colysis in intact and digitonin-permeabilized bloodstream trypomastigotes of Trypanosoma brucei

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Abstract:

Digitonin has been used to permeabilize bloodstream trypomastigotes of Trypanosoma brucei. Such permeabilized parasites revealed a fully-functional glycolytic pathway which catabolized glucose and some phosphorylated glycolytic intermediates. Glucose-starved bloodstream trypomastigotes revealed saturation kinetics with a glucose Km = 0.6 mM and Vmax = 150 natom O/min per 10(8) for intact parasites; Km = 4 mM and Vmax = 100 natom O2/min per 10(8) for permeabilized parasites. Glucose oxidation in intact parasites was stimulated 40% by addition of 3 micrograms digitonin/10(8) parasites. Higher concentrations of digitonin than this inhibited the glucose oxidation. Ten millimolar phosphoenolpyruvate (PEP) inhibited the rate of O2 consumption by permeabilized trypanosomes respiring on glucose under aerobic conditions by 50%. It is proposed that glucose oxidation is apparently limited by transport across trypanosomal plasma membrane, and phosphofructokinase is regulated by PEP levels. It is concluded that permeabilization of trypanosomes with digitonin might offer a closer physiological condition for the study of the regulation of glycolysis by using glycolytic intermediates and other chemical compounds which would otherwise not be transported across the membrane(s).