Pasteur Effect, Malonate Pasteur Effect and Crabtree Effect in Ehrlich Ascites Tumor Cells

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In confirmation of earlier reports by others, it was found that ascites tumor cells exhibit a clear cut Pasteur effect, *i. e.*, they have a lower aerobic than anaerobic glucose utilization. A similar type effect was also demonstrated in malonate blocked cells, incubated aerobically. Under these conditions, fumarate stimulated the oxygen uptake markedly and reduced the glucose uptake as well as the lactic acid formation (malonate Pasteur effect). In the absence of malonate, fumarate had no such action upon glycolysis and respiration.

It has been shown that the concentration of inorganic phosphate in the incubation medium is limiting for the rate of aerobic glycolysis in the Ehrlich ascites cells. The same was found when the cell suspensions were incubated in partial vacuum, as well as in the presence of malonate. It has further been demonstrated that the concentration of inorganic phosphate within the cells decreased to immeasurable values during aerobic glycolysis. An almost complete depletion of intracellular inorganic phosphate was also found to accompany the malonate Pasteur effect.

The Pasteur effect as well as the malonate Pasteur effect decreased by increasing the phosphate concentration of the medium. More ^{32}P was taken up by the cells during aerobic glycolysis than during anaerobic glycolysis. In the latter case, phosphate was given off from the cells to the medium.

An accumulation of acid soluble phosphate esters, including fructose-diphosphate, glucose-6-phosphate and adenosine-triphosphate (ATP), did only accompany aerobic glycolysis. The ATP concentration increased at the expense of ADP and AMP. The amount of acid labile phosphate esters which accumulated, declined by reducing the phosphate concentration of the medium, in contrast to the acid stable phosphate esters. In the presence of iodoacetic acid (10^{-4} M) glucose further reduced the ADP and AMP, a reduction which was not counteracted by the corresponding increase in ATP.

The data indicate that the accumulation of phosphate esters during aerobic glycolysis further restrict the intracellular availability of

inorganic phosphate, resulting in a reduced rate of aerobic glycolysis, *i. e.* a Pasteur effect.

The aerobic accumulation, especially of acid labile phosphate esters in the presence of glucose and phosphate, may also have some bearing upon the Crabtree effect. As this effect was more or less counteracted by removal of phosphate from dilute suspensions of glycolyzing cells, it is probably not directly caused by intracellular phosphate limitation. The Crabtree effect may possibly be explained by a depletion of mitochondrial phosphate acceptors, due to the ATP accumulation during aerobic glycolysis.

Chromatographic Separation of Urinary Corticoids

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Dirscherl *et al.*¹ have found that individual steroid hormones can be separated by means of partition-chromatography on cellulose columns.

This principle has been applied to the fractionation of urinary corticosteroids, and several individual hormone metabolites have been separated and identified.

The procedure involves hydrolysis of the urinary steroid conjugates, either with acid or by means of β -glucuronidase. The steroids are then extracted with ether, the ether extract washed with 0.1 M NaOH and with water. After evaporation of the ether, the residue is dissolved in 2 ml toluene saturated with 1,2-propylene glycol. This solution is chromatographed on a cellulose column impregnated with 1,2-propyleneglycol. Elution fluid: toluene saturated with 1,2-propyleneglycol. The corticoids are determined as reducing steroids.

By means of this method we have determined the loss of several individual corticosteroids when the urine is hydrolyzed with acid and with β -glucuronidase, respectively.

Likewise we have determined the percentage of free and glucuronic acid bound urinary corticoids.

1. Dirscherl, W., Korus, W. and Schriefers H. Hoppe-Seyler's *Ztschr. f. physiolog. Chemie* 305 (1956) 116.