interareal differences detected. However, concentration differences were greater in the cirrhotic liver studied than in all but the carcinomatous liver tissue, where areas that contained cancer cells had a higher B_{12} concentration than other adjoining, but normal, liver tissue obtained from the same subject. Without additional investigations, an exact meaning cannot be assigned to this observation. It is worth mentioning in this connexion, however, that it has been reported that mouse tumour cells are able to synthesize vitamin B_{12} (ref. 13).

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Effects of Thiols on *Escherichia coli* β-Galactosidases

IN the extensive experimentation that has involved the assay of β-galactosidase activity during the past 5 years it has been common practice to use thiols, and particularly β-mercaptoethanol. In our method of preparing this enzyme[1] we use thioglycolate for the primary purpose of reducing large aggregates to a minimum. The substitution of β-mercaptoethanol in the preparative procedure, using strain *ML*308 as a source of β-galactosidase, has resulted in a variety of effects, one of which is a progressive inactivation.

In exploring the action of β-mercaptoethanol on β-galactosidase we have verified the observation that, under assay conditions, the addition of this thiol (at concentrations greater than 10^{-3} M) increases the apparent activity by about 50 per cent. However, the thiol effect on our enzyme preparations is a function of the Mg^{++} (or Mn^{++}) concentration as well as *p*H. A typical example is shown in Fig. 1. At *p*H 7, both inactivation and activation by thiols may be seen, depending on the presence of Mg^{++} or Mn^{++}. At a *p*H value of 8·6 these effects are greatly diminished and the rates are reduced.

Highly purified preparations of β-galactosidase from *ML*308 lose activity readily but may be stabilized either by storing in 50 per cent glycerol or by removing Mg^{++} by dialysis against ethylenediamine tetraacetic acid (EDTA). The latter technique is successful only for dilute solutions of protein. However, changes occur in more concentrated solutions due to the fact that such a protein preparation cannot be eluted off DEAE columns even at high salt concentration.

We interpret these results as indicating that β-galactosidase has several conformational and association modes and that the most active form enzymatically is not the most stable thermodynamically. In the presence of β-mercaptoethanol and Mg^{++} the structure is permitted more possibilities to associate with the substrate. In the absence of Mg^{++} other associations occur which are enzymatically inactive. Numerous ultracentrifuge investigations have failed to show readily reproducible differences between active and thiol-inactivated enzyme. In gel-electrophoresis, the latter moves at about 0·9 times the rate of the former.

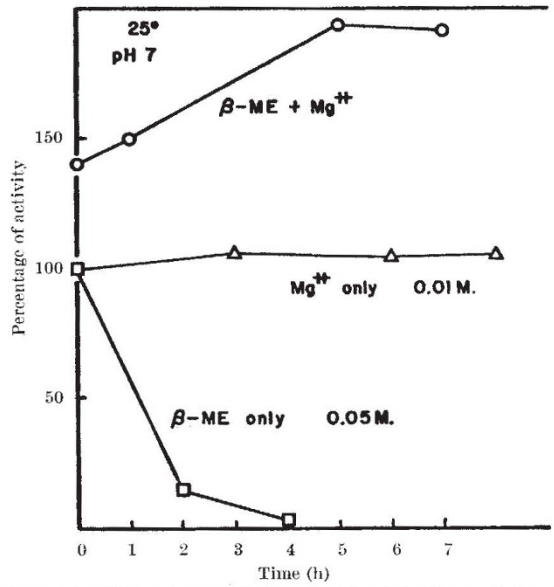

Fig. 1. The effect of β-mercaptoethanol on β-galactosidase activity when under assay conditions. Substrate solution: 9 ml. 0·05 M sodium phosphate buffer *p*H 7 plus 1 ml. *o*-nitrophenyl β-D-galactoside (30 mg in 10 ml.). The release of *o*-nitrophenol from the substrate by enzymatic action was plotted (420 mμ) by a 'Cary' spectrophotometer. Points on the graph correspond to slopes, linear under assay conditions

Beta-galactosidase isolated from strain *K*12 does not behave toward thiols as described here. Rather they seem necessary for retention of activity[2]. Presumably this is due to conformational and sequential differences in the two proteins.

Effects of β-mercaptoethanol such as we have observed in the enzyme isolation process have also been noted in the case of fatty acid synthetase[3]. The phenomenon of superactivity, or the marked enhancement of activity by β-mercaptoethanol, has been seen recently in experiments on lactate dehydrogenase[4]. The interrelation of divalent ions to β-mercaptoethanol activation has been recorded for histidinol dehydrogenase[5]. Whether or not all these effects have the same chemical basis remains to be determined.

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