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X-Ray Diffraction by Hairs and Feathers. Part I

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INTRODUCTION

The use of x-ray diffraction to investigate the details of fiber structure has been most important among the techniques of a predominantly physical type in this field. Such x-ray fiber diagrams were reported by Herzog and Jancke,[1] and similar photographs of human hair, wool, horn, etc. were obtained by Astbury and collaborators.[2,3] The hair and wool fibers are found to be decidedly nonhomogeneous, and these fibers produce patterns of α- and β-keratin in the normal unstretched and stretched states, respectively. Suitable molecular configurations were proposed by Astbury and Bell[4] and later on by Pauling and Corey[5] to account for the x-ray interferences. In the present investigation the authors have studied the x-ray diffraction by the tail hairs of a dozen mammals, both domestic and wild, and the feathers of a few birds. A comparative study of the two has also been attempted.

EXPERIMENTAL

The specimen was kept inside a Lindemann glass capillary tube mounted at the center of a circular camera of diameter 114.6 mm. All the fibers used in the investigation had been washed in alcohol to remove natural grease and then introduced into the glass capillary tube in bundles. The incident monochromatic beam falls perpendicular to the meridional axis of the fiber. The radiation used was that from a copper target tube filtered of $K\beta$ by nickel foil. All the specimens were exposed for 3 hr. under identical conditions.

RESULTS AND DISCUSSION

The patterns due to the hairs of common domestic animals and some wild animals were obtained. In both cases similar patterns were obtained. The patterns consisted of a spot on either side and a diffuse halo in all cases except in the case of buffalo hair, where 5 or 6 Debye-Scherrer rings were also recorded. In no other case have these rings been recorded. A pattern due to back reflection was obtained in no case, and this may be due to the very poor reflecting power of the fibers studied. Even though the patterns of cow's and horse's hair were obtained previously they have been taken again for comparison.

The microphotometer curves for all the patterns were taken, and the distances between the spots and the diameter of the halo determined from the curve. The spacings corresponding to the spots and halo were calculated by use of the well-known Bragg relation $n\lambda = 2d \sin \theta$, taking $\lambda = 1.542$ A.

TABLE I
Hairs from Domestic Animals

Specimen	Distance between spots, cm.	Spots θ	Spots d, A.	Diameter of halo, cm.	Halo θ	Halo d, A.
Horse	1.889	4° 42′	9.409	4.652	11° 37′	3.829
Sheep	1.938	4° 50′	9.150	4.700	11° 45′	3.786
Ox	1.889	4° 42′	9.409	4.531	11° 19′	3.929
Goat	1.889	4° 42′	9.409	4.361	10° 45′	4.133
Cow	1.889	4° 42′	9.409	4.458	11° 27′	3.883
Ass	1.744	4° 21′	10.164	4.337	10° 50′	4.102

TABLE II
Hairs from Wild Animals

Specimen	Distance between spots, cm.	Spots θ	Spots d, A.	Diameter of halo, cm.	Halo θ	Halo d, A.
Lion	1.884	4° 42′	9.409	4.281	10° 42′	4.153
Leopard	1.859	4° 39′	9.510	4.281	10° 42′	4.153
Elephant	1.859	4° 39′	9.510	4.111	10° 16′	4.326
Porcupine (quill)	1.884	4° 42′	9.409	4.380	10° 57′	4.059
Brown bear	1.884	4° 42′	9.409	4.380	10° 57′	4.059
Himalayan bear	1.811	4° 31′	9.790	4.331	10° 49′	4.108
Giraffe	1.762	4° 24′	10.049	4.208	10° 31′	4.089
Hyena	1.762	4° 24′	10.049	4.331	10° 49′	4.108

TABLE III
Buffalo Hair

Distance between spots, cm.	Spots		Diameter of halo, cm.	Halo			Ring	
	θ	d, A.		θ	d, A.		θ	d, A.
1.801	4° 30′	9.826	4.601	11° 30′	3.867			
						1	14° 45′	3.028
						2	18° 15′	2.440
						3	19° 59′	2.254
						4	22° 59′	1.973
						5	25° 15′	1.807
						6	29° 15′	1.578

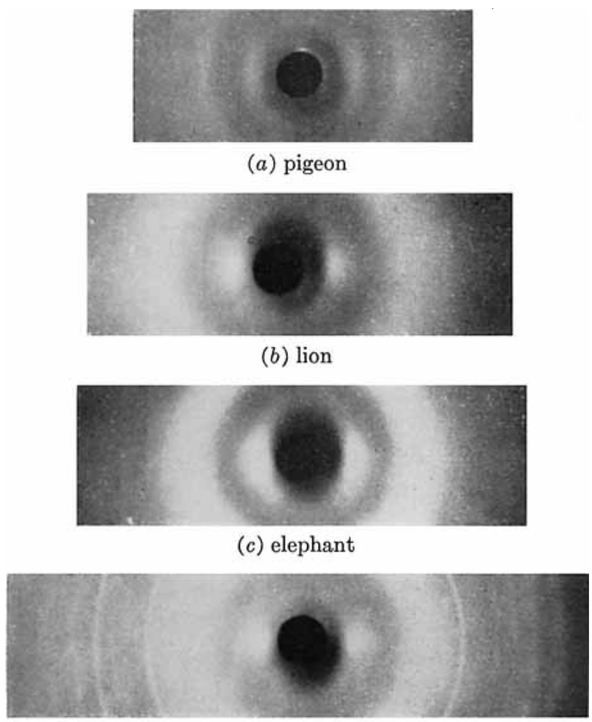
The *d* values for the hairs studied are given in Tables I–III.

The x-ray photographs of hairs are not sufficiently well defined to permit anything more than a description of their salient features. The equator is characterized by two groups of reflections, the most prominent being the disproportionately large spot. This is definitely not due to a single reflection. It is spread over and should include reflections from three or four overlapping planes. The second equatorial reflection is the diffuse halo of spacing approximately equal to 4 A. In no case did we obtain a halo of high spacing obscuring the center as obtained by Astbury in certain cases. He determined the dimensions of the unit cell of α-keratin and has calculated the spacings for different planes. Our *d* values agree fairly well with Astbury's values.

In all the patterns obtained for the various hairs it was observed that the diffuse halo starts at about 4 A. The lengthy exposure required for good hair photographs is an indication that only a fraction of the hair substance is in an approximately true crystalline state. Elliott[6] has studied the infrared spectra of fibers in both the unstretched and stretched states. His findings also support the above conclusion. Amorphous halos tend to reveal the most probable inter- or intramolecular distances in a noncrystalline system. The noncrystalline fraction of hair, being built of combinations of α-amino acids which must have certain relatively small spacings in common, would naturally yield such a halo.

We have seen earlier that the buffalo hair, in addition to the usual pattern, shows a few Debye-Scherrer rings. Astbury and Street have recorded one very weak powder ring due to the (001) plane. The positions and the spacings in our pattern show that no ring corresponds to the plane (001). The presence of powder rings definitely shows that there is a more crystalline arrangement in buffalo hair and that the crystalline protein is randomly oriented about the fiber axis. One question automatically now presents itself, viz., whether this crystalline protein of buffalo hair is made up of α-keratin alone or some other additional proteins.

To determine this, the hair was hydrolyzed in 6*N* hydrochloric acid for 24 hr. in a reflux condenser. A chromatographic study of the hydrolyzed product revealed the presence of one additional compound. The following amino acids were identified: (*1*) leucines (leucine and isoleucine); (*2*) phenylalanine; (*3*) valine; (*4*) proline (suspected); (*5*) tyrosine (black pigmentation); (*6*) alanine; (*7*)



(*a*) pigeon

(*b*) lion

(*c*) elephant

(*d*) buffalo

Fig. 1. Patterns of hairs and feathers.

threonine; (*8*) glycine; (*9*) serine; (*10*) glutamic acid; (*11*) aspartic acid; (*12*) arginine; (*13*) asparagin; (*14*) ornithine; (*15*) cystein. In addition, the chromatograph revealed the presence of another amino acid in the buffalo hair which could not be identified. For comparison a chromatograph of sheep hair was taken, and this did not show the presence of the additional amino acid. The powder rings of buffalo hair may be attributed to this compound which exists as crystallites arranged in a perfectly random manner.

In feathers, the spots in the x-ray pattern were elongated into arcs. There were two arcs on either side and two or three comparatively less intense but slightly diffuse rings. The rings were superimposed on the arcs. The rings in the case of feathers were not so well defined as those due to buffalo hair. The patterns obtained from tail hairs of lion, elephant, and buffalo and the feather of pigeon are reproduced in Figure 1. The spacings obtained for feathers studied are given in Table IV.

TABLE IV
Feathers

	Arcs (pair I)		Arcs (pair II)		Halo	
Specimen	θ	d, A.	θ	d, A.	θ	d, A.
Parrot	4° 50′	9.150	9° 41′	4.583	13° 8′	3.393
Pigeon	5° 5′	8.676	9° 34′	4.638	13° 6′	3.402
Ostrich	5° 0′	8.841	9° 50′	4.514	14° 23′	3.103
Swan	4° 52′	9.081	9° 51′	4.514	14° 23′	3.103
Crane	4° 52′	9.081	9° 51′	4.514	13° 15′	3.363
Emu	4° 45′	9.311	9° 51′	4.514	12° 45′	3.502
White peacock	4° 52′	9.081	9° 45′	4.578	12° 45′	3.502
Kingfisher	4° 50′	9.150	9° 22′	4.737		
Crow	5° 1′	8.816	9° 22′	4.737		
Kite	4° 58′	8.905	9° 33′	4.647		

The feather keratin gives an entirely different pattern. The patterns obtained in this investigation closely resemble that of β-keratin. The arcs lie along the equatorial line, and their spacings are approximately 9 and 4.6A. These two values are typical of protein structures, in general, the first being the lateral side chain spacing and the second the backbone spacing of an extended polypeptide chain.

CONCLUSIONS

From the results obtained in this investigation the following conclusions may be drawn.

(*1*) In both the cases we recorded spots along the equatorial axis. This may be attributed to random orientation of the crystallites about the meridional axis.

(*2*) The presence of a number of powder rings in the case of buffalo hair may be due to the fact that the crystallites are present in all possible orientations about all possible axes in the fiber.

(*3*) A comparison of Tables I–III with Table IV clearly brings out that the side-chain spacing of feather keratin is slightly smaller than that of hair keratin while the backbone spacing is larger to a small extent. Thus the polypeptide chain, is elongated along the fiber axis and thinner in the side chain direction. As has been reported by Astbury and Marwick,[7] this may be explained by assuming that, even during the formation, the feather keratin is subjected to lateral pressure and the chain is in an extended state and any further large extensions, as in the case of hairs, may not be possible.

(*4*) The halo produced by feathers is comparatively less diffuse, thus showing that there is a more crystalline arrangement in feathers.

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Synopsis

A study of the x-ray diffraction of tail hairs of mammals, both domestic and wild, and feathers of birds has been made. The pattern due to buffalo hair is unique in the sense that about six Debye-Scherrer rings have also been recorded in addition to the usual pattern of α-keratin. The rings have been explained as due to crystallites in the fiber arranged at random about the fiber axis. The chromatographic record in the case of buffalo hair revealed the presence of a new amino acid. The d values have been calculated. The feather pattern resembles more that of β-keratin. A comparison of the study of the hairs and the feathers has been attempted.

Résumé

Le étude de la diffraction aux rayons-X de poils de mammifères domestiques et sauvages et de plumes d'oiseaux a été effectuée. Le spectre répondant aux poils de buffle est unique en ce sens que environ six anneaux Debye-

Scherrer ont été trouvés en plus de la photo normale de l' α-kératine. Les anneaux seraient dus à la présence de cristallites dans la fibre, arrangés statistiquement autour de l'axe de la fibre. L'examen chromatographique dans le cas des poils de buffle révèle la présence d'un nouvel acide aminé. Les valeurs *d* ont été calculées. L'image des plumes ressemble plus à celle de la β-kératine. Une comparaison de l'étude des poils et des plumes a été entreprise.

Zusammenfassung

Eine Untersuchung der Röntgenbeugung von Schwanzhaaren von Säugetieren, und zwar sowohl von Haustieren als auch von wilden Tieren, sowie der Federn von Vögeln wurde durchgeführt. Das Diagramm von Büffelhaar ist insofern einzigartig, als etwa sechs Debye-Scherreringe, zusätzlich zum normalen α-Keratindiagramm erhalten werden. Die Ringe wurden auf Kristallite in der Faser zurückgeführt, die statistisch um die Faserachse angeordnet sind. Die Chromatographie zeigte im Falle von Büffelhaar die Anwesenheit einer neuen Aminosäure. Die *d*-Werte wurden berechnet. Das Federdiagramm hat grössere Ahnlichkeit mit dem von β-Keratin. Ein Vergleich der Untersuchung an Haaren und Federn wurde versucht.

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