without oxime, while curves 2–8 illustrate the effect of increasing concentrations of pyridine-4-aldoxime methiodide. In all these experiments the luminol reaction was catalysed by hæmoglobin. The results clearly demonstrate that the maximal intensities of chemi-luminescence as well as the total amount of light (area under each curve) were reduced when increasing amounts of oxime were used.

Essentially the same results were obtained when other oximes (inhibitors) and other catalysts of luminol reaction were applied. Table 1 shows molar concentrations of various oximes which decrease the chemi-luminescence to a value of 50 per cent at various concentrations of hæmoglobin. It is evident that oximes inhibit the luminol reaction effectively.

These findings present not only an additional proof for the mechanism of reactivation of cholinesterase with oximes, but also the method described in this communication provides a means for a simple evaluation of oximes as reactivators of cholinesterase.

Further details of these and other experiments on related subjects will be published elsewhere.

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Inactivation and Reactivation of Chymotrypsin

In previous work on the immediate effects of acylation of trypsin it was shown[1] that the enzyme can be obtained in a fully inactive form by acylation with various anhydrides at *p*H 8–9, and that partial instantaneous reactivation can be effected by treatment with alkaline hydroxylamine[2].

In an extension of this work it was found that chymotrypsin behaves similarly to trypsin on acylation at higher *p*H values, complete inactivation occurring at *p*H 8. Even though a reactivation was observed on incubation, it was almost negligible in the course of 2 hr. The acylations were carried out using an automatic titration unit (Polarad Electronics Corp.), at various constant *p*H values. In Table 1 are recorded the percentages of inactivation and reactivation in terms of proteolytic activity obtained on acetylation at 0–5° C. at different *p*H values, whereby 60 equiv. of acetic anhydride were used per mole of enzyme. The activity was assayed at *p*H 7·8, using the usual hæmoglobin substrate. Any inactivation rapidly reversed at that *p*H [3,4] could accordingly not have been observed.

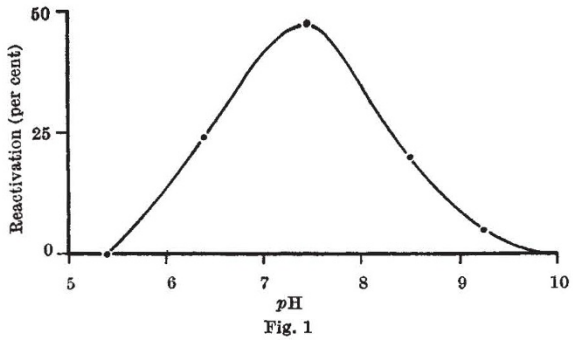

Fig. 1

Table 1. Inactivation of Chymotrypsin at Different *p*H Values

Time (min.)	Activity (per cent) 5	6	7	8
0	100	100	100	100
8	65	56	31	0
20	64	58	35	0
120	67	60	38	0

Butyric and propionic anhydrides gave similar results. As observed in the case of trypsin, citraconic anhydride gave rise to an inactivation amounting to only about 50 per cent.

Even though the spontaneous reactivation of acetylchymotrypsin is much slower than in the case of acetyltrypsin, an instantaneous reactivation was obtained on treatment with hydroxylamine. In Fig. 1 is shown the extent of reactivation recorded at various *p*H values on treatment of the inactivated chymotrypsin for a period of 8 min. with 4 equiv. of hydroxylamine per equivalent of anhydride added. It can be seen that maximum reactivation takes place in the region of *p*H 7·4–7·8. The reactivation bears a striking resemblance to the reaction of inactive acetyltrypsin with hydroxylamine[2] except that the *p*H optimum of reactivation is shifted to lower *p*H values by about one *p*H unit.

The inactive monoacetyl chymotrypsin obtained by other investigators[3,4] was reactive with hydroxylamine in the region of *p*H 5–6. In reaction with nitrophenylacetate there is also a great similarity between trypsin and chymotrypsin and the monoacetyl enzymes obtained were more stable at *p*H 5–6, easily undergoing hydrolysis at *p*H 7–8. However, in the case of the inactive acetyl derivatives obtained at *p*H 8, it was found that they were more stable at *p*H 7–8 than at *p*H 5–6. The hypothesis[2] that there are two susceptible sites in the active centre of trypsin, acetylated at *p*H 5 and *p*H 8 respectively, appears to be supported in the case of chymotrypsin. The small deviations in the *p*H optima of reactivation with hydroxylamine could be attributed to secondary differences between the two enzymes.

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Activity of Glutamic Acid Decarboxylase in Insect Nerve Tissue

As compared with the nerve tissue of mammals, that of insects is known to be particularly rich in acetylcholinesterase[1] and in choline-acetylase[2]. On the other hand, nothing has been reported so far about the occurrence in insects of another enzyme which, in mammals, is characteristic of nerve tissue, namely, glutamic acid decarboxylase. Its reaction