STUDIES ON THE STRUCTURE OF KERATIN

IV. THE MOLECULAR STRUCTURE OF SOME MORPHOLOGICAL COMPONENTS OF KERATINS

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SUMMARY

The keratins of feather and whisker of lion and tiger have been examined by (a) oxidation with peracetic acid and followed by fractionation using dilute alkali, (b) X-ray diffraction and (c) optical microscopy particularly using polarised light.

Feather (calamus) was shown to be polyphase in structure, consisting of an interior layer with molecular orientation along the axis, and an exterior layer with orientation at right angles to the axis. It is considered that the X-ray diffraction pattern of calamus is composite, consisting of two pictures at approximately right angles to each other. X-ray diffraction patterns of chemical fractions raise doubts as to whether native feather keratin is in an exclusively β-configuration and it is considered possible that it contains some α-protein.

In the case of the whiskers, however, which are composed of α-keratin, the interior layer round the medulla showed orientation at right angles to the axis, with the exterior molecules orientated longitudinally. It is considered that the function of these structures is to give mechanical strength to prevent the feather calamus or tactile whisker from splitting during flexing.

INTRODUCTION

A study has been made of the molecular structure of some chemical and morphological components of the keratins of feather and the whiskers of lion and tiger. Chemical fractionation was carried out by alkali extraction after oxidation of the keratin with peracetic acid[1]. The molecular structure of these materials was examined by X-ray diffraction and optical microscopy using normal and polarized light.

Feather was examined because although it is generally accepted that it is a β-keratin, preliminary work suggested that it may not be exclusively in this configuration. The whiskers, which are in an α-configuration, exhibited three points of interest. The fibre after oxidation underwent a reversible change in length with pH, a phenomenon previously reported by PAUTARD AND SPEAKMAN[2] for horse hair. Further, the wiskers had a complex multi-medullated structure when viewed in cross-section, and exhibited strong birefringence in a discrete ring which shows that they possess a molecular structure hitherto unknown in animal hairs.

EXPERIMENTAL

Materials

White goose wing feathers were dissected with scissors into barbs, calamus, rachis, medulla (pith) and the pulp cap which lies at the root-end. The medulla was rejected. Lions and tigers whiskers were shed by the adult animal or obtained post mortem. Prior to oxidation, the feathers were scoured in a warm dilute solution of soap and ammonia, washed with water, air-dried and then Soxhlet extracted with alcohol and then ether. The whiskers were degreased in hot acetone.

Isolation of fractions

The whiskers were oxidized with peracetic acid and separated into α-keratose (soluble in ammonia, precipitated by acid), β-keratose (insoluble in ammonia) and γ-keratose (soluble in ammonia, not precipitated by acid)* by methods described previously[3,4].

In the case of feather the bulkiness of the material necessitated the use of a 500% excess of a 2% solution of peracetic acid for oxidation (calculated for —S—S— → 2 — SO_3H), the α-keratose fraction being precipitated by acidifying the ammoniacal extract to normality with hydrochloric acid. The γ-keratose was recovered from solution by neutralizing with ammonia and adding six times its volume of acetone, when the γ-fraction separated slowly as a flocculent precipitate.

Optical examination of materials

Cross-sections of the feather components and whiskers were obtained by hand-sectioning with a razor blade or by the use of a microtome. Cross-sections of whiskers were taken very near the root, but the actual root was avoided. Specimens were mounted in xylene and viewed in ordinary and polarised light.

X-ray examination

X-ray diffraction photographs were obtained using a flat plate camera together with CuKα radiation from a Raymax rotating anode crystallographic unit operating at 40 mA and 40 kV. Where comparison photographs were required, a quadrant type cassette was used.

RESULTS AND DISCUSSION

Feather

The barbs gave values of 31%, 18% (11% on repeated treatment) and 35% for α-, β- and γ-keratose respectively, whereas for the calamus the values were 65%, 13% and 23%. Although the latter are similar to the corresponding values for wool, in the case of the barbs the α-keratose fraction is much smaller.

It is generally accepted that feather is a β-keratin and the soluble α-keratose, when cast from ammonia solution into a film, which was incapable of being orientated, showed X-ray spacings of 4.61 Å and 9.1 Å suggestive of a β-configuration. In addition, however, a strong sharp reflection at 4.4 Å was present which is characteristic of neither an α- or β-configuration. It is difficult to understand the significance of

* This nomenclature is adhered to throughout the paper, although in the case of feather keratin the prefixes α and β do not correspond with main-chain configurations as shown by X-ray diffraction.

this reflection, but when wool is oxidized with permolybdic or pertungstic acids[5] and extracted with ammonia solution, both the α- and β-keratose fractions show this reflection. In the case of the insoluble β-keratose fraction, which again could not be orientated, two of the most prominent reflections were at 2.70 Å and 4.26 Å, which are very similar to those given by the weaker reflections of α-keratin.

Although it can be asserted that the soluble α-fraction is in a β-configuration, the residue insoluble in ammonia is definitely not in this configuration and corresponds more to an α-structure. Since on chemical treatment of keratin a $\beta \rightarrow \alpha$ transformation is unknown, it is possible that native feather is not exclusively in a β-configuration, and contains some α-keratin. It is relevant that the thin membrane that constitutes the pulp cap is known to give an α-type X-ray diffraction pattern[6]. The orientation of a cap varied along its length, being parallel to the quill axis at the base, and at right angles at the rachis end. The membrane was too friable to determine if it would undergo an $\alpha \rightarrow \beta$ transformation on stretching.

The outside third of the calamus wall exhibited very strong birefringence colours when a cross-section was viewed in polarised light, this area being separated from the remainder by a sharp boundary line. The interior two-thirds showed strong birefringence when viewed longitudinally, and it must therefore be concluded that whereas these molecules are laid down approximately parallel to the calamus axis, the exterior layer of molecules lies at right angles to the axis.

KEIGHLEY[7], in an infra-red absorption spectrographic study of goose wing-feather calamus, found dichroic ratios for the NH, CH and CO stretching frequencies of 1.18, 1.12 and 1.24 respectively, with a value for the NH deformation of 0.89. The NH and CO stretching frequencies were 3300 cm^{-1} and 1635 cm^{-1}, corresponding to a β-configuration, and therefore the dichroic ratios are consistent with the molecules being orientated perpendicular to the length of the feather. It would appear that, since feather calamus is polyphase in structure, KEIGHLEY's results are applicable to the outer layer only. Preliminary X-ray diffraction work on the two components isolated mechanically has shown that each gives the same pattern, but they are approximately at right angles to each other. The contribution that each makes to the composite picture of the intact calamus will depend on the relative proportions of the two components present.

An examination of the cross-section of the rachis showed that only about a seventh of its outer wall was birefringent. The rachis is the external continuation of the calamus which lies below the skin, and there is, therefore a reduction in the width of the outer layer, which exhibits molecular orientation at right angles to the axis, from the root to the tip end of the feather. ASTBURY AND BELL[8] observed that the molecular orientation of a thin outer layer of quill was at right angles to the feather axis.

In general, a biological structure is related to the function it has to undertake. Feathers, particularly those of the wing, may be subjected to severe flexing, and an outer layer of material orientated at right angles to the feather axis, will prevent the longitudinal splitting that might occur if the structural elements were laid down exclusive parallel to the axis.

Lion's and tiger's whiskers

Untreated whiskers gave a well-defined α-keratin X-ray diffraction pattern which was sharper than that given by wool and more nearly resembled that for porcupine

quill[9]. Oxidation with peracetic acid followed by alkali extraction, gave α-, and β-keratose fractions similar in size to those of wool.

After treatment with peracetic acid, the α-pattern persisted but showed a lower

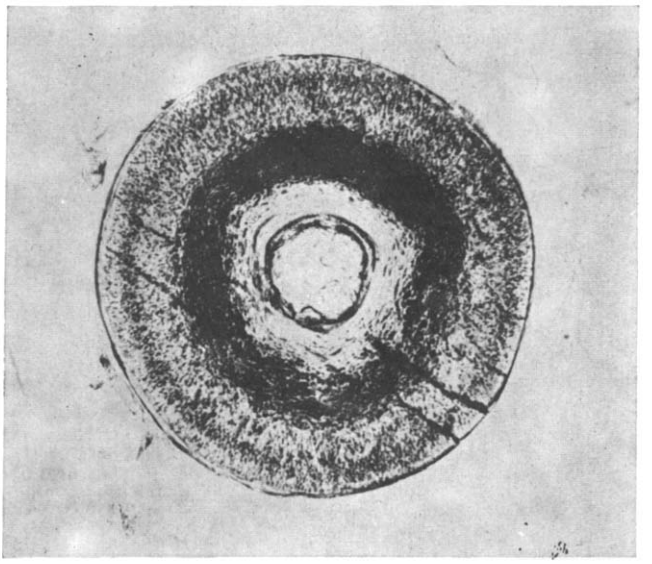

Fig. 1. Untreated lion whisker viewed in normal light (Cross Section × 100).

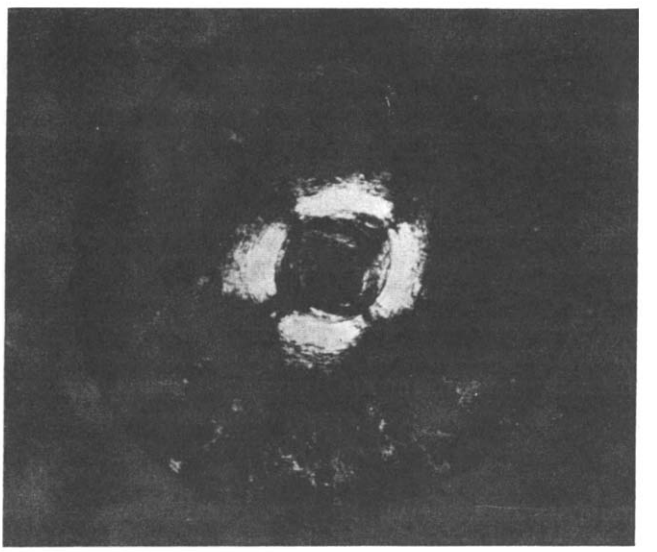

Fig. 2. Untreated lion whisker viewed in polarized light (Cross Section × 90).

degree of orientation. On placing the treated whisker in ammonia some outer membranes peeled off (*vide infra*) and these were found to have an amorphous structure. In the ammonia solution the whisker extended in length by approx. 100%. On removing from the solution and drying, the wisker reverted to its original length and gave a α-type X-ray diffraction photograph. This behaviour has been reported previously for horse-hair by PAUTARD AND SPEAKMAN[2]. Although it is possible that extension in ammonia solution might produce a β-configuration, reverting to an α-

structure on removal and drying, it has not been possible to test this hypothesis since it has proved difficult to obtain a satisfactory X-ray diffraction pattern of the fibre in the wet state.

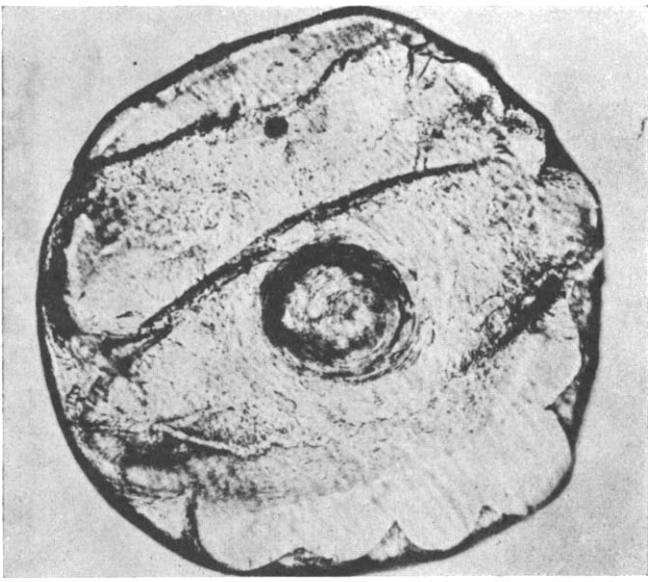

Fig. 3. Lion Whisker treated with peracetic acid and ammonia, (Cross Section × 100).

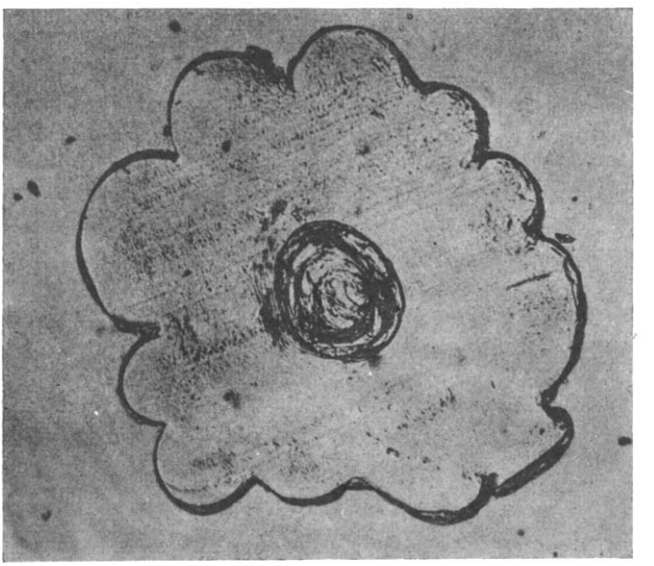

Fig. 4. Lion whisker treated peracetic acid and ammonia showing the outer membrane to have peeled away (Cross Section × 100).

A cross-section of untreated lion's whisker in normal light (Fig. 1) showed six discrete concentric regions. From the interior to the outside of the fibre these are a medulla 1, a small membrane surrounding the medulla 7, three broad rings 3–5 and finally an exterior very thin cuticle 6, which readily broke away. Between crossed Nicols, this cross-section showed very strong interference colours in region 3, ex-

hibiting a black maltese cross on the illuminated ground (Fig. 2). The cross remained stationary when the specimen was rotated, but the width of the arms fluctuated. The cross rotated, however, as the analyser moved. After treatment with peracetic acid,

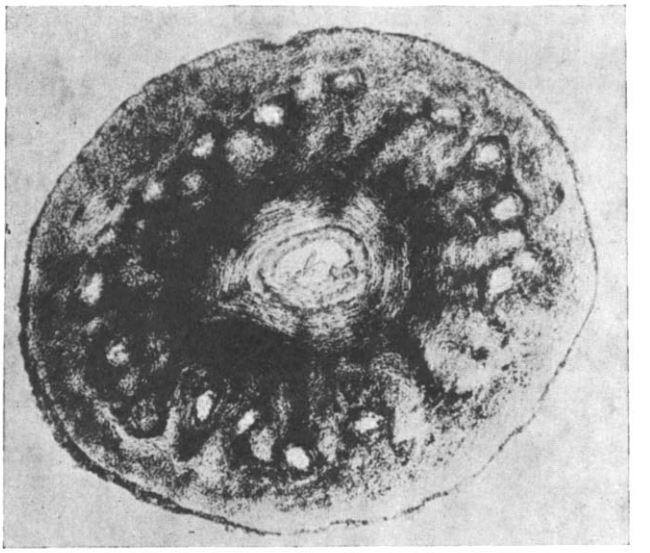

Fig. 5. Tiger whisker viewed in normal light (Cross Section × 50).

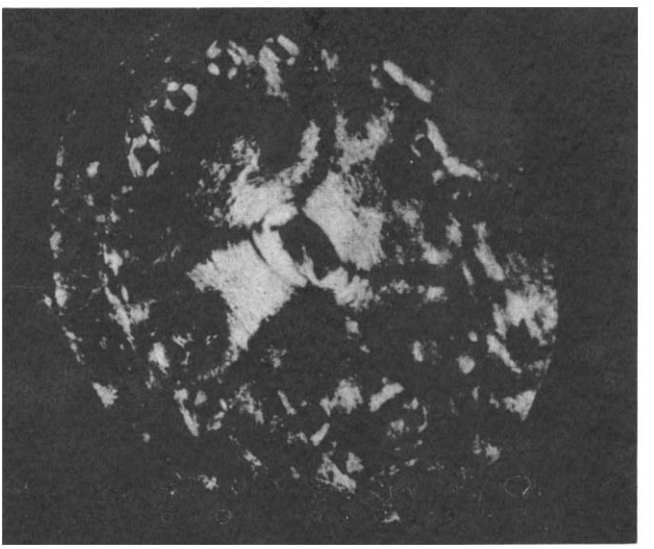

Fig. 6. Tiger whisker viewed in polarized light (Cross Section × 80).

the cuticle, 6, became detached when the fibre was placed in ammonia solution (Fig. 3) and finally peeled off (Fig. 4).

Tiger's whisker was remarkably complex in cross-section (Fig. 5) and showed all the structural features of lion with the addition of a number of sub-medullae arranged on a circle in ring 4. In crossed-Nicols, region 3 was again strongly birefringent

exhibiting the black maltese cross, and the region round each sub-medulla also exhibited its own cross (Fig. 6). Cat's whisker very closely resembled Lion's whisker and in polarised light exhibited the same phenomena. It must be pointed out that under high magnification, region 4 was seen to be extremely complex and although the small medullae are nearly always absent in the case of lion, in point of fact this region is very similar in the whiskers of lion and tiger.

Crystallographically, two explanations of the optical phenomena are possible. Either a uniaxial crystal system is present with the axis parallel to the whisker axis, or the molecular structure is orientated at right angles to the axis (in the plane of the section) parallel to the whisker wall. The first explanation must be rejected because keratin is too imperfectly crystalline. Supporting evidence for the second explanation is found from the microscopical examination of cross-sections, which show that the macro-structural elements in region 3 appear to be laid down concentrically in the plane of the section. In general, the macro structure of fibrous materials reflects the molecular structure. The fluctuation of the dimensions of the cross on rotating the specimen can be explained by the fact that the cells comprising region 3 are not laid down in perfectly concentric circles but show a scalloped pattern. This is supporting evidence for correlating the maltese cross with molecular orientation in the plane of the section. The function of this structure is probably similar to that of feather and would prevent the thick tactile whisker from splitting during flexing.

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