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Research Article

STUDIES OF THIAMINE METABOLISM IN MAN. I. THIAMINE BALANCE. THE NORMAL REQUIREMENT OF VITAMIN B_1 AND THE RÔLE OF FECAL THIAMINE IN HUMAN NUTRITION [1]

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Although the Committee on Food and Nutrition of the National Research Council (1) has stated that 1.8 mgm. is the optimal daily intake of thiamine for a moderately active man whose caloric intake is 3000 calories, there is actually little agreement on the human requirements for the vitamin (Table I), as is clearly shown by Melnick (2) in his review. One reason for the disagreement is the fact that various observers have used different criteria in arriving at their figures. Several investigators (2 to 6) rely upon evaluation of the feeling of well being of the subjects or of their ability to perform work. This approach is clinical. In addition, it is questionable whether the minimal requirement should be considered as merely that amount which will prevent clinical evidence of deficiency. Amounts larger than those needed to maintain a state of well being may be necessary to prevent physiological and biochemical derangements which cannot be detected clinically in studies of short duration.

Many studies (2 to 7) were made on subjects whose thiamine intake was restricted. Objections may be raised to this approach because such conditions are abnormal. Reducing the thiamine intake may affect body metabolism and, secondarily, its thiamine requirements. Similar criticism may be directed against those experiments (7) in which the diet was essentially synthetic.

Some investigators (2, 4, 6, 7) selected the maintenance of a certain level of urinary thiamine excretion as a criterion of values for the minimal requirements of vitamin B_1. Since the same level of urinary thiamine excretion was not maintained by all observers, it is not surprising that their values do not agree. Furthermore, the full significance of urinary thiamine in relation to vitamin B_1 metabolism awaits clarification.

Some earlier studies of thiamine requirements may also be criticised because of the failure of all observers to consider the fecal excretion and the intestinal biosynthesis of this vitamin. The feces of animals and man contain considerable amounts of B_1 (8 to 11). Most of it probably arises from bacterial synthesis in the gut (8, 12, 13), although some may result from incomplete absorption of oral thiamine, since oral test doses greater than 5 mgm. result in large increases in fecal thiamine (9 to 11).

In brief, many studies of minimal thiamine requirements in man are open to criticism. Perhaps most of the criticisms are referable to various interpretations of the word "minimal." The simplest and most precise definition of "minimal requirement" is that amount which is consumed, or chemically altered, in metabolic processes, plus what is unavoidably lost from the body. The present study is designed to measure this "minimal" requirement.

Little is known concerning the metabolism of thiamine. That some of the vitamin is consumed in connection with its rôle in intermediary metabolism is likely, but the extent to which this process takes place and the nature of the end-products of thiamine breakdown require further elucidation. The pyrimidine moiety of the thiamine molecule, 2-methyl-5-hydroxymethyl-6 aminopyrimidine, is obtained by hydrolysis of the vitamin and may be excreted in the urine following thiamine cleavage in the body (14, 15).

This substance, its ethyl derivative, and thiamine have the property of accelerating yeast fermentation. Urine normally contains a substance, or group of substances, other than thiamine, which likewise stimulate fermentation. The concentration of this material in the urine increases following the oral or parenteral administration of thiamine supplements (16 to 18). It is therefore

[1] This study was aided by a grant from the Josiah Macy, Jr. Foundation.

TABLE I

Minimal daily requirement of thiamine as reported in the literature

Author	Condition and method of experiment	Criteria	Minimal daily requirement	
			total mgm.	*mgm. per 1000 calories*
Williams, Mason, Smith and Wilder (3)	Restriction in B_1 intake	Clinical. Development of symptoms of deficiency		0.22 to 0.50
Williams, Mason and Wilder (4)	Restriction in B_1 intake	Clinical. Symptoms of deficiency. Impairment in CHO metabolism and low urinary B_1 excretion.		0.45
Melnick (2)	Study in normal and deficient subjects. Diet intake calculated.	Clinical evaluation. Maintenance of, or restoration to, "normal" urinary B_1 excretion.		0.35
Elsom *et al.* (5)	Restriction of B_1 and other factors of the B complex	Clinical. Development of symptoms of deficiency.		0.35
Holt (7)	Synthetic diet. Thiamine restriction.	Amount of B_1 necessary to give barely detectable B_1 excretion in the urine.	0.47	0.26 to 0.31*
Keys *et al.* (6)	Restriction in B_1 intake.	Ability to work and think. Maintenance of normal CHO metabolism. Urinary B_1 excretion.		0.23 to 0.33

* As calculated by Melnick (2).

probable that this substance is related to the metabolism of vitamin B_1. Simultaneous estimation of the urinary excretion of thiamine and the pyrimidine which accelerates yeast fermentation (PAYF) may be expected to give valuable information concerning the metabolism of the vitamin.

METHOD

The subject was a normal 35-year-old man, weighing 180 lbs., who was eating a well-balanced diet of approximately 2400 calories. Complete dietary freedom was permitted, and the subject continued his usual daily activities throughout the experiments.

Two experiments were done. The first consisted of a one-week balance study in which the thiamine and pyrimidine contents of the food were determined while the excretion of these substances in the urine and feces was measured. An equal portion of all the food consumed was collected in a jar containing 50 ml. of 10 per cent hydrochloric acid and 10 ml. of toluene. At the end of each day the contents of the jar were homogenized in a Waring Blendor and weighed. An aliquot was analyzed for total (free plus phosphorylated) thiamine by a specific chemical method previously described (10). The thiamine content of the food was also calculated from standard tables.[2]

The 24-hour urines were collected and their thiamine content determined chemically (19). The stools were collected and analyzed for free and phosphorylated thiamine as described previously (10, 19), with the following modification: The stools were homogenized in dilute hydrochloric acid. Aliquot samples were placed in a boiling water bath for 10 minutes in order to kill most of the organisms present; the aliquots were then stored in the refrigerator under toluene until analyzed. In the analysis the precipitate resulting from the addition of lead acetate was removed *before* the addition of sodium carbonate. From this point the routine procedure was followed. In this way almost all the fecal pigment carried down was removed before some of the precipitate could be dissolved by sodium carbonate. The recovery of thiamine was considerably improved.

In order to test the possibility that most of the fecal B_1 exists within the bodies of fecal organisms, a stool was analyzed as follows: The stool was collected, weighed immediately and mixed by mechanical stirrer with sufficient water to make a total of 1 kgm. An aliquot was quickly centrifuged and the supernatant filtered through a coarse filter paper. The filtrate was passed through a Seitz filter, immediately acidified with a few drops of concentrated hydrochloric acid and analyzed for free thiamine. The result was compared with that obtained on an aliquot of the stool treated and analyzed in the usual way.

The pyrimidine content of food, urine and feces was calculated by estimating the total thiamine and pyrimidine from the total yeast fermentation accelerating power in the Warburg apparatus (14), and then subtracting the values for thiamine and cocarboxylase obtained by chemical analysis; a figure for the pyrimidine accelerating yeast fermentation (PAYF) was obtained.

[2] Applied Dietetics. Frances Stern. Williams and Wilkins Co., Baltimore, 1943.

TABLE II

Thiamine and pyrimidine balance

Date	Intake			Output				
	Total thiamine*		PAYF†	Urine		Feces		
	Calc.‡	Anal.		Free B_1	PAYF†	Free B_1	Cocarb.†	PAYF†
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
2/28 to 2/29	1.25	1.21	0.54	0.38	0.91	0.081	0.563	0.74
2/29 to 3/1	1.32	1.18	0.66	0.26	0.92	0.200	1.010	0.70
3/1 to 3/2	1.08	0.93	0.76	0.23	0.99	0.121	0.840	0.49
3/2 to 3/3	1.74	1.23	0.78	0.14	1.14	0.060	0.870	0.39
3/3 to 3/4	1.30	1.00	0.82	0.21	0.92	0.220	1.210	1.15
3/4 to 3/5	2.47	2.47	0.49	0.34	1.18	0.110	0.870	0.83
3/5 to 3/6	1.33	1.09	0.83	0.14	0.85	0.023	0.504	0.74
Total	10.49	9.11	4.88	1.70	6.91	0.815	5.867	5.04
Average per day	1.50	1.30	0.70	0.24	0.99	0.116	0.838	0.72

* Total thiamine = free thiamine plus cocarboxylase.
† Expressed in terms of thiamine.
‡ Calculated from standard tables.

During the second experiment, which consisted of a 51-day period preceded by 9 days of control observation, the subject received a total of approximately one gram of thiamine intramuscularly in daily doses which varied from 0.5 mgm. to 145 mgm. The daily thiamine and pyrimidine in the urine were measured, and the oral intake of the vitamin was calculated from standard tables.[3]

To study the claim of Najjar and Holt (13) that thiamine can be absorbed from the rectum and large bowel, the subject underwent the following experiment: After collection of two 24-hour urines and one 24-hour stool, the subject received a retention enema of 230 ml. containing twice as much cocarboxylase and free thiamine as is in the average 24-hour stool. To assure maximum penetration of the enema, the subject alternately raised the legs and hips while lying on his back, turned on his left side, then to the right, and repeated this procedure several times for 10 minutes after the enema was instilled. The urge to defecate persisted for only a short time and the enema was retained for 24 hours. The urine excreted during these 24 hours and the stool passed at the end of this period were collected and analyzed.

RESULTS

Thiamine and pyrimidine balance. During the one week balance experiment (Table II), the average daily intake of total thiamine[4] determined chemically was 1.3 mgm. This was 0.2 mgm. less than the figure obtained by calculation for the same food. The difference might be attributed to inadequate allowance made in our calculation for losses incurred in the preparation of the food. The average intake of PAYF was 0.70 mgm.[5] per day.

During this period the daily urinary thiamine averaged 0.24 mgm. The amount of ingested thiamine which could not be accounted for as urinary B_1 was 1.06 mgm. The daily PAYF excretion was 0.99 mgm.[5]

The daily urinary PAYF exceeds the ingested PAYF. If this excess, which probably arises from thiamine breakdown, is subtracted from the amount of thiamine unaccounted for, a value of 0.7 mgm. is obtained which represents the amount of thiamine which cannot be accounted for either as thiamine or as PAYF.

During the 51-day study (Table III) the total thiamine intake was 1.086 grams; the average daily oral intake (calculated) was 1.5 mgm. When this is adjusted for the difference between calculated and analytical values (0.2 mgm. per day) the total corrected intake is 1.076 grams; 887 mgm. were recovered in the urine as thiamine and 189 mgm. could not be accounted for. Consequent to the parenteral administration of 1.01 grams of thiamine over this period, 134 mgm. of PAYF were excreted in the urine over and above the amount which would have been excreted on the oral intake alone (39 mgm.). By subtraction of this extra pyrimidine from thiamine unaccounted for, 55 mgm. or 1.09 mgm. per day are left; this

[3] Applied Dietetics. Frances Stern. Williams and Wilkins Co., Baltimore, 1943.

[4] Free thiamine plus cocarboxylase.

[5] Expressed in terms of thiamine.

represents the amount of thiamine unaccounted for as urinary B_1 or as urinary pyrimidine resulting from thiamine supplements.

Fecal thiamine, cocarboxylase and pyrimidine. The concentrations of thiamine and cocarboxylase in feces indicate that the stool is one of the richest known sources of vitamin B_1. The 24-hour stool contains 105 mcg. of free thiamine and 803 mcg.[5] of cocarboxylase (Tables III, IV) on the average. The concentrations of both of these substances are remarkably constant and are in the same ratio to each other as in tissues (10). Analysis of a Seitz filtrate of stool revealed that 53 per cent of the free thiamine remained behind on the filter (Table IV).

The average PAYF in the 24-hour stool was 0.72 mgm. (Table IV).

Urinary and fecal thiamine following retention enema. An enema containing approximately twice the amount of thiamine and cocarboxylase in the average daily stool was retained for 24 hours. During this time the urinary thiamine did not increase above the control level, and all of the administered thiamine and cocarboxylase was recovered in the feces (Table V).

TABLE III

Fifty-one day study of thiamine and pyrimidine balance during administration of parenteral thiamine

	Daily	Total
	mgm.	*mgm.*
Oral thiamine intake	1.3*	66.5*
Parenteral thiamine intake	0.5 to 145	1009
Total		1076
Total thiamine excreted in urine		887
Thiamine unaccounted for		189
	mgm.	*mgm.*
Total pyrimidine excreted	173	
Basal pyrimidine excretion†	39	
Pyrimidine from thiamine supplements	134	134
Total amount of B_1 unaccounted for as thiamine or as pyrimidine arising from thiamine supplements		55
Amount unaccounted for per day		1.09

* These values are based upon calculations from standard tables and have been corrected for the difference between calculated and analytical values.

† This figure was obtained by multiplying the average daily pyrimidine excretion during the 9-day control period before parenteral thiamine administration by 51.

TABLE IV

Fecal thiamine, cocarboxylase and pyrimidine

Date	Stool weight	Thiamine content		Cocarboxylase content*		PAYF content*	
		Per gram	Total	Per gram	Total	Per gram	Total
	grams	*mcg.*	*mcg.*	*mcg.*	*mcg.*	*mcg.*	*mcg.*
2/28 to 2/29	100	0.81	81	5.63	563	7.4	740
2/29 to 3/1	231	0.87	200	4.37	1010	3.0	700
3/1 to 3/2	125	0.97	121	5.22	840	3.9	490
3/2 to 3/3	74	0.81	60	11.70	870	5.4	390
3/3 to 3/4	295	0.75	220	4.10	1210	3.8	1150
3/4 to 3/5	174	0.63	110	5.00	870	4.8	830
3/5 to 3/6	83	0.28	23	6.10	504	8.9	740
1/2/45	108	1.12	121	5.18	559		
1/2/45†	108	0.53	57				
Average per day		0.78	105	5.90	803	5.3	720

* Expressed in terms of thiamine.

† Analysis of a Seitz filtrate.

DISCUSSION

Progressive restriction of oral thiamine intake results in a decrease in its urinary excretion to the point where the concentration is too small to be detected by most methods (16, 20). Under such conditions considerable amounts of thiamine and cocarboxylase can still be found in the tissues (21). This has been attributed (22) to the function of the kidney in conserving thiamine when its exogenous source becomes critically curtailed. In computing the daily minimum requirement of thiamine, it is justifiable to deduct the urinary thiamine from the intake, since urinary thiamine arises from thiamine excess and is not an unavoidable loss.

Whether this reasoning can be applied also to the urinary pyrimidine is questionable. Supplementing the intake of thiamine by oral (17) or parenteral (16, 23) administration results in substantial increases in both urinary PAYF and thiamine; the pyrimidine which arises from thiamine is proportional to the thiamine intake within certain limits (1.3 to 20 mgm.) (23). Under these conditions urinary pyrimidine results from administration of excessive amounts of B_1 and consequently may be deducted from thiamine intake in evaluating the minimal requirement of the vitamin.

In the 51-day balance study where thiamine supplements were administered the urinary PAYF in excess of the basal excretion of this substance was accordingly deducted from the thiamine in-

TABLE V

Urinary and fecal thiamine following retention enema

	24-hour urine thiamine	24-hour fecal thiamine and cocarboxylase					
		Free B_1	Expected B_1*	Recovery of expected B_1	Cocarboxylase†	Expected* cocarbox.	Recovery of expected cocarbox.
	mcg.	*mcg.*	*mcg.*	*per cent*	*mcg.*	*mcg.*	*per cent*
Before enema	116 108	121			559		
After enema	107	490	418	117	3050	2690	118

* Calculated on the basis of stool weight and the average concentration of free thiamine and cocarboxylase in stools of same subject (Table IV); also included is the amount of thiamine (0.25 mgm.) and cocarboxylase (2.0 mgm.) in the retention enema.

† Expressed in terms of thiamine.

take. Thus 55 mgm. of B_1, or 1.09 mgm. per day, could not be accounted for as thiamine or as pyrimidine arising from a daily thiamine intake of more than 1.3 mgm.

Although there are no studies on urinary pyrimidine following reduction of thiamine intake to less than 1.3 mgm. daily, it is probable that the PAYF arising from thiamine will continue to decrease as the intake of the vitamin is progressively curtailed, since the available data (16, 17) indicate that the urinary PAYF is lowest in those subjects whose dietary thiamine is small. Under such circumstances the urinary PAYF may arise from food PAYF rather than from thiamine cleavage in the body, for it has been shown (15) that a related compound, 2-methyl-6-amino-5-ethoxymethyl pyrimidine, when fed to rats, is rapidly and quantitatively excreted in the urine. Probably PAYF behaves similarly. To determine how much ingested thiamine is converted to pyrimidine, the amount of PAYF in the food should therefore be deducted from the PAYF excreted.

Since little is known about the factors which influence or control the conversion of thiamine to pyrimidine and since there is no direct evidence that urinary PAYF will decrease as thiamine intake is reduced below 1.3 mgm., this alteration is best regarded as a form of thiamine utilization. Any value proposed for the minimum requirement of thiamine must therefore cover the conversion of thiamine to pyridimine at this level of B_1 intake.

There are four possible explanations for the disappearance of thiamine. Some of it may go to replenish tissue stores. In the first experiment storage probably did not occur, since the subject had taken thiamine in the form of vitamin B complex for some time up to 10 days before the experiment. Under such circumstances one could assume that his body stores were saturated and did not take up any of the ingested thiamine.

Thiamine can also disappear by degradation in connection with its function in intermediary metabolism. Almost nothing is known about the end products of thiamine utilization. Whether thiamine conversion into pyrimidine, presumably by simple cleavage, is related to the function of thiamine in metabolism is obscure. Borsook *et al.* (21) found that when thiamine tagged with radioactive sulfur is administered to man, a considerable fraction of the radioactive sulfur is excreted in the urine as neutral sulfur or as ethereal and inorganic sulfate. This proves that the thiazole moiety which would be the other cleavage product suffers further degradation in the body, but it throws no light on whether pyrimidine also is altered.

A third way in which ingested thiamine may be lost is in the sweat. Five to 15 per cent of the thiamine intake may thus be excreted and under the stimulus of heat as much as 0.15 mgm. per day may be lost (24). Since our observations were made during the winter season, the amount of thiamine lost in the sweat was probably less. On the basis of the above figures, it may be assumed that approximately 0.1 mgm. of thiamine per day (8 per cent of the intake) was lost in this way.

Thus far fecal B_1 has been disregarded. Whether this is justifiable remains to be proven,

for the origin of thiamine in the feces is obscure. Some of it may arise from incomplete absorption of the ingested vitamin. When doses of 5 or more mgm. of thiamine are taken orally, a considerable fraction appears in the stools (9 to 11). Although smaller amounts may be absorbed quantitatively from the normal bowel, any study of thiamine utilization must include incomplete absorption as a fourth way by which ingested B_1 may be lost.

The occurrence of thiamine in feces may also be consequent to excretion of the vitamin into the gastrointestinal tract. This is unlikely, since even after large doses of thiamine given parenterally there is only a slight increase in fecal B_1 (8 to 10).

If the fecal thiamine and cocarboxylase were to be considered as part of unabsorbed and/or excreted thiamine, the discrepancy between ingested and excreted thiamine would be negligible. This would indicate no utilization or other loss of ingested thiamine which, as shown above, is impossible. It is probable that most of the fecal thiamine arises as a result of intestinal biosynthesis. Most of it seems to exist within the bodies of fecal organisms, since 53 per cent does not pass through a Seitz filter. Furthermore, it is found even in subjects who have been on a thiamine-free diet (13). The fecal concentrations of thiamine in our experiments were remarkably constant, which is in accord with the observations of Leong (8). Also, the ratio of free to total thiamine is the same in feces as in tissues (10).

That the thiamine resulting from intestinal biosynthesis plays a rôle in the nutrition of man is unlikely. Thiamine and cocarboxylase given in a retention enema not only failed to appear in the urine, but also were recovered quantitatively in the feces. This is not in accord with the findings of Najjar and Holt (13), who observed rises in urinary thiamine in two subjects following the administration of 50 mgm. of the vitamin daily for two successive days in retention enemata. It must be pointed out, however, that the conditions were not physiological. Fifty mgm. of thiamine is an enormous amount compared with that normally present in feces. Yet even after such large doses only small amounts (1.5 and 5.0 mgm.) appeared in the urine (13). Most of the fecal thiamine, furthermore, is in the form of cocarboxylase (Table IV), whereas Najjar and Holt (13) administered only the free form of the vitamin. In view of the difficulty with which phosphorylated compounds pass through cell membranes, it is doubtful whether cocarboxylase can be absorbed as such. It is also unlikely that the dephosphorylating enzymes necessary to convert cocarboxylase into free thiamine exist to any great extent in the large bowel (25).

In the one-week balance experiment where the daily thiamine intake was 1.3 mgm., 1.06 mgm., or 0.44 mgm. per 1000 calories, were found to be the minimal daily requirement for a normal, moderately active, well nourished, male subject on a 2400 calorie diet. When large amounts of supplementary thiamine were provided, practically the same figure (1.09 mgm.) was obtained. This agreement indicates that in a normal subject both the requirement of thiamine and its utilization in metabolism are independent of the concentration of the vitamin in the tissues between the limits provided by intakes of from 1.3 to 145 mgm. daily.

The value of the minimum requirement presented above covers (a) thiamine destruction in connection with its function in body metabolism, (b) losses resulting from thiamine conversion to pyrimidine when the intake of the vitamin is 1.3 mgm. or less, (c) thiamine losses in sweat[6] and (d) possible losses incurred by incomplete intestinal absorption. This value agrees with that of Williams, Mason and Wilder (4), but is somewhat higher than those reported by other investigators (5 to 7, 20). As pointed out above, it is likely that all the urinary PAYF which results from thiamine may also be subtracted from the intake. This would lower the minimal thiamine requirement from 0.44 mgm. to 0.32 mgm. per 1000 calories. Further work is necessary, however, to establish the validity of this calculation.

CONCLUSIONS

1. The term "minimal requirement" has been defined as that amount of any material which is utilized or otherwise altered in body metabolism plus what is needed to cover all uncontrollable losses from the body.

2. The difference between ingested thiamine and thiamine excreted into the urine is the mini-

[6] Nothing is known about thiamine excretion in sweat when the intake of the vitamin is restricted.

mal requirement of thiamine as defined above. When urinary PAYF rises in response to a thiamine intake larger than 1.3 mgm., the increase in PAYF can also be deducted, since it represents a controllable loss.

3. The minimal requirement in a normal male whose caloric intake was 2400 calories was found to be 0.44 mgm. per 1000 calories.

4. Values are given for the fecal excretion of thiamine and cocarboxylase. The concentrations of these substances in feces are remarkably constant and are in the same relationship as in tissues. Fecal pyrimidine values are also given.

5. Most of the fecal thiamine probably results from intestinal biosynthesis and exists largely within the bodies of fecal organisms.

6. Thiamine and cocarboxylase when administered in physiological amounts by retention enema were not absorbed from the large bowel.

7. Values are also given for pyrimidine contents of foods.

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