the intake and excretion of thiamine increased."

There is also a marked difference in the thiamine excretion levels between the two larger subjects, CAS and HAL, and the two smaller subjects, MLW and RBD, Figure 2. That body size is a factor in thiamine requirement of man has been reported by several investigators. Jolliffe and co-workers (30, pp.198-211) found indications that smaller subjects excreted more thiamine than larger ones when maintained on the same intake level. Daum, Tuttle, and Wilson (12, pp.398-403) stated that "there is reason to surmise that differences in the mass of active metabolizing tissue would be reflected in differences in the thiamine requirement of individuals.... Increased body size, especially in mass of active, metabolizing tissue, would call for increasing absolute amounts of thiamine in man." In contradiction to these statements is the finding of Elsom, et al. (15, pp.569-577) that the output of thiamine was independent of the body weight of the subjects on their study.

The thiamine excretion values of these subjects can be compared to the values obtained in other experiments. Mason and Williams

(36, pp.247-255), having their subjects on 1000 mcg. daily thiamine intake level, stated that if the output was 100± 10 mcg., the thiamine intake was sufficient for minimal needs. Melnick, Field, and Robinson (42, pp.593-610) maintained their subjects on the same intake level of 1000 mcg. daily and obtained excretion values from 237 to 248 mcg. which compare with the higher values of this study. Gifft and Hauck (18, pp.635-645) gave vitamin B-complex tablets to their subjects for 6 days prior to the study. The subjects were then maintained on intake of 600 mcg. per 1000 calories which is higher than the intake for the first period of this study. Their excretion values ranging from 195 to 210 mcg. were no higher than the mean values of the two smaller subjects of this study during the first period. Hathaway and Strom (25, pp.1-8) fed 3 normal adult women a synthetic diet with a daily thiamine intake of 1000 mcg. The average excretions were 113, 116, and 147 mcg. daily. These are lower values than most reported in literature for subjects on comparable intake levels. Jolliffe and co-workers (30, pp.198-211) reported daily thiamine output values of 319-676 mcg. when the intake level of the subjects was 564 mcg. per 1000 calories. They also stated that the deficiency symptoms did not appear before the output was below 100 mcg. daily. The intake level on Jolliffe's study corresponds approximately with the intake during the first period of this study, whereas the excretion range is considerably higher. However, the subjects of Jolliffe had prior to the study had a "control period" during which they received large amounts of thiamine,

so that they were in a stage of saturation when the experiment was started.

As indicated in the case of RBD, tissue stores of thiamine cause variation in the excretion and higher levels of output. The low excretion values of HAL may be caused by poor thiamine nutrition previously. That the previous dietary intake of thiamine is an important factor in thiamine excretion on controlled diet has been shown frequently in literature. Mason and Williams (36, pp.247-255) came to the conclusion that evidence of tissue storage of thiamine may be found in excretion as long as four weeks after restriction of the intake to 400-600 mcg. daily. Daum, and co-workers (13, p.1049) had a group of subjects on 140 mcg. daily thiamine intake level until they were depleted. When transferred to a level of 1340 mcg. thiamine daily, their excretion did not increase until the third month. Melnick (39, pp.139-151) compared the urinary thiamine excretion before and after tissue saturation of 6 normal adults maintained on 1000 mcg. daily intake. The daily excretion was 145 mcg. before and 317 mcg. after saturation on the same intake. Oldham, Davis and Roberts (47, pp.163-180) had young women on different thiamine intake levels. When the intake was 510 mcg. per 1000 calories, the average excretion was 107 mcg. before and 196 mcg. after saturation. Robinson, Melnick, and Field (50, pp.399-408) observed 2 groups of hospital patients, males and females, divided according to their estimated previous thiamine nutrition. The daily excretion of the group considered to be on normal levels was 90 mcg.

or more for males and 60 mcg. or more for females, the corresponding values being 66 mcg. or less and 43 or less, respectively, for the group which had had poor thiamine nutrition.

The daily thiamine intake during the last period of this study compares approximately with the intake of 625 mcg. daily, received by the subjects of Daum, Tuttle, Wilson, and Rhodes (13, pp.1049). These authors reported that the daily thiamine excretion of the subjects on this level diminished for the three first months of the study averaging 31 mcg. which suggested that this intake was not sufficient. Oldham, et al. (47, pp.163-180) maintained their subjects on the intake level of 360 mcg. per 1000 calories which is somewhat higher than the level of the last part of this study. Their excretion values averaged 107 mcg. daily which is considerably higher than the average values obtained in this study. Mason and Williams (36, pp.247-255) reported that when their subjects were maintained on 600 mcg. daily thiamine intakes, the excretion of those with good stores of thiamine ranged from 196 to 233 mcg. and of those with poor stores from 52 to 71 mcg. daily. The first values are high on this intake level if compared with others on comparable intake.

According to the investigations reviewed above, it may be assumed that 90 to 100 mcg. is the critical level of daily urinary excretion of thiamine. Since the average output of the four subjects on this study was above this level during the first half of the experiment, it indicates that the intake of 0.5 mg. per 1000 calories was enough to meet the requirement of these subjects, although the

larger subjects, CAS and HAL, might have needed higher intakes for optimal thiamine nutrition. The average excretions during the last period of the study fell below the critical level of 90 mcg. showing that the intake level of 0.3 mg/1000 calories daily was not adequate to meet the thiamine requirement of any of these subjects. It should also be noticed that during this low thiamine period the thiamine excretion of each subject was constantly diminishing.

DAILY THIAMINE EXCRETIONS IN PERCENTAGE OF INTAKE

As shown in Table 7, the daily thiamine excretion in percentage of the intake ranged from 9.8 to 26.8 per cent for the first period and from 5.7 to 12.5 per cent for the second period of the study. These figures may be compared to similar values in the literature. The subjects of Gifft and Hauck (18, pp.635-645) were in a state of saturation when the study was started. They received 600 mcg. of thiamine per 1000 calories and the excretion values in percentage of intake were from 9 to 17 per cent which is surprisingly low if compared to values obtained in this study on a lower thiamine intake. Their subjects were stated to have sufficient thiamine nutrition. Melnick, Field, and Robinson (42, pp.593-610) reported higher excretion levels. Their subjects on 600 mcg. daily intake excreted 9-17 per cent of the intake, and when the intake was increased to 1000 mcg., the excretion also increased, ranging from 21 to 25 per cent of the intake. The subjects of Jolliffe and co-workers (30, pp.198-211), on an intake level of 564 mcg. of thiamine per 1000

calories which approximately compares with the intake level of the first part of this experiment, excreted 13.1 to 25.7 per cent of the intake.

When considered in comparison to the reviewed results above, the excretion values of this study seem to fall within the same range if the first period of the study is considered. Even the lowest percentage, that of HAL, compares with those stated as normal by Gifft and Hauck. The range of the percentage on the low intake level is markedly below that of Melnick's subjects on a comparable level of intake, and may be considered as subnormal. Hence, according to these evaluations of thiamine nutrition by determining the excretion in percentage of the intake, the intake of thiamine during the first period of the study was sufficient, but during the second period it was inadequate for all of the subjects.

TABLE 7.

MEAN THIAMINE EXCRETIONS IN MCG. AND IN PERCENTAGE OF INTAKE FOR BOTH PERIODS OF STUDY

Subject	Intake mcg.	Output mcg.	Output %
MLW	1028	268.0	26.1
	628	78.5	12.5
HAL	1028	100.5	9.8
	628	35.6	5.7
CAS	1028	132.5	12.9
	628	48.5	7.7
RBD	1028	275.6	26.8
	628	71.5	11.4

RECOVERY OF THE TEST DOSE

A 5 mg. oral test dose was given to the subjects the last day of the study. The recovery in the 24-hour urinary excretion was determined. The percentage of the recovery was calculated in relation to the total day's intake. Table 8. shows the recovery values.

TABLE 8.

RECOVERY OF A 5 MG. ORAL TEST DOSE
IN MCG. AND IN PERCENTAGE OF INTAKE

Subject	Total Intake mcg.	Recovery in 24 Hours mcg.	%
MLH	5628	194.7	3.5
HAL	5628	130.8	2.3
CAS	5628	141.5	2.5
RED	5628	375.7	6.7

The subjects whose thiamine excretion had been lowest during the study also had the lowest recovery values showing that they were more depleted in thiamine than the others. These results may be compared to results of similar experiments as stated in the literature. Robinson, Melnick and Field (50, pp.399-408) gave a 5 mg. oral test dose of thiamine to two groups of subjects. The first group who had adequate thiamine intake previously excreted more than 7.5 per cent of the test dose during 24 hours, and the second group whose thiamine intake previously had been inadequate

excreted less than 7 per cent of the test dose. Melnick and Field (41, pp.131-138) also considered that an individual who excreted less than 7 per cent of a 5 mg. oral test dose of thiamine was deficient in the vitamin. Goldsmith and Sarett (31, p.129) gave the same test dose to 7 subjects on adequate thiamine intake, 150 mcg. was excreted during the first 4 hours. Gifft and Hauck (18, pp.635-645) obtained recoveries of 15 to 22 per cent of the same test dose in 24 hours. They stated that the subjects had adequate thiamine nutrition. A comparison with these findings shows that all the subjects of this study had inadequate thiamine levels. Even the highest value, 6.7, is below the critical level of 7 per cent. Hence, the recovery of the test dose indicates that the intake of 0.3 mg. per 1000 calories which was consumed during the last half of the study was not adequate for these subjects.

DAILY THIAMINE EXCRETIONS IN RELATIONSHIP TO CREATININE EXCRETIONS

The daily thiamine excretion values were also calculated in mcg. per gram of creatinine. The values are shown in Table 6. Adamson, et al. (1, p.25) used this method of expression when surveying the nutritional status of a population in Newfoundland. According to their statement, 150 mcg. excretion of thiamine per gram of creatinine indicates normal thiamine nutrition, while 50 mcg. or less of thiamine per gram of creatinine indicates a state of deficiency. The mean thiamine excretion per gram of creatinine of the subjects in this study ranged from 80.2 to 249.9 mcg. for the first and from 27.5 to 71.0 mcg. for the second period of the study. These figures, according to the

statement of Adamson and co-workers, show that the subjects during the first period of the study had adequate levels of thiamine, at least even the lower values were above the 50 mcg. excretion level. During the second period, two of the subjects dropped below the 50 mcg. level and the other two came quite close to it. This indicates that the thiamine intake during the second period was not adequate for the subjects. These indications of thiamine nutrition of the subjects check approximately with those obtained by total daily thiamine excretion values. There are only slight variations as the above mentioned fact that only two subjects during the second period dropped below the critical excretion level whereas, when considered in relation to the total mcg. of thiamine excreted in 24 hours, all the subjects showed inadequate thiamine nutrition. This variation in result, however, may be just a matter of interpretation, and it seems to be quite valid to express the thiamine excretion in mcg. per gm. of creatinine.

If body size or the amount of muscle tissue, as indicated on page 38, was the determining factor in thiamine requirement of an individual, it should follow the rule that a subject with higher creatinine excretion should require more thiamine than a subject with lower creatinine excretion, and hence, excrete less on the same intake. This was true in this experiment with the exception of thiamine excretion values of HAL. The smallest subject, RBD, excreted the least creatinine and the most thiamine. The next was MLW, but in case of CAS and HAL the order was reversed. CAS was

larger and excreted more creatinine, but her thiamine excretion also was higher than that of HAL, although the difference was never very big. Hence, some other factors must be involved besides the amount of muscular tissue in determining the thiamine requirement of an individual. One of them, as mentioned before, may well be the previous dietary history, but other factors might well also be involved. Between the daily excretion values of thiamine and creatinine for a given subject, there did not seem to be any detectable relationship according to the values obtained in this study. An increase in creatinine excretion did not necessarily cause a decrease in thiamine excretion or vice versa. (Figure 2.) The creatinine coefficients which were calculated for this study, values given in Table 9, do not seem to have any direct relationship to the amount of thiamine excreted by these subjects.

DETERMINATION OF THIAMINE PER GRAM OF CREATININE IN SEPARATE VOIDINGS

When the study was over, the subjects were asked to preserve one normal day's voidings separately for thiamine and creatinine determinations. The diet of that day was not controlled. The purpose of this procedure was to gain more information as to whether the relationship of thiamine excretion to creatinine excretion is constant in different voidings of the day. According to the report of Adamson, et al. (1, p.25), as mentioned previously, it was assumed to be fairly constant since they expressed thiamine excretion per gram of creatinine by analysing an individual voiding of urine.

TABLE 9.

CREATININE COEFFICIENTS OF FOUR SUBJECTS ON CONTROLLED DIET

Day of experiment	MLW	HAL	CAS	RBD
Preliminary	19.82	18.50	21.79	22.74
1	18.92	18.97	22.37	22.53
2	20.36	19.44	22.22	22.32
3	18.56	20.06	22.22	23.79
4	18.20	20.06	21.65	21.26
5	20.00	19.59	*	23.16
6	20.18	*	21.93	23.16
7	20.18	18.81	20.49	23.37
8	20.18	19.75	20.92	22.95
9	20.18	19.28	21.07	23.16
10	20.36	18.97	20.92	22.95
11	20.54	20.06	21.21	22.74
12	21.80	20.06	22.08	24.42
13	20.72	19.75	22.08	24.84
14	20.90	19.59	21.79	22.95
15	21.26	20.22	21.65	21.89
16	20.72	19.91	21.36	26.11
17	21.08	20.22	21.79	22.74
18	20.36	20.22	22.66	22.74
19	19.82	19.91	22.94	23.58
20	19.82	19.91	22.66	22.74
21	19.28	20.06	22.51	22.74
22	20.18	19.91	22.51	23.16
23	20.36	19.91	22.51	*
24	19.64	21.16	22.37	23.37
25	20.54	20.69	23.38	23.58
26	20.36	19.75	22.00	22.74
27	19.64	20.53	22.22	22.53
28	20.36	21.16	22.51	23.16
29	19.28	20.06	22.08	21.89
30	20.00	20.69	21.36	22.32
Average for 30 days	20.13	19.96	22.01	23.07

* Value missing because of some loss of the urine.

The primary reason why these separate voidings of one day were analyzed was that, as shown in Table 6. and Figure 2., there was some loss of urine on certain days. The part of the urine preserved those days was analyzed for thiamine and creatinine and an attempt was made to estimate the day's thiamine excretion by calculating it in relation to the average creatinine excretion of the subject. The calculated values were as follows: HAL, the 6th day of the study, 117 mcg.; CAS, the 5th day of the study, 176 mcg.; and RBD, the 23rd day of the study, 101 mcg. These results do not seem to differ very much from the thiamine excretion values obtained in the days close to these particular ones except that they are somewhat higher, that of RBD being considerably higher than the values for the other days during the same period of the study. (Table 6.)

The values obtained in this experiment are shown in Table 10. They show considerable variation from voiding to voiding in the ratio of mcg. of thiamine per gram of creatinine. And as an indication of the total daily output the separate values vary greatly. However, any voiding of a given subject could give an approximate illustration of the thiamine level of the subject. The first value of RBD is an exception being unduly high in comparison with her other values and the daily total excretion of thiamine. The results of this particular experiment seem to indicate that on lower excretion levels, there is less variation from voiding to voiding in thiamine excretion values per gram of creatinine excreted in the same

TABLE 10.

THIAMINE, CREATININE, AND THIAMINE PER GRAM OF CREATININE IN SEPARATE VOIDINGS OF 24-HOUR PERIOD

Subject	Time	Volume of preserved urine	Thiamine	Creatinine	Thiamine per gram creatinine	Mean thiamine per gram creatinine
		ml.	mcg.	mg.	mcg.	mcg.
MLW	12:00 A.M.	194	51.5	.17	302.9	
	6:25 P.M.	224	69.9	.21	332.9	
	8:45 P.M.	178	51.9	.14	370.7	
	7:00 A.M.	423	79.9	.29	275.5	320.5
Total		1019	253.2	.81		
HAL	12:00 A.M.	275	22.3	.22	101.4	
	5:00 P.M.	186	23.7	.22	107.7	
	11:30 P.M.	247	31.2	.30	104.0	
	6:50 A.M.	536	37.1	.35	106.0	104.8
Total		1244	114.3	1.09		
CAS	9:10 A.M.	337	57.6	.23	250.4	
	1:00 P.M.	342	51.4	.24	214.2	
	6:45 P.M.	211	50.4	.35	144.0	
	7:00 A.M.	845	129.6	.75	172.8	195.4
Total		1835	289.0	1.57		
RBD	11:00 A.M.	219	76.2	.15	508.0	
	2:25 P.M.	338	48.8	.18	271.1	
	5:15 P.M.	362	37.2	.19	195.8	
	11:15 P.M.	212	65.9	.36	183.1	
	7:00 A.M.	411	63.3	.29	218.3	275.5
Total		1542	291.4	1.17		

voiding. More data would be needed before making any conclusions in this field.

SUMMARY AND CONCLUSION

Four normal adult women were maintained for 30 days on a controlled diet which furnished 2000 calories per day. For the first period of 15 days, 0.5 mg. of thiamine per 1000 calories was given to the subjects. For the last period of 15 days, thiamine intake was reduced to 0.3 mcg. per 1000 calories.

The average values for 24-hour urinary excretion of thiamine for the first period ranged from 100.5 to 275.6 mcg. or from 9.8 to 26.8 per cent of the intake, and for the last period from 35.6 to 78.5 mcg. or 5.7 to 12.5 per cent of the intake.

The 24-hour response to a 5 mg. oral test dose of thiamine given the last day of the study ranged from 130.8 to 375.7 mcg. or 2.3 to 6.7 per cent of the total intake of the day.

There seemed to be evidence that, in general, thiamine excretion was related to creatinine excretion. The subjects with lower excretion levels of creatinine had higher thiamine excretion levels and vice versa when the intake was the same.

The daily thiamine excretion values as calculated per gram of creatinine averaged from 80.2 to 249.9 mcg. per gram of creatinine for the first period and from 27.5 to 71.0 mcg. per gram of creatinine for the last period of the study.

The values for excretion of thiamine per gram of creatinine in 24-hour separate voidings were determined for one day. The variation of this ratio from voiding to voiding was considerable for each subject, but each value might still give a very approximate estimation

of daily thiamine excretion of the subject. The data obtained in this short study do not give sufficient material for any conclusion.

The Food and Nutrition Board of the National Research Council's recommended daily allowance of 0.5 mg. of thiamine per 1000 calories proved to meet the needs of these four subjects as measured by usual criteria. It was none too high an intake for the subjects with larger amounts of muscular tissue. The intake of 0.3 mg. per 1000 calories was not adequate for any of these subjects. The body size and the previous thiamine nutrition of the individual seemed to be determining factors in thiamine requirement, but other factors may be involved.

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