the activity continued to rise linearly. In addition, ADP could not replace P-P as an activator, nor could ATP act as its own activator.

The upper curve (△) of Fig. 1 illustrates the results of adding P-P to the EDTA-NH_4^+ system: P-P did not increase the activation, but at higher concentration prevented maximal activation. The two curves approach each other at approximately 0.04 *M* P-P.

In the EDTA-NH_4^+ system containing no P-P, the maximum velocity was obtained with 0.008 *M* ATP. Fig. 2 shows the effect of ATP or inorganic pyrophosphate added to the optimal system. It is clearly seen that the substrate inhibition arising from over optimal amounts of ATP is exactly the same as that obtained by addition of equivalent quantities of P-P. ADP did not show this effect. Inhibition by ATP was not detectable in the P-P-NH_4Cl system lacking EDTA.

No simple explanation of the effects recorded above can be offered. At present, the effects of other triphosphorylated nucleotides and other chelating agents are being investigated; and all of the agents are being further studied using H-meromyosin as the enzyme.

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Esterification of protein and amino acid carboxyl groups by mustard gas and related compounds

From chemical considerations mustard gas and related sulphur compounds (S-mustards) can react with any of the polar groups occurring in proteins and amino acids provided these groups are present in the necessary ionic state. At physiological pH values the carboxyl groups of proteins and amino acids would be in the ionised state and thus would be able to combine with these compounds. Although esterification of the anions of various organic acids by S-mustards and some of the related nitrogen compounds (N-mustards) has been conclusively demonstrated[1,2], evidence for this reaction with protein carboxyl groups is indirect in nature, being obtained either from changes in the titration curves of proteins following treatment with S-mustards[3,4] or from examination of the pH stability of bonds formed between S-mustards and proteins[5,6].

The nucleophilic reagent, hydroxylamine, reacts very readily with esters to liberate an alcohol molecule and form a hydroxamic acid which can be determined by the coloured complex it forms with Fe^{+++} ions. The possibility of using this reagent to follow O-alkylation reactions of S-mustards with protein and amino acid carboxyl groups has therefore been investigated.

Hide powder collagen was treated with radioactive (^{35}S) di-(2-chloroethyl) sulphide (H) essentially as described by PIRIE[5]. The treated collagen was exhaustively Soxhlet-extracted with acetone and ether to remove any unbound radioactive material. Samples of the treated collagen were then incubated for 24 h at pH 7.5 and 38° in the presence of 1.2 *M* hydroxylamine. This treatment resulted in the loss of 56% of the bound H as determined by radioactivity measurements whereas incubation under the same conditions but in the absence of hydroxylamine resulted in the liberation of only 3% of the bound H. When incubation was carried out at pH 11.9, however, 60% of the bound H was liberated in the absence of hydroxylamine. There is thus close agreement between the bound H which is alkali-labile and that which can be liberated by hydroxylamine treatment, as would be expected if hydroxylamine were attacking ester-type linkages between H and collagen.

A further series of experiments was carried out using glycine and several S-mustards. 2 mmoles mustard were allowed to react with 240 mmoles glycine at room temperature in 200 ml water. The pH was maintained at 6.0 to avoid the possibility of any N-alkylation reactions. The large excess of glycine was employed in an attempt to eliminate loss of the reagents by

hydrolysis[7]. Aliquots of the reaction mixtures were removed at intervals, treated with hydroxylamine and the Fe^{+++} ion complex of the resulting hydroxamic acid formed as described by HESTRIN[8]. The absorption spectra of the complexes corresponded to that obtained with glycine hydroxamic acid and the intensity of the colour, measured at 500 mμ, was found to obey Beer's law. The amount of hydroxamic acid formed by treating the aliquots could therefore be determined from a calibration curve prepared from a standard solution of glycine hydroxamic acid and from this value the amount of ester formed by the reagent could be calculated. With ethyl 2-bromoethyl sulphide, benzyl 2-bromoethyl sulphide and H, the amount of esterification was found to increase with time to a maximum and to remain constant thereafter. The maximum amount of esterification produced by these reagents is shown in Table I. A similar experiment was carried out with the difunctional N-mustard di-(2-chloroethyl) methylamine and it was found that the rate of ester formation was much slower and the maximum amount formed much less than with the S-mustards. The maximum amount of esterification observed was only 27% of that produced by H under similar conditions. The ester formed with this N-mustard seemed much more labile since the amount of ester fell off rapidly after reaching a maximum, a finding which is of interest in view of the failure of FRUTON, STEIN AND BERGMANN[9] to demonstrate ester formation between sodium acetate and this mustard.

TABLE I

ESTERIFICATION OF GLYCINE BY VARIOUS MUSTARDS

Reagent	*pH of reaction*	*Maximum esterification observed: moles ester produced / moles mustard added*
Ethyl 2-bromoethyl sulphide	6.0	0.91
Benzyl 2-bromoethyl sulphide	6.0	1.02
Di-(2-chloroethyl) sulphide	6.0	1.98
Di-(2-chloroethyl) sulphide	7.5	1.51
Di-(2-chloroethyl) methylamine	6.0	0.54

From the results in Table I it would appear that at pH 6.0, one mole of each of the unifunctional S-mustards had reacted with one mole of glycine whereas one mole of H had reacted with two of glycine. One experiment was carried out to study the amount of ester produced between H and glycine at pH 7.5. In this instance the molar ratio of ester formed to H originally present was 1.51, suggesting that at physiological pH values the main reaction between H and glycine is esterification.

These results confirm that S-mustards can react with the carboxyl groups of proteins. The hypothesis has been put forward that mustards and other radiomimetic compounds bring about their biological effects by cross-linking genetic material[10]. The findings obtained with H are therefore of interest as they suggest that one way in which this agent may form cross-links is by diester formation with two neighbouring carboxyl groups.

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