and ^{32}P labelled compounds can also be distinguished in this way as ^{14}C is also a soft beta-emitter (0.155 MeV) and darkens only one side of the film.

The region of the paper chromatogram corresponding to band B on the radiogram was cut out, and eluted with water. The eluate contained fairly pure CoA of high specific activity. Biological experiments can be performed by the addition of carrier CoA.

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Received July 29th, 1955

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The enzymic hydrolysis of *iso*maltose. Its localization by means of paper electrophoresis

The assay for limit dextrinase as reported by BACK and co-workers[1] is long and tedious and any simplification of the method would be advantageous. An ideal substrate would be a simple carbohydrate in which hydrolysis could be measured by an increase in reducing power. STINSON[2] recently reported that *iso*maltose (6-O-α-D-glucopyranosyl-D-glucose) is rapidly hydrolyzed by partially purified limit dextrinase isolated from the culture medium of *Aspergillus niger* NRRL 330, and suggests that this disaccharide might serve as a substrate for limit dextrinase. Though the chemical structure of limit dextrin is not well known, it is recognized that this material does possess D-glucosidic linkages in the 1,6-position[3]. In addition, TSUCHIYA *et al.*[4] reported that *iso*maltose was hydrolyzed by a culture filtrate of *Aspergillus niger*. This note presents a brief report on the localization of the area on an "ionogram" which is responsible for the hydrolysis of *iso*maltose.

Filter-paper electrophoresis experiments[5] were performed on exhaustively dialysed culture filtrates of *Aspergillus niger* P.R.L. 558. The electrophoretic investigations were carried out in acetate buffer (pH 3.5, ionic strength 0.05) on a crude sample containing approximately 7.5 mg of protein. After the completion of a run, the paper strip was cut into 1 cm sections and extracted with 5 ml of pH 4.9 acetate buffer. Protein and enzymic activity were determined for each section.

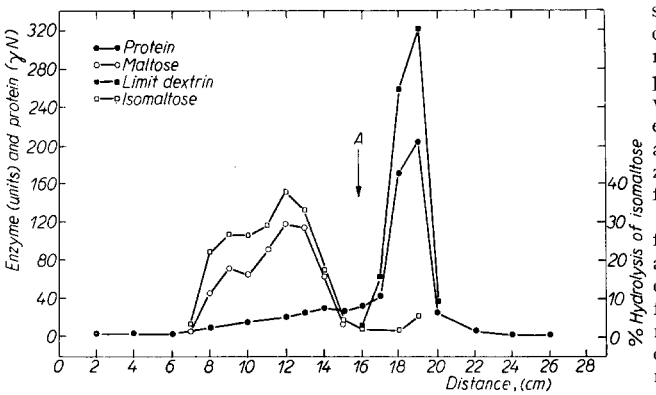

Fig. 1. A plot of the protein and enzymic zones on a paper electrophoresis showing the protein area (●——●) and the zones in which the hydrolysis of maltose (○——○), *iso*maltose (□——□), and limit dextrin (■——■) occurred.

Fig. 1 presents the data for a typical experiment. The arrow "A" represents the point of application after correction for electroosmotic effects. The major fraction is negatively charged at pH 3.5 while the minor ones carry a positive charge. The main protein component contains the limit dextrinase activity with a trace of maltase. From the figure it can be seen that the major portion of the maltase is found in the minor fraction. Though not shown in the figure, all the invertase activity was found in the minor protein component as well.

The *iso*maltose used in this work was prepared from a sample of *iso*maltose octaacetate kindly supplied by Dr. M. L. WOLFROM. The enzyme reaction was conducted at 30° C in acetate buffer (pH 4.6, 0.2 *M*) with a substrate concentration of approximately 0.01 *M*. The extent of hydrolysis was measured by an increase in reducing power after a reaction time of 3 h. The zone in which the hydrolysis of *iso*maltose took place is shown in Fig. 1 as percent hydrolysis. The hydrolysis occurred in the same region as that found for maltose. There is no significant cleavage of *iso*maltose in the region of maximum limit dextrinase activity. From this experiment one can make the following observations: one, *iso*maltose cannot be substituted as a substrate for limit dextrin; two, that maltase may be responsible for the breakdown of *iso*maltose, and three, it might be inferred since there is no hydrolysis of *iso*maltose in the limit dextrinase zone, that in the hydrolysis of limit dextrin by limit dextrinase the 1,6-linkages are not attacked.

The observation that maltase attacks *iso*maltose is not too surprising as the only difference between the two disaccharides concerns the glucosidic linkage which is attached to C_6 of the aglucon group. The α-linkage appears to be important as β-gentiobiose (6-O-β-D-glucopyranosyl-D-glucose) is not hydrolyzed by maltase[6] thus indicating that *iso*maltose is attacked by an α-glucosidase. The possibility that the *iso*maltose breakdown is caused by an enzyme other than maltase has not been eliminated and must await further purification of maltase.

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Issued as N.R.C. No. 3727.

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Received July 29th, 1955