its effect is of no importance. Chlorogenic and caffeic acids are thus not the only anti-fungal factors in the potato plant.

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# The Assay and Specific Activity of Crystalline Alcohol Dehydrogenase of Horse Liver

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Crystalline alcohol dehydrogenase was first prepared from horse liver by Bonnichsen and Wassén [1]. Modifications of the procedure were reported by Bonnichsen [2] and Bonnichsen and Brink [3]. Assay of the enzyme, based on spectrophotometric measurements of the reduction of diphosphopyridine nucleotide (DPN) coenzyme by excess ethanol substrate at alkaline pH, was described by Theorell and Bonnichsen [4,3]. A modification of this assay method is described below.

*Reagents.* 0.1 M glycine-NaOH buffer, pH 10.0. DPN solution 0.83 mg/ml (1 mg/ml »cozymase 90», Sigma Chemical Co.). Ethanol solution, 1 ml 96 % to 100 ml with water.

*Procedure.* The reagents and the cell compartment of the spectrophotometer are kept at 23.0°. 1.85 ml buffer, 1.0 ml DPN solution and 0.15 ml ethanol solution are mixed in a cuvette of 1 cm optical depth, and the extinction at 340 m$\mu$ ($E_{340}$) is measured. A small volume (1 to 10 $\mu$l) of enzyme solution is introduced into the reaction mixture on a stirrer, and the time ($t_{0.2}$) required for an increase of 0.200 in $E_{340}$ ($\lambda_{max}$DPNH) is measured.

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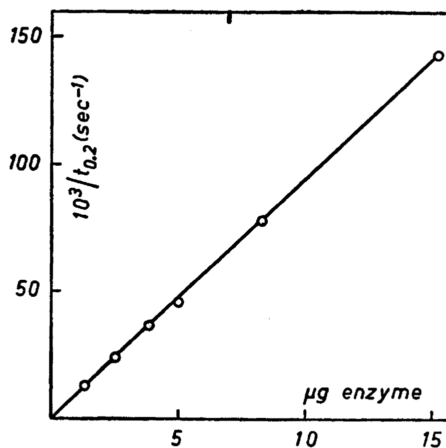


*Fig. 1.*

Theoretically, the enzyme concentration should be more closely proportional to the reciprocal of $t_{0.2}$, the time required for the reduction of a definite fraction of the DPN, than to the amount of DPN reduced in a fixed time ($E_{340}$ in 3 min) measured in the earlier method [3], provided that the enzyme is stable in the test mixture [5]. This was found to be the case, as shown in Fig. 1 by a plot of measured values of $1/t_{0.2}$ against the weight of enzyme used in the test, obtained by adding different volumes (0.9 to 10 $\mu$l) of the same enzyme solution. The plot is a satisfactory straight line passing through the origin, *i.e.* the product, $t_{0.2} \times$ amount of enzyme, is constant to within 2 %. The method has proved consistently reproducible to this extent.

The ethanol concentration (9 mM) is the optimum value [6]; the concentration of 300 mM used originally causes considerable inhibition. Neither the ethanol nor the DPN concentration (0.42 mM) is critical, variations of 50 % in either causing only 10 % variation in the results. Semicarbazide has no effect, as at pH 10.0 the reduction of DPN goes practically to completion with these reactant concentrations [4].

*Specific Activity.* The enzyme used for the assays of Fig. 1 had been recrystallized with ethanol to constant activity/$E_{280}$ ratio. The enzyme was prepared as described by Bonnichsen and Brink [3]; after removal of the haemoglobin, the enzyme

was precipitated twice with 30 % (v/v) ethanol and then recrystallized 5 times with 7–10 % ethanol. The relative values of the activity/$E_{280}$ ratio obtained for the 5 crystalline products were, successively, 0.80, 0.89, 0.97, 1.00, 0.98 and 0.99.

A solution of the final product, after thorough dialysis against 0.01 M phosphate, pH 7.3, was found by dry weight to contain 15.5 mg protein/ml, and was diluted 10 fold for activity and $E_{280}$ measurements. From the latter a value of 0.450 was obtained for the extinction of 1 mg/ml in 1 cm optical depth, in good agreement with Bonnichsen's [2,3] value of 0.455. From the activity measurements (Fig. 1) the relationship between the volume in ml ($v$) of an enzyme solution used in the assay, the value of $t_{0.2}$ in seconds, and the concentration of the enzyme solution is mg/ml $= 1.05/(t_{0.2} \times v)$. If $E_{280}$ is measured on the same solution in 1 cm optical depth, the % purity of the sample relative to the above preparation will be $0.47/(E_{280} \times t_{0.2} \times v)$. These data have been reproduced to within a few per cent with many other crystalline preparations.

In the activity test of Theorell and Bonnichsen [4], our enzyme preparations gave an increase of $E_{340}$ of 0.058 in 3 min per $\mu$g enzyme per ml, which is significantly higher than values previously reported (0.036[4], 0.045[5]). The corresponding value of the maximum initial rate of the reaction of ethanol and DPN at pH 10.0 was $k_2 = 8.0$ sec$^{-1}$, compared with 5.5 sec$^{-1}$ obtained by Theorell and Bonnichsen [4]. In spite of the high activity, evidence of inhomogeneity in the present preparations was obtained in prolonged electrophoresis, indicating the presence of about 10 % of a second component. Further work on the preparation and properties of the enzyme is in progress.

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# The Sedimentation Constant of Crystalline Horse Liver Alcohol Dehydrogenase

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The sedimentation constant of crystalline alcohol dehydrogenase (ADH) prepared from horse liver [1,2] was found by Pedersen to be $S^0_{20} = 4.9$ $S$ as the mean of two runs in the oil driven ultracentrifuge at Uppsala, with *ca.* 10 mg protein/ml at pH 7.8 [3]. This gives 4.5 $S$ after correction as suggested by Pedersen [4]. In connection with the interpretation of marked effects of pH and chloride ion concentration on the kinetics of the enzyme reaction, DPNH + acetaldehyde + H$^+$ $\rightleftharpoons$ DPN + ethanol, reported by Theorell and his collaborators [5,6]. it is of interest to test the possibility that the enzyme itself undergoes some change, such as dissociation or polymerisation, with change of ionic milieu. Such changes might be revealed by variations of the sedimentation constant.

*Table 1.* Sedimentation constants of liver alcohol dehydrogenase.

| ADH mg/ml | NaCl M | Buffer (ionic strength = 0.1) | pH | $S^0_{20}$ Svedberg units |
|---|---|---|---|---|
| 7.8 | 0.075 | phosphate | 7.15 | 4.91 |
| 7.8 | 0.15 | phosphate | 7.15 | 4.90 |
| 7.8 | 0.30 | phosphate | 7.15 | 4.85 |
| 13.3 | 0.075 | phosphate | 7.15 | 4.69 |
| 13.3 | 0.30 | phosphate | 7.15 | 4.71 |
| 7.8 | 0.15 | phosphate | 5.85 | 4.93 |
| 7.8 | 0.15 | glycine | 9.9 | 4.82 |

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