STUDIES ON THE STRUCTURE OF FEATHER KERATIN

I. X-RAY DIFFRACTION STUDIES AND OTHER EXPERIMENTAL DATA

R. SCHOR *and* S. KRIMM

From the Harrison M. Randall Laboratory of Physics, University of Michigan, Ann Arbor. Dr. Schor's present address is Department of Physics, University of Connecticut, Storrs

ABSTRACT Previous data as well as new results are examined with a view to determining the boundary conditions which present experimental information places on a satisfactory polypeptide chain model for the structure of feather keratin. Our studies indicate these conditions to include the following: (1) A 189 A identity period, with a pseudoidentity of 94.5 A; (2) characteristic fiber axis periodicities of 23.6$_4$ A and 18.9 A; (3) a meridian reflection of 2.96 A, but none in the 1.0 A region; (4) a strong, but sensitive, equatorial reflection of about 33 A spacing, with a possible equatorial reflection near 50 A; (5) perpendicular infrared dichroism of ν (NH) of at least 5:1; (6) a limited extensibility along the fiber axis direction; (7) the natural accommodation of about 12 per cent of proline residues in the structure; (8) the possibility of breaking down the structure into units of about 10,000 molecular weight. The implications of these conditions with respect to a satisfactory model are considered.

INTRODUCTION

The structure of feather keratin has posed a problem which so far has not been satisfactorily resolved. Whereas our present ideas on polypeptide chain conformations have permitted the elucidation of several fibrous protein structures, the feather keratin structure has not been amenable to such solution. The question thus immediately arises as to whether ordered polypeptide chain conformations other than those known at present can occur in proteins. Or is it just that we have not yet been clever enough in relating feather keratin to the known types of structures? The structure is also of interest from another standpoint. It has been shown recently (1) that a particular mutation in fowl, the Frizzle mutation, is correlated with certain structural alterations in the feather keratin, as manifested by changes in the x-ray diffraction pattern. At present, the source of these changes can only be inferred

tentatively. The details of this structural change might be better understood if we had more detailed knowledge of the structure of normal feather keratin.

The feather keratin x-ray diffraction pattern, which is also given by some reptilian keratins (2), was discussed by Astbury and Marwick (3) in terms of "a rather bewildering elaboration" of the extended form of a polypeptide chain, such as occurs in silk fibroin and β-keratin. Soon after, Astbury and Lomax (4) noted that there were certain similarities between the diffraction patterns of feather keratin and crystalline pepsin. This led them to suggest the possibility that the feather keratin structure might arise from an aggregation of corpuscular units. This idea was again proposed on the basis of the observed similarity between the diffraction patterns of feather keratin and of F-actin (5), and was incorporated by Bear and Rugo (6) into a micellar model for the structure of feather keratin.

The first detailed model of the polypeptide chain conformation in feather keratin was that of Pauling and Corey (7), who proposed a fibrous structure consisting of α-helices alternating with sheets of extended chains. This structure failed to give significant agreement with the observed x-ray diffraction pattern, and was later replaced (8) by a suggested model made up of α-helices in a coiled-coil arrangement. The calculated diffraction pattern for the latter model was again in poor agreement with the observed pattern (9). The question brought up by these proposals is nevertheless of importance, *viz.*, is the feather keratin structure based on an aggregation of small micellar units, or is it similar to extended polypeptide chain fibrous structures such as silk, collagen, etc.? The former viewpoint is supposedly favored by chemical studies (10–12), which indicate that feather keratin can be solubilized to yield a unit of about 10,000 molecular weight from which a material similar to the native keratin can be reconstituted. The inferences from this work are only suggestive, however, rather than conclusive. Models of both the former type (13) and the latter type (14) have formed the basis for discussions of the structure of feather keratin.

The purpose of the present paper is to reexamine the boundary conditions which experimental information, both previous data and new results to be discussed, places on a model for the structure of feather keratin. In terms of these conditions a new polypeptide chain model, briefly proposed previously (14), will be considered in some detail in the succeeding paper.

X-RAY DIFFRACTION STUDIES OF FEATHER KERATIN

1. *Experimental.* Standard x-ray fiber diffraction patterns were obtained using a Norelco diffraction unit and a Unicam single crystal goniometer. Nickel-filtered copper Kα radiation was used, giving a resolution of spacings up to about 50 A. When it was desirable to eliminate air scattering, the goniometer was enclosed in an evacuable chamber.

Diffraction patterns of the rachis and calamus of many different kinds of birds were

studied. These patterns were all basically the same. Most of the work was done with sea gull and with turkey feathers. The diffraction pattern from benzene-extracted turkey calamus, taken in an evacuated camera, is shown in Fig. 1. As compared with the pattern of an air-dried specimen, the layer line spacings are the same but the equatorial spacings are slightly smaller than for the air-dried sample. The turkey pattern differs from that of the sea gull feather primarily in not having the well developed row line of spots centered at the 33 A equatorial reflection and extending out to layer line spacings of about 10 A which is present in the diffraction pattern of sea gull keratin.

2. *X-ray Diffraction Pattern.* The measured layer line and equatorial spacings in the diffraction pattern of air-dried turkey calamus are given in Tables I

TABLE I
LAYER LINE SPACINGS IN TURKEY CALAMUS DIFFRACTION PATTERN

This study				Kraut (9)	Rudall (2)	Bear (15)	Astbury and Bell (16)	Corey and Wyckoff (17)
Spacing	Intensity	l	c_0					
95.1	vvw	2	190.2					
48.5	vs	4	194.0	50.7		47.6	47.0	
23.6_4*	vvs	8	189.0	23.7	23.6	23.7	23.4	21.3
18.6	mw	10	186.0	19.3		18.9		
15.9	w	12	190.8			15.5		
13.5	w	14	189.0			13.7		
11.7_6	w	16	188.0	11.9	11.9	11.9		
10.4*	w	18	187.2	10.4	10.4_8	10.5	10.4	
9.38	s	20	187.7			9.52	9.1	9.08
8.51	mw	22	187.2			8.61		
7.60	mw	25	190.0	7.94		7.83		
7.09	mw	27	191.3			7.24		
6.30(*)	s	30	189.0	6.26	6.30	6.32	6.26	6.20
5.89	mw	32	188.5	5.54	5.53	5.55		
4.97*	s	38	189.0	4.96	4.98	4.99	4.93	4.90
4.47	mw	42	188.0	4.45	4.45	4.46	4.42	4.37
3.94*	vw	48	189.1	3.99				3.95
3.80*	vvw	50	190.0	3.80				
3.57*	w	53	189.2	3.57	3.54		3.54	3.52
3.37	vvw	56	188.9	3.40				
3.25*	vvw	58	188.7	3.25	3.29		3.29	3.22
3.15	s	60	189.2	3.09	3.08		3.08	3.07
2.96*	mw	64	189.5	2.97	2.94		2.94	
2.77*	mw	68	188.3	2.75	2.74		2.74	
				2.56				
2.39*	vw	79	189.0	2.34				
2.25*	vw	84	189.1					
2.10*	vw	90	189.0	2.13				
				2.04				

vs = very strong, s = strong, m = medium, w = weak.
* Meridian reflection.

TABLE II
EQUATORIAL SPACINGS IN TURKEY CALAMUS DIFFRACTION PATTERN

This study		Kraut (9)	Rudall (2)	Astbury and Bell (16)	Corey and Wyckoff (17)
Air-dried	Vacuum-dried				
				115	115
				81.8	81.8
49.5. s	48.8 s	55		51	51
32.6 vs	31.2 vs	33.5	34	33.3	33.3
16.6 w	16.1 mw	17.3	17.6	17.6	17.1
11.2 mw	10.6 vs	11.2	11.3	11.3	11.0
8.71 w	8.5 w	8.84	8.8	8.8	8.56
5.88 w	5.7 w	5.82	5.8	5.8	
		4.90			
4.66 s(broad)	4.68 s	4.50	4.50	4.50	4.68
	4.35 w				
		3.90			
3.38 w		3.50			
		3.25			
		2.28			

and II. Measurements by previous workers (2, 9, 15–17) are included for comparison, although their values differ slightly because in several cases the specimen used was sea gull feather (9, 15). The meridian spacings for sea gull are essentially the same as for turkey, but the equatorial spacings are up to a few per cent larger. A reciprocal lattice diagram corresponding to the diffraction pattern is shown in Fig. 2. Before discussing the pattern in more detail, several general remarks are appropriate.

First, while the normal feather keratin pattern (*e.g.*, that in Fig. 1) is given by the calamus and rachis, it is interesting to note that near the base of the feather this pattern appears with different orientation. For an average size turkey feather, the normal pattern appears beyond 6 to 8 mm from the base, but at about 2 mm from the base it is oriented with its "fiber axis" perpendicular to the quill axis, while at about 4 mm both orientations are present. It is not known whether or not this reflects orientational changes in the cellular structure associated with the growth of the feather.

Second, at a macroscopic level the feather protein appears to be homogeneous: diffraction patterns of longitudinal sections, each representing about one-third of the thickness of the calamus, are essentially the same. This, of course, tells nothing about the homogeneity, or lack thereof, at the microscopic level.

Third, although all orientations of the crystalline regions about the fiber axis are equally probable (as evidenced by the uniform rings obtained when the x-ray beam

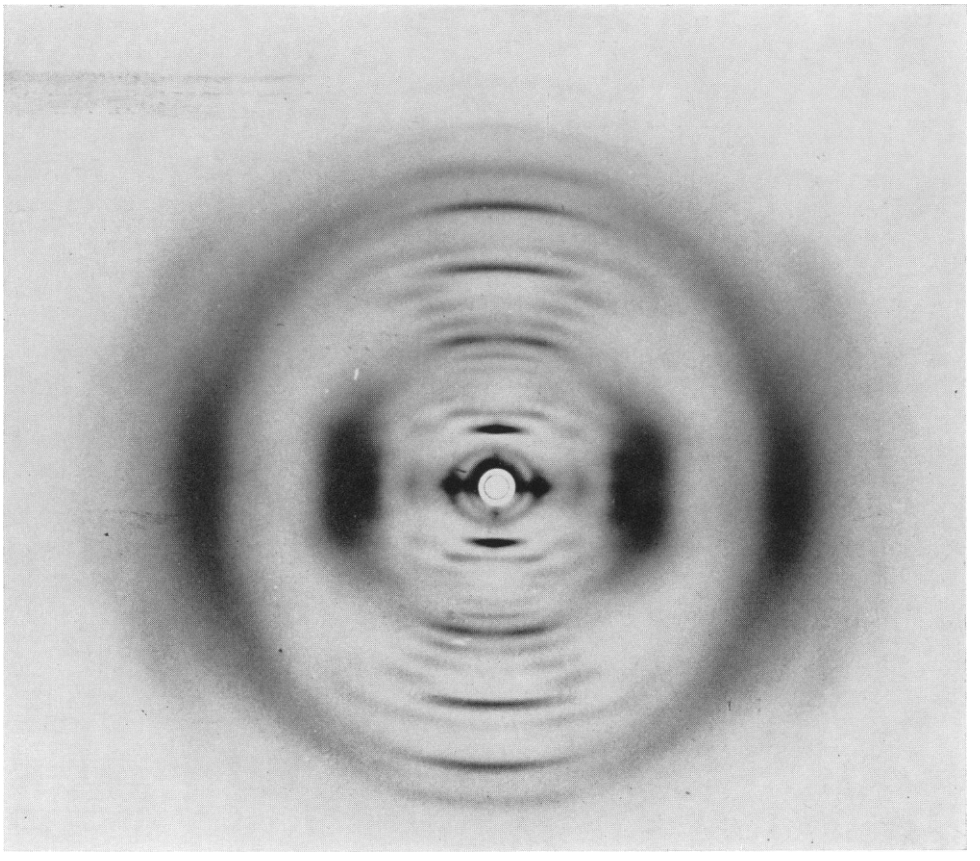

FIGURE 1 X-ray diffraction pattern of turkey calamus. Cu Kα radiation. Beam perpendicular to fiber axis and parallel to surface. Specimen-to-film distance 11.56 cm, evacuated cylindrical camera, 250 hour exposure.

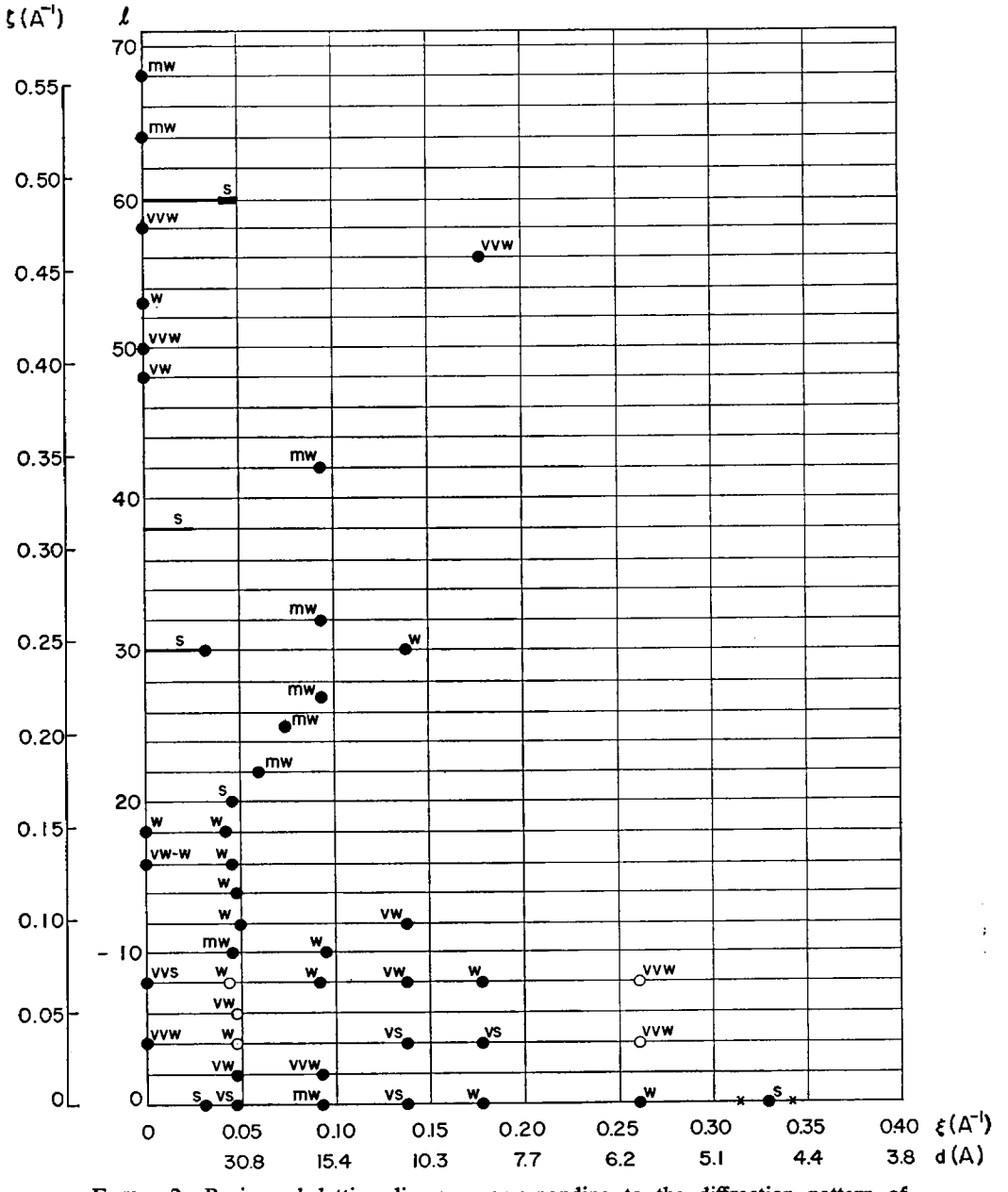

FIGURE 2 Reciprocal lattice diagram corresponding to the diffraction pattern of feather keratin. ●, reflections found in turkey calamus; ○, additional reflections present in sea gull.

is incident parallel to the fiber axis), there is a certain degree of preferred orientation. When a diffraction pattern is obtained with the beam perpendicular to the fiber axis, there is a smaller angular spread to the spots when the beam is parallel than when it is perpendicular to the surface of the feather. This may also reflect the cellular organization of the fibrous protein, since it is usually possible to peel off thin layers of material successively from a specimen of calamus. Attempts to produce double orientation (for example, by rolling a specimen which had been immersed in hot water) were unsuccessful.

Turning now to the diffraction pattern itself, several new features have emerged from our studies.

(*a*) *Identity Period.* All previous analyses of the feather keratin diffraction pattern have indicated a fiber axis identity period of about 95 A, although it has been noted (9) that some multiple of this value may be required to account for all the reflections. Our indexing of the pattern shows that an identity period of 189 A is necessary. In particular, reflections occur on layer lines representing the 25th and 27th orders of 189 A, so that an identity period of half this amount is not possible. On the other hand, most of the reflections (23 out of the 27 observed layer line and meridian spacings) fall on even order layer lines, indicating that the structure has a pseudoidentity period along the fiber axis of 94.5 A. A satisfactory model for the structure must account for these two observations.

(*b*) *The 3.1 A "Meridian" Reflection.* In all previous studies, the intense reflection at about 3.08 A "on the meridian" was indexed as a 31st order of the 95 A fiber axis period, and was interpreted as representing the projected amino acid residue repeat. We have examined this reflection somewhat more carefully by taking a diffraction pattern with the sample tilted into the beam at an angle designed to bring this reflection just into the surface of the reciprocal sphere. Such a pattern is shown in Fig. 3. It is evident from this pattern that the reflection in question is not meridional, and on this basis it is found to be more properly indexed as the 60th order of the 189 A repeat. A true meridian reflection is found nearby on the 64th layer line, and is much weaker than the 60th order spot. In this connection, we also suggest that a more satisfactory indexing of the layer line reflection previously given at 5.55 A, which we have been able to resolve into two spots in our patterns, is to the 32nd order of the 189 A repeat rather than to the 17th order of a 95 A period.

(*c*) *Other Meridian Reflections.* A search was made for meridian reflections which are characteristic of certain polypeptide chain configurations. The diffraction pattern of feather keratin shows no meridian reflection near 5.1 A, which is characteristic of the α class of keratins, but nevertheless a search was undertaken for the other reflection characteristic of this group, *viz.*, that at 1.5 A (18). A possible weak reflection was observed at 1.49 A, but its intensity was orders of magnitude less than the corresponding reflection for the α-keratins. The β-keratin structures exhibit a characteristic meridian reflection at 1.10 to 1.16 A (19). Oscillation

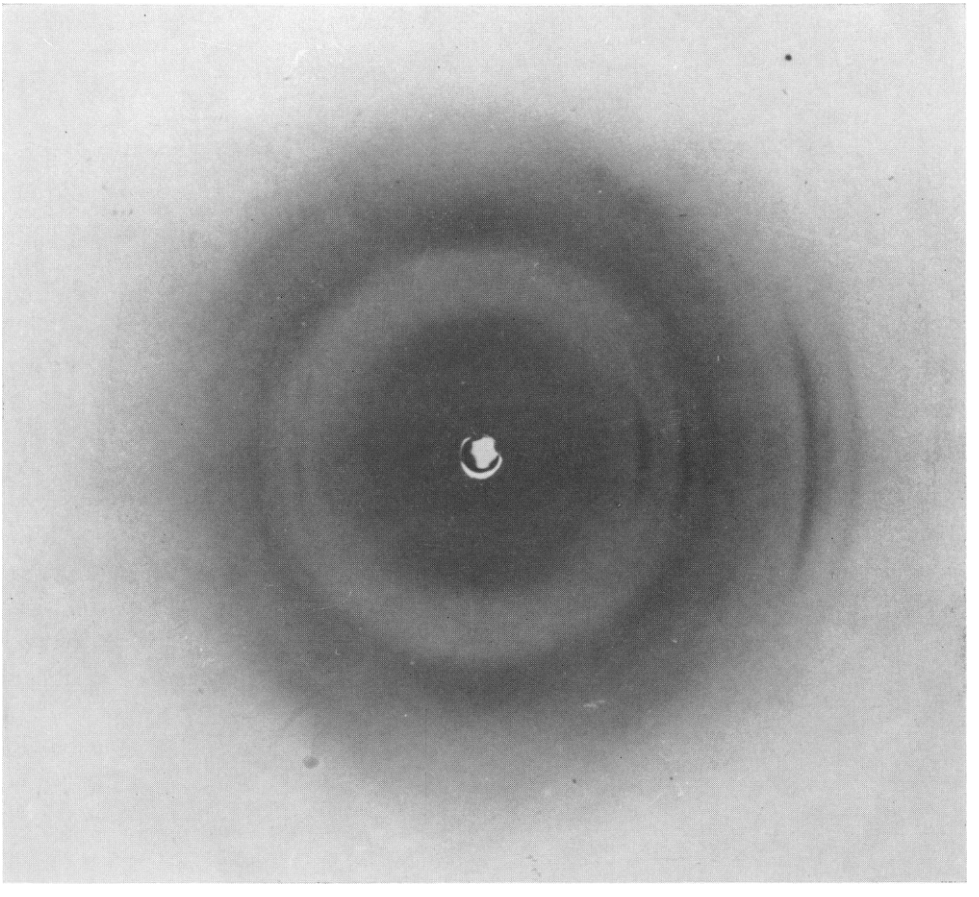

FIGURE 3 X-ray diffraction pattern of turkey calamus, tilted at an angle designed to examine the "3.1 A meridian reflection."

photographs of feather were taken which were designed to detect reflections down to a spacing of 0.9 A, but no reflection in the region of 0.9 to 1.3 A was observed.

(*d*) *Equatorial and Row-Line Reflections.* Although there has been essential agreement among all workers on the equatorial reflections of spacing about 33 A and lower, there has not been a coincidence of opinion concerning the presence of equatorial reflections with higher spacing. Equatorial reflections at 115 A, 81.8 A, and 51 A were first reported by Corey and Wyckoff (17), and later by Astbury and Bell (16). Bear subsequently suggested (15) that these three reflections were spurious, resulting from "radiation artifacts" associated with the nearby intense 33 A spot. However, the $\sim$ 50 A spot seems to appear on some of his photographs (6), and we have seen it quite clearly on our diffraction patterns. In one case (that of a heterozygous Frizzle feather), this reflection was the most intense in the pattern, the 33 A spot being practically absent. We therefore think that at least this $\sim$ 50 A spot on the equator is real, and should be accounted for by a proposed structure. A unit cell with one lateral dimenison larger than the 33 A previously suggested (6) is therefore required.

Although no satisfactory indexing of the equatorial pattern to give the lateral unit cell dimensions has as yet been proposed, it has been suggested (6) that all the intense equatorial and row line reflections can be indexed with one index; *i.e.*, the first four ξ values are all simple multiples of $\xi \cong 0.046$ A^{-1}. A closer examination of the pattern (see Fig. 2) shows that this is not strictly true. First, such indexing is, of course, not possible if the $\sim$ 50 A spot is real. Second, it is clear from the present, as well as earlier (6), work that not all the reflections on the first prominent row line have exactly the same ξ coordinate. The maximum variation in ξ (at the $\xi \cong 0.046$ row line) is about 0.005, and is undoubtedly significant. Third, the prominent row lines do not all have ξ coordinates that are simple multiples of the first strong row line. This is especially true, both on our photographs and those of previous workers, of what has been labeled the 4th row line. For example, from Fig. 2 it will be seen that although the predicted position of this equatorial reflection is $4 \times 0.047_3 = 0.189$ it is found at $\xi = 0.177$. Finally, if we admit the reflections on the 22nd and 25th layer lines as *bona fide* reciprocal lattice reflections, then again a simple indexing with one index is no longer feasible. This suggests that the necessity for requiring a sheet type of structure (6, 13) or a cylindrical lattice (20) must be accepted with caution.

e. Non-Crystalline Scattering. Although it seems not to have been specifically remarked on before, it is clear from the x-ray diffraction pattern that a non-crystalline component is present in feather keratin. This is indicated by the diffuse scattering appearing in several parts of the pattern, particularly the diffuse ring centered on the equator at a spacing of about 4.6 A. Two remarks about this non-crystalline component are pertinent. First, it is partially oriented, as indicated by the concentration of intensity in the 4.6 A ring near the equator. Second, it seems to correspond

to a normal β type of fibrous protein. This is supported by the observation that the diffuse areas of scattering centered near $\xi = 0.34$ and $\zeta = 0.23$, reflections which are characteristic of the β keratins, correspond to a repeat distance of about 6.6 A. While this distance is not observed in the main part of the feather diffraction pattern, it does correspond to that found in typical β-keratins. This emphasizes the point that at the molecular level the structure may be somewhat heterogeneous. The component contributing to the major portion of the diffraction pattern, however, appears to be homogeneous.

3. *Alterations in the Diffraction Pattern.* The x-ray diffraction pattern of feather keratin can be altered by both mechanical and chemical treatment of the feather, and these changes give insight into various features of the structure.

(*a*) *Effects of Stretching.* It had been observed (3) that feather can be mechanically stretched (up to a maximum of about 6 per cent before rupture), that this extension is reversible, and that it is accompanied by an increase in the 3.1 A meridional spacing. We have made measurements on all the meridional and equatorial spacings of stretched feather, and find that all the meridional spacings increase uniformly, while there is no significant change in the equatorial spacings. For a sample of turkey calamus stretched macroscopically by 5.5 per cent, the following percentage increases in measured meridional spacings were observed: 3.15 A: 4.9 ± 0.7; 4.98 A: 4.8 ± 0.4; 6.30 A: 5.1 ± 0.3; 23.6 A: 4.9 ± 0.4. The stretching was not accompanied by any significant change in the relative intensities of the reflections in the pattern. Thus, it appears that a definite, though limited, extension in the fiber axis direction can be achieved for feather, this extension being associated only with a change in the scale of the structure at the molecular level. This is in contradistinction to the α to β change in α-keratins, in which there is a major change in the conformation of the polypeptide chain upon stretching.

(*b*) *Effects of Chemical Treatments.* Several chemical treatments lead to marked changes in certain features of the x-ray diffraction pattern of feather keratin. Perhaps the most striking is that due to the action of water. As was observed by Bear and Rugo (6), if we compare the diffraction patterns of thoroughly dry feather (in our case, vacuum-dried) with that of a feather that has been soaked in water, two marked differences will be seen. First, there is a general increase of several per cent in the equatorial spacings of the wet feather, without any significant change in the meridional spacings. Second, there is a remarkable change in the intensities of some of the equatorial reflections: the very intense reflection at $\xi = 0.047$ in dry feather drops to practically zero intensity in a wet specimen, and we have also observed that the reflection at $\xi = 0.177$ becomes relatively more intense in comparison with the reflection at $\xi = 0.138$. Furthermore, these changes are completely reversible. The equatorial reflection at $\xi = 0.047$ is also sensitive to certain other treatments: it increases markedly in intensity when osmium is deposited in the feather (13), while the meridional reflections are not significantly affected. Probably

the most significant characteristic of the meridional pattern is the persistence of the 24 A meridian reflection. Treatments which result in a degradation of detail in the pattern do not initially affect this reflection, and it is the last to disappear prior to the total degradation of the pattern to that of a disordered protein (6). The 24 A meridian reflection and the 33 A equatorial reflection are thus unique features of the structure, the former because of its insensitivity to external influences, the latter because of its marked sensitivity to such treatments.

4. *Cylindrical Patterson Function.* The cylindrical Patterson function, $P(r, z)$, which represents the weighted two-dimensional interatomic vector density distribution, contains all the information which is obtainable from a fiber diagram without the introduction of special assumptions. This information, while not capable of providing a unique determination of the structure, can indicate the presence of significant interatomic vectors and the presence or absence of certain known types of chain conformations, and can also be used as a check on proposed models. Since from such data it may also be possible to generate reasonable hypotheses concerning the chain structure, we have computed the cylindrical Patterson for feather keratin.

The cylindrical Patterson function for a fibrous structure with periodicity along its fiber axis and random orientation about this direction is given by (21):

$$P(r, z) = \sum_l A_l(r) \cos\left(\frac{2\pi lz}{c}\right) \tag{1}$$

$$A_l(r) = K \int_0^\infty H(l, \xi) J_0(2\pi\xi r)\xi \, d\xi \tag{2}$$

where r is the radial distance of a point in the structure from the fiber axis, c is the fiber axis identity period, ξ is the radial coordinate in reciprocal space, $H(l, \xi)$ is the intensity distribution on the lth layer line in reciprocal space, J_0 $(2\pi\xi r)$ is the zero order Bessel function of the first kind, and K is a constant of proportionality.

Intensity measurements were made with a recording microphotometer on x-ray diffraction patterns of turkey calamus obtained in an evacuated 11.56 cm radius cylindrical camera. Two photographic films, one behind the other, were exposed simultaneously in order to be able to measure adequately the intensities of the weakest as well as the strongest spots. The intensity distribution above background was used, uncorrected by Lorentz or polarization factors (which were determined to be small compared to the "temperature factor" correction). In several cases the intensities were corrected in terms of a temperature factor of $e^{-7.5R^2}$, which represented a compromise between that used for a highly oriented fiber pattern, *viz.*, nylon (22), and that used for a relatively disoriented fiber pattern; *viz.*, collagen (23). Because of the presence of non-crystalline scattering in the pattern particularly in the region of the 4.66 A equatorial reflection, an attempt was made to correct for this effect by suitable reduction of the intensity in this region. The numerical calculations were performed on MIDAC (Michigan Digital Automatic Computer),

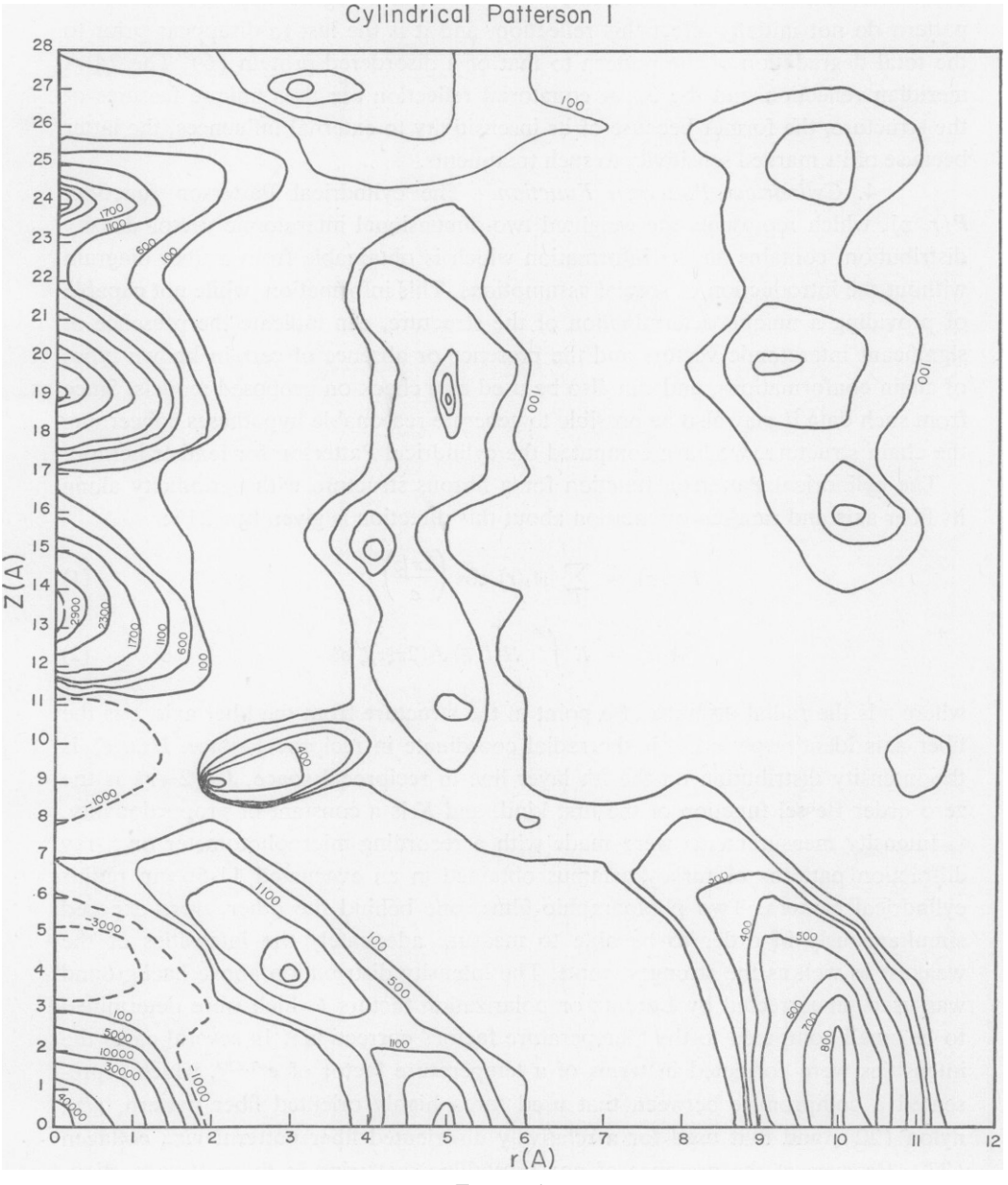

FIGURE 4 *a*

FIGURE 4 Cylindrical Patterson functions for feather keratin. (*a*) $P_1(r,z)$, (*b*) $P_3(r,z)$, (*c*) $P_4(r,z)$. (See text for explanation.)

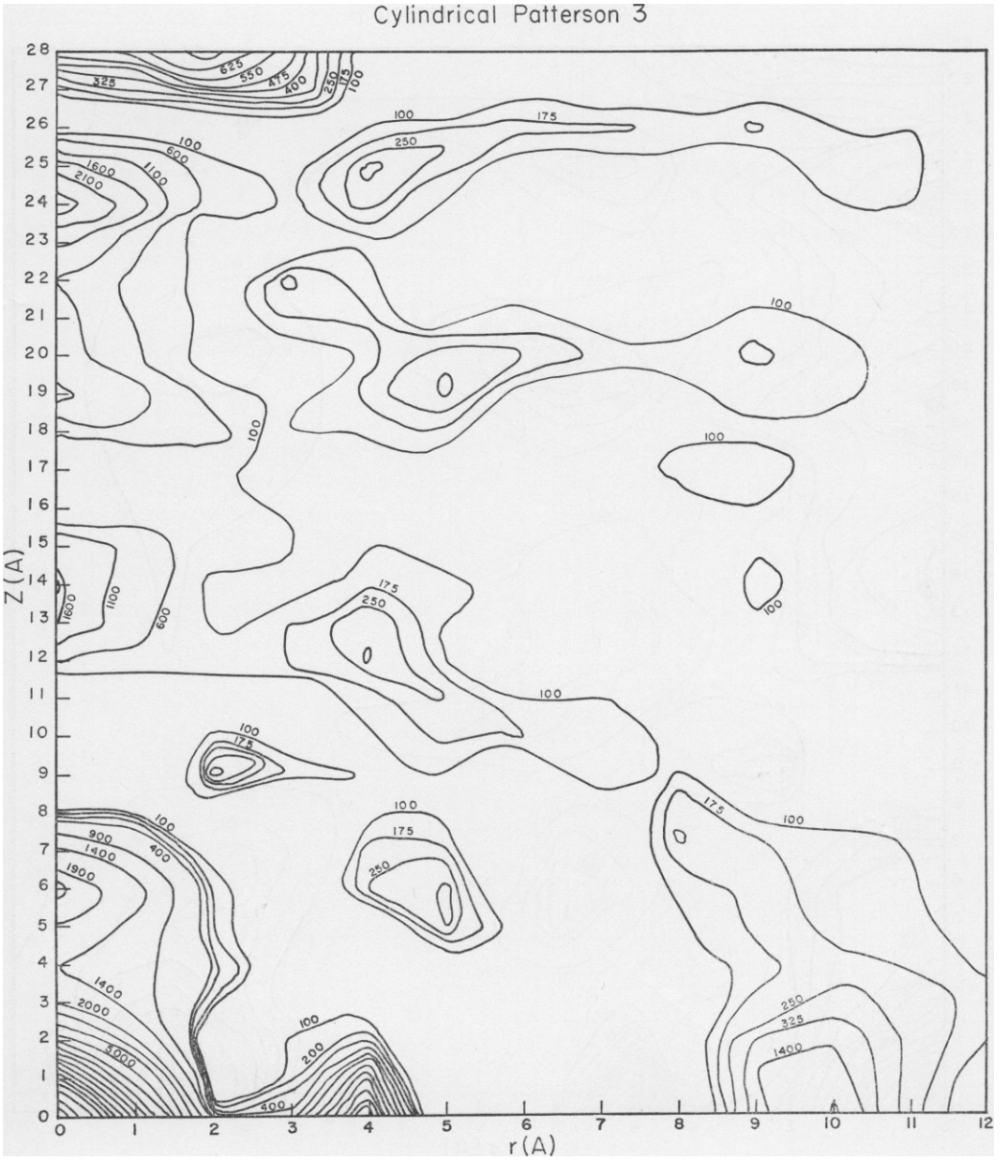
Cylindrical Patterson 3
Z(A)
r(A)
0
1
2
3
4
5
6
7
8
9
10
11
12
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
100
175
250
325
400
475
550
625
600
1100
1600
2100
900
1400
1900
2000
2400
200
400

FIGURE 4 *b*

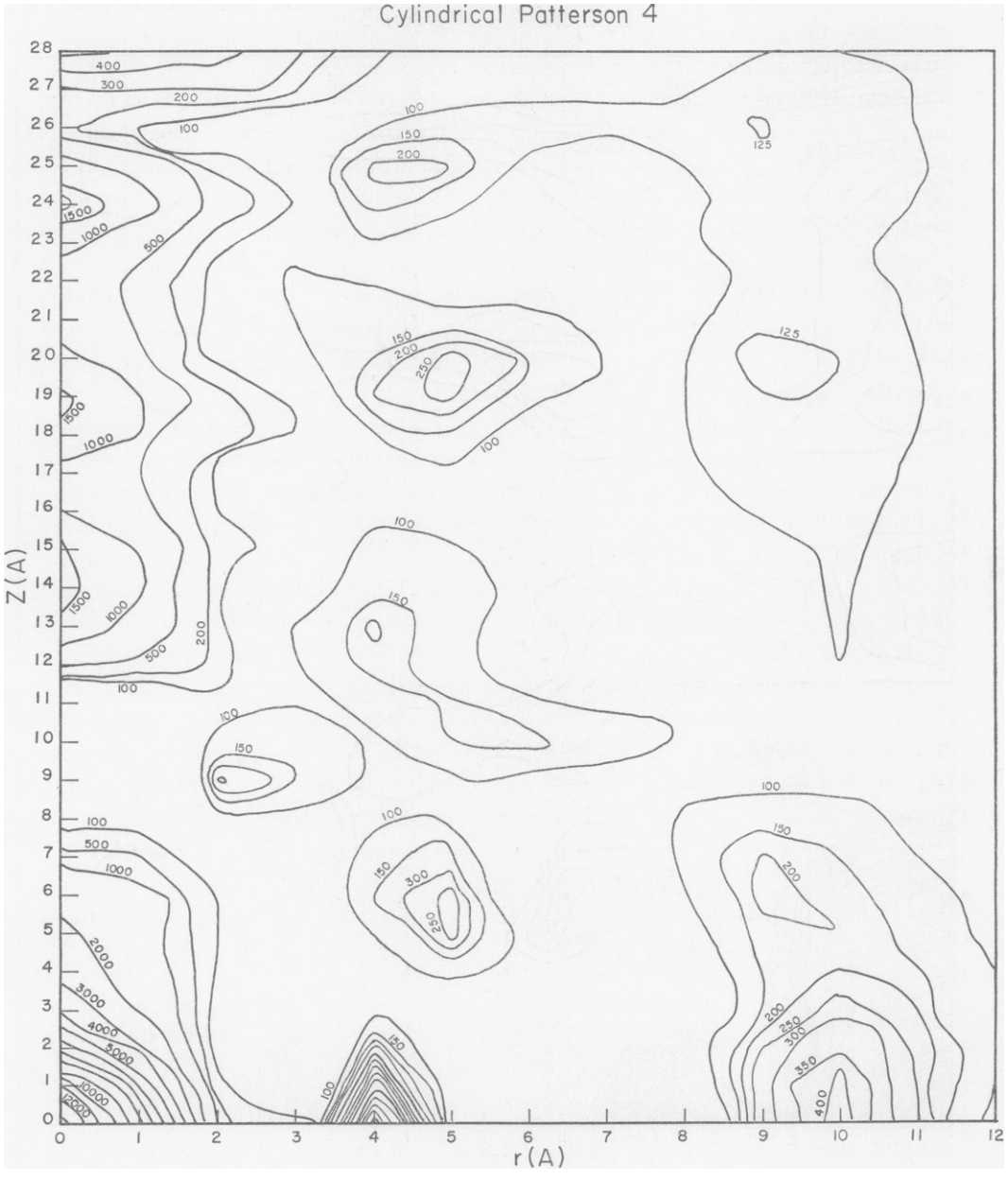

FIGURE 4 *c*

the function $P(r, z)$ being evaluated in the range of $r = 0$ to 12 A and $z = 0$ to 28 A with a grid interval of 1 A in both directions.

In order to make an effort at evaluating the effect of the uncertainties in the corrected intensities, five different Patterson functions were computed: P_1 (r, z), in which the uncorrected relative intensities were multiplied by $e^{+7.5R^2}$; P_2 (r, z), the same as P_1 (r, z) except that the intensity of the 4.66 A equatorial reflection was cut down by a factor of one-half on $l = 0$ and diminished monotonically to zero at $l = 8$; P_3 (r, z), the same as P_1 (r, z) except that the 4.66 A reflection was cut down by a factor of one-fourth; P_4 (r, z), in which the same relative intensities were used as in P_3 (r, z) but no temperature factor correction was used; and P_5 (r, z), in which the same relative intensities were used as in P_3 (r, z), but a correction factor of $e^{-8.35R^2}$ was used in order to avoid series termination errors. Several of these Patterson functions, P_1 (r, z), P_3 (r, z), and P_4 (r, z), are shown in Fig. 4; P_5 (r, z) is almost the same as P_4 (r, z), and P_2 (r, z) and P_3 (r, z) show essentially the same features.

Although the resolution in the cylindrical Patterson functions is not especially good, certain general conclusions can be drawn from the essentially constant features of P_1 (r, z) to P_5 (r, z). First, there is no indication of the presence of the α-helix in the feather structure. For the α-helix, strong peaks are found (23) in the cylindrical Patterson at (r, z) values of (3, 2), (3, 5), (3, 8), and (3, 12), regions in which feather shows no peaks at all. Second, although there are some features which the cylindrical Patterson of collagen (23) has in common with that of feather, such as peaks near (5, 0) and (5, 12), there are not enough similarities to suggest a common conformation for the collagen and feather keratin polypeptide chains (24). Third, many peaks in the Patterson remain relatively constant despite the various approximations to the diffracted intensities, and these probably represent significant features of the feather structure. Along the line $(0, z)$ there are large peaks at about (0, 19) and (0, 24), with somewhat less sharply localized peaks at (0, 6–7) and (0, 13–15). Along the line $(r, 0)$ large peaks are found at (4–5, 0) and (9–10, 0). In addition, peaks occur near (2, 9), (4, 25), (5, 6), (5, 13), (5, 19–20), (9, 6), (9, 20), and (9, 26). Possible structural implications of these Patterson peaks will be considered later.

CHEMICAL AND INFRARED STUDIES OF FEATHER KERATIN

In addition to x-ray diffraction studies, chemical and infrared studies of feather keratin provide information which is highly pertinent to considerations of the structure of this protein. We summarize below the main results of such studies.

1. *Amino Acid Composition of Feather Keratin.* The amino acid composition of feather keratin represents the first stage in the determination of its chem-

ical structure. In Table III we give the results of two such analyses, the one by Schroeder (25) being done by chromatographic techniques and ours by a microbiological assay method (26). The results of the former are accurate to ± 2 or 3 per cent, of the latter to ± 5 per cent. It must be remembered that these represent gross analyses. If, as we have indicated, feather keratin contains more than one molecular species, it is possible that these will have different amino acid compositions. There also seem to be slight differences in the amino acid compositions of feathers from different kinds of birds (25).

TABLE III

AMINO ACID COMPOSITION OF TURKEY CALAMUS

Amino acid	Residue *per cent*	
	Schroeder (25)	This study
Alanine	8.8	6.4
Arginine	4.2	3.7
Aspartic acid	5.8	2.9
Cystine	3.8	4.7
Glutamic acid	6.5	7.7
Glycine	14.0	14.5
Histidine	0.4	0.4
Isoleucine	3.3	4.1
Leucine	7.4	8.7
Lysine	0.7	1.7
Methionine	0.3	0.4
Phenylalanine	3.8	4.2
Proline	10.5	11.5
Serine	15.7	13.7
Threonine	4.4	4.3
Tryptophane	—	0.4
Tyrosine	2.4	1.9
Valine	7.9	8.6

Despite these differences, certain general features stand out. One is the high percentage of proline, perhaps up to 13 per cent (12). Among the fibrous proteins only collagen has a higher percentage of imino acids, *viz.*, about 22 per cent (27). It is of interest to note that the percentage of proline is constant among the various parts of a feather (calamus, rachis, barbs) and also between feathers of different species (25). The small residues of glycine, alanine, and serine comprise about 40 per cent of the total. About 40 per cent of the residues contain side chains with polar groups such as OH, NH, NH_2, and COOH.

The next step in the elucidation of the chemical structure is the determination of amino acid sequences. Relatively little has been done in this area. Schroeder (28)

has, in a preliminary analysis, isolated and identified 59 peptides from the partial acidic hydrolysates of turkey calamus. The only significant conclusions that can be drawn from these incomplete amino acid sequence studies are: (1) in a sequence such as X-proline, X tends to be an amino acid with a relatively short side chain, *e.g.*, glycine, serine, threonine; (2) no proline-proline sequences have been identified; (3) cystine has glutamic acid, serine, and proline as close neighbors.

2. *Solution Studies.* By employing special techniques, it is possible to solubilize feather keratin, and in some cases to reconstitute a film which exhibits some of the x-ray spacings of the original feather. Several experiments of this type have been done, and attempts made to obtain information on the homogeneity, molecular weight, size and shape, and end-groups and amino acids of the solubilized material.

The first studies of this type were done by Ward, High, and Lundgren (29), who obtained a soluble keratin by digesting whole feathers in a solution containing $NaHSO_3$ and Naconol NRSF (a detergent). The particle weight of the protein portion of the protein-detergent complex was estimated at between 34,000 and 40,000. The preparation, however, was polydisperse, so that no firm structural conclusions can be drawn from these early studies.

Using a buffered urea-bisulfite system as a solvent, Woodin (11) solubilized 80 to 85 per cent of whole feathers. Experiments were done on this reduced material, SH-keratin, and on a preparation, cysteic acid–keratin, prepared by oxidizing SH-keratin with performic acid. The cysteic acid–keratin migrated with a single boundary in both electrophoresis and ultracentrifugation experiments, which was thought to indicate a homogeneous preparation. The number average molecular weight, obtained from osmotic pressure measurements on the SH-keratin, was $M = 9800 \pm 250$, and the weight average molecular weight, obtained from light-scattering measurements, was $11,000 \pm 1000$. Woodin concluded that these values were sufficiently close to preclude the possibility of a large distribution of particle weights. Viscosity measurements led to the conclusion that the particle, if it could be treated as an unhydrated prolate ellipsoid of revolution, had an axial ratio of 13.2.

Rougvie (10) reported similar results on a preparation of whole feathers solubilized by reduction with thioglycol, as well as a cysteic acid–keratin produced by oxidation with peracetic acid. Both a monomer and dimer were reported, the molecular weight of the former (from sedimentation-diffusion) being 9300 ± 700, and that of the latter 19,300. Both preparations gave single peaks in the ultracentrifuge. On the assumption of a prolate ellipsoid shape, the following axial ratios were computed for the monomer: (a) from sedimentation-diffusion data: 9.74 (for zero hydration), 6.83 (for 30 per cent hydration); (b) from viscosity measurements: 12.3 (zero hydration), 9.77 (30 per cent hydration); (c) from Scheraga-Mandelkern theory: 22. The dimensions of the monomer unit range from 100 A by 14.7 A

(for an axial ratio of 6.83) to 148 A by 12.0 A (for an axial ratio of 12.3). The dimer is approximately twice as long for the same minor axis dimension, based on sedimentation-diffusion data.

A comparison of the results of Woodin and of Rougvie indicates agreement on two important points: (1) solubilization leads to the presence of a homogeneous unit in solution, of molecular weight approximately 10,000; (2) on the assumption of a prolate ellipsoid, this protein unit has an axial ratio significantly greater than unity. Although point one seems to be established, caution is required in accepting point two. Discrepancies in the sedimentation data were already noted by Woodin (11), and the wide variation in the axial ratios obtained by Rougvie suggests the questionable validity of the prolate ellipsoid assumption. In fact, the data seem to be more consistent with the assumption of a random coil. If we use the Flory-Fox equation to determine the r.m.s. end-to-end length, $(\overline{r^2})^{1/2}$, for a random coil from intrinsic viscosity, $[\eta]$, namely

$$[\eta]_M = \Phi(\overline{r^2})^{3/2} \tag{3}$$

where Φ is a constant whose most probably value is 2.5×10^{21} (30, p. 617), we find, using Rougvie's value of 0.135 for $[\eta]$ and $M = 9300$, that $(\overline{r^2})^{1/2} = 79.5$ A. On the other hand, $(\overline{r^2})^{1/2}$ can be computed from sedimentation data by combining equation (3) with the following relationship (30, p. 627):

$$s_0[\eta]^{1/3}M^{-2/3} = \frac{\Phi^{1/3}(1 - \bar{v}\rho)}{5.11\eta_0 N} \tag{4}$$

where s_0 is the sedimentation constant, $\bar{v}$ the partial specific volume of the protein, ρ the density of the solvent, η_0 the viscosity of the solvent, and N is Avogadro's number. Taking Rougvie's values of $s_0 = 1.06 \times 10^{-13}$, $\bar{v} = 0.725$, and $\rho = 1.00$ and $\eta_0 = 0.01$, we compute a value of $(\overline{r^2})^{1/2} = 78.5$ A. Thus, viscosity and sedimentation data yield essentially the same random coil dimensions, but give axial ratios for prolate ellipsoids which differ by the order of 30 per cent from each other. This indicates that a random coil conformation may be the best approximation to the shape of the soluble feather keratin unit.

Woodin (12) has examined the solubilized cysteic acid–keratin to determine what, if any, end-groups it exhibits and what its amino acid composition is. He finds 0.1 equivalent of N-terminal amino acids per mole of keratin (molecular weight taken as 10,000), and concludes that, there being no evidence for masking of amino groups, therefore a cyclic structure is implied. No N-terminal proline was found by the fluorodinitrobenzene method. The carboxypeptidase method yielded 1 equivalent of C-terminal amino acid per mole of protein, but this was distributed among seven or eight different amino acids. Woodin concluded that this is most probably due to the stepwise hydrolysis of an impurity. The amino acid composition of cysteic acid–keratin is found to be (in equivalents/mole of keratin): ala, 4.0,

arg, 3.9, asp + glu, 10.2, cysteic acid, 6.1, gly, 9.8, lys, 0.1-0.2, phe, 3.2, pro, 13.0, ser, 13.7, thr, 5.0, val + leu, 19.0. It is interesting to note that the proline of the feather is concentrated in the soluble unit.

Rougvie (10) was able to reconstitute oriented films of both SH-keratin and cysteic acid–keratin which showed some of the x-ray diffraction spacings of the native feather. In particular, the SH-keratin gave meridian reflections at 22.1, 16.1, 6.20, 5.40, and 3.05 A, and equatorial reflections at 33.5, 9.48, and 4.63 A. The cysteic acid–keratin, while exhibiting meridian reflections at about 22.1 and 6.20 A, and equatorial scattering in the 9.5 and 4.6 A regions, failed to show an equatorial reflection at 33.5 A. On the basis of these results, it has been concluded (10, 12) that the soluble unit is responsible for the x-ray diffraction pattern of the native feather. This conclusion, of course, does not necessarily follow unambiguously from the data. There has been no demonstration that the soluble unit goes over without change of shape or form into the reconstituted keratin, or that the solubilization of the native material is accomplished without alteration of the polypeptide chain conformation. In fact, as we have seen, there is good evidence to believe that the soluble unit is in the form of a random coil, which would indicate that a change in chain conformation is a likely consequence of solubilization and reconstitution. The supposed cyclic nature of the soluble keratin, which conclusion may itself not be without ambiguity, also does not necessitate the presence of cyclic polypeptide chains in the native material. In fact, using the suggested dimensions of such a unit (12), it is not possible to make any reasonable correlation with the spacings shown in the x-ray diffraction pattern.

We conclude that, while solubilization studies indicate that feather keratin can be easily broken down into units of molecular weight around 10,000, no good evidence exists for supposing that these units are other than random coils in solution.

3. *Infrared Studies.* Infrared spectra of feather keratin have been used to obtain information on the orientation of the constituent polypeptide chains and on their conformation.

While the perpendicular dichroism of the ν (NH) and ν (CO) modes is quite low in native feather keratin, it has been shown (31) that deuteration of the feather results in a marked increase in the perpendicular dichroism of the ν (NH) mode, from 1.8 : 1 to 4.8 : 1. The evidence suggests that this remaining high dichroism is to be associated with the hydrogen atoms of the peptide groups, and therefore indicates the presence of fairly well oriented, essentially extended, polypeptide chains.

The presence of a double absorption band in the 1645 cm^{-1} region of the infrared spectrum was taken (32) to indicate that, in addition to a β or extended component, there was some α, or folded, protein present. This was based on the attempted empirical correlation (33) of a band near 1630 cm^{-1} with a β-protein and a band near 1660 cm^{-1} with an α-protein. However, as was pointed out by Krimm (34), there

is no good reason for correlating this frequency with the over-all conformation of the polypeptide chain; it is more probably associated with the local structure of the hydrogen bonded units. On this basis, we have not considered that the infrared spectrum necessitates postulating the presence of ordered structures other than an essentially extended polypeptide chain. It is interesting to note that subsequent work (35, 36) has required the rejection of the above empirical correlation, since it has been shown that polypeptide chains in other than the α-helical form, *e.g.*, in the disordered state, also give rise to a band near 1660 cm^{-1}. This is, of course, consistent with our previous observation concerning the presence of non-crystalline material in feather keratin.

DISCUSSION

The above data on feather keratin, although they do not permit the unique deduction of a model for the structure, allow some fairly definite remarks to be made about the limitations which must be placed on considerations of a satisfactory model. In particular, these data serve to eliminate from consideration the presently known polypeptide chain structures, and to suggest some of the features which must be part of a satisfactory structure. We wish to consider these points now in somewhat greater detail.

As is evident from the x-ray data and the cylindrical Pattersons, the α-helix is not a component of feather keratin. None of its characteristic spacings appear either in the diffraction pattern or in the Patterson. The high perpendicular infrared dichroism of ν (NH) is also inconsistent with any kind of model containing α-helices whose axes are predominantly parallel to the fiber axis of the feather. It is likely that the infrared results also exclude other types of folded polypeptide chain structures.

It has been suggested (24) that the structure of feather keratin is based on the three-chain model for the structure of collagen (37, 38). There are a good many reasons which suggest that this is unlikely. First, the characteristic meridional spacing related to the amino acid repeat is found at 2.86 A in collagen (39), but appears at 2.96 A in feather keratin. Second, as we noted earlier, while the cylindrical Patterson of feather keratin shares several peaks with that of collagen, the resemblance between the two does not extend sufficiently beyond this to suggest a common structure as the basis for these two fibrous proteins. Third, collagen exhibits none of the long periodicities found to be so characteristic of the diffraction pattern of feather keratin. Our stretching experiments indicate that these long spacings are an integral part of the feather keratin structure. Fourth, some of the characteristic infrared absorption bands of collagen are at significantly different frequencies from those of feather keratin. For example, the ν (NH) mode, which is found at 3300 cm^{-1} in α- and β-proteins, occurs at 3315 cm^{-1} in feather keratin and 3330 cm^{-1}

in collagen (32). Since these frequencies probably reflect the local environment of the hydrogen bond in the peptide group (34), it is likely that the above difference in frequency is at least indicative of a difference in local structure around the peptide group. Finally, the amino acid compositions of these two proteins are so widely different in certain significant features as to make it questionable, in view of the way in which the collagen structure is related to its composition, that the two chain conformations are the same. For example, while feather has about 12 per cent proline, collagen has 22 per cent of proline plus hydroxyproline; and while glycine comprises just over one-third of the residues in collagen, where it occupies a very structurally significant position, it accounts for only 14 per cent of those in feather keratin. Furthermore, whereas proline-hydroxyproline sequences are common in collagen (40, 41), no proline-proline sequences have been identified in feather (28). Since proline is undoubtedly a significant factor in the determination of the polypeptide chain conformation, this fact alone would make it seem very unlikely that collagen and feather keratin have similar structures.

The most reasonable assumption concerning the feather keratin structure would appear to be that it is related to the β, or extended polypeptide chain, class of fibrous proteins. This is indicated by the strong perpendicular dichroism of its ν (NH) absorption band in the infrared spectrum (31), as well as by the presence of a perpendicular ν (CO) band at a frequency characteristic of the β-proteins, *viz.*, about 1642 cm^{-1} (32). It is further supported by the x-ray diffraction data: the fiber axis repeat of about 3 A is close to values characteristic of the pleated sheet structures (42), and the equatorial scattering near 4.6 A corresponds to the hydrogen-bonded distance between chains which is found in such structures. An additional check on this is provided by a comparison of the one-dimensional Patterson projections of silk, computed from the data of Marsh, Corey, and Pauling (43), and that of feather keratin, computed from our data. Both functions show peaks near $r = 5$ A and $r = 10$ A, with a minimum at $r = 3$ A. And yet, the feather keratin pattern exhibits features not common to those of the usual extended chain structures, particularly in the presence of its long spacings. We will consider a possible resolution of this problem in the following paper.

SUMMARY

The present work indicates that certain requirements must be met by a satisfactory model for the structure of feather keratin. These are considered to be the following: (1) The structure must have an identity period of 189 A, with a pseudoidentity period of 94.5 A. (2) A characteristic fiber axis periodicity of 23.6_4 A must emerge, and in such a manner that it is relatively insensitive to external influences. Another fiber axis periodicity, *viz.*, of 18.9 A (manifest both in the x-ray diffraction pattern and in the cylindrical Patterson), should also be evident. (3) The structure should

give rise to a 2.96 A meridian reflection, while at the same time not having a strong reflection in the 1.0 A region on the meridian. (4) A characteristic equatorial reflection of about 33 A spacing should be predicted, which is of such a nature that it is highly sensitive to external influences. Equatorial reflections of about 50 A spacing should be accommodated by the structure. (5) The perpendicular infrared dichroism of ν (NH) should be at least of the order of 5:1. (6) The structure should be such that a finite but limited extension of it along the fiber axis direction is possible, and in such a manner that all of its dimensions along this direction increase uniformly without any change taking place in the over-all conformation. (7) Of the order of 12 per cent of the residues in the structure must be proline, and their incorporation must occur in a natural fashion. (8) It should be possible to break down the structure in such a way that protein chains of molecular weight about 10,000 result, and with a possibility of partially reforming the original structure upon reconstitution of this solubilized material.

The only model which satisfies these requirements is the one previously proposed by us (14), and considered in detail in the following paper.

Support is gratefully acknowledged from United States Public Health Service grants H-2740 and A-2830.

This work is based in part on a thesis submitted by R. Schor in partial fulfillment of the requirements for the Ph.D degree, University of Michigan, 1958.

Received for publication, March 13, 1961.

REFERENCES

1. KRIMM, S., *J. Molecular Biol.*, 1960, **2**, 247.
2. RUDALL, K. M., *Biochim. et Biophysica Acta*, 1947, **1**, 549.
3. ASTBURY, W. T., and MARWICK, T. C., *Nature*, 1932, **130**, 309.
4. ASTBURY, W. T., and LOMAX, R., *Nature*, 1934, **133**, 794.
5. ASTBURY, W. T., PERRY, S. V., REED, R., and SPARK, L. C., *Biochim. et Biophysica Acta*, 1947, **1**, 379.
6. BEAR, R. S., and RUGO, H. J., *Ann. New York Acad. Sc.*, 1951, **53**, 627.
7. PAULING, L., AND COREY, R. B., *Proc. Nat. Acad. Sc.*, 1951, **37**, 256.
8. PAULING, L., and COREY, R. B., *Nature*, 1953, **171**, 59.
9. KRAUT, J., Ph.D. thesis, California Institute of Technology, 1954.
10. ROUGVIE, M. A., Ph.D. thesis, Massachusetts Institute of Technology, 1954.
11. WOODIN, A. M., *Biochem. J.*, 1954, **57**, 99.
12. WOODIN, A. M., *Biochem. J.*, 1956, **63**, 576.
13. FRASER, R. D. B., and MACRAE, T. P., *J. Molecular Biol.*, 1959, **1**, 387.
14. KRIMM, S., and SCHOR, R., *J. Chem. Physics*, 1956, **24**, 922.
15. BEAR, R. S., *J. Am. Chem. Soc.*, 1944, **66**, 2043.
16. ASTBURY, W. T., and BELL, F. O., *Tabulae Biologicae*, 1939, **17**, 90.
17. COREY, R. B., and WYCKOFF, R. W. G., *J. Biol. Chem.*, 1936, **114**, 407.
18. PERUTZ, M. F., *Nature*, 1951, **167**, 1053.
19. BAMFORD, C. H., BROWN, L., ELLIOTT, A., HANBY, W. E., and TROTTER, I., *Nature*, 1953, **171**, 1149.
20. RAMACHANDRAN, G. N., and SASISEKHARAN, V., *Arch. Biochem. and Biophysics*, 1956, **63**, 255.

21. MACGILLAVRY, C. H., and BRUINS, E. M., *Acta Cryst.*, 1948, **1**, 156.
22. BUNN, C. W., and GARNER, E. V., *Proc. Roy. Soc. London, Series A*, 1947, **189**, 39.
23. YAKEL, H. L., and SCHATZ, P. N., *Acta Cryst.*, 1955, **8**, 22.
24. RAMACHANDRAN, G. N., *Acta Cryst.*, 1957, **10**, 816.
25. SCHROEDER, W. A., KAY, L. M., LEWIS, B., and MUNGER, N., *J. Am. Chem Soc.*, 1955, **77**, 3901.
26. HENDERSON, L. M., and SNELL, E. E., *J. Biol. Chem.*, 1948, **172**, 15.
27. TRISTRAM, G. R., *in* The Proteins, (H. Neurath and K. Bailey, editors), New York, Academic Press, Inc., 1953, **1**, pt. A.
28. SCHROEDER, W. A., KAY, L. M., MUNGER, M., MARTIN, N., and BALOG, J., *J. Am. Chem. Soc.*, 1957, **79**, 2769.
29. WARD, W. H., HIGH, L. M., and LUNDGREN, H. P., *J. Polymer Research*, 1946, **1**, 22.
30. FLORY, P. J., Principles of Polymer Chemistry, Ithaca, Cornell University Press, 1953.
31. PARKER, K. D., *Biochem. et Biophysica Acta*, 1955, **17**, 148.
32. AMBROSE, E. J., and ELLIOTT, A., *Proc. Roy Soc., London, Series A*, 1951, **206**, 206.
33. AMBROSE, E. J., and ELLIOTT, A., *Proc. Roy. Soc., London, Series A*, 1951, **205**, 47.
34. KRIMM, S., *J. Chem. Physics*, 1955, **23**, 1371.
35. ELLIOTT, A., HANBY, W. E. and MALCOLM, B. R., *Nature*, 1957, **180**, 1340.
36. ELLIOTT, A., HANBY, W. E., and MALCOLM, B. R., *Discussions Faraday Soc.*, 1958, **25**, 167.
37. RAMACHANDRAN, G. N., and KARTHA, G., *Nature*, 1955, **176**, 593.
38. RICH, A., and CRICK, F. H. C., *Nature*, 1955, **176**, 915.
39. COWAN, P. M., NORTH, A. C. T., and RANDALL, J. T. *in* Nature and Structure of Collagen, (J. T. Randall, editor) London, Butterworth & Co., Ltd., 1953, 241.
40. SCHROEDER, W. A., KAY, L. M., LEGETTE, J., HONNEN, L., and GREEN, F. C., *J. Am. Chem. Soc.*, 1954, **76**, 3556.
41. KRONER, T. D., TABROFF, W., and MCGARR, J. J., *J. Am. Chem. Soc.*, 1955, **77**, 3356.
42. PAULING, L., and COREY, R. B., *Proc. Nat. Acad. Sc.*, 1951, **37**, 729; 1953, **39**, 253.
43. MARSH, R. E., COREY, R. B., and PAULING, L., *Biochim. et. Biophysica Acta*, 1955, **16**, 1.