The effect of 3 different incubation media on the rate of pyruvate synthesis from D-glucose metabolism in *T. brucei* cells permeabilized with 60 /micro/g digitonin/10^8 trypanosomes was investigated. Pyruvate synthesis was maximal in the medium containing K^+ and Mg^{2+}, while it was very low in the medium containing Na^+ as the only metal cation. By titrating glycolysis in *T. brucei* incubated in the medium containing K^+ and Mg^{2+} withadenosine triphosphate (ATP) pyruvate synthesis was stimulated but later inhibited when the molar ratio of ATP to Mg/superscript/2/superscript/+ was greater than unity; when D-glucose was replaced by fructose 1,6-diphosphate (FDP) as substrate, adenosine diphosphate (ADP) could replace ATP. This confirms the requirement for ATP in the conversion of D-glucose to FDP and the requirement of ADP during substrate-level phosphorylation in glycolysis. Addition of 1 mM exogenous reduced nicotinamide adenine dinucleotide (NAD^+) to the incubation medium had no significant effect on the metabolism of D-glucose to pyruvate. The results of this investigation indicate that permeabilization of *T. brucei* cells with digitonin causes the leakage of some Mg^{2+}, K^+, ATP and ADP, but not NAD^+. It is concluded that ADP, Mg^{2+} and ATP have a different microenvironment in the cell from that occupied by NAD^+. Some advantages for this cellular organization are discussed.