several weeks, and a mixture of insoluble enzyme with cellulose powder could be used to digest proteins in a chromatographic column. Fig. 1 shows how such a column gave a constant degradation of a casein solution, which was slowly flowing through the column for a period of 10 h. The insoluble enzyme derivatives were observed to degrade casein, clupein, gelatine, *N*-benzoyltyrosine ethyl ester and toluene sulphonyl-L-arginine methyl ester with relative rates of splitting similar to the relative rates obtained with the unmodified subtilisin.

Amino acid analysis, performed after 24 h of hydrolysis with 6 M HCl at 110°, indicated the content of all amino acid residues to be unchanged except that of lysine (Table II). The content of this amino acid was decreased from 11 residues per mole in unmodified enzyme to 7 residues per mole after reaction with glutaraldehyde, suggesting that the insolubility was due to the reaction between glutaraldehyde and the amino groups of lysine, in this way introducing cross-links between the molecules.

The retention of only around 15% of the original enzymatic activity might perhaps be explained by the relatively smaller surface area which the polymerized molecules have available for contact with the solvent containing the substrate molecules. The insoluble enzyme particles represented a variety of particle sizes, as seen from a striation in the precipitates during the centrifugation used in the purification. Further experiments are required before uniform preparations of insoluble enzymes can be prepared by the glutaraldehyde method.

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Resistance of alkaline phosphatase of human serum to the protease of *Streptomyces griseus*

ARFORS, BECKMAN AND LUNDIN[1] described a genetically controlled variation of the alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) present in human blood serum. Type 1 phosphatase appears in starch-gel electrophoresis as a single zone of activity. The electrophoretic mobility of this enzyme is decreased after incubation with neuraminidase (mucopolysaccharide *N*-acetylneuraminylhydrolase, EC 3.2.1.18)[2]. Type 2 phosphatase exhibits an additional zone of activity which is resistant to neuraminidase[2]. The evidence which indicates that the latter phosphatase component is derived from the intestine has been recently

TABLE I

EFFECT OF *S. griseus* PROTEASE ON HUMAN SERUM ALKALINE PHOSPHATASE ACTIVITY

After incubation for 18 h, the samples were dialyzed for 24 h against two changes of 4 l 5 mM Tris-HCl (pH 8) and then analyzed. The assay reaction mixture contained 0.5 ml 0.01 M glycine–NaOH buffer (pH 10.5) with 0.001 M $MgCl_2$, 0.5 ml 0.012 M *p*-nitrophenyl phosphate ,and 0.1 ml of the sample. After the samples and appropriate blanks were incubated at 37° for 30 min, 10 ml of 0.02 M NaOH was added, and the $A_{410\ m\mu}$ measured. The results were compared with *p*-nitrophenol standards. The alkaline phosphatase activity is expressed as μmoles *p*-nitrophenol formed under the assay conditions.

Sample	*Components (ml) of incubation mixture*				*Alkaline phosphatase activity*
	Serum	*Distilled water*	*1 M Tris–HCl (pH 8.65)*	*Pronase (4 mg/ml) in 1 M Tris–HCl (pH 8.65)*	
A	1	1	---	—	0.046
B	1	—	—	1	0.050
C	1	—	1	—	0.054
D	—	1	—	1	0.003

reviewed[3]. Alkaline phosphatase of swine kidney[4] and undissociated alkaline phosphatase of *Escherichia coli*[5] are known to resist enzymatic proteolysis. It seemed desirable, therefore, to determine whether the two components of alkaline phosphatase of serum would exhibit similar resistance or show differential susceptibility to proteolysis. We have found that both components are resistant to the protease of *Streptomyces griseus*. Because of the potential value of this property of serum phosphatases to

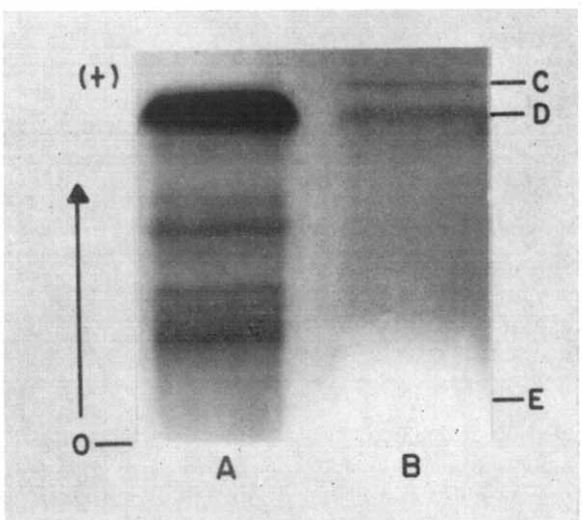

Fig. 1. Starch-gel electrophoresis of control serum (A) and Pronase-treated serum (B). The gel, stained for protein with amido-black, illustrates that in Pronase-treated serum all of the serum proteins except two, one with the migration of a pre-albumin (C), and another with the migration of albumin (D), are absent. E indicates an area of clearing of the gel due to the action of Pronase on the native proteins of the gel. O indicates the origin, and the arrow indicates the direction of electrophoretic migration.

investigators interested in the purification of these enzymes, we report here the results of our experiment.

Pronase (*S. griseus* protease, 45 000 proteolytic units/ml, Calbiochem) was dissolved (4 mg/ml) in 1 M Tris–HCl (pH 8.65). Equal parts of this solution and serum were incubated for 18 h at 37°. The buffer, which is near the optimal pH for the protease, has the capacity to control the pH during proteolysis. Compared with the starting serum, the final solution was lighter in color and slightly turbid.

Assays, performed after the incubation, revealed between 90 and 100% recovery of the phosphatase for both type 1 and type 2 sera. The results of a typical experiment are given in Table I. The digested sera and control sera were also subjected to horizontal starch-gel electrophoresis and stained for protein and for alkaline phosphatase[6]. Only two protein bands remained after the digestion (Fig. 1). The phosphatases, which migrate roughly one-half as far as albumin, are not stained by the protein stain. On the gels stained for phosphatases, they were found to have migrated slightly faster than controls. This increased mobility may be related to alterations in the phosphatases, or changes produced in the gel by the protease[7], or other factors. Since the phosphatase stains do not photograph well in black and white, they are not reproduced here.

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