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Research Article

TISSUE THIAMIN CONCENTRATIONS AND URINARY THIAMIN EXCRETION [1]

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INTRODUCTION

Abnormalities of thiamin excretion are observed chiefly in patients in whom the diagnosis of deficiency is fairly obvious on the clinical grounds of history and physical examination (1 to 4). The deviations from normal excretion in individuals suffering from mild or subclinical deficiency are usually equivocal. These observations suggest that the changes in tissue thiamin concentrations in human deficiency are not great or that the changes which occur have a relatively slight effect upon the urinary excretion of this vitamin. In the following study, the concentrations of thiamin in human tissue have been determined and the variations observed have been compared with the changes found in thiamin excretion in individuals with overt deficiency. Supplementary animal experiments have permitted direct observation of the relationship between tissue changes and changes in vitamin excretion.

METHODS

a. Tissue analyses

The thiochrome method has been used essentially as in earlier studies of the thiamin content of tissues (5). The small amount of thiamin in human tissue has required the extraction of somewhat larger quantities of tissue and has proportionally increased the error due to the inclusion of irrelevant fluorescent materials in the final thiochrome measurement.

Human tissues are finely divided in a Waring Blendor. Five gram aliquots are extracted with 50 milliliters of 0.1 normal sulfuric acid for 1 hour at 100 degrees centigrade with an occasional stirring. The pH is adjusted to 4.5 with sodium acetate and the extracts are cooled and centrifuged. The solid material is discarded and the supernatant is incubated overnight at 37 degrees centigrade with 1 gram of takadiastase to complete hydrolysis of the cocarboxylase. Five milliliter aliquots of the incubated extract are treated with alkaline ferricyanide, and the thiochrome extracted with 15 milliliters of isobutyl alcohol. The concentration of thiochrome in the butanol is estimated with a photoelectric fluorometer.

Calculations of the thiamin content of the tissues are based upon the recovery of thiamin obtained in duplicate aliquots of extract to which known amounts of thiamin have been added. The errors in the method are of the order of minus 15 per cent, due to incomplete extraction of thiamin from the tissue, and plus 30 per cent, due to inclusion of irrelevant fluorescent materials in the final thiochrome measurement. These errors may be decreased by repeating the extraction procedure and by using permutit columns to diminish the amount of irrelevent fluorescent material (6, 7).

b. Urine analyses

The thiochrome method has been used essentially as in earlier studies of thiamin excretion (7).

All patients and animals are fasted overnight before being tested.

Urine collections of short duration in animals are secured by tying the urethra under novocaine anesthesia at the beginning of the collection period. At the conclusion of the experiment the animal is killed by decapitation, the bladder is removed in toto, and the urine transferred to a small collecting vessel. Urine collections of greater duration than 4 hours are obtained by the use of small metabolism cages.

For tolerance tests in patients, a base line of excretion is established from a urine specimen taken immediately preceding the test. In animal experiments, the base line is estimated from the excretion observed in control animals, normal and deficient, that have been given saline or water instead of vitamin.

For the per oram test in man, 5 milligrams of thiamin hydrochloride are administered with the mid-day meal. The procedures for parenteral tests in man are indicated in Table V.

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For the *per oram* test in animals, 160 micrograms of thiamin and 160 micrograms of riboflavin are administered by stomach tube in 4 milliliters of water. Urine is collected for 24 hours, the animals fasting, with water *ad lib.* For the subcutaneous test, 40 micrograms of thiamin and 40 micrograms of riboflavin are injected subcutaneously in the right axilla in 1 millilter of normal saline solution. Urine is collected for 3½ hours, the animals fasting, with water *ad lib.* For the intravenous tolerance test, 40 micrograms of thiamin and 40 micrograms of riboflavin are injected intravenously in 1 milliliter of normal saline solution. Four milliliters of water are administered by stomach tube and urine is collected for 1½ hours. Control experiments indicate that these periods are sufficient to permit the excretion of thiamin to return to the basal level. Measurement of riboflavin excretion (8) serves in the tests as a control for variables of absorption and renal function.

c. Diets

The deficient diet used in the study of the psychiatric patient was the basal diet without supplementary vitamins, described by Williams *et al.* (9).

The control diet for the animal experiments was that described by Peters and Rossiter (10), except that autolyzed yeast (Vegex) was substituted for their dried yeast. The deficient diet was obtained by autoclaving the autolyzed yeast for 2 hours at 20 pounds pressure before mixing it with the other ingredients.

RESULTS

a. Thiamin content of human tissues

A comparative study with the yeast fermentation method (11), in which the sources of error are different from those in the thiochrome technique, has verified the approximate validity of the thiamin analyses (Table I).

A study of the effect of post-mortem autolysis upon the thiamin content of tissues has failed to show significant change, plus or minus 10 per cent, following 24 hours' incubation at 37 degrees centigrade, or 48 hours' preservation at 0 degrees centigrade. Corroborative evidence for the validity of post-mortem analyses is obtained from a comparison of autopsy and biopsy analyses of brain and muscle of human subjects (Table II).

The range of thiamin concentrations found in human tissues is illustrated in Table III. The patients have been grouped on the basis of their probable nutritional status as judged by clinical circumstances and dietary histories. In general the concentrations of thiamin found in the tissues are in agreement with the grouping. Tissues from young individuals appear to contain more thiamin per gram than do tissues from older persons. A possible exception is cerebral cortical tissue which in immature infants contains very little thiamin (12).

Symptoms of thiamin deficiency were recognized in only one patient in Table III. In the group with poor nutrition, the alcoholic with tuberculosis complained of pain and paresthesia in the lower extremities during the period when he was still ambulatory. The other patients were not ambulatory at a time when exercise might have produced symptoms of deficiency.

TABLE I

Comparison of yeast fermentation method and thiochrome method of determining the thiamin content of human tissue

Calculations expressed as micrograms thiamin per gram fresh tissue.

Heart		Brain		Liver		Kidney		Skeletal muscle	
T*	Y†	T	Y	T	Y	T	Y	T	Y
				1.4	1.3				
1.4	2.2			1.0	0.9	1.0	1.1	0.4	0.3
				1.2	0.8			0.5	0.4
1.9	3.1	1.0	1.2	1.0	1.1	1.3	1.5	0.4	0.5
2.2	3.0								
1.9	3.0								
1.9	1.9								
3.5	3.6								
2.0	2.5	1.2	1.3						
3.5	2.8							1.8	1.2
2.3	2.3								
4.9‡	4.9‡								
2.0	1.8								
1.2	1.1								
1.1	1.1								
1.7	2.0								
2.7	3.6								
4.1	4.2			2.4	2.0				
1.4	1.0								

* T = Thiochrome method.
† Y = Yeast fermentation method.
‡ 5.0 by rat curative assay, done through the courtesy of Dr. W. L. Sampson, Merck Institute for Therapeutic Research, Merck and Company, Rahway, New Jersey.

TABLE II

Comparison of concentrations of thiamin found in human tissues at operation and autopsy

Tissue	Source	Thiamin per gram tissue
		micrograms
Brain	Autopsy	1.1
Brain	Biopsy	1.2
Skeletal muscle	Autopsy	0.6
Skeletal muscle	Autopsy	0.4
Skeletal muscle	Biopsy	0.5
Skeletal muscle	Biopsy	0.4

TABLE III

Concentration of thiamin in human tissue

Calculations expressed as micrograms thiamin per gram tissue.

Subject	Age in years	Sex	Heart	Skeletal muscle	Liver	Kidney cortex	Cerebral cortex	Remarks
Presumably *normal* individuals	0	Female	1.8	1.4	1.1			6 months fetus
	0	Female	1.8*	1.5*	0.9*	0.9*	0.5*	8 months fetus
	10	Male	3.5	1.2	1.4	2.1		Food in stomach, traumatic death
	30	Male	2.4	0.4	1.0	1.8	1.1	Negro, traumatic death
	52	Male	2.0	0.4	1.2	1.3	0.8	Traumatic death, survived 24 hours
Patients with *good* nutrition	0	Female		1.3*			0.3*	Omphalocele
	0	Female	3.5	2.7	1.5	2.8		Meningoencephalocele
	7 weeks	Male			1.3		0.6*	Pneumonia 2 weeks
	10 months	Female	3.3	1.6	1.1	3.1		Fulminating sepsis
	5 years	Male	3.2	1.0	1.1	1.4		Leukemia 6 months
	6	Female	3.8		1.9	1.9	1.0	Neuroblastoma of adrenal
	37	Male		0.4	1.0	1.0		Brain tumor
	48	Male	2.4	0.5	1.4	1.3		Cerebral metastases
	51	Male	2.3	0.4	1.1	1.7	1.0	Brain tumor
	58	Male	1.7	0.4	1.1	1.0	1.1	Brain tumor
	61	Female	1.9	0.4	1.0	1.3	1.0	Brain tumor
Patients with *fair* nutrition	26	Male	1.3	0.4	0.7	1.2		Acute rheumatic carditis
	47	Female	1.4	0.2	0.7	0.7		Heart failure with anasarca
	50	Male	1.9	0.5	1.1	1.2	1.0	Alcoholic coma, ?cerebral injury
	54	Male	1.3	0.2	1.0	1.2		Cirrhosis, gastric hemorrhage
	68	Male	1.4	0.4	1.0	1.0		Calcified aortic valve, cardiac failure
Patients with *poor* nutrition	34	Female	0.9	0.2	0.7	1.0		High cord injury, sepsis 3½ months
	37	Male	0.5*	0.1*	0.5*	0.5*	0.6*	Alcoholism, tuberculosis
	38	Female	0.6	0.0	0.3	0.4	0.5	Active tuberculosis of spine and adrenals; high fever
	49	Female	0.5	0.1	0.6	0.3		Lymphosarcoma, uremia; high fever for 3 weeks

* Permutit column analyses.

The thiamin concentrations observed in adult patients, after the administration of large quantities of thiamin, are presented in Table IV. The concentration in some tissues appears higher than the usual normal.

b. Thiamin excretion in patients

The tendency of thiamin deficient patients to excrete less thiamin than normal subjects is illustrated in Table V. Since the observations were made on patients with different degrees of deficiency, the sensitivities of the various tests cannot be compared.

The effect of a restricted intake of thiamin, 300 micrograms per day, was studied in an ambulatory psychiatric patient in good physical and fair mental condition. The daily excretion of thiamin fell promptly from an initial level of 60 micrograms to a level of 5 to 10 micrograms. The excretion of parenterally administered vitamin changed but little. At the beginning of the experiment, 5 per cent of a subcutaneous tolerance test of 200 micrograms was excreted in 4 hours. Two days later 11 per cent of a subcutaneous test of 600 micrograms was excreted in 4 hours. After 2 weeks of deficient diet the tests were repeated. Four per cent of the 200 microgram test was excreted and 10 per cent of the 600 microgram test. The excretion of orally administered thiamin, on the other hand, had become frankly abnormal. Less than 10 per cent of a 5 milligram test was excreted in a 24 hour period (2). With the ad-

TABLE IV

Concentration of thiamin in tissues of patients receiving thiamin therapy

Calculations expressed as micrograms thiamin per gram fresh tissue.

Age in years	Sex	Heart	Skeletal muscle	Liver	Kidney cortex	Cerebral cortex	Remarks
59	Male	2.6	0.8	1.3	2.1		Carcinomatosis. 10 mgm. thiamin intravenously, each day *ad* 25
76	Female	1.3	0.7	1.2	1.2		Carcinoma of mouth. 3 mgm. thiamin by mouth for 3 weeks, none for 2 weeks
75	Male	2.3					Carcinoma of stomach; cachexia. B complex intramuscularly for 10 days
40	Female					1.2	Cerebral tumor. 10 mgm. B_1 by mouth, each day, for 1 week
18	Female	4.9					Encephalitis? 50 mgm. thiamin each day, 3 weeks; 100 mgm. nicotinic acid each day, for 1 week, up to day of death
38	Male	4.3		2.4			Cirrhosis. 30 grams Brewer's yeast each day, 30 days; 20 mgm. B_1 intramuscularly or subcutaneously each day, 21 days; 5 cc. liver extract intramuscularly weekly up to 2 days before death

TABLE V

A comparison of the amounts of thiamin excreted by normal individuals and by individuals whose diets have been deficient in thiamin

Micrograms thiamin excreted		Type of test
Normal subjects	Deficient subjects	
150 109 283	44 20 0	24 hour excretion.
252 280 230	15 50	24 hour excretion following subcutaneous injection of 0.5 mgm. B_1 to normals and 1.0 mgm. B_1 to deficients.
206 136	78 52	3 hour excretion following intramuscular injection of 1 mgm. B_1 and B_2.
315 300 350	250 250 150	1 hour excretion following intravenous injection of 1.2 mgm. B_1 and B_2.
Per cent of tolerance test excreted		**Type of test**
Normal subjects	Deficient subjects	
22 26 25 22 21	9 6 12	1 hour excretion following intravenous injection of 0.02 mgm. B_1 and B_2 per kgm. body weight.

ministration of repeated 5 milligram tests, the excretion of orally administered thiamin gradually returned to normal (Figure 1).

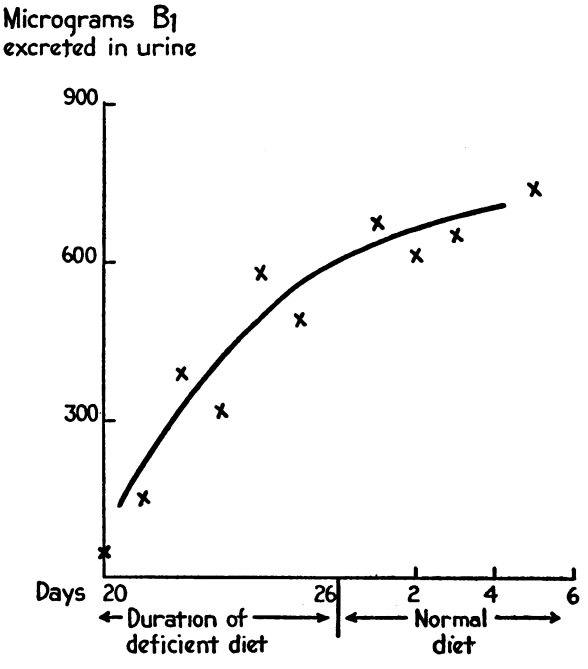

FIG. 1. AMOUNT OF THIAMIN EXCRETED IN THE DAILY URINE BY A DEFICIENT SUBJECT IN RESPONSE TO SUCCESSIVE TOLERANCE TESTS CONSISTING OF 5 MGM. OF THIAMIN HYDROCHLORIDE ADMINISTERED WITH EACH MIDDAY MEAL

The lower limit of normal for this type of test is an excretion of about 500 micrograms (2).

c. Thiamin content of rat tissues

The effect of a thiamin deficient diet upon the

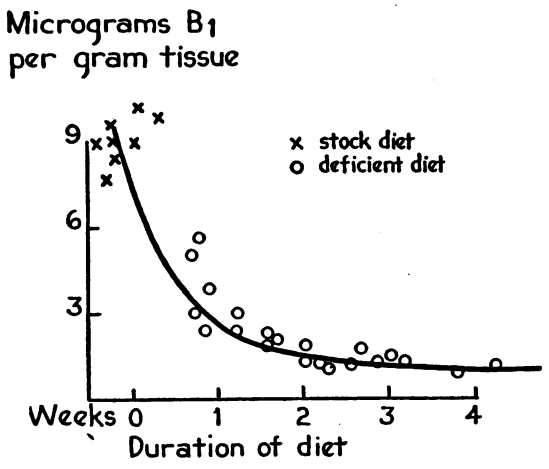

FIG. 2. EFFECT OF THIAMIN DEFICIENT DIET UPON CONCENTRATION OF THIAMIN IN LIVER OF RATS

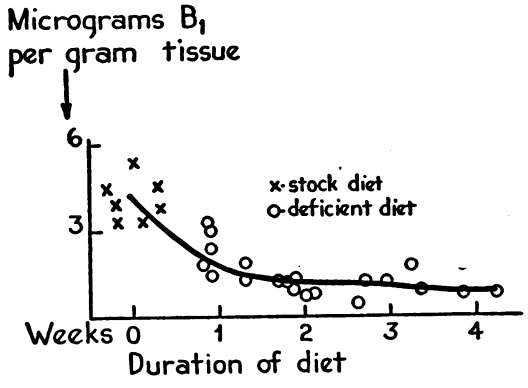

FIG. 3. EFFECT OF THIAMIN DEFICIENT DIET UPON CONCENTRATION OF THIAMIN IN KIDNEY OF RATS

thiamin content of the livers and kidneys of 180 gram male Wistar rats is illustrated in Figures 2 and 3. Muscle concentrations also fell from 1 microgram per gram to 0.5 microgram after 2 weeks of deficient diet. Symptoms of thiamin deficiency were not observed until the third week.

The effect of thiamin administration is illustrated in Figures 4, 5, and 6. In normal animals little increase in thiamin concentrations occurs (Figures 4 and 5). In deficient animals, the thiamin concentrations promptly return to normal (Figure 6).

d. Thiamin excretion in rats

In Figures 7 and 8 and in Table VI, the excretion of thiamin by normal animals is compared with the excretion of thiamin by thiamin deficient animals following tolerance tests of various types.

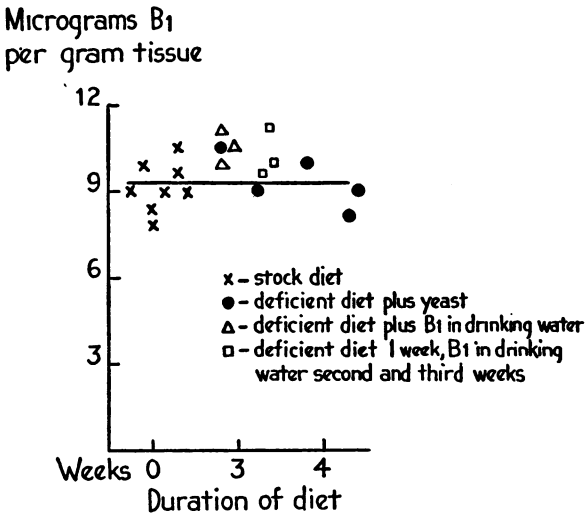

FIG. 4. EFFECT OF HIGH THIAMIN DIET UPON CONCENTRATION OF THIAMIN IN LIVER OF RATS

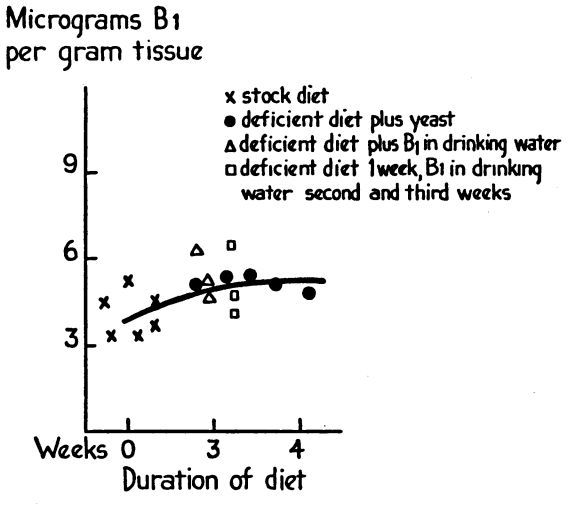

FIG. 5. EFFECT OF HIGH THIAMIN DIET UPON CONCENTRATION OF THIAMIN IN KIDNEY OF RATS

Differentiation of the normal animals from the deficient animals by means of these tests is generally possible. The chief confusion occurs when the intravenous test is applied to severely deficient animals that have lost 50 per cent of their body weight (Figure 7).

DISCUSSION

Our estimations of the concentrations of thiamin in rat tissues and their variations with diet are in agreement with observations made by other investigators (10, 13, 14). In the case of human

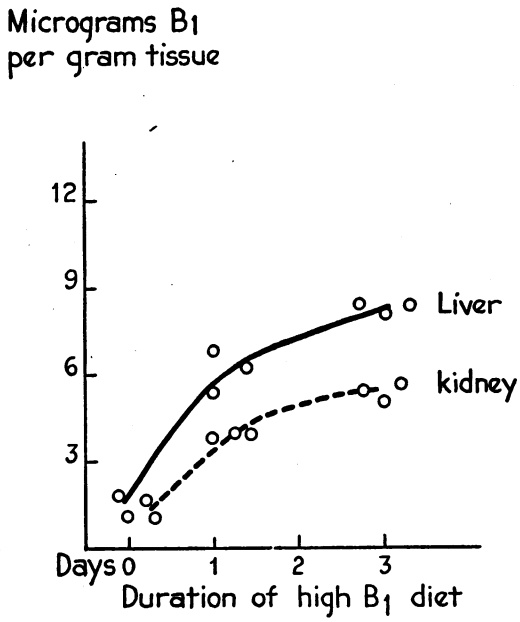

FIG. 6. EFFECT OF HIGH THIAMIN DIET UPON THE CONCENTRATION OF THIAMIN IN LIVER AND KIDNEYS OF RATS THAT HAD BEEN ON A DEFICIENT DIET FOR 2 WEEKS

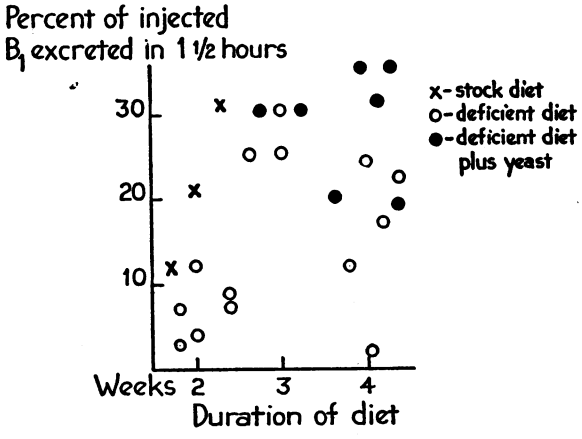

FIG. 7. EXCRETION OF THIAMIN BY NORMAL AND DEFICIENT RATS FOLLOWING THE INTRAVENOUS INJECTION OF 40 MICROGRAMS OF B_1 AND B_2

tissue, values with which our results might be compared have not appeared. The concentrations which we have found in man are of the order of 2 or 3 micrograms of thiamin per gram of heart muscle, 0.5 microgram per gram of skeletal muscle, and 1 microgram per gram of liver, kidney, and brain; the total for the average person being about 25 milligrams of thiamin. Concentrations significantly below these may be found in individuals suffering prolonged febrile illnesses, with dietary restrictions of an order sufficient to cause symptoms in active patients. Conversely, concentrations somewhat higher than the usual adult normal may be found in children and in patients who have recently received large quantities of the vitamin. In general, however, as with animals (15, 5), the ability to store added thiamin appears limited to short periods.

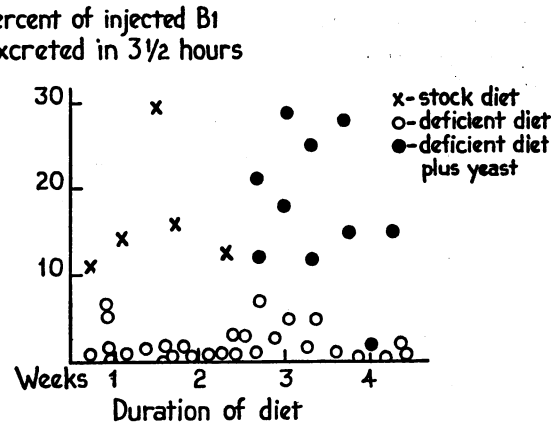

FIG. 8. EXCRETION OF THIAMIN BY NORMAL AND DEFICIENT RATS FOLLOWING THE SUBCUTANEOUS INJECTION OF 40 MICROGRAMS OF B_1 AND B_2

TABLE VI

A comparison of the micrograms of thiamin excreted in the urine during several tolerance tests performed on normal rats and on rats maintained for 2 weeks on a thiamin deficient diet

	Normal animals	Deficient animals	Normal controls*	Deficient controls*
Per oram test. 24 hours	9.9	1.7	4.3	1.0
	18.6	0.8	3.1	1.3
	10.8	8.0	3.1	0.8
	6.0	2.0	2.4	1.3
Subcutaneous test. 3½ hours	6.9	0.5	0.6	0.3
	15.9	0.4	0.6	0.3
	6.0	0.5	0.7	0.3
		0.5	0.9	0.3
Intravenous test. 1½ hours	5.0	1.7	0.3	0.1
	8.6	1.5	0.6	0.2
	12.6	3.0	0.3	0.2
		4.8		

* Received water or saline instead of thiamin solution.

The tissue concentrations at which symptoms of deficiency develop are probably different in different tissues and probably vary with the amount of physiologic activity demanded of the tissue at the time of its deficiency. It is therefore not

surprising that in bedridden patients and in caged animals a considerable loss of thiamin may occur before symptoms definitely indicative of deficiency appear. Fortunately for the clinician, the amount of dietary restriction necessary to produce overt deficiency is usually sufficient to attract attention and to suggest the advisability of supplementary thiamin therapy. The occurrence of minor variations of thiamin concentrations in ambulatory patients, on the other hand, the so-called subclinical deficiencies, presents a complicated problem. Discussion of this type of deficiency will not be critical until biopsy and other studies permit an accurate appraisal of the interrelationships of dietary intake, tissue concentrations, and work performance.

The correlation between changes in tissue thiamin concentrations and changes in urinary thiamin excretion observed in our experiments appears definite but not particularly acute. In conformity with previous experiences (1 to 4), the excretion of individuals with clinically recognized deficiency was found sufficiently abnormal to permit their differentiation from normal subjects. In the single study of experimental deficiency in man (Figure 1) the *per oram* tolerance test appeared more sensitive than the parenteral test in detecting a minor degree of deficiency. However, a number of factors interfere with the general application of *per oram* tests (16, 17).

The quantitative relationship of the tissue changes and the excretory changes in our patients is difficult to evaluate without biopsy analyses. The tissue concentrations associated with frankly abnormal thiamin excretion might be estimated on clinical grounds to be of the order of the concentrations found in the autopsies of patients with poor nutrition (Table III). Past experience with measurements of thiamin excretion in patients with fair nutrition, such as those listed as "fair nutrition" in Table III, would not lead us to anticipate marked excretory changes in the latter group.

It has been a general experience that as the clinical evidence for thiamin deficiency becomes less secure, the differentiation afforded by the excretion test becomes proportionately less certain (1 to 4). The underlying difficulty appears to be that thiamin excretion is not a simple threshold phenomenon. The dependence of rate and amount of excretion upon the size of the test dose and the route of administration (16), clearly indicates that the quantity excreted is not determined solely by the patient's nutritional status. The failure of normal subjects to excrete more than 20 to 40 per cent of the test vitamin is probably associated with a marked though temporary increase in the concentration of thiamin in their tissues (15, 5). The ability of tissue, both normal and deficient, to phosphorylate and hold thiamin for a few hours until it can be destroyed, considerably diminishes the working margin of tolerance tests. In the rat experiments, for example, striking changes in tissue concentrations were associated with differences in excretion that amounted to but a small percentage of the thiamin administered. In sick patients, evaluation of similar slight changes is complicated by the knowledge that physiologic variations, of the type exhibited in passing from the fasting to the absorptive state, may affect thiamin excretion as much as deficiency itself (16, 17). It is evident, therefore, that while excretion may be correlated with tissue concentrations under standard conditions (18), it is also unfortunately dependent upon renal function (3) and upon a number of variables which affect the rate at which thiamin is absorbed and distributed to the tissues, and the rate at which the tissues in turn can phosphorylate the vitamin, bind it to their protein (19), or destroy it. Control of these variables constitutes a major difficulty in the clinical application of tolerance tests.

SUMMARY AND CONCLUSIONS

1. The concentrations of thiamin in human tissue are of the order of 2 to 3 micrograms per gram for heart muscle, 0.5 microgram per gram for skeletal muscle, and 1 microgram per gram for brain, liver and kidney.

2. These concentrations may be temporarily increased by thiamin therapy, or they may be considerably reduced by inadequate diets.

3. Under comparable circumstances, deficient subjects tend to excrete less thiamin than normal subjects.

4. This tendency is not sufficient to permit recognition of small changes in tissue thiamin concentrations by measurements of thiamin excretion.

The writers wish to acknowledge their indebtedness to a number of individuals who helped materially in the completion of this work. The analyses by the Yeast Fermentation Method (Table I) were carried out at the Fleischmann Laboratories, Standard Brands, Inc., New York City, through the kindness of Dr. C. N. Frey, Dr. A. S. Schultz, and Dr. L. Atkin. The dietary study (Figure 1) was carried out at the New York Psychiatric Institute through the kindness of Dr. M. M. Harris. The biopsies of human brain and the studies of the effect of thiamin therapy upon tissue thiamin concentrations were obtained at the Neurological Institute, New York City, through the cooperation of Dr. A. Stowell. Material assistance in carrying out animal experiments and in making routine analyses was obtained from Dr. Norman Molomut, Mrs. Lillian Stout, Miss W. Greenspan, and Miss M. H. Carleen. Supplies of thiamin, riboflavin, cocarboxylase, and thiochrome were generously furnished by Merck & Co., Rahway, N. J.

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