mate dehydrogenase, while inhibition of decarboxylase and transaminases was insignificant. Thus, recognition of phosphonic and phosphinic analogues as being similar to glutamic acid might be a peculiar property of glutamine synthesizing enzymes. This might be due to the similarity of inhibitors to the intermediate γ-glutamyl phosphate.

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Effect of cholinesterase on the chemiluminescence of luminol

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Summary. The chemiluminescent oxidation of luminol was activated by cholinesterase. Physostigmine and dimethoate inhibited the luminescence, TEA had no effect. These results indicate that the esteratic site is responsible for the activating action of the enzyme.

The chemiluminescent oxidation of luminol with hydrogen peroxide in aqueous alkaline solution occurs to a significant extent only in the presence of an 'activating agent'[1]. The activating action of organophosphorous cholinesterase (ChE) inhibitors on the oxidation of luminol was first described by Goldenson[2]. Weber and co-workers have extensively studied the activating action of DFP, tabun, sarin[3] and various organophosphorous insecticides[4,5]. In our previous papers, we reported that cholinomimetic agents (acetylcholine and pilocarpine) also activate the oxidation of luminol[6–8].

This study was undertaken to determine whether ChE exerts any action on the luminescence of luminol, and if so, to examine the interaction between the enzyme and its inhibitors.

Material and methods. Luminescence intensity was recorded as a function of time with a luminophotometer described by Weber[9]. Maximum luminescence intensity (Φ_m) was plotted against the concentration of the reagent investigated. All experiments were performed with a reaction mixture containing 0.4 mM luminol, 45 mM NaOH and various concentrations of H_2O_2. The total volume of reaction mixture was 50 ml, the pH 12.2 and the temperature 20 °C. Pseudocholinesterase (4 units/mg) was obtained from Calbiochem, and luminol from Koch-Light. All other reagents were of analytical grade.

Results and discussion. Upon addition of ChE to the reaction mixture an intense luminescence was immediately evident, indicating that the enzyme activates the oxidation of luminol. A linear relationship exists between maximum luminescence intensity and enzyme concentration. The intensity of light produced is markedly affected by the amount of H_2O_2. Figure 1 shows the effect of H_2O_2 concentration on maximum luminescence intensity in the

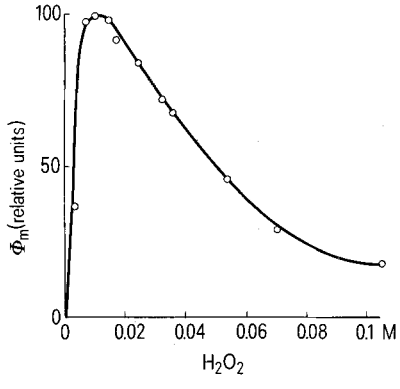

Fig. 1. Maximum luminescence intensity vs H_2O_2 concentration. Activator: ChE 0.04 mg%. Conditions: $4 \cdot 10^{-4}$ M luminol, reaction pH 12.2.

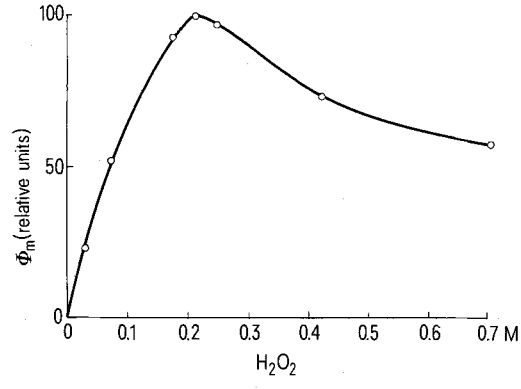

Fig. 2. Maximum luminescence intensity vs H_2O_2 concentration. Activator: dimethoate 0.1 mM. Conditions: $4 \cdot 10^{-4}$ M luminol, reaction pH 12.2.

presence of 0.04 mg% ChE. Maximum effect is obtained with 0.01 M H_2O_2, while further increases in H_2O_2 concentration cause a steady decrease of maximum luminescence intensity. The effects of 3 ChE inhibitors, tetraethylammonium iodide (TEA), physostigmine sulphate and an organophosphorous insecticide, dimethoate, have been studied. The enzyme was incubated with the inhibitor for 5 min prior to addition to the reaction mixture. In the experiments with TEA and physostigmine the concentration of ChE was 0.02 mg% and of H_2O_2 0.007 M. No significant change in light emission was observed with the addition of TEA to ChE. The maximal concentration of TEA investigated was 0.01 M. The addition of physostigmine to ChE markedly inhibited luminescence. The molar concentration of the inhibitor which inhibited the maximum luminescence intensity by 50% (I_{50}) was $4 \cdot 10^{-3}$ mM of physostigmine.

Dimethoate itself activates the oxidation of luminol[5]. Before investigating the interaction of this compound and ChE the effect of H_2O_2 concentration in the presence of dimethoate as the activator was determined. Figure 2 shows that dimethoate requires higher concentrations of H_2O_2 than ChE, to exert its activating action. Experiments with the combination of ChE and dimethoate as the activator were carried out with 2 different concentrations of H_2O_2. With 0.007 M H_2O_2 in the reaction mixture ChE is maximally effective, while dimethoate is completely ineffective as activator. The addition of dimethoate to ChE results in an inhibitory effect of the organophosphorous agent, with $I_{50} = 0.018$ mM. The curve of inhibition is shown in figure 3. With 0.176 M H_2O_2 in the reaction mixture dimethoate is maximally effective, while the action of ChE is negligible. When various concentrations (up to 0.16 mg%) of ChE were added to dimethoate, there was only a slight increase of luminescence intensity, but no inhibition.

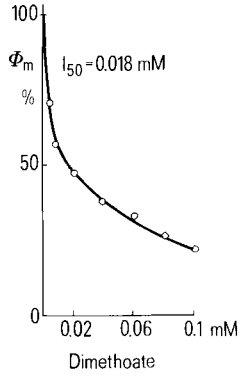

Fig. 3. Inhibition of luminescence by dimethoate. Activator: ChE 0.02 mg%. Conditions: 0.007 M H_2O_2, $4 \cdot 10^{-4}$ M luminol, reaction pH 12.2.

The present results show that ChE activates the oxidation of luminol with H_2O_2. The part of the enzyme molecule involved in this reaction could be deduced by examining the effect of the various inhibitors. It is well known that TEA combines with ChE only at the anionic site of the enzyme, physostigmine is attached to the enzyme at both the anionic and esteratic sites, while the reaction between ChE and the organophosphorous inhibitors which do not possess a quaternary nitrogen, such as dimethoate, occurs only at the esteratic site. Since physostigmine and dimethoate, and not TEA, had inhibitory action on the chemiluminescent activated by ChE, it could be suggested that the esteratic site is involved in the activating action of the enzyme.

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Selection at the Adh locus in *Drosophila melanogaster:* Adult survivorship-mortality in response to ethanol[1]

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Summary. Functionally significant biochemical properties of the naturally occurring electrophoretic variants at the Adh locus (ADH^{Fast} and ADH^{Slow}) are correlated with the adult flies' ability to utilize and survive in an ethanol environment. The results are consistent with the idea that an environmentally dependent form of balancing selection is responsible, at least in part, for the maintenance of the polymorphism at this locus.

The application of biochemical techniques to population genetics has provided a means of addressing the neutralist-selectionist controversy by directly examining the functional properties of the gene products in question. This approach has been applied to many gene-enzyme systems in a variety of organisms with varying degrees of success[3]. One such example is the alcohol dehydrogenase system of *Drosophila melanogaster.* A number of biochemical properties of the 2 major electrophoretic variants, ADH^{Fast} and ADH^{Slow}, have been examined by a large number of researchers in an attempt to understand, in a functional sense, the polymorphic nature of this locus. Description of the variation in temperature stability[4,5], enzyme activity[6], enzyme concentration[7], and kinetic parameters[8] have been used to establish a correlation between these functional properties of the gene products and organismal phenomena, life stage mortality[9,10], the existence of gene frequency clines[11] etc. We report here, a study in which there exists a direct correlation between properties of the alcohol dehydrogenase gene products in *D. melanogaster* and adult flies ability to utilize and survive in an alcohol environment.

Materials and methods. Adult survivorship-mortality studies