I. 5. A HIGH-SULFUR PROTEIN FROM WOOL

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For a long time the insolubility of wool keratin has hindered investigation of the properties of its constituent proteins, but recent developments in the fractional extraction of these proteins with alkaline solutions of thioglycolic acid, followed by coupling with iodoacetate, have enabled many significant advances to be made (*1–4*).

About 65% of wool can be dissolved in this way and it can be shown electrophoretically that there are at least six proteins in these extracts of which two (about 40% of wool) have been obtained in a purified form. They give single boundaries on electrophoresis and ultracentrifugation at low protein concentrations, but their degree of homogeneity is difficult to establish because of aggregation. These two proteins are low in sulfur (around 1% as *S*-carboxymethyl cysteine, compared with wool having 3.5% as cystine), and are fairly similar in physical and chemical properties. In solution they show a high aggregating tendency giving molecular weights ranging up to several hundred thousand, although the monomeric units may have molecular weights as low as 9000 (*5*). They precipitate between pH 4.5 and 5.0 and in general are insoluble in dilute salt solutions at pH values below these. It is thought that the more readily extractable of these two proteins originates in the ortho segment of the wool fiber, whereas the more difficultly extractable (*S*-carboxylmethyl kerateine 2) comes from the para region. From the general similarity between these two proteins it might be suggested that they perform a similar function in the architecture of the two segments.

From the more easily extractable protein material there has been isolated also, a fraction (10% of wool) rich in sulfur (6.5%) which shows three peaks on electrophoresis at both pH 7 and 11 (Fig. 1), but only one on ultracentrifugation at pH 7 (Fig. 2) (S_{20_w} 1.5). These proteins show no evidence of aggregation at pH 7 and have a molecular weight by the Archibald method of about 16,000, which is constant with time. These results suggest that this high-sulfur fraction consists of proteins which are of about the same size but which differ slightly in charge, perhaps due to differing quantities of *S*-carboxylmethyl cysteine residues. They are highly

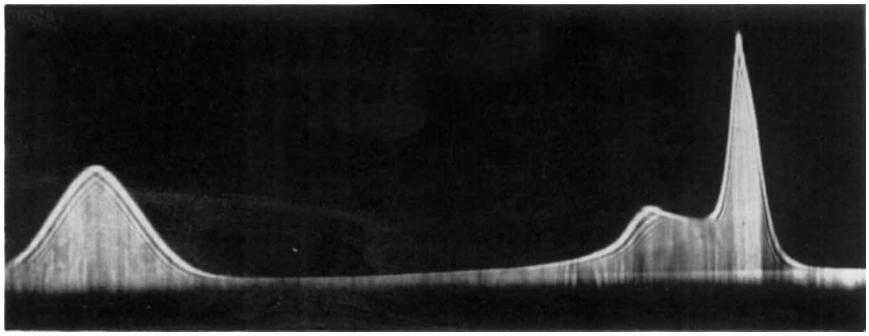

FIG. 1. Ascending electrophoresis pattern of high-sulfur wool proteins run at pH 7 in 0.1 $\frac{\Gamma}{2}$ imidazole-HCl buffer. Direction of movement, left to right.

acidic proteins with a minimum solubility at pH 2.9 being soluble on either side of this value.

Attempts are now being made to separate the components in this fraction but the low molecular weight of the proteins and the small differences in charge between them is making this a difficult task to accomplish. Fractional precipitation with ammonium sulfate and with acetone or ethanol have proved unsuccessful but the use of the anion exchanger *N*-*N'*-diethylaminoethylcellulose (EK 7392) developed by Peterson and Sober (*6*) appears to be more promising for this purpose.

Before use, the resin is repeatedly washed with 2 *M* NaCl to remove

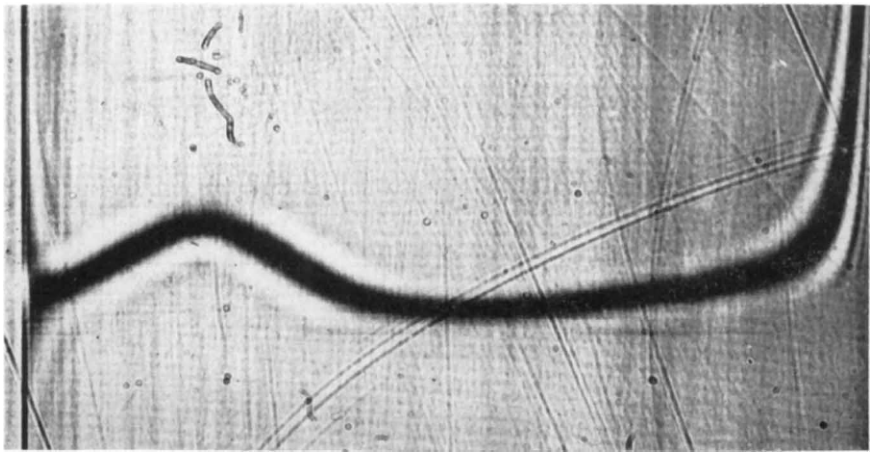

FIG. 2. Ultracentrifuge pattern of high-sulfur wool proteins run at pH 7 in 0.05 $\frac{\Gamma}{2}$ imidazole-HCl, 0.15 *M* KCl buffer, for 128 minutes at 59,780 r.p.m. Direction of movement, left to right.

material absorbing at 277 mμ and then equilibrated with 0.005 ionic strength imidazole-HCl buffer of pH 7. A column 10 × 100 mm. is used with the resin bed supported on a sintered glass disk, 20 mg. of protein in 2 ml. of buffer is added and washed in with 2 ml. of buffer. A gradient elution technique is employed in which the concentration of sodium chloride rises linearly to 1 *M* with the volume of eluant passing through the column. The effluent is collected in a series of equal volume samples and the protein content estimated from the absorption at 277 mμ. A typical elution curve is shown in Fig. 3. It can be seen that this is similar to the electro-

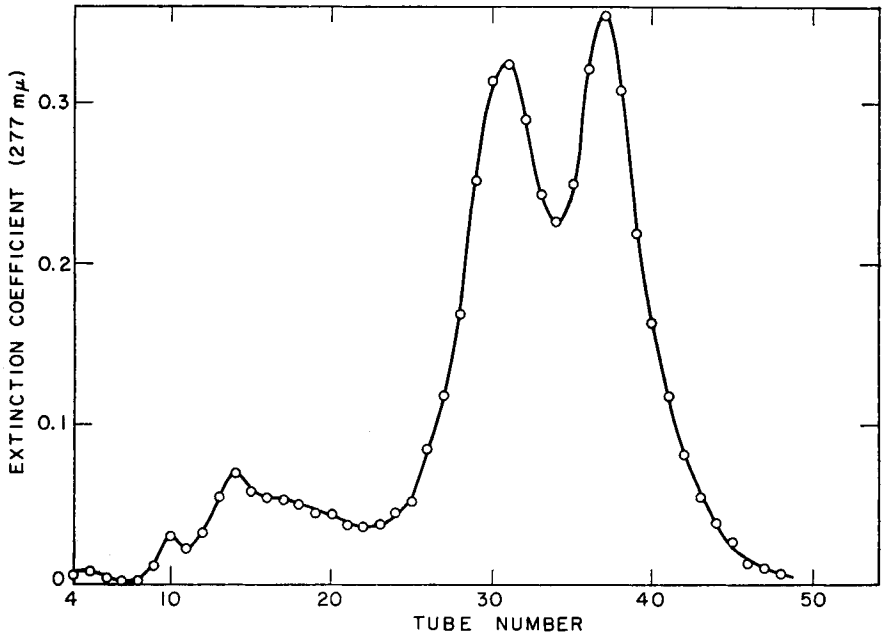

FIG. 3. Gradient elution of high-sulfur wool proteins from *N*-*N'*-diethylaminoethylcellulose.

phoretic pattern, showing two main components and several minor ones, with the materials of highest charge coming off the column last and thus to the right of the graph. Isolation of the two major components in sufficient quantities for further study is now being carried out.

It is of interest that at least 40% of the wool fiber is made up of two very similar proteins with a comparatively low sulfur content (the residue undissolved by alkaline solutions of thioglycolic acid has a similar amino acid composition to whole wool) which may be the major constituents of the microfibrils. Much of the sulfur appears to be concentrated in a com-

paratively small amount of protein which may constitute a part of the high-sulfur interfibrillar matrix proposed by Mercer on histological grounds (*7*). In view of the observation (*8*) that the proteins of the wool root are lower in sulfur than the wool fiber, it is tempting to speculate that the high-sulfur proteins are introduced into the wool fiber at a late stage in development.

References

1. J. M. Gillespie and F. G. Lennox, *Biochim. et Biophys. Acta* **12,** 481 (1953).
2. J. M. Gillespie and F. G. Lennox, *Australian J. Biol. Sci.* **8,** 97 (1955).
3. J. M. Gillespie, *Australian J. Biol. Sci.* **10,** 105 (1957).
4. J. M. Gillespie, *Biochim. et Biophys. Acta* **27,** 225 (1958).
5. B. S. Harrap, *Australian J. Biol. Sci.* **10,** 116 (1957).
6. E. A. Peterson and H. A. Sober, *J. Am. Chem. Soc.* **78,** 751 (1956).
7. E. H. Mercer, *Proc. Intern. Wool Textile Research Conf., Melbourne, 1955* **F,** p. 215 (1956).
8. W. J. Ellis, J. M. Gillespie, and H. Lindley, *Nature* **165,** 545 (1950).

Discussion

HAUROWITZ: Are the proteins digested like keratin?

GILLESPIE: I don't know about the high-sulfur protein, but the low-sulfur proteins are digestible.

JENSEN: I would like to close this session by putting in a word for an industry that has not yet been represented in relation to sulfur in proteins. We have heard from the wool people and the hair treatment people. This morning we heard about clots from serum proteins. Yesterday Dr. Benesch demonstrated for you a sulfhydryl-flavored Jello. But nobody has put in a word for the poultry industry. I would like to close by saying that by proper manipulation of the factors involved in the gelation and clotting of proteins, you can, if you wish, insure that your boiled egg when you open it in the morning will be transparent. You can easily make sure whether you have one or two yolks. I brought a couple of these along. This has perhaps special interest around the time of Easter.