Permeability of Ehrlich ascites cells and spectrophotometric assay of the sub-cellular enzymes

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Summary. A permeabilization method which allows the assay of intracellular enzymes of the Ehrlich ascites cells is described. The developmental changes in the activity of lactic dehydrogenase and glucose-6-phosphate dehydrogenase of toluene-treated Ehrlich ascites cells were studied.

When yeast[2] and *E. coli*[3,4] cells were treated with small quantities of toluene, several intercellular enzymes became directly accessible to external substrates and thus allowed the study of the enzymes under the situation, which closely resembles the physiological state of the enzyme. The properties of the enzymes were found to be essentially the same as in cell-free extracts[2]. So, it was thought to be of interest to see the effect of toluene on Ehrlich ascites cells (EAC) and subsequent assay of glucose-6-phosphate dehydrogenase (G-6-PD) and lactic dehydrogenase (LDH) of the toluene-treated cells.

Material and methods. Ehrlich ascites cells were aspirated out of the peritoneal cavity of the mice. The cells were washed in chilled 0.1 M sodium-potassium phosphate buffer, p^H 7.2 containing 5 mM $MgCl_2$ and finally resuspended in 1:10 v/v proportion in this buffer. The cold suspension was taken in a test-tube and vigorously agitated on a Vortex mixer. While the agitation was continued, 0.01 vol. of toluene-ethanol (1:9 v/v) mixture was added to the suspension and agitation was continued for 5–10 min. The suspension was then kept in ice and finally centrifuged at 100,000 × g for 30 min. The supernatant was kept for enzyme assay.

G-6-PD was assayed spectrophotometrically[5] by following the increase of OD at 340 min. LDH was assayed after the method of Kornberg[6]. Protein was determined according to Lowry et al.[7].

Results. The activity of both G-6-PD and LDH was quite marked in the supernatant fraction. The pellet, after centrifugation at 100,000 × g, was resuspended in buffer, and no enzyme activity was found in the resuspended pellet.

Agitation of the cell-suspension during toluene-ethanol treatment was continued for different periods: 5 min and 10 min were found to be most effective for permeabilization of the cell-wall for LDH and G-6-PD respectively igure 1) when the maximum pool of the enzymes occur in the supernatant.

Different dilutions of the cell-suspension were used for the toluene treatment; 10 times dilution of the cells in buffer to a case of 30 mg/dry wt/ml proved to be effective for permeabilization of the cell wall for the assay of both the dehydrogenases.

The packed cell volume was taken as the measure of tumour growth. The ascites cells were taken from the animals after 4th day of transplantation till the 10th day, and the supernatant was taken for the enzyme assay after being toluene-treated. Figure 2 shows that the activity of both G-6-PD and LDH is directly proportional with the tumour growth.

Discussion. As the structure of the cell-wall may change with the growth phase of different organisms, it appeared to work out for each particular situation. Though no enzyme activity was detected in the supernatant after low-speed centrifugation of yeast[2] cell-suspension, indicating that the enzyme remained within the boundaries of the cell, in case of Ehrlich ascites cells the supernatant showed quite a good activity of both dehydrogenases. The results indicated the proportionality between the tumour growth and the changes in activity of G-6-PD and LDH, which is justified as glycolysis and shunt pathway are quite predominant in tumour tissues.

Freezing and thawing after toluenization which increased accessibility of *E. coli*[4], proved unnecessary in the case of EAC cells. It could be said that it may not be possible to study all enzymes by this simple method of permeabilization, but perhaps one may try several enzymes in many types of cells.

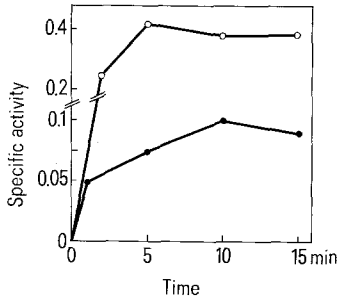

Fig. 1. Time course of the permeabilization for glucose-6-phosphate dehydrogenase and lactic dehydrogenase. (●—●) glucose-6-phosphate dehydrogenase; (○—○) Lactic dehydrogenase.

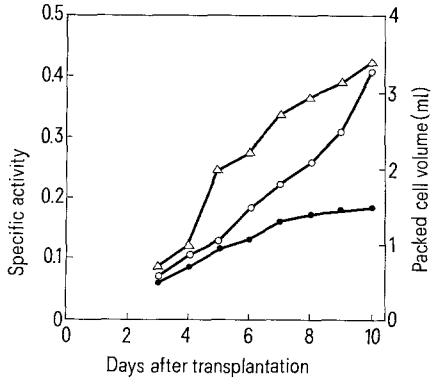

Fig. 2. Developmental changes of glucose-6-phosphate dehydrogenase and lactic dehydrogenase activity in toluene – treated Ehrlich ascites cells. (●—●) glucose-6-phosphate dehydrogenase; (△—△) lactic dehydrogenase; (○—○) packed cell volume of Ehrlich ascites cells.

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