The Effect of Heat and Moisture on the Small Angle X-Ray Diffraction Diagram of Feather Keratin

by

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I. INTRODUCTION

A. Literature on Feather Keratin

1. X-ray diffraction studies

The earliest X-ray diffraction studies on feather keratin were reported by Astbury and Marwick (1,2). Feather keratin was also the first of all the protein fibers to be examined at low angles of diffraction: a consequence, probably, of its comparatively small fiber-axis period. The diagram published was made consonant with Astbury's existing (3) theories on keratin structure, based on wide-angle observations, by identifying it as only a more complex form of the pattern given by natural silk (fibroin) and stretched hair (β-keratin).

The wide-angle diffraction patterns of the fibrous proteins had been shown to be of two types: the keratin-myosin-epidermis-fibrinogen (k-m-e-f hereafter) group, fibers of which show long-range elasticity; and the collagen group, fibers of which are relatively inelastic. The k-m-e-f group includes the fibrous proteins of the epidermis of mammals, amphibians and certain fishes; the fibrous structures such as hair, horns, nails, spines, whalebone, etc., which arise from mammalian epidermis; the principal muscle protein, myosin; and fibrinogen and fibrin of mammalian blood. All these proteins in their normal,
unstretched form give a characteristic wide-angle pattern called the \( \alpha \)-keratin type. On stretching, however, they all are transformed in such a way as to give another characteristic wide-angle pattern, called the \( \beta \)-keratin type. The transformation is reversible on release of tension and may be repeated indefinitely without visible change.

The prototype of the \( \beta \)-keratin pattern has been taken to be the diffraction-rich diagram given by natural silk. Meyer and Mörk (4) suggested that the silk pattern derives from a structure in which fully extended polypeptide chains are aligned parallel to the fiber axis. Because the wide-angle patterns arising from stretched mammalian keratin (i.e., \( \beta \)-type) and from silk resemble each other, Astbury (3) has inferred that the \( \beta \)-configuration of keratin is also one of fully extended polypeptide chains. On the basis of X-ray and other data, the unstretched, \( \alpha \)-type keratin has been referred (5) to a molecular structure in which the polypeptide chains are folded in a regular manner. These interpretations of wide-angle fiber diagrams in terms of proteins have been extensively reviewed (6).

For comparison with the hypotheses outlined above it should be noted that Huggins (7) has proposed several structures for fibrous proteins for which he claims better
agreement with the experimental data than is the case with structures advanced by others. Whatever objections may be raised to Astbury's theory of keratin structure, however, it is supported by three separate lines of experimental evidence: (a) retention of the α-pattern during minor contraction must be taken as indicative of some kind of regular folding, (b) thermodynamic analysis requires the conclusion that elasticity of these fibers is not due to entropy change (the reverse of the random disorientation involved in rubber contraction), but does involve change in internal energy (8), and (c) electron microscope studies (9) indicate that fibrils follow a straight course whether contracted or relaxed.

While the chief structural protein of the vertebrate epidermal tissues gives X-ray patterns of the α-type, the patterns given by the harder epidermal structures of birds and reptiles, such as feathers, beaks, claws, scales, etc., in their native state indicate the polypeptide chains of these proteins to be in an approximate β-configuration. Because this type of pattern was first found in feather, it is called "feather keratin"; it is designated the prototype of the β-subgroup of the k-m-e-f group of fibrous proteins from the X-ray point of view (2,10,11). It is clear, however, from the diffraction diagrams published of feather (1,2 for example)
that the similarity between the structures of feather and stretched hair does not extend to the large periodicity responsible for the low-angle reflections. It is also evident that if the indices assigned by Astbury, from which he derived an orthorhombic unit cell (13), are correct, feather must either have an additional component to account for the apparently lower orders observed or be assigned more specific classification than that which can be based on its wide-angle assignment to the β-subgroup.

The assignment of feather to the β-keratin subgroup was qualified by a report (11) that the feather pattern arose from a structure that was contracted about 10% from the normal β-configuration in which the polypeptide chains are inferred to be fully extended. As evidence for this contracted state there were reported: (a) that the feather pattern had a prominent spacing at 3.1Å, instead of the 3.4Å spacing given by stretched hair, and (b) that the 3.1Å spacing could be increased to 3.3Å by stretching the feather to just below the point of breaking.

Astbury (14) reported Marwick's resolution of the 24Å meridional spacing of the feather pattern into a very close pair and assigned them as the 12th and 14th orders of a 309Å fundamental translation of a grouping of 100 amino-acid residues. Later (13) this value was
corrected on unexplained grounds to "at least 70A, but perhaps as high as 304A."

Clark et al (15) reported measuring an equatorial spacing of 81A in the pattern of a specimen identified only as "keratin" and stated, without giving a reference, that this measurement confirmed a prediction by Astbury that a lateral spacing of approximately 90A should exist. Corey and Wyckoff (16) reported their highest meridional spacing on patterns from chicken feather as 23.1A (strong), which may be considered as confirmation of Astbury and Warwick's results with sea-gull "quill" (2). Corey and Wyckoff also reported from the same patterns equatorial spacings corresponding to 51.0 (faint), 81.8 (medium) and 115A (medium) which have not been confirmed to date. Bear has suggested (14) that general radiation artifacts associated with the intense fourth meridional order and the equatorial 34A arc were probably the source of the long spacings reported by Corey and Wyckoff, and that another type of artifact may have caused Astbury (13,14) to suspect that the fundamental fiber-axis translation might be as high as 309A. The doublet reported by Astbury (14) for the intense 24A meridional arc in support of this large spacing Bear (17) ascribes to the Cu Kα satellite which is almost invariably recorded even with otherwise adequate Ni filtering.
Bear (17,18) has reported low-angle fiber-axis spacings of feather keratin based on measurements of 18 orders of meridional and near-meridional reflections, which gave an average fiber-axis period of 94.6A. From the Bernal rotation diagram coordinates, $\xi$ (xi) and $\xi$ (zeta), given it was clear that many of the row-line coordinates, $\xi$, were those of the prominent row line ($\xi = 0.045$), corresponding to a lateral spacing of 34A. The paucity of reflections on this row line and the poor accuracy with which they could be measured made it impossible at that time to determine the dimensions of the macrocell other than the fiber-axis primitive translation noted above.

Astbury and Lomax (19), from wide-angle patterns of keratins, from Bernal and Crowfoot's (20) patterns of crystalline pepsin and from fibrin patterns published by Katz and DeRooy (21), inferred that the structure of feather keratin arose by the polymerization of a linear array of corpuscular units. While admitting that proof of this point was wanting, they quote some experimental sources as evidence (22). Bear (17) in considering complete diffraction patterns given by several types of protein fibers, including feather, finds that a common feature is the increased sharpness and greater number of diffractions in the meridional direction as compared to those in transverse directions in both the wide- and
small-angle regions. He states that it is difficult to conclude definitely on the basis of existing data whether the structural order indicated by these observations arises from construction of the constituent fibrils from longitudinally oriented polypeptide chains or from long arrays of particles attached end-to-end.

Astbury has described (6) the feather keratin diagram as one consisting of the pattern (wide-angle) of the β-configuration, characteristic of the structure of component units, upon which is superimposed a "giant pattern" (low-angle) deriving from a particular combination of those structural units. In support of this inference, he makes the observation that prolonged exposure of feather to steam destroys the definition of the higher (low-angle) pattern leaving only that of the β-configuration. This appears to suggest that he would assign the low-angle pattern to a different unit of structure than that from which the wide-angle pattern is derived.

Bear (17) has discussed the relationship of wide-angle to low-angle scattering of X-rays by protein fibers in detail and concludes that the fine structure of the wide-angle diffraction supports the view that they are aggregates of high orders of large spacings. Because exact assignment of such large indices is impossible in these patterns, he advises that the short spacings (wide-angle reflections) given by protein fibers be considered as
related to small pseudo-unit cells which are only part of the macrocell. He also explains that large struc-
tural patterns (low-angle reflections) are more sus-
ceptible to disorienting influences and, therefore, may escape recording by X-rays.

Although Astbury has often stated that his general theory concerning keratin structure would be confirmed if members of all classes could be demonstrated to give pat-
terns ascribable to the three postulated states of peptide chain disposition (see for example 23), he has not re-
ported success in transforming the feather pattern to the \( \alpha \)-type by any kind of treatment to date.

Rudall (24) has studied extensively the "com-
parative biophysics" of the keratins. Using low-angle X-ray diffraction he has been able to relate the occurrence of the various types of keratin structure with the phylogeny of the animal forms in which each type is found. In this manner, for example, he has found independent physical evidence of a close relationship between the birds and the reptiles. His studies have also made clear the manner in which the typical feather keratin is laid down in parallel with the \( \alpha \)-type by the feather-generating cells of birds.
2. Other evidence on chemistry and structure

Block has stated (25) that the keratins are characterized by a ratio of their component basic amino acids histidine:arginine:lysine of 1:12:4 and by their high stability and insolubility. The ratio of the basic amino acids indicated by Block as a general property, however, has not been born out (26). The keratins are also known to have a typically high sulfur and cystine content (27). It has been observed by K. H. Meyer (4) that the high cystine content of the keratins can be linked with their insolubility, and it has been assumed that the polypeptide chains of the keratins are held together by S-S bridges. Speakman and his co-workers, in a long list of papers (28), have implemented these assumptions in part, for wool at least, and demonstrate as a basic bond the co-valent S-S bridge between the two halves of the cystine residues of contiguous chains of peptides. He has also presented evidence for the linking of adjacent polypeptide chains by salt linkages between the dicarboxylic amino acids (aspartic and glutamic acids) on one chain with basic amino acids (lysine and arginine) on another.

Goddard and Michaelis (29) concluded that dispersion of chicken feather keratin required not only the reduction of disulfide bonds, but also that the salt
linkages of the molecule must be broken by alkali. These conclusions were used as a basis for work by Jones and Mecham on extensive dispersion of chicken feathers by sodium sulfide (30) and by a variety of other reducing agents acting on the S-S bond in neutral solution (31). The selective susceptibility of feather keratin to dispersion in comparison to other keratins could not be explained by these authors.

Lundgren (32) prepared fibers from solutions of chicken feather dispersed in neutral solutions of reducing agents in the presence of detergents containing long-chain polar groups. When the fibers were stretched they gave a typical β-keratin pattern without any of the rich low-angle detail typical of patterns from untreated feather (32) and undistinguished from that of a fiber made similarly from egg albumin (33). It was shown (34) that the dispersed feather keratin in solution had an average molecular weight of 75,100 from the sedimentation and diffusion constants. Later Lundgren, Stein, Koorn and O'Connell (35) presented evidence from studies on alcohol-water-salt mixtures and feather keratin to prove that the fiber network is stabilized by salt linkages, which are affected by inorganic ions; non-electrostatic forces (presumably H-bonds), which are influenced by alcohols (activity of which increases with salt); and S-S cross-links, which are broken by reducing agents.
The tendency for amino acids to repeat periodically in protein chains which was first intimated by Kossel and Schenk (36) and Waldschmidt-Leitz and Kofranyi (37) has been extended by Bergmann. In the hypothesis advanced by Bergmann and Niemann (38) on the arrangement of amino acids in the protein molecule there is the basic assumption that the amino-acid residues occur in fixed and invariant periodicity along the peptide chain. It was further postulated that all proteins have 288 \(2^{m}3^{n}\) residues or a whole number multiple thereof and that the molecular weight classes reported by Svedberg (39) are a reflection of the 288 unit.

Aside from damage done the theory by the now well-established fact that the Svedberg hypothesis is untenable, many other objections have been raised. These objections have been summarised especially by Chibnall (40) and Bull (41) and fall into these main groups: (a) The choice of the number 288 simultaneously provides the greatest purely arithmetic chances of fitting the data, gives a very high probability that random distribution of amino acids will accord with the \(2^{m}3^{n}\) formula, and requires the least violence to analytical figures to make them fit the theory. Further, the gaps in the \(2^{m}3^{n}\) series are small except in two places. (b) Analytical values for amino acid content which were used as a basis for the hypothesis and for its extension to other proteins have not been sufficiently
reliable to provide any critical check on the hypothesis.
(c) Clear X-ray evidence for regular and invariant
periodicity is lacking. (d) Calculation of the average
residue weight involves sufficient uncertainty to give a
significant error in the frequency value calculated.
(e) In the case of gelatin, for which extensive amino
acid analyses are available, glycine and proline would
compete for the 21st positions along the chain. (f) In
the case of several other proteins in which the number
of residues have been derived from both physical methods
and analysis, the Bergmann value cannot be calculated.
(g) Lacto-globulin and insulin, by titration and Svedberg
molecular weight values, give a number of residues dif-
ferent from 288n. (h) Enzyme studies do not support the
hypothesis.

While Astbury (42) has often discussed keratin
structure from the stoichiometric point of view, it would
appear that the best amino-acid composition values avail-
able to date for feather (26,27) (Table I), are far from
a sufficient basis for testing any periodicity theory in
detail.

In connection with the reaction of water and α-
keratin it has been stated (43) that water causes a lon-
gitudinal swelling of about 1% of the fiber length and
that chemical treatment, such as with dilute aqueous sodium
sulfide, can cause marked changes in the affinity of wool
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<th>Amino Acid Composition of Representative Keratins (%)</th>
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<tr>
<td></td>
</tr>
<tr>
<td>Glycine</td>
</tr>
<tr>
<td>Alanine</td>
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<tr>
<td>Phenylalanine</td>
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<tr>
<td>Leucine</td>
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<tr>
<td>Valine</td>
</tr>
<tr>
<td>Proline</td>
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<tr>
<td>Glutamic acid</td>
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<tr>
<td>Aspartic acid</td>
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<td>Arginine</td>
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<td>Lysine</td>
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<td>Histidine</td>
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<td>Threonine</td>
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<td>Methionine</td>
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<td>Cystine</td>
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<td>Tryptophane</td>
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<td>Tyrosine</td>
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(1) Values given in Block and Bolling (27), as best averages from the literature (except as noted).  
(2) From Salo (26) (except as noted). Data on barb material only, rachis or calamus not included.  
(3) Block and Bolling, loc. cit., value for gull feather. (4) Block and Bolling, loc. cit., value for unknown type. (5) Salo, loc. cit., for Canada porcupine, Erethizon dorsatus.  
(6) Includes isoleucine.
and hair keratin for water. Lateral swelling due to water is approximately ten times as great as the longitudinal effect. It has been shown by Speakman (44) that water adsorption was not the result of reaction with free amino groups and that (45) the swelling is due predominantly to intermicellar adsorption on the basis of Katz' (46) definition which is based on failure to change the X-ray pattern by swelling. Water penetration between molecular chains was said by Astbury and Lomax (47) to explain the continuous increase in lateral spacing; the drawing out of reflections along layer lines as a result of treatment with steam being said to indicate a unidirectional disturbance parallel to the side-chains.

Lloyd and Phillips (48) correlated the insolubility of the fibrous proteins with their preponderance of short-chain aliphatic amino acids and stated that as oriented structure developed in proteins a stability range toward hydrating influences appears between pH 4 and 8. The tendency for keratin hydration to increase with reduction in pH below the isoelectric point was correlated by Speakman and Hirst (49) with the concomitant increased ease of stretching. Above 55°C. it was concluded by Speakman and Cooper (50) that the S-S bonds of wool were attacked and hydrolyzed by water vapor. The products of hydrolysis reacted with Hg vapor to form mercuric sulfide in a period of two days. In the absence of Hg vapor volatile sulfur-
containing compounds were evolved.

In an extension of the hydration studies Speakman (51), using sodium sulfide, was led to conclude that the micelles of wool are lamellar in shape. Senti, Copley and Nutting (52), in a paper on artificial fiber made from corpuscular proteins, state that the crystallites are much longer in the fiber-axis direction than in the side-chain direction.

Closely related to the question of hydration is the combination of the keratins with acids. Speakman and Stott (53) have presented evidence that swelling of wool is greater at any given pH with weak acids like mono-chloroacetic and formic than with strong acids. Steinhardt, Fugitt and Harris (54), as part of an extended study on wool, have shown that weak acids combine with keratin in the undissociated form as well as the ionic form, and that the amounts combined in undissociated form may far exceed that bound from ionic form.

From the titration curves of feathers from several different kinds of birds Speakman and Townsend (55) determined the isoelectric point of feather keratin as pH 4.6. The feathers had been extracted to remove fats and waxes.

The reactions of proteins with chemical reagents has been summarized by Herriott (56) including iodination of tyrosyl and other residues and reduction of S-S bonds.
Clcott and Fraenkel-Conrat (57) summarize specific group reagents for proteins, such as phenylmercuric chloride for -SH groups and iodine for tyrosyl groups. Of the reactions indicated in these references, the only application in tagging specific groups of keratin for X-ray diffraction studies has been the use of mercuric acetate by Rudall (58).

On the basis largely of electron micrographs, Farrant et al. (59) have suggested, from work on the amorphous matrix material of wool cortex, that the keratin molecule is a well-ordered discrete unit similar to that postulated by Astbury for globular proteins. They further state that fibrous keratin does not consist of long chains, but of strings of globular molecules as for clam muscle protein (60), later (61) known as paramyosin. This is not specifically extended to feather keratin, but a close connection is implied.

B. Theoretical Considerations

1. Paramyosin lattice and generalization

Bolduan and Bear (62) described criteria by which low-angle X-ray diffraction diagrams may be used to determine, at the level of large units of structure, the degree of order characteristic of submicroscopic fibrous protein diffractors. This method involves predominantly consideration
of shapes of diffractions, observation of movement of reflections with tilt of specimens, and determination of the number of lattice translations necessary to account for all diffractions observed. By use of this method the large structure of collagen was demonstrated to be ordered in only one dimension. By the same technic α-keratin (porcupine quill tip) and β-keratin (feather rachis) are probably three-dimensional while muscle fibrils (paramyosin and "myosin") apparently are two-dimensional.

Evidence has been reported, on the basis of electron micrographs of clam muscle, that paramyosin has a two-dimensional lattice of submicroscopic structural elements (60). Hall, Jakus and Schmitt (60) analyzed the paramyosin lattice in terms of two integers, one of which describes the way in which parallel planes passed through equivalent nodes of the structure, orthogonal to the fiber axis, divide the fundamental spacing; the other integer being related to an angle at which equivalent nodes cut the fiber axis. There results the possibility of describing the lattice in terms of a simple net of primitive symmetry, with one axis obliquely inclined to the fiber axis, forming non-primitive cells.

There follows an example from the analysis of the paramyosin lattice as determined by electron microscope studies: Figure 1 is the diagrammatic lattice derived
Figure 1. Diagrammatic lattice of Venus paramyosin showing geometrical relations and dimensions between stained regions (from Schmitt, Bear, Hall and Jakus (61)).
from electron micrographs of *Venus mercenaria* (62) adductor muscle, later called paramyosin (61), stained with phosphotungstic acid. The lattice geometry has been described in terms of \( d \), the spacing between adjacent like cross bands; \( s \), the lateral distance between centers of adjacent spots; and the angles \( \alpha \) and \( \beta \), thus:

\[
5d \tan \alpha = 2s, \quad 5d \tan \beta = 3s, \quad (1)
\]

and

\[
\tan \alpha / \tan \beta = 2/3 \quad (2)
\]

Numerical values for all quantities in (1) and (2) were obtained from enlarged micrographs. The numerical values in Fig. 1 are averages for measurements of \( d \) and \( s \) for six fibrils and calculated values for the angles.

The equations found advantageous in the above example, because they include the characteristic integral values and contain only readily measurable quantities, may be put into more general form for use in analyzing other lattices of the same general type, where \( m \) and \( n \) are any integers:

\[
\tan \alpha = (m/n) \cdot s/d, \\
\tan \beta = [(n-m)/m] \cdot s/d \quad (3)
\]

and

\[
\tan \alpha / \tan \beta = m/(n-m) \quad (4)
\]

For use with measurements derived from X-ray diffraction data Bear (63) developed selection rules
analogous to the expressions used above for the electron microscope case. The selection rules are used in determining what diffractions may be observed from a given lattice and are derived from the structure factor to permit prediction of intensities of diffractions:

\[
\widehat{F}_{hk} = \int \sum_{m=0}^{n=p+q-1} e^{i2\pi [n \frac{p_n}{p+q} + k \frac{q}{p+q}]} \]

where \( f \) is the structure factor common for all equivalent positions of the lattice and tells the number of electrons about each equivalent position, the exponential factor expresses the phase factor, \( n \) is an integer numbering equivalent positions in the cell, \( h \) and \( k \) are analogous to Miller indices and \( pn/p+q \) and \( n/p+q \) are coordinates for the \( n \)th equivalent position of the structure. This may be transformed to:

\[
\widehat{F}_{hk} = \int \sum e^{i \frac{2\pi}{p+q} (hp + k)n} \]

and through an expanding series identity to:

\[
\widehat{F}_{hk} = \int \left[ \frac{e^{i2\pi(hp+k)}}{e^{i2\pi(hp+k)}/(p+q)} - 1 \right] \]
Since this is an amplitude, it must be multiplied by its complex conjugate to give an intensity expression:

\[
\sqrt{f_{hk} f_{hk}^*} = \sqrt{\frac{\sin^2 \pi (hp+k)}{\sin^2 \pi (hp+k)}} \frac{\sin^2 \pi (hp+k)}{(p+q)}
\]

In substituting in equation (8) a random choice of integers for \( h, p, \) and \( k \) will give a numerator equal to zero. Diffractions are, therefore, given only when

\[
\frac{hp+k}{p+q} = m,
\]

where \( m \) is any integer. The index \( h \) is constant on any row line; \( h \) cannot be distinguished from \( -h \) so that solving for \( k \) yields:

\[
k = (p+q)m+hp,
\]

\( k \) then represents the layer lines on which diffractions at the row line of \( h \) can be expected in the pattern of the structure. In the case of the two-dimensional paramyosin net treated above for use with electron micrographs, when applied to X-ray diffraction use, \( p = 2 \) and \( q = 3 \) and

\[
k = 5m+2h,
\]

which selection rule is observed to hold to a fair degree of precision on paramyosin diffraction patterns.
By a similar treatment Bear (63) found that for three-dimensional structures of corresponding type, having at least the monoclinic degree of symmetry (as shown by row lines being perpendicular to layer lines when observed) the selection rule can be expressed in terms of three integers as follows:

$$k = Pm\pm(hp + lq),$$

(12)

where $k$ is the layer-line index, $h$ and $l$ the row line indices, $P$ expresses the number of equivalent positions per non-primitive unit cell, and $p$ and $q$ determine the orientations of two sets of planes relative to the $xy$ and $yz$ planes, and $m$ is any integer. When the non-primitive cell dimensions and angle are known, the selection rule governing the intensity of reflections along layer lines and row lines will permit the dimensions of the primitive unit cell to be calculated.

2. Nature of feather keratin pattern

On the basis of considerations outlined above, some preliminary conclusions may be drawn on the structure of feather keratin through the use of published data (17,18). Since the row lines observed in a feather pattern appear to be normal to the layer lines, it may be concluded for three-dimensional crystallites that the fiber axis is normal to the other two axes. This signifies at least monoclinic symmetry.
Tilting experiments by Bear and Bolduan (64) with feather gave movement of the reflections on the row lines, but not of those on the meridian. These observations provide presumptive evidence for the existence of three-dimensional order. It is to be noted, however, that if the feather lattice were ordered in only two dimensions and the third dimension were sufficiently large, the tilting behavior of a three-dimensional system would be approached.

The necessity of having to fall back, as a result of the tilting experiments described above, onto the number of lattice translations that would be necessary to account for all the observed diffractions is also fraught with intrinsic difficulty. Diffuseness of reflections in their lateral dimensions, possibly due to crystallite disorientation, has made it impossible to determine whether all the diffractions which appear to be on the strong 34A row line actually constitute only one row line.

With the diffractions observed no overall fit of the selection rule stated above for the three-dimensional case, equation (12), can be achieved. One may note, however, some approximate relations of this type for the innermost spots. Bear's data (17) indicate that the only meridional diffractions at low-angles are \( k = 4 \) and 8 and that in the lowest "subpattern," the diffractions lie
closely on the apparently complex principal row line at k indices 0, 2 and 4. Values of $\xi$ included in the same reference indicate that two values recur for spots on the prominent row, i.e., 0.041 and 0.046. If one of these values is taken as a row line $h = 1$, $l = 0$ and the other as $h = 0$, $l = 1$, the selection rule above, equation (12), reduces to:

$$k = Pm, \quad k = Pm + p, \quad k = Pm + q$$

(13)

for the meridian and the two possible row lines indicated.

Substituting in these equations for the sub-patterns nearest the equator it is found that $P = 4$ since the innermost meridional spots have indices equal to multiples of 4.

Further use of the selection rule for inner row-line spots suggests $p = 2$ and $q = 4$. When $q = P$, a third set of planes defining the lattice nodes may be taken parallel to the fiber axis. In the case of the postulated 3-dimensional lattice, the $q = P$ value signifies a stack of lamellae made by translation of 2-dimensional planes. Figure 2 is a diagram of a possible lattice of this type.

Further progress in testing the adequacy of this or other possible cells depends on obtaining satisfactory patterns, from which separation of close row lines can be secured, and measurements permitting calculation of the dimensions of the primitive cell can be derived.
Figure 2. Diagram of lattice consisting of "stacked" two-dimensional lamellae (as indicated by selection rule analysis of Bear's data, where the circles indicate equivalent nodes of electron density in the structure).
3. Statement of problem

Since sufficiently well defined patterns have not been available for the unequivocal determination of the macrocell, the major task is the improvement of experimental technique toward the end of producing well-resolved and accurately measurable films. This will include especially the design of cameras with suitable resolution and improvement of specimen preparation method.

To aid in crystallographic indexing of diagrams, chemical and physical manipulation of specimens is indicated with a view to changing the distribution of intensities among diffractions, increasing the number and sharpening the quality of the reflections obtained, diminishing disorientation of submicroscopic crystallites, increasing separation of row lines by increased resolving power of cameras or by swelling the structure through reduction of S-S bonds or loosening of other lateral linkages, "tagging" specific residues of the keratin molecules, and changing both wide- and low-angle patterns by swelling or stretching the structure.

Confirmation and refinement of published values for the fiber-axis translation and establishment of the second translation of the unit cell are primary goals.
II. EXPERIMENTAL METHODS

The usual procedures for photographing the diffraction patterns of fibrous organic materials with Cu Kα radiation were employed. The long exposures (of the order of days to weeks) involved in optimal resolution of the long feather keratin spacings made necessary refinements in camera design and use. The normal disorientation of diffracting elements in feather required improvement of specimen preparation techniques. Crystallographic indexing of the complex diffraction patterns given by feather keratin is at once extremely difficult, and of profound necessity in the elucidation of structure. In an attempt to provide aids to indexing, the present work included physical and chemical treatments of the specimen material which were aimed at enlightening modification of the diagrams. There follows a description of the more important experimental equipment and procedures.

A. Radiation Characteristics and Specimen Thickness

The X-ray tubes were of the North American Philips Co. Inc., Cu-target sealed type with mica windows. To monochromatize the Cu radiation while maintaining a useful level of Cu Kα radiation a single Ni foil (0.015 mm. thickness) was used.
When, as in this study, the specimen is wider than the cross-section of the incident X-ray beam, the optimum thickness has been shown to be $1/\mu$, where $\mu$ is the linear absorption coefficient of the specimen material for X-rays (65). For proteins having the approximate elementary composition of feather keratin (66) the value of $\mu$ for Cu Kα radiation is of the order of $10.\text{cm}^{-1}$, which corresponds to a specimen thickness of about 1 mm. Specimens were cut to this thickness or, when necessary, were built up by combining platelets under a dissection microscope.

B. Camera Design

In accordance with principles reviewed by Bear (67), adequate angular resolution and suitable specimen-to-film distances were selected on the basis of previous measurements on the long spacings of feather keratin (17,18), the precision of measurement desired, and permissible length of exposure. For relatively rapid preliminary examination not intended for accurate measurement, camera construction was based on pinhole dimensions easily obtainable by good instrument-making practice. For cameras capable of resolving the fiber-axis spacings and permitting an adequate precision of measurement, but without prolonging exposure time unduly, more critical design was required. In these cases pinhole dimensions and collimating tube lengths
were calculated from expressions derived for the design of optimum cameras by Bolduan and Bear (68) and constructed with the aid of a microscope. In the alignment of fine pinholes precision was attained with the aid of a high-intensity point source of light and the light diffraction patterns produced.

Given below are the critical dimensions of the cameras employed in the work here reported. The proximal aperture (p), distal aperture (r), distance between proximal and distal apertures (v), specimen–tc–film distance (s), guard aperture (q), and the distance between distal and guard apertures (w) are given under the indicated symbols and refer to Figure 3.

Table II

Critical Dimensions of X-Ray Diffraction Cameras
(millimeters)

<table>
<thead>
<tr>
<th>Camera</th>
<th>p</th>
<th>r</th>
<th>v</th>
<th>s</th>
<th>q</th>
<th>w</th>
<th>Actual Resolving Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ways</td>
<td>.30</td>
<td>.30</td>
<td>57</td>
<td>50</td>
<td>.6</td>
<td>13</td>
<td>110-125A</td>
</tr>
<tr>
<td>R-1</td>
<td>.118</td>
<td>.071</td>
<td>57</td>
<td>70</td>
<td>.242</td>
<td>15</td>
<td>330A</td>
</tr>
<tr>
<td>R-2</td>
<td>.274</td>
<td>.164</td>
<td>65.6</td>
<td>80</td>
<td>.324</td>
<td>16.4</td>
<td>ca. 170A</td>
</tr>
</tbody>
</table>
Resolution shown in the last column of Table II was calculated on the following practical basis. If consecutive orders of diffractions are considered to be at the limit of visual resolution when their adjacent edges are coterminous, it is evident that the practical resolving power of the camera in which they were recorded would be given by the relation

$$R.P. = d \cdot \frac{y}{b}$$

(14)

where R.P. (actual resolving power) is in Angstrom units when d is the structure period giving rise to the diffractions in Angstrom units, y is the distance between centers of reflections on consecutive layer lines in millimeters, and b is the size of the image on the film of the undiffracted beam in millimeters. In this case the structure period was approximated at 95A (see Table III) and y and b were average values of many measurements made on several films in each case.

All of the cameras described above were operated in air. Lead beads were provided, just before the photographic film, of such dimensions in each case as to intercept the undiffracted beam, without stopping the diffracted beam corresponding to the lowest observable order.

To make most efficient use of the radiation, cameras were in all cases aligned to a critical angle in respect to the plane of the target. Adjustment was initially
visual with a fluorescent screen and finally photographic on fine-grain X-ray film (Eastman-Type A).

C. Photography

Diffraction diagrams were recorded photographically on Eastman Nb-Screen or Type K film mounted in plane type cassettes. All films were developed in 10 minutes in Eastman X-Ray Developer at 20°C, fixed 10 minutes in Eastman Liquid X-Ray Fixer at the same temperature, washed in running tap water (temperature from 18° to 26°C) for 1 hour, immersed for approximately 30 seconds in a dilute solution of a surface active agent and hung to dry under atmospheric conditions. When wide variations in intensities between different orders of reflections on the same specimen were anticipated, the cassette was loaded with a pair of films one behind the other. This practice facilitated examination by recording all reflections at near-optimal density with a single exposure.

D. Specimen Preparation

In the preparation of specimens considerable effort was expended in the selection of material and its manipulation to insure the production of patterns of the greatest possible orientation and clarity. Bear (17) had agreed with Astbury and others (13) in having obtained the best
diffraction results from sea-gull samples. On the basis of their experience, cast-off primary feathers of unknown species of gulls of local habitat were used throughout this study. In accordance with Bear's technique (17), essentially flat strips were cut from the ridges of the ventral cortex of the rachis on either side of the ventral groove. The length of the strips was determined in each case by the length of the specific feather from a point about 1/2 cm. distal to the superior umbilicus to the point, further distal, at which the normal taper of the rachis made the strip too narrow for practical use. After excision, each strip was trimmed in width, on the stage of the dissecting microscope, to remove any portions in which the fibers did not seem to be parallel to the axis. The final width was not allowed to deviate significantly from 1 mm. The broad faces of the strips were then scraped superficially to remove adhering remnants of the white spongy medulla from the internal surface, and any dis-oriented fibers from the external surface.

As a possible guide to the orientation of the crystallites, attempts were made to determine the packing of keratin cells in the cortex by histological techniques. Several experiments, in which segments of rachis were im-bedded in paraffin or celloidin, sectioned with a micro-tome, and examined microscopically, failed to yield sig-nificant information on this point.
Some improvement over specimens made by published techniques was essayed by more radical scraping of both faces to remove surface layers of disoriented \( \alpha \)-keratin. The existence of these layers had been first reported by Rudall (58) in connection with his phylogenetic study of the keratins by X-rays. His diagram, based on the earlier histological studies of Lillie and Wang, is given in Fig. 4. This treatment had the incidental effect of making the strips flat and of a final thickness of approximately 1/10 mm. The strips were then cut into platelets 1 cm. long, in which form they were of appropriate size for combination into specimens.

The 1/2 x 1/10 x 10 mm. platelets were stacked on their broad faces until 1 mm. thick, in accordance with the optimum thickness considerations outlined above, and held together either with a small amount of cellulose acetate cement or by tying with fine (15 denier) nylon filament or silk thread. The specimen was then cemented firmly on the specimen holder of the camera in such a manner as to prevent movement during prolonged exposure. The amount of cement in the path of the X-rays was held to a minimum to reduce extraneous absorption and incoherent scatter.

Initially, in keeping with earlier techniques of this Laboratory, specimens were exposed with the X-ray
Figure 4. Diagram of growing feather calamus; —— feather keratin; ——α-keratin; col. collar; r.c. regeneration cells (from Rudall after Lillie and Wang).
beam perpendicular to the broad faces of the specimen's component platelets (and perpendicular to the fiber axis). Later, when the specimen was oriented with these faces parallel to the beam, improved orientation of the diffraction pattern resulted. All later diagrams were made with specimens oriented in this manner.

In several experiments, a study was made of the effect of end-to-end orientation of the platelets relative to one another in the final specimen. Since this factor did not introduce significant change in the patterns, it was concluded that this refinement was not productive. In all later specimens end-to-end placing of platelets was allowed to be random. The patterns reproduced in Fig. 5 illustrate the effects of the improved techniques outlined above.

E. Chemical Procedure

Once the clarity and orientation of patterns had been improved to the practical limit of physical manipulation, further changes in patterns depended on chemical procedures. These are outlined below.

1. Extraction of fats and waxes

The first step was to extract specimens exhaustively to remove fats and waxes. The purpose of this
Figure 5. X-ray patterns of feather rachis illustrating effects of improved techniques. (a) with original technique, beam perpendicular to face of specimen platelets, (b) with improved preparation, beam perpendicular to face, and (c) with improved preparation, beam parallel to face. It will be noted that (b), while of a generally more diffuse appearance, bears indications of improved crystal-lite orientation in that the "arcing" of reflections along layer lines has been reduced; (c) shows improved resolution along each of the "diffuse" outer row lines.
procedure was two-fold: (a) to eliminate the effect of these substances in impeding the transfer of polar solvents between the specimen and its medium in any future treatment, and (b) to eliminate any diffuse scattering of X-rays which might be caused by these substances. Feather samples, either just before or after the cleaned strips were cut into segments, were extracted by refluxing with successive increments of various organic solvents at 45°C for 1 1/2 to 18 hours in each case. 5:95 absolute ethyl alcohol:benzene, 20:80 water:dioxane, absolute methyl alcohol and chloroform were the solvents used. The order in which the solvents were used was varied slightly in different batches. In each case, after decanting one solvent, the sample was washed thoroughly with fresh increments of the same solvent before proceeding with the next.

For comparison, one set of feather samples was extracted with the same group of solvents as before, but using a Soxhlet apparatus in place of the batch technique. When the extraction was completed the samples were stored either dry in a clean, stoppered Pyrex test tube or under absolute methyl alcohol in a tightly-sealed screw-cap vial.

None of the variations noted above introduced any detectable difference in the patterns obtained. A crude gravimetric analysis on one batch indicated that the material extracted by all solvents together was equal to approximately 0.1% of the total initial weight of sample.
The extracted material was not further investigated. All samples from this point were extracted in the manner outlined above.

2. Wetting and drying

The effects of wetting on the structure of feather keratin were tested by making diffraction patterns of specimens immersed in distilled water. Evaporation of the water was prevented by enclosing the specimen in a closely-fitting glass capillary heat-sealed at both ends. To minimize absorption and scattering by the capillary, Pyrex glass of wall thickness not exceeding .018-.025 mm. was used. To prevent heating the specimen during the sealing operation, considerable care was used in manipulating a small and intensely hot oxygen flame. Checks for cracks which would cause leakage were made in each case by microscopic examination. Despite this precaution there were occasional cases in which undetected micro-cracks permitted loss of water during exposure. In all such cases the patterns were rejected. Preliminary experiments indicated that specimens came into equilibrium with water rapidly and that no special precautions were necessary in this connection.

Dry specimens were prepared and protected from atmospheric rehydration by heating in a vacuum oven and sealing in a capillary. Before entering the oven, the
specimen was mounted in a capillary of the type described above, one end of which had been sealed. The capillary was then affixed to a specimen holder for ease of manipulation and to aid in heat transfer. After drying 24–36 hours at 80°C over phosphorus pentoxide, the vacuum was relieved slowly through a tube containing anhydrous CaCl₂ and the specimen immediately removed to a desiccator for cooling. Once cool, the capillary was sealed with an oxygen flame as before.

Breakage and X-ray absorption and scatter were sources of difficulty despite the great care exercised in making and handling capillaries. In an effort to solve this problem, some capillaries were made of methyl methacrylate (lucite) polymer by dipping cleaned and polished wires into a chloroform solution of the plastic and withdrawing them at a controlled and constant rate. In this manner capillaries could be made of any predetermined bore and wall thickness without breakage or waste. Capillaries made of this material were flexible and could be heat sealed at a temperature sufficiently low as to avoid danger of heat damage to the specimen. Preliminary exposures, however, showed some slight scattering by the lucite. These capillaries were, therefore, used in preliminary experiments only in this investigation; but the technique merits further development for use in diffraction studies.
There is also the possibility of preserving specimens by polymerizing methyl methacrylate monomer or dimer around them. It appears possible to modify techniques in current use for metallographic samples toward this end.

3. Heat treatment

As an extension of hydration experiments the effect of heat on chemical modification of feather structure was studied by exposing uniform specimens to controlled temperatures: dry and in the presence of water, with and without other reagents. In all cases the specimens were protected from extraneous effects by suitable techniques. That most commonly used was to enclose the specimen and any reagent(s) in ampoules made by sealing chemically-clean Pyrex test tubes before heating.

For heating, an air oven, water thermostat, refluxing system or steam autoclave was used, as appropriate and convenient to the requirements of each experiment. Heat treatment was in all cases followed immediately by air or water cooling (as suitable) to room temperature. Where indicated, a desiccator was used for air cooling.

4. Reduction with sodium sulfite

Previous workers had reported marked effects of reducing agents on the structure of feather keratin
(29-32, 35, 69) in terms of the properties of dispersed protein, but X-ray diffraction studies on the intact structure were not included. Feather was treated with 2% aqueous sodium sulfite, in conjunction with the heat treatment outlined above, in a preliminary survey of the effects on the X-ray patterns.

5. Phenylmercuric chloride tagging

In early experiments there were indications that volatile sulfur compounds could be produced from feather by simple means in a water medium without the complicating presence of additional reagents. This was concluded to be due to S-S bond breakdown. From this and the premise that S-S bond breakage in other keratins has been reported to liberate reactive -SH groups (for example 29,70), the possibility arose of using reagents that would combine specifically with -SH groups and "tag" them for detection. Evidence of the validity of this argument was obtained when microscopic examination showed that feather had reacted when treated with the colored sulfhydryl reagent reported by Bennet et al (71). It was attempted to extend the results of this experiment by "tagging" with a compound which would scatter X-rays strongly in addition to being specific for -SH groups. In consequence, specimens of feather were treated with phenylmercuric chloride in aqueous, butanol and butanol-water solutions at various
temperatures for X-ray examination. Phenylmercuric chloride was used because it would confer high electron density, is reported to be highly specific for -SH groups (57), is easily available and inexpensive. As a result of preliminary experiments, it was thought that because of its low solubility in water, a sufficient concentration of the mercury salt was not available for the reaction. To avoid this problem n-butanol, in which phenyl-HgO1 is relatively soluble, was used. The compound used throughout was that listed by Eastman Kodak Company as phenylmercuric chloride, MP 254-255°C. In all cases solutions were saturated in respect to the mercury salt in the specific solvent.

6. Butanol

As a result of using butanol as a solvent, as indicated in the section on phenylmercuric chloride, the effects of butanol on the action of water and other reagents on feather were studied at various temperatures. The Eastman Kodak Company n-butyl alcohol, BP 116-118°C., was used in all experiments. In cases where water was used, its concentration was such as to saturate the butanol at room temperature.

7. Iodine "tagging"

On the premise of identifying the tyrosyl residues of the feather structure by X-ray methods, preliminary
efforts were made to tag them with iodine. Feather samples were maintained at 75°C in a closed vessel in contact with I₂ vapor for several days. To eliminate the possibility of scattering or absorption by uncombined iodine that may have been sublimed on the surface, specimens were removed from the vessel and kept in an unconfined space at 75°C for several days. As a control for the effects of dry heat alone, a parallel sample was heated at 75°C in an air oven for a length of time equal to that of the entire iodine treatment.

8. Tilting experiments

One of the methods available for determining the degree of order of large-period fibrous protein diffractions is that of observing movement of low-angle reflections with tilt of specimen, as described by Bolduan and Bear (62). Their tilting apparatus was used in testing tentative feather keratin lattices. The tilting specimen holder was adapted to the "Ways" camera of Table II. To detect tilt, successive photographs were taken of the same specimen with all elements of the apparatus kept unchanged and constant from diagram to diagram, except the angle at which the X-ray beam impinged on the specimen.
III. EXPERIMENTAL RESULTS

A. X-Ray Diffraction Photographs

Figure 6 shows the patterns of extracted, vacuum-dried and extracted, wet sea-gull feather rachis photographed at small to moderate angles at 8 cm. specimen-to-film distance. There are to be seen on the pattern of dry feather, in addition to those diffractions reported by Bear (17) on the first four layer lines, those with odd indices (k = 1, 3). They are so weak, however, that they may be seen in these photographs only with difficulty.

On the original pattern, however, these new spots are of sufficient intensity to be measured with adequate precision. While the fourth order of the fiber-axis period appears to be the innermost spot on the meridian due to its extremely high intensity, close inspection of the original patterns has made it possible to measure the (k = 3) meridional diffraction without equivocation. Despite the high resolution evident in these patterns, there is wanting any evidence for the double nature assigned the intense 24A meridional arc (k = 4) by Astbury (14). Since the fainter Cu Kβ satellite of this diffraction cited by Bear (17) is clearly resolved, the lack of evidence at any place on the entire pattern for a fundamental period higher than 95A must be taken as
confirmation of Bear's analysis (see section on literature of feather keratin X-ray studies).

Reflections appearing on the dry pattern (Fig. 6(a)), but not previously reported, are those with indices \((k = 11, 12, 20, 24, 26, 27\) and 30) on the meridian, and \((k = 15, 16)\) on the innermost prominent row line \((\xi = 0.045)\).

Comparison of the patterns of dry and wet feather indicates the most significant difference between them is an alternation of the intensities of certain diffractions. The most apparent of these are the \((k = 0, 4)\) spots on the inner prominent row line. In the dry pattern the equatorial spot is extremely intense and the fourth order is of much less intensity than the fifth; in the wet pattern the equatorial spot has almost disappeared and the intensity of the \((k = 4)\) spot has increased until about equal to that of \((k = 5)\).

In the small-angle pattern especially (spacings of lower index than the sixth order), there is less arcing along layer lines of all reflections on the wet pattern. On the more diffuse row lines there also appears to be better resolution of individual spots. There is no evidence of any change in fiber-axis spacing or other extensive change in structure.

Figure 7 includes patterns of sea-gull feather rachis heated in the presence of water. Figure 7(a) shows
Figure 6. Small to moderate angle X-ray diagrams of sea-gull feather rachis: (a) extracted vacuum-dried; (b) extracted, wet. The fiber axis of the rachis is vertical. Diagrams were photographed in camera "R-2" of Table II at 8 cm. specimen-to-film distance, but have been enlarged in reproduction to about 1.3 diameters of the original. Fundamental fiber-axis period is about 95A. Note diffuse row-line fine structure not hitherto demonstrated.
that a distinctive change can be produced in the X-ray pattern of untreated feather by heating the specimen at 120°C in aqueous butanol for 15 minutes. (See for comparison Fig. 6(a).) While it is clear that the diffractions of the lateral wide-angle pattern have become much more diffuse and that much of the wide-angle meridional pattern remains essentially unaltered; it is quite as evident, even after reproduction, that a new orderliness has been revealed in the low-angle pattern. The new and characteristic symmetry results from the appearance of diffractions on layer lines of even indices \( (k = 0, 2, 4, 6, 8, 10, 12) \) alternately on the meridian and the first prominent row line \( (\xi = 0.045) \). Thus spots are clearly visible on the meridian at layer lines \( (k = 4, 8, 12) \) and on the row line at \( (k = 2, 6, 10) \) with marked enhancement of the intensity of many of them. Meanwhile spots of odd layer-line indices \( (k = 1, 3, 5, 7, 9, 11) \) have almost or quite disappeared.

The type of pattern shown in Fig. 7(a) has been called the "two-dimensional net" here for reasons related to the tentative analysis of the structure which this type of pattern has made possible. This aspect is discussed later in this report in more detail. The "two-dimensional net" type of pattern results from controlled heating under other conditions than those given above. Further details
concerning this are given in the section on pattern changes below.

Figure 7(b) is an example of the effect of a type of heat treatment which has caused more advanced deterioration of the typical feather keratin pattern than that resulting in the "two-dimensional net." In this case sea-gull rachis was heated to 100°C for 4 hours in the presence of water alone. The form of the resultant pattern has been called the "oriented β-type" in this report since it resembles the diagrams which Astbury and others on many occasions (3, for example) have associated with the structure of the β-keratins. In the "oriented β-type" pattern, which is reproduced here, however, there appears to be more diffraction detail at low angles and along the meridian at wider angles than is exactly typical of Astbury's β-keratin pattern.

Still further advanced deterioration of the pattern than that reported above resulted from heating feather in the presence of water at 120°C for 30 minutes or more. Here the pattern degenerated to two diffuse rings, the spacings of which correspond to those identified by Astbury (3) with the disoriented β-keratin structure. In this report, therefore, this pattern has been called the "disoriented β-type." Since excellent photographs of this kind of pattern have been published widely and since they do not lend themselves to the quantitative
Figure 7. Small to moderate angle X-ray patterns of feather rachis heated in the presence of water: (a) at 120°C for 15 minutes in the presence of n-butanol, example of "two-dimensional net" type; (b) at 100°C for 4 hours in water alone, example of "oriented α-type."

Diagrams were photographed in camera designated "Ways" in Table II at 5 cm. specimen-to-film distance; magnification due to reproduction is about 1.3X. Note in (a) enhancement of reflections in the form of a square net; in (b) low-angle and meridional detail not hitherto reported in β-keratin type patterns.
analysis which is a primary goal of this work, they have not been reproduced here.

B. Fiber-Axis Period

The measurements that could be made on all patterns with the greatest precision were those related to the fiber-axis period, as would be expected in a fiber-type diagram. Tables III, IV and V list in column Y all diffractions related to the fundamental spacing system which were of sufficient intensity to be measured with adequate precision. Y is synonymous with Bernal's rotation-diagram axis coordinate, $\xi$, in reciprocal lattice space (72). Both the layer-line coordinates, $\xi$, and the true meridional reflections were used in arriving at values for Y by applying the relation

$$Y = \frac{k\lambda}{b_0}$$

The Y values corresponding to the average fundamental fiber-axis translations of 94.6 and 94.7A for the two types of diagram shown in Fig. 6, e.g., dry and wet sea-gull feather rachis, are listed in Tables III and IV, respectively. The Y values of Table V, with corresponding average fiber-axis period of 96.1A, are referred to the diagram in Fig. 7(a): the "two-dimensional net." Calculations for Y values were checked graphically at as many points as possible and by independent repetition.
The film measurements from which all values in Tables III, IV and V were calculated were made in a uniform manner. Distances were measured on films on which the diffractions were observed by transmitted light with the aid of fine-pointed bow dividers. These were read on an accurately-machined millimeter scale provided with a vernier. Five independent values for each measurement were recorded for each distance, the average of the five being used in further calculations. The use of a microphotometer, provided with a traveling stage, the translation of which could be read to one micron, did not furnish additional precision of measurement on this type of pattern.

C. Second Unit-Cell Translation

In addition to the fiber-axis spacing discussed above, Tables III, IV and V list diffractions related to the second translation of the unit-cell in reciprocal space, in the column headed X. X is synonymous with Bernal's rotation-diagram coordinate $\xi$ (72). In the lower section of each table are values of X for the more diffuse reflections on row lines of higher indices than those of spacing $\xi = 0.045$. Although Bragg spacings calculated from these data appear to cluster rather closely around 3.5A, unequivocal assignment of row-line indices is not possible on the basis of available diffraction patterns.
Table III
Diffractions by Extracted, Vacuum-Dried Sea-Gull Feather Rachis at Small to Moderate Angles.

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>k</th>
<th>b₀, Å</th>
<th>I</th>
</tr>
</thead>
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<tr>
<td>0.047</td>
<td>0.0000</td>
<td>0</td>
<td>89.5(?)</td>
<td>9</td>
</tr>
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<td>1</td>
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<td>0.0336</td>
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<td>94.7</td>
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<td>10</td>
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<td>3</td>
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<td>6</td>
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</tr>
<tr>
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<td>0.111</td>
<td>7</td>
<td>94.2</td>
<td>2</td>
</tr>
<tr>
<td>0.000</td>
<td>0.130</td>
<td>8</td>
<td>94.7</td>
<td>4</td>
</tr>
<tr>
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<td>94.7</td>
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<td>0.166</td>
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<td>9</td>
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<td>11</td>
<td>93.6</td>
<td>2</td>
</tr>
<tr>
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<td>0.194</td>
<td>12</td>
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<td>2</td>
</tr>
<tr>
<td>0.065</td>
<td>0.199</td>
<td>12</td>
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<td>0.215</td>
<td>13</td>
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<td>27</td>
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<td>30</td>
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<td>3</td>
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Average 94.6

"Diffuse" Reflections:

<table>
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<tr>
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<th>h</th>
<th>Layer lines present</th>
<th>I</th>
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<td>2</td>
</tr>
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<td></td>
<td></td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>1</td>
</tr>
<tr>
<td>0.138</td>
<td>3</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>2</td>
</tr>
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<td></td>
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<td>0.176</td>
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</tr>
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<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>2</td>
</tr>
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<td></td>
<td></td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>0.263</td>
<td>6</td>
<td>streak</td>
<td>-</td>
</tr>
<tr>
<td>0.31</td>
<td>7</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td>0.36</td>
<td>8</td>
<td>&quot;</td>
<td>-</td>
</tr>
</tbody>
</table>

(see note, page 55)
Table IV
Diffractions by Extracted, Wet Sea-Gull Feather Rachis at Small to Moderate Angles

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>k</th>
<th>$b_0, , \mathbf{k}$</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.045</td>
<td>0.0000</td>
<td>0</td>
<td>-</td>
<td>2</td>
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<tr>
<td>0.046</td>
<td>0.0174</td>
<td>1</td>
<td>88.5(?)</td>
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<td>0.045</td>
<td>0.0328</td>
<td>2</td>
<td>93.2</td>
<td>4</td>
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<td>0.024</td>
<td>0.0598</td>
<td>3</td>
<td>92.8(?)</td>
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</tr>
<tr>
<td>0.000</td>
<td>0.0651</td>
<td>4</td>
<td>91.6</td>
<td>10</td>
</tr>
<tr>
<td>0.042</td>
<td>0.0651</td>
<td>4</td>
<td>91.6</td>
<td>6</td>
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<tr>
<td>0.042</td>
<td>0.0826</td>
<td>5</td>
<td>93.2</td>
<td>5</td>
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<td>0.0988</td>
<td>6</td>
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<td>2</td>
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<tr>
<td>0.046</td>
<td>0.113</td>
<td>7</td>
<td>93.5</td>
<td>2</td>
</tr>
<tr>
<td>0.046</td>
<td>0.130</td>
<td>8</td>
<td>91.6</td>
<td>4</td>
</tr>
<tr>
<td>0.000</td>
<td>0.148</td>
<td>9</td>
<td>93.7</td>
<td>3</td>
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<tr>
<td>0.037</td>
<td>0.116</td>
<td>9</td>
<td>95.1</td>
<td>2</td>
</tr>
<tr>
<td>0.046</td>
<td>0.163</td>
<td>10</td>
<td>94.6</td>
<td>5</td>
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<td>0.178</td>
<td>11</td>
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<td>2</td>
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<td>0.070</td>
<td>0.178</td>
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<td>2</td>
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<tr>
<td>0.097</td>
<td>0.209</td>
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<td>95.7</td>
<td>3</td>
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<td>0.227</td>
<td>14</td>
<td>95.1</td>
<td>1</td>
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<td>0.212</td>
<td>15</td>
<td>95.9</td>
<td>6</td>
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<td>0.078</td>
<td>0.258</td>
<td>16</td>
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<td>3</td>
</tr>
<tr>
<td>0.000</td>
<td>0.277</td>
<td>17</td>
<td>94.1</td>
<td>2</td>
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<tr>
<td>0.000</td>
<td>0.306</td>
<td>19</td>
<td>95.7</td>
<td>6</td>
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<td>0.000</td>
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Average 94.7

"Diffuse" Reflections:

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<th>I</th>
</tr>
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</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>1</td>
</tr>
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<td>0.130</td>
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<td>0</td>
<td>3</td>
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<td>2</td>
<td>3</td>
</tr>
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<td>0.168</td>
<td>4</td>
<td>0</td>
<td>3</td>
</tr>
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<td></td>
<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>0.258</td>
<td>6</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>0.335</td>
<td>7-8</td>
<td>streak</td>
<td>-</td>
</tr>
</tbody>
</table>

(see note, page 55)
Table V

Diffractions by Sea-Gull Feather Rachis Heated in the Presence of Moisture at Small to Moderate Angles ("Two-Dimensional Net")

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>k</th>
<th>b₀, A</th>
<th>I</th>
</tr>
</thead>
<tbody>
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<td>0.0316</td>
<td>2</td>
<td>97.5</td>
<td>8</td>
</tr>
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<td>0.022</td>
<td>0.0493</td>
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<td>93.7</td>
<td>1</td>
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<tr>
<td>0.000</td>
<td>0.0639</td>
<td>4</td>
<td>96.4</td>
<td>10</td>
</tr>
<tr>
<td>0.039</td>
<td>0.0805</td>
<td>5</td>
<td>95.7</td>
<td>3</td>
</tr>
<tr>
<td>0.045</td>
<td>0.0974</td>
<td>6</td>
<td>94.9</td>
<td>4</td>
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<tr>
<td>0.031</td>
<td>0.112</td>
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<td>95.9</td>
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<td>0.000</td>
<td>0.128</td>
<td>8</td>
<td>96.4</td>
<td>6</td>
</tr>
<tr>
<td>0.015</td>
<td>0.159</td>
<td>10</td>
<td>96.4</td>
<td>3</td>
</tr>
<tr>
<td>0.000</td>
<td>0.192</td>
<td>12</td>
<td>96.4</td>
<td>2</td>
</tr>
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<td>0.000</td>
<td>0.211</td>
<td>15</td>
<td>95.9</td>
<td>7</td>
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<tr>
<td>0.115</td>
<td>0.255</td>
<td>16</td>
<td>96.7</td>
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<td>0.276</td>
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<td>95.0</td>
<td>3</td>
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<td>0.304</td>
<td>19</td>
<td>96.4</td>
<td>7</td>
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<td>0.000</td>
<td>0.377</td>
<td>24</td>
<td>97.9</td>
<td>3</td>
</tr>
<tr>
<td>0.000</td>
<td>0.419</td>
<td>26</td>
<td>95.5</td>
<td>2</td>
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<tr>
<td>0.000</td>
<td>0.479</td>
<td>30</td>
<td>96.4</td>
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Average 96.1

"Diffuse" Reflections:

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</tr>
</thead>
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<tr>
<td>0.073</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>0.133</td>
<td>3</td>
<td>0, 2</td>
<td>7</td>
</tr>
<tr>
<td>0.161</td>
<td>4</td>
<td>0, 2</td>
<td>7</td>
</tr>
<tr>
<td>0.257</td>
<td>6</td>
<td>streak</td>
<td>-</td>
</tr>
<tr>
<td>0.309</td>
<td>7</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td>0.353</td>
<td>8</td>
<td>&quot;</td>
<td>-</td>
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</tbody>
</table>

(see note, page 55)
Footnote to Tables III, IV and V

X and Y in all tables are synonymous with Bernal's rotation diagram coordinates xi and zeta (72), k is the layer-line index, h is an approximate row-line index and b₀ is the fiber-axis spacing given by b₀ = kλ/Y. Meridional reflections are those for which X = 0 and the equatorial diffraction has Y = 0. Intensities indicated in the last column are relative on an arbitrary scale; no implication of precision is intended. Average b₀ value does not include values followed by (?). Measurements from which the values in these tables were calculated were made on films photographed at specimen-to-film distances as follows: Tables III and IV, 80 mm. with camera "R-2"; Table V, 50 mm. with camera "Ways."
Therefore, values for lateral spacings of diffuse diffractions could not be brought to bear directly on the present problem. In view of this limitation further quantitative consideration is confined to X values derived from diffractions apparently lying on the innermost prominent row line.

The first step in structure analysis by X-rays, the determination of the unit cell dimensions, depends upon proper indexing of observed reflections. With a single crystal the a₀ and c₀ primitive translations of the lattice may be determined by using patterns in which the crystal has been rotated about the x and z axes. Since this procedure cannot be used with fibers, it is necessary in the case of feather to resort to indirect analysis of X-values to reach conclusions concerning dimensions of the unit cell, other than the b₀ spacing already determined.

Since the prominent row lines of the patterns of feather keratin are normal to the equator, it is assumed that the crystallites of this structure are of at least monoclinic symmetry. Orthogonality of the fiber axis to the plane of the other two axes of the unit cell is intrinsic to this symmetry. In the pattern given by a structure with an orthogonal fiber axis, all points with the same X value are on the same plane of the reciprocal lattice
of the structure. Consequently, the segregation of equal X value's is a necessary first step. To aid in this segregation, frequency plots of the X values in Tables III, IV and V were made for each type of pattern measured. These plots are reproduced in Fig. 8. The values of the layer-line index, k, at which any given spacing occurred are shown above the value of that spacing.

D. Pattern Changes

1. Enhancement of reflections

The effect of wetting in changing the pattern given by feather keratin which has been vacuum dried is shown in Fig. 6. It is pointed out that the main difference between the two types of pattern is the loss in intensity caused on hydration of the very strong (k = 0) spot on the inner prominent row line of the dry-feather pattern. In general, it may be said that dry specimens are better diffractors than wet.

When feather is heated in the presence of water, with or without other reagents, there are more marked consequences in the resulting pattern. The most advanced deterioration of reflection intensity results in a pattern of two diffuse rings which is called the "disoriented β-type." A less drastic form of this change, resulting
Figure 8. Frequency plot of $X$ values for feather keratin. The values from which these plots were derived are those listed in Tables III, IV and V, respectively. The numbers above the plotted values of $X$ denote the layer-line index, $k$, at which each value of $X$ occurs. The numbers below the base line of the plots are the plotted $X$ values.
from slightly different treatment, gives the "oriented β-type" pattern shown in Fig 7 (b). The experimental conditions of specimen treatment which give rise to these patterns are summarized in Table VI. An experiment not reported in Table VI demonstrated that concentration of carbon dioxide dissolved in the water had no effect on the degeneration of pattern structure associated with heating.

2. "Two-dimensional net"

Feather specimens may be treated under controlled conditions (given in Table VI) in such a manner that they will give rise to a pattern called the "two-dimensional net." From the photograph given in Fig. 7(a), it is noted that the distinctive symmetry of this pattern is a preferential enhancement of reflections on even-numbered layer-lines, alternately on the meridian and prominent row line in a net of a high order of symmetry. There is a simultaneous loss of intensity of reflections with odd layer-line indices, including disappearance of some.

Tentative inferences were made concerning the structure from which such a pattern could arise. The development on which they are based is given in the discussion section of this report.

3. Tilting behavior

In testing the inferences based on the "two-dimensional net" pattern, specimens were tilted and fiber-
axis spacings recorded. Tilt angles were varied from 0° to 20° (measured from normal to the beam). Patterns of two complete series of specimens were measured with the precision attainable on the microphotometer previously mentioned. The results indicated that tilting the specimen caused no change in the fiber-axis spacings on the "two-dimensional" pattern.

E. Macroscopic Physical Changes

In conjunction with the primary object of photographing X-ray diffraction patterns, observations were made of macroscopic physical changes which occurred in specimens incident to manipulation. The results of these observations are also summarized in Table VI.

F. "Tagging" Behavior

Patterns given by samples of sea-gull feather treated with phenylmercuric chloride or iodine were inspected carefully, but bore no indication that either reagent had formed a complex with the feather keratin. Several sharp rings corresponding to the powder pattern of the respective reagent indicated, however, that an adequate quantity of the uncombined compound was present. Degeneration of the normal feather pattern to a point intermediate between the "oriented β" and "disoriented β"
Table VI

Effects of Heating in the Presence of Water and Other Reagents on the X-Ray Diffraction Pattern and Physical Properties of Sea-Gull Feather Rachis

<table>
<thead>
<tr>
<th>Medium</th>
<th>Temp. °C</th>
<th>Time</th>
<th>Type pattern produced</th>
<th>Macroscopic physical condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>air</td>
<td>75-100</td>
<td>77 hrs.</td>
<td>normal feather</td>
<td>normal</td>
</tr>
<tr>
<td>air, I₂</td>
<td>75</td>
<td>77 hrs.</td>
<td>degenerated &quot;oriented β&quot;</td>
<td>dark iodine color</td>
</tr>
<tr>
<td>water</td>
<td>45</td>
<td>days</td>
<td>normal feather</td>
<td>normal</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4 hrs.</td>
<td>&quot;oriented β&quot;</td>
<td>normal</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>15 mins.</td>
<td>unknown</td>
<td>slight contraction; sl. elasticity when wet</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sl. more contraction; sl. more elasticity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25 mins. unknown</td>
<td>contracts 85%; DR lost. regained on stretching vol. sulfur cpds. prod.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 mins. &quot;disoriented β&quot;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 hrs. &quot;disoriented β&quot;</td>
<td>same as 30 min., but color yellow; structurally v. weak</td>
</tr>
<tr>
<td>water, Na₂SO₃</td>
<td>45</td>
<td>24 hrs.</td>
<td>unknown</td>
<td>normal</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>15 mins.</td>
<td>unknown</td>
<td>same as water, 120°, 4 hrs exc. weaker; DR loss irreversible</td>
</tr>
<tr>
<td>water, phenyl-HgCl</td>
<td>100</td>
<td>5 hrs.</td>
<td>&quot;oriented β&quot;</td>
<td>normal</td>
</tr>
<tr>
<td>water, phenyl-HgCl, butanol</td>
<td>120</td>
<td>85 mins.</td>
<td>&quot;oriented β&quot;</td>
<td>normal</td>
</tr>
<tr>
<td>water, butanol</td>
<td>120</td>
<td>15 mins.</td>
<td>weak</td>
<td>turned black</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>15 mins.</td>
<td>&quot;2-dimensional&quot;</td>
<td>normal</td>
</tr>
<tr>
<td>butanol, phenyl-HgCl</td>
<td>120</td>
<td>85 mins.</td>
<td>normal feather</td>
<td>normal</td>
</tr>
<tr>
<td></td>
<td>5 1/2 hrs.</td>
<td>normal feather</td>
<td>slight darkening</td>
<td></td>
</tr>
</tbody>
</table>
types was associated with iodine treatment. A control eliminated the possibility that the heating involved had been responsible for this change.

It is clear that in these preliminary attempts the proper conditions were not found for tagging selected residues of the feather keratin structure. The results of these experiments did, however, provide useful signposts for other studies.
IV. DISCUSSION AND CONCLUSIONS

A. Effects of Hydration

1. Structure orientation and degeneration

The wetting of the structure of feather keratin at room temperature is shown by Fig. 6 to cause characteristic change in intensity of some of its low-angle X-ray diffractions. The change appears to be an early phase of a series of structure transformations some stages of which are characterized by distinctive patterns. Two of these are reproduced in Figure 7.

Diminished "spreading" along layer lines, especially in the low-angle pattern, is evidence that wetting is associated with improved orientation of crystallites. In this connection, the wet pattern is also characterized by improved resolution of spots on the more diffuse row lines. Neither of these effects could be brought about by simple increase in resolving power of the camera. Consequently, it must be concluded that wetting causes improved orientation of the feather structure. In this there lies a clue to the direction in which treatment of specimens may be developed for producing patterns on which more precise measurements of lateral spacings may be made. Loss of intrinsic capacity to diffract X-rays attendant on hydration of feather is a factor with which there must be a reckoning,
however. Resolution of presently diffuse lateral dif-
fractions would clearly separate the details of row-line
structure for which there is presumptive evidence.

That treatment in the presence of water may also
cause extensive degeneration of feather structure is clear
from the "two-dimensional," "oriented β" and "disoriented β"
type patterns reported. Heat appears to be necessary for
producing these changes, but to an extent which is a function
of the type of reagent present during heating. The rate and
extent of hydration varies with temperature and other factors.

Controlled treatment of feather specimens with water
and butanol at elevated temperatures for short periods of
time (Table VI) results in the production of a distinctive
pattern which has been called the "two-dimensional net" in
this report. The factors involved in the treatment giving
rise to this pattern are considered to have implications in
terms of the feather structure.

From Table VI it may be seen that there is a corre-
lation between the type of X-ray pattern given by feather
specimens and their macroscopic physical properties. De-
generation of pattern is parallel to loss of structural
characters, advanced states of which are seen in contraction
of length, high elasticity, loss of birefringence and loss
of tensile strength. For example, the "disoriented β"
pattern has not been found except when the specimen itself
had contracted and become very elastic when wet. These characters have potential utility as signposts to future practical experimental methods and as adjuncts to X-ray evidence. In addition they indicate that drastic dis-orientations may be introduced into the structure of a protein, normally considered highly oriented and rigid, through the action of water and comparatively moderate heating in the absence of other reagent.

Factors in Hydration

1. Heating

In Table VI there is clear evidence for the conclusion that heat alone in the absence of water or other reagents, at least at temperatures up to 100°C, is not sufficient to alter the structure to the extent indicated by any change in pattern. In addition to experiments in which water was the medium, there is evidence from one experiment, for example, that the effect on feather of iodine in air at a temperature of 75°C is to cause considerable dis-orientation, while heating at 75°C in the absence of iodine causes no change in the pattern. In the presence of water alone increase in temperature has a progressively dis-orienting effect on the feather pattern. Additional evidence for this point also exists in the fact that under
identical conditions of temperature, heating in water may cause complete disorientation of pattern while heating in the presence of butanol has no effect on the normal feather pattern.

The degree of disorientation of the pattern attained in water alone appears to be roughly characteristic of that temperature. At 100°C under the experimental conditions, for example, the "oriented β" type pattern cannot be further degenerated by heating alone.

The effect of increasing time of heating is to increase degeneration of the pattern until the maximum state is reached. At 120°C, for example, it may be concluded from the reported data that a significant decrease in pattern orientation occurs between 20 and 30 minutes of heating.

2. Phenylmercuric chloride

In the experiments in which water was the medium and phenyl-HgCl was present, the pattern remained at the "oriented β" stage even if the heating was prolonged and as high as 120°C. In experiments in which the medium was aqueous butanol, however, the pattern remained at the "two-dimensional" stage even in the presence of this mercury salt.
Since the normal feather pattern is not altered by heating in the presence of butanol and phenyl-HgCl, it is probably safe to extend the observations above by concluding that heating in butanol alone would not change the feather pattern from the normal type.

3. Butanol

When feather rachis is heated in a water medium containing butanol at 100° or 120°C, it is evident from Table VI that the "two-dimensional" type pattern appears. Comparison of the effect of aqueous butanol against that of water alone indicates that the presence of butanol impedes degeneration. It seems doubtful that the function of butanol is anything but to reduce the activity of water, so that at these temperatures degeneration of the pattern is limited. The possibility of the existence of an equilibrium structure in a butanol-water medium is suggested in these observations.

4. Sodium sulfite

By correlating macroscopic effects with pattern types and applying that information to the effect of sodium sulfite, it is seen that the presence of this salt may be associated with more rapid degeneration. It is probable that sodium sulfite acts in this fashion by virtue of being a reducing agent.
C. The "Two-Dimensional" Pattern

1. Selection rule evidence

The regular structure of the "two-dimensional" pattern, Figure 7(a), suggested the possibility that it arose from a structure lattice in which two dimensions were ordered. To test this possibility the selection rule method of analysis, using equation (13), was applied to determine which diffractions would be given by a two-dimensional structure lattice. Through application of the selection rule to previous data (17) it was found that, for the feather lattice, equation (13) reduced to

\[ k = 4m + 2h \]  \hspace{1cm} (16)

for the \( h = h, \lambda = 0 \) row line since \( P = 4 \) and \( p = 2 \) (see section on the nature of the feather keratin pattern under Theoretical Considerations). In the case of the "two-dimensional" pattern the \( q = \frac{1}{4} \); system of the general case, equation (12), has disappeared.

When small integers are substituted in a systematic fashion for \( m \) and \( h \) in equation (16) permitted values for \( k \) are found. In this manner it is possible to determine the indices of layer lines on which the reflections corresponding to a given row line will fall. The result of this calculation can be shown to yield the information that when the index of the row line, \( h \), is an even integer, the
reflections fall on layer lines with the indices \( k = 0, 4, 8, 12, 16, \) etc. Similarly, when \( h \) is odd \( k = 2, 6, 10, 14, \) etc.

The "two-dimensional" diffraction pattern for feather rachis reproduced in Fig. 7(a) shows enhanced reflections on the meridian \( (h = 0) \) when \( k = 4, 8, 12 \) and on the first prominent row line \( (h = 1) \) when \( k = 2, 6, 10. \) There is also some vague indication on the pattern that enhanced reflections occur on \( h = 2 \) at \( k = 0 \) and on \( h = 3 \) at \( k = 2, 6. \) Spots on layer lines with odd indices occur at greatly reduced intensity or have quite disappeared from the pattern.

The agreement between the observed enhancement of diffractions on this pattern with the predictions of the selection rule argues that the previous model lattice was correct with respect to the \( p = 2 \) aspects, i.e., that the structure of feather keratin contains the prominent lamellae shown in Figure 2. This is the basis upon which the pattern was named "two-dimensional net."

2. Tilting evidence

It has been reported in an earlier section that specimens were tilted to provide evidence for degree of order of the structure, by the method of Bolduan and Bear (62). It was also stated that no movement of low-angle meridional reflections could be observed when a
specimen giving rise to the "two-dimensional" pattern was tilted through 20° from normal to the beam.

According to the criteria of Bolcuan and Bear the failure of spots to move with tilt allows of two possible interpretations: Either (1) the fibril structure is that of a two-dimensional net, with appreciable thickness normal to the plane of the net; disorientation of fibrils explaining persistence of spots with tilt, or (2) the structure is a three-dimensional lattice, with the most intense diffractions being derived from planes normal to the prominent net; persistence with tilt again being explained by disorientation.

Both possibilities are similar if it be assumed that hydration causes either randomness or a leveling of electron density peaks in the third dimension, in a structure well ordered in two dimensions, thus giving a more or less homogeneous structure normal to the plane of the net. Of the fashions in which such quasi-homogeneity of structure might arise there is the possibility of chemical alteration in some of the integral groupings of the protein structure. The removal of a loosely-bound substance not integral with the protein itself, such as a lipid for example, would be another.

3. Evidence in other keratins

It is seen in this report that the "two-dimensional net" is evident in the pattern of sea-gull feather rachis
only after the material has been treated with heat and water. It is of interest to note that some keratins possess normally a structure more like a two-dimensional net than that of untreated feather. Rudall (58) has reported, for example, the enhancement of even-numbered meridional orders with layer-line indices of \( k = 4, 8, 12, 16, 24, 32 \) in the pattern given by the untreated claw of the lizard *Varanus niloticus*. Although Rudall indicates this sequence to be a point of difference between *Varanus* and feather, it is clearly the parallel of the "two-dimensional net" reported here for feather. It is of interest to note in this connection that the fiber-axis spacing in *Varanus* is slightly longer than in feather (98.5A to 94.6A, respectively) while it is evident from Table V that the fundamental period given by meridional spacings of the "two-dimensional net" (96.1A) is also longer than that of untreated feather (94.6A). That these similarities may not be altogether fortuitous is supported by the close relationship between *Varanus* claw and feather rachis indicated by the fact that both have several odd orders (\( k = 15, 17, 19, 21 \)) with similar relative intensities.
D. Lattice Constants

1. Fiber-axis period

In Table III there is evidence that the fundamental fiber-axis spacing of sea-gull feather rachis is 94.6 Å in the dry state. This value is the average of a large number of measurements of rather well resolved spots and confirms the value reported by Bear (17).

This value is not altered significantly by wetting at room temperature (Table IV), hence it may be presumed that this degree of wetting, reflected by the change in intensities of some spots, is not of such nature as to affect the fiber-axis translation. Heating in the presence of water and butanol does, however, appear to effect a slight increase in spacing (Table V). The pattern from which these values were derived had fewer reflections which could be measured with accuracy. Therefore, this increase must be considered as a tentative value, the precision of which may be improved.

2. Second lattice translation

The importance of determining second and third lattice translations as a necessary step in structure analysis has been pointed out. There have also been presented, in Fig. 8, frequency plots of the X values occurring on all three major types of feather pattern which
have yielded accurate spacing measurements.

The upper part of Fig. 8 was derived from the dry-feather pattern shown in Fig. 6. This plot shows that the principal value of X, in terms of frequency of occurrence, is 0.046. Comparison of this value of X with the plots for wet feather and the "two-dimensional net" shows that this translation is relatively unaffected by hydration of the structure. In this connection it can be seen that reflections with layer-line indices $k = 0, 2, 6, 10$ are prominent in being maintained at X values between 0.044 and 0.046 in the wet pattern. The average of these is calculated to be 0.046. The effect of this translation on the pattern is greatly intensified by heating, as the frequency plot of the "two-dimensional net" shows. The persistence in the "two-dimensional" pattern, on layer lines with indices $(k = 2, 6, 10)$ of reflections corresponding to $X = 0.046$ is additional evidence that this translation should be taken as a prominent value for X in the reciprocal lattice of feather keratin.

That $X = 0.046$ row line may represent the second-order reflections of a larger translation is evidenced by the appearance of reflections at $X = 0.024$ and 0.022 in the wet and "two-dimensional" patterns, respectively, for spots of layer-line index $k = 3$. Additional evidence from spots on other layer-lines is the case of $k = 13$ in which
translations from the dry pattern (0.093) and the wet pattern (0.097), when considered as second-order diffractions, corroborate the persistence of the 0.046 translation in the hydrated specimen.

3. Evidence for double row line

In addition to \( X = 0.046 \), there is a possible \( X = 0.041-0.043 \) translation, as may be seen in the Fig. 8 frequency plot for the dry-feather pattern. The possibility of measuring this translation allows the demonstration of the double nature of the innermost prominent row line of the feather keratin pattern, the existence of which has not been previously reported. The weighted average value of 0.043 is used henceforward. This translation is represented by reflections with layer-line indices \( k = 4, 5, 9 \). There is additional evidence for this value of \( X \) if \( k = 8 \), 16 spots are plotted as of second-order origin. The shift of this translation with moisture may conceivably be greater than was the case with the 0.046 translation; but the most remarkable aspect of this row line \( (h = 0, \ell = 2) \) is its increase in intensity on wetting.

4. Attempt to index feather pattern

Of the accurately measured row-line spacings the greater number were available from the dry-feather pattern.
Therefore, all numerical data on row-line translation from this point forward were derived from Table III (i.e., from the dry-feather pattern).

The other tentative values for reciprocal lattice vectors which may be selected through use of Fig. 8 are \( X = 0.054 \) (from \( k = 7, 11 \)) and \( X = 0.066 \) (from \( k = 12 \)).

Having collected together all available \( X \) values into groups of equal magnitudes; e.g., \( X = 0.046, 0.043, 0.054 \) and 0.066; the next step is to assign indices to these values. Here the analytical method has been used to resolve the index indeterminateness intrinsic to fiber-pattern spacings. This reduces to fitting the \( X \) values, tentatively derived above, to the quadratic relation

\[
x^2 = Ah^2 + Bl^2 + Chl,
\]

in which \( h \) and \( l \) are Miller indices of the diffraction planes, and \( A, B \) and \( C \) are constants depending on the \( a_0, c_0 \) and \( b \) dimensions of the unit cell.

Application to equation (17) of the available \( X \) values gives the simplest fit when indices are assigned in the following manner:

<table>
<thead>
<tr>
<th>( X )</th>
<th>( (hl) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.046</td>
<td>20</td>
</tr>
<tr>
<td>0.043</td>
<td>02</td>
</tr>
<tr>
<td>0.054</td>
<td>22</td>
</tr>
<tr>
<td>0.066</td>
<td>31</td>
</tr>
</tbody>
</table>
Under these circumstances the values of the constants are found to be:

\[ A = 0.000534 \]
\[ B = 0.000453 \]
\[ C = -0.000270 \]

5. Tentative unit cell

By substituting these values in the relations

\[ A = \left( \frac{\lambda}{a_0 \sin \beta} \right)^2 \]

\[ B = \left( \frac{\lambda}{c_0 \sin \beta} \right)^2 \]

\[ C = \frac{-2}{a_0 c_0} \frac{\cos \beta}{\sin^2 \beta} \]

the values for \( a_0 \), to \( c_0 \) and \( \beta \) may be found.

On the basis of the development outlined above, the suggested tentative unit cell dimensions for feather keratin are

\[ a_0 = 69.3A \]
\[ b_0 = 94.6A \]
\[ c_0 = 75.2A \]
\[ \beta = 74.03^\circ \]

It is to be noted, however, that the number of spacings from which these values are derived is the minimum permissible if index indeterminateness is to be eliminated.
Low intensities of many reflections have prevented observation and intrinsic disorientation of crystallites has reduced precision of measurement below the point that permits definitive statement of the unit cell dimensions at this time. The above dimensions are presented in the most tentative sense only.

In the foregoing discussion of the "two-dimensional net" it was found that selection rule analysis was in agreement with enhancement observed in diffractions on the row-line with index \( h = 1 \). It was concluded in that discussion that the agreement argued for the correctness of the previous model lattice (shown in Fig. 2) in the respect that the structure of feather keratin contains prominent lamellae.

In terms of the tentative unit cell proposed above this would mean that the structure consisted of lamellae lying in planes parallel to the fiber axis, stacked along a direction perpendicular to the fiber axis. These lamellae would be rectangular and of the dimensions \( b_0 \times a_0 \) (parallel and perpendicular to the fiber axis, respectively). In the stack (along an axis perpendicular to the fiber axis, but not to the planes of the lamellae) the lamellae would repeat at intervals of \( c_0 \).

In fitting available X values to the quadratic equation (17), it was found that the best index that could
be assigned to the $X = 0.046$ value was $h = 2$. On this premise, it is apparent that the lateral dimensions of the net must be doubled, i.e., that the $xy$ face of Fig. 2 should be pictured as doubled along the $x$ direction. However, as Bernal and Fankuchen (73) noted with tobacco mosaic virus, it may be necessary in thin diffractors (thin along the direction in question) sometimes to use half-integral indices. The use of half-integral indices would explain the faint first order row line ($X = 0.023$) evidence observed in the wet and "two-dimensional" patterns and odd values $h = 1, 3$ (present assignment). Also it must always be kept in mind that if a lattice has, here and there, slightly different electron densities at otherwise equivalent positions, such as might come from an imperfectly distributed extra substance (see section on tilting evidence), one might find "forbidden" lines weakly developed (74).

E. Future

In the determination of the structure of feather keratin the outstanding problem is that of the unequivocal determination of the unit cell dimensions. It is evident from the progress reported here that further attempts must be made to improve the orientation of feather patterns. The purpose in this is to increase the precision of spacing measurements, especially those of row lines. Additional
data may also be derived from improved orientation, since a large number of diffractions would then become sufficiently discrete to be measurable. A careful study of heating in an aqueous-butanol medium, or other medium in which the activity of water would be suppressed, appears to be outstanding in potential productiveness.

The evidence presented for the double nature of the innermost prominent row line points out the importance of continuing work in the direction of separating the two components.

Swelling through the use of weak acids, as reported by Speakman and Stott (53) and Steinhardt, Fugitt and Harris (53), is clearly a direction of high priority. Treatment with cuprammonium hydroxide, as by Whewell and Woods (75) for hair, might serve a dual purpose by altering lattice constants and by tagging. Controlled action of cupriethylenediamine, which Coleman and Howitt (76) used in solubilizing silk fibroin, may have a similar effect. The use of alcohol-water-salt mixtures, following the work of Lundgren et al (35,69) in the solubilization of chicken feathers, has the presumed advantage of allowing selection of the type of bond to be loosened.

Although the preliminary application of phenyl-mercuric chloride and iodine was unsuccessful as reported here, there is every indication that this approach can be productive. For the use of mercury salts and organic
arsenicals for tagging -SH groups there are many reports (58,77) in the literature as applied to the most diverse systems. The most highly specific of these is p-chloromercuribenzoate, but it may be that the use of the smaller mercuric acetate will prove necessary. It is highly probable that repeated short alternate treatments with a reducing agent and metal salt would avoid problems contingent on extensive molecular disorientation by limiting breakdown to a few S-S bonds at one time.

There is some evidence (78) that iodine as used here may not be in its most reactive form. There are also reports of successful iodination of silk (79), and conditions have been defined for limiting iodination to the tyrosyl residues (80) of some proteins. From these it should be possible to determine methods for introducing iodine into the feather keratin molecule as desired.

Other compounds such as the methyl mercuric iodide used by Hughes (81) for serum albumin or the methyl iodide which reacted with wool (82) may also be adaptable for use in this problem.
V. SUMMARY

Developments in specimen preparation techniques have yielded X-ray diagrams of sea-gull feather rachis which give evidence of improved crystallite orientation in and reduced absorption and scattering by the specimens.

X-ray diffraction photographs of extracted, vacuum dried and extracted, wet sea-gull feather rachis and sea-gull feather rachis heated in the presence of water are presented. They include fine structure, especially at low diffraction angles, not previously demonstrated.

Tables of the more important spacings summarize careful measurements of small to moderate angle diffractions which derive from the structure of normal sea-gull feather rachis and which show the effects of hydration and heating on that structure. In these tables it has been possible to state the fundamental fiber-axis translation with more precision than has hitherto been reported.

There are reported the effects on the feather pattern of treating the specimens with selected reagents at various temperatures.

The "two-dimensional net" type of pattern given by treated feather rachis is demonstrated. The development in this investigation of this pattern has made possible a definitive statement of the second translation
of the feather keratin unit cell.

Existing difficulties in the determination of the lattice of feather keratin are discussed and suggestions are made of paths in which future investigations of this problem could be directed.
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