sample from the prolonged digestion of sinigrin by myrosin in the presence of ascorbic acid at pH 3.0 gave a positive modified nitrile test, whereas a positive test for neither nitrile nor sinigrin was observed with a sample from a similar enzyme digestion conducted at pH 5.8.

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Further Observations on the Immunologic Reactions of the Old Yellow Enzyme

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The OYE* and its apoprotein were found to react identically with specific antibodies against the enzyme in agar gel diffusion experiments [1]. The antiserum inhibited the enzymatic activity [2], but no influence was noticed upon the association reaction between the apoprotein and the coenzyme FMN which was studied by fluorimetric technique. The inhibition was non-competitive with regard to the substrate TPNH. These results indicate that the antibodies are bound to other parts of the enzyme molecule than the coenzyme or the substrate.

Atabrine and promazines have been found to inhibit the activity of the OYE after preincubation with the apoprotein [3]. Fluorimetric studies showed that the inhibitors did not prevent the binding of FMN to the apoprotein but slowed down the rate of this reaction. It is suggested that the inhibitors are bound to the apoprotein molecule close to the sites of the FMN. The two different types of inhibitors, drugs and antibodies, thus seemed to exert their effect on different parts of the enzyme molecule. The inhibition studies did not allow any conclusions, however, as to whether the drugs are exclusively bound to sites where they interfere with the coupling of the coenzyme, or whether they affect other parts of the enzyme molecule as well.

In the present work the inhibition of the OYE by anti-serum has been studied at different FMN concentrations. The quantitative precipitation reactions of the OYE and its apoprotein with antibodies, and the influence of atabrine upon these reactions, has been investigated. The effect of the promazines could not be studied, since it was found that precipitation occurred upon mixing promazine or chlorpromazine with rabbit serum.

Materials and methods. The OYE used had been recrystallized once, and the apoprotein was obtained from the same preparation. Immunization with OYE and collection of serum was carried out as described before [1]. The manometric technique used for the inhibition studies has already been described in detail [3]. Quantitative precipitin reactions were performed after the serum had been inactivated at +56°C for 20 min. Varying amounts of antigen in 0.3 ml 0.15 M sodium phosphate buffer pH 7.4 were added to 0.25 ml serum and incubation performed at +37°C for 30 min, followed by 24 h at +4°. After being washed several times in buffer and distilled water, the precipitates were dissolved in 0.3 ml 1 N NaOH and the protein content determined according to Lowry *et al.* [4] The absorption values were compared to those of a bovine serum albumin (Armour) standard. In some experiments antigen solutions containing 0.25 mg OYE or apoprotein per ml were incubated with 2×10^{-4} M atabrine at +37°C for 30 min before being added to the serum. The concentration of atabrine in the antigen-serum mixture was 1.1×10^{-4} M.

* Abbreviations: OYE = old yellow enzyme, FMN = flavin mononucleotide, TPNH = reduced triphosphopyridine nucleotide.

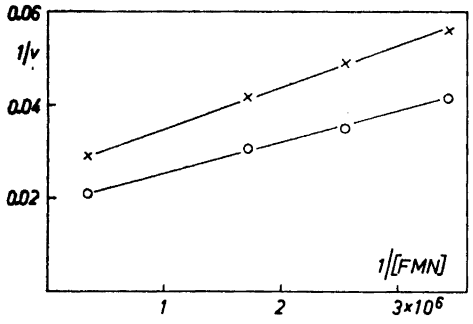

Fig. 1. The inhibition of the OYE activity by antiserum at different concentrations of FMN, measured by Warburg technique. Along the ordinate $1/v$ is marked, where v means the oxygen uptake during 30 min. The total volume of the reaction mixture was 3.0 ml, containing 1.9×10^{-2} M glucose-6-phosphate, 1 Kornberg Unit of Zwischenferment, 4.5×10^{-4} M TPN, 2×10^{-7} M apoprotein, 2.7×10^{-3} M KCN, 0.13 M sodium phosphate buffer pH 7.4, and varying concentrations of FMN, which are marked along the abscissa. Points marked × indicate experiments where the reaction mixture contained 0.5 ml rabbit anti-OYE serum, and points marked O experiments where 0.5 ml normal rabbit serum was added. The reactions were started by introducing the apoprotein from the sidearm.

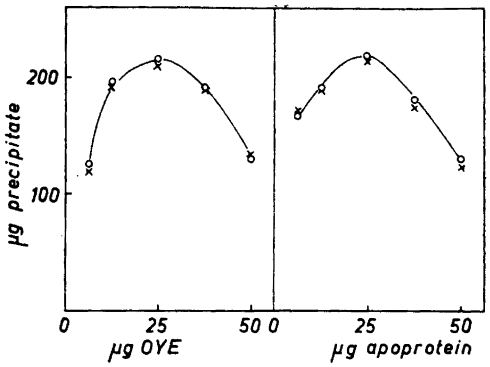

Fig. 2. Two experiments where the influence of atabrine on the quantitative precipitin reaction between the OYE and antiserum was studied. To the left is an experiment where the holoenzyme was used as antigen. O marks results obtained in the absence of atabrine, and × results obtained in the presence of 1.1×10^{-4} M atabrine. To the right is a similar experiment with the same marking, where the antigen used was the apoprotein of the OYE. The same serum, obtained after immunization with OYE, was used in both experiments.

Fig. 1 shows a non-competitive inhibition of the OYE by antiserum, when the concentration of FMN was varied in the reaction system. Essentially identical results were obtained in the quantitative precipitin reactions when either holoenzyme or apoprotein was used as antigen (Fig. 2). These results thus confirm previous conclusions that the antibodies and FMN are bound to different parts of the molecule. Fig. 2 also shows that the presence of atabrine did not disturb the reaction of the enzyme with antibodies. Atabrine was only slightly soluble in the reagent used for the protein analysis, and tests using a saturated solution showed no influence on the readings of the standard. The failure of atabrine to influence the reaction between enzyme and antibody supports the conclusions of the inhibition studies that different binding sites are concerned in the reactions of the drug and the antibodies with the enzyme.

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