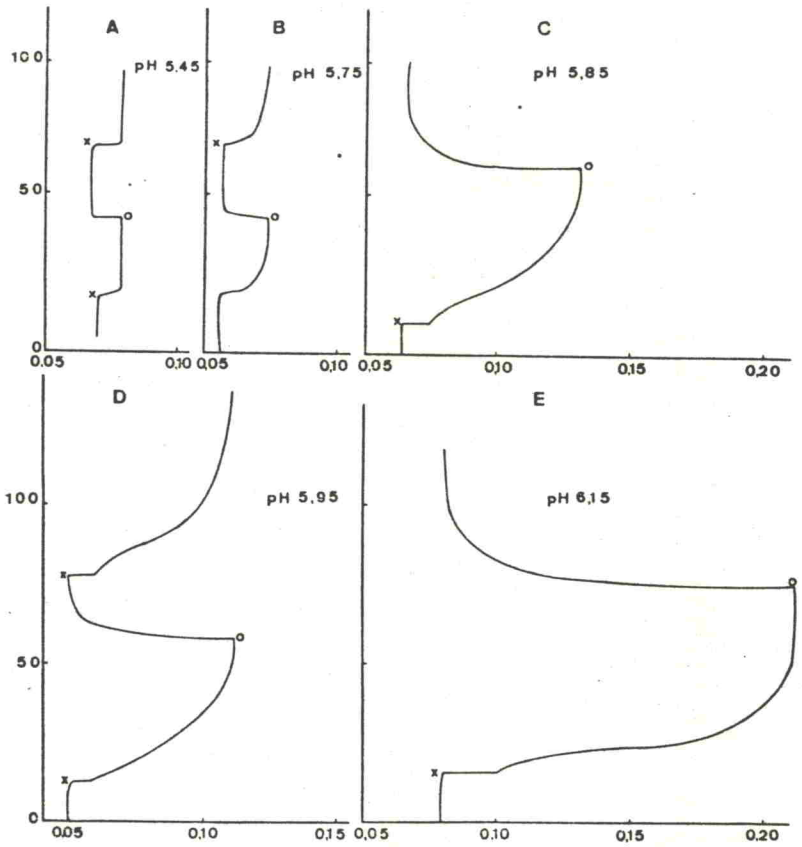

Fig. 1. Influence of pH on the polymerization of fibrin monomers in 1 M NaBr; abscissa, photomultiplier current in μA; ordinate, time in min; ○, application of pressure of 2,500 kg/cm²; ×, release of pressure. The solutions were initially depolymerized by application of pressure.

of protein on the light scattering changes under pressure is shown in Fig. 2. Turbid clots[8], prepared by 20 fold dilution of 10 mg/ml. fibrin monomer solutions in phosphate buffer ($I = 0{\cdot}2$, $p\mathrm{H} = 6{\cdot}0$), depolymerized equally well under a pressure of 2,500 kg/cm².

Because the polymerization is very pH sensitive and the pH of the acetate buffer decreases under pressure (about 0·4 pH units at 3,000 kg/cm²) it was tested whether the depolymerizing effect of pressure could be ascribed to pH changes. The experiments were therefore repeated in a 0·05 M ammonium acetate buffer (decrease of about 0·1 pH units at 3,000 kg/cm²) and in 0·05 M MES (N-morpholino - ethane - sulphonic acid) buffer (increase of about 0·25 pH units at 3,000 kg/cm²). In all cases an apparently complete depolymerization was obtained at 2,500 kg/cm².

Finally, it was tested whether the pressure effect could be ascribed to an alteration of the protein during the preparation of monomers. Native plasma, diluted with buffer to a concentration of 1 mg fibrinogen/ml. and clotted with thrombin, behaved identically under pressure.

On the basis of the complete reversibility of the depolymerization and polymerization reactions and in view of the fact that the phenomenon is the same in buffers with different pH dependence on pressure, we conclude that the polymerization is accompanied by an increase in volume. This volume increase is not explained by the hypothesis of hydrogen bonding between histidyl and tyrosyl or ε-amino groups as the only polymerization mechanism for this reaction in the pH range investigated should be accompanied by a volume decrease (resulting from hydrogen bonding and from electrostriction around the liberated protons).

In fact, our results indicate that during polymerization an additional interaction occurs with a significant volume increase sufficient to overcome the volume decrease due to hydrogen bonding and electrostriction. This seems to point in the direction of the existence of additional hydrophobic bonding or ion pair bonds (salt linkages)[9]. The role of hydrophobic bonding seems to be small in view of the depolymerizing effect of temperature and electrolyte concentration. Attempts to show the presence of hydrophobic sites on the surface by binding the ligand

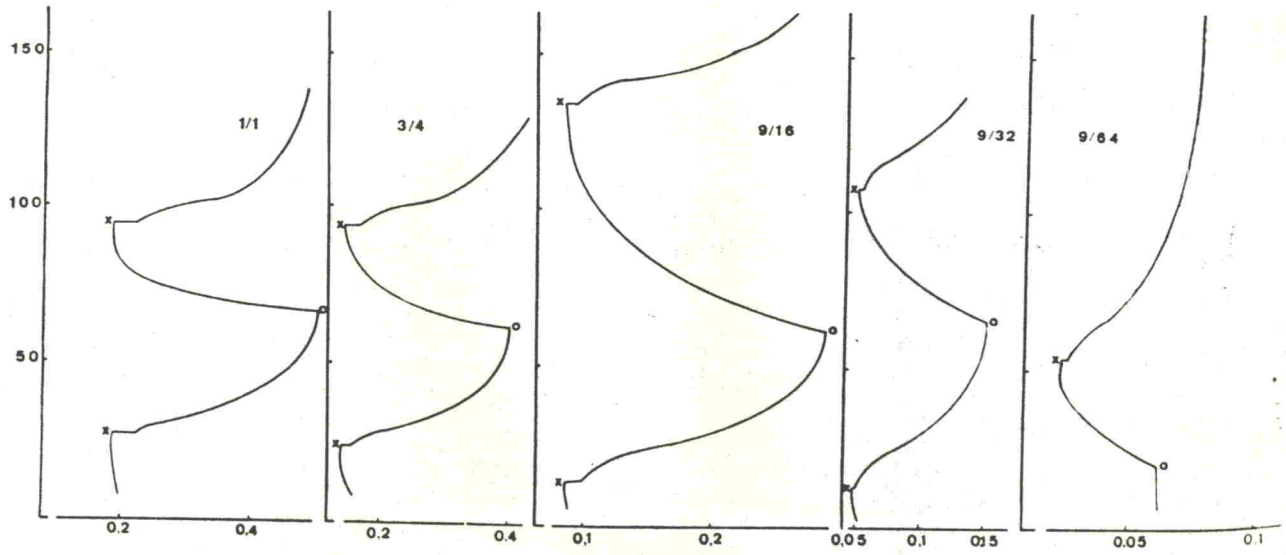

Fig. 2. Influence of concentration of protein on the polymerization in 1 M NaBr, pH 6·1. Abscissa, photomultiplier current in μA; ordinate, time in min; ○, application of pressure of 2,500 kg/cm²; ×, release of pressure. The solutions were initially depolarized by application of pressure. Dilution 1/1 = 10 mg fibrin monomers per ml.