AN ACCEPTOR EFFECT IN GLYCOLYSIS

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In order to observe maximal glycolytic rates in tissue homogenates, especially in liver, it is advisable to include fluoride in the medium in order to inhibit the loss of adenine nucleotides. This technique requires the bypassing of enolase (Potter, 1957). It was thought that rather than using the fluoride technique, the addition of an acceptor system, such as is routinely used in oxidative phosphorylation might be feasible.

The normal acceptor system of oxidative phosphorylation cannot be used, but the addition of hexokinase and 2-deoxyglucose does result in a workable system. This couple is feasible since the product of phosphorylation, 2-deoxyglucose-6-phosphate, is a relatively poor substrate for mouse liver glucose-6-phosphatase. It has the additional advantage that 2-deoxyglucose-6-phosphate not only cannot be metabolized further, but that it inhibits the utilization of residual glycogen via glycolysis or shunt (Wick et al., 1957).

As experimental animals Swiss strain mice were used, starved twelve to eighteen hours before the experiment. The livers were homogenized in ten volumes 0.25 M sucrose, 0.05 M in potassium chloride. The Warburg flasks had the following additions: in the sidearm 12 μM

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fructose-di-phosphate (FDP) and 15 units of yeast hexokinase (Darrow and Colowick, 1957). In the main compartment were included: 1.5 μM diphosphopyridine nucleotide, 3.0 μM adenosine-triphosphate, 50 μM sodium potassium phosphate, 20 μM magnesium chloride, 120 μM nicotinamide, 0.03 μM cytochrome c, and 0.3 ml homogenate in a final volume of 3.0 ml. Final pH 7.6, gas phase air, with 0.2 ml 20 per cent potassium hydroxide in the center well. Bath temperature 37°C. After 10 minutes equilibration the sidearm contents were tipped in, and incubation was continued for an additional forty minutes.

Including the deoxyglucose and hexokinase has a profound effect on the end products of FDP glycolysis. Table I shows the result of typical experiments. (1) It accelerates oxygen uptake reproducibly by about twenty per cent. (2) It decreases lactate formation. (3) It decreases pyruvic acid formation by at least ninety per cent. Pyruvic acid accumulates without an acceptor system. The identity of the acid was verified by paper chromatography of the 2,4-dinitrophenylhydrazone. The oxygen uptake is more sensitive to the acceptor system than the pyruvate decrease; in experiment #2, Table I, in another vessel the inclusion of only 3 units of hexokinase gave an oxygen uptake of 2.7 μM, but still a formation of 1.29 μM pyruvate. (4) The glycolysis is not very sensitive to decreased phosphate concentrations, but the oxygen uptake is.

Intact cells, even in the resting state, have a continuous drain on synthesized ATP for synthetic processes, active transport and conversion to storage forms of high energy such as creatine phosphate or polyphosphates. The introduction of such an acceptor system to imitate the normal drain on ATP, especially by the 2-deoxyglucose-hexokinase couple which is not directly connected with carbohydrate metabolism, institutes again a

Table I

The effect of an acceptor system on the end products of aerobic fructose-diphosphate glycolysis

Experiment	Omission from vessel	Δ O_2 (μM)	Δ lactate (μM)	Δ pyruvate (μM)	Δ phosphate (μM)
1	None	-2.83	+3.40	+0.15	- 9.2
	-2-deoxyglucose	-2.19	+3.70	+5.19	+ 7.4
2	None	-2.30	+5.82	-0	-10.43
	-hexokinase	-2.02	+7.20	+4.76	+10.41
3	None	-2.42	+4.70	+0.41	- 5.44
	-phosphate*	-0.23	+5.60	-0	- 0.37

* 0.58 μM endogenous phosphate was present.

property of whole cells, not normally found in homogenates. Applications of this system will be published elsewhere.

References

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