## Effect of Yeast Pyruvate Decarboxylase on Ehrlich Ascites Carcinoma *in vivo*

THIS communication reports on the inhibition of the reduction of pyruvate to lactate, catalysed by lactate dehydrogenase with NADH as coenzyme. This is of interest in an attempt to block glycolysis in tumour cells[1,2].

Pyruvate decarboxylase, commonly known as carboxylase[3], catalyses the following reaction in plants:

$$\text{pyruvate} \longrightarrow \text{acetaldehyde} + \text{carbon dioxide}$$

The addition of pyruvate decarboxylase to an *in vitro* reaction system containing pyruvate, LDH and NADH has been found to result in decreased LDH activity within the system[4]. The pyruvate decarboxylase was extracted as an ammonium sulphate paste from dried brewers yeast by the method of Holzer *et al.*[5]. LDH activity was determined spectrophotometrically at a physiological *p*H of 7·4 and 25° C by measuring the change in extinction at 366 mμ, which is a function of the NADH concentration. The production of acetaldehyde was confirmed by spectrophotometric measurement of alcohol dehydrogenase activity.

This inhibitory effect on the rate of one enzyme reaction caused by the addition of a second enzyme to the system which reacts with the same substrate (a "competing enzyme" inhibition) was thought to have potential value in inhibiting tumour cell glycolysis. If the foreign enzyme protein could enter the cell by means of phagocytosis or pinocytosis, it could then exert a detrimental effect on the anaerobic energy production. This type of inhibition of the LDH reaction would not result in a block in the chain of glycolysis, but rather open up a new pathway of pyruvate metabolism.

$$\text{acetaldehyde} + \text{carbon dioxide} \xleftarrow{\text{pyruvate decarboxylase}} \text{pyruvate} \xrightarrow[\text{LDH}]{\text{NADH}\longrightarrow\text{NAD}} \text{lactate}$$

Nevertheless, under anaerobic conditions, a decreased lactate production would be accompanied by a decrease in NAD available for 3-phosphoglyceraldehyde metabolism resulting in turn in less NADH available for the LDH step. Thus the intermediate ATP producing reactions would be in turn more and more inhibited. Previous experiments using less enzyme and fewer mice showed that pyruvate decarboxylase did inhibit the early growth of Ehrlich ascites after one enzyme injection. Mice with five or six days' advanced tumour growth likewise showed a transitory decrease in tumour growth rate.

In the present experiment, adult *NMRI* (U.S. Naval Medical Research Institute Strain) male mice were divided at random into three groups of ten. The mice were weighed to the nearest 0·1 g and injected intraperitoneally under aseptic conditions with 0·25 ml. of ascites fluid containing approximately $5 \times 10^8$ cells. Therapy was begun on the second day following tumour injection. One test group received two intraperitoneal injections of 0·25 ml. of holoenzyme solution containing 0·07 IU of activity. The other test group received two intraperitoneal injections of 0·25 ml. of apoenzyme solution equivalent to 0·07 IU of activity when recombined with the coenzyme thiamine pyrophosphate. The control group received similar injections of saline. On the following day each of the three groups received one injection.

The holoenzyme solution was prepared by dissolving the enzyme-ammonium sulphate paste in sterile water, then dialysing for 2 h against continuously changed distilled water at 4° C to remove ammonium sulphate. The dialysed enzyme solution was then sterilized by bacterial filtration.

The apoenzyme was extracted from the enzyme-ammonium sulphate paste by the method of Holzer and Goedde[6]. The apoenzyme was then dissolved in sterile water, dialysed, and filtered in a similar manner to the holoenzyme.

The mice were housed and fed under similar conditions and weighed daily. The average daily change in weight for each group is shown in Fig. 1.

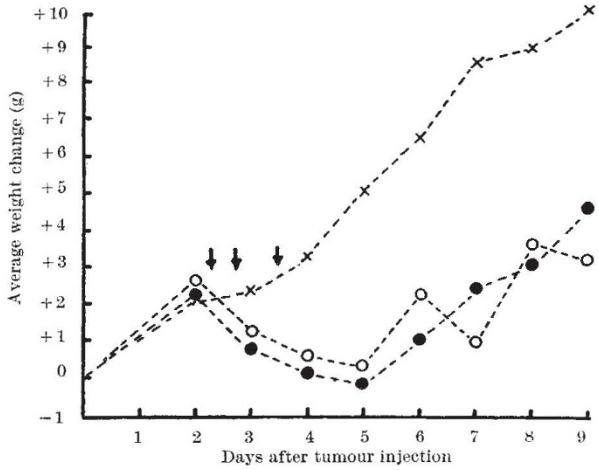


Fig. 1. ●, Group receiving holoenzyme; ○, group receiving apoenzyme; ×, group receiving saline. Arrows indicate therapy.

All mice developed the tumour. After treatment with holoenzyme and apoenzyme, the two test groups show a similar inhibition of tumour growth followed two days after discontinuance of therapy by further tumour growth. This would seem to indicate that experiments using more intensive and prolonged therapy are needed.

Because of the pyruvate present in the ascitic fluid, it is difficult to assess the extent of tumour cell damage caused by acetaldehyde produced in the ascites fluid. As a result, the question of enzyme entrance into the cell as well as other theoretical considerations remains unproved. Anaerobic investigations *in vitro* using apoenzyme, which is active only when recombined with the coenzyme thiamine pyrophosphate, however, have shown that the amount of pyruvate converted to acetaldehyde is much greater in ascites fluid containing cells than in cell free ascites fluid. Because a large amount of pyruvate is also found in cell free ascites fluid, this difference cannot be attributed to lack of substrate, but rather to lack of TPP available for activation of the apoenzyme. Considered in the light of the *in vivo* tumour inhibition also brought about by apoenzyme, there is some indication that the apoenzyme may have combined with intracellular TPP. Whether the diffusion rate of TPP out of the cell is great enough to account for this has not been determined.

These preliminary results appear to indicate that further investigations using solid tumours and more intensive enzyme administration by various routes are called for. The basic principle of "competing enzyme" inhibition may also find application in other areas of research concerned with metabolic disease caused by disruption or imbalance in cellular and extracellular enzyme reactions.

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