SUBCLINICAL VITAMIN DEFICIENCY.[1] IV. PLASMA THIAMIN

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INTRODUCTION

The possibilities for assaying directly by laboratory measurements the nutritional status of an individual with respect to his thiamin nutrition may be divided into several categories: determinations of intracellular thiamin, determinations of extracellular thiamin, and determinations of thiamin excretion. Determinations of thiamin excretion have been used extensively (1 to 5), determinations of extracellular and intracellular thiamin much less frequently (6 to 11). The following investigation represents an effort to explore the possibility of using measurements of extracellular thiamin, in this case plasma thiamin, as an index of thiamin nutrition.

Because the major portion of blood thiamin is bound in the cellular constituents (12), and in view of the recognized limitations of methods previously utilized (7, 9), the micro-yeast fermentation method was used to determine the plasma thiamin (10). In using this method, it is necessary to distinguish between "true" thiamin and non-thiamin yeast-stimulating substances (12). This is particularly true where there is a wide range of thiamin values of low magnitude and where the non-thiamin moiety represents as much as 65 per cent of the total activity measured.

The necessity for determining the blank was recognized early in this investigation. Preliminary trials with the sulfite technic (13) proved unsatisfactory. Attempts were made to destroy the plasma thiamin using a purified carp "anti-thiamin enzyme," prepared from dried carp intestines (14). This method failed because of the introduction of new yeast-stimulating substances in the enzyme preparations used.[2] It was finally necessary to revert to the sulfite procedure, herein described, in spite of its unsatisfactory characteristics. The justification for this course lies in the fact that while an error of 20 per cent may result, the clinical variations sought are two or three times as great.

METHOD

Care is taken to avoid hemolysis of the samples. Blood is drawn into tubes containing heparin, and immediately centrifuged at high speed. Gorham *et al.* (12, p. 163), in a study of leukocyte thiamin, have shown that although there is fragmentation of cells, no liberation of thiamin into plasma takes place. Significant hemolysis would yield falsely high values. Two or 3 cc. of plasma from the

TABLE I

Cubic millimeters of carbon dioxide produced in one hour by yeast suspension in thiamin standard samples and in plasma samples

Sample	Manometer readings		Corrected Δ in pressure	Constant	CO_2
	Initial	Final			
	mm.	*mm.*	*mm.*		*c.mm.*
Blank	151	191	42	1.57	66
Blank	151	195.5	46.5	1.39	65
2 millimicrograms thiamin	151	212	63	1.47	93
2 millimicrograms thiamin	150	212	64	1.39	89
3 millimicrograms thiamin	151	220	71	1.43	102
3 millimicrograms thiamin	150	224	76	1.33	101
4 millimicrograms thiamin	150	222	74	1.54	114
4 millimicrograms thiamin	150	227	79	1.42	112
5 millimicrograms thiamin	151	229	80	1.53	122
Diluted plasma (0.2 cc. plasma)	151	215	66	1.55	102
Diluted plasma (0.2 cc. plasma)	151	221	72	1.45	104
Diluted plasma (0.2 cc. plasma)	151	219	70	1.49	104
Barometer	150	148			

method. An aliquot of the same solution, suitably diluted, always gave some yeast stimulation above the control values.

[1] Aided by a grant from the Williams and Waterman Fund, Research Corporation, New York City.

[2] These carp preparations destroyed all thiamin in 10 and 25 microgram samples, incubated one half hour, at 45° C. at pH 7.4, as tested by the thiochrome fluorescence

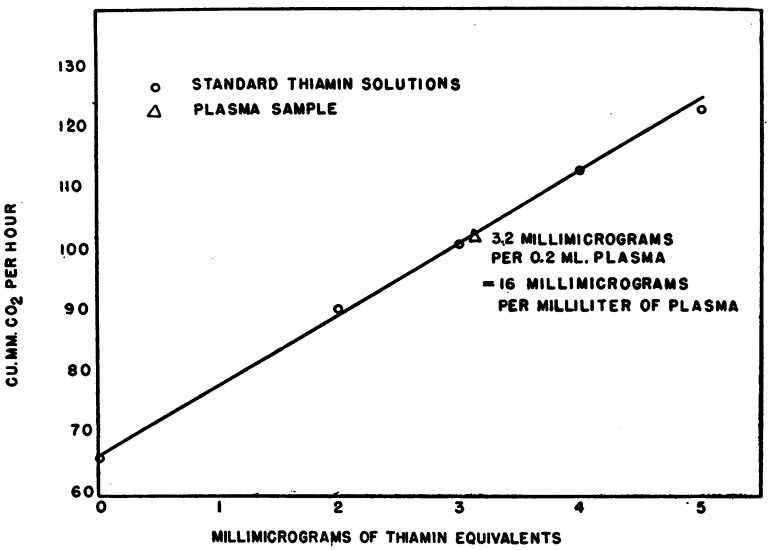

FIG. 1. C. MM. OF CO_2 PRODUCED IN ONE HOUR BY STANDARD THIAMIN SOLUTIONS

The plasma sample was diluted so that 1 ml. of the diluted plasma used equalled 0.2 ml. of the original plasma.

heparinized blood are acidified with 0.1 normal sulfuric acid to pH 4.5 and diluted with 1 to 4 volumes of water, the dilution factor varying with the estimated nutritional status of the patient. Duplicate or triplicate 1 cc. samples of the diluted and acidified plasma are incubated with special yeast suspensions in Warburg vessels (10), the carbon dioxide evolution measured, and the thiamin equivalence calculated from standard curves as previously described (15) (see Table I and Figure 1).

Correction for yeast-stimulating material, other than thiamin, in plasma is obtained by destroying the thiamin in duplicate aliquots of plasma with sulfite and determining the residual yeast-stimulating activity. Five cc. aliquots of plasma are acidified, sulfited, and heated as in the procedure described by Schultz, Atkin, Frey and Williams (13). This treatment, applied to plasma, results in the formation of a heavy protein coagulum, which is removed by suction filtration. The procedure for removal of excess sulfite from the solution (13) is then completed on the clear filtrate, the acidity adjusted to pH 4.5, and 1 cc. samples incubated, as described above. By difference, the "true" thiamin content of plasma can then be calculated.

The coagulum of plasma proteins is a probable source of error in this procedure. Up to the present time, we have not found suitable conditions under which sulfiting may be conducted without the precipitation of protein. On the basis of our experience, we estimate that there is introduced an error of 20 per cent, due to adsorption of yeast-stimulating material on the coagulum.[3]

[3] This investigation was terminated because of other war work.

RESULTS

A number of experiments were performed to assay the stability of the yeast-stimulating materials in human plasma. The stability in contact with red cells, that is, in heparinized whole blood, was first investigated.

Plasma that was separated immediately from a sample of freshly drawn, heparinized, whole blood had a yeast-stimulating activity equivalent to 8.0 millimicrograms of thiamin per cc. of plasma. Plasma, separated after the heparinized whole blood had stood for 30 minutes at room temperature, had a yeast-stimulating activity equivalent to 7.8 millimicrograms of thiamin per cc. of plasma. It would therefore appear unnecessary to work with more than moderate expedition in collecting samples of blood for plasma thiamin analyses.

The stability of the yeast-stimulating materials in plasma, after removal of the blood cells, was next determined. The activity of untreated plasma was found to decrease on standing in the refrigerator. The activity of samples, acidified to pH 4.5 with 0.1 normal sulfuric acid, remained constant for periods of several days (Table II).

The greater portion of the yeast-stimulating

material of plasma appeared to be freely diffusible at pH 5 to 7, in other words, probably free to exchange between plasma and general extracellular fluid. Five cc. samples of plasma were dialyzed in cellophane sacs against 200 cc. of 0.9 per cent sodium chloride, at pH 5, 6, and 7, for periods of 20 hours, at 5° C. By this period of dialysis, 86 ± 6 per cent of the plasma activity was removed (Table III).

The plasma of subjects who are in the habit of taking vitamin tablets containing thiamin was found to have a higher yeast-stimulating activity than that observed in the usual normal individual. The yeast-stimulating activity fell to normal ranges promptly upon the cessation of thiamin medication and became less than normal in individuals subsisting upon an experimental diet deficient in thiamin (16) (Table IV).

In a heterogeneous group of samplings (Table V), the "true" thiamin content of plasma was found to range between 1.7 and 13.5 millimicrograms per cc., the variations appearing in gross coordination with the thiamin nutrition of the subjects. Individuals known to be taking vitamin tablets containing thiamin, or to have recently ingested food of high thiamin content, seemed to have higher plasma thiamin values than subjects whose thiamin nutrition was less generous. In this connection, the low value of 1.7 millimicrograms of thiamin per cc. of plasma observed in the last subject, T, is interesting. This individual, a young woman in good physical condition, had been told independently by an outside physician that she was "lacking in B vitamins." This diagnosis had been made on the basis of a glossitis with moderate atrophy of the marginal papillae and hypertrophy of the fungiform papillae.

The blanks, or plasma activities after sulfiting, are given in Table V. As can be seen in this

TABLE II

Effect of pH upon the preservation at 5° C. of the yeast-stimulating activity of human plasma

Activity expressed in thiamin equivalents, millimicrograms per cc. of plasma

Period of preservation	pH of preservation	Yeast stimulating activity
days		*millimicrograms*
0		6.5
1	7.4	5.3
0		6.6
1	7.4	4.1
0		6.1
2	4.5	6.0
4	4.5	6.0
0		5.7
5	4.5	5.9

TABLE III

Diffusibility of yeast-stimulating materials of human plasma dialyzed against normal saline at pH 5, 6, or 7

Yeast-stimulating activity expressed in thiamin equivalents, millimicrograms per cc.

Sample	pH of dialysis	Thiamin equivalents before dialysis	Thiamin equivalents after dialysis	Per cent removed by dialysis
W	7	13.2	1.7	87
W	6	16	3.2	80
W	6	7.5	0.8	89
N	7	8.8	1.2	87
N	6	8.8	0.7	92
H	6	8.3	1.0	87
H	5	8.3	.7	91

TABLE IV

Yeast-stimulating activity of plasma of individuals of varying nutritional status

Activity expressed in thiamin equivalents, millimicrograms per cc. of plasma

Subject	Sex	Nutritional status	Plasma activity
P	F	Normal diet plus 6 vitamin tablets o.d.	16.0
W	M	Normal diet plus 3 vitamin tablets o.d.	13.2
W	M	Normal diet plus 3 vitamin tablets o.d.	16.0
W	M	Normal diet without vitamin tablets for 1 week	7.5
W	M	Normal diet without vitamin tablets for 1 month	8.5
B	M	Normal diet plus 2 yeast cakes o.d.	9.3
B	M	Normal diet without yeast for 1 week	7.3
N	M	Normal diet	8.8
H	F	Normal diet	8.3
R	F	Normal diet	6.9
T	F	Usual diet (? thiamin deficient)	5.0
Bt	M	Thiamin deficient diet, 1 week. Usual exercise	4.1
S	M	Thiamin deficient diet, 1 week. Walked 15–20 miles a day	2.5
Ko	M	Thiamin deficient diet, 2 weeks. Usual exercise	3.9
Ki	M	Thiamin deficient diet, 2 weeks. Walked 15–20 miles a day	2.9

table, the blanks fluctuate in a narrow range from 2.4 to 5.5 millimicrograms. Since thiamin deficient plasma activity is almost wholly due to blank substances, the importance of determining the blank value is obvious. The data available at present are insufficient to evaluate the relationship of the blank to normal, subclinically deficient, or deficient plasma thiamin.

Comparison of the yeast-stimulating activity of plasma and the 24-hour urinary excretion of thiamin by the thiochrome method (17) reveals a rough parallelism between these two measurements of thiamin nutrition (Table VI). The quantitative aspect of the parallelism is interesting in that it affords some information on the physiology of thiamin excretion. Thus, in a fasting individual, the glomerular filtration rate for plasma was found by mannitol clearance [4] (18) to be 76 cc. per minute. The thiamin excretion at the same time was 130 millimicrograms per minute. The yeast-stimulating activity of the plasma during the experiment was equivalent to 5.6 millimicrograms of thiamin per cc. of plasma. Assuming a plasma blank of the order of 3, the theoretical glomerular excretion of thiamin might be calculated as (5.6–3) × 76 or 198 millimicrograms per minute. This calculation, while inaccurate because of the assumption of the size of the plasma blank, appears to preclude the possibility of an extensive tubular reabsorption of thiamin.

TABLE V

Yeast-stimulating activity of human plasma before and after treatment with sulfite

Activity expressed in thiamin equivalents, millimicrograms per cc. of plasma

Sample	Activity of plasma before sulfiting	Activity of plasma after sulfiting	"True thiamin"		Nutritional status
			mμ* per cc. of plasma	Percentage of total activity	
P	16.0	2.5	13.5	84	Normal diet plus 6 vitamin tablets o.d.
B	12.2	2.4	9.8	80	Normal diet (sample taken 1 hour after noon meal)
H	10.6	3.0	7.6	72	Normal diet
L	10.0	4.4	5.6	56	Normal diet (1 vitamin tablet on previous evening)
W	8.5	5.5	3.0	35	Normal diet
T	5.0	3.3	1.7	34	Usual diet (? thiamin deficient)

* 1 mμ equals 1 millimicrogram.

[4] We are indebted to Dr. W. C. Bridges, Peter Bent Brigham Hospital, Boston, for this measurement.

TABLE VI

Comparison of the yeast-stimulating activity of plasma and the 24-hour urinary excretion of thiamin

Yeast-stimulating activity expressed in thiamin equivalents, millimicrograms per cc. of plasma

Subject	Yeast stimulating activity of plasma	Thiamin content of 24-hour urine sample	Nutritional status
	millimicrograms	*micrograms*	
W	16.0	500	Normal diet plus 3 vitamin tablets o.d.
B	9.3	190	Normal diet plus 2 yeast cakes o.d.
W	8.5	125	Normal diet without vitamin tablets for 1 week
B	7.3	100	Normal diet without yeast for 1 week
Ko	3.9	41	Thiamin deficient diet for 2 weeks, usual exercise
Ki	2.9	27	Thiamin deficient diet for 2 weeks, walked 15–20 miles o.d.

DISCUSSION

Evidence has been presented to indicate that measurements of plasma thiamin are technically feasible, using the yeast-fermentation method of thiamin assay (10, 15) and the sulfite cleavage method of distinguishing thiamin from other materials having yeast-stimulating activity (13).

Dialysis experiments have indicated that a major portion, at least 80 to 90 per cent, of the yeast-stimulating material of plasma is freely diffusible at pH's 5 to 7 and hence, *in vivo*, probably is in free exchange with similar material, notably thiamin in the general extracellular fluids. This observation is important in lending support to the idea that changes in plasma thiamin concentration may be taken as an index of changes in the thiamin content of the general cellular environment.

Since the functional thiamin enzymes are, on the whole, intracellular components of considerably greater concentration than that obtained by the thiamin of the extracellular fluids (ratio about 100 to 1) (5) and since it is inadequacy of these intracellular enzymes which, in all

probability, determines the symptoms of thiamin deficiency, it would be desirable to ascertain to what extent variations in plasma thiamin concentrations may be indicative of changes in intracellular thiamin concentrations (10). Preliminary experiments to this purpose have been performed (16). From the observations reported in this paper, it is evident, however, that plasma thiamin concentrations, and, for that matter, total plasma yeast-stimulating activities, are quickly responsive to changes in thiamin nutrition, the responses being in many ways similar to those observed in urinary thiamin excretion. Thus, the plasma thiamin level, like the urinary thiamin excretion, appears to have a large degree of freedom in the range above the level of clinical thiamin deficiency, rising to several times normal value under generous thiamin administration and falling rapidly to subnormal levels with degrees of thiamin deprivation scarcely sufficient to produce signs or symptoms of thiamin deficiency.

SUMMARY AND CONCLUSIONS

(*1*) The yeast-stimulating activity of plasma was determined by the yeast-fermentation method and recorded in terms of thiamin equivalence.

(*2*) The yeast-stimulating activity due to thiamin in the plasma was destroyed by sulfite cleavage and the residual yeast-stimulating activity determined.

(*3*) The "true" plasma thiamin was calculated from (*1*) and (*2*) by difference.

(*4*) It was found that the yeast-stimulating materials in plasma, thiamin and others, were freely diffusible at pH's 5 to 7, from which it was concluded that they were probably free to exchange *in vivo* with similar materials in the general extracellular fluids.

(*5*) The yeast-stimulating activity of plasma was found to vary with the level of thiamin nutrition, with the level of thiamin excretion, and with the "true" plasma thiamin concentration.

(*6*) It was therefore concluded that measurements of the yeast-stimulating activity of plasma could be used as indices of thiamin nutrition. However, since some variation in activity, due to materials other than thiamin, was observed, calculation of "true" plasma thiamin appeared preferable, particularly in plasma of low thiamin content.

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