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X-Ray Diffraction Studies on Protein Fibers. II. Feather Rachis, Porcupine Quill Tip and Clam Muscle

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The first paper of this series[1] reported evidence regarding the diffractions exhibited at small angles by fibers from collagenous tissues. The present article deals with similar studies of keratin and muscle preparations, brief descriptions of which have appeared in previous communications.[2,3]

Feather keratin has the oldest history of any of the protein fibers with respect to small-angle diffractions, probably because of its relatively small fiber-axis period (95 Å.). Astbury and Markwick[4] observed the important meridional spacing at 24 Å. and suggested that the fundamental period is larger. Corey and Wyckoff[5] gave data for both feather and porcupine quill keratins, but offered no interpretation of their information.

The first patterns for porcupine quill possessing adequate angular resolution were those of MacArthur,[6] who concluded that the fiber-axis macroperiod of this form of α-keratin is either 198 or 658 Å. The larger figure was favored because it was thought to agree with certain conceptions of Astbury,[7] but it has been pointed out[2] that existing diffraction data do not require a period larger than 198 Å.

While it has been known that fibers of vertebrate muscle and myosin exhibit diffractions at low angles,[6,8] the large fiber-axis period of clam muscle escaped diffraction study until Jakus, Hall and Schmitt[3] drew attention to it with the electron microscope. Electron micrographs of osmic acid-treated fibrils disclosed an axially banded structure, the distance between like bands being given as 360 Å. X-Ray diffraction examination of intact dried clam muscles yielded a small-angle pattern which is undoubtedly the most remarkable one found to date with protein fibers. The true fiber-axis period was thus found to be about 725 Å., or twice the value observed for the osmic acid-treated fibrils with the electron microscope.

Experimental Methods

The diffraction techniques were essentially the same as those described earlier.[1] In preparing material for examination particular attention was given to securing material which yielded the greatest clarity of pattern and the best obtainable orientation. Thus far little consideration has been given to determining the effects on the diffraction patterns of chemical or physical manipulation.

Specimen Sources.—It was considered desirable to investigate at least one protein fiber whose short spacings are of the β-keratin type. Some attempts were made with human hair, maximally stretched in hot water, but this was abandoned in favor of the diffraction-rich feather keratin, whose wide-angle diffractions are those of a somewhat less extended β-keratin.[4a] Best results were obtained from sea-gull samples, agreeing with the experience of the Leeds investigators.[9] Small plates were cut from the ridges on the ventral surface of the rachis, for here the bulk of the protein fibrils seem to run predominantly parallel.

As MacArthur also found,[6] the best α-keratin for small-angle work is that of the porcupine quill tip. Quills from Canadian and African porcupines were examined, the latter specimens being kindly furnished by the Harvard Museum of Comparative Zoölogy. The African quills are preferable, since they yield smooth, woody preparations of fair fiber orientation and of some size. The diffraction patterns of both types have thus far shown no differences.

The quill tips are objectionable in two respects: the constituent fibers are not strictly parallel, coming together somewhat in forming the tip; also the conical shape of the tips makes it difficult to combine them to obtain optimum thickness (see ref. 1). Attempts to overcome these difficulties by using thin rectangular plates from the quill walls were not promising, since it seemed likely that the material of the tips is intrinsically better organized than that of the wall.

The clam muscles used were those from the adductor muscles of the clams *Mya arenaria; Venus mercenaria* and *Anodonta implicata*. In the last two instances white and pink portions of the muscle can be distinguished, of which the former was selected for study. All samples gave nearly the same patterns, but since Venus samples seemed best, results from this material form the basis of the present discussion. During drying preparatory to X-ray examination muscle strips were left in place connecting portions of the upper and lower shell halves, parts of which were broken away to permit removal of the clam body, including excess muscle. In this way the preparations were kept at normal length during drying and damage was avoided.

The fiber-axis period (7 Å.) of silk fibers is not too different from the corresponding short pseudo-period of β-keratin, a fact which has profoundly influenced interpretations of the β-keratin pattern.[10] For this reason, incidental to this study, raw silk fibers of the usual *Bombyx mori* and the wild type, Tussah, were examined, using samples kindly furnished by Mr. Kenneth R. Fox of the Textile Division of this Institute. Bundles were formed by combining several hundred fibers. A particularly good source for silk diffraction patterns was found to be silkworm "gut," sold by surgical supply houses.

Experimental Results

Excellent diffraction photographs of feather keratin and of porcupine quill tip have been published.[11] Since the present aim is to reconsider and to extend the interpretation of the data for these materials, further reproductions

(1) R. S. Bear, THIS JOURNAL, **66**, 1297 (1944).

(2) R. S. Bear, *ibid.*, **65**, 1784 (1943).

(3) M. A. Jakus, C. E. Hall and F. O. Schmitt, *ibid.*, **66**, 313 (1944).

(4) (a) W. T. Astbury and T. C. Markwick, *Nature*, **130**, 309 (1932); (b) Astbury, *Trans. Faraday Soc.*, **29**, 206 (1933).

(5) R. B. Corey and R. W. G. Wyckoff, *J. Biol. Chem.*, **114**, 407 (1936).

(6) I. MacArthur, *Nature*, **152**, 38 (1943).

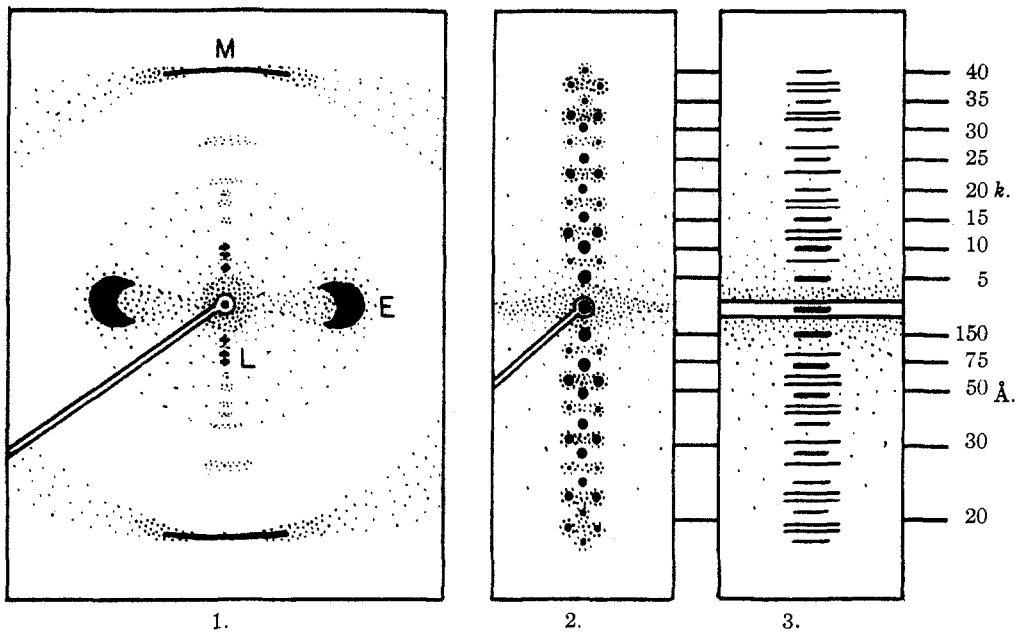
(7) W. T. Astbury, in "Advances in Enzymology," Interscience Publishers, Inc., New York, N. Y., 1943, Vol. III, p. 63.

(8) O. Kratky, A. Sekora and H. H. Weber, *Naturwissenschaften*, **31**, 91 (1943)

(9) W. T. Astbury and F. O. Bell, *Tabulae Biologicae*, **17**, 90 (1939).

(10) W. T. Astbury, *Trans. Faraday Soc.*, **29**, 193 (1933).

(11) See W. T. Astbury, *J. Chem. Soc.*, 337 (1942).



Figs. 1, 2, and 3 are drawings of the respective wide-angle, pinhole small-angle and slit small-angle patterns of clam (Venus) muscle. The original photographs after which these drawings were made will be reproduced elsewhere. The fiber-axis direction in all patterns is vertical, and original specimen-to-film distances were 7, 20 and 26 cm., using Cu K α radiation and the respective diffraction systems 2, 3 and 4 (described in ref. 1), which possess increasing angular resolution. In reproduction the magnifications have been altered so that now Figs. 2 and 3 are at the same angular magnification, which is about 4 times that of Fig. 1. M is the prominent composite meridional arc at 5.1 Å., E the equatorial diffraction at 9.6 Å., these being the characteristic α-pattern diffractions at wide angles referred to in the text. L of Fig. 1 marks the small-angle series of diffractions which are indicated in greater detail in Figs. 2 and 3. Only the chief diffractions are shown in Fig. 3, which distorts the intensity relationships somewhat. Better conception of intensities can be gained from Table III. To the right are given the layer-line indices, k, and the approximate scale of the first order Bragg spacings applicable to Figs. 2 and 3.

are omitted. Figures 1, 2 and 3 are sketches of typical patterns of clam muscle.

On the patterns of all of these materials there are numerous diffractions, many of them being meridional orders of the large fiber-axis periods. Others are more or less removed from the meridian, being on the equator of the pattern or forming prominent vertical row lines. These non-meridional interferences supply evidence that for each protein fiber there is one outstanding moderately large spacing transverse to the fiber axis, ranging from 34 Å. for feather keratin to about 325 Å. for clam muscle.

The presence of these large lateral spacings complicates the determination of the still larger fiber-axis periods. Row lines are crowded close to the meridian, so that any lack of perfect orientation of the fibrils of a sample causes arcing of the off-meridian spots onto the meridian. Furthermore, to resolve all diffractions requires fine pinhole collimations in order to maintain adequate angular resolution in all directions of the pattern; this in turn makes exposure times unusually long.

Feather Rachis.—With feather samples the fiber-axis period and the spacing transverse thereto are not so large that the necessary collimation prevents registration of patterns in convenient times. Table I lists the principal small-angle diffractions, or rather those which are most obviously related to the large-spacing system, some of them extending to moderately wide angles.

Both of Bernal's rotation diagram coördinates, ξ and ζ, are given in Table I. The layer-line coordinates, ζ, and the true meridional diffractions agree in arriving at a value of 95 Å. for the fiber-axis period of feather keratin. Many of the ξ coördinates are those of the prominent row line ($\xi = 0.045$), related to a lateral spacing of 34 Å. Those diffractions which are not on this row line are so few in number, and the accuracy with which the coördinates can be measured is so poor that further progress in determining the dimensions of the macrocell, other than the fiber-axis primitive translation, is not yet possible.

Of the first four layer lines only those with even indices ($k = 0, 2, 4$) are represented on the pattern. The innermost meridional spot is the fourth order of the fiber-axis period and is the most intense diffraction on the pattern. Failure thus far to register the first three orders of the 95 Å. spacing indicates that these must be very weak. Both the fourth meridional order and the equatorial 34 Å. arc are so strong that general

TABLE I

DIFFRACTIONS BY SEA-GULL FEATHER RACHIS AT SMALL TO MODERATE ANGLES

ξ and ζ are Bernal's rotation diagram coördinates,[10] k is the layer-line index, and b_0 is the fundamental fiber-axis period given by $b_0 = k\lambda/\zeta$. Meridional spots are those for which $\xi = 0$, while the equatorial arc has $\zeta = 0$. Relative intensities are indicated in the last column by arbitrary figures, tρ which precise quantitative significance is not to be attached.

ξ	ζ	k	b_0, Å.	I
0.045	0.0000	0	..	8
.046	.0324	2	95.1	2
.000	.0651	4	94.4	10
.041	.0645	4	95.5	3
.045	.0815	5	94.5	4
.046	.0994	6	93.0	2
.044	.1128	7	95.6	2
.000	.1293	8	95.2	3
.040	.1290	8	95.5	3
.000	.1473	9	94.1	3
.035	.1460	9	94.9	3
.045	.1620	10	95.1	5
.060	.179	11	94.6	3
.079	.197	12	94.0	3
.101	.213	13	94.0	3
.000	.244	15	94.5	6
.000	.278	17	94.0	3
.000	.309	19	94.6	6
.000	.346	21	93.4	4
		Average	94.6	

radiation artifacts related to them (located within these spots and streaking toward the center) often confuse the central regions of the pattern. This is probably the source of the 51, 82, and 115 Å. equatorial spacings reported by Corey and Wyckoff,[5] and may have caused Astbury[4b] to suspect that the fundamental meridional spacing might be as high as 309 Å. The double nature of the intense 24 Å. meridional arc, cited by Astbury in this connection, is undoubtedly due to the fact that a fainter satellite of this diffraction arises from Cu Kβ radiation, even with fairly heavy Ni filtering.

Porcupine Quill Tip.—The intrinsic imperfections and larger spacings of the porcupine quill tips make it difficult to separate both ξ and ζ coördinates with any satisfaction. It is possible, however, to treat both meridional and near-meridional diffractions together in determining the large fiber-axis period. For this purpose it is useful to note that at small diffraction angles both the Bragg law applied to the meridional spots and the calculations based on the layer lines reduce to very nearly the same expression: approximately, $d = k\lambda/\Phi$, where d is the primitive translation along the fiber axis, λ the X-ray wave length, and Φ the diffraction angle. Even at moderately wide angles one can use the Bragg law for both meridional and near-meridional diffractions without great error.

Accordingly, slit collimations were employed to photograph the diffractions quoted in Table II as evidence for the fiber-axis period of 198 Å. possessed by African porcupine quill tips. Angular resolution was thus maintained in the meridional direction. Under these conditions arcing of near-meridional spots caused by poor orientation has the effect of diffusing and increasing the apparent diffraction radii of some of the orders involved. Though this results in slightly reduced calculated values for d in such instances, the evidence for the 198 Å. period seems reliable and is in essential agreement with the data of MacArthur.[6]

TABLE II

SMALL-ANGLE MERIDIONAL AND NEAR-MERIDIONAL REFLECTIONS OF AFRICAN PORCUPINE QUILL TIP

d_1 is the spacing calculated from the first order Bragg law. Otherwise the symbols have the same significance as in Table I, b_0 being calculated from $b_0 = kd_1$. Intensity figures indicate, in effect, relative layer-line enhancements near the meridian.

d_1(Å.)	k	b_0(Å.)	I
66	3	198	6
49	4	196	1
39	5	195	2
27.4	7	192	4
24.5	8	196	2
22.0	9	198	2
19.8	10	198	4
18.06	11	197.8	3
15.2	13	198	1
13.2	15	197	1
12.36	16	197.8	4
10.40	19	197.6	3

As with feather keratin, the lower orders (k = 1, 2 in this case) have not been observed for porcupine quill, and the most intense meridional spot is the innermost one (66 Å.), corresponding to the third order of the fundamental period. The diffraction angles below that for the third order have been examined, using angular resolutions capable of examining spacings as high as 700 Å. or more, without discovering further signs of diffraction. The evidence on this point is not as conclusive as might be desired, however, because of a tendency of the preparations to yield objectionable diffuse background scattering at low angles.

The prominent large lateral spacing of porcupine quill tip is most clearly seen in the equatorial diffractions at low angles. Three such spots become progressively more diffuse as they are displaced from the central beam and possess intensities which can be respectively designated 10, 6 and 4 on the intensity scale of Table II. These diffractions appear to be the first three orders of a fundamental period of 83 Å. within the accuracy with which they can be measured.

Clam Muscle.—The orientation of the clam muscle diffractions is about as good as could be desired, so that even 325 Å. row lines to either side of the meridian can be distinguished clearly. The chief difficulty with this material is that of

securing sufficiently intense pinhole patterns capable of resolving very large spacings in all directions of the pattern simultaneously. With present equipment two types of pattern have been relied upon: pinhole patterns (Fig. 2) able to resolve the row lines (diffraction system 3 of ref. 1), and slit patterns (Fig. 3) capable of determining the large fiber-axis period (systems 4 and 5 of ref. 1).

The pinhole patterns (Fig. 2) may be described as follows: On the meridian are to be seen only a series of intense, evenly spaced diffractions, which by themselves would indicate a fiber-axis period of 145 Å. On layer lines which appear to be halfway between the positions of the strong meridional spots are found moderately intense, slightly more diffuse spots constituting the row lines, one row being on each side of the meridian. These row-line spots would appear to indicate a doubling of the fiber-axis period to 2 × 145 or 290 Å., but this is not the case as is shown below. The lateral displacements of the row lines yield evidence for the prominent 325 Å. spacing transverse to the fiber axis, though the value of this spacing cannot be determined as accurately as can that of the fiber-axis period.

Slit patterns (Fig. 3) show that the row-line spots are in reality double in the meridional direction. Furthermore, new faint diffractions become apparent. The data given in Table III,

TABLE III

SMALL-ANGLE MERIDIONAL AND NEAR-MERIDIONAL REFLECTIONS OF CLAM (VENUS) MUSCLE FIBERS

For explanation see Table II.

d_1, Å.	k	b_0, Å.	I
147	5	735	10
89.2	8	714	2
81.0	9	729	1
72.9	10	729	10
66.2	11	728	1
59.8	12	718	4
55.5	13	722	4
48.5	15	728	6
42.2	17	717	1
40.1	18	722	2
36.4	20	728	4
34.5	21	725	1
33.1	22	728	1
31.5	23	725	4
29.1	25	728	5
26.9	27	726	3
24.4	30	732	4
22.7	32	726	4
22.1	33	729	2
21.3	34	724	1
20.8	35	728	3
20.2	36	727	1
19.7	37	729	3
19.2	38	730	3
18.2	40	728	4
		Average 726	

when analyzed as described above for porcupine quill, indicate that the true fiber-axis period of clam muscle is close to 5 × 145 or 725 Å.

The manner of intensification of the diffractions is unusual. Considering roughly the over-all layer-line enhancements, one can briefly describe the intensities by considering the expression $k = 5m + p$, where m and p are integers, the latter being restricted to the values 0 through 4. Layer lines whose indices (k) are represented by $p = 0$, with m having the values 1 through 8, usually possess considerable intensity, chiefly from the true meridional diffractions. With $p = 1$ or 4 and $m = 1$ through 7 the layer lines are weak, and with the same m values and $p = 2$ or 3 the layer-line intensification is usually moderate, much of it resulting from the row-line diffractions. Values of k for which $m = 0$ and $p = 1$ through 4 (the first four layer lines) are close to the central beam, and diffuse background makes them difficult to study.

Beyond the fortieth layer line the intensities are weak, though many of them are present up to about $k = 80$, some even apparently reaching index values as high as a little over 100. Further details regarding the clam muscle small-angle diffractions await the application of improved apparatus now under construction.

Silk Fibers.—Fibers of silk protein (fibroin) are said to lack distinct small-angle diffractions.[5] The present experiments confirm this fact for both the usual variety of silk (Bombyx) and Tussah. This result seems best established from the photographs of silkworm "gut," which were particularly excellent at wide angles but failed to disclose any sign of discrete diffractions at small angles, when techniques were employed which were adequate with other protein fibers.

Failure to register a small-angle pattern for fibroin has only the force of a negative result. This is particularly to be remembered in protein long-spacing studies since, as was shown with collagen,[1] for example, distortions of the fibrils introduce variations in the macroperiod which have the effect of obliterating much of the discrete small-angle diffraction. Silk fibers are formed by a rapid spinning process in contrast to the slow formation of collagen, keratin and muscle fibers in the animal body, which means that sufficient "crystallization" time may be lacking for the production of large spacings with fibroin.

Short-Spacing Patterns.—The wide-angle diffractions of protein fibers are well known.[9] Here will be considered only a phenomenon observed at wide angles but closely related to small-angle effects: the short-spacing diffractions of feather and porcupine quill keratins and of clam muscle exhibit evidences of fine structure. By this is meant that the individual spots, which appear diffuse on patterns of poor angular resolution, are observed with improved resolution to possess an appearance such as would be expected if they

were produced by intensification of a group of neighboring high-order diffractions of the large fiber-axis and lateral periods.

The fine structure of the diffuse spots is in most instances so indistinct, yet detailed, as to defy clear reproduction or precise numerical description. Nevertheless, the origin of the component of a structured spot seems evident from the following observations: Pursuant to the fact that the feather keratin large periods, both longitudinal and lateral, are smaller than the corresponding spacings of porcupine quill and clam muscle, the structure of the feather keratin diffractions at wide angles is easier to demonstrate. Also, since the lateral spacing (34 Å.) of feather keratin is smaller than the longitudinal one (95 Å.), as is the case also with porcupine quill and clam muscle, the structuralization is more easily resolved transverse to the meridian of a given pattern than it is in the direction of the meridian.

These phenomena are most easily shown with the equatorial diffractions of feather keratin, being visible in published reproductions.[11] For example, the "side chain" equatorial spacing of 10 Å. is laterally divided into two components related to 11.3 and 8.8 Å. spacings,[9] and these components in turn extend vertically over distances corresponding to the positions of the first half dozen layer lines of the 95 Å. period, thus resembling portions of diffuse, unresolved row lines. MacArthur[6] noted a similar fine structure for the side-chain equatorial spacing of porcupine quill patterns, specifying the stronger lateral components at 10.5 and 9.2 Å.

The 5.14 Å. meridional arc of the α-keratin pattern (porcupine quill) illustrates another type of structured, wide-angle diffraction. Lateral components[6] give it a characteristic appearance on low resolution photographs, that of a poorly oriented but fairly sharp non-circular arc. Improved angular resolution indicates that the sharpness in the meridional direction is also illusory, some photographs suggesting that the individual components are also composed of a number of fine, meridionally displaced arcs (range 4.9–5.2 Å.).

On low resolution patterns the wide-angle diffractions of porcupine quill and clam muscle are very similar, being both of the α-type. With improved resolution differences become apparent. No fine structure has been noted for the corresponding wide-angle diffractions of clam muscle, except as the shapes of the principal spots are influenced. Thus, as is indicated in Fig. 1, the meridional arc (5.10 Å.) is characteristically non-circular though relatively sharp, while the diffuse equatorial spot (9.6 Å.) resembles a first-quarter moon with "horns" turned toward the center of the pattern, due presumably to an extension of intensified fine structure off the equator and toward the center. This structure is not resolved because of the very large size of the lateral (325 Å.) and longitudinal (725 Å.) periods.

Fine structure of collagen and silk wide-angle diffractions has not been observed, corresponding to the facts that the collagen fiber-axis period is very large (640 Å.), that this material does not possess a well-defined lateral macroperiod, and that silk exhibits no large spacings whatsoever. Comparisons of the above type have been made using patterns with comparable angular resolution (diffraction system 2, ref. 1), which is about that required to resolve the 198 Å. macroperiod of porcupine quill. Present techniques do not photograph the short-spacing diffractions adequately beyond this magnification.

Discussion

General Features of the Complete Diffraction Diagrams of Protein Fibers.—It is apparent that a fairly distinct division of the diffractions found on each type of pattern is possible: some are obtained at wide angles, corresponding to spacings less than about 20 Å., and others occur at small angles, being derived from large structural periods along and transverse to the fiber axis. Departures from this generality arise from the circumstances that silk apparently does not possess macroperiods, only the fiber axis has a large period in collagen, and feather keratin's two large periods are not great enough to avoid overlapping of the wide- and small-angle diffraction regions.

A second common feature of the protein patterns is a better development, both in the sharpness and in the number of diffractions extending in the meridional direction as compared to those situated in transverse directions. This applies to both wide- and small-angle regions of the patterns. Such facts argue for greater order along the constituent protein fibrils than across them. At the moment it is difficult to conclude definitely whether this order arises from construction of the fibrils from longitudinally oriented polypeptide chains or from long arrays of particles attached end-to-end. The relative sharpness of the meridional in contrast to the lateral diffractions is undoubtedly due in part to the thinness of the fibrils compared to their length (*cf.* the electron microscope results of Schmitt, Hall and Jakus for collagen[12]). Particularly for the small-angle diffractions is it necessary to keep in mind the limited thickness of the fibrils, which may make it impossible to employ the usual crystallographic considerations with regard to the lateral large periods (*cf.* Bernal and Fankucken on plant virus "intramolecular" diffractions[13]).

Classification of Protein Fiber Patterns.—Astbury[14] has frequently pointed out that the fibrous proteins may be classified according to

(12) F. O. Schmitt, C. E. Hall and M. A. Jakus, *J. Cell. Comp. Physiol.*, **20**, 11 (1942).

(13) J. D. Bernal and I. Fankuchen, *J. Gen. Physiol.*, **25**, 147 (1941).

(14) W. T. Astbury, *Trans. Faraday Soc.*, **34**, 377 (1938); Astbury and S. Dickinson, *Proc. Roy. Soc.* (London), **B129**, 307 (1940).

their wide-angle diffractions. The small-angle interferences permit a similar classification, as now appears.

Feather keratin exhibits the β-type, while porcupine quill and clam muscle yield the α-type of wide-angle pattern. According to Astbury's criteria, therefore, all three belong to the "keratin-myosin" group. Their small-angle diffractions, however, are far from being even approximately identical (*cf.* the otherwise similar patterns of porcupine quill and clam muscle).

Consideration of this situation discloses that the criteria for classification at small angles cannot be the complete or nearly complete identity of the diffractions of the several materials involved. Feather, porcupine quill and clam muscle are, however, alike in exhibiting extensively developed meridional systems of diffractions *and* at least one unmistakable lateral period. On the other hand collagen, with its characteristically different wide-angle pattern, is distinguished also by its lack of any definite large lateral spacings, apparently possessing only the large fiber-axis period.[1]

Astbury[7] has pointed out that the silks, the "keratin-myosin" group and collagen are chemically as well as structurally different. It is worthwhile to pursue this matter further in attempting to understand the general characteristics of the complete diffraction patterns of protein fibers.

Accompanying the chemical simplicity of silk there is unusually good development of wide-angle diffractions and a lack of small-angle interferences. Among the other protein fibers the effects of increased chemical complexity are of two sorts, as shown by the keratins and clam muscle on the one hand and collagen on the other. Both types of complex protein, however, are alike in suffering deterioration of their wide-angle diffractions (in distinctness and number of diffractions) with the concomitant development of small-angle evidences for large structures.

Further interesting relations can be noted for feather, porcupine quill and clam muscle. Arranged in the order given, their fiber-axis macroperiods (95, 198 and 725 Å.), their innermost intense meridional diffractions (24, 66 and 145 Å.), and their large lateral spacings (34, 83 and 325 Å.) all progressively become larger. In fact, the ratio between the lateral and longitudinal fundamental spacings is remarkably constant, being nearly 0.4 in all cases, which indicates almost proportional changes in both large periods. The observations on the relative ease with which the fine structures of the wide-angle diffractions can be made out for these three proteins also support the comparative order given.

It may be significant that the fibers *normally* possessing the α-type of wide-angle pattern (porcupine quill and clam muscle) have also the largest fundamental periods (contrast with Astbury's belief that the β-type of diagram is given by more extended molecular chains).[15] From the present comparative standpoint the wide-angle β-pattern of feather keratin is the better one, since it possesses a fairly definite layer-line structure related to a fiber-axis pseudo-period of about 6.2 Å. The α-patterns often lack clear evidence of wide-angle layer-lines.

Relations between the Wide-Angle and Small-Angle Diffractions.—The comparisons given above suggest the rough generalization for protein fibers that as chemical complexity increases wide-angle patterns deteriorate and the structures responsible for the low-angle diffraction increase in size. At one extreme is silk with fairly extensive wide-angle diffractions and no large spacings. Clam muscle, on the other hand, has the poor α-type of short-spacing pattern, but shows very large spacings at small angles.

Nevertheless, a number of facts point to a certain amount of independence of these two varieties of diffraction. An example of this was previously noted in the relative constancy of the wide-angle diffractions of collagen despite considerable variability of the large period.[1]

Leading to a similar conclusion is the fact that porcupine quill and clam muscle both yield α-type wide-angle patterns, though their small-angle diagrams are quite different. Astbury[7] and MacArthur[6] considered that the 5.14 Å. meridional arc of the porcupine quill wide-angle pattern is the 128th order ($128 = 2^7 3^0$) of their supposed 658 Å. fiber-axis period. Aside from the fact that there is no reason to believe that the 658 Å. period is preferable to one of 198 Å. for porcupine quill, it is clear that clam muscle has a fundamental repeating pattern of 725 Å., yet possesses a similar wide-angle meridional diffraction at 5.10 Å. In clam muscle this arc would be about the 141st order of the large fiber-axis period, though it undoubtedly possesses the same structural significance as the so-called 128th order of porcupine quill.

The fine structure of the wide-angle diffractions indicates the correctness of regarding the short-spacing spots as high orders of the large spacings though a range of indices is required to describe a given wide-angle diffraction, rather than a single index set. For this reason employment of large indices becomes of doubtful value. It would seem better to consider the short spacings of protein fibers as related to small pseudo-cells belonging to a restricted portion or portions of the total matter entering into the structure of the macrocell.

With this possibility in mind, the nature of the relation between the wide-angle and small-angle patterns becomes more clearly focussed. When the chemical composition is simple and the differ-

(15) It is true that the α- and β-patterns are not mutually exclusive, since one can often be transformed into the other in a single given material, but the nature of the microperiods after such transformation remains to be demonstrated.

ent kinds of side chain are few in number, as in silk, the situation approaches that of other natural high polymers, such as cellulose, starch amylose, chitin and rubber, in each of which only one type of residue is involved, so that repeated structures are developed which are of the order of size of the residues. As constitution becomes more complex new local configurations or types of order become necessary. This phenomenon does not occur to the same degree everywhere in the protein fiber, so that there result various regions of two or more kinds differing in the degree of crystalline or quasi-crystalline order.

Presumably one kind of ordered region contributes most heavily to the wide-angle diffractions, but since it comprises but a portion of the protein the wide-angle diffraction suffers in intensity as well as by a certain amount of loss in detail due to decreases in perfection of order. The different varieties of ordered (or disordered) region apparently are not randomly distributed in space, but are built into large patterns of structure. Increase of chemical complexity may increase the number or variety of "regions" and hence the size of the large "patterns."

It is to be expected that the large structural patterns should be more susceptible than the local ordered regions to distortion by chemical or physical means. Consequently, the large spacings may either be destroyed or become so distorted that they escape attention with X-rays, even when the wide-angle diffractions remain apparently unaffected (*cf.* results with oriented gelatin and macerated collagen[1]).

That the large fundamental periods are in evidence at all under favorable circumstances is testimony to a fair degree of regularity of the distribution of amino-acid types. To what extent this regularity extends to extreme detail, such as the original Bergmann–Niemann theory supposed, or is of a statistical sort, is an open question at present.

Lotmar and Picken,[16] using a preparation similar to the clam muscle of the present experiments (they employed the adductor muscle of *Mytilus edulis*), obtained a particularly excellent wide-angle pattern, which unfortunately could not be reproduced at will. With the new information they were able to propose a more detailed structure for this sort of fibrous protein than was previously possible, suggesting that the longitudinal extension per amino-acid residue is about 2.8 Å., which can be compared to the corresponding figure of 1.7 Å. derived by Astbury[17] for proteins in the normal α-configuration. Since it is unreasonable to expect an amino-acid residue of a fully extended polypeptide chain to be more than about 3.5 Å. in length, Lotmar and Picken could not explain the long range extensibility of protein fibers after the manner of Astbury. They expressed the view that the extensibility is derived in large part from non-crystalline portions of the fiber. The non-crystalline portions of Lotmar and Picken become the poorly ordered regions of the present explanation for the general diffraction effects of protein fibers.

Diffraction Intensities.—The relative intensities of the meridional diffractions at small angles offer the possibility of securing one-dimensional plots of electron density as a function of position along the fibril. At this point only a few readily obtained qualitative facts of this sort will be mentioned for the keratin and clam muscle fibers.

The collagen longitudinal density plot should possess a dual nature, being composed of a slowly varying large cycle of density repeating every 640 Å. along the fibril, with smaller fluctuations superimposed thereon, most important being sub-periods of the order of 70 to 110 Å.[1] The keratin and clam muscle density distributions must differ from that of collagen in one important respect: in none of the keratin and muscle instances is the meridional first order very intense, so that the large density cycle of period equal to that of the total fiber-axis repeating pattern is missing for these fibrils. The intense innermost meridional orders are those representing sub-periods of pronounced density fluctuations equal to 24, 66, and 145 Å., respectively, for feather, porcupine quill and clam muscle, which sub-periods are more nearly of the order of magnitude of the important collagen ones.

It is to be expected, therefore, that electron micrographs of the keratin and clam muscle fibrils may most readily detect density variations whose extensions along the fibrils are smaller than the true periods. This is indeed the case with clam muscle, the first electron micrographs[3] having indicated a longitudinal period of 360 Å. A repeating sub-pattern of this magnitude is not easily reconciled with the X-ray diffraction intensities, but subsequent electron micrographs, taken under improved conditions, have resolved a fine structure which includes both the 145 and 725 Å. spacings.[18] Thus the electron microscope and X-ray data may be considered to be in essential agreement.

The pronounced development on the clam muscle diffraction diagrams of the meridional orders with indices 5, 10, 15, 20, 25, 30, 35 and 40 argues for a very regular intensification of electron density at fifths of the fundamental period of 725 Å. The intensification of orders whose indices are multiples of five departs from Astbury's expectation[7] that indices having factors of 2 and 3 should be prominent for protein fibers. Striking "selection rules" of this sort are not observed for the keratin low-angle diffractions.

As has been mentioned, the keratin and clam muscle diffractions are characteristically different

(16) W. Lotmar and L. E. R. Picken, *Helv. Chim. Acta*, **25**, 538 (1942).

(17) W. T. Astbury, *Chem. and Ind.*, **60**, 491 (1941).

(18) F. O. Schmitt, "Advances in Protein Chemistry," Vol. I, p. 60, Academic Press, New York, N. Y., 1944.

from those of collagenous fibers with respect to the exhibition of large spacings transverse to the fiber axis. With the former group the pronounced evidences of lateral spacings indicate that the denser layers distributed along the fibrils (every 145 Å. in the case of clam muscle) in turn are composed of denser and less dense concentrations of electrons distributed transversely in each layer. In such instances two-dimensional (corresponding to radially symmetrical fibrils) or three-dimensional density distributions will be required to explain the diffraction effects, whereas for collagen the one-dimensional longitudinal plots are all that present diffraction data require.

Summary

Studies of the X-ray diffractions exhibited at small angles by feather and porcupine quill keratins and by clam muscle fibers have disclosed for these materials two principal kinds of large spacing: one represents the repeated pattern along the fiber axis and the other is a prominent spacing transverse thereto. The measured values are, respectively, 95 and 34 Å. for feather, 198 and 83 Å. for porcupine quill, and about 725 and 325 Å. for clam muscle. The observation that silk fibers lack discrete small-angle diffractions has been confirmed.

The keratin and clam muscle fibers exhibit in their wide-angle diffractions phenomena which suggest that the diffuse short-spacing spots are in reality clusters of higher diffraction orders of the large fundamental structural pattern. Fibers whose wide-angle patterns at low angular resolution are almost identical (porcupine quill and clam muscle) may differ considerably in their diffraction effects at small angles, hence also in their wide-angle fine structure.

A comparison of existing data regarding the complete diffraction patterns of protein fibers indicates that as chemical complexity increases wide-angle patterns deteriorate and the structures responsible for low-angle diffraction increase in size. It is tentatively suggested that the complex fibrous proteins possess local regions of two or more kinds differing in the degree of crystalline or quasi-crystalline order. Only a fraction of the better ordered regions contribute predominantly to the wide-angle diffractions, while larger structural patterns, embracing a number of the smaller regions of all kinds, are in evidence in the small-angle systems of interferences.

Astbury's keratin–myosin class of fibrous proteins is recognizable at small diffraction angles by the following general characteristics: the possession of both longitudinal (fiber-axis) and lateral (transverse) large spacings, and the predominant intensification of meridional diffraction orders higher than the first (in contrast to collagenous fibers which possess a distinctively different wide-angle pattern, lack a definite lateral macroperiod, and intensify the first meridional order most strongly).

The remarkable clam muscle diffractions at small angles exhibit a striking near repetition of layer lines in index cycles of five, the meridional orders whose indices are multiples of five being most intense (through the fortieth order). This is evidence for the location of dense layers of matter (relative to background) every 145 Å. along the muscle fibrils. Prominent row lines to either side of the meridian suggest that these layers in turn possess structure laterally.

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[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

The Structure of Copolymers. II[1]

BY FREDERICK T. WALL

When dealing with the structure of ordinary polymers (those obtained from a single kind of monomer unit), one does not usually encounter any serious problems with respect to chemical composition, except possibly from the effect of end groups. In the case of copolymers, however, the question of composition becomes one of great importance. This is true not merely because the average monomer ratio in a copolymer can be varied, but also because different polymer molecules obtained from the same batch can have quite different compositions for various reasons to be set forth later.

A simple quantitative theory which recognized this possibility was given earlier by the author.[1] The important point is that when a mixture of monomers undergoes polymerization, the polymer formed at any instant does not necessarily have the same composition as the monomer mixture from which it is derived. Accordingly if one starts with a given monomer charge and carries the polymerization to completion, the resulting copolymer will in general exhibit substantial heterogeneities in composition.

The theory originally advanced[1] has not been found to be entirely satisfactory on a quantitative basis although the work of Marvel and coworkers[2] has given qualitative support to the theory. Moreover, certain patents have been issued[3] which indicate that cognizance has been

(1) For the first paper of this series see F. T. Wall, THIS JOURNAL, **63**, 1862 (1941).

(2) Marvel, Jones, Mastin and Schertz, *ibid.*, **64**, 2356 (1942); Marvel and Schertz, *ibid.*, **65**, 2054 (1943).

(3) Finkentscher and Hengstenberg, U. S. Patent 2,100,900 (1937).