It seems likely that this mechanism consists of direct chemical interaction between MeHb and GSH. In order to reconcile the present tentative conclusions with the often emphasized fact that methemoglobinemia and glutathione instability are not necessarily correlated *in vivo* [7], it will be necessary to investigate more closely the mechanisms which, in the intact cell, may protect GSH from the oxidizing effect of MeHb, or may be able to replenish the losses of GSH effectively. It may well be that ultimately it will depend on the effectiveness or failure of these mechanisms whether an increased rate of formation of MeHb will lead to actual loss of GSH.

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Isolation of β-Fructofuranosidase from Yeast by Ion Exchange Chromatography on Diethylaminoethylcellulose

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The mechanism of the splitting of sucrose into α-D-glucopyranose and β-D-fructofuranose by invertase from yeast has been investigated by numerous workers. This has given rise to a great number of papers describing purification procedures of the enzyme. One of the most successful is that of Fischer and Kohtes [1], which using precipitation with picric acid and acetone, followed by adsorption to $Al(OH)_3$ obtained a preparation containing 4–5 % N with an activity of 4 000 units/mg N.

The development of cellulose derivatives for separation of proteins by ion exchange chromatography [2,3] has opened new possibilities for a further purification of the enzyme.

In the present work the β-fructofuranosidase from yeast has been separated on a N,N-diethylaminoethylcellulose (DEAE) column. The starting material was »Invertan.K.B.» (commercial invertase preparation, manufactured by De forenede Bryggerier, Copenhagen.) After dialysis against distilled water at 4°C for 24 h the solution was added to the column, previously equilibrated with 0.005 M phosphate buffer, pH 6.5. Eluation was made with increasing concentrations of sodium chloride (0–0.25 M). A sharp separation was obtained between the active component and the other proteins in the solution. After freeze-drying the nitrogen content was determined to give a value of 11.02 %. The enzyme activity was determined according to a method described in a previous publication [4]. Converted to the units of Fisher and Kohtes the activity was 22 000 units/mg N to be compared with the 4 000 units/mg N obtained by these authors. Electrophoresis, which at the present time has only been carried out at one pH, indicates one single protein component. Determination of the molecular weight at the optimal pH (4.75), using the osmometer designed by Christiansen and Jensen [5] gave the value 116 000. Calculation of the enzyme activity as number of substrate molecules reacting per min per molecule of enzyme gives a value of 260 000.

A more detailed description of the isolation will be given.

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