DECREASE OF APOENZYME ACTIVITIES IN VITAMIN-DEFICIENT ANIMALS

II. DECREASE OF OXIDATIVE PHOSPHORYLATION IN VITAMIN B₁-DEFICIENT RATS

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In the previous paper, the authors reported the studies on the decrease of pyruvate-apooxidase activity in thiamine-deficient rats (1). Further researches were made to elucidate whether thiamine activated oxidative phosphorylation (Fig. 1) in the pyruvate oxidase system of thiamine-deficient rats, and it was

FIG. 1 Relationship between the Electron-transferring System and Hexokinase

first confirmed that thiamine contributed to the increase of oxidative phosphorylation in the pyruvate oxidase system of rats. In further experiments, other oxidation systems were tested using α -ketoglutarate, L-glutamic acid and L-proline as substrates. In these cases, the decrease of oxygen uptake Q_{O_2} (N) and of P/O ratio were always observed¹.

EXPERIMENTAL AND RESULTS

1. Oxidative Phosphorylation of Pyruvate

Methods for producing thiamine-deficient animals and for preparing washed liver homogenates were the same as described in the preceding paper (1). Pyruvate oxidase system is given in Table I. The O_2 uptake by pyruvate in the presence of the homogenate was estimated using the Warburg apparatus, and the decrease of inorganic phosphate was determined colorimetri-

¹ Preliminarily reported at the 116th Vitamin B Research Committee (2).

TABLE I
Reaction System for Pyruvate Oxidation in Rat Liver Homogenate

Constituent	Volume ml
Washed liver homogenate ^a	1.0
0.2 M niacinamide	0.1
0.002 M DPN	0.1
0.00025 M ATP	0.1
0.0001 M cytochrome c	0.1
0.01 M cocarboxylase	0.1
0.01 M glutathione	0.1
0.001 M CoA	0.1
0.001 M FAD	0.1
0.002 M pyridoxal-5-phosphate	0.1
1 μ g/ml B ₁₂	0.1
0.01 M lipoic acid	0.1
0.04 M MgCl ₂	0.1
1.0 M NaF	0.1
0.1 M D-glucose	0.1
15 mg/ml hexokinase ^b	0.2
0.02 M fumaric acid	0.1
0.1 M substrate	0.2
1/15 M phosphate buffer	0.3
20% KOH	0.3
Total volume	3.5

^a The content of nitrogen was determined by micro-Kjeldhal method, yielding 2–4 mg/ml.

^b Obtained by Okunuki's method.

cally by Takahashi's method using molybdic acid and L-ascorbic acid in Kotaki Spectrophotometer Type SP-GI, with a filter S 70 (Fig. 2). The values of P/O for the oxidation of pyruvate are given in Table II.

2. Oxidative Phosphorylation of α -Ketoglutarate, L-Poline, L-Glutamic Acid and L-Valine

The uptake of O₂ by substrates were determined in the same way as mentioned above, and the decrease of inorganic phosphate was also calculated by Takahashi's method as described above. The data are summarized in Tables III and IV.

1 ml reaction mixture
 ↓ Deproteinized with 8% trichloroacetic acid
 Filtrate
 | 1 ml of 2.0% Na-molybdate and 1 ml of 1.5N
 | H₂SO₄ are added.
 ↓ Agitated for 10 seconds with 4 ml of
 ↓ isobutanol
 Isobutanol phase
 ↓
 2 ml of the isobutanol phase
 | 2 ml of 0.5% L-ascorbic acid and 1 ml of
 | ethanol are added.
 ↓ Kept at 35° for 30–40 min.
 Coloration
 ↓ After cooling to room temperature
 Photometry ($\lambda_{\max}$ 700 m μ)

FIG. 2 Determination of Inorganic Phosphate

SUMMARY

1. In the present paper it was studied whether thiamine activated the

TABLE II
Oxidative Phosphorylation of Pyruvic Acid^a

No. of exp.	B ₁ -positive group			B ₁ -deficient group		
	Uptake of O ₂	Decrease of Pi	P/O	Uptake of O ₂	Decrease of Pi	P/O
	<i>atoms</i>	<i>moles</i>		<i>atoms</i>	<i>moles</i>	
1	3.4	5.7	1.67	2.7	2.1	0.78
2	8.0	9.1	1.14	2.9	1.7	0.59
3	4.2	5.6	1.33	1.3	1.6	1.23
4	2.3	3.3	1.43	1.8	1.0	0.56
5	6.7	8.8	1.26	2.8	2.7	0.96
6	5.0	11.2	2.24	3.0	3.4	1.13
7	2.8	2.6	0.93	1.7	0.41	0.24
Average			1.43			0.78

^a The uptake of O₂ and the decrease of inorganic phosphate per mg nitrogen of the washed liver homogenate are given in the Table.

TABLE III
QO₂ (N) for the Phosphorylation of Various Substrates in the Presence of Washed Liver Homogenate

Substrate	B ₁ -positive control	B ₁ -deficient group
Pyruvic acid	121.6	52.4
α -Ketoglutaric acid	100.0	34.7
L-Proline	240.0	187.0
L-Glutamic acid	51.0	42.0
L-Valine	20.0	13.0

TABLE IV
P/O Ratios for Various Substrates

Substrate	B ₁ -positive control	B ₁ -deficient group
Pyruvic acid	1.43	0.78
α -Ketoglutaric acid	2.27	1.02
L-Proline	0.36	0.19
L-Glutamic acid	1.51	0.61
L-Valine	0.74	0.66

oxidative phosphorylation of various substrates in the presence of the washed liver homogenate of thiamine-deficient rats.

2. To the reaction system pyruvic acid, α -ketoglutarate, L-proline, L-glutamic acid, and L-valine were added. The decrease of P/O ratio was seen in thiamine-deficient homogenate, but no such phenomenon occurred in thiamine-positive control animals.

REFERENCES

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