The Crystal Structure of Silk Fibroin

By J. O. Warwicker

British Cotton Industry Research Association, Shirley Institute, Didsbury, Manchester 20, England

An X-ray investigation of a precipitate obtained by the action of pancreatic enzymes on an aqueous solution of silk fibroin suggests that the precipitate has essentially the same crystal structure as the silk fibre. Since the precipitate consists almost entirely of glycine, alanine and serine residues, it is concluded that only one crystalline phase, composed of these three amino-acids, is present in silk fibroin.

The precipitate, which is more highly crystalline than silk fibroin, has a density of 1·40 g.cm.$^{-3}$. This value is taken as an approximation to the density of the crystalline regions of the silk fibre.

From photographs obtained when silk fibres are oscillated in the X-ray beam, it is concluded that the fibroin structure possesses a twofold screw axis.

Consideration of all the available evidence suggests an orthorhombic unit cell and the space group $P22_12_1$ for silk fibroin. A detailed structure is proposed, and it is shown that intensities of X-ray reflexions calculated from it are in reasonable agreement with those observed.

Introduction

The diffraction of X-rays by silk, first observed by Herzog & Jancke (1920), has been frequently used as a means of investigating the structure of silk fibroin. Brill (1923) studied silks of various types, and concluded that they all had essentially the same ortho-rhombic crystal lattice and that the only amino-acids that could be present in the crystalline component were glycine and alanine. Meyer & Mark (1928), who were the first to interpret the crystal structure of silk in terms of long polypeptide chains, suggested the possibility that the crystallites might have a mono-clinic, instead of an orthorhombic, unit cell; the space groups assigned to the two forms were $P2$ and $P222$ respectively. The view that the crystalline regions were built up from glycine and alanine residues was supported by the work of Goldschmidt & Strauss (1930), who, by oxidizing silk with alkaline hypo-bromite, obtained a resistant fraction which contained only glycine and alanine but gave the same X-ray diagram as the unoxidized silk. Meyer, Fuld & Klemm (1940) later made the suggestion that serine units might take the place of some of the alanine units in the crystalline regions. A further X-ray investigation of silks of various types was carried out by Kratky & Kuriyama (1931), who made use of highly oriented films of fibroin prepared from the glands of the silk-worm (Kratky, 1929). These workers showed that the X-ray diagram given by silk of the type *Bombyx mori* could be accounted for by eleven different monoclinic unit cells, but, by rejecting those which gave obviously improbable values for the density of the crystalline regions, they were able to reduce this number to six. Brill (1942) drew attention to the similarity between the X-ray diagrams of silk fibroin and synthetic polyamides, and, on the basis of analogy with structures suggested for the latter, he proposed both a unit cell and a structure for silk. The mono-clinic unit cell which he adopted is closely related to two of those deduced by Kratky & Kuriyama.

It is assumed in all the work reviewed above that silk fibroin contains only one crystalline phase. The suggestion that there might be two crystalline phases was first put forward by Trogus & Hess (1933) as a possible explanation of their observation that certain X-ray reflexions were the same for all silks whereas others varied with the type of silk. Recently an inter-pretation of the X-ray diagram of silk fibroin in terms of two crystalline phases has been advanced by Hap-pey & Hyde (1952), who suggest that one of the phases is composed of glycine and alanine residues, with some replacement of alanine by serine, and the other is associated with tyrosine residues.

The foregoing review shows that no general agree-ment has yet been reached about the unit cell of the fibroin lattice, much less about the details of the structure, and it is clear that the evidence hitherto available is insufficient for a solution of the problem. The present paper gives some new experimental evidence bearing upon the structure of silk of the type *Bombyx mori*. Part of this evidence is derived from the study of a fraction obtained by the action of pancreatic enzymes on silk fibroin; the remainder comes from new X-ray data obtained with fibrous silk. On the basis of this new information and current views about the configuration of the polypeptide chain, a unit cell and space group are assigned to the fibroin lattice and a possible arrangement of the polypeptide chains is suggested.

X-ray diagrams of silk fibroin and a fraction obtained from it

In an investigation made in these laboratories (Drucker & Smith, 1950; Drucker, Hainsworth & Smith,

Table 1. *Comparison of X-ray data for silk fibroin, regenerated fibroin, and the insoluble fraction*

| | Powder diagrams | | | | | | | | | Fibre diagram; silk | | |
| | Fibrous silk | | | Regenerated fibroin | | | Insoluble fraction | | | | | |
Ring	Sin θ	d (Å)	Intensity	Sin θ	d (Å)	Intensity	Sin θ	d (Å)	Intensity	Spot	Sin θ	Intensity
R_1	0·083	9·26	m; diffuse	0·083	9·26	m; diffuse	0·085	9·00	s	A_1	0·083	s
R_2		Diffuse haze			Diffuse haze		0·165	4·67	vs	A_2	0·164	s; diffuse
R_3	0·178	4·33	vs	0·178	4·33	vs	0·181	4·26	vvs	A_3	0·179	vvs
R_4	0·215	3·59	s	0·215	3·59	s	0·213	3·61	s	$\left.\begin{array}{l}\text{II}_0\\\text{I}_3\end{array}\right\}$	$\left\{\begin{array}{l}0\text{·}218\\0\text{·}208\end{array}\right.$	$\begin{array}{l}m\\vs\end{array}$
R_5	0·236	3·26	w	0·236	3·26	vvw; diffuse		Not observed		II_1	0·234	m
R_6		Not observed			Not observed		0·254	3·03	s	A_4	0·252	m–s; diffuse
R_7	0·280	2·76	m	0·280	2·76	m; diffuse	0·278	2·77	s	II'_2	0·272	w
										II_2	0·286	m
R_8		Not observed			Not observed		0·330	2·34	w	I_4,II_3	0·335, 0·333	mm
										A_5	0·324	m
R_9	0·342	2·25	m	0·340	2·26	m; diffuse	0·342	2·25	m	III_1	0·339	m
R_{10}	0·379	2·04	s	0·379	2·04	s; diffuse	0·379	2·04	s	III_2	0·367	m
R_{11}	0·418	1·84	w	0·418	1·84	vw	0·418	1·84	w	III_3	0·423	w
R_{12}	0·479	1·61	vvw		Not observed		0·479	1·61	vvw	IV_1	0·464	w
R_{13}	0·499	1·54	w; diffuse	0·494	1·56	w	0·499	1·54	w–m	A_6	0·495	w
R_{14}	0·551	1·40	vvw		Not observed		0·551	1·40	vvw	V_0	0·551	m
R_{15}	0·595	1·29	w	0·585	1·32	w	0·595	1·29	w	III_4	0·597	vw
R_{16}	0·669	1·15	m	0·669	1·15	m	0·669	1·15	m	VI_0	0·656	s

1953a, b) it has been shown that the action of pancreatic enzymes on silk fibroin in aqueous solution leads to the formation of a precipitate consisting mainly of glycine, alanine and serine residues, with only a small proportion of tyrosine residues. This composition suggested that the precipitate might be similar in structure to the crystalline regions in silk, and a comparison of the X-ray diagrams of silk fibroin and the precipitate was therefore made. For this purpose it was necessary to use powder diagrams. Silk fibroin was examined in two forms, viz. silk fibres cut to as short a length as possible, and the powdery material obtained by the regeneration of fibroin from its aqueous solution by the addition of ethyl alcohol. The X-ray photographs are shown in Fig. 1, and the data obtained from them, together with data from the fibre diagram of silk, are recorded in Table 1.

Inspection of the photographs and reference to the table show that the diagrams given by the silk and the regenerated fibroin are essentially the same. The diagram given by the precipitate bears a strong general resemblance to those obtained with fibroin, but is considerably sharper. However, a closer comparison reveals other differences: the diffraction rings R_2, R_6, and R_8 which are given by the precipitate do not appear in the fibroin diagram, and the ring R_5 is given by the fibroin but not by the precipitate. It will be shown that these differences are not inconsistent with the views that the precipitate has essentially the same crystal structure as fibroin, but before this question is discussed it is necessary to consider some features of the fibre diagram of silk and the probable mechanism of the formation of the precipitate.

In the X-ray fibre diagram given by silk the reflexions fall into two distinct groups, viz. sharp and

diffuse. The diffuse reflexions probably arise from distortion of the lattice, caused either by imperfect packing of side-chains within the lattice or by the presence of bulky side-chains on the fringes of the crystalline regions. According to this view, the planes which give rise to the diffuse reflexions might be expected to lie at right angles to the direction of the weakest bonds in the structure.

It is assumed (Drucker *et al.*, 1953a, b) that in the attack on fibroin by the pancreatic enzymes the peptide chains are severed in such a way as to produce chain molecules consisting only of glycine, alanine and serine units except for a tyrosine unit at one end, and that these shortened chain molecules, containing no bulky side-chains except that of the terminal tyrosine unit, are able to crystallize from the solution. In these circumstances it would be expected that the packing of the chains would be more nearly perfect than in the original fibre, and that the resulting precipitate would therefore contain larger crystallites and a smaller proportion of amorphous material than the original silk. The effect of the recrystallization following attack by pancreatic enzymes should thus be to sharpen all the X-ray reflexions, particularly those that were diffuse in the fibroin diagram. Further, the reflexions that were originally diffuse should tend to occur at slightly higher Bragg angles with the precipitate than with fibroin because of the smaller interplanar spacings which result from the improved packing of the polypeptide chains in the precipitate.

The fulfilment of these expectations is most clearly seen in connection with the diffraction ring R_1. This reflexion, subsequently indexed as 100, is seen to be sharper, and the corresponding interplanar spacing is found to be slightly less, with the precipitate than with
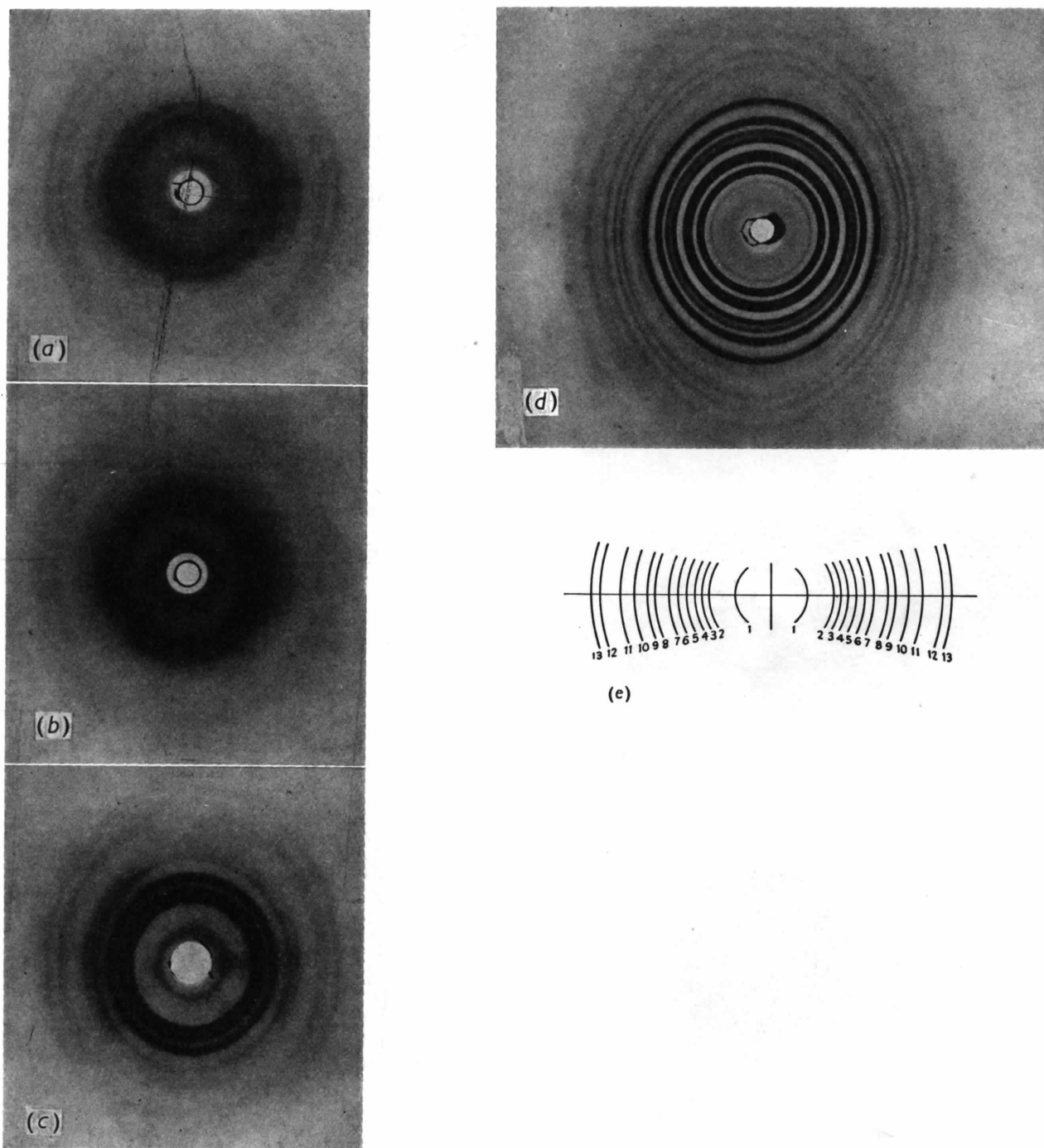
Fig. 1. Powder photographs of (a) silk, (b) regenerated fibroin, (c) precipitate obtained by the action of pancreatic enzymes on aqueous solution of fibroin, and (d) DL-alanyl-glycine. (e) Key to (a), (b) and (c).

fibroin. This observation suggests that the main distortion of the fibroin lattice is perpendicular to the (100) planes, and it would therefore be expected that higher-order reflexions from this set of planes, as well as reflexions from closely connected planes, would show similar features. Thus the reflexions A_2 and A_4, indexed as 200 and 300 respectively, which are diffuse in the fibre diagram, are not distinguishable in the powder diagram of fibroin but do contribute to the powder diagram given by the precipitate. The composite spot (A_2+A_3) shows as a single diffraction ring in the powder diagram of fibroin, but as a result of the recrystallization process it shows as two rings in the powder photograph given by the precipitate, the extra ring being R_2. Similarly, the ring R_5, which is visible in the powder diagram of fibroin only because of the overlapping of II_1 and A_4, shifts to the position R_6 mainly because of sharpening of the reflexion corresponding to A_4. The absence of R_6 from the fibroin diagram and the absence of R_5 from the diagram given by the precipitate are thus explained. In a similar way, the presence of R_8 in the diagram from the precipitate can be explained as the result of the reinforcement of the A_5 reflexion by the sharpening of the reflexion I_4 and II_3 from the (401) and (302) planes respectively. Thus it is possible to account for all the differences between the powder diagrams given by fibroin and the precipitate, and it is concluded that the precipitate has the same crystal structure as silk, differing from it only in being more highly crystalline. That the precipitate has a high degree of crystallinity is shown by a comparison of its X-ray diagram with that given by the crystalline dipeptide DL-alanyl-glycine (Fig. 1).

The X-ray diagram given by the precipitate can also be used to test Happey & Hyde's hypothesis that silk fibroin contains two crystalline phases. According to these workers, the first phase is composed of glycine and alanine units, with some replacement of alanine by serine, and has a monoclinic unit cell, whereas the second phase, which is associated with the tyrosine residues, has an orthorhombic unit cell. The reflexions in the fibre diagram of silk may be indexed on the basis of these two unit cells, with the results shown in Table 2. It should be noted that since A_1 and A_2 are the fundamental reflexions for the second phase, any alteration in this phase should result in a corresponding change in these reflexions. It is evident that A_1 is more clearly defined (as R_1) in the diagram from the precipitate than in the powder diagram from fibroin, in spite of the fact that the precipitate contains only about 1% of tyrosine, supposedly the most important constituent of the second phase. Moreover, the rings R_6, R_7 and R_{10} would also have to be indexed as reflexions from the second phase, and they are all present in the diagram given by the precipitate. The evidence provided by the precipitate is thus strongly against the two-phase structure for silk suggested by Happey & Hyde.

Density and unit cell of fibroin crystallites

It has already been mentioned that Kratky & Kuriyama (1931) specified eleven different unit cells that were compatible with the results of their X-ray examination of silk. Each of these unit cells is distinguished by a different density, and consequently a choice of the most probable unit cell for silk could be made if the actual density of the crystalline regions were known. If it is accepted that the precipitate described above has essentially the same structure as the crystalline portions of silk, then the density of this precipitate should provide a good approximation to that of the crystallites in the silk fibre. The density of the precipitate was found to be 1·398 g.cm.$^{-3}$. (According to Goodings & Turl (1940), the density of silk is 1·353 g.cm.$^{-3}$.) Since the precipitate is not perfectly crystalline, it may be presumed that the density of the crystallites in silk is slightly greater than 1·40 g.cm.$^{-3}$.

Of the possible unit cells listed by Kratky & Kuriyama, only the first gives a theoretical density in the required region, viz. 1·40 g.cm.$^{-3}$; the modified form of this cell adopted by Brill gives a density of 1·375 g.cm.$^{-3}$. These values are derived on the assumption that the crystallites are built up from alternating glycine and alanine residues, and they will be increased if some of the alanine units are replaced by serine units. Smith and his collaborators give the molar ratios of glycine, alanine and serine in the precipitate as 3:2:1, and if this is accepted as the composition of the crystallites in silk then the average weight of the repeating unit increases from 128, the value for a glycyl-alanyl unit, to 133; on this basis the theoretical density of Kratky & Kuriyama's first cell becomes 1·459 g.cm.$^{-3}$ and that of the Brill cell 1·429 g.cm.$^{-3}$. The choice appears to lie between these two unit cells, and it will be shown later that a modified form of the Kratky–Kuriyama unit cell accords well with all the available evidence.

Evidence from X-ray fibre photographs

Evidence about the symmetry elements present in the fibroin structure may be obtained from a consideration of fibre diagrams given by silk. Fig. 2 shows the normal fibre photograph, taken with a stationary vertical bundle of fibres, and two photographs taken with a horizontal bundle which was oscillated through an angle of 15° during the exposure.

The two oscillation photographs are of particular interest. The first of these (Fig. 2(b)), obtained when the fibre bundle was set at an angle of 15° to the X-ray beam, clearly shows the 002 reflexion, but gives no indication of the presence of 001 or 003 reflexions. (The fibre axis is taken as the c axis.) The second (Fig. 2(c)), for which the fibre bundle was set at an angle of 30° to the beam, shows, in the original, the 004 and 006 reflexions, but is rather inconclusive with respect to the 005 reflexion; it is probable that what

appears to be a weak 005 reflexion is caused by departures from the ideal arrangement in the side groups, leaving the main structure unchanged (cf. the interpretation by Legrand (1952) of the 030 reflexion in the cellulose X-ray diagram). It may therefore be concluded that 00l reflexions occur only when l is even, and hence that the fibroin structure possesses a twofold screw axis.

The formation of a twofold screw axis from the polypeptide chains present in the crystalline portions of fibroin requires some discussion. In hydrolysates from fibroin Levy & Slobodian (1952; Slobodian & Levy, 1953) found the dipeptides alanyl-glycine and glycyl-alanine, but very little glycyl-glycine or alanyl-alanine, and they concluded that the sequence alanyl-glycyl-alanyl is an important one in this protein. If it is accepted that only glycine, alanine and serine residues occur in the crystalline regions of silk, then it seems probable that the repeating unit in the chain is glycyl-alanyl, with occasional replacement of alanyl by seryl units. With such an arrangement it is impossible for a single polypeptide chain to possess a twofold screw axis, and it must therefore be inferred that each twofold screw axis involves at least two separate chains.

Arrangement of the polypeptide chains

Before considering possible arrangements of the molecular chains in the unit cell it is necessary to discuss the form of the individual chains. Until recently it was generally accepted that the polypeptide chains in silk were in the fully extended form. However, it has been shown by Corey & Donohue (1950) that this form requires a repeat distance of 7·27 Å, whereas the fibre period in silk is 6·95 Å. It has therefore become clear
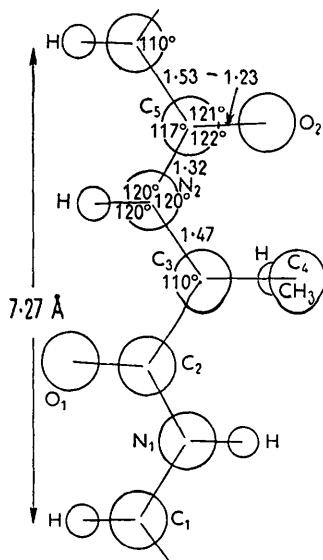
that the polypeptide chains in the crystalline regions of silk cannot be fully extended.

A study of possible configurations of the polypeptide chain was made with the help of an accurately constructed molecular model. The fully extended form of the chain was modified by rotations around bonds to the α carbon atoms (Pauling, Corey & Branson, 1951), the amide group being kept planar. It was found that a small rotation about the C_2–C_3 bond (cf. Fig. 3) caused the CH_3 group of the alanine residue to stand almost at right angles to the plane of the adjacent amide group, and that a small rotation about the C_3–N_2 bond made the CO groups point in parallel but opposite directions. These operations caused the chain to bend in such a manner that the repeat distance along it was 6·95 Å. Fig. 4 shows the resulting atomic
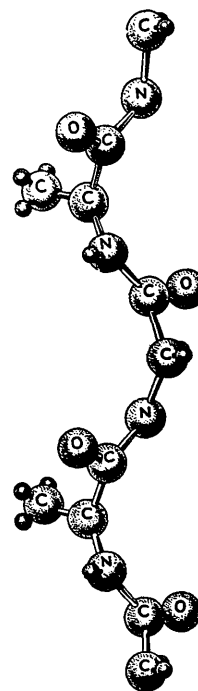

Fig. 4. Perspective drawing of modified polypeptide chain.

arrangement, which resembles the unit used by Pauling & Corey (1953) in the formulation of their pleated-sheet structure for β-type proteins and thought by them to be plausible for silk fibroin.

If the first unit cell of Kratky & Kuriyama (1931) is accepted provisionally, the simplest space group consistent with the evidence is $P2_1$, with two asymmetric units per cell. The polypeptide chains, bent in the manner discussed above, must then be arranged so that each chain is related to its neighbour by a twofold screw axis, and this arrangement can be easily achieved, with a reasonable hydrogen-bond distance of 2·85 Å and a distance of 4·6 Å between chain axes. As is shown diagrammatically in Fig. 5, such an arrangement fits the diagonal of the chosen cell and conforms to the space group $P2_1$.

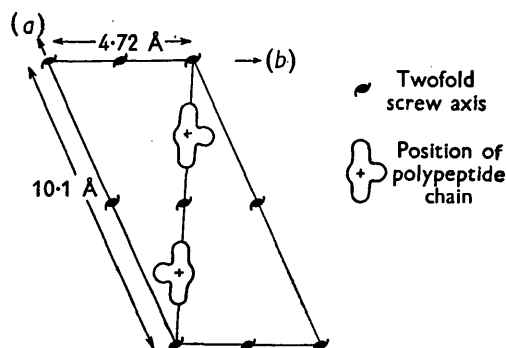Fig. 3. Dimensions of the fully extended polypeptide chain (Corey & Donohue).

Fig. 5. Unit cell and possible arrangement of chains in conformity with space group $P2_1$.

At first sight this structure appears to be a satisfactory solution to the problem, but difficulties arise when it is considered in relation to evidence provided by the work of Kratky & Kuriyama (1931) with oriented silk films. These workers found that when the X-ray beam was perpendicular to the direction of stretching but parallel to the film surface the diagram showed spots A_1, A_2, and A_4 on the equator; a photograph taken with the X-ray beam at right angles to the surface of the film showed only the spot A_3 on the equator. The structure outlined above would require the (110) planes to be parallel to the film surface in these experiments if it is assumed that rolling or stretching orients the sheets of polypeptide chains parallel to this surface. With this orientation, consideration of the conditions of reflexion shows that the proposed structure would lead to results exactly the reverse of those obtained by Kratky & Kuriyama. Furthermore, the most diffuse reflexions would be expected to be 110 and 220, i.e. the part A_3 of the composite spot (A_2+A_3) and the spot A_5, because the weakest forces of the structure are perpendicular to these planes; similar arguments show that the 100, 200 and 300 reflexions, i.e. the spot A_1, the part A_2 of the composite spot (A_2+A_3), and the spot A_4, should be sharp. Again the predicted results are the reverse of those found experimentally, particularly for the reflexions A_1, A_4 and A_5. Thus these considerations strongly suggest that the molecular chains are approximately at right angles to the chosen position.

If the molecular chains are turned through 90° the plane of the sheets of chains becomes parallel to the b axis, with the hydrogen bonding also in this direction. Since alternate chains are not equivalent, this change necessitates the doubling of the b dimension of the unit cell, and in consequence the number of asymmetric units per cell becomes four, and the space group $P2_1$ is no longer valid. The conditions of reflexion now become hkl for $k = 2n$, $hk0$ for $k = 2n$, and $00l$ for $l = 2n$. The first condition is not consistent with any monoclinic space group, so that the most probable space group indicated is $P2_1/b$, where hkl appears for all orders. In the polypeptide chain only

the residues of one of the two optical isomers of alanine are present so that there cannot be any planes of symmetry in the structure. Thus no arrangement of polypeptide chains with a glycyl–alanyl repeating unit can be formed with a glide plane of symmetry involving a mirror plane perpendicular to the axis of the chains, and hence it is impossible to fit such polypeptide chains into the space group $P2_1/b$. No other monoclinic space group involving at least one twofold screw axis and having a cell with four asymmetric units could be found to fit the available evidence, and it was therefore necessary to seek another solution.

Proposed structure for silk fibroin

A new unit cell with $\gamma = 86°$ can be formed from the (100) and (110) planes of the Kratky–Kuriyama cell as shown in Fig. 6. When it is borne in mind that the dimensions of the original cell depend on measurements made on fibre diagrams, and hence are subject to considerable error, it is not unreasonable to take the angle of the derived cell as 90°. This immediately suggests orthorhombic symmetry for the crystallites of silk fibroin, a possibility noted by Kratky & Kuriyama (1931) but not discussed in detail.

The orthorhombic unit cell proposed has the following dimensions:

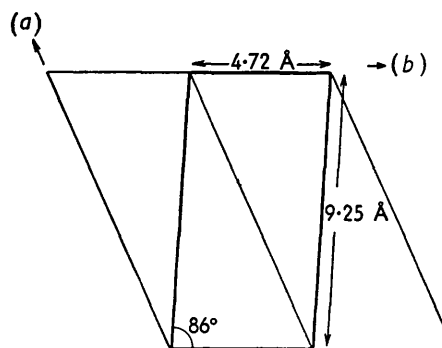$$a = 9.29, \quad b = 9.44, \quad c = 6.95 \text{ Å}; \quad z = 4.$$

Fig. 6. Derivation of proposed unit cell.

The theoretical density of this cell is virtually the same as that of the Kratky–Kuriyama cell from which it is derived, and hence consistent with the evidence given earlier. All the reflexions can be indexed on the basis of the new cell, as is shown by Table 2, and the conditions of reflexion are hkl for all orders, $0k0$ for $k = 2n$, and $00l$ for $l = 2n$. The suggested space group is thus $P22_12_1$, which requires four asymmetric units in general positions.

Pauling & Corey (1953) have suggested that a pleated sheet of polypeptide chains in antiparallel arrangement is possible for silk fibroin. Such a pleated sheet, formed with polypeptide chains having the configuration discussed above, can easily be fitted into the proposed cell if it is placed perpendicular to

Table 2. *Observed principal reflexions in fibre diagrams of silk fibroin*

Spot	Sin θ	d (Å)	Intensity		Happey & Hyde Phase I	Happey & Hyde Phase II	Proposed orthorhombic cell
A_1	0·083	9·29	s;	diffuse	—	010	100
A_2	0·164	4·68	s;	diffuse	—	{ 100 020	200
A_3	0·179	4·30	vvs;	sharp	010	—	120, 020, 200
A_4	0·252	3·06	ms;	diffuse	—	030	300
A_5	0·324	2·38	m;	sharp	120	—	040, 400
A_6	0·495	1·56	w;	sharp	130	—	060, 600, 160
I_1	0·133	5·78	w;	diffuse	—	011	101, 011
I_2	0·197	3·91	w;	diffuse	—	101 021	201
I_3	0·208	3·70	vs;	sharp	011	—	021, 121, 201
I_4	0·335	2·30	m;	sharp	121	—	041, 401
II_0	0·218	3·53	m;	(diatrope)	—	002	—	002
II_1	0·234	3·29	m;	sharp	—	012	102, 012
II_2'	0·272	2·83	w;	diffuse	—	102 ⎫	202, 122, 022
II_2	0·286	2·69	m;	sharp	—	022 ⎬	
II_3	0·333	2·31	m;	sharp	—	032	302, 032
III_1	0·339	2·27	m;	(pseudo diatrope)	—	013	103, 013
III_2	0·367	2·10	m;	sharp	—	103 023	203, 023
III_3	0·423	1·82	w;	sharp	—	033	303, 033
III_4	0·597	1·29	vw		133	—	063, 603, 163
IV_0	0·435	1·77	w;	(diatrope)	—	004	—	004
IV_1	0·464	1·66	w		—	104 024	204, 024, 124
IV_2	0·489	1·57	w		—	034	304, 034
V_0	0·551	1·40	m;	(pseudo diatrope)	—	105	105, 015
VI_0	0·656	1·17	s;	(diatrope)	—	006	—	006

* The fibre axis is taken as the c axis.

the a axis. If a hydrogen-bond length of 2·80–2·85 Å is assumed, the distance between chain axes is 4·72 Å, so that two chains of the sheet fit in the b dimension of the unit cell. The repeat distance along the chains coincides with the c dimension of the cell. If a second sheet is placed in the cell so that it is related to the first by a twofold screw axis parallel to the fibre axis, another screw axis parallel to the b axis is also generated. It will be shown later that this can be done, with reasonable van der Waals distances between successive sheets, so as to give the a dimension of the proposed unit cell. This arrangement thus fits into the proposed cell; it also conforms with the space group $P22_12_1$ because it possesses the required twofold axis parallel to the a axis and twofold screw axes parallel to both the b and c axes. These arguments will be clarified by reference to Fig. 7, which shows three views of the proposed structure.

This structure is consistent with the evidence of Kratky & Kuriyama (1931) if the reasonable assumption is made that in a highly oriented fibroin film the pleated sheets, i.e. the (100) planes, lie parallel to the surface of the film. On the assumption that the dispersion of planes is 15–20°, as expected by Kratky & Kuriyama, a photograph taken with the X-ray beam parallel to the film surface should show the reflexions A_1, A_2 and A_4, indexed as 100, 200 and 300 respectively, but not the reflexion A_3, indexed as 020 and 120.

A photograph taken with the beam at right angles to the film surface could show the 010, 020, 030 and 120 reflexions, assuming the same degree of dispersion. The 020 and 120 reflexions have very similar Bragg angles, and they appear as the spot A_3. The fact that the 010 and 030 reflexions do not appear is in full accord with the proposed space group. Kratky & Kuriyama expected the planes contributing to the spot A_3 to be inclined at an angle of at least 40° to the planes giving the reflexions A_1, A_2 and A_4; consideration of the unit cell shows that the (120) planes make an angle of 63°, and the (020) planes an angle of 90°, with the (100) planes.

The proposed structure is also in agreement with the evidence advanced by Brill (1942). His unit cell* can be modified in a manner similar to that described above so as to give a cell with only slight dimensional differences from that proposed here. Moreover, Brill's arrangement of the molecular chains bears a general resemblance to that given above, so that it would be expected that the analogies between silk and synthetic polyamides which Brill noted should be preserved in the new structure.

A final test of the structure was made by comparing calculated and observed intensities. For the purpose

* It should be noted that the a direction in Brill's unit cell corresponds to the b direction in the proposed cell.
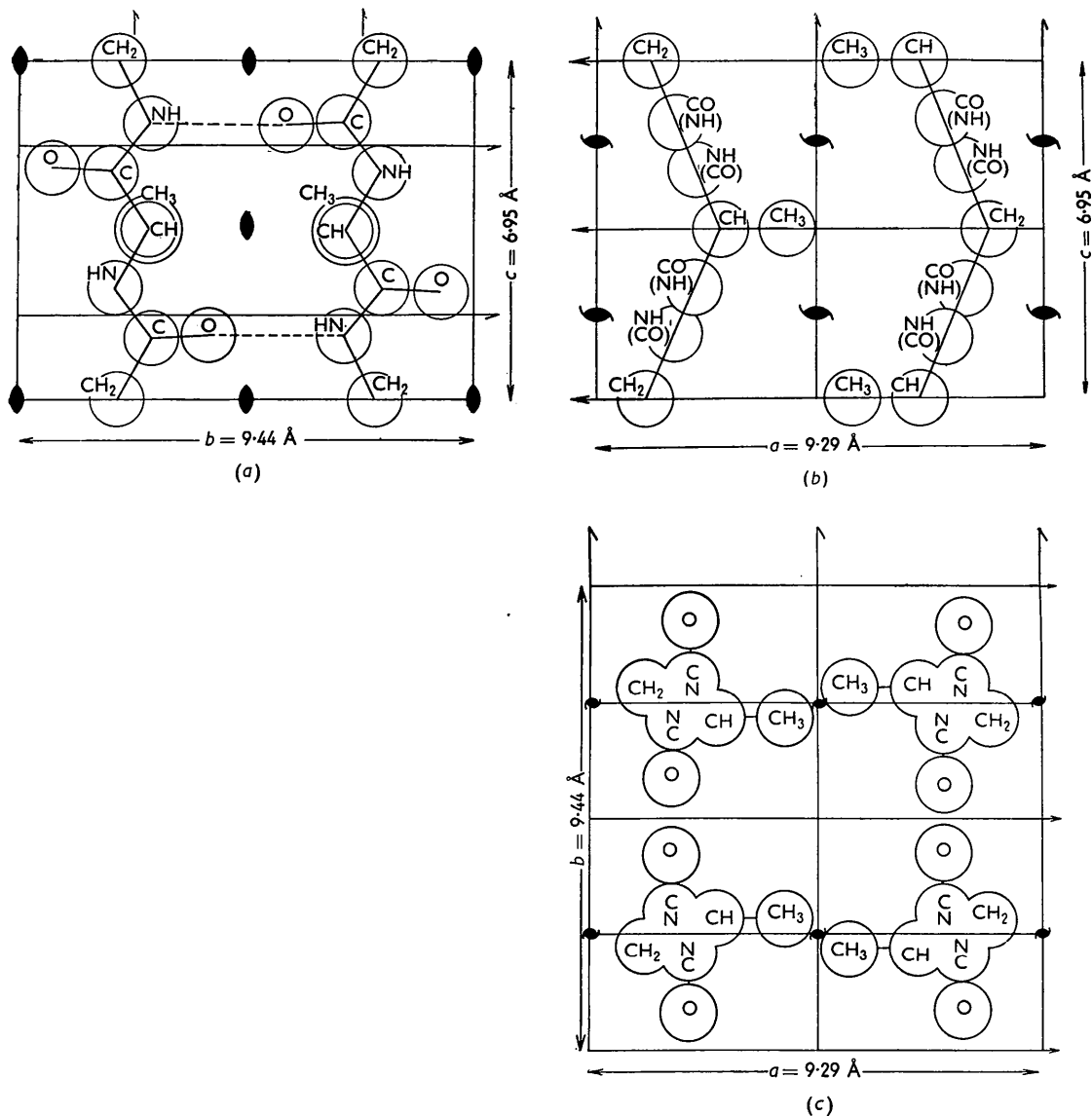
Fig. 7. Projections of proposed structure (a) on (100), (b) on (010), (c) on (001).

of the calculation of the intensities it was necessary to assume that the polypeptide chains consisted of alternate glycine and alanine residues, the effect of the replacement of some of the alanine residues by serine being neglected. The effect of any distortion of the crystal lattice had also to be left out of account.

The co-ordinates of the various atoms in the structure were obtained by placing an accurate model of the molecular chain in parallel light, plotting two projections at right angles to each other, and then deriving the required co-ordinates from the two projections. A check of the accuracy of these co-ordinates was made by using them to calculate the bond lengths between atoms and comparing the values obtained with those used in the model. An overall mean error of approximately 1% was found, and this was considered

to be well within the accuracy of the X-ray data.

The chain was placed in the unit cell with its axis at $x = 0.2$ and $y = 0.25$ and the CH_2 group at $z = 0$. This position permits the symmetry operations required by the space group $P22_12_1$, and each sheet fits into the folds of the preceding sheet with values of 4.0 and 3.7 Å for the distances

$$>CH.CH_3 \ldots >CH_2 \quad \text{and} \quad >CH_2 \ldots >CH.CH_3$$
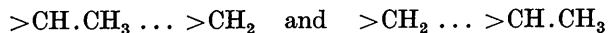
respectively; these values are reasonable for the operation of van der Waals forces. The atomic co-ordinates are given in Table 3.

The structure factors for all the principal planes contributing to the normal fibre diagram were computed from the appropriate equations for the space group $P22_12_1$ (*International Tables*, 1952). The atomic

Table 3. *Co-ordinates of atoms in proposed orthorhombic cell*

Atom	X/a	Y/b	Z/c
C_1	0·12	0·22	0
C_2	0·22	0·20	0·33
C_3	0·28	0·28	0·50
C_4	0·44	0·28	0·51
C_5	0·18	0·29	0·82
C_6	0·12	0·22	1·00
O_1	0·22	0·07	0·32
O_2	0·18	0·42	0·81
N_1	0·18	0·29	0·18
N_2	0·22	0·21	0·68

scattering factors used in these calculations were taken from graphs drawn from the data for carbon, nitrogen and oxygen in *Internationale Tabellen* (1935), but no temperature correction was applied. The relative intensities of reflexions were calculated from the formula

$$I \propto LF^2/D_1 ,$$

where F is the structure factor, L, given by the expression $(1+\cos^2 2\theta)/\sin 2\theta$, is the combined Lorentz and polarization factor, and D_1, given by the expression $\sqrt{\{\cos^2 \varphi - \sin^2 \theta\}}/\cos \theta$, is the geometrical correction factor of Cox & Shaw (1930) which allows for the inclination of the reflecting planes, φ being the angle between the plane and the axis of rotation. No allowance was made for film absorption since appropriate data were not available, but the error produced by neglecting it is expected to be very small. The results of these calculations are compared with the observed intensities in Table 4(a).

Some discussion of the intensities of the reflexions A_2 and A_3 is necessary. It is found that the Bragg angles of the 200, 020 and 120 reflexions are so similar that the reflexions will overlap in a fibre photograph, producing one very intense spot identified as A_3. However, some distortion of the lattice in a direction perpendicular to the (100) planes is to be expected, and this will have the effect of rendering the reflexions from these planes broad and diffuse. Thus the second-order reflexion from the (100) planes, i.e. the reflexion indexed as 200, appears as a fringe to the reflexion A_3 and is identified as A_2. For this reason the reflexions A_2 and A_3 have been treated as a single spot in the calculations. Similar arguments apply to I_2 and I_3. When these considerations are taken into account, the agreement between the observed and calculated intensities may be regarded as satisfactory for the equatorial reflexions and fairly good for the other reflexions.

Similar calculations were made for the 00l reflexions found on the equator of the photographs taken with a horizontal bundle of fibres. Table 4(b) shows that here also the agreement between observed and calculated intensities is as good as could be expected.

Calculations of intensities were also made for about a hundred reflexions which theoretically could occur but are not visible in the fibre photographs. The calculated intensity, expressed as a percentage of that of the strongest observed reflexion, was 1–5 % for nine reflexions, viz. 500, 501, 502, 402, 301, 241, 053, 221 and 232, and less than 1 % for all the rest. In view of the fact that the calculations take no account of lattice distortion and the presence of serine residues in the polypeptide chains, these results are not inconsistent with the validity of the structure.

The proposed structure thus provides a reasonably satisfactory explanation not only of the positions but also of the intensities of the X-ray reflexions from silk fibroin, and it may therefore be regarded as at least an approximation to the structure of the crystalline portions of the silk fibre.

Table 4. *Calculated and observed intensities of reflexions in fibre diagrams of silk fibroin*

(a) Fibre bundle vertical

Spot	Reflexions included	Obs.	Calc.	
A_1	100	s; diffuse	370	s
A_2	200	s; diffuse		
A_3	020, 120	vvs; sharp	1335	vvs
A_4	300	m–s; diffuse	396	s
A_5	040, 400	m; sharp	64	m
A_6	600, 060, 160	w; sharp	38	w–m
I_1	101, 011	w; diffuse	33	w–m
I_2	201, 211	w; diffuse		
I_3	021, 121	vs; sharp	376	s
I_4	041, 401, 321, 231, 141	m; sharp	93	m
II_0	002	m	12	w
II_1	102, 012	m; sharp	63	m
II_2	202, 122, 022	m {diffuse edge to spot	184	s
II_3	302, 032, 132	m; sharp	71	m
III_1	103, 013	m	493	vs
III_2	203, 023, 213	m; sharp	99	m
III_3	303, 033, 313	w; sharp	111	m–s
III_4	063, 603, 163	vw; diffuse	5	vw

"Relative intensity" spans the Obs. and Calc. columns.

(b) Fibre bundle horizontal

00l Reflexions

Spot	Reflexion	Obs.	Calc.	
II_0	002	m	12	w
IV_0	004	w	10	w
VI_0	006	s	57	m

"Relative intensity" spans the Obs. and Calc. columns.

Experimental

Determination of density

The density of the insoluble fraction obtained by the action of pancreatic enzymes on silk fibroin was determined in benzene at 20° C. by means of a specific-gravity bottle. The measurement was made on material that had been dried over phosphorus pentoxide.

X-ray photographs

The X-ray photographs were taken with nickel-filtered copper radiation from a tube working at 45 kVp. and 15 mA., and recorded on Ilford Industrial B X-ray film. The collimator consisted of a lead-glass capillary tube, the bore of which was 0·25 mm. for the powder photographs and 0·5 mm. for the fibre photographs.

For the powder photographs a sample of the material weighing 20 mg. was compressed into a pellet and mounted on the goniometer head of a 3 cm. cylindrical camera; the specimen was kept stationary during the exposure. Confirmatory photographs were taken with cylindrical specimens, 1 mm. in diameter, which were obtained by moulding the powdery materials after they had been moistened with a 0·5% aqueous solution of gum arabic; in these cases the specimens were rotated. The time of exposure was 3 hr. for all the materials examined.

The normal fibre photograph was taken with a vertical bundle of degummed silk fibres kept under tension by means of a special holder adapted for use with the 3 cm. cylindrical camera; the diameter of the bundle was about 1 mm. After satisfactory photographs had been obtained the fibre bundle was moistened with a 2% aqueous solution of gum tragacanth and allowed to dry while still under tension. A new photograph showed that this treatment caused no detectable change in the X-ray diagram. A short portion was then cut from this fibre bundle with a sharp razor-blade, and it was attached to the end of a glass fibre and mounted on the goniometer head of the cylindrical camera with the fibre axis horizontal; the method of mounting and adjustment was essentially that used for single crystals. Oscillation photographs were then taken at various settings of the fibre bundle relative to the X-ray beam, the amplitude of the oscillation being 15°. An exposure of 1–1·5 hr. was required.

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