these and related experiments will be given in due course in the Collection of Czechoslovak Chemical Communications.

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On the importance of α-glycerophosphate dehydrogenase in glycolysing insect muscle*

The DPN-linked α-glycerophosphate dehydrogenase catalyzes the reduction of dihydroxyacetone phosphate according to the reaction:

$$\text{Dihydroxyacetone phosphate} + \text{DPNH} \rightleftarrows \alpha\text{-glycerophosphate} + \text{DPN}^+$$

This reaction is of little significance in vertebrate-muscle glycolysis because it is the reduction of pyruvate which is linked with the oxidation of glyceraldehyde-3-phosphate. However, it is known that glycolysis in insect muscle lacks the proper stoichiometry[1,2], which may be due to the impaired activity of lactic dehydrogenase[3].

The activities of lactic and α-glycerophosphate dehydrogenases in both coxal and thoracic muscles of the American cockroach, *Periplaneta americana*, are seen in Table I.

The results clearly show that the lactic dehydrogenase level in both types of muscle is unusually low. On the other hand, that of α-glycerophosphate dehydrogenase is extraordinarily high. Consequently, most of the DPN is probably regenerated by the reduction of dihydroxyacetone phosphate.

In order to check the physiological significance of these activities, the formation of lactate, pyruvate and α-glycerophosphate from hexose diphosphate was studied under anaerobic conditions. Typical results presented in Table II indicate the stoichiometry of hexose diphosphate conversion.

For every mole of HDP utilized, one mole of α-glycerophosphate and pyruvate was formed. The slower rate of accumulation of pyruvate suggests that the reactions producing pyruvate *via* the Embden-Meyerhof scheme appear to be operating but one or more may be rate-limiting.

The conversion of HDP required DPN which could not be replaced by TPN. ADP alone and ADP plus inorganic phosphate had a slightly stimulatory effect on the metabolism of HDP.

In summary, therefore, the reductive step represented by lactic dehydrogenase appears to be of negligible importance in glycolysis of coxal muscles of the roach. Instead, the α-glycerophosphate dehydrogenase system assumes the major role in regenerating DPN from DPNH. The predominant end-products of roach-muscle glycolysis appear to be α-glycerophosphate and pyruvate. Consequently we have here a normal situation similar to Neuberg's so-called second form of fermentation which may be also induced in muscle with the aid of "steering substances"[8].

* The following abbreviations are used: DPN, diphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; ADP, adenosine diphosphate; HDP, hexose diphosphate; TPN, triphosphopyridine nucleotide; Tris, tris(hydroxymethyl)aminomethane.

TABLE I

ACTIVITY OF LACTIC AND α-GLYCEROPHOSPHATE DEHYDROGENASES IN THE MUSCLES OF *Periplaneta americana*

Lactic dehydrogenase: The reaction cuvette contained 2.0 μmoles Na pyruvate; 0.2 μmole DPNH; 100 μmoles phosphate buffer, pH 7.4; 1.0 ml enzyme preparation (approximately 17 mg tissue/ml) and distilled water to 3.0 ml. Blank cuvettes contained no substrate. The activity in the test cuvettes was corrected for the appreciable activity in the blanks.

α-Glycerophosphate dehydrogenase: Each test cuvette contained 1.8 μmoles dihydroxyacetone phosphate; 0.2 μmole DPNH; 100 μmoles Tris buffer pH 7.4, 0.10 ml enzyme preparation (approximately 5 mg tissue/ml) and distilled water to a vol. of 3.0 ml. At this tissue concentration no activity could be detected in the blanks during the period of measurement.

The activities of both enzymes were assayed by the rate of oxidation of DPNH at 340 mμ. All activities were proportional to the enzyme concentration. A unit of enzyme is defined as that amount which causes an initial rate of oxidation of 0.01 μmole DPNH/min. All readings were taken at 30 sec intervals for 5 min. The reactions were run at 25° and measurements were made in a Beckman model DU spectrophotometer.

The tissue was prepared by grinding 170 mg of muscle for the lactic dehydrogenase and 50 mg for the α-glycerophosphate dehydrogenase study. The homogenization was carried out in a Potter-Elvejhem homogenizer for 2 min in 10 ml of ice-cold 0.9% KCl. The brei was then centrifuged for 10 min at approximately 1° at 16,000 × *g* in a Servall angle-head centrifuge. The bulk of the enzyme activity remains in the supernatant[1].

Tissue	*Activity (units/g wet wt./min)*	
	lactic dehydrogenase	*α-glycerophospate dehydrogenase*
Coxal muscles	10	3206
Thoracic muscles	21	4824

TABLE II

STOICHIOMETRY OF HEXOSE DIPHOSPHATE CONVERSION

Each Thunberg tube contained 95.0 μmoles Tris buffer, pH 7.6; 48.6 μmoles $MgCl_2$; 30.0 μmoles magnesium HDP, pH 7.6; 2.0 μmoles DPN and 2.0 ml enzyme. Total vol. 6.0 ml.

The muscle extract was prepared as described previously. The tubes were evacuated for 5 min with a water aspirator. After deproteinization by boiling for 5 min, appropriate aliquots were used for analysis of HDP[4], lactate[5], pyruvate[6] and α-glycerophosphate[7]. Similar results were obtained with homogenates which were not centrifuged.

Time of incubation (h)	*HDP utilized (μmoles/g tissue)*	*Products appearing (μmoles/g tissue)*		
		lactate	*pyruvate*	*α-glycerophospate*
1	230.7	18.4	21.8	200.8
2	465.6	10.0	48.4	427.3
8	532.8	10.4	452.4	533.1

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