Anaerobic Glycolysis in Fortified Cell-free Homogenates of Tapeworm Tissue.* (18656)

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There is evidence that the anaerobic degradation of carbohydrate in the tapeworm, *Hymenolepis diminuta,* occurs by processes involving the transfer of phosphate radicals (1). The mechanisms are probably similar to those occurring in vertebrate animals.

The present communication is concerned with a study of the glycolytic activity of fortified cell-free preparations of tapeworm tissues. LePage(2) described a cell-free system in which he was able to maintain glycolysis and an over-all esterification of inorganic phosphate using mammalian tumor homogenates as the source of enzymes. In the present study LePage's system was used as a basis, and the individual ingredients of the system were varied in an attempt to determine optimal conditions for lactate production and phosphate esterification.

Procedure. The tapeworm, *Hymenolepis diminuta,* was maintained in the laboratory in albino rats(1). The following procedure was used in all experiments: Reaction vessels were prepared before the tissue was homogenized. All reactants were added except the homogenate, and the vessels kept in cracked ice until the addition of the homogenate. The basic system contained .0024 M K_2HPO_4, .025 M $KHCO_3$, .040 M nicotinamide, .00033 M adenosine triphosphate (A.T.P.), .00020 M diphosphopyridine nucleotide (D.P.N.), .002 M hexose diphosphate, .005 M pyruvate, .0066 M $MgCl_2$, .010 M KF, .010 M glucose, and .2 ml of 25% homogenate. The total volume of the reaction mixture was 3 ml. The worm tissue was homogenized in a chilled all-glass homogenizer after the addition of 3 parts (by weight) of ice-cold .154 M KCl (made alkaline by adding 8 ml of .02 M $KHCO_3$ per liter). Preliminary experiments using water homogenates yielded highly irregular results. The use of alkaline KCl in homogenizing greatly improved reproducibility. Completeness of homogenization was checked microscopically and in all preparations at least 90% of the cells were broken. The pH of the reaction mixtures in all experiments was 7.4. After addition of the homogenate the vessels were gassed with pure nitrogen and incubated at 38°C for 30 minutes. The reaction was terminated by the addition of .2 ml of 100% trichloracetic acid, with mixing, to each vessel. After neutralization with strong KOH (to pH 8.2) and rapid centrifugation, aliquots of the medium were analyzed for lactic acid(3) and inorganic phosphate (4). In all experiments unincubated reaction mixtures were similarly analyzed. From these data it was possible to calculate the lactate formed and phosphate esterified under the varying conditions. The results obtained in these experiments are summarized in Table I.

In attempting to find an optimal system for sustaining phosphate bond energy and lactate production some compromise is necessary since several enzymes are concerned in the reactions. Addition of fluoride to the medium probably blocks the reactions at the stage of phosphoglyceric acid and limits phosphatase activity. Addition of fluoride causes accumulation of phosphoglyceric acid in tapeworm tissue extracts to which H.D.P. has been added(1) and this tapeworm possesses a fluoride-sensitive, magnesium-activated A.T.P.-ase(5). Because of fluoride-blocking, pyruvate was added as hydrogen acceptor. However, the evidence that pyruvate functions

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TABLE I. Effect of Varying Concentration of Cofactors on Lactate Production and Phosphate Esterification by Tapeworm Homogenates.

Exp.	Component varied	P esterified, μM	Lactate formed, μM
	Pyruvate		
1	.005 M	+2.33	1.52
	0	−1.74	0.34
2	.005	+2.06	1.61
	0	−1.48	0.24
	Hexose diphosphate		
3	.002 M	+1.81	1.95
	.001	+1.52	1.55
	0	+1.20	0.88
4	.002	+2.01	1.69
	.001	+1.65	1.12
	0	+1.08	0.64
	$MgCl_2$		
5	.0033 M	+2.39	1.24
	.0066	+1.94	1.73
	.0099	+1.84	0.95
6	.0033	+2.09	1.19
	.0066	+1.89	1.80
	.0099	+1.41	0.71
	A.T.P.		
7	.0002 M	+3.75	1.24
	.0004	+4.48	2.03
	.0008	+2.52	1.55
8	.0002	+3.53	1.49
	.0004	+4.63	2.14
	.0008	+2.07	1.78
	D.P.N.		
9	.0002 M	−0.38	0.81
	.0004	+1.62	1.67
	.0006	+3.81	1.98
10	.0002	−0.68	0.24
	.0004	+1.87	1.79
	.0006	+3.90	2.13
	Nicotinamide		
11	.02 M	+1.42	1.36
	.04	+2.16	2.27
	.08	+3.58	2.61
12	.02	+1.31	1.14
	.04	+2.02	1.88
	.08	+3.44	2.89
	Inorganic P		
13	.0010 M	+0.11	0
	.0015	+1.41	0.65
	.0025	+2.94	1.78
14	.0010	+0.10	0.09
	.0015	+1.07	1.12
	.0025	+2.61	1.94
	KF		
15	.0066 M	+2.97	0.95
	.0100	+1.62	2.02
	.0200	+1.00	1.61
16	.0066	+2.48	0.65
	.0100	+1.95	1.87
	.0200	+1.09	1.30

as a hydrogen acceptor in the present experiments is only presumptive. When A.T.P. or hexose diphosphate were not added to the reaction mixture a negligible amount of lactic acid was produced and the amount of inorganic phosphate esterified was significantly lower. The lower net uptake of inorganic phosphate at higher concentrations of A.T.P. is probably due to increased phosphatase activity. However, it is difficult to account for the lowered lactate production with higher A.T.P. concentrations. The decrease in inorganic phosphate esterified at higher concentrations of magnesium is probably due to phosphatase activation(5), but again the lowered lactate production under these conditions is peculiar. It would be of interest to determine whether calcium ion which antagonizes magnesium activation of phosphatase in this animal(5) would have an effect on the glycolytic activity of the present system.

Obviously, the optimum concentration of D.P.N. was not determined in these experiments, but it is also obvious that D.P.N. is necessary for phosphate esterification and lactate production. Nicotinamide probably functions in protecting D.P.N. from the activity of enzymes which destroy it(2).

These experiments may furnish additional evidence that the reactions, glucose→lactate, proceed by processes similar to those in vertebrate tissues. This is not meant to imply that lactate is the only product of glycolysis in tapeworms. This work indicates that anaerobic glycolysis can be maintained in a cell-free system, with the tissues of this tapeworm as the source of enzymes.

LePage's system which is optimal for mammalian tumor tissues is not optimal for worm tissue homogenates. The data indicate that a more nearly optimal system contains the following: .005 M pyruvate, .002 M hexose diphosphate, .0033 M $MgCl_2$, .0004 M A.T.P., .0006 M D.P.N., .08 M nicotinamide, .0025 M phosphate, .0066 M KF, .025 M $KHCO_3$, .010 M glucose, and the homogenate. Further study of the system will be necessary to establish that these conditions are actually optimal in all respects.

Summary. Lactate production and phosphate esterification were followed in dilute cell-free homogenates of the rat tapeworm,

Hymenolepis diminuta. The system was fortified with bicarbonate, magnesium chloride, nicotinamide, diphosphopyridine nucleotide, adenosine triphosphate, hexose diphosphate, pyruvate, and glucose. Fluoride was used to inhibit phosphatase activity. Semi-optimal conditions were determined. The data support the idea that glycolysis in tapeworms proceeds by processes similar to that in vertebrate tissues.

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