AN INVESTIGATION OF THE STRUCTURE OF SILK FIBROIN*

by

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I. INTRODUCTION

Of all the fibrous proteins, silk fibroin might appear to be most suitable for a determination of structure by the methods of X-ray diffraction analysis. It is the simplest of the fibrous proteins in that it contains a preponderance of small amino-acid residues. Chemical analyses now account for 98% of its constituent residues; of these, glycine, the simplest of the amino acids, comprises about 44% and glycine, alanine, and serine, the three simplest, 82%. Its amino-acid composition, as given in the compilation of TRISTRAM[1], is shown in Table I. The X-ray diffraction pattern of silk fibroin indicates a highly ordered structure in comparison with most other fibrous proteins. Since the first observations of HERZOG AND JANCKE[2] in 1920, many attempts have been made to explain the X-ray diffraction patterns of fibroin in terms of groupings of polypeptide chains arranged approximately parallel to the fiber axis. All of these attempts have been qualitative rather than quantitative: proposed units of structure have been derived from the X-ray fiber diagram and suggestions have been made regarding the probable arrangement of extended polypeptide chains within the units, but none of these structures has been precisely formulated and hence the validity of none of them has been tested by comparison of observed and calculated intensities of the X-ray reflections.

In the present X-ray investigation of silk fibroin advantage has been taken of current knowledge of the dimensions and configurations of polypeptide chains in proteins[3] to assign definite positions to the C, N, and O atoms of the chain. From their positional coordinates the contributions of these atoms to the intensities of the X-ray reflections have been calculated. The critical discussion of the structure is therefore based upon the quantitative agreement between the observed and calculated X-ray diffraction patterns as well as upon other physical and chemical evidence.

The first attempt to derive the structure of silk fibroin from its X-ray diffraction pattern was made by BRILL[4] in 1923. He assigned indices to about 20 spots in the fiber diagram in terms of an orthogonal unit with identity distances of 7.0 A along the fiber axis and 9.3 and 10.4 A perpendicular to it. He concluded that silk fibroin consisted of at least two proteins, one of which was crystalline, and that the crystalline component was composed of equimolar quantities of glycine and alanine. For the composition of this

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TABLE I

AMINO-ACID COMPOSITION OF SILK FIBROIN (TRISTRAM[1])

Amino acid	*Moles/10^5 g protein*	*Residue %*
Glycine	581.0	44.7
Alanine	334.0	25.7
Serine	154.3	11.9
Tyrosine	70.7	5.4
Valine	30.8	2.4
Aspartic acid	20.75	1.6
Phenylalanine	20.4	1.6
Glutamic acid	14.7	1.1
Threonine	13.45	1.0
Isoleucine	8.40	0.6
Leucine	6.95	0.5
Proline	6.43	0.5
Arginine	6.33	0.5
Lysine	4.66	0.4
Histidine	2.32	0.2
Total	1275.2	98.1

crystalline component he suggested polymerization products of glycylalanine or alanylglycine and pointed out that the unit cell would contain one glycylalanyl portion of each of four chains. In a discussion based on BRILL's data, MEYER AND MARK[5] presented more definitely some possible arrangements of four polypeptide chains of alternating glycine and alanine residues. They suggested that the peptide chains were strongly attracted to one another by forces between the CO and NH groups of adjacent chains, and also that there might be occasional replacement of glycyl or alanyl by other amino-acid residues.

Double orientation of the silk fiber by rolling was first reported by HERZOG AND JANCKE[6] in 1929. Photographs of rolled silkworm gut showed marked differences in the relative intensities of the equatorial and layer-line reflections depending on whether the X-ray beam were perpendicular or parallel to the plane of rolling. This effect was further investigated by KRATKY[7]. He prepared his oriented specimens by moistening the freshly extracted silk gland with dilute acetic acid and then immediately deforming the contents of the gland by stretching and rolling it. He reported equatorial reflections corresponding to the following values of interplanar spacings: 9.4, 4.62, 4.26, and 3.04 A. Of these reflections, the first, second, and fourth are diffuse and arise from planes which are approximately parallel to the plane of the rolled sheet; the third is sharp and its plane apparently makes an angle of between 55° and 90° with the sheet. A few spots on the first layer line appeared to be related to diffuse and sharp spots on the equator. These observations on oriented specimens completely invalidated BRILL's earlier assignment of axes. KRATKY called attention to the possible implications of the broad, diffuse reflections, all apparently occurring from planes with a particular orientation—either the crystallite was very flat, or, more likely, the repetition in one direction was not exact. He pointed out that if the latter implication is true, it has especial significance with reference to the suggestions made by MEYER AND MARK[5] regarding the chemical constitution of silk, namely, the possibility of the occasional replacement of residues of alanine and glycine by residues of other amino acids.

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In a more extensive investigation, KRATKY AND KURIYAMA[8] reported that *Bombyx mori* and tussah silk gave similar but not identical X-ray patterns, and that four other silks gave patterns which resembled one another but were not closely related to that of *Bombyx mori*. Possible unit cells for *Bombyx mori* silk fibroin were derived from the diffraction patterns by selecting sets of three interplanar equatorial vectors corresponding to three reciprocal lattice vectors mutually related as the sides of a triangle. In their paper they concluded that the plane of the sharp equatorial reflection (4.26 A) must make an angle of between 60° and 75° with the plane of the rolled sheet. Of ten possible unit cells, four were eliminated because the densities calculated for them on the assumption of a molecular weight of 128 for each of four chains (alanylglycyl) failed to agree with the expected density of silk, 1.3 to 1.5 g/cm^3. No further selections were made among the six remaining unit cells. HUGGINS[9] has shown that of these six only two permit the polypeptide chains to be arranged in ways which are not in conflict with the X-ray data.

TROGUS AND HESS[10] compared the X-ray patterns, chemical analyses, and other properties of several varieties of silk. From measurements of their excellent X-ray patterns they obtained numerical data which were more accurate than those previously published; furthermore, the patterns of tussah silk, spider silk, and others differed somewhat from that of *Bombyx mori*. No attempt was made to derive new unit cells or to propose new structures for the crystalline constituent of fibroin. TROGUS and HESS suggested that silk may contain more than one crystalline component, one of which is common to all silks, and also an amorphous component.

In a paper published in 1943[11], BRILL suggested for silk fibroin a unit of structure having the dimensions $a = 9.65$ A, $b = 10.40$ A, $c = 6.95$ A (fiber axis), $\gamma = 62.4°$, and containing four alanylglycyl residues. Neighboring chains, arranged approximately in the *ac* plane, run in opposite directions and are held together by hydrogen bonds between CO and NH groups; methyl groups extend in the general direction of *b*, and probably require some rotation of adjacent chains in order to give satisfactory packing. No atomic parameters were assigned. BRILL emphasized the necessity for an adequate discussion of the X-ray intensities before this proposed structure could be considered to be other than hypothetical.

In discussing the composition of the crystalline component of fibroin BRILL raised the question which has bothered investigators from the beginning, namely, whether tyrosine can be included in the unit of structure, or must be relegated to a non-crystalline component. Unlike KRATKY, he preferred to explain the diffuseness of the reflections from planes making small angles with the *ac* plane as arising from the probable small dimensions of the micelle in directions roughly perpendicular to this plane, rather than from a randomness of intermolecular spacing due to the inclusion of side chains other than methyl. He concluded quite definitely that the benzene nucleus of a tyrosine residue requires so much room that tyrosine cannot be present in the crystalline portion of fibroin. In 1941 MEYER, FULD AND KLEMM[12] had reported that coupling of silk with diazobenzenesulfonic acid caused no change in the X-ray diagram, from which they concluded that tyrosine and presumably all amino-acid residues other than glycine, alanine and serine are present in silk in a noncrystalline component. Recently, similar conclusions were drawn by DRUCKER AND SMITH[13, 14]: tryptic digestion of "renatured" silk dissolved in cupriethylenediamine solution produced a precipitate which they reported to be a polypeptide of molecular weight 7000 consisting of glycine, alanine,

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and serine. On the other hand, FRIEDRICH-FREKSA, KRATKY AND SEKORA[15] had reported that silk in which most of the tyrosine residues were iodinated gave three new, very faint meridional reflections that could be correlated with a period of 70 A, suggesting that in the crystalline component of fibroin every twentieth residue is tyrosine. ZAHN, KRATKY AND SEKORA[16] reported in 1951 that nitration of silk in a manner which transformed the tyrosine into 3-nitrotyrosine and 3,5-dinitrotyrosine resulted in the introduction into the X-ray pattern of the first-order meridional reflection (6.95 A) and also two additional layer lines corresponding to spacings of 10.4 and 20.9 A. ZAHN[17] explained these results by assuming that the tyrosine residues occur at specific positions on the outer surface of the crystallites or between them. The conclusions drawn from experimental observations are thus conflicting, so that the presence or absence of tyrosine in crystalline portions of silk fibroin is as yet not satisfactorily established.

II. EXPERIMENTAL

The investigation into the structure of silk fibroin was begun at this Institute in 1940 by Dr. MAX ROGERS. Dr. ROGERS prepared a large number of samples of silkworm gut (*Bombyx mori*) many of which were doubly oriented by stretching and rolling in the manner described by previous workers[6,7,8]. Dr. ROGERS photographed these samples in a cylindrical vacuum camera with 3-cm radius using Kα radiation from both copper and cobalt targets. More recently, we have rephotographed many of the same samples in a cylindrical camera with 10-cm radius using Ni-filtered Cu Kα radiation. In this camera an atmosphere of helium was used in order to eliminate fogging of the film by air scattering. Photographs were taken with the X-ray beam perpendicular to the fiber axis and both parallel and perpendicular to the plane of rolling of the doubly-oriented samples. Typical photographs are reproduced in Figs. 1 and 2. The double orientation of these samples was also recorded on Weissenberg photographs taken with the X-ray beam perpendicular to the fiber axis.

Accurate values for the relative intensities and interplanar spacings of the equatorial reflections from silk fibroin were obtained by the use of an X-ray spectrometer. For the purpose seven strands of commercial silkworm gut were arranged in a cylindrical bundle about 1.5 mm in diameter which was bathed in a beam of crystal-monochromatized Cu Kα radiation. The equatorial spectrum was recorded by means of a Geiger counter, measurements being made at intervals of 10 minutes of arc. The intensity of the scattered radiation at each position of the counter was taken to be proportional to the counting rate—the reciprocal of the time required to accumulate 10,000 counts. The scattering curve for the equatorial spectrum, reproduced in Fig. 3, was obtained by plotting counting rate as a function of the scattering angle 2θ. The background scattering, estimated empirically as indicated in Fig. 3, was subtracted from the total scattering and the corrected counting rates thus obtained were used to derive the positions and the intensities of the diffraction maxima.

The positions of the centers of the four maxima 1, 4, 5, and 8 (Table II) were obtained by the following procedure. The assumption was made that the corrected counting rates in the vicinity of a diffraction maximum can be fitted to a Gaussian curve $R_x = R_0 \exp(-Ax^2 - Bx)$, in which R_0 is the counting rate at some arbitrary origin $x = 0$ near, but not necessarily coincident with, the center of the maximum, and R_x is the counting rate at the distance x, measured in degrees, from this origin. The center of the maximum

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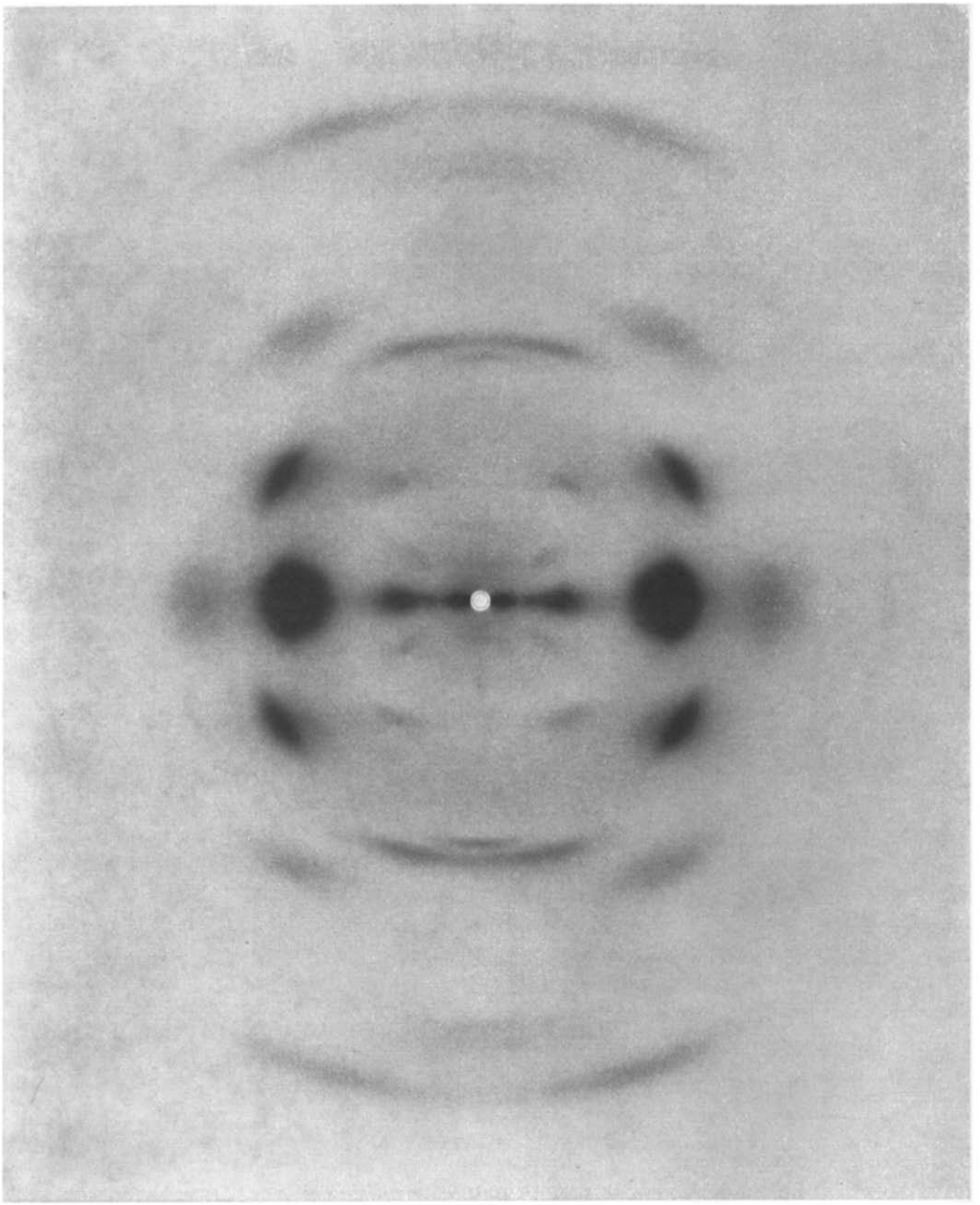

Fig. 1. X-ray diffraction pattern for silk fibroin.

is then given by $x_0 = -B/2A$, best values of A, B, and R_0 being determined from solution of the normal equations of a least-squares treatment*. In order to test the validity of the assumption that the observed counting rates representing individual maxima

* See, for example, *The Calculus of Observations* by E. T. WHITTAKER AND G. ROBINSON, London, 1937, Chapt. IX.

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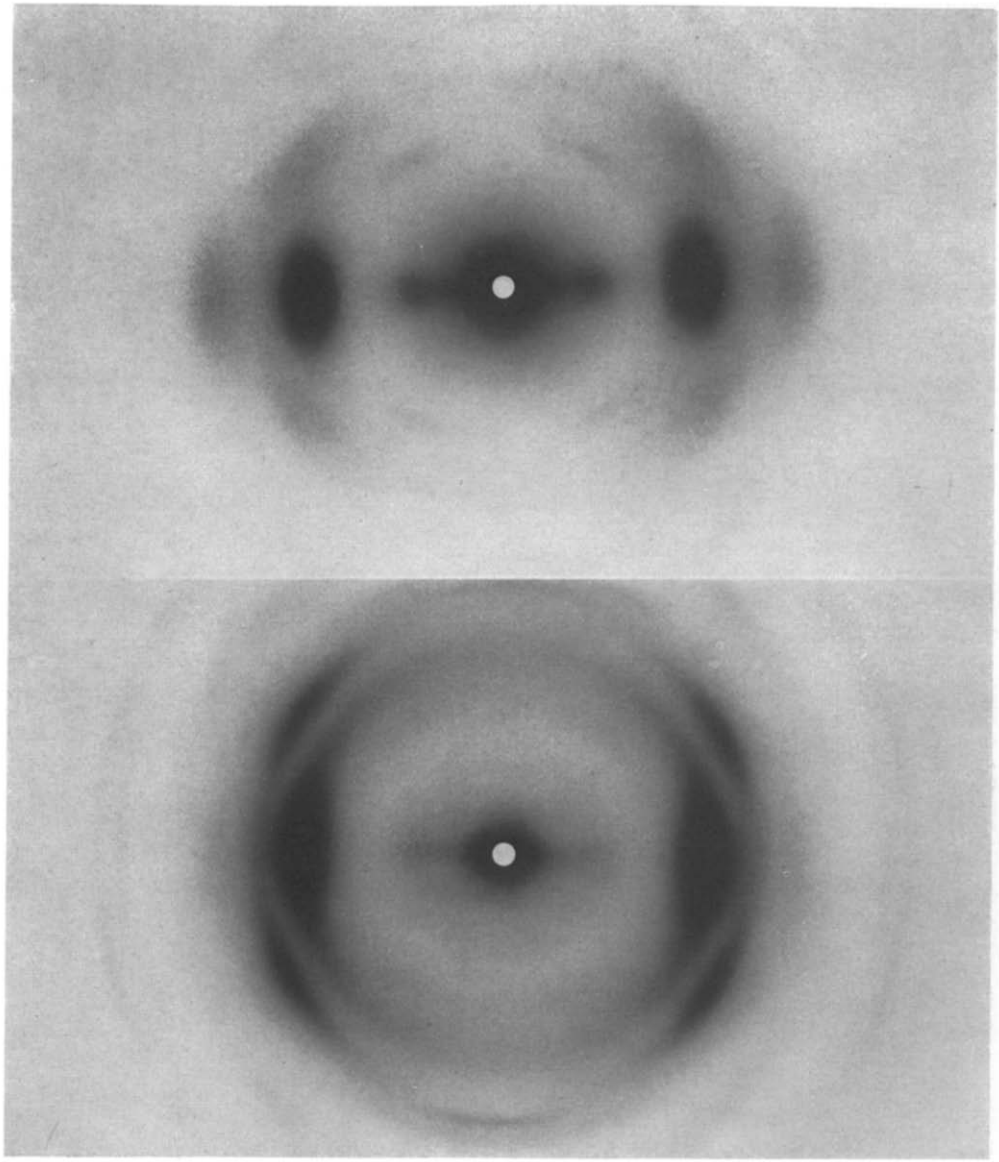

Fig. 2. X-ray photographs of doubly-oriented fibroin.
(above) X-ray beam parallel to plane of rolling.
(below) X-ray beam perpendicular to plane of rolling.

could be closely fitted to a Gaussian curve, values of R_i were calculated from these values of A, B, and R_0; for each of the four maxima, agreement with the observed values was found to be within the limits of error imposed by the statistics of the observed counting rates.

In our spectrometric measurements the two maxima 2 and 3, representing spacings of 4.7 and 4.2 A, could not be satisfactorily resolved. We were unable to obtain spectrometric data from the doubly-oriented specimens of silk fibroin; therefore, the positions

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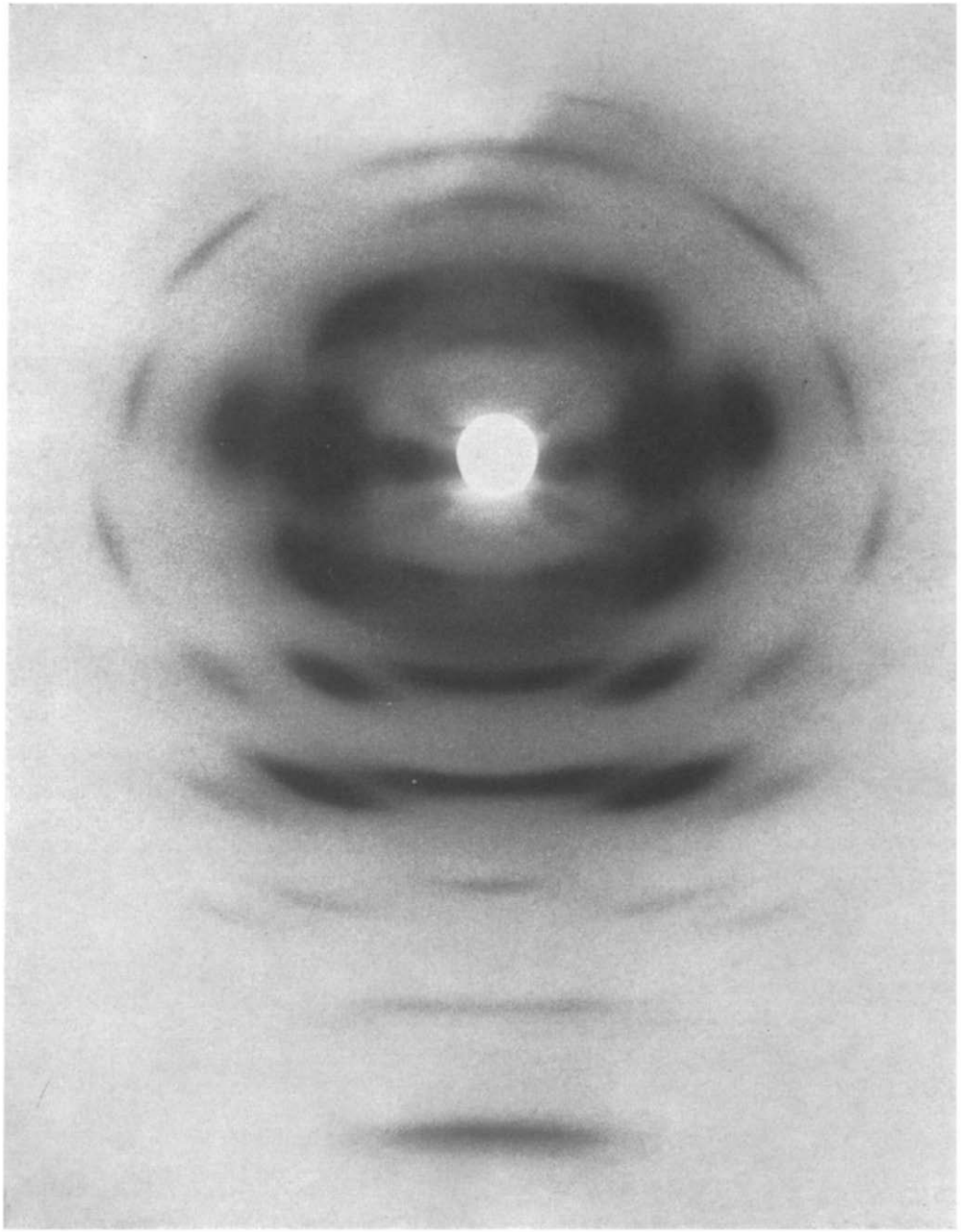

Fig. 4. Photograph of stretched silkworm gut taken in 3-cm radius camera with fiber axis horizontal and making an angle of 60° with the X-ray beam.

of these maxima were derived from measurements of photographs of doubly-oriented specimens taken in the 10-cm radius camera.

Final values for the spacings of the equatorial reflections are given in column 2

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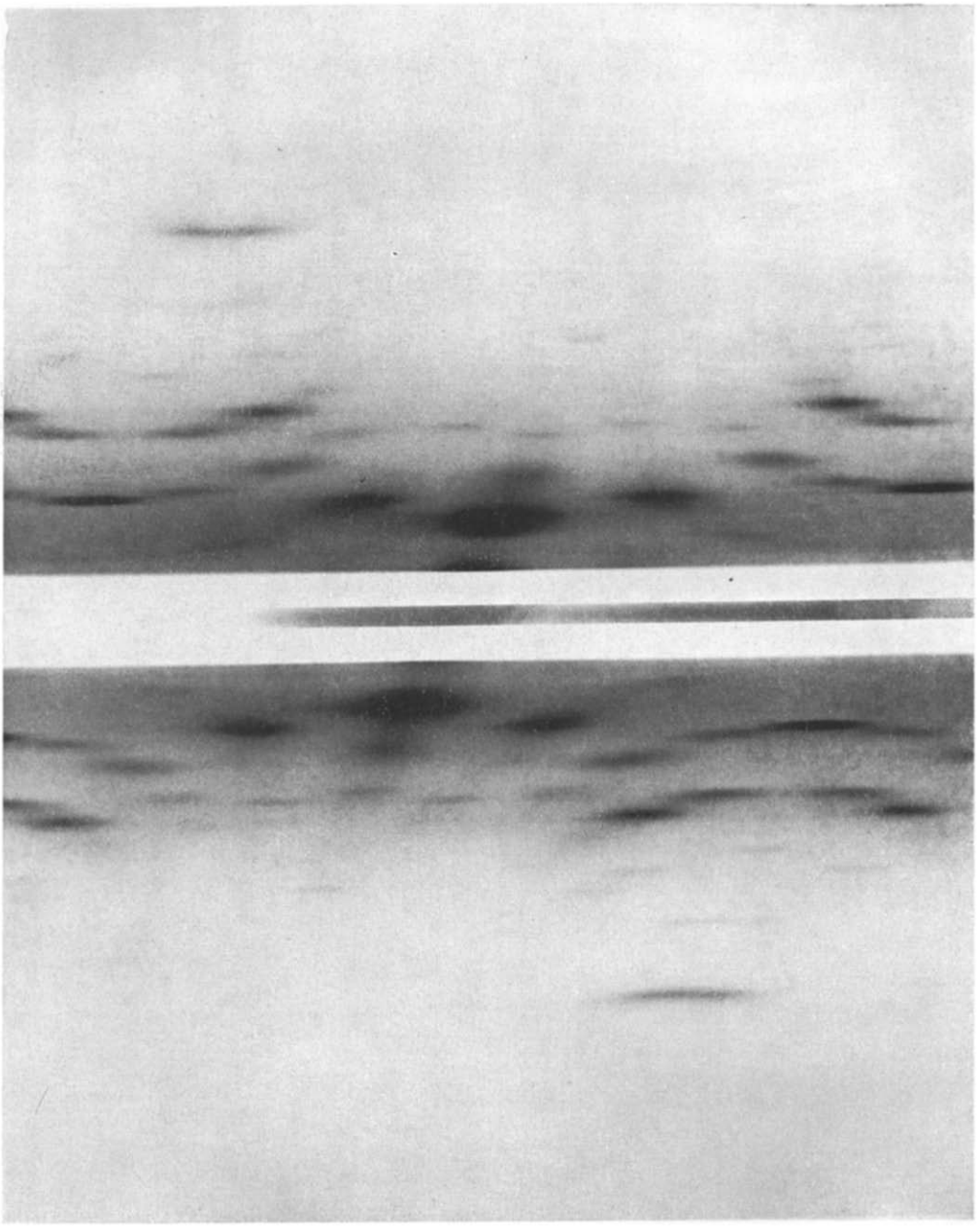

Fig. 5. Weissenberg photograph of stretched silkworm gut. The sample was rotated around an axis perpendicular to the fiber axis.

of Table II. The uncertainties given in the table are estimated limits of error (three times the standard deviations). For reflections 1, 4, 5, and 8, the limits of error as calculated from the residuals of the least-squares treatment were increased by approximately 50% to allow for small systematic errors in the experimental procedure. The limits of error for reflections 2 and 3 were estimated from the deviations of values

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obtained from measurements made on several photographs; reflections 6, 7, 9, and 10 were observed only on long exposures and no uncertainties for them are reported. For comparison, the spacing values reported by KRATKY AND KURIYAMA[8] are listed in column 4. The agreement with our values is satisfactory with the exception of reflection 1; the value of 9.28 A reported by KRATKY AND KURIYAMA for this reflection differs from our value of 9.7 A by an amount considerably greater than the limit of error of our determination.

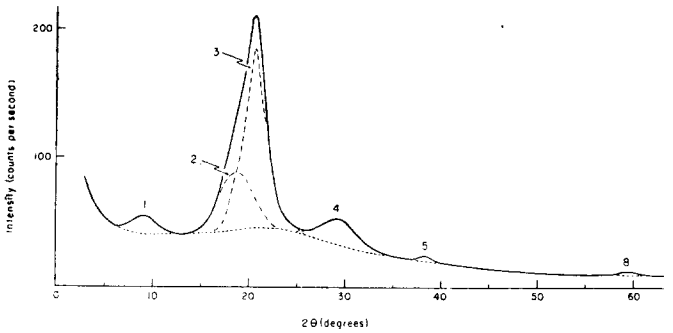

Fig. 3. Equatorial spectrum of silk fibroin obtained from spectrometric measurements.

TABLE II

EQUATORIAL REFLECTIONS FROM SILK FIBROIN

	This investigation		KRATKY AND KURIYAMA[8]			
(1) *No.*	*(2)* *Spacing (A)*	*(3)* *Relative intensity*	*(4)* *Spacing (A)*	*(5)* *Relative intensity*	*(6)* *Appearance*	*(7)* *Orient.**
1	9.70 ± 0.25	90	9.28	S	Broad	∥
2	4.70 ± 0.20	450	4.59	S	Broad	∥
3	4.25 ± 0.15	900	4.33	V S	Med. Sharp	*ca.* 70°
4	3.05 ± 0.02	180	3.07	M	Broad	∥
5	2.35 ± 0.01	20	2.38	W	Sharp	⊥
6	2.10 ± 0.05	< 5 VW				
7	1.80 ± 0.05	< 5 VVW			Broad	∥
8	1.56 ± 0.01	18			Sharp	⊥
9	1.20 ± 0.05	< 5 VW			Sharp	⊥
10	0.95 (?)	< 5 VVW				

* Orientation of diffraction planes relative to the plane of rolling of the doubly oriented samples.

The relative intensities listed in column 3, Table II, were obtained from graphical integration of the spectrometric curve after correction for background scattering. On this curve, which is reproduced in Fig. 3, reflections 2 and 3 are represented by a single maximum, and it was necessary to separate this maximum empirically into its two components before performing the graphical integration. For this reason it is probable that the intensities reported for reflections 2 and 3 are less reliable than those reported for the other reflections. The reported intensities are not corrected for absorption, or for Lorentz or polarization factors. In general, the agreement with the qualitative estimates of intensity reported by KRATKY AND KURIYAMA (column 5) is satisfactory; again, the only serious discrepancy is in reflection 1, which we have observed to be considerably

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weaker than reported by KRATKY AND KURIYAMA. All of the photographs prepared in these Laboratories, as well as the spectrometric data, have indicated that reflection 1 is much weaker than reflection 2.

The relative sharpness or diffuseness of each equatorial reflection is indicated in column 6; the apparent orientation of the corresponding diffraction plane relative to the plane of rolling of the doubly-oriented sample is indicated in column 7. The diffuse reflections 1, 2, 4, and 7 apparently arise from sets of planes oriented very nearly parallel to the plane of rolling, the sharp reflections 5, 8, and 9 appear to arise from sets of planes oriented nearly perpendicular to the plane of rolling, and the medium sharp reflection 3 appears to arise from sets of planes oriented at an angle of about 70° from the plane of rolling. These observations are in complete agreement with the observations of KRATKY AND KURIYAMA for reflections 1 through 5; reflections 6 through 10 were not reported by them.

In addition to the reflections reported in Table II, two other regions of significant darkening were observed on our best photographs, both apparently being due to equatorial reflections arising from sets of planes oriented nearly parallel to the plane of rolling of the doubly-oriented samples. One is a moderately strong diffuse reflection corresponding to a spacing of 30 to 40 A; on the small-angle side of this reflection a continuous blackening extends to the limit of observation (78 A). The other appears to be a diffuse reflection of low intensity with a spacing of about 15 A; on poorer photographs this reflection merges with the stronger 9.7 A reflection.

The spacing along the fiber axis was determined from the layer-line separations on photographs of stretched silk-worm gut taken in cylindrical cameras with 3-cm and 10-cm radius. In addition, a photograph (Fig. 4) was taken in the 3-cm radius camera with the fiber tilted at an angle of 60° with the X-ray beam in order to obtain reflections of high order along the fiber axis, especially the strong sixth order. The value 6.97 $\pm$ 0.03 A was derived from measurements of these photographs; it is in close agreement with the value 6.94 A reported by BAMFORD *et al.*[18]. In long exposures made with stretched commercial silkworm gut two intermediate layer lines were observed corresponding to the first and second orders of a 21-A identity distance along the fiber axis. These two weak layer lines were also observed by KRATKY and co-workers[16,19] on photographs of nitrated and of alkalized silk.

Non-equatorial reflections were recorded on photographs taken in the two cylindrical cameras, in a flat-film camera with the sample-to-film distance equal to 10 cm, and in a Weissenberg camera (5.73 cm diameter). Measurements of the photographs taken in the 10-cm radius cylindrical camera and in the flat-film camera were used to compute the horizontal components, d_{hol}, of the interplanar spacings for reflections occurring on the first three layer lines, together with rough visual estimations of the relative intensities. For comparison, the spacing and intensity data of KRATKY AND KURIYAMA are also included. The higher layer lines were recorded on a photograph taken in a 3-cm radius cylindrical camera with the fiber axis of the sample perpendicular to the axis of the camera and making an angle of 60° with the direction of the X-ray beam (Fig. 4). The complete X-ray diffraction pattern was also recorded on a Weissenberg photograph taken with the sample (stretched but not rolled) rotated around an axis perpendicular to its fiber direction (Fig. 5).

Values of d_{hol}, together with some estimated probable errors, are listed in Table III; rough visual estimations of the relative intensities are also listed. For comparison the spacing and intensity data of KRATKY AND KURIYAMA[8] are included.

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TABLE III

SPACING AND INTENSITY DATA FOR NON-EQUATORIAL REFLECTIONS

	This investigation		KRATKY AND KURIYAMA	
	d_{hol} (A)	*Int.*	d_{hol}	*Int.*
1st Layer	9.6 ± 0.4	W	9.6	W
	4.7 ± 0.2	W	4.73	M
	4.2 ± 0.1	S	4.24	S
	3.0 ± 0.1	VW		
	2.35 ± 0.03	M	2.38	W
	2.12 ± 0.03	W		
	1.8	VVW		
	0.95 (?)	VW		
2nd Layer	∞	VS	∞	M
	9.6 ± 0.8	MS	10.4	M
	5.1 ± 0.5 / 4.4 ± 0.4	MS	4.59	M
	3.0 ± 0.3	M	3.13	W
	2.3 ± 0.1	W		
3rd Layer	9	MS	8.9	W
	7	VW		
	4.1 ± 0.7	S	4.5	S
	3.0 ± 0.5	W		
	2.3	VW		
	1.85	VW		
	1.54	MW		
	1.32	MW		
	1.17	VW		
4th Layer	∞	MW		
	5	MW		
	3	W		
5th Layer	9	MW		
	5	W		
6th Layer	∞ / 9	S		

The effect of double orientation of the sample is less apparent on the layer-line reflections than on the equatorial reflections. Nevertheless, as can be seen in Fig. 2, the effect is great enough in some cases to permit a direct correlation of certain layer-line reflections with equatorial reflections having the same values of d_{hol}.

III. DERIVATION OF THE STRUCTURE

1. *The pleated-sheet configurations*

All previous attempts to derive an acceptable structure for silk fibroin were made before the fundamental dimensions of the polypeptide chain had been established and before specific configurations of these chains had been systematically investigated and precisely described. The method consisted in deriving probable unit cells from the X-ray fiber diagrams and proposing possible arrangements of polypeptide chains within these unit cells which were in rough qualitative agreement with the general features of the

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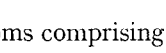
diffraction patterns. No atomic coordinates were assigned from which the intensities of X-ray reflections could be calculated.

Recent X-ray analyses of crystals of amino acids, simple peptides, and related substances have provided precise data from which the interatomic distances and bond angles in polypeptide chains have now been derived[3]. These X-ray studies have also established two structural principles of fundamental importance in arriving at the most probable configurations of polypeptide chains in fibrous and globular proteins: (1) the coplanarity of the atoms comprising the amide group,  , and (2) the formation of close to the maximum possible number of N—H···O=C hydrogen bonds. The application of these structural principles and the use of accurate values for interatomic distances and bond angles led to the discovery and the exact description of several possible configurations of the polypeptide chain.

Of these possible configurations, the first to be discovered was the α-helix[20,21,22]. This configuration has been shown experimentally to be a principal structural constituent of many fibrous and globular proteins and synthetic polypeptides[23]. Another configuration of great interest was discovered in the course of a systematic survey[24] in which the arrangement of the amide groups was restricted to certain orientations around the C—C and C—N single bonds. The most significant feature of this configuration is the orientation of the >C=O and >N—H groups. The >C=O and >N—H groups of successive residues protrude from opposite sides of the helix; their orientation, nearly perpendicular to the axis of the helix, is particularly favorable to the formation of lateral C=O···H—N hydrogen bonds between adjacent helixes. A succession of helixes bonded together in this manner can build up two sheet structures, the parallel-chain and the antiparallel-chain pleated sheets, corresponding to a parallel or antiparallel arrangement of adjacent helixes. These two structures were originally formulated on the basis of specific, presumably favored orientations around the C—C and C—N single bonds[24]; subsequently[25] they were revised so as to make all N—H···O hydrogen bonds linear. Although this revision involved departure from the previous orientations around the single bonds, the new structures were considered to be probably more stable. The fiber-axis identity distance calculated for the new parallel-chain pleated sheet is 6.50 A and that for the antiparallel-chain pleated sheet is 7.00 A. The lateral displacement between equivalent chains (adjacent chains) in the parallel-chain pleated sheet is 4.85 A; between equivalent chains (alternate chains) of the antiparallel-chain pleated sheet this distance is 9.50 A. These dimensions gave rise to the suggestion[24] that the β-keratin proteins, for which the fiber-axis identity distance is about 6.6 A, probably have a structure based upon the parallel-chain pleated sheet, and that silk fibroin, for which the observed fiber-axis identity distance is 7.0 A, probably has a structure based upon the antiparallel-chain pleated sheet. A drawing of the antiparallel-chain pleated sheet is reproduced in Fig. 6.

Several features of the X-ray data give confirmation to the choice of an antiparallel-chain pleated sheet as the basic structural component of silk fibroin. The fact that silk fibroin may be doubly oriented by a rolling process indicates that a sheet, rather than an individual helix, forms the basis for the structure. The agreement between the calculated fiber-axis identity distance (7.0 A) and that obtained experimentally (6.97 A) has already been mentioned. The spacing of the C, O, and N atoms at an approximately uniform interval along the fiber axis might be expected to give rise to a sixth-order

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meridional reflection corresponding to a spacing of 1.17 A for the antiparallel-chain structure and 1.08 A for the parallel-chain structure; a strong 1.16-A meridional reflection is indeed observed. The three sharp equatorial reflections—the only reflections shown by the photographs of doubly-oriented specimens to arise from planes perpendicular to the plane of rolling—correspond approximately to fourth, sixth, and eighth orders of a 9.4-A spacing; the spacing of alternate (equivalent) chains calculated for the antiparallel-chain pleated sheet is 9.5 A. We have accordingly assumed that in silk fibroin the polypeptide chains are arranged in the form of antiparallel-chain pleated sheets and that the plane of rolling of the doubly-oriented samples is the plane of the pleated sheet.

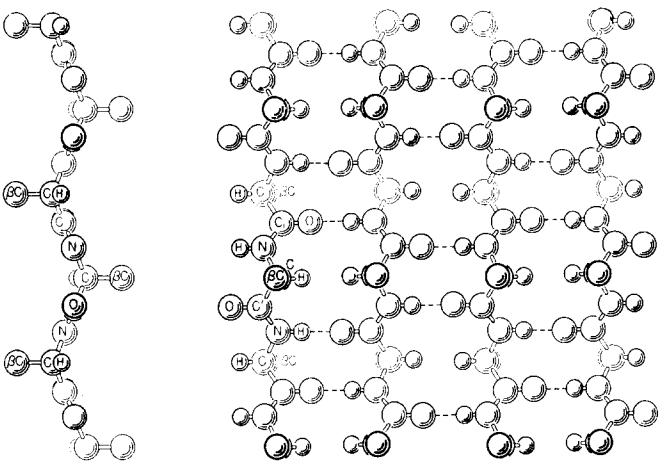

Fig. 6. A drawing of the antiparallel-chain pleated sheet.

The reasons for choosing the antiparallel-chain in preference to the parallel-chain pleated sheet should perhaps be clarified by additional brief discussion. It is evident that the observed reflections corresponding to the fourth, sixth, and eighth orders of a 9.4-A spacing between alternate chains of an antiparallel-chain pleated sheet could as readily be interpreted as second, third, and fourth orders of a 4.7-A spacing between adjacent chains of a parallel-chain sheet. On what, then, does the choice depend? The fundamental basis is our confidence in the reliability of the dimensions of the polypeptide chain in proteins as determined from precise X-ray analyses of crystals of amino acids, peptides, and related compounds[3], and of the observed lengths and directional characteristics of N—H···O hydrogen bonds as determined in the same analyses. The experimental evidence indicates that stable structures of peptides and other compounds related to proteins involve the formation of close to the maximum number of N—H···O hydrogen bonds, and that generally these bonds are approximately 2.8 A long and close to linear, that is, the N—H···O angle is generally close to 180°. Indeed it was the convincing nature of this experimental evidence which led to the conclusion that in the formation of stable pleated sheets from parallel and antiparallel assemblies of polypeptide chains the approximate linearity of the N—H···O hydrogen bond is of primary importance[25]. Polypeptide chains incorporating dimensions based on the best experimental data and extended so as to correspond to the observed 6.97-A fiber-axis identity

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distance of silk fibroin assume a configuration such that the C=O and N—H bonds are oriented almost exactly perpendicular to the fiber axis. Separated by 4.7 A and arranged in the antiparallel sense, these chains can form linear N—H···O hydrogen bonds 2.76 A in length and build up antiparallel-chain pleated sheets of dimensions compatible with the data from silk fibroin. Arranged in the parallel sense, on the other hand, these chains can, at best, form hydrogen bonds which are 2.97 A long and which depart from linearity by 20°. There seems to be little reason to doubt that antiparallel-chain pleated sheets involving short, linear hydrogen bonds would be significantly more stable than parallel-chain sheets involving longer and distorted hydrogen bonds. Accordingly the antiparallel-chain pleated sheet has been chosen as the structural basis for silk fibroin.

This argument, which is based on the observed fiber-axis identity distance 6.97 A for silk fibroin, does not rule out the parallel-chain pleated sheet for β-keratin, for which this distance is 6.6 A.

2. A pseudo unit of structure

Attempts were first made to derive possible unit cells from the spacings of the equatorial reflections and to fit antiparallel-chain pleated sheets into these unit cells so as to give satisfactory packing. As a simplification—one which has been attractive to previous investigators also—it was assumed that the crystalline portion of silk fibroin which gives rise to the X-ray diffraction pattern contains only the smallest amino-acid residues, glycine, alanine, and serine, and that larger residues, such as tyrosine, leucine, etc., are present only in a non-crystalline component. The orientational requirements indicated by photographs of doubly-oriented samples imposed further restrictions on the choice of a possible unit.

The only simple unit cell that we have found which is able to accommodate the the antiparallel-chain pleated sheet and which is also reasonably compatible with the positions and orientations of the equatorial reflections is an orthogonal one with dimensions $a_0 = 9.40$ A, $b_0 = 6.97$ A, $c_0 = 9.20$ A. The b axis of this unit cell is in the direction of the fiber axis; the a and c axes lie in the basal plane perpendicular to the fiber axis and are parallel and perpendicular, respectively, to the plane of rolling of the doubly-oriented samples. On the basis of this unit cell the equatorial reflections may be indexed as shown in Table IV. Reflections apparently arising from sets of planes oriented approximately perpendicular to the plane of rolling would be expected to be axial reflections of the type $h00$; the reflections arising from planes oriented parallel to the plane of rolling would be expected to be reflections of the type $00l$. The 4.25-A reflection apparently arises from sets of planes oriented at an angle of about 70° from the plane of rolling; this angle is consistent with crystallographic planes of the forms $\{201\}$ and $\{20\bar{1}\}$. The only discrepancy between the observed and calculated interplanar spacings is in the values 9.7 A reported and 9.20 A calculated for reflection No. 1; this discrepancy will be discussed in Part 3 of this section.

If one assumes that antiparallel-chain pleated sheets are arranged parallel to the plane of rolling of the doubly-oriented samples, then the a and b axes of the proposed unit cell must lie in the plane of the pleated sheet. The b axis is parallel to the axis of the polypeptide chains; its identity distance represents the distance occupied by two residues along each chain. The a axis is parallel to the lateral hydrogen bonds within the sheets; its identity distance represents the distance between alternate polypeptide chains within the sheets. Thus, four amino-acid residues of a pleated sheet can be fitted

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TABLE IV

INDICES OF EQUATORIAL REFLECTIONS BASED ON PSEUDO UNIT CELL

No.	Interplanar spacing		hol	Orientation	Intensity
	Observed (A)	Calculated (A)			
1	9.70 ± 0.25	9.20	001	∥	90
2	4.70 ± 0.20	4.60	002	∥	450
3	4.25 ± 0.15	4.19	201, 20$\bar{1}$	*ca.* 70°	900
4	3.05 ± 0.02	3.07	003	∥	180
5	2.35 ± 0.01	2.35	400	⊥	20
6	2.10 ± 0.05	2.09	402, 40$\bar{2}$		< 5
7	1.80 ± 0.05	1.84	005	∥	< 5
8	1.56 ± 0.01	1.57	600	⊥	18
		1.54	601, 60$\bar{1}$		
9	1.20 ± 0.05	1.18	800	⊥	< 5

into this unit cell; the number of sheets within the *c*-axis identity distance may be derived from the following considerations.

As stated previously, this pseudo unit cell is assumed to contain only the glycine, alanine, and serine residues in silk fibroin. In Table V there are listed the densities of these three amino acids and of some simple peptides of glycine and alanine. From a consideration of these densities, it seems reasonable to predict that a polypeptide containing only glycine, alanine, and serine in the proportions in which they occur in silk (about 3:2:1) would have a density of about 1.45 g/cm^3. Similarly the average residue weight of a compound containing glycine, alanine, and serine residues in the proportions 3:2:1 would be 67. Assuming a density of 1.45 and an average residue weight of 67, the number of residues within the pseudo unit cell is calculated to be 7.9. Thus, since four residues of each pleated sheet are contained within the pseudo unit, these calculations indicate that there are two pleated sheets per cell.

TABLE V

DENSITIES OF CRYSTALS OF SELECTED AMINO ACIDS AND PEPTIDES

Compound	Density (g/cm^3)	Reference
glycine	1.607	26
D,L-alanine	1.40	27
D,L-serine	1.537	28
α-triglycine	1.57	29
D,L-alanylglycine	1.429	30
glycyl-D,L-alanine	1.425	30
D,D-dialanine	1.280	31
L,L,L-trialanine	1.333	31

Information concerning the spacing of pleated sheets in the direction of the *c* axis can be obtained from the intensities of the equatorial reflections representing orders of (001). Four reflections of this type are observed, but the intensity of 005 was too low for spectrometric measurement. Relative values of F^2 for the reflections 001, 002, and 003 were obtained by correcting the observed intensities for Lorentz and polarization factors.

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These values of F^2 can be used for the computation of a one-dimensional Patterson function.

$$P(w) = \sum_l F^2_{00l} \cos 2\pi lw$$

A plot of this function is shown in Fig. 7.

Besides the peak at the origin, the only features of this function are the two related maxima at about 3.7 and 5.5 A from the origin. This Patterson projection can be interpreted as indicating that in the structure of silk fibroin the distances between adjacent pleated sheets are alternately 3.7 A and 5.5 A. The details of the packing of the pleated sheets in accordance with this interpretation may be derived from the following structural considerations.

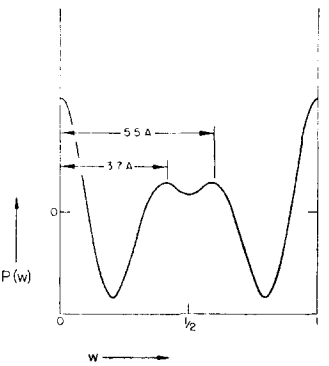

Fig. 7. The Patterson function $P(w)$ calculated from the intensities of the first three orders of (001). The vertical scale is arbitrary.

A feature of the pleated-sheet structures is that the bonds between the α-carbon atoms in the main polypeptide chains and the adjoining β-carbon atoms of the side chains are oriented approximately perpendicular to the plane of the sheet. As a consequence, the way in which adjacent sheets are packed together is determined almost entirely by the sizes and shapes of the side chains of the component amino-acid residues. Pleated sheets containing only glycine residues, in which β-carbon atoms are replaced by hydrogen atoms, would be expected to pack together much more closely than would pleated sheets consisting of alanine or of larger amino-acid residues. Indeed, construction of accurate scale models[32] of antiparallel-chain pleated sheets shows that two sheets consisting solely of glycine residues may pack together at a distance of about 3.5 A, whereas sheets containing only alanine or serine residues may pack together efficiently at a distance of about 5.7 A; larger amino-acid residues require larger packing distances.

An additional feature of the pleated-sheet structures is that within each polypeptide chain the side chains of adjacent amino-acid residues protrude from opposite sides of the sheet (Fig. 6). Thus, polypeptide chains consisting of an alternation of glycine and alanine residues may be arranged in such a way as to form a sheet having only the hydrogen atoms of the glycine residues protruding from the front side and only the methyl groups of the alanine residues protruding from the back side. Two sheets of this type can pack together efficiently either front-to-front at a distance of 3.5 A or back-to-back at a distance of 5.7 A; a succession of these sheets would pack together at distances alternately 3.5 and 5.7 A. An arrangement of this sort would be in approximate agreement with the positions of the maxima in the Patterson function $P(w)$; it would also be consistent with a composition of four glycine, three alanine, and one serine residue in the unit cell.

The structure which we propose as a pseudo unit for silk fibroin is shown diagrammatically in Fig. 8. Two unit cells are shown, each containing eight amino-acid residues comprising portions of two antiparallel-chain pleated sheets. For simplicity, the atoms in each pleated sheet are represented as being coplanar. The methyl or hydroxymethyl side chains of the alanine or serine residues are labeled "R"; they protrude, along the *c* axis, backward from the front plane and forward from the rear plane, interlocking so

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as to give efficient packing. These two planes are 5.7 A apart. The hydrogen atoms of the glycine residues protrude from the front of the forward plane and from the back of the rear plane; thus, the distance between the front plane of one unit cell and the rear plane of the next unit cell is 3.5 A.

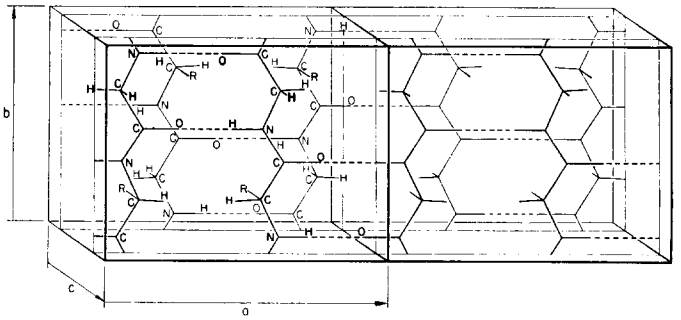

Fig. 8. A diagrammatic representation of the arrangement of the polypeptide chains within the pseudo unit of structure.

The assumption of the value 3.5 A for the distance between pairs of pleated sheets presenting glycine side chains (hydrogen atoms) toward one another is supported by the results of intensity calculations, and also by the consideration of the X-ray pattern of polyglycine. We have pointed out[23] that the powder X-ray pattern of polyglycine is compatible with a pleated-sheet structure. The strongest line of this pattern gives the spacing between adjacent pleated sheets, and its value is 3.47 A.

A schematic representation of the proposed pseudo structure projected along the *b* axis is shown in Fig. 9. In this drawing the backbones of the polypeptide chains are represented as roughly elliptical; they are bonded together by hydrogen bonds to form sheets parallel to the *a* axis. The large circles between these sheets represent methyl or hydroxymethyl side chains of alanine or serine residues and the small circles represent hydrogen atoms of glycine residues. The arrangement of the sheets at alternately 3.5 and 5.7 A is clearly shown.

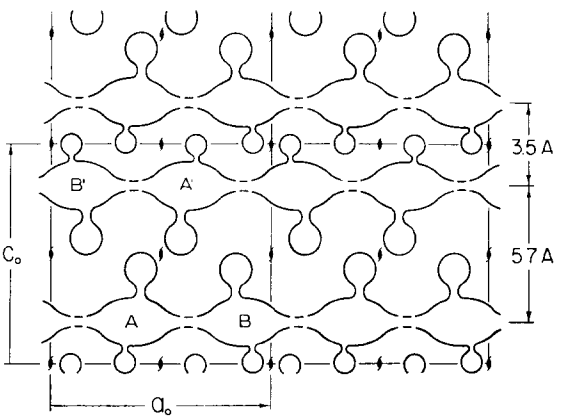

Fig. 9. A schematic representation of the proposed pseudo unit of structure projected along the fiber axis.

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Packing drawings of the structure viewed along the y and z axes are shown in Figs. 10 and 11 respectively.

Although the unit cell is orthogonal, the symmetry of the proposed pseudo structure is that of the monoclinic space group $P2_1$; furthermore, the presence of the two-fold screw axes is dependent on the assumption that the serine and alanine side chains are

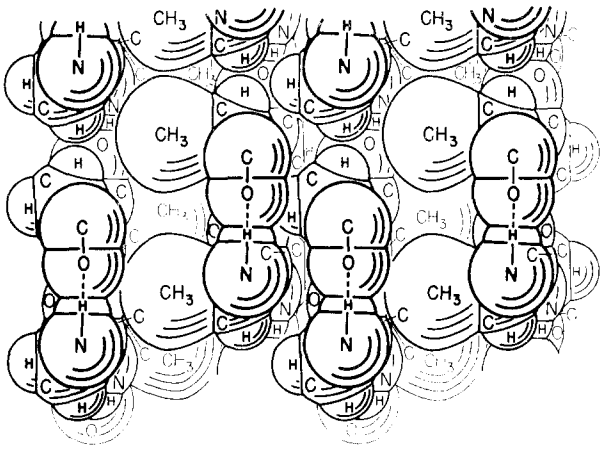

Fig. 10. A packing drawing of the structure viewed along the fiber (y) axis.

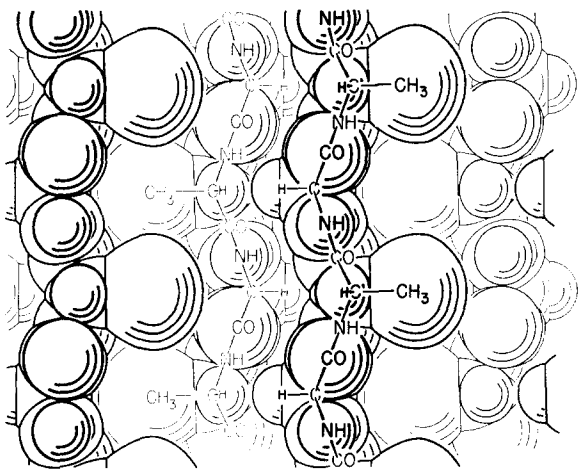

Fig. 11. A packing drawing of the structure viewed along the z axis, perpendicular to the fiber axis and parallel to the plane of the pleated sheets.

structurally equivalent. The operation of the two-fold screw axes, whose positions in the unit cell are shown in Fig. 9, is a rotation of 180° followed by a translation parallel to the b axis by an amount equal to $b_0/2$; by this operation each polypeptide chain A is transformed into a chain A′ and each chain B is transformed into a chain B′. Thus, the side chains of a pleated sheet A′ B′ are displaced from those of its neighbor A B by an amount equal to $b_0/2$; side chains from adjacent pleated sheets thus interlock.

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The detailed packing of the side chains is determined by the displacement along the a axis of the polypeptide chains A′ and B′ relative to chains A and B. In the pseudo structure, as shown in Fig. 9, the chains are staggered so that the center of chain A′ is midway between the centers of chains A and B. This arrangement is in agreement with the observed strength of the reflection 400.

Since the symmetry of the proposed structure is monoclinic, no restriction is placed on the size of the angle β between the a and c axes. We have chosen this angle to be 90°. The justification for this choice rests in the success of this pseudo unit of structure in explaining both the positions and the intensities of the equatorial X-ray reflections. In particular, if the 1.56-A reflection (No. 8) is correctly indexed as being a superposition of reflections 601 and $60\bar{1}$, in agreement with the intensity calculations, its small angular spread limits the value of β to 90° within a few minutes of arc.

On the basis of the proposed pseudo structure atomic positional parameters have been assigned to all of the main-chain atoms except hydrogen and to the β-carbon atoms of the alanine and serine residues; they are listed in Table VI. No parameters have been assigned to the oxygen atom of the serine residue.

TABLE VI

ATOMIC POSITIONAL PARAMETERS FOR THE PSEUDO STRUCTURE

Space group: $P2_1$
Equivalent positions: x, y, z (Chains A and B)
$\bar{x}, \frac{1}{2} + y, \bar{z}$ (Chains A′ and B′)
$a_0 = 9.40$ A
$b_0 = 6.97$ A
$c_0 = 9.20$ A
$\beta = 90°$

Chain	*Residue*		*N*	*C*	*C′*	*O*	*βC*
A	I	x	0.404	0.340	0.422	0.553	
		y	0.074	0.252	0.426	0.426	
		z	0.163	0.113	0.173	0.173	
A	II	x	0.346	0.410	0.328	0.197	0.410
		y	0.574	0.752	0.926	0.926	0.752
		z	0.215	0.265	0.205	0.205	0.430
B	III	x	0.904	0.840	0.922	0.053	0.840
		y	0.926	0.748	0.574	0.574	0.748
		z	0.215	0.265	0.205	0.205	0.430
B	IV	x	0.846	0.910	0.828	0.697	
		y	0.426	0.248	0.074	0.074	
		z	0.163	0.113	0.173	0.173	

The bond distances and angles calculated from the positional parameters are listed in Table VII. All of the distances and angles are in satisfactory agreement (within 0.02 A and 3°) with the accepted values for the fundamental dimensions of polypeptide chains[23].

The atomic positional parameters listed in Table VI were used to calculate structure factors for those reflections on the equator and first three layer lines which have inter-

TABLE VII

INTERATOMIC DISTANCES AND BOND ANGLES FOR THE PROPOSED PSEUDO STRUCTURE

	Calculated from parameters in Table VI	*Accepted values*[3]
	Bonded distances	
N—C	1.45 A	1.47 A
C—C′	1.54	1.53
C′—O	1.23	1.24
C′—N	1.31	1.32
C—βC	1.52	1.54
N···H···O	2.76	2.8
	Bond angles	
N—C—C′	110.5°	110°
C—C′—O	120.1°	121°
C—C′—N	116.8°	114°
O—C′—N	123.0°	125°
C′—N—C	122.5°	123°
C′—N···O	122.3°	123°
C—N···O	115.1°	114
N—C—βC	108.5°	109.5°
C′—C—βC	111.0°	109.5°

planar spacings greater than 1.0 A. The structure factor expression for the space group $P2_1$ is $F = A + iB$, where

$$\left.\begin{aligned} A_{hkl} &= \sum_j f_j \cos 2\pi(hx_j + lz_j) \cos 2\pi k y_j \\ B_{hkl} &= \sum_j f_j \cos 2\pi(hx_j + lz_j) \sin 2\pi k y_j \end{aligned}\right\} \text{for } k = 2n$$

and

$$\left.\begin{aligned} A_{hkl} &= -\sum_j f_j \sin 2\pi(hx_j + lz_j) \sin 2\pi k y_j \\ B_{hkl} &= \sum_j f_j \sin 2\pi(hx_j + lz_j) \cos 2\pi k y_j \end{aligned}\right\} \text{for } k = 2n + 1$$

The summations extend over all atoms j in the four residues (I, II, III, IV, Table VI) comprising the asymmetric unit: 10 carbon atoms, 4 oxygen atoms, and 4 nitrogen atoms. Atomic form factors f_j for carbon, nitrogen, and oxygen were taken from the tables of JAMES AND BRINDLEY[33]. The calculated values of $F^2 = A^2 + B^2$ are listed in Table VIII. Because of the orthogonality of the pseudo unit cell each reflection of the type *hkl* is superimposed on a corresponding reflection $hk\bar{l}$; for simplicity values of F^2_{calc} listed in the table comprise the sum of the contributions of the two planes *hkl* and $hk\bar{l}$. The observed intensities of reflections occurring on the equator and the first three layer lines are also listed in Table VIII. In cases where an observed reflection could not be unambiguously indexed from photographs of doubly-oriented samples, brackets have been used to include all planes compatible with the measured interplanar spacings. The observed intensities of the equatorial reflections have been corrected for Lorentz and polarization factors to give values of F^2_{obs}.

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TABLE VIII

INTENSITY DATA OBTAINED FROM SILK FIBROIN COMPARED WITH VALUES OF F^2 CALCULATED FROM THE PARAMETERS LISTED IN TABLE VI

h	l	d_{hol}	$k=0$			$k=1$		$k=2$		$k=3$	
			I_{obs}	F^2_{obs}	F^2_{calc}	I_{obs}	F^2_{calc}	I_{obs}	F^2_{calc}	I_{obs}	F^2_{calc}
0	0	—	—	—	14,400	—	0	vs	123	—	0
1	0	9.40			3		50	ms	16	ms	361
0	1	9.18	90	380	697	w	79		166		4
1	1	6.56			0		148		38	vw	639
2	0	4.70			0		55		0		21
0	2	4.59	450	3940	2460	w	424	ms	372	s	7
2	1	4.19	900	8700	2100	s	1015		33		88
1	2	4.12			17		186		52		454
2	2	3.28			208		336		43		6
3	0	3.08			10		17		214		182
0	3	3.07	180	1192	2725	vw	180	m	346	w	74
3	1	2.97			34		28		464		250
1	3	2.92			56		139		41		369
3	2	2.59			23		68		404		98
2	3	2.57			0		108		74		2
4	0	2.35	20	385	502	m	296		9		3
0	4	2.30			0		21		3		21
4	1	2.27			18		96	w	13	vw	8
1	4	2.23			66		84		55		279
3	3	2.19			4		99		284		88
4	2	2.09	< 5	< 110	116	w	348		30		26
2	4	2.07			180		552		45		10
5	0	1.88			9		35		17		166
4	3	1.87			162		332		45		8
3	4	1.85			10		102		231	vw	66
0	5	1.84	< 5	< 135	708	vvw	369		317		71
5	1	1.84			42		30		73		211
1	5	1.81			64		79		63		165
5	2	1.74			33		8		91		56
2	5	1.71			24		78		60		6
4	4	1.64			0		10		2		10
5	3	1.60			0		21		46		59
3	5	1.59			0		147		185		19
6	0	1.57			0		1		0	mw	1
6	1	1.54	18	615	612		38		47		2
0	6	1.53			61		38		266		164
1	6	1.51			86		74		54		80
6	2	1.48			238		20		47		6
2	6	1.46			32		196		152		2
5	4	1.45			24		4		89		45
4	5	1.45			20		246		99		4
6	3	1.39			46		10		23		4
3	6	1.38			22		188		100		6
7	0	1.34			7		0		17		630
7	1	1.33			17		45		91		977
5	5	1.32			7		51		119		11
0	7	1.31			1		10		110	mw	85
1	7	1.30			126		43		35		56
6	4	1.30			308		76		59		30
7	2	1.29			0		127		120		587
4	6	1.28			14		40		104		34
2	7	1.27			22		106		125		198
7	3	1.23			32		136		86		505
3	7	1.21			90		149		38		7
6	5	1.19			10		12		13		4

(continued overleaf)

TABLE VIII (continued)

h	l	d_{hkl}	$k=0$			$k=1$		$k=2$		$k=3$	
			I_{obs}	F^2_{obs}	F^2_{calc}	I_{obs}	F^2_{calc}	I_{obs}	F^2_{calc}	I_{obs}	F^2_{calc}
5	6	1.19			19		126		54		24
8	0	1.18	< 5	< 250	250		1		29		1
8	1	1.17			46		2		16		0
7	4	1.16			43		80		152	W	423
0	8	1.15			150		12		90	W	586
4	7	1.15			16		8		57		16
1	8	1.14			129		19		35		46
8	2	1.14			184		16		44		0
2	8	1.12			4		18		4		24
8	3	1.10			274		16		61		14
6	6	1.10			44		48		39		30
7	5	1.08			29		180		207		187
3	8	1.08			102		90		19		6
5	7	1.08			111		81		2		22
8	4	1.05			0		0		0		2
9	0	1.04			5		23		55		102
9	1	1.04			24		20		132		127
4	8	1.03			0		16		65		158
0	9	1.02			0		9		0		27
9	2	1.02			22		9		137		28
1	9	1.02			100		16		37		31
7	6	1.01			149		303		108		32
6	7	1.01			44		40		33		104
2	9	1.00			0		58		38		178

In general there is good agreement between calculated and observed values of F^2 for the equatorial reflections. Indeed, the agreement would be excellent if the calculated values of F^2 were corrected with an anisotropic temperature factor of the type $\exp(-\alpha h^2 - \gamma l^2)$, where γ is considerably larger than α. A temperature factor of this type would be appropriate in view of the apparent disorder in the direction of the c axis as manifested by the diffuseness of observed reflections of the type $00l$.

The observed and calculated intensities for non-equatorial reflections are in general qualitative agreement. The most conspicuous discrepancy occurs for the meridional reflection 020; whereas this reflection is observed as being very strong, its calculated value of F^2 is only moderate. The low value of F^2 calculated for this reflection is due to the regular spacing of atoms along each polypeptide chain; the contributions of the two carbon atoms and one nitrogen atom in the chain nearly cancel one another, and the contributions of the oxygen atoms are to some extent canceled by the contributions of the β-carbon atoms. Since the extreme strength of the sixth-order meridional reflection is strong evidence in support of the basic arrangement of the polypeptide chain, it would appear that the strength of the 020 reflection must be due, in large part, to the contributions of side-chain atoms.

3. An extension of the pseudo unit and the structure of silk fibroin

Early in the course of our investigations of silk fibroin it was recognized that the complete and detailed structure of this substance could not be satisfactorily described on the basis of a unit cell as simple as the pseudo unit which we have discussed above. Nevertheless, it has seemed profitable thus far to describe the fundamental features of

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the structure in terms of the pseudo unit; indeed, the general agreement between observed values of intensities and interplanar spacing and those calculated for the pseudo unit is so striking that there can be little doubt that the real structure is closely related to the pseudo structure. It is the purpose of this section to discuss those ways in which the pseudo unit of structure must be revised and extended in order to approach more closely to the true structure of silk fibroin.

There are two major reasons why the pseudo unit cannot be the true unit of structure of silk fibroin. In the first place, the observed spectrum of the diffuse equatorial reflections—those reflections which arise from diffracting planes oriented parallel to the plane of rolling of the doubly-oriented samples, and which have been indexed as orders of 001—cannot be satisfactorily explained by a *c* axis identity distance as small as 9.2 A. The presence of diffuse reflections at spacings of about 35 A and 15 A is strong evidence that the identity distance is probably very much greater than 9.2 A; furthermore, the observed spacing of the 9.7 A reflection differs from the assumed value, 9.2 A, by an amount greater than the limit of error of our observations.

A second objection to the pseudo structure is that it takes into account only the three smallest amino-acid residues present in silk fibroin—glycine, alanine, and serine; the remaining 18% of the residues, including about 5% of tyrosine, remain unaccounted for. There has been a tendency among previous investigators to ignore this 18% of the larger residues on the grounds that the X-ray diffraction pattern of silk fibroin is so simple relative to those of other proteins that only small, well-ordered amino-acid residues can be present in the crystalline portion. This viewpoint has found some support in various chemical experiments as discussed in the introduction to this paper; however, there is also considerable contradictory evidence, and at the present time there seems to be insufficient justification for assuming that the larger amino-acid residues occur only in a non-crystalline portion of the protein. It seems more likely that the diffraction pattern of silk fibroin is representative of all of the residues present in the protein.

It is reasonable to assume that the true structure of silk fibroin is based on the pseudo unit of structure so modified and extended as to satisfactorily account for the diffuse equatorial reflections and to include the larger as well as the smaller amino-acid residues. The diffuse reflections have already been interpreted as being representative of the method of packing of the antiparallel-chain pleated sheets, so that changes in the distances between adjacent sheets would affect the positions and intensities of these reflections. The distances between adjacent sheets are determined primarily by the sizes and shapes of the side chains of the amino-acid residues; in particular, the side chains of the larger amino-acid residues present in silk fibroin would require inter-sheet distances considerably larger than the 3.5 and 5.7 A distances in the pseudo structure. It seems probable, then, that the X-ray diffraction pattern of silk fibroin can be explained by assuming that the majority of the small amino-acid residues glycine, alanine, and serine are arranged in accordance with the proposed pseudo unit; the remaining 18% of the residues, which are too large to be accommodated by the pseudo unit, are nevertheless present in the crystalline component of silk fibroin and are responsible for deformations of the pseudo unit. These deformations occur in the direction of the *c* axis of the pseudo unit, and hence have a relatively large effect on the spacings and intensities of the 00*l* reflections.

Measurements made on models of antiparallel-chain pleated sheets show that a

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sheet containing the side chains of tyrosine or phenylalanine protruding from one side may pack with an adjacent sheet consisting of glycine residues at a distance of approximately 8 to 9 A. In view of the fact that the van der Waals diameter of a phenyl group is about 7 A, this distance is, at first glance, somewhat shorter than might be expected. However, since the bond connecting the α-carbon atom of the main polypeptide chain with the corresponding β-carbon atom of the side chain is oriented perpendicular to the plane of the pleated sheet, the bond connecting the β-carbon atom to the phenyl group makes an angle of only 20° with the plane of the sheet; thus, the phenyl group can conveniently lie nearly parallel to the plane of the sheet. Consequently the packing is determined predominantly by the van der Waals thickness rather than the diameter of the phenyl group.

Inter-sheet distances required by the other large amino-acid residues present in silk fibroin are more difficult to predict since there are fewer restrictions on the relative positions of the various side-chain atoms. It appears that efficient packing can be obtained if the distances are approximately the same as that required by the tyrosine residues—about 8 or 9 A if the adjacent pleated sheet consists of glycine residues or about 10 A if the adjacent sheet consists of alanine or serine residues. Thus, the introduction of the larger amino-acid residues into the crystalline part of silk fibroin would require that the strict alternation of inter-sheet distances between the values of 3.5 and 5.7 A, as derived for the pseudo unit, be interrupted occasionally by distances of about 9 A.

One might expect that definite information concerning the sequence of distances between adjacent pleated sheets, and in particular the way in which pleated sheets containing the larger residues are packed in relation to the sheets containing the smaller residues, could be derived from the positions and intensities of the reflections of the type 00*l*. However, there are so few reflections of this type that no precise conclusions as to the packing can be drawn; indeed, the diffuseness of these reflections may be taken as an indication that perhaps there is no well-ordered method of packing of the sheets, and therefore no true identity distance in the direction perpendicular to the sheets. A minimum identity distance roughly compatible with the observed spacings of all of the diffuse equatorial reflections is about 58 A.

Some additional information regarding the packing of the sheets may be derived from considerations of density. Since the larger amino-acid residues are to be included in the structure, the density of the material giving rise to the X-ray diffraction pattern must be taken as the density of silk fibroin rather than the density of a pseudo-silk containing only glycine, alanine, and serine. The most reliable value for the density of silk fibroin appears to be that reported by HEERTJES[34], 1.34 g/cm^3. From this value for the density and the average residue weight of 76.5 reported by TRISTRAM[1], the volume occupied by one residue is calculated to be 94.8 A^3. The area occupied by four residues within each pleated sheet is given by the *a* and *b* identity distances of the pseudo unit cell—$9.4 \times 6.97 = 65.5$ A^2; from these figures the average distance between adjacent sheets may be computed. The resulting value is 5.8 A, as compared with the value of 4.6 A derived for the pseudo structure. These calculations indicate that if there is a definite *c*-axis identity distance in silk fibroin it is a multiple of 5.8 A.

In an attempt to derive some conclusions as to the sequence of pleated sheets in silk fibroin, we have made intensity calculations for several different arrangements of the sheets. The method used for each of these calculations is as follows. First, an

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identity distance normal to the plane of the pleated sheets was assumed; the distances actually chosen were 58 A and 174 A, the value of 58 A having been previously derived as being the minimum spacing compatible with the positions of the diffuse equatorial reflections. Within the chosen identity distance there were placed pleated sheets consistent in number with the density calculations; thus, ten pleated sheets were placed in the assumed identity distance of 58 A and thirty in the distance 174 A. For the actual positioning of these sheets it was assumed that approximately two-thirds of the sheets are packed in accordance with the pseudo structure, that is, with inter-sheet distances alternating between the values 3.5 and 5.7 A. The remaining sheets were placed at distances of about 9 A, representing the packing distance required by pleated sheets containing the larger amino-acid residues. For each calculation it was assumed that all of the scattering material present in each pleated sheet is located in the plane of that sheet; thus, the effect of the side chains as well as that of the slight puckering of the sheets were neglected. These assumptions imply that the distance between any two adjacent sheets must remain the same throughout the structure. This implication, which arises from the necessity of performing computations on simplified models, should not be regarded as defining a structural feature of silk fibroin. It is probable that the sequence of amino-acid residues in silk fibroin requires that the distance between any two adjacent sheets vary considerably from region to region; the models upon which these calculations are based should, accordingly, be regarded as representing localized methods of packing which occur frequently throughout the structure.

In order to simplify the calculations, each sheet was represented by a point on the c axis and these points were arranged symmetrically about the origin. The structure factor expression for each plane $00l$ is then of the form

$$F_{00l} = \sum_i \cos 2\pi l \, (z_i/c_0)$$

where z_i is the distance in Ångströms of each point i from the origin and c_0 is the assumed identity distance.

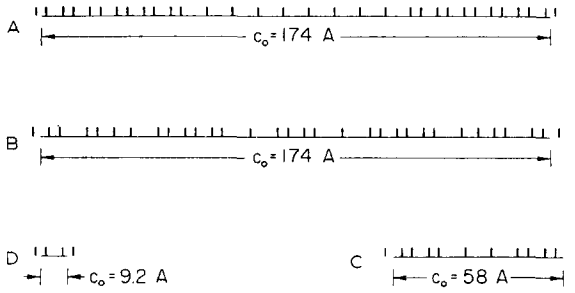

Fig. 12. Arrangements of pleated sheets assumed for intensity calculations.

Typical arrangements of pleated sheets for which intensity calculations were made are described in Table IX and Fig. 12. The results of the intensity calculations are shown diagrammatically in Fig. 13, in which the calculated values of F^2_{00l}/n^2 are plotted against sin θ/λ. For purposes of normalization, the calculated values of F^2 were divided by n^2, where n is the number of sheets within the assumed identity distance c_0. For comparison, the observed spectrum of the diffuse equatorial reflections is shown at the bottom of Fig. 13.

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TABLE IX

PARAMETERS z_j, IN ÅNGSTRÖMS, FOR ARRANGEMENTS OF PLEATED SHEETS SHOWN IN FIG. 12

A ($c_0 = 174$)		*B* ($c_0 = 174$)		*C* ($c_0 = 58$)	*D* ($c_0 = 9.2$)
1.75	91.37	2.85	89.85	2.85	1.75
7.45	100.09	6.31	93.31	6.35	7.45
10.95	108.81	15.75	102.75	12.05	
16.65	117.53	19.21	112.18	15.55	
20.15	126.25	24.91	115.64	24.55	
25.85	129.75	30.61	121.34	33.45	
29.35	135.45	40.04	124.80	42.45	
35.05	138.95	43.50	130.50	45.95	
38.55	144.65	49.20	133.96	51.65	
44.25	148.15	52.66	143.39	55.15	
47.75	153.85	58.36	149.09		
56.47	157.35	61.82	154.79		
65.19	163.05	71.25	158.25		
73.91	166.55	80.69	167.69		
82.63	172.25	84.15	171.15		

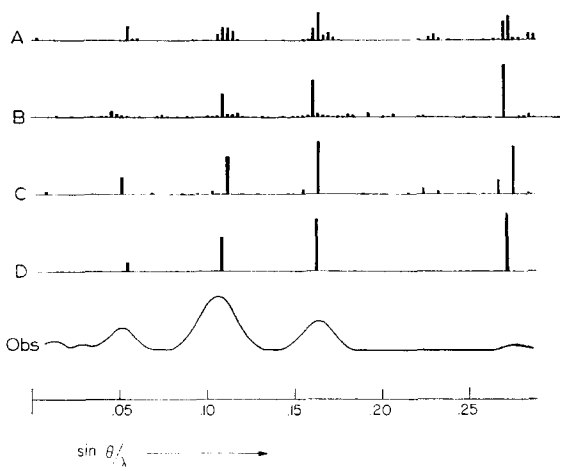

Fig. 13. Observed spectrum of diffuse equatorial reflections and values of F^2/n^2 calculated for various arrangements of pleated sheets (See Fig. 12 and Table IX).

Arrangement D represents the pseudo unit of structure in which there are two pleated sheets within the identity distance $c_0 = 9.2$ A and there is a strict alternation of inter-sheet distances between the values 3.5 and 5.7 A. It has already been pointed out that in this arrangement no provision is made for amino-acid residues other than glycine, alanine, and serine, that its density would be expected to be significantly higher than that observed for silk fibroin, that it will not explain satisfactorily the observed position of the 9.7-A reflection (sin $\theta/\lambda = 0.053$), and that it will not account for the presence of the weak reflections at smaller diffraction angles. Arrangement C is based upon an identity distance of 58 A containing 10 pleated sheets; arrangements A and B are based on an identity distance of 174 A containing 30 pleated sheets. These three arrangements are compatible with the observed density. It should be emphasized again that these

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arrangements are not presented as being possible structures of silk fibroin, but rather as a means of exploring the effect on the diffuse equatorial reflections of the introduction of the larger residues.

Of the intensity plots shown in Fig. 13, that of arrangement C probably shows the most satisfactory agreement with the observed positions and intensities of the diffuse equatorial reflections. However, it is evident that the observed spectrum, including the weak reflections at small diffraction angles, can be approximated by the intensities calculated for any of several different arrangements of the pleated sheets and that no "best" arrangement can be decided upon.

Better agreement with the observed spectrum could be obtained by applying a temperature factor of the type $e^{-B(\sin\theta/\lambda)^2}$ to the calculated intensities; a "temperature" factor of this sort would compensate for general disorder in the packing of the sheets and also take into account, to some extent, the "pleating" of the sheets and the effects of side-chain atoms.

It should be noted that the introduction of the larger amino-acid residues can be accomplished without major changes in the *0kl* spectrum, and that their presence would have even less effect on the intensities of the general reflections of the type *hkl*. It therefore seems reasonable to conclude that the significance of the general agreement between the observed intensities and those calculated for the pseudo structure (Table VIII) may be extended to more complete structures which include all of the amino-acid residues known to be present in silk fibroin.

Two conclusions may be drawn from the agreement between observed and calculated intensities as represented in Table VIII and Fig. 13. In the first place, the diffraction pattern of silk fibroin can be explained on the basis of an arrangement of antiparallel-chain pleated sheets wherein the strict alternation of inter-sheet distances as derived for the pseudo structure is interrupted occasionally by larger spacings representative of the larger amino-acid residues; in the second place, no information as to the exact dispositon of these larger spacings within the structure can be derived. Indeed, it is reasonable to suppose that these large inter-sheet spacings do not occur in a well-defined manner; it is probable that the detailed structure in any region is not determined *a priori*, but adapts itself to the requirements imposed by the chemical composition of the polypeptide chains within that region. One further point should be stressed: it seems improbable that the overall amino-acid composition of any one particular pleated sheet within the structure differs significantly from the composition of any other sheet. It is likely that there are some regions within a given sheet where essentially all of the residues are those of the smaller amino-acids and other regions where there is a concentration of the larger residues; but there seems to be no reason and there certainly is no need to assume that one pleated sheet—or one polypeptide chain within a sheet—consists entirely of small amino-acid residues and that another pleated sheet contains the larger residues. Thus, the method of packing of adjacent pleated sheets is determined by the composition of regions within the sheets rather than by the composition of the sheets as a whole.

The structure of silk fibroin may, then, be described as consisting of antiparallel-chain pleated sheets packed together in a manner determined by the sequence of amino-acid residues within the polypeptide chains. In particular, the sequence –G–X–G–X–G–, where G is glycine and X is alanine or serine, must occur with high frequency; this is the only sequence which will account for the alternation of inter-sheet distance between the

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values 3.5 and 5.7 A. which predominates in the structure. The larger amino-acid residues such as tyrosine are also present within the crystalline portion of silk fibroin, although the precise manner in which they are arranged in the structure cannot be derived.

Just as a satisfactory description of the packing of the pleated sheets requires a modification of the *c*-axis identity distance of the simple pseudo unit of structure, so a complete picture of the distribution of the amino-acid residues within the sheets, either along the fiber axis of the polypeptide chains or in the direction perpendicular to the fiber axis, requires an abandonment of the corresponding *b*- and *a*-axis identity distances 6.97 and 9.4 A. The values for b_0 and a_0 as deduced from the X-ray diffraction pattern cannot be regarded as true identity distances in the strict crystallographic sense, but rather as repeat distances of the back-bone atoms of the polypeptide chains; true identity distances would be determined by the disposition of side-chain atoms. In view of the variety of the amino-acid residues present, it is likely that identity distances in the strict crystallographic sense do not exist in silk fibroin. The occurrence on heavily exposed photographs of weak layer lines representing first and second orders of a 21-A repeat distance in the *b* direction is undoubtedly due to some kind of ordering of side-chain atoms. It is tempting, although probably not justified, to regard these layer lines as arising from a regular repetition of serine residues in a sequence such as –G–A–G–A–G–S–.

IV. DISCUSSION OF THE STRUCTURE

The structure of silk fibroin which has been derived in the preceding section lacks complete definition in the sense that it does not assign specific coordinates to all of the atoms in the protein. Nevertheless, it is characterized by very specific structural features which are so well defined as to merit further examination and discussion.

1. Physical properties and the orientation of hydrogen bonds

The proposed structure of silk fibroin consists essentially of a lateral packing of pleated sheets composed in turn of polypeptide chains bound together in the plane of the sheet by N—H···O hydrogen bonds normal to the fiber axis. Supplementary evidence concerning the validity of this structure may be sought in studies of silk fibroin made with the electron microscope and by the techniques of infrared spectroscopy.

Mr. EDWARD D. HENDERSON of these Laboratories has made several electron micrographs of silk fibroin prepared in the following manner. Degummed natural silk fibers were cut into short lengths and were further disintegrated in water suspension by prolonged treatment with a Waring Blendor. The suspension was allowed to settle for several hours, after which time portions of the supernatant liquid containing the finer particles were removed for examination with the electron microscope. Fig. 14 shows a typical electron micrograph obtained in this way. The flat ribbonlike form of the silk fibers is similar to that reported by other workers (ZAHN[35], HEGETSCHWEILER[36]). By means of tryptic digestion, MERCER[37] obtained thin "ribbon-like fragments" of silk fibroin which in electron micrographs showed what seemed to be "long, thin, parallel microfibrils". All of this evidence appears to be favorable to a structure for silk fibroin composed of laterally packed sheets of polypeptide chains; it cannot, of course, be construed as proof of such a structure.

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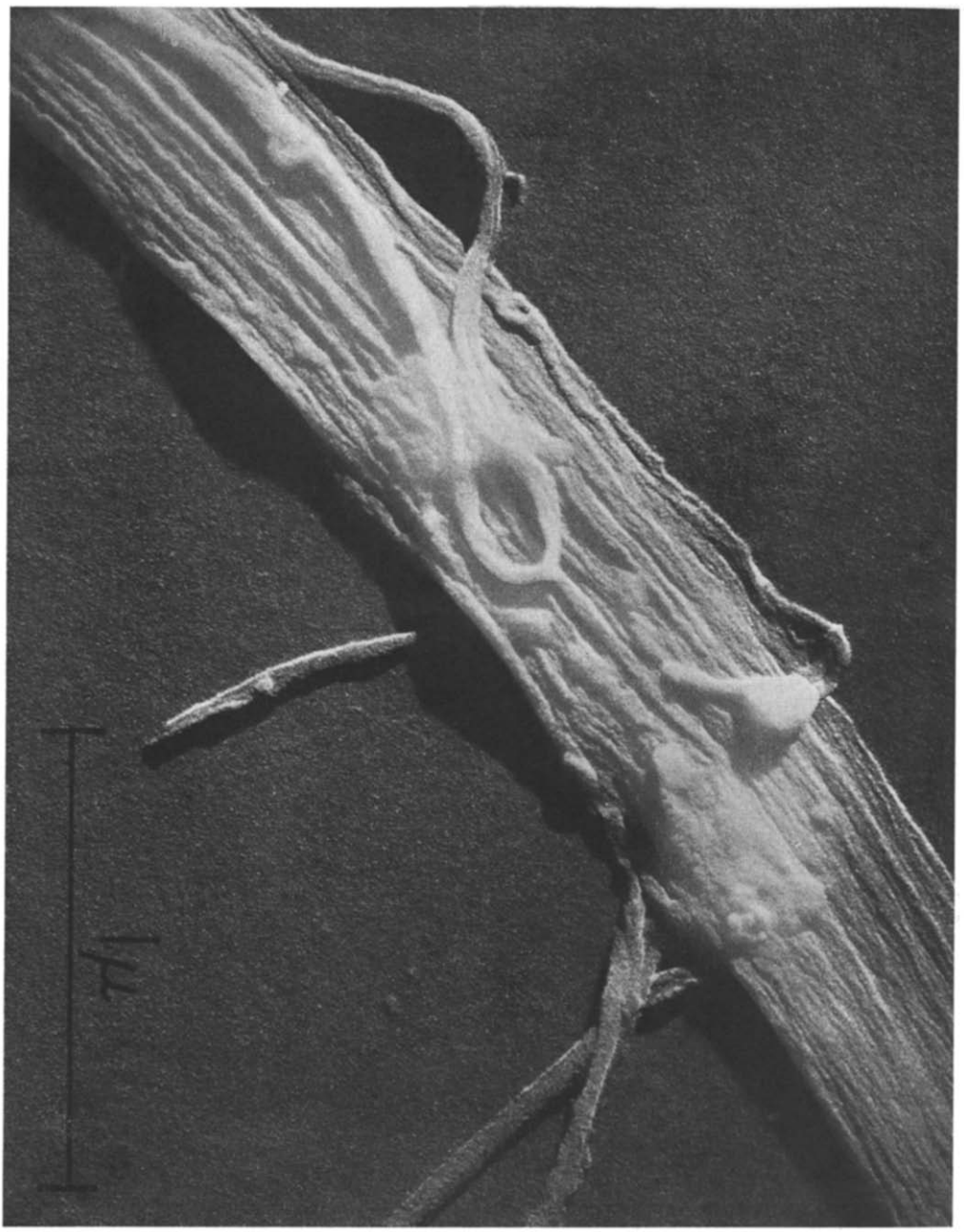

Fig. 14. An electron micrograph of a silk fiber shadowed with a 15-A film of gold-palladium alloy at the angle $\tan^{-1} = 1/3$.

The results of infrared spectroscopic measurements, especially measurements of infrared dichroism, can be interpreted more definitely in terms of structural features of silk fibroin. The approximate orientation of the C=O and the N—H bonds in fibers

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of synthetic polypeptides and in fibrous proteins have been determined by measuring the dichroic ratio observed for polarized infrared radiation (AMBROSE AND ELLIOTT[38]). By this means, AMBROSE AND ELLIOTT[39] were able to decide that these bonds are oriented approximately parallel to the fiber axis in the α-proteins such as hair and approximately perpendicular to the fiber axis in the β-proteins, including silk suture. They interpreted their infrared data as also showing that silk suture contains a component whose chains are folded in a fashion similar to that of the α-proteins. The use of dichroic ratios for determining unambiguously the precise orientations of the C=O and N–H bonds in silk fibroin and other fibrous proteins is complicated by problems of interpretation which have apparently not yet been satisfactorily solved[10, 11, 12].

2. Chemical composition and sequences of amino-acid residues

Unlike structures previously proposed for silk fibroin, which accounted for only the glycine, alanine, and serine residues, the present structure provides for the presence of nearly all of the amino-acid residues shown by analysis to be present in the protein. The only amino-acid residue which cannot be accommodated is proline, which, because of the steric restrictions imposed by the five-membered ring, would cause major distortions or interruptions of the pleated sheets. However, the proline content of silk fibroin—0.5 residue per cent—is probably too small to have a determining effect upon the structure.

A fundamental feature of the proposed structure is the frequent occurrence in the polypeptide chains of the sequence –G–X–G–X–G–X–, in which G represents glycyl and X represents alanyl or seryl residues. Evidence concerning the occurrence of this sequence is to be sought in the identity and relative amounts of the peptides isolated from hydrolysates of silk fibroin.

From chromatographic analysis of acid hydrolysates of fibroin, LEVY AND SLOBODIAN[13, 14] report the presence of large amounts of the dipeptides alanylglycine and glycylalanine, but only very small amounts of glycylglycine. In a later paper[15], these authors reported the presence of large amounts of the tripeptide glycylalanylglycine. More complete chromatographic analyses of partial hydrolysates of silk fibroin have recently been reported by KAY AND SCHROEDER[16]. The principal dipeptides found by these authors are alanylglycine, glycylalanine, serylglycine, tyrosylglycine, and glycyltyrosine; no glycylglycine or serylalanine and only small amounts of alanylalanine were reported. They also separated a number of tripeptides, the most prominent of which they believed to be serylglycylalanine, glycylalanylglycine, alanylglycylalanine, and glycylvalylglycine. The total amounts of dipeptides and tripeptides found by them account for approximately 50% of the glycine, alanine, serine, and tyrosine present in silk fibroin. Two tetrapeptides, alanylglycylanalylglycine and serylglycylalanylglycine, have also been isolated from large-scale chromatograms[17].

From the relative amounts of alanylglycine and glycylalanine which they obtained, LEVY AND SLOBODIAN proposed the sequence $-G-X_1-A-G-A-G-X_2-$ (G = glycine, A = alanine, X = other residue) as a minimum repeating unit for silk fibroin. However, KAY AND SCHROEDER raise some question as to the significance of this proposal; in particular, they point out that the effect of the relative ease of hydrolysis of the bonds X_1–A and G–X_2 might be rather great in determining the amounts of alanylglycine and glycylalanine in the hydrolysates. Indeed, if X_2 were a serine residue, the extreme case of hydrolysis of the glycyl-seryl bond[18] would be expected to increase the observed

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amounts of alanylglycine and glycylalanylglycine. Similarly, if the assumption is made that the sequence –A–G–A–G–S–G– occurs frequently in silk fibroin, the dipeptides formed on hydrolysis would be expected to contain considerably more alanylglycine than glycylalanine, and the tripeptides would contain considerably more glycylalanylglycine than alanylglycylalanine. The observational data of KAY AND SCHROEDER are in accord with the results of this assumption; indeed, the relative amounts of the dipeptides and tripeptides of alanine, glycine, and serine obtained by them can be satisfactorily explained if one assumes that the sequence –G–X–G–X–G–, where X may be either alanine or serine, occurs frequently in silk fibroin.

Among the heavier amino-acid residues, the principal peptides isolated by KAY AND SCHROEDER are tyrosylglycine, glycyltyrosine, and gylcylvalylglycine. The presence of these sequences would indicate that the larger amino-acid residues in silk fibroin are surrounded by glycine residues; thus, in those portions of the protein which contain the larger amino-acid residues, the sequence is apparently –G–X–G–X–G–, where X may be any of the larger residues. This sequence is compatible with the proposed structure. Polypeptide chains containing sequences of this type could form antiparallel-chain pleated sheets in which the hydrogen atoms of the glycine residues protrude from one side and the side chains of the large residues protrude from the other side; two sheets of this sort could pack together front-to-front at a distance of 3.5 A, back-to-back at a distance of about 11 A, or front-to-back at a distance of about 8 A. On the basis of the present evidence, there appears to be no way to choose between the latter two arrangements; in the packing models for which we have calculated intensities of the 00*l* reflections, we have chosen a value of approximately 9 A to represent the distance between sheets containing the larger amino-acid residues without regard as to whether these sheets pack front-to-front or front-to-back, or as to whether or not the large side chains protrude from both sides of a single pleated sheet. The chemical evidence of KAY AND SCHROEDER might be considered to indicate that, in general, the large residues protrude from only one side of the sheets. On the other hand, SANGER[49] concludes from a study of the works of various investigators that "there are tetrapeptide sequences in fibroin that contain no glycine or alanine"; if this is the case, it would mean that there are at least small regions in the structure where the larger side chains protrude from both sides of a single pleated sheet.

3. Concluding Remarks

Previous investigators have proposed structures for silk fibroin comprising extended polypeptide chains oriented parallel to the fiber axis and held together by lateral hydrogen bonds[5,9,11], but no previous attempt has been made to assign definite coordinates to carbon, nitrogen, and oxygen atoms. The present structure, based on many more data, and in particular on quantitative spectrometric measurements of the intensities and interplanar spacings of the equatorial reflections, is superficially similar. Because of the greater accuracy of the data, however, it has been possible to arrive at a definite picture of the way in which the chains are packed together, and for the pseudo unit, at least, even to assign positional parameters to the principal atoms comprising the chains.

The validity of these parameters has been tested by comparison of calculated and observed X-ray spectra.

This picture fails to provide a complete and accurate description of the structure of silk fibroin in terms of the precise positions of all of the amino-acid residues and the

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coordinates of all atoms related to a definite unit of structure. Indeed, it seems reasonable to believe that the structure of silk fibroin involves some randomness and that it is not a simple repetition of identical structural units which can be precisely defined. The present clarified but still incomplete picture of the structure of silk may therefore be close to a possible limit of precision which is imposed by the nature of the material and the available chemical and X-ray data.

One definite implication of the present structure is especially interesting because of its failure to agree with a suggestion which has been put forward in many studies dealing with the structure of silk fibroin. This suggestion, which was originally made by X-ray workers, is to the effect that silk fibroin consists of at least two components, a crystalline component containing only glycine, alanine, and serine residues which is responsible for the characteristic X-ray diffraction pattern, and an amorphous component containing the larger amino-acid residues (especially tyrosine) in addition to the three residues mentioned above. By chemical and enzymatic treatment of silk DRUCKER AND SMITH[13,14] have isolated products which have been interpreted as corresponding to such crystalline and noncrystalline components. As already mentioned in this paper, the small units of structure derived from earlier X-ray data were unable to accommodate tyrosine, which comprises 5.4 residue percent of silk, or the other relatively large amino-acid residues, which make up an additional 10.4 residue percent. As pointed out in the derivation of the present structure, the recent, more accurate X-ray data are not compatible with a simple unit but require the presence of longer interplanar spacings in a way which permits ample provision to be made for tyrosine and other large residues. Quite aside from chemical reasoning, the new X-ray data alone point to the inclusion in the crystalline portion of all of the amino-acid residues, and in approximately the proportion in which they are known to occur. The present X-ray data provide no basis for conclusions concerning an amorphous component in silk fibroin.

ACKNOWLEDGEMENTS

We are indebted to Dr. MAX ROGERS for the preparation of doubly-oriented specimens of silk fibroin and for making these specimens available to us. We are also indebted to Mr. EDWARD D. HENDERSON for the preparation of electron micrographs of silk. We wish to thank Miss ANN SHERIDAN and Warner Brothers Studio for silkworms from which many of our specimens of silk were obtained.

SUMMARY

An investigation based on new X-ray diffraction data, including quantitative spectrometric measurements of X-ray intensities, has led to the derivation of the fundamental structural features of silk fibroin. The structure consists of extended polypeptide chains bonded together by lateral N—H···O hydrogen bonds to form antiparallel-chain pleated sheets. The sequence –G–X–G–X–G–X– in which G represents glycyl and X alanyl or seryl residues predominates throughout the structure, so that adjacent sheets pack together at distances of about 3.5 and 5.7 Å. Longer inter-sheet distances are explained by the presence in the structure of the larger amino-acid residues, such as tyrosine.

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RÉSUMÉ

Une nouvelle étude de la diffraction des rayons X, comportant des mesures spectrométriques quantitatives des intensités des rayons, permet de proposer une structure fondamentale pour la fibroïne de la soie. Cette structure consiste en chaînes polypeptidiques allongées reliées entre elles par des liaisons hydrogène N—H···O latéraux pour former des lames plissées à chaînes antiparallèles. La séquence –G–X–G–X–G–X–, dans laquelle G représente un résidu de glycocolle et X un résidu d'alanine ou de sérine, prédomine le long des chaînes, de telle façon que des lames adjacentes sont écartées d'environ 3.5 et 5.7 A. Des distances plus élevées entre les lames s'expliquent par la présence dans les chaînes d'amino-acides plus encombrants, tels que la tyrosine.

ZUSAMMENFASSUNG

Eine auf neuen Röntgenstreuungsergebnissen, einschliesslich quantitativen spektrometrischen Intensitätsmessungen, beruhende Untersuchung hat zur Aufstellung des Strukturbildes von Seidenfibroin geführt. Die Struktur besteht aus gestreckten Polypeptidketten, die durch seitliche N—H···O Wasserstoffbrücken zusammengehalten werden und plissierte Schichten (pleated sheets) antiparalleler Ketten bilden. Die Reihenfolge –G–X–G–X–G–X–, in der G Glycin- und X Alanin- oder Serinreste darstellen, herrscht in der gesamten Struktur vor, so dass benachbarte Schichten auf einen Abstand von etwa 3.5 und 5.7 A zusammengepackt sind. Grössere Zwischenschichtabstände werden durch die Anwesenheit von grösseren Aminosäureresten in der Struktur, wie Tyrosin, erklärt.

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The article by J. O. WARWICKER entitled "The Crystal Structure of Silk Fibroin" (*Acta Crystallographica*, 7 (1954) 565) has just come to our attention. The structure described by WARWICKER resembles the structure of our pseudo unit in that it is based on antiparallel-chain pleated sheets packed together at spacings alternately 3.7 and 5.6 A (our values being 3.5 and 5.7 A); also, alternate residues in the polypeptide chains are glycine, the others being alanine or serine. These features of our structure were described in a report to the Solvay Congress on proteins in April, 1953 (L. PAULING, "The Configuration of Polypeptide Chains in Proteins", in *Les Protéines*, R. Stoops, Editeur, Bruxelles, 1953). However, WARWICKER's structure differs from our pseudo unit in that it is based on an orthorhombic space group. At an early stage of our investigation we had considered this orthorhombic structure and had been forced to reject it because of its failure to give even approximate agreement between the observed and calculated intensities of principal equatorial reflections. The strongest reflection on the pattern of silk fibroin is the medium-sharp equatorial reflection with spacing 4.3 A (reflection No. 3 of Table II). From photographs of doubly-oriented specimens prepared by KRATKY AND KURIYAMA (1931) and by us this reflection has been definitely shown to arise from sets of planes making an angle of approximately 70° with the plane of rolling. This reflection can be unambiguously indexed as 120 (on the basis of WARWICKER's unit cell). The intensity of 120 calculated with the parameters listed in WARWICKER's paper is practically zero; thus WARWICKER's structure does not explain the occurrence of this very strong reflection, and it must be rejected.

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