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Matrix-Microfibril Texture in α -Keratin as observed by X-ray Diffraction and Electron Microscopy

THIN sections of keratin fibres can be "stained" by reaction with solutions of the salts of some heavy metals, notably silver and osmium. Electron microscopy shows a texture caused by the heavier staining of the matrix material surrounding the microfibrils. Such treatments also effectively enhance the small-angle equatorial X-ray diffraction, which has the character of the scattering from an assembly of roughly cylindrical holes (the microfibrils) in a continuous matrix of comparatively large electron density. It consists of peaks, E_1 , E_2 , E_3 , and with equivalent Bragg spacings $d_1=80 \text{ \AA}$, $d_2=42 \text{ \AA}$, and $d_3=27 \text{ \AA}$, of which the first may be chiefly ascribed to intermicrofibrillar interference effects, and the other two to the first two diffraction fringes from a single microfibrillar hole¹. The intensity ratio I_2/I_3 of E_2 to E_3 should in theory be approximately 4.2:1, and this agrees well with the observed value in the most studied case of silver staining².

A wide variety of heavy metal staining techniques has recently been examined here in the search for improved electron microscope contrast. Most of them have proved ineffective in comparison with silver or osmium, even though the small-angle X-ray diffraction intensity is usually enhanced considerably. This implies a texture with grain of the order of 100 \AA in the heavy metal atom distribution. The diffraction results for a number of Lincoln wool samples variously treated are given in Table 1. The small-angle enhancement index in the third column is proportional to I_2/I_p , where I_p is the intensity of the main protofibril (coiled coil) peak in the 10 \AA region, the constant of proportionality being chosen to make the index unity for untreated fibres.

Table 1. X-RAY DIFFRACTION AND ELECTRON MICROSCOPY OF LINCOLN WOOL FIBRES

| Treatment | I_2/I_3 | Small-angle enhancement | E.M. contrast | Reference |
|---------------------------------------|-----------|-------------------------|---------------|-----------|
| Untreated | 1.0 | 1.0 | Absent | |
| R + OsO ₄ | 2.8 | 7.4 | Good | 3 |
| R + Hg(OAc) ₂ | 1.8 | 40 | Absent | 3 |
| Uranyl acetate | <1 | — | Absent | 4 |
| R + Ag(NO ₃) ₂ | 5.1 | 60 | Good | 2, 3, 4 |
| R + phenylmercuric hydroxide | 3.8 | 20 | Moderate | |
| Ox + Pb(OH) ₂ | 0.8 | 1.8 | Absent | 6 |
| Gold chloride | 4.1 | 24 | Moderate | 5 |
| Phosphotungstic acid | 1.7 | 21 | Absent | 5, 7 |

R, Reduction; Ox, oxidation.

Only four of the treatments listed in Table 1 lead to well marked contrast between matrix and microfibrils in the electron microscope. It is significant that these have the highest values for the ratio I_2/I_3 . Some untreated keratin fibres, for example, human hair, have high values for this ratio which cannot therefore form the sole diffrac-

tion criterion of electron microscope contrast. But such contrast will almost certainly be accompanied by a high small-angle enhancement, from which it seems that electron microscope contrast will be developed when both the ratios I_2/I_3 and I_2/I_p are large compared with their values in the untreated material. When this happens the spacing ratio d_2/d_3 is significantly reduced².

These results imply that electron microscope contrast will be well developed when the metal distribution is such as to lead to scattering of the conventional "hole in a uniform matrix" type. There is still no solution of the problem of the metal distribution in cases, such as those of mercuric acetate and phosphotungstic acid, where there is considerable small-angle enhancement but poor electron microscope contrast. The diffraction results imply that the major discontinuities in metal distribution may be associated with the protofibrils rather than with the microfibrils. The unexpected insensitivity of the electron microscope to the electron density variations might then be another instance of the complexities of image formation which have been discussed by Johnson and Sikorski^{8,9}.

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Alkali Halides: Relationship between Heat of Fusion, Melting Point, Electronic Polarizability and the Ionic Radius Ratio

FUSION is a complex phenomenon for which there is no clear theoretical basis. If heat of fusion could be independently related to the measurable physical properties and structural features of a substance, it should be possible to predict the change of mutual forces acting between molecules (or ions) of the same species when it melts.

The Clapeyron-Clausius equation and Richard's law for pure substances seem to describe the necessary type of relation. No attempt has been made here to derive equations from fundamental laws and/or properties of substances. Rather, the data were examined empirically in order to limit the number of properties which have influence on fusion.

In this communication, I wish to point out that if for alkali halides one plots $H_f/RT_m\alpha^{1/3}$ against the ratio of cation to anion r^+/r^- and connects points for a given cation, a set of almost straight lines is obtained for the sequences LiX, NaX, KX, RbX ($X=F, Cl, Br$ and I). Here H_f , T_m and α are heat of fusion¹, melting point² and electronic polarizability³ of the salt, respectively.

Pauling⁴ expected that the irregular variances of melting points (Fig. 2) for a sequence LiX, NaX, KX, RbX, CsX were to be attributed to the radius-ratio effect. He argued that the hypothetical alkali halide shows the expected regularity when corrections are made for the