obtained by heart puncture 6 days after the final injection.

Table 1 gives the agglutinative titer of the substrains in sera prepared against the stock strain, while Table 2 shows the results of agglutinin absorption tests performed with samples of serum, each of which had previously been absorbed with one of the substrains.

During the course of the work there was no observable change in cultural or fermentation reactions.

Of the substrains derived from a stock culture apparently stable in agglutinative potency, only those cultivated in the presence of 10% antiserum gave any evidence of change in agglutinogenic property. While the loss in titer was not great, the fact that the parent strain had been maintained for a number of years without change in antigenic characteristics may give reason to believe that further cultivation in the presence of antiserum might produce greater change. This possibility is indicated by the fact that the strain which had gone through 44 generations showed greater loss than the one which was observed through only 7 generations. Should further changes occur during prolonged cultivation the results would confirm the report of Evans (*4*), who attributed the spontaneous appearance of an R variant of a Type III stock strain to the presence of antibody in the human blood used for the medium on which it was cultivated.

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The Inactivation of Invertase by Tyrosinase

IRWIN W. SIZER[1]

Department of Biology,
The Massachusetts Institute of Technology

The problem of the inactivation of an enzyme by the action of oxidases on certain groupings in the enzyme protein was raised by Sizer (*5*), who demonstrated that a fraction of the tyrosyl groups of certain proteins could be oxidized by tyrosinase. In view of the importance of tyrosyl groups for the activity of most biologically active proteins (*4*), it seemed possible that certain enzymes might be oxidatively inactivated by tyrosinase. No effect of tyrosinase on enzyme activity could be demonstrated (*2, 5*), however, for the proteases pepsin, trypsin, and chymotrypsin. Despite these negative results, it seemed wise to investigate further the possible control of one enzyme system by another. In the initiation of this problem we have studied the action of mushroom tyrosinase on yeast invertase.

[1] The author is grateful to Mr. John Fenessey and Miss Janette Robinson for their assistance in this study.

In a typical experiment, 0.5 ml of tyrosinase (Treemond, 3,500 Miller and Dawson units/ml) is incubated with 0.1 ml of diluted invertase[2] (*1*), 0.5 ml of M/20 phosphate buffer, pH 6.0 and 0.5 ml of water plus toluene for 18 hrs at 37° C. A control experiment, run simultaneously, is identical except for the fact that the tyrosinase has been inactivated by boiling. In certain experiments both control and experimental solutions were placed in Cellophane sacs and dialyzed continuously during the reaction against the phosphate buffer. After incubation with tyrosinase the residual activity of the invertase was measured. The samples were diluted to 5 ml with buffer (at 37° C) and at zero time were rapidly mixed with 5 ml of 12% sucrose. One-ml samples were removed at successive time intervals and added to 3 ml of dinitrosalicylic acid reagent for reducing sugars (*6*). The intensity of color developed after heating the solutions in the usual way (*6*) was measured at 575 mμ with the Coleman Universal Spectrophotometer and converted to milligrams of invert sugar. Typical results are presented in Fig. 1, from which it appears that the liberation of invert sugar from sucrose by invertase follows zero-

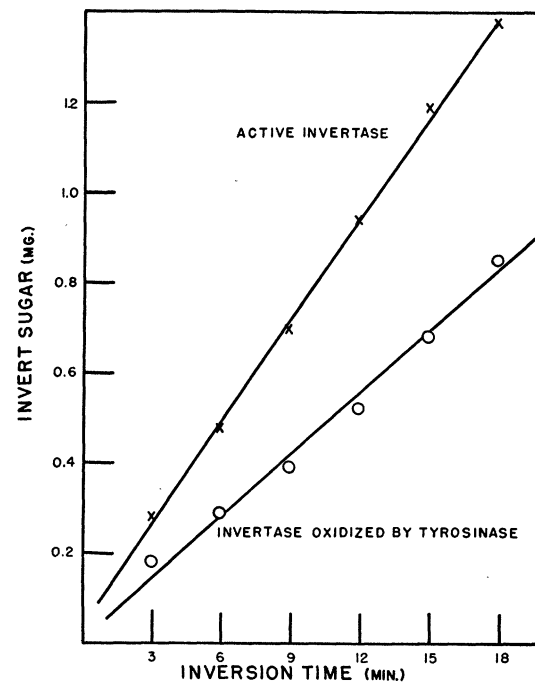

FIG. 1. The formation of invert sugar from 6% sucrose at pH 6.0 at 37° C in the presence of 0.004% Dieu invertase is plotted as a function of the inversion time. The "active invertase" had been treated previously with boiled Treemond tyrosinase for 18 hrs at 37°, while the "invertase oxidized by tyrosinase" had been incubated with active tyrosinase for the same time.

[2] The author is most grateful to Hector Dieu, University of Liège, for a generous sample of high-purity invertase. The invertase solution had a time value of about 0.30 min and contained 0.20 mg of nitrogen and 0.40 mg of carbohydrates/ml. It was diluted 250 times before use.

order kinetics reasonably well during the initial phase of the hydrolysis. Rates are calculated from the slopes of the straight lines fitted to the points and, when compared, show that the invertase oxidized by active tyrosinase has only 57% of the activity of the control invertase which had been treated with boiled tyrosinase.

In more than 100 experiments using purified preparations of tyrosinase the invertase was inactivated 10–40% when compared with the control invertase treated with boiled tyrosinase. While all yeast invertase preparations, whether crude or highly active, can be inactivated by tyrosinase, it is found that not all tyrosinase preparations are effective. Crude tyrosinase does not inactivate invertase, and purified tyrosinase of high "cresolase" activity has little effect, while purified tyrosinase of high "catecholase" activity[3] (*3*) is most effective.

The question of whether the tyrosinase directly inactivates the invertase, or converts by oxidation impurities (or products of protein autolysis) to invertase inhibitors, cannot be satisfactorily answered at this time. Since the phenomenon is equally apparent if continuous dialysis of the solutions of tyrosinase plus invertase occurs during the inactivation, it is clear that the results cannot be accounted for by an action of tyrosinase on dialyzable potential inhibitors of invertase. The fact that invertase in many different stages of purification may be inactivated by tyrosinase makes it more likely that invertase is inactivated directly rather than by a high-molecular-weight impurity, which becomes an inhibitor after the oxidation of its phenolic groups by tyrosinase.

The results might conceivably be explained on the hypothesis that the tyrosinase preparations merely contain an invertase inhibitor which is destroyed by boiling. To test this idea it is necessary to employ some means other than boiling to prevent the action of tyrosinase in the control experiment. This can be done by excluding oxygen from the control by bubbling nitrogen through the control solution and then evacuating it at the water pump. An equally effective method is to bubble continuously through the control solution hydrogen activated with platinized asbestos.[4] Results with both techniques are comparable to those with the usual method using boiled tyrosinase and show that the inactivation of invertase by tyrosinase is not explained by the tyrosinase preparation acting as an inhibitor. These experiments also show the dependence upon oxygen of the inactivation reaction. Experiments are now in progress to correlate the loss in activity of the invertase oxidized by tyrosinase with chemical and physical changes in the invertase molecule.

These preliminary results show that yeast invertase can be partially inactivated by incubation with mushroom tyrosinase. This inactivation is best explained on the basis of an oxidation of essential tyrosyl groups in the invertase molecule by tyrosinase.

[3] Both the "cresolase" and the "catechlase" preparations of tyrosinase were generously supplied by C. R. Dawson, of Columbia University.

[4] This technique is not applicable to highly purified Dieu invertase, which is partially inactivated by activated hydrogen.

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Mammary Carcinoma in Female Rats Fed 2-Acetylaminofluorene[1]

R. W. Engel and D. H. Copeland[2]

Laboratory of Animal Nutrition,
Alabama Agricultural Experiment Station,
Alabama Polytechnic Institute, Auburn

In connection with studies concerning the relation of nutrition to cancer it was observed that mammary tumors developed consistently in from 3 to 6 months in all female rats receiving diets containing .03% 2-acetylaminofluorene. This observation has been confirmed in several experiments, results of which are reported in this paper.

Piebald female rats of the Alabama Experiment Station (AES) strain were used for these studies. The animals were placed on the experimental diets at 23 days of age, at weights of 35 to 50 gm. They were caged individually on screen floors and fed daily *ad libitum*.

The composition of the diets used in these studies is given in Table 1. Two dietary modifications—omission of choline and the addition of iodinated casein—were tried, but these did not influence the results, as can be seen from the summary data in Table 2.

A period of about 16 weeks was required for the animals to attain a body weight of approximately 200 gm. This amount of gain is normally made in 6–8 weeks when these diets are fed without carcinogen.

Twenty-four of the 25 female rats fed the carcinogenic diets developed palpably detectable mammary tumors between the 95th and 181st day of the experiment. One animal died of unknown causes during the third month of the experiment, at which time no tumors were detectable in any of the animals. The animals were killed and autopsies were made, usually within one or two weeks

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[2] The authors are indebted to H. L. Stewart, senior pathologist, National Cancer Institute, for assistance in the interpretation of the microscopic material.