(Steven, 1964) was precipitated from acetic acid dispersion by exposure to ammonia vapour. The gel was allowed to form in the interstices of a nylon mesh, either in the unstretched state or during 20 cycles (3h) of repeated extension (33.5%) and relaxation. Both forms of gel were maintained for a further 16h in the atmosphere of ammonia and the cyclical extension was continued in the one case for this period.

After this period of 'aging' the acetic acid-solubility of the unstretched gel fell from $93.2 \pm 3.6\%$ to $46.6 \pm 8.2\%$ and that of the stretched gel to $15.0 \pm 2.3\%$ ($P = 0.005$). Stretching also induced a 25% decrease in the susceptibility of the gel to collagenase but increased its susceptibility to elastase by 117%.

A decrease in solubility and in susceptibility to collagenase such as is observed could indicate the formation of an increased number of cross-linkages during the stretching process. However, since Hall, Tunbridge & Wood (1953) and Banga (1953) have demonstrated that denatured collagen is more susceptible to elastase, the results with this enzyme would not appear to be in agreement with the hypothesis.

This apparent paradox can, however, be resolved if one considers the groups that are essential for binding elastase to its substrate (Hall & Czerkawski, 1961; Tolnay & Bagdy, 1959). Both carboxyl and hydroxyl groups are required in a free state on the substrate, but the blocking of the latter permits increased adsorption of enzyme without subsequent elastolysis. It may therefore be that elastase is bound on to unstretched collagen in such a fashion that the hydroxyl groups are too far distant from the carboxyl groups for active complex-formation, but that on stretching realignment places these essential groups in the desired spatial relationship to one another and proteolysis ensues.

Factors such as these may account for the extra-cellular production of an elastase-susceptible material (pseudo-elastin; Hall, 1969) in those aging tissues such as skin and vascular wall that during life have been subjected to marked cyclical stress.

Banga, I. (1953). *Nature, Lond.*, **172**, 1099.

Gross, J. (1958). *J. exp. Med.* **107**, 247.

Hall, D. A. (1969). *Expl Geront.* **3**, 77.

Hall, D. A. & Czerkawski, J. W. (1961). *Biochem. J.* **80**, 128.

Hall, D. A., Tunbridge, R. E. & Wood, G. C. (1963). *Nature, Lond.*, **172**, 1099.

Steven, F. (1964). *Ann. rheum. Dis.* **23**, 300.

Tolnay, P. & Bagdy, D. (1959). *Biochim. biophys. Acta*, **31**, 566.

Isolation of Ovomucoid by using an Insoluble Trypsin Derivative

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In a study of the biosynthesis of the egg-white glycoprotein ovomucoid it was necessary to isolate small quantities of completed protein from tissue extracts. To meet this need the interaction of ovomucoid with an insoluble trypsin derivative has been examined as a micropreparative procedure. Chicken ovomucoid is known to be an inhibitor of trypsin but not of chymotrypsin (Rhodes, Bennett & Feeney, 1960).

Trypsin, in the presence of 50mM-benzamidine to minimize autolysis, was linked covalently to Sephadex G-200 that had been activated by reaction with cyanogen bromide (Axén, Porath & Ernback, 1967). Proteolytic activity was measured with casein as substrate (Northrop, Kunitz & Herriott, 1948) in stirred suspensions. The activity of Sephadex G-200-trypsin was equivalent to $12.5 \mu\text{g}$ of soluble trypsin/mg dry wt. Of this activity 71% was inhibited by ovomucoid at pH 7.6. The residual activity was not affected by the presence of a large excess of ovomucoid and apparently arises from enzyme molecules that are not accessible to inhibitor.

The insoluble trypsin-ovomucoid complex was stable at pH 7.6, but could be dissociated at pH 1.5 with the release of ovomucoid into solution and the restoration of 100% of the initial tryptic activity. Isoelectric focusing on polyacrylamide gel of the released product did not reveal any new components, indicating that the procedure did not degrade ovomucoid. On isoelectric focusing ovomucoid is separated into five closely related components (Beeley, 1969).

The Sephadex G-200-trypsin has been used to isolate inhibitors directly from egg white and from homogenates prepared from the washed oviducts of laying hens. In each case isoelectric focusing of the isolated material showed it to contain the characteristic pattern of three major and two minor ovomucoid components. However, an additional component of higher isoelectric point was also present. This substance also bound to Sephadex G-200-chymotrypsin and may therefore be both a chymotrypsin inhibitor and a trypsin inhibitor. The isolation procedure was made specific for ovomucoid by including a preliminary precipitation of the crude extract with 1 vol. of 10% (w/v) trichloroacetic acid adjusted to pH 3.5 with sodium hydroxide (Fredericq & Deutsch, 1949).

The experiments reported indicate that very small quantities of ovomucoid can be prepared rapidly and with a high degree of purity by a method

involving the use of an insoluble trypsin derivative. Further, it has been shown that multiple species of ovomucoid exist in the hen oviduct before their secretion.

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- Axén, R., Porath, J. & Ernback, S. (1967). *Nature, Lond.*, **214**, 1302.
 Beeley, J. G. (1969). *Abstr. FEBS 6th Meet., Madrid*, no. 809.
 Fredericq, E. & Deutsch, H. F. (1949). *J. biol. Chem.* **181**, 499.
 Northrop, J. H., Kunitz, M. & Herriott, R. (1948). *Crystalline Enzymes*, 2nd ed., p. 308. New York: Columbia University Press.
 Rhodes, M. B., Bennett, N. & Feeney, R. E. (1960). *J. biol. Chem.* **235**, 1686.

The States of Aggregation of Citrate Lyase

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Citrate oxaloacetate-lyase (EC 4.1.3.6), also known as citrate lyase, cleaves citrate with the formation of oxaloacetate and acetate. The enzyme has been shown to exist as octamer, tetramer, dimer and monomer with molecular weights of 575 000, 273 000, 137 000 and 74 000 daltons respectively (Mahadik & SivaRaman, 1968). Only the octamer is enzymically active and requires Mg^{2+} , or other bivalent ions, for its activity and stability (Blair, Datta & Tate, 1967). The molecular weights referred to above were determined by the transient-state method in the ultracentrifuge and are therefore sensitive to conditions pertaining to the air/solution meniscus. This renders the method of particular value for the detection of size heterogeneity (Creeth & Pain, 1967).

The purpose of the present study was to re-examine the molecular weight of 314 000 reported by Bowen & Rogers (1963), who used the Ehrenberg modification of the transient-state method. In addition, use was made of the high-speed meniscus-depletion method for sedimentation equilibrium capable of yielding molecular weights independent of conditions at the meniscus and at much lower protein concentrations where thermodynamic conditions closer to ideality are possible (Van Holde &

Baldwin, 1958; Yphantis, 1960). The enzyme was prepared by a method recommended by S. P. Datta (personal communication), and a partial specific volume of 0.735 ml/g (Bowen & Rogers, 1963) was assumed. First, the conditions used (Bowen & Rogers, 1963) were closely repeated with a protein concentration of 4.5 mg/ml in a buffer containing 50 mM- KH_2PO_4 adjusted to pH 7.4 with 2M-KOH, containing 0.15M-KCl but no Mg^{2+} . The approach to equilibrium was studied at 8500 rev./min at 20°C. The Trautman plot constructed was mainly linear with a slope corresponding to a molecular weight of 290 000 daltons. However, a small region of greater slope for the earlier photographs indicated that the sample contained a heavier component, probably the octamer (Yphantis, 1959), which would be absent from the meniscus for the later photographs. Secondly, a repeat of the experiment in a buffer composed of 50 mM-tris adjusted to pH 7.4 with M-HCl containing 1 mM-EDTA and 5 mM-MgCl₂ gave a molecular weight of 570 000 from a Trautman plot, with no sign of the slight curvature mentioned above. In this experiment the simultaneous use of Mg^{2+} and EDTA was as in the purification scheme recommended by S. P. Datta (personal communication) although EDTA alone acts as an inhibitor.

The high-speed equilibrium meniscus-depletion method was applied to protein concentrations of 0.5 and 0.65 mg/ml, respectively yielding results of 573 000 and 570 000 daltons. In both cases the runs were carried out at 12°C in the Mg^{2+} -EDTA buffer described above at 11 272 rev./min.

The present results closely agree with those reported by Mahadik & SivaRaman (1968) for the active octameric form of the enzyme, although the experimental conditions were varied considerably.

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- Blair, J. Mc.D., Datta, S. P. & Tate, S. S. (1967). *Eur. J. Biochem.* **1**, 26.
 Bowen, T. J. & Rogers, L. J. (1963). *Biochim. biophys. Acta*, **67**, 633.
 Creeth, J. M. & Pain, R. H. (1967). *Prog. Biophys. molec. Biol.* **17**, 217.
 Mahadik, S. P. & SivaRaman, C. (1968). *Biochem. biophys. Res. Commun.* **32**, 167.
 Van Holde, K. E. & Baldwin, R. L. (1958). *J. phys. Chem.* **62**, 734.
 Yphantis, D. A. (1959). *J. phys. Chem.* **63**, 1742.
 Yphantis, D. A. (1960). *Ann. N.Y. Acad. Sci.* **88**, 586.