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Research Article

THIAMINE AND COCARBOXYLASE CONCENTRATION IN HEART, LIVER, AND KIDNEY, OF PATIENTS WITH HEART FAILURE

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In patients with congestive heart failure, thiamine deficiency has been demonstrated by the test-dose method (1, 2), by measurement of the lactate-pyruvate ratio of the blood (3), and by determination of blood cocarboxylase (4). The oxidation of pyruvate to acetate requires thiamine in the form of cocarboxylase (thiamine pyrophosphate). Olson, Pearson, Miller, and Stare have shown that thiamine deficiency in experimental animals (rats and ducks) resulted in a decrease in the ability of cardiac muscle slices to oxidize pyruvate (5). The present investigation was thus undertaken to determine the thiamine and cocarboxylase content of cardiac muscle in patients with heart failure as compared with that of non-cardiac patients. To our knowledge no data are available on this point in the literature.

METHODS

Patients were selected from the Philadelphia General Hospital and Temple University Hospital under treatment for congestive heart failure. In order to study the influence of long-standing heart disease upon the thiamine and cocarboxylase content of heart muscle only those patients who had failed to respond to therapy and expired with manifestations of cardiac decompensation were analyzed. There were twelve cases from whom tissues were obtained at post-mortem examination including seven with hypertensive cardiovascular disease, four with arteriosclerotic heart disease, and one with rheumatic heart disease. There were six males, and six females; their ages ranged from 39 to 81 years. The duration of treatment for heart disease in eight patients ranged from two and one-half years to eight years in the cardiac clinics or by private physicians; in four patients the duration of the disease could not be accurately determined. In each instance, treatment included the use of digitalis, mercurial diuretics or ammonium chloride, and salt restriction diets. Despite these regimens, symptoms of dyspnea, orthopnea due to pulmonary congestion, dependent edema, venous hypertension, hepatomegaly, ascites and hydrothorax (two cases) persisted, necessitating hospitalization. The patients received no supplementary vitamins but were treated with diuretic agents, digitalis, oxygen, bed rest, and restricted sodium intake. The patients expired with manifestations of advanced heart failure including Cheyne-Stokes respiration, pulmonary congestion, and general signs of anoxia. The tissues obtained for analysis of thiamine and cocarboxylase content included heart (ventricle), liver, and kidney. In the entire group, these determinations were made on wet tissues; in six cases, thiamine and cocarboxylase were measured in relation to the nitrogen content per gram of tissue.

Similar analyses were made on corresponding tissues from ten patients dying of non-cardiac causes. There were five who expired of cerebrovascular accident, one of pulmonary embolism, one of gangrene of foot, one of obstructive uropathy, one of coronary occlusion of two days' duration, and one of brain tumor. None of these patients received thiamine prior to death. Tissues from normal control subjects were unobtainable so that non-cardiac patients were used as the control group; in addition to determinations on wet tissue in each instance, analysis was made for thiamine and cocarboxylase content based on the dry weight and compared to the nitrogen content per gram of tissue in six non-cardiac cases.

Within one hour after death, the body was placed in an ice box at temperature of 38° F. and within five hours after death tissues were removed, kept frozen at minus 10° F. until immediately before they were analyzed. Pieces of tissue weighing about 100 grams were dissected from tendon and fat. A sample of tissue weighing approximately 50 grams was homogenized in distilled water (about 1 weight tissue and 2 weight water) using a Waring Blender. Analysis was made of this homogenate for free thiamine using a modified method of Hochberg and Melnick (6). The mixture was permitted to stand for one hour at room temperature and filtered through No. 40 Whatman filter paper. Sodium acetate 2 N was added to the mixture to adjust the pH to 4.5. It was passed through Zeolite (Decalso column) filter tube. Boiling distilled water was passed through Decalso column and free thiamine was determined utilizing the reaction between thiamine and p-amino-acetophenone. A second sample of tissue was used for analysis of total thiamine and cocarboxylase, using a modification of the method of Hochberg, Melnick, and Oser (7). Approximately 50 grams of the tissue were macerated in a Waring Blender with 0.1 N H_2SO_4, and the volume was brought to 250 ml. with 0.1 N H_2SO_4. The mixture was digested for one hour at 100° C.; and then cooled to room temperature. The mixture was adjusted to the pH of 4.5 with 2 N sodium acetate. Clarase®,[1] 3 per cent by

[1] We are indebted to Takamine Laboratory for the supply of Clarase®.

weight of tissue, was added and incubated at 37° for 12 hours. This completed the hydrolysis of cocarboxylase to thiamine. It was filtered and aliquot parts (100 ml.) passed through the Zeolite (Decalso column) filter and thiamine was determined (7). The cocarboxylase was calculated by the difference between free thiamine of the first homogenate and the total thiamine of the second homogenate. Total nitrogen was determined by the micro-Kjeldahl method.

RESULTS

The analyses of heart, liver, and kidney tissues for total thiamine and cocarboxylase are presented in Tables I and II. These data express the values obtained as micrograms of these substances per gram of the wet weight of tissues. The mean values for the cardiac cases were for thiamine 0.60 microgram per gram of tissue and for cocarboxylase 0.36 microgram per gram of tissue; in the non-cardiac cases, the mean values for thiamine were 1.37 micrograms per gram of tissue and for cocarboxylase 0.84 micrograms per gram of tissue.

The difference between the means of total thiamine and cocarboxylase in patients with car-

TABLE I

Cardiac patients—concentration of total thiamine and cocarboxylase in heart, liver, and kidney *

Patient	Age	Sex		Heart	Liver	Kidney	Remarks
A. O.	60	F	Th	0.52	0.54	0.87	Hypertensive cardiovascular disease. Cardiac failure. Died on 5th hospital day.
			Coc.	0.34	0.48	0.71	
R. S.	66	F	Th	0.63	0.85	0.81	Arteriosclerotic heart disease. Residual left hemiplegia. Cardiac failure. Died on 17th hospital day.
			Coc.	0.50	0.75	0.68	
A. C.	81	F	Th	0.88	0.27	0.71	Hypertensive cardiovascular disease. Thrombophlebitis—right lower extremity. Cardiac failure. Died on 35th hospital day.
			Coc.	0.59	0.23	0.60	
J. T.	70	M	Th	0.89	1.07	0.77	Arteriosclerotic heart disease. Apical myocardial infarct; myocardial failure. Died on 10th hospital day.
			Coc.	0.70	0.84	0.59	
S. B.	39	F	Th	0.86	0.75	1.9	Malignant hypertension. Cardiac failure. Died on 14th hospital day.
			Coc.	0.45	0.56	1.49	
W. R.	80	M	Th	0.43	0.41	1.03	Hypertensive cardiovascular disease. Pulmonary edema. Died after 6 hours hospitalization.
			Coc.	0.33	0.23	0.77	
F. C.†	63	M	Th	0.81	0.33	0.53	Hypertensive cardiovascular disease. Cardiac failure. Died on the 12th hospital day.
			Coc.	0.52	0.28	0.45	
K. H.†	70	F	Th	0.29	0.52	1.10	Arteriosclerotic heart disease. Congestive failure. Died on the 3rd hospital day.
			Coc.	0.06	0.30	0.78	
P. F.†	66	F	Th	0.76	0.38	0.67	Hypertensive cardiovascular disease. Died on 3rd hospital day.
			Coc.	—	0.27	0.51	
J. C.†	78	M	Th	0.69	0.40	2.32	Arteriosclerotic heart disease. Died on the 11th hospital day.
			Coc.	0.23	0.16	1.26	
M. F.†	77	M	Th	0.39	0.41	0.66	Hypertensive cardiovascular disease. Cardiac failure. Died on the 1st hospital day.
			Coc.	0.23	0.34	0.54	
R. M.†	73	M	Th	0.02	0.16	0.16	Rheumatic heart disease. Congestive failure. Died on 6th hospital day. Three previous admissions for cardiac failure.
			Coc.	0	0.16	0.16	
Means of tissue			Th	0.60	0.51	0.96	
			Coc.	0.36	0.38	0.71	

* Results are expressed in micrograms per gram of wet tissue.
† Measurements are also available per gram of tissue nitrogen.

TABLE II

Non-cardiac patients—concentration of total thiamine and cocarboxylase in heart, liver, and kidney *

Patient	Age	Sex		Heart	Liver	Kidney	Remarks
D. K.	70	F	Th	1.43	1.35	1.45	Hypertension. Cerebral hemorrhage. Died on the 14th hospital day.
			Coc.	1.16	0.94	0.54	
M. S.	74	F	Th	1.2	1.7	1.2	Cerebral thrombosis. Bronchopneumonia. Died on the 8th hospital day.
			Coc.	0.82	1.43	0.82	
E. H.	52	M	Th	0.99	0.89	1.1	Pulmonary embolism; drug addiction; diverticulosis; transverse colostomy. Died on the 18th hospital day.
			Coc.	0.81	0.71	0.85	
M. S.	65	F	Th	1.21	1.08	2.26	Cerebral hemorrhage due to hypertension. Died on the 11th hospital day.
			Coc.	0.69	0.57	1.40	
M. H.†	57	F	Th	1.67	0.95	0.79	Cerebrovascular accident. Died on the 10th hospital day.
			Coc.	0.55	0.60	0.30	
I. P.†	75	M	Th	1.39	0.79	1.46	Amputation of left leg for gangrene. Died on the 3rd hospital day.
			Coc.	0.85	0.48	1.18	
E. G.†	69	M	Th	1.27	0.72	1.27	Cerebral hemorrhage. Died on the 3rd hospital day.
			Coc.	0.52	0.16	0.52	
T. J.†	62	M	Th	1.62	0.95	1.20	Obstructive uropathy. Bronchopneumonia. Died on the 5th hospital day.
			Coc.	1.47	0.64	0.80	
T. C.†	59	F	Th	1.88	1.04	1.87	Coronary occlusion. No previous history of heart disease. Died on the 2nd hospital day.
			Coc.	0.90	0.81	0.80	
S. M.†	58	F	Th	1.01	0.31	0.99	Brain tumor. Died on the 5th postoperative day.
			Coc.	0.58	0.10	0.59	
Means of tissue			Th	1.37	0.98	1.36	
			Coc.	0.84	0.64	0.78	

* Results are expressed in micrograms per gram of wet tissue.
† Measurements are also available per gram of tissue nitrogen.

TABLE III

Statistical evaluation of total thiamine and cocarboxylase in wet tissues of twelve cardiac and ten non-cardiac patients*

	Heart		Liver		Kidney	
	Thiamine *Micrograms per gram*	Cocarbox. *Micrograms per gram*	Thiamine *Micrograms per gram*	Cocarbox. *Micrograms per gram*	Thiamine *Micrograms per gram*	Cocarbox. *Micrograms per gram*
Cardiac cases						
Mean	0.60	0.36	0.51	0.38	0.96	0.71
Standard deviation	0.26	0.21	0.25	0.22	0.57	0.34
Control cases						
Mean	1.37	0.84	0.98	0.64	1.36	0.78
Standard deviation	0.27	0.28	0.35	0.36	0.41	0.31
Mean of control cases minus mean of cardiac cases	+0.77	+0.48	+0.47	+0.26	+0.40	+0.07
Probability of "t" for difference between means	Less than .001	Less than .001	Less than .01	.06	.1	.6
	Highly significant	Highly significant	Highly significant	Non-significant	Non-significant	Non-significant

* Data for cocarboxylase in heart missing from one cardiac case.

diac failure and the means in the control patients was tested by means of the "t" test. The probabilities of the "t" are listed in Table III. The conventional 0.05 level of significance was used. It is apparent that in the case of the heart tissue, there was a highly significant difference between the two groups of patients both for total thiamine and cocarboxylase ($P < .001$). In the liver tissue, the difference between the two groups was highly significant for total thiamine but not significant for cocarboxylase. In the kidney tissue from the control group both thiamine and cocarboxylase were higher than in the kidney tissues from the cardiac group. The difference between the means, however, was statistically not significant.

In six of the cardiac patients and in six control patients, the total thiamine and cocarboxylase expressed in micrograms per gram of tissue nitrogen are presented in Tables IV and V. The mean values for the cardiac patients for thiamine were 17.25 micrograms per gram of nitrogen of heart tissue, and for cocarboxylase 7.14 micrograms per gram of nitrogen of heart muscle, and in the non-cardiac cases 56.7 micrograms of thiamine per gram of nitrogen of heart muscle and 32.03 micrograms of cocarboxylase per gram of heart tissue nitrogen. The statistical evaluation of the total thiamine and cocarboxylase per gram of tissue nitrogen in both cardiac and non-cardiac cases is presented in Table VI. It is evident that the results are similar in the ashed tissues to those obtained using the wet tissues.

DISCUSSION

The results of this study tend to indicate that all three types of tissues had a lower concentration of both total thiamine and cocarboxylase in patients with cardiac failure as compared with those who died of other causes. The differences, however, were statistically significant for total thiamine and cocarboxylase in heart only and for total thiamine alone in liver tissue.

The results are in agreement with experimental data reported by several investigators that dietary restriction of thiamine results in a decrease of tissue concentration of both thiamine and cocarboxylase (8, 9). We and others have suggested that patients with heart failure are apt to show thiamine deficiency because of inadequate intake of thiamine in the food, poor absorption of ingested thiamine, and also as a result of the use of mercurials for elimination of edema (2, 3, 10).

TABLE IV

*Concentration of total thiamine and cocarboxylase in tissues from six cardiac patients**

Name	Age	Sex		Heart	Liver	Kidney	
F. C.	63	M	Thiamine	26.9	11	20.1	Hypertensive cardiovascular disease. Cardiac failure. Died on the 12th hospital day.
			Cocarbox.	17.3	9.3	17.1	
K. H.	70	F	Thiamine	9.9	15.8	43.7	Congestive heart failure. Arteriosclerotic heart disease. Died on the 3rd hospital day.
			Cocarbox.	2.0	9.4	31.0	
P. F.	66	F	Thiamine	28.3	12.5	31.3	Hypertensive cardiovascular disease. Cardiac failure. Died on the 3rd hospital day.
			Cocarbox.	—	8.9	23.9	
J. C.	78	M	Thiamine	22.5	12.08	78.65	Arteriosclerotic heart disease. Died on the 11th hospital day.
			Cocarbox.	7.52	5.17	42.72	
M. F.	77	M	Thiamine	15.02	14.02	30.29	Hypertension. Cardiac failure. Died after 14 hours hospitalization.
			Cocarbox.	8.86	11.63	24.78	
R. M.	73	M	Thiamine	0.88	5.17	7.62	Rheumatic heart disease. Cardiac failure. Three previous admissions. Died on the 6th hospital day.
			Cocarbox.	0	5.11	7.62	
Means of tissue			Thiamine	17.25	11.76	35.28	
			Cocarbox.	7.14	8.25	24.52	

* The results are expressed in micrograms per gram of tissue nitrogen.

TABLE V

*Concentration of total thiamine and cocarboxylase in tissues from six non-cardiac patients**

Name	Age	Sex		Heart	Liver	Kidney	
M. H.	57	F	Thiamine	76.0	66.4	58.5	Cerebrovascular accident. Died on 10th hospital day.
			Cocarbox.	25.4	41.9	22.2	
I. P.	75	M	Thiamine	53.8	30.5	70.2	Amputation of left foot for gangrene. Died on 3rd hospital day.
			Cocarbox.	32.9	18.5	56.8	
E. G.	69	M	Thiamine	50.4	30.1	50.4	Cerebral hemorrhage. Died on 3rd hospital day.
			Cocarbox.	31.4	23.4	31.4	
T. J.	62	M	Thiamine	51.35	25.18	51.48	Obstructive uropathy. Bronchopneumonia. Died on 5th hospital day.
			Cocarbox.	46.59	16.96	35.32	
T. C.	59	F	Thiamine	70.50	36.19	72.74	Coronary occlusion. Died after 48 hours' hospitalization. No previous history of heart disease.
			Cocarbox.	33.75	28.18	31.11	
S. M.	58	F	Thiamine	38.58	10.17	34.16	Brain tumor. Died on 5th postoperative day.
			Cocarbox.	22.15	2.95	20.36	
Means of tissue			Thiamine	56.77	33.09	56.25	
			Cocarbox.	32.03	21.98	32.87	

* The results are expressed in micrograms per gram of tissue nitrogen.

Our analyses were done on tissues obtained at the post-mortem table. The results of the total thiamine are probably valid because of the fact that post-mortem autolysis has an insignificant effect upon thiamine content of tissues according to Ferrebee, Weissman, Parker, and Owen (11). The evaluation of cocarboxylase in our post-mortem material is difficult. Apparently no data are available on the stability of thiamine pyrophosphate under these conditions but analyses in both cardiac and control cases on tissues obtained under similar conditions permit a meaningful comparison. In this connection the findings of Ferrebee, Weissman, Parker, and Owen (11) are of interest. In their analysis of the thiamine content of heart and liver tissues obtained from per-

TABLE VI

*Statistical evaluation of the total thiamine and cocarboxylase in tissues of six cardiac and six non-cardiac patients**

	Heart		Liver		Kidney	
	Thiamine	Cocarbox.	Thiamine	Cocarbox.	Thiamine	Cocarbox.
Cardiac cases						
Mean	17.25	7.14†	11.76	8.25	35.28	24.52
Standard deviation	7.94	6.06	3.32	2.37	22.32	10.91
Control cases						
Mean	56.77	32.03	33.09	21.98	56.25	32.87
Standard deviation	12.70	7.72	16.94	11.81	13.02	11.93
Mean of control cases minus mean of cardiac cases	+39.52	+24.89	+21.33	+13.73	+20.97	+ 8.35
Probability of "t" for difference between means	Less than .001	Less than .001	.04‡	.05+‡	.1	.3
	Highly significant	Highly significant	Significant	Non-significant	Non-significant	Non-significant

* The mean values are expressed as micrograms per gram of tissue nitrogen.
† Data for cocarboxylase in heart tissue were missing for one cardiac case.
‡ Adjusted for unequal variances.

sons in poor nutritional status with respect to this vitamin, values were found which correspond closely to those obtained in heart and liver tissues of our patients who died of heart failure.

On the other hand, total thiamine in heart tissue of autopsy material from three subjects who met accidental death ranged between 2.5 to 4.5 micrograms per gram of tissue (average 3.7 micrograms per gram) (12). These values are considerably higher than were found in our control cases. A possible explanation for this is the better state of nutrition of subjects who met accidental death.

Whether the thiamine content of heart muscle in the cardiac cases was at such a low level as to impair the utilization of pyruvate we are unable to state. In several studies of the respiration and utilization of substrate by slices of cardiac muscle from experimental animals it was shown that large decreases of heart muscle thiamine were required before impairment of pyruvate utilization occurred but below 2.5 micrograms of thiamine per gram fresh weight of cardiac muscle the conversion of pyruvate to non-lactate products was a function of the thiamine content (13). Whether a similar metabolic behavior obtains in human heart is not established. Manifestations of thiamine deficiency were not observed in these patients with the exception of one who had malnutrition, smooth red tongue, and hyporeflexia (Case R. M.). It is well known that tissue thiamine levels at which symptoms of thiamine deficiency develop vary with the amount of physiologic activity but it is reasonable to assume that the low total thiamine and cocarboxylase content of the myocardium had a deleterious effect on cardiac muscle metabolism. Whether this factor played a role in the refractoriness of patients to cardiac therapy is a point for further study.

The liver tissues from the cardiac cases showed a significantly lower total thiamine content than from the control cases. This may be explained by the fact that in cardiac failure liver damage is of frequent occurrence (14) and as Borson (15) has suggested hepatic dysfunction may lead to a decreased phosphorylation of thiamine with a consequent elimination of the vitamin in the urine.

In attempting to explain the small difference between thiamine and cocarboxylase content of the kidney tissue in the groups of patients, we must bear in mind that in both groups the thiamine stores were below normal. In thiamine depletion the low tissue thiamine concentration may increase the rate of recovery of thiamine from urine in order to replete body stores of this vitamin (16). Such a process would be expected to increase the thiamine content of renal tissue.

SUMMARY

Heart, liver, and kidney tissues were analyzed for thiamine and cocarboxylase content in 12 cardiac and 10 non-cardiac patients. The analyses were made in wet tissues in the entire series and in six of each group the results were also related to the nitrogen content of the tissues.

In all tissues examined, there were lower concentrations of both total thiamine and cocarboxylase in patients with cardiac failure than in patients who died of other causes. The differences were statistically significant for both total thiamine and cocarboxylase in heart tissue and for total thiamine in liver tissue.

The finding of the significantly reduced thiamine and cocarboxylase contents of heart muscle is worthy of further investigation as to its possible influence upon myocardial metabolism in heart failure.

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