RESTORATION OF PYRUVATE BREAKDOWN IN PIGEON MUSCLE HOMOGENATES IMPAIRED BY THIAMINE DEFICIENCY

by

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According to experiments carried out in this laboratory by MONFOORT[1], the anaerobic formation of acetoin from pyruvate added to homogenates of various muscles from pigeons, which had received a diet free of thiamine and rich in carbohydrate[2] for 12 days, is markedly diminished as compared to the formation of this compound by homogenates prepared from the corresponding muscles of normal pigeons. The acetoin formation was raised to the normal level again by adding thiamine pyrophosphate (TPP) *in vitro*.

We now wish to give a preliminary report of experiments which have demonstrated that the oxidation of pyruvate by muscle homogenates is likewise impaired in thiamine deficiency. In order to observe the decrease of pyruvate oxidation it is quite immaterial whether potassium ferricyanide or molecular oxygen is present as an electron acceptor. However, while the oxidation with ferricyanide is raised approximately to the normal level again by adding TPP *in vitro* (see Table I), the sole addition of TPP enhances the oxidation by oxygen but little. This is shown in Table II. This table also shows that a supernatant, prepared by centrifuging a homogenate of normal pigeon breast muscle, which in itself is completely inactive in pyruvate oxidation and contains much less TPP than the amounts added in the experiments referred to above, is much more effective in restoring pyruvate oxidation. The greatest effect, however, by which pyruvate oxidation by the deficient tissue is indeed raised to the normal level, is observed in the presence of both the supernatant of a normal muscle homogenate and TPP.

TABLE I

BREAKDOWN OF PYRUVATE BY PIGEON BREAST MUSCLE HOMOGENATES IN THE PRESENCE OF POTASSIUM FERRICYANIDE AS AN ELECTRON ACCEPTOR

In main compartment of Warburg vessel: 2.0 ml 10% homogenate in 0.1 *M* sodium potassium phosphate buffer, pH 6.8, containing 0.01 *M* $MgCl_2$. Amount of TPP added 10 γ. Tipped in from side-arm: 100 μM potassium ferricyanide and 12.5 μM sodium pyruvate in 0.3 ml phosphate buffer. 3 hours at 37° C. Within the experimental error the quotient pyruvate utilized/CO_2 formed was 1. The amounts of acetate tabulated were calculated from the averages of the amounts of pyruvate utilized and acetoin formed. 20 normal and 10 pigeons after 12 days on a thiamine free carbohydrate rich diet were examined. The figures between brackets are the minimum and maximum values.

	TPP	*Normal*	*12 d def.*
Pyruvate utilization (μM)	—	6.6	2.6
		(5.4–7.6)	(2.0–3.5)
	+	9.0	8.0
		(7.1–11.4)	(6.4–9.5)
Acetoin formation (μM)	—	0.8	0.15
		(0.6–1.0)	(0.1–0.2)
	+	1.3	1.0
		(1.1–1.7)	(0.8–1.2)
Acetate formation (μM)	—	5.1	2.3
		6.5	6.0

REED AND DEBUSK's experiments[3,4] with an apo-pyruvic dehydrogenase prepared from a lipothiamide deficient *E. coli* mutant have shown that anaerobic decarboxylation of pyruvate and oxidation of pyruvate by ferricyanide are activated by TPP, whereas the oxidation by oxygen is only activated by lipothiamide pyrophosphate. We believe that the results obtained by MONFOORT[1] and ourselves with deficient pigeon muscle homogenates bear close resemblance to those of REED

TABLE II

OXIDATION OF PYRUVATE IN PIGEON MUSCLE HOMOGENATES BY OXYGEN

1.4 ml 0.1 M sodium phosphate buffer or supernatant (SN) of normal breast muscle homogenate, pH 7.4, containing 100 mg tissue per Warburg vessel; 2 hours at 37° C. Amount of TPP added 10 γ. 10% KOH in center well. 20 μM sodium pyruvate tipped in from side arm. The phosphate buffer and the SN always contained potassium magnesium versene complex in 0.01 M concentration. SN at the top of a column means that muscle from a thiamine deficient pigeon had been homogenized in the supernatant, prepared from a 10% homogenate of normal breast muscle in 0.1 M sodium potassium solution, pH 5.6, by centrifuging at 15,000 *g* and adjusting the pH after separation to 7.4. The data tabulated concern only muscles of pigeons after 12 days on a thiamine free, carbohydrate rich diet. Normal breast muscle homogenates (7 pigeons): O_2 uptake 16.0–27.7 μM, average 21.5 μM; pyruvate utilization 6.1–11.9 μM, average 9.6 μM. Normal heart muscle homogenates (3 pigeons): O_2 uptake 11.5–16.8 μM, average 15.0 μM; pyruvate utilization 7.3–10.6 μM, average 9.0 μM. No effect of adding TPP and only 10–30% increase by SN in these homogenates.

Pigeon No.		*Breast muscle*				*Heart muscle*			
		Control	*TPP*	*SN*	*SN + TPP*	*Control*	*TPP*	*SN*	*SN + TPP*
1	O_2*	7.6	8.8	21.8	23.9				
	Pyruvate**	2.4	3.9	7.3	8.9				
2	O_2	3.0	2.8	7.9	10.9	2.2	2.9	5.5	8.9
	Pyruvate	3.8	3.8	5.7	7.1	0.8	0.0	2.8	5.3
3	O_2	6.3	7.8	12.1	17.6	3.9	4.1	8.7	8.7
	Pyruvate	2.6	3.6	5.1	7.3	5.6	6.7	6.9	8.6
4	O_2	15.7	17.8	39.4	38.8	3.6	3.8	13.5	15.4
	Pyruvate	5.4	5.5	10.2	10.7	0.4	0.0	8.5	11.2
5	O_2	7.0	9.3	22.2	20.3	2.6	2.9	6.3	15.9
	Pyruvate	2.8	4.6	5.3	7.3	1.4	1.4	4.8	10.1
6	O_2	11.2	13.0	18.5	21.2	3.3	3.3	9.6	11.6
	Pyruvate	6.3	5.0	9.0	10.3	2.6	1.8	7.7	9.4

* μM O_2 uptake; ** μM pyruvate utilization.

and DeBusk, if we postulate that the pyruvic dehydrogenase not only loses its TPP in thiamine deficiency, but also part of its lipoic acid, and that the normal muscle supernatant contains lipothiamide pyrophosphate and/or lipoic acid besides TPP. As we did not dispose of the former substances the final proof could not yet be given. Moreover a system containing tissue homogenate is so much more complicated than a system containing only purified apo-enzymes and coenzymes that we wish to abstain from a full discussion at this moment.

This work forms part of investigations on the metabolism and physiological function of thiamine carried out by H. G. K. Westenbrink and collaborators.

REFERENCES

[1] C. H. Monfoort, *Biochim. Biophys. Acta*, 14 (1954) 291.
[2] M. Gruber, *Nature*, 166 (1950) 78; *Biochim. Biophys. Acta*, 10 (1953) 136.
[3] L. J. Reed and B. G. DeBusk, *J. Am. Chem. Soc.*, 75 (1953) 1261.
[4] L. J. Reed, *Physiol. Rev.*, 33 (1953) 544.

Received April 12th, 1954