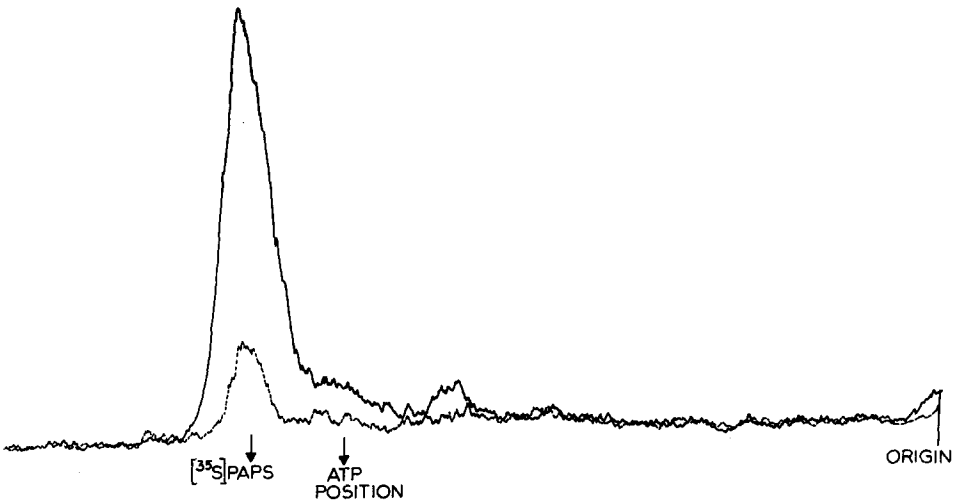

Fig. 1. Influence of chondroitin on PAPS formation. Scan of paper electrophoretogram of nucleotide fraction from incubation as in Expt. 1 of Table I. Electrophoresis was carried out in 0.025 *M* citrate buffer, pH 5.5, at 2.7 V/cm for 16 h. ——— Control; --------- Addition of 2 μmoles repeating disaccharide unit chondroitin.

These results indicate that it is likely that sulphation takes place at some stage in the biosynthesis of CSA before the formation of the final polysaccharide molecule. Further work is proceeding to try and determine at which stage sulphation occurs.

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The effect of residual peroxide on enzymic digestion of oxidized papain

In connection with studies of the amino acid sequence of papain it has been repeatedly observed that the protein after oxidation with performic acid exhibits considerable variability in its susceptibility to trypsin. In fact, one preparation of oxidized papain was found to be completely resistant to trypsin, chymotrypsin and even activated papain. This relative or complete resistance to proteolysis is now known to be caused by residual peroxide in the oxidized protein. Inasmuch as oxidation followed by

proteolytic digestion is now an important standard procedure in the study of protein structure, we wish to report two methods of removing residual peroxide.

SANGER[1] used performic acid oxidation for cleavage of the disulfide bonds of insulin and since that time others[2-4] have studied the oxidative reaction with proteins and polypeptides. All have stressed the importance of removing peroxide. THOMPSON[2] demonstrated that the presence of chloride during the oxidation or of peroxide during subsequent HCl hydrolysis resulted in formation of 3-chlorotyrosine and 3,5-dichlorotyrosine. According to THOMPSON, peroxide could be removed best by repeated freeze-drying. Similar observations were made by MUELLER *et al.*[3] and by HIRS[4]. In these cases the oxidized materials were water soluble. Oxidized papain is almost completely insoluble in aqueous media below pH 10[5].

Crystalline papain or mercuripapain in 88% formic acid is oxidized at 0° to −5° with preformed performic acid[4]. After 2–3 h, the reaction mixture is diluted with 10 times its volume of deionized water and freeze-dried 3 times. After the first drying the protein is highly insoluble and very difficult to wet. Consequently, it is suspended in water for the next two dryings. The oxidized protein is homogenized in a Potter-Elvejhem homogenizer in order to prepare a uniform suspension. Crystalline trypsin is added to this suspension and digestion is allowed to proceed at room temperature in a pH stat regulated at pH 7.8. The amount of 0.2 *N* NaOH necessary to maintain the pH at this value gives an estimate of the rate of proteolysis. The rate at which trypsin or chymotrypsin will digest different preparations of oxidized papain has been variable although the method of oxidation and freeze-drying was the same in all cases. As noted above, one preparation was completely resistant to proteolysis. In exploring possible reasons for this resistance it was found that the preparation contained residual peroxide[6]. Pretreatment of a suspension of this particular preparation with a minute amount of catalase at pH 7 for 30 min, prior to addition of trypsin, rendered the protein susceptible to proteolysis. When this experiment was repeated with other preparations of oxidized papain, it was found that the rate of tryptic digestion was always greater if the protein was pretreated with catalase. A typical result with a preparation of oxidized papain which had been dried 3 times is shown in Fig. 1.

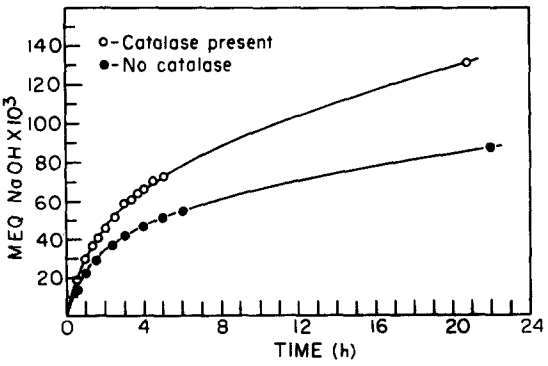

Fig. 1. The effect of catalase on the rate of digestion of oxidized papain by trypsin. A suspension of 500 mg oxidized papain in 100 ml water was digested with 2.5 mg crystalline trypsin at pH 7.8 in a pH stat. A duplicate suspension was pretreated for 30 min with 1 mg crystalline catalase before addition of trypsin. The rate of uptake of 0.2 *N* NaOH is given as a measure of the rate of proteolysis.

It is apparent from the data that repeated freeze-drying does not remove all H_2O_2 from oxidized papain. In all probability the insolubility of this protein after freeze-drying is responsible.

Further experience has shown that precipitation with trichloroacetic acid[7] can

be substituted for the catalase treatment. The protein is precipitated from the oxidation mixture by addition of an equal volume of 20% trichloroacetic acid. The precipitate is removed by centrifugation and washed 4 times with 10% trichloroacetic acid, followed by 2 washes with absolute alcohol and 2 washes with ether. The final product is air-dried at room temperature. Oxidized papain which is treated in this manner gives a negative test for peroxide and is digested as rapidly by trypsin as the catalase-treated preparations.

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