Short Communications and Preliminary Notes

ON THE RATIO OF THIAMINE PYROPHOSPHATE CONTENT AND RATE OF SUCCINIC SEMIALDEHYDE PRODUCTION IN HOMOGENATES OF VARIOUS MUSCLES OF NORMAL AND THIAMINE-DEFICIENT PIGEONS

by

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In a previous communication[1] we were able to show that large differences exist between various types of muscles regarding (1) the rate of production of acetoin from pyruvate added to homogenates, and (2) the rate at which the acetoin-producing enzyme, the pyruvic decarboxylase, disappears from these muscles upon thiamine privation. The experiments were carried out with homogenates of the large breast muscle (pectoralis major), the muscle of the left heart ventricle and the leg muscles of normal pigeons and of pigeons which had been on a thiamine-free diet for 4 and 12 days. For details see our earlier communication[2].

In this preliminary note the results will be given of the investigation along similar lines of another thiamine pyrophosphate (TPP) dependent enzyme, the α-ketoglutaric decarboxylase, which catalyses the reaction

$$\alpha\text{-ketoglutaric acid} \rightarrow \text{succinic semialdehyde} + CO_2.$$

The succinic semialdehyde (SSA) formed from α-ketoglutarate added to the muscle homogenate was determined according to a method based on the principle given by OCHOA[3].

The results are assembled in Table I. Q = the amount of SSA formed per 200 mg tissue per 3 hours, divided by the amount of TPP per 200 mg tissue.

The following conclusions may be drawn (Q_B, Q_H and Q_L denote Q for breast muscle, heart muscle and leg muscle, respectively):

1. *Normal pigeons.* The rate of SSA formation declines in the same order as the rate of acetoin formation (see[1]) and as the TPP contents: breast muscle > heart muscle > leg muscle.

$Q_B = Q_H > Q_L$. This means—provided that the assumptions, mentioned in Ref. 1 are valid—that in breast muscle and heart muscle the same percentage of the total TPP is present as the prosthetic group of α-ketoglutaric decarboxylase, and that this percentage is higher than that in leg muscle.

2. *Influence of thiamine deficiency.* The TPP content decreased during the whole period of thiamine privation in every type of muscle to the same extent as in our previous experiments[1].

In the case of the breast muscle the production of SSA also decreases during the first 4 days of deficiency, but it remains constant during the next 8 days. The ratios of the changes of TPP content and SSA production are such, that Q_B remains constant during the first 4 days and then increases very considerably.

In heart muscle, however, there is no change of α-ketoglutaric decarboxylase activity in the first 4 days, while it diminishes slightly in the next 8 days. This implies that Q_H is about doubled in both periods.

In leg muscle there is hardly any influence of thiamine deficiency on the activity of the α-ketoglutaric decarboxylase. Therefore Q_L increases considerably.

In general one may conclude that with the exception of breast muscle during the first 4 days of deficiency, where the pyruvic decarboxylase appears to be little affected by the disappearance of TPP from the tissue, the α-ketoglutaric decarboxylase is much better maintained during deficiency than the pyruvic decarboxylase.

TABLE I

THIAMINE PYROPHOSPHATE CONTENT AND SUCCINIC SEMIALDEHYDE PRODUCTION IN HOMOGENATES OF PIGEON MUSCLE

B = breast muscle, H = heart muscle, L = leg muscle.
TPP expressed in γ per 200 mg, SSA in γ per 200 mg in 3 hrs.

$$Q = \frac{\gamma \text{ SSA per 200 mg in 3 hrs}}{\gamma \text{ TPP per 200 mg}}$$

Numbers of muscles examined:

	Normal	*4 d. def.*	*12 d. def.*
B	7	9	9
H	7	8	9
L	7	8	9

All standard deviations mentioned are standard deviations of the means.

	Normal			*4 days def.*			*12 days def.*		
	TPP	*SSA*	*Q*	*TPP*	*SSA*	*Q*	*TPP*	*SSA*	*Q*
B	1.50±0.10	728±47	486±13	0.94±0.04	473±37	503±21	0.62±0.06	493±40	800±62
H	1.29±0.07	588±37	462±27	0.77±0.05	620±49	825±82	0.30±0.07	466±17	1637±125
L	0.45±0.04	117±16	255±21	0.22±0.01	78±3	355±16	0.14±0.02	85±10	628±68

P values calculated according to "STUDENT"'s method:

B	$P_{Q(N-4d.)} > 0.1$	$P_{Q(12d.-N)} < 0.001$	$P_{Q(12d.-4d.)} < 0.001$
	$P_{SSA(N-4d.)} < 0.001$		$P_{SSA(4d.-12d.)} > 0.1$
H	$P_{Q(4d.-N)} < 0.001$	$P_{Q(12d.-N)} < 0.001$	$P_{Q(12d.-4d.)} < 0.001$
	$P_{SSA(N-4d.)} > 0.1$	$P_{SSA(N-12d.)} < 0.01$	$P_{SSA(4d.-12d.)} < 0.01$
L	$P_{Q(4d.-N)} < 0.01$	$P_{Q(12d.-N)} < 0.001$	$P_{Q(12d.-4d.)} < 0.01$
	$P_{SSA(N-4d.)} < 0.05$	$P_{SSA(N-12d.)} > 0.05$	$P_{SSA(12d.-4d.)} > 0.05$

Increase and decrease of Q indicate that the enzyme studied disappears more slowly or more rapidly, respectively, than the other TPP dependent enzymes collectively. If Q is constant the enzyme studied and the latter enzymes disappear at equal rates.

Upon comparison of the change of the Q values for α-ketoglutaric acid decarboxylation with the change of Q values for pyruvic acid decarboxylation (see[1]), it appears that there exist great differences between the disappearance of α-ketoglutaric decarboxylase and pyruvic decarboxylase in relation to the loss of TPP.

During the first 4 days of deficiency the pyruvic decarboxylase disappears more slowly than the other TPP-dependent enzymes collectively (including the α-ketoglutaric decarboxylase). The same applies to the disappearance of α-ketoglutaric decarboxylase and the other TPP-dependent enzymes collectively (including the pyruvic decarboxylase). During the next 8 days of deficiency, however, the pyruvic decarboxylase disappears at a higher rate than the other TPP-dependent enzymes collectively, while the α-ketoglutaric decarboxylase continues to disappear at a lower, occasionally at a much lower, rate.

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

REFERENCES

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