The protein III contains much more glutamic acid, probably in amide form, and a lesser amount of arginine than α-amylase.

The results described above and the results presented in a previous paper[1] strongly suggest that the protein III is not merely "cross-reacting material"[5–7], but it must be a real α-amylase precursor.

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The use of urea-starch-gel electrophoresis in studies of reductive cleavage of an α_2-macroglobulin

The presence of several protein components of high molecular weight in human serum is well established[1]. There are two major groups of proteins with sedimentation coefficients between 17 S–20 S. One of these groups is comprised of proteins belonging to the γ_1-globulins[2,3]; the other is composed mainly of proteins belonging to the α_2-globulins[4]. It has been postulated that these macromolecules represent polymeric proteins[5,6]. DEUTCH AND MORTON[7] have shown that the addition of mercaptoethanol and cysteine result in a fall in sedimentation rate from 18 S to 7 S and that stable monomeric units are formed in the presence of iodoacetamide.

This report is concerned with the effects of reductive cleavage with mercaptoethanol in urea on the protein of high molecular weight belonging to the α_2-globulins. This protein is variously known as 'heat-labile glycoprotein"[8], the "alpha 2 macroglobulin"[4], and as "slow alpha 2 globulin"[9,10]. SCHONENBERGER *et al.*[11] studied the physicochemical properties of this protein in great detail and described dissociation of the protein into two components (11 S and 15.7 S) in the presence of 5 *M* urea. Reaggregation to a single component of 18.1 S occurred after removal of the urea with and without the presence of iodoacetamide. Comparable results were obtained by ISLIKER[12], who measured the change in viscosity of the protein after the addition of cysteine, cysteamine and thioglycollate. Ultracentrifugal analysis revealed a 6 S component after treatment of the proteins with these components.

A method is described using starch-gel electrophoresis for the separation and

demonstration of products obtained by reductive cleavage with mercaptoethanol in urea and after stabilization of the –SH groups by addition of iodacetamide. Gels for this purpose are conveniently prepared by mixing 60–65 g of Starch Hydrolysed (Connaught Medical Research Laboratories, Toronto, Canada) and 240 g of urea. Small portions of the mixture are then rapidly poured into a flask (2000 ml) containing 300 ml of buffer and vigorously stirred by hand between each addition.

The highly viscous solution is heated in a water bath (70°) with occasional stirring for 10 min. After removal of the trapped air, the gel is poured into a tray, with occasional stirring for 10 min, similar to that used for vertical gel electrophoresis[13] and allowed to settle for 24 h.

A preparation of the α_2-macroglobulin (generous gift of Dr. H. E. SCHULTZE, Behringwerke, Germany) was used in all experiments. Starch-gel electrophoresis in the discontinuous system of buffers[14] disclosed that this preparation contained one major band and a minor less rapidly migrating component. An 0.5 % solution of the protein was prepared in: (a) 0.1 *M* borate buffer, (b) 8 *M* urea (recrystallized) and 0.1 *M* borate buffer, (c) the same as (b) with the addition of mercaptoethanol, final concn. 0.02 *M*, (d) the same as (c) with the addition of iodoacetamide, final concn. 0.05 *M*. The solutions, after standing for 2–3 h at room temperature, were then subjected simultaneously to electrophoresis for 24 h at a voltage gradient of 6 V/cm in starch gels prepared in: (a) borate buffer[9], (b) borate buffer and 8 *M* urea[15], (c) formate buffer (0.05 *M* formic acid, 0.01 *M* NaOH; pH3.1) and 8 *M* urea (SMITHIES, personal communication). After completion of the experiment the gel was stained in the usual manner with Amido Black 10B. The greatest separation was achieved in the formate urea gel as shown in Fig. 1.

Five heavy and two faint bands can be observed in the control (S α_2 dissolved in borate buffer alone) as well as in borate buffer and 8 *M* urea (Fig. 1A and B). The two patterns are similar though pattern 1B shows clearer separation of the bands, probably due to the equilibrium achieved between the urea in the gel and that of the applied material.

Mercaptoethanol-treated material separated into four main bands as shown on Fig. 1C. However, some of the protein did not migrate into the gel as judged by the amount of stained material left at the origin (visible as heavy line on the photograph). The iodoacetamide-treated material was also separated into four bands (Fig. 1D) and four additional minor bands were seen though they are not readily shown by photography. If the reactions are allowed to proceed at 10° for 24 h a similar pattern is obtained with additional separation of the slowest component of the iodoacetamide-treated material. In contrast to the results obtained using mercaptoethanol alone (1C), complete entry of the protein into the gel occurred when iodoacetamide was added (1D). The findings suggest the probability that slow α_2-globulin contains subunits linked, at least in part, by disulfide bonds. Other linkages, however, cannot be excluded[11]. Ultracentrifugal studies on the isolated components are in progress. It was observed that the separated components exhibit a much greater avidity for the Amido Black dye than the original material, owing to the additional groups made accessible to the dye by the opening of side chains. These results indicate that the subunits of the slow α_2-globulin may be successfully separated using the technique of urea-starch-gel electrophoresis. Application of this method appears useful in structural studies on a variety of purified proteins.

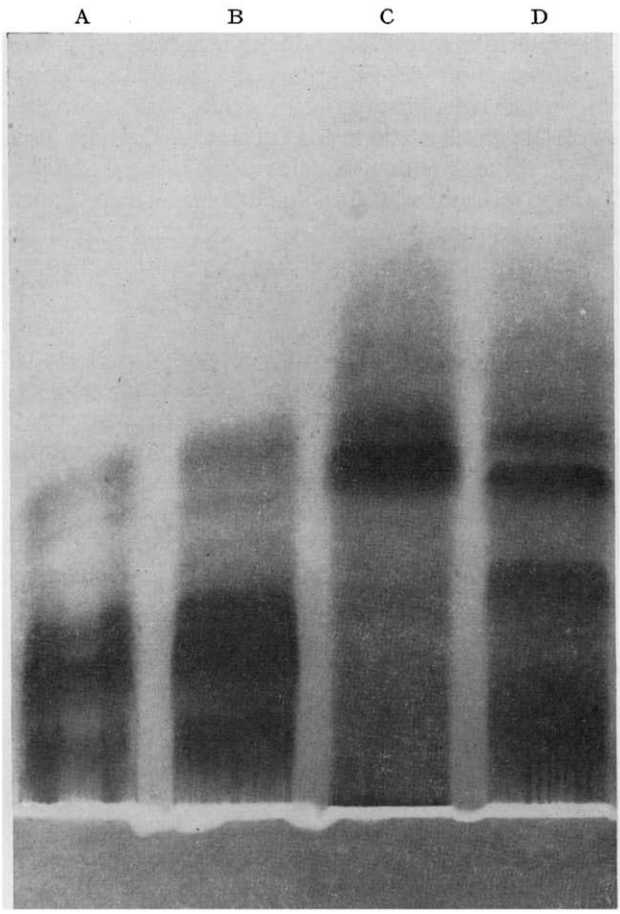

Fig. 1. Vertical urea-starch-gel electrophoresis of S α_2-globulin after reductive cleavage. Starch gel is prepared in 8 *M* urea and formate buffer (0.05 *M* formic acid, 0.01 *M* NaOH; pH 3.1) and electrophoresis conducted at 6 V/cm for 24 h. A. Slow α_2, borate buffer. B. Slow α_2, borate and urea. C. Slow α_2, borate, urea and mercaptoethanol. D. Slow α_2, borate, urea, mercaptoethanol and iodoacetamide.

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Polarographic identification of the reactive form in the hydrolysis of pyridoxal 5-phosphate

The monoanion of phosphoric acid monoalkyl and monoaryl esters undergoes hydrolysis more easily than the undissociated form or the dianion[1]. In view of its biochemical importance, the hydrolysis of pyridoxal 5-phosphate was studied, using

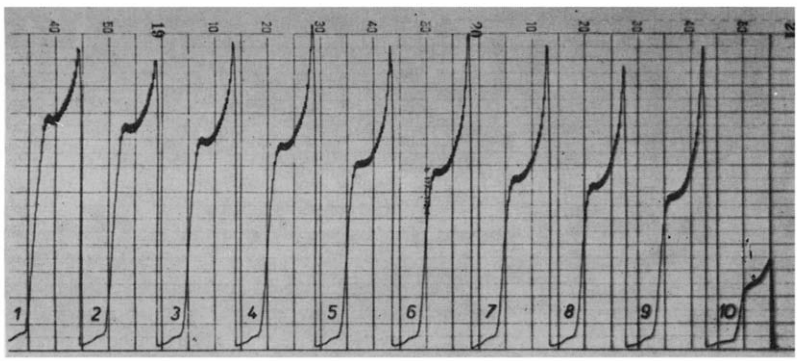

Fig. 1. Polarographic record of the course of hydrolysis of pyridoxal 5-phosphate in 0.01 M $HClO_4$ at $I = 1.0$, and at 79°. $5 \cdot 10^{-4}$ M pyridoxal 5-phosphate after: (1) 15; (2) 23; (3) 41; (4) 50.5; (5) 79; (6) 94.5; (7) 110; (8) 125 (9) 152 min. Curve 10, $5 \cdot 10^{-4}$ M pyridoxal. Curves were registered on a pen-recording polarograph, constructed by Institut für Gerätebau DAW, Berlin, D.D.R. Full scale deflection, 10 μA.

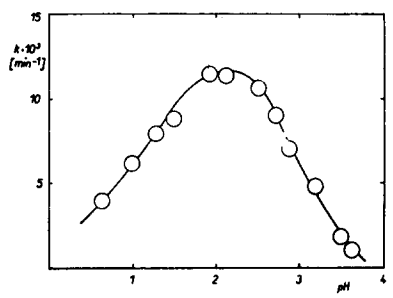

Fig. 2. The dependence of the rate constant of hydrolysis of pyridoxal 5-phosphate on pH at 87.8° and $I = 1.0$.