



PLATE 1. Electron micrographs of fractionated soluble derivatives of feather keratin. Experimental details are given in the text. (a) $\times 120\,000$. (b) $\times 200\,000$.

APPENDIX

X-Ray-Diffraction and Electron-Microscope Observations on Soluble Derivatives of Feather Keratin

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It has been shown (Fraser & MacRae, 1963) that solutions of unfractionated feather-keratin derivatives can be dried down into films that give well-oriented X-ray-diffraction patterns containing many of the features associated with the microfibrillar structure of the native material. It was found that similar films could be obtained from the fractions described by Harrap & Woods (1964). In most cases spontaneous birefringence developed around the edge of the film during drying. The X-ray-diffraction patterns yielded by these films when the X-ray beam passed parallel to the surface were similar to those of the unfractionated derivatives at low angles of diffraction, with a prominent meridional reflexion at 23 Å and equatorial reflexion at 33 Å. At wider angles the fractionated materials show strong layer lines at 4.8 and 2.4 Å and a series of equatorials which index as orders of a 33 Å spacing.

The electron micrographs shown in Plate 1 were obtained by spraying a 1:1 (v/v) mixture of 0.05% protein solution and 1% phosphotungstate, pH 5.6, on to grids covered with a carbon-collodion film and using the negative-staining method of Brenner & Horne (1959). It is apparent from Plate 1 that, on drying, the fractionated derivatives spontaneously polymerize to form fibrils. The apparent

thickness of the fibrils varies from 40 to 60 Å at their narrowest part (arrow in Plate 1b) to approx. 130 Å at their widest part, suggesting that they are helical with a pitch of approx. 1500 Å. At first sight the fibrils appear to consist of two filaments coiled around each other to give a two-strand rope, but a ribbon containing three strands cannot be excluded at present. A feature is the tendency of the fibrils to aggregate laterally in precise register.

At the present time it is not clear whether the individual filaments about 40 Å in diameter in the fractionated proteins are structurally similar to the microfibrils in the native feather material (Rogers & Filshie, 1962; Filshie & Rogers, 1962; Fraser & MacRae, 1963), although the similarity between the low-angle X-ray patterns supports this view.

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Soluble Derivatives of Feather Keratin

2. MOLECULAR WEIGHT AND CONFORMATION

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The preceding paper (Harrap & Woods, 1964) details the preparation, amino acid analysis and electrophoretic heterogeneity of feather proteins. Previous estimates of homogeneity with regard to size (Rougvie, 1954; Woodin, 1954) have suggested

that feather keratin is composed of fairly homogeneous units of mol.wt. 10000. Since, in contrast with Woodin (1954), we have found soluble feather proteins to be electrophoretically heterogeneous, it was important also to re-examine the molecular-