tions. These experiments have shown that at least one factor (Factor V) is not a zymogen, and that it behaves as an accelerator for the enzymatic conversion of prothrombin by activated Factor X. The chemistry of the conversion was described in detail by S. Magnusson (University of Cambridge). He showed that prothrombin, which is a protein composed of a single chain, was converted into a smaller molecule, thrombin, which possesses two chains of unequal length. These two chains were joined through a disulphide bridge. Hence the conversion of this zymogen to its activated form required the fission of two peptide bonds. In blood coagulation, there is apparently no shortage of activators for the various zymogens.

The prorennin-rennin transformation takes place, however, in the absence of an enzyme. B. Foltmann (University of Copenhagen), describing this transformation, made a plea for reverting to the old name of chymosin, rather than rennin, since this would avoid confusing rennin with renin. Dr Foltmann showed that prochymosin is stable under weakly alkaline conditions, and that under more acid conditions at pH 5 it is converted into chymosin. This is accompanied by the release of a peptide. The kinetics of the conversion at this pH show that the reaction was partially autocatalytic. Under more acid conditions of pH 3 the conversion is very rapid and there is no suggestion of autocatalysis. To explain this it is suggested that zymogen is stabilized in its inactive form by electrostatic interactions. Under acidic conditions when the acidic side chains are discharged, the zymogen rearranges into an active configuration, which subsequently undergoes irreversible activation on the loss of the peptides.

The milk clotting activity of chymosin was shown to be due to a limited proteolysis of the κ fraction of casein, during which a Phe-Met bond is hydrolysed. This results in the release of the hydrophobic moiety of κ -casein, which then aggregates.

The fourth speaker, Professor Werle (Munich), sounded a note of caution when he described the diverse results obtained on the specificity of the kallikreins prepared from different sources. The kallikreins form a part of sequences of enzymes similar to the coagulation system and both the sequences have a common starting point, namely Factor XII. The kallikreins are responsible for the enzyme cleavage of kininogen to give the pharmacologically active kinins.

The kallikreins, like trypsin, are serine proteases, which are readily inhibited by heavy metals. Kinetic studies with synthetic substrates, such as the arginine esters of different alcohols, showed that the rate limiting step in these reactions was the acylation reaction rather than the deacylation reaction. Professor Werle proposed that the kallikrein catalysis commences with a nucleophilic attack of the substrate.

PHOTO-OXIDATION

Photons, Protons and Proteins

from our Molecular Biology Correspondent

REAGENTS specific for particular groups in proteins have all too often proved a mirage. Dye-sensitized photo-oxidation with the aim of destroying particular residues in enzymes has been attempted in many

laboratories, but has always seemed a blunt instrument which might be expected to annihilate various side chains in a fairly indiscriminate manner. This has now been shown to be by no means inevitable, and two papers have appeared which show that in the right conditions a remarkable precision of aim can be achieved.

Jori, Galiazzo and Scoffone (*Biochemistry*, **8**, 2868; 1969) have followed up their earlier work, which showed that photo-oxidative attack, sensitized by dyes, could be essentially confined to methionine if the reaction was carried out in an acetic acid solvent. The necessity for such denaturing conditions is clearly a drawback, but this has now been overcome. Porphyrins are well known sensitizers, which are thought to act through their triplet states. Under the action of chlorophyll, Jori *et al.* find that tryptophan, tyrosine, histidine and methionine are all rapidly oxidized at pH 6. With other porphyrins the reaction is slower and more selective: in the presence of haematoporphyrin photo-oxidation is limited to tryptophan and methionine. The reaction will proceed at a pH of up to 6.5 or in solutions containing acetic acid. Moreover, the reaction of the tryptophan side chain seems to depend on the presence of the α -amino group, for tryptophan peptides are completely resistant. It thus seems that photo-oxidation in these conditions displays a high selectivity for methionine, and this is borne out in the reaction of native lysozyme. Irradiation in the presence of haematoporphyrin leads to a first-order loss of some 50 per cent of the activity, after which there is no further reaction. Amino-acid analysis shows that one of the two methionine residues has been converted to the sulfoxide. By cleavage of the chain and identification of the fragments, the reactive residue was identified as met-12, which occurs at the surface of the protein. Met-105, on the other hand, is inaccessible, but when the reaction is carried out in 80 per cent acetic acid, the protein unfolds, and both methionines react, with irreversible loss of activity.

A second example of a specific photo-oxidation involves the novel strategy of using a protein-bound sensitizer as a means of directing the reaction towards the required target. Rippa and Pontremoli (*Arch. Biochem.*, **103**, 112; 1969) have studied the enzyme 6-phosphogluconate dehydrogenase, using the chromophore of pyridoxal phosphate as sensitizer. This molecule, which contains an aldehyde function, binds to some amino groups of proteins, and three molecules in all will attach themselves to the enzyme in question. Two of them bind to essential lysine residues of the active site (or sites, for there is evidence that there may be two subunits), with complete loss of activity. If the substrate is present these two lysines are protected, and do not react. When the pyridoxal phosphate is attached to the active site, irradiation leads to loss of activity, which is revealed on removal of the pyridoxal. The only amino-acids to be oxidized are two histidines and three cysteines, but the latter are invulnerable to attack if they are first blocked with a thiol reagent. When the pyridoxal chromophore is added to the solution, but not bound to the active site, there is no photochemical reaction. It thus seems that excitation of the pyridoxal group leads to photo-oxidation of the histidine in its immediate vicinity in the active site, and it therefore functions as a kind of molecular lightning rod.