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Probabilistic Aspects of the Structure of the Collagen Fibril

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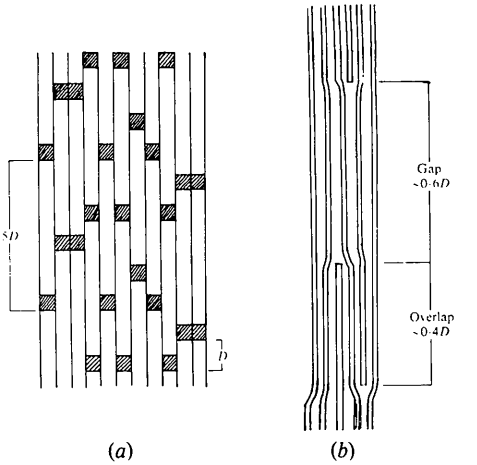
The purpose of this paper is to improve the explanation, given by Woodhead-Galloway & Machin [*Acta Cryst.* (1976), A**32**, 368–372] for the marked diffuse near-equatorial X-ray scattering seen in the diffraction patterns of rat-tail tendon and other collagens. That paper suggested a lateral liquid-like disorder among the molecules, but the treatment involved a number of simplifications, the most important being its restriction to an equatorial projection. This paper elaborates on the treatment and provides two different versions each dealing with the problem in three dimensions. The first approach derives the crystallographic consequences of the random-stagger model for the fibril proposed by Grant, Cox & Horne [*Nature* (*London*), (1965), **207**, 822–826]. The second approach relaxes the strict crystallography assumed in the treatment of the model of Grant *et al.* (1965); instead it suggests a stochastic analogy with the theory of Woodhead-Galloway [*Acta Cryst.* (1977), B**33**, 1212–1218] for the off-equatorial reflections seen in the diffraction pattern of rat-tail tendon. In addition, the opportunity is taken to remove a discrepancy between theory and experimental results by introducing the fanning of the diffraction pattern (which has its origins in molecular tilting or in fibril shearing).

1. Introduction

It is worth beginning by rehearsing briefly the theory of the collagen fibril advanced by Hodge & Petruska (1963): they demonstrated that the 67 nm (=D) axial period of the fibril was a consequence of any two molecules in the fibril being related axially by an integral multiple of this distance (Fig. 1*a*). The molecular length (L) is close to 300 nm, *i.e.* about 4·5D, and it follows that in axial projection the period consists of two distinct regions containing molecular segments in the ratio of 5 (overlap) to 4 (gap) (Fig. 1*b*). Any more detailed model of the arrangement of molecules in the fibril is subject to that constraint.

The low and medium-angle X-ray diffraction pattern obtained from rat-tail tendon includes relatively sharp Bragg reflections together with a marked continuous diffuse scatter close to the equator (Miller & Wray, 1971). These two features indicate the presence of long-range order and considerable disorder in the disposition of the molecules; deterministic and probabilistic aspects of the structure respectively.

The origins of the diffuse scatter have been suggested recently to lie in an irregular liquid-like lateral packing of single molecules (Woodhead-Galloway & Machin, 1976*a*,*b*). However, *a priori* the fibril might be expected to suffer two further sorts of disorder: first, irregular 'tilting' of the molecules resulting in the near-equatorial diffuse scatter (and that, for example, on the 0·9 nm layer line) being fanned. Shearing of the fibrils would produce a more or less identical effect near the equator (Tomlin & Ericson, 1960). § 2 of the present paper



(*a*) (*b*)

Fig. 1. Schematic arrangements of molecules in the collagen fibril used as bases for the calculations of this paper. (*a*) A random distribution of discrete gaps (see text of § 3). If the five different axial relations among molecular strands are present with equal frequency, then in axial projection the fibril has a strict D periodicity. (*b*) Molecular segments in both the gap and overlap regions fill the available volume uniformly. In the model of Woodhead-Galloway (1977), the segments occupy lattice points in the two regions. In the approach described in § 5, the space is filled isotropically and irregularly. (Tilting of the molecules has been neglected in the diagrams.)

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shows the consequences for the distribution of diffuse intensity of combining such a tilting with the theory of Woodhead-Galloway & Machin (1976*a*,*b*).

A less trivial sort of disorder is one involving the axial relations among the molecules. Grant, Cox & Horne (1965) drew attention to the possibility that the set of relations is distributed randomly (Fig. 1*a*). Some possible (circumstantial) evidence for this view was obtained from an analysis of the amino-acid sequence of collagen by Hulmes, Miller, Parry, Piez & Woodhead-Galloway (1973). Conceptually (and technically), a random-stagger model is the simplest one possible which may be combined readily with a lateral disorder. More realistic models probably involve near-randomness in the relations, but a discussion of the crystallographic consequences is beyond the scope of the paper, although the formalism developed in § 3 to deal with a random model is quite general enough to provide a rigorous framework for the calculations.

2. Effect of irregular molecular tilting on the equatorial diffuse scattering

Woodhead-Galloway & Machin (1976*a*,*b*) simplified considerably the problem of the structure of the fibril in proposing a model for the origins of the near-equatorial diffuse scatter. The fanning of the pattern was not considered; molecules were treated as uniform straight cylinders parallel to the fibril axis. The treatment was also limited to that of a projection on the equator – the molecules further approximated by uniform discs and the lateral packing of the molecules by a gas of hard discs. An acceptable account of the diffuse intensity was achieved although there were important discrepancies, notably at very small angles.

With no tilting, the intensity, $I(k')$, is confined strictly to the equator and is accounted for, theoretically, by the expression

$$I(k') \propto S(k')f^2(k') \qquad (2.1)$$

where $f(k')$ is the transform of the molecule in projection and $S(k')$ is the interference function for an irregular, liquid-like packing of molecules [$k' = (k_x^2 + k_y^2]^{1/2}$. Woodhead-Galloway & Machin (1976*a*,*b*) have shown how $S(k')$ may be calculated for a simple model where the only two parameters defining the function are the molecular radius, R, and the packing fraction, $\eta = n_0\pi R^2$ (n_0 is the number density of molecules in equatorial projection).

If, however, rather than straight and parallel to the axis, the molecules suffer a degree of random tilting up to a maximum small angle of $\pm\varphi_{max}$ from the axis, then each point defined by k' on the equator spreads into an arc of length $2k'\varphi_{max}$ and the relative intensity of an equatorial tracing becomes

$$I_0(k') \underset{\text{relative}}{\propto} S(k')f^2(k')\,k'^{-1}. \qquad (2.2)$$

Fig. 2 shows a comparison between an experimental equatorial intensity trace from wet rat-tail tendon and a curve calculated through (2.2), $S(k')$ being obtained by the method referred to above, where (originally) values of $2R = 1{\cdot}18$ nm and $\eta = 0{\cdot}608$ gave the best fit with experiment. The value of $2R$ is not appreciably altered by the modification of (2.2) but a good fit with experiment seems to demand a slight increase in η to 0·648.*

Fig. 3(*a*) shows experimental intensity curves obtained from diffraction patterns of elastoidin (see also Woodhead-Galloway & Knight, 1977), together with Fig. 3(*b*) showing theoretical curves for comparison.

The closeness of the fit between theory and experiment is, to some extent, fortuitous, given the number of approximations made in the service of expediency. Conceptually, three of these are of little significance, *viz* approximating the molecule in projection by a simple uniform disc, employing the hard-disc gas approach to

* The series solution for $S(k')$ developed by Woodhead-Galloway & Machin (1976*a*,*b*) had to be extended to ensure good convergence at the higher density and two further terms were calculated in the direct correlation function $C(k')$ related to $S(k')$ by $S(k') = [1 - C(k')]^{-1}$, bringing the total to ten.

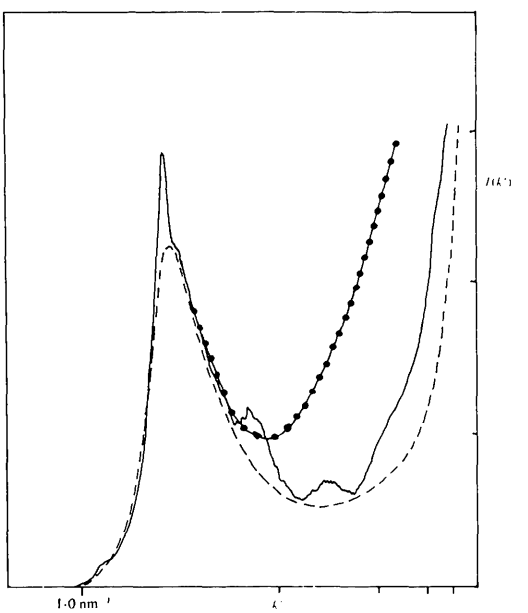


Fig. 2. Equatorial densitometer traces for wet rat-tail tendon. —— Experimental curve. – – – Theoretical curve of relative intensity from (2.2) fitted to the experimental curve by varying R and η. It is worth speculating that the sort of explanation offered here might also be offered for the origins of similar scattering in the diffraction pattern of the silk of *Apis melifera* (Atkins, 1967). •–•–•–• Theoretical curve of relative intensity from (2.2) with (3.15), (3.16) and (2.1). The intensity includes, therefore, contributions from layer lines close to the true equator. The value of $2R = 1{\cdot}18$ nm and that of $\eta = 0{\cdot}648$ are used for both theoretical curves. For a possible explanation of the discrete reflections superposed on the continuous trace, and which account for a large part of the diffracted intensity from rat-tail tendon, see, for example, Woodhead-Galloway (1977).

calculate $S(k')$ and adopting the trivially simple treatment of the equatorial fanning. All are susceptible to refinement without adding much to our understanding of the problem. This is not true of the other major simplification, however – limiting the treatment to an equatorial projection.

Fig. 1(*a*) and 1(*b*) represent schematically the two (possible?) sorts of three-dimensional molecular arrangement treated in the present paper. It has already been pointed out that the feature common to all possible models is the ratio of 5 : 4 between the number of molecular segments in the 'overlap' and 'gap' regions respectively. Thus, different models are better distinguished by the distribution of space – the *missing* molecular segment. In the former, the space is in discrete units – gaps in otherwise continuous molecular strands.

In Fig. 1(*b*) the space is distributed more evenly – the molecular strands occupy the available volume in gap and overlap regions uniformly.

3. Effect of random staggers on the diffraction pattern

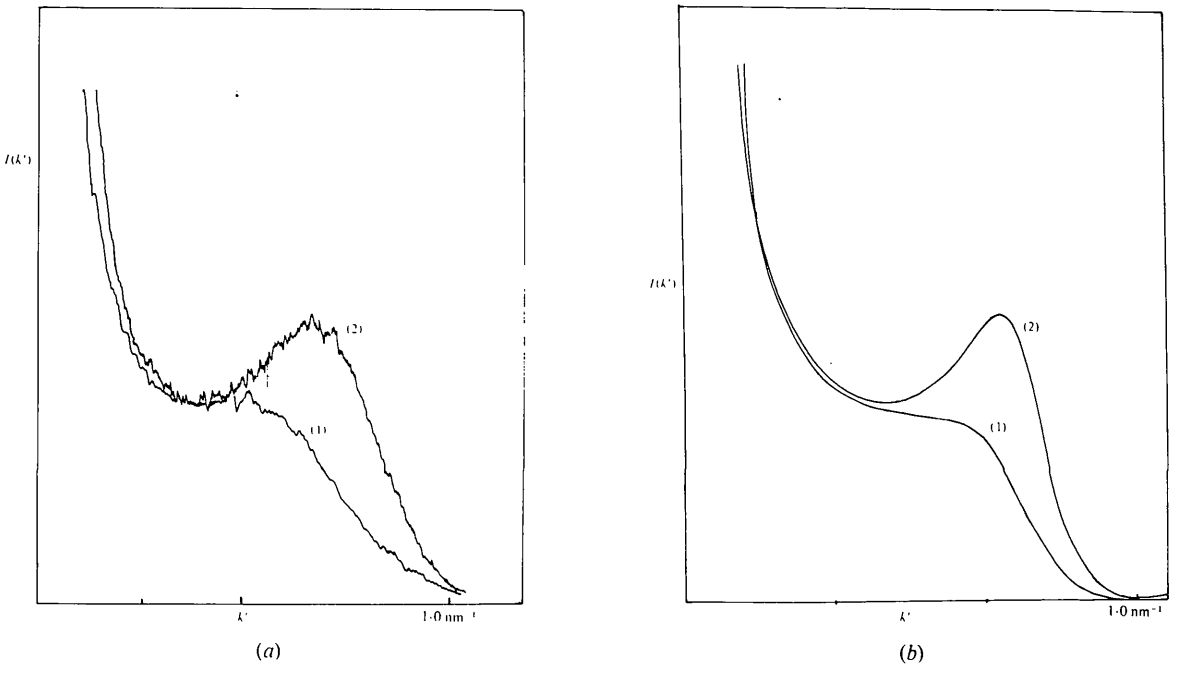
It is clear that, in general, for models (involving straight molecules) like that of Fig. 1(*a*) intensity in the diffraction pattern is confined to layer lines (l) spaced by $(5D)^{-1}$; for truly crystalline models intensity is also confined to row lines (h,k). If, rather than possessing long-range lateral order, the side to side packing is irregular, then clearly such row lines are not present but the intensity is distributed continuously on each layer line (?).

The model of Woodhead-Galloway & Machin (1976*a*,*b*) neglected intensity on layer lines other than $l = 0$, the true equator; equivalently they ignored the presence of gaps altogether. This section develops a formalism to describe the off-equatorial intensity and explicitly calculates it for gaps which are distributed at random.

Details of molecular structure will not seriously affect intensities very close to the equator, so if attention is limited to the first few layer lines, a simple cylinder model for the molecule may well be adequate, the Fourier transform of which is

$$f(k', k_z) \sim 2\pi R \frac{J_1(k'R)}{k'} \cdot 2 \sin\left(\frac{k_z L}{2}\right) \Big/ k_z \qquad (k' \to 0, k_z \to 0) \quad (3.0)$$

where the length of the molecule, L, is known to be approximately $4{\cdot}5D$. The first factor is independent of the layer-line order; Table 1 shows the square of the second factor to illustrate how intensity varies with L following the appropriate substitution $k_z = l2\pi/5D$.



(*a*) (*b*)

Fig. 3. (*a*) Experimental equatorial intensity traces obtained from X-ray diffraction patterns of dog-fish fin elastoidin: (1) as reported by Woodhead-Galloway & Knight (1977); (2) curves of this sort are also obtained sometimes (see, for example, Wray, 1972). The two curves represent different states of hydration of the material which in its natural state contains a good deal of water. (*b*) Theoretical curves calculated as for Fig. 2 but with lower values of the packing fraction, η: (1) $\eta = 0{\cdot}452$, (2) $\eta = 0{\cdot}550$. $2R = 1{\cdot}2$ nm.

It is clear from Table 1 that, other things being equal, intensity near the equator is dominated by the contribution from $l = 0$ suggesting that neglect of other intensity is not likely to lead to serious error, the total intensity contributed by the first four layer lines amounting to only 10% of that on the equator. What follows, however, indicates that for the simple model used in § 2 the fit between theory and the experimental results is somewhat fortuitous.

Table 1. $F(k_z) = 4[\sin(k_z L/2)/k_z]^2$ *calculated for the first few layer lines* (l) *for two values of the molecular length* L. $k_z = 2\pi l/5D$

l	$L = 4{\cdot}4D$	$L = 4{\cdot}5D$
0	1·000	1·000
±1	0·018	0·012
±2	0·015	0·011
±3	0·012	0·009
±4	0·008	0·007
±5	0·005	0·005
±6	~0·003	~0·003

A theory has been given recently in a quite different context which may be modified to treat the problem in hand (Leung, Stott & Young, 1976), and that paper should be consulted for any details of the underlying theory which are not obvious from the treatment given here. The formalism of the problem will be presented in a quite general way, although the explicit solution given is that for the problem of random staggers where, laterally, we shall presume that the molecules are actually on the points of an underlying one-dimensional lattice; this latter restriction may be readily relaxed (see § 5). The structure of the fibril will be formally defined to consist of molecular strands of period $5D$ related to one another by random axial translations (Fig. 1*a*). It is therefore clear that intensity will be confined to layer lines (l) spaced by $1/5D$. The problem is to discover the intensity distribution on such layer lines. Formally, we treat this as a five-component system [corresponding to the five distinguishable axial translations; the treatment of Leung *et al.* (1976) was for a two-component system]. We further presume that there is a total of N strands where N is a multiple of five to comply with the requirement of strict periodicity so that there are $N/5$ of each translation, and we may define for parallelism with other treatments a concentration C_α of each component, suitably normalized so that $C_\alpha = 1/5$ and $\alpha = 0, 1, 2, 3, 4$. We notice that in this treatment, the contribution to the diffracted amplitude from all molecular strands having the same axial relation contains the explicit phase factor

$$V_\alpha = \exp(2\pi i \alpha l/5). \tag{3.1}$$

For the contribution to the interference function $|Q(k_x)|^2$ from the distributions of the five different components we define a set of partial structure factors $S_{\alpha\beta}(k_x)$ where

$$C_\alpha C_\beta N(S_{\alpha\beta}(k_x) - 1) = \left\langle \sum_{\substack{n_\alpha n_\beta \\ \text{appropriate } n}}^{N/5,\ N/5} \exp|ik_x a(n_\alpha - n_\beta)| \right\rangle. \tag{3.2}$$

In terms of such partial structure factors, an expression for the interference function $|Q(k_x)|^2$ may be written:

$$\begin{aligned} |Q(k_x)|^2 = N[&|\overline{V}|^2 - |\bar{V}|^2 + C_0^2|V_0|^2 S_{00} \\ &+ C_1^2|V_1|^2 S_{11} + \ldots C_4^2|V_4|^2 S_{44} \\ &+ C_0 C_1(V_0^* V_1 + V_1^* V_0) S_{01} \\ &+ \text{all other similar cross terms}], \end{aligned} \tag{3.3}$$

where

$$\begin{aligned} \bar{V} = \tfrac{1}{5}[1 &+ \exp(2\pi i l/5) + \exp(4\pi i l/5) + \exp(6\pi i l/5) \\ &+ \exp(8\pi i l/5)]. \end{aligned} \tag{3.4}$$

Thus

$$\left.\begin{aligned} \bar{V} &= 1 \text{ if } l \text{ is a multiple of five} \\ &= 0 \text{ if } l \text{ is not a multiple of five} \end{aligned}\right\} \tag{3.5}$$

and

$$|\overline{V}|^2 = \tfrac{1}{5}(1 + 1 + 1 + 1 + 1) = 1, \text{ independent of } l. \tag{3.6}$$

Thus, as always in problems involving short-range order, calculation centres on the explicit evaluation of the $S_{\alpha\beta}(k_x)$. In the context of collagen one such evaluation has been suggested in an attempt to discuss the lateral irregularity (Woodhead-Galloway & Machin 1976*a*,*b*) and here we now present a further one. By analogy with Leung *et al.* (1976) we define

$$\xi(k_x) = \frac{1}{N} \sum_n^{N/5} \exp(inak_x) \tag{3.7}$$

where the summation is over all sites of the underlying lattice, and for the case of complete randomness, this may be shown to be related to $S_{\alpha\beta}(k_x)$ by

$$S_{\alpha\beta}(k_x) = N|\xi(k_x)|^2. \tag{3.8}$$

From (3.7)

$$\left.\begin{aligned} &\xi(k_x) = 0 \text{ if } k_x \neq \text{a reciprocal-lattice vector of the underlying lattice} \\ &\xi(k_x) = 1 \text{ if } k_x \text{ is such a vector, } \textit{i.e.} \text{ if } k_x = \frac{2\pi\gamma}{a} \text{ where } \gamma \text{ is any integer.} \end{aligned}\right\} \tag{3.9}$$

Thus

$$\left.\begin{aligned} &S_{\alpha\beta}(k_x) = 0 \text{ if } k_x \text{ is not a reciprocal-lattice vector} \\ &S_{ab}(k_x) = N \text{ if } k_x = \frac{2\pi\gamma}{a}. \end{aligned}\right\} \tag{3.10}$$

These results may now be substituted into the expression for the interference function which we may write more concisely as

$$|Q(k_x)|^2 = N\left\langle \overline{|V|^2} - |\bar{V}|^2 + \sum_{\alpha\beta} C_\alpha C_\beta V_\alpha V_\beta^* S_{\alpha\beta}(k_x) \right\rangle \tag{3.11}$$

and

$$\left.\begin{aligned} \sum_{\alpha\beta} C_\alpha C_\beta V_\alpha V_\beta^* S_{\alpha\beta}(k_x) &= 0 \text{ if } k_x \neq \frac{2\pi\gamma}{a} \\ &= N|\bar{V}|^2 \text{ if } k_x = \frac{2\pi\gamma}{a}. \end{aligned}\right\} \tag{3.12}$$

Thus

$$\left.\begin{aligned} |Q(k_x)|^2 &= N[1 - |\bar{V}|^2] \text{ if } k_x \neq \frac{2\pi\gamma}{a} \\ &= N[1 - |\bar{V}|^2 + N|\bar{V}|^2] \text{ if } k_x = \frac{2\pi\gamma}{a}. \end{aligned}\right\} \tag{3.13}$$

Recalling the results of (3.5) and combining them with those of (3.13) yields immediately the final results of this analysis:

$$|Q(k_x)|^2_{\text{configurational average}} = \begin{array}{c|c|l} l = 5m & l \neq 5m & \\ \hline N^2 & 0 & k_x = \dfrac{2\pi\gamma}{a} \\ 0 & N & k_x \neq \dfrac{2\pi\gamma}{a} \end{array} \tag{3.14}$$

Fig. 4(*b*) sums up these findings to compare with the expected diffraction pattern from the Hodge–Petruska model. It could, of course, have been shown in a simpler way that the intensity for $l = 5m$ and $k_x = 2\pi\gamma/a$ is invariant under different stagger models.

We have discussed in detail the case of the one-dimensional lateral geometry, this being more convenient to demonstrate. But the formalism generalizes readily. For the two-dimensional lateral case, the general result (equation 3.3) still stands except now the $S_{\alpha\beta}$ are the appropriate two-dimensional structure factors and it is to be understood that k_x is replaced by (k_x, k_y). In the special isotropic case when the structure factors depend only on $(k_x^2 + k_y^2)^{1/2}$, the work links up directly with that of Woodhead-Galloway & Machin (1976*a*,*b*) who interpreted this function *via* a two-dimensional theory of classical liquids.

An answer to a theoretical model which includes random axial staggers and liquid-like lateral relations follows directly. The substitutional model of Faber (1972) will be valid, and all the $S_{\alpha\beta}$ of (3.2) will be the same [$S(k')$, say]. Equation (3.2) then becomes

$$|Q(k')|^2 = N[\overline{|V|^2} - |\bar{V}|^2 + |\bar{V}|^2 S(k')] \tag{3.15}$$

or explicitly, since (3.5) and (3.6) still apply,

$$\left.\begin{aligned} |Q(k')|^2 &= N\,S(k') \text{ if } l \text{ is a multiple of five,} \\ &= N \text{ otherwise.} \end{aligned}\right\} \tag{3.16}$$

It is worth observing that Faber's (1972) results are sometimes used in liquid-metal theory, but we believe that the present application is *a priori* more valid and realistic.

4. Comparison with experiment

A combination of the considerations leading to equations (2.2) and (3.16) suggests that roughly speaking, contributions to the diffracted intensity arising from layer lines (l), I_l, are given by

$$\left.\begin{aligned} I_l &\sim N\,S(k')f^2(k_1', k_z)\,k'^{-1} \text{ if } l \text{ is a multiple of five} \\ I_l &\sim N f^2(k_1', k_z)\,k'^{-1} \text{ otherwise} \end{aligned}\right\} \tag{4.1}$$

where $f(k_1', k_z)$ is given approximately by (3.0) and $k' = (k_x^2 + k_y^2)^{1/2}$ and $k_z = 2\pi l/5D$.

Because $5D$ is very large (>330 nm), layer lines are closely spaced, and there is considerable overlap of intensity among them. Thus, the resultant intensity on the true equator is given by

$$I_{\text{resultant}} = I_0 + \sum_{\text{relevant } l} (I_{+l} + I_{-l}). \tag{4.2}$$

The meaning of 'relevant l' over which the summation is to be taken is vague; no attempt will be

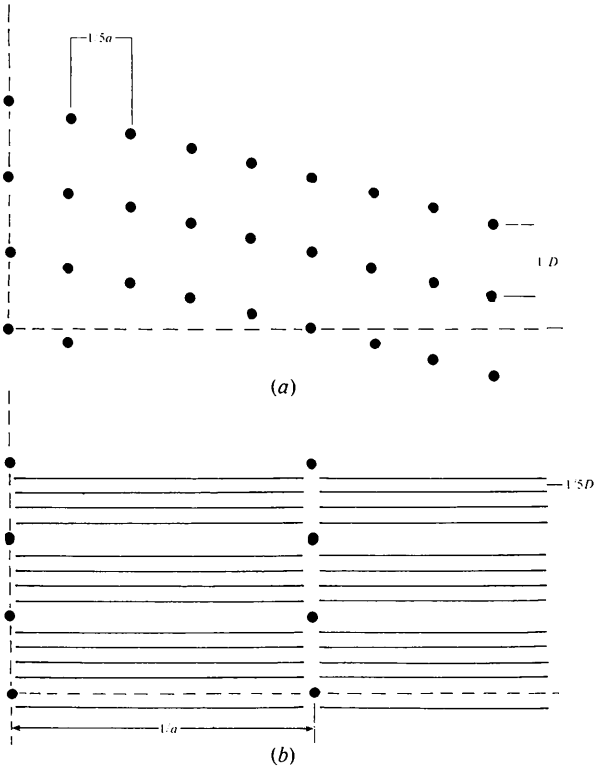


Fig. 4. The effect of randomizing axial relations on the theoretical X-ray diffraction interference function: (*a*) pattern predicted by regular Hodge & Petruska (1963) scheme, (*b*) diagram showing relations (3.14), discrete reflections and continuous intensity.

made to treat this part of the problem rigorously. Table 1 shows that intensity if falling off rapidly with increasing $|l|$ and a summation over the first four layer lines estimates the effect qualitatively. Fig. 2 shows (4.2) calculated with these layer lines and $S(k')$ calculated for $\eta = 0{\cdot}648$ as already shown in the figure.

Theory seriously overestimates the low-angle scattering and the comparison with experiment seems to rule out a random-stagger model as formulated here.

5. A less formally crystallographic approach

The failure of the random-stagger model to recover the experimental details adequately may imply that there is a greater degree of correlation in axial relations and the defining and solving of such models is not uninteresting. We shall, however, rather than pursuing that line, attempt a rather different approach suggested by Woodhead-Galloway (1977) in an attempt to account for the strong off-equatorial Bragg reflections in the diffraction pattern of rat-tail tendon. The essential idea is illustrated in Fig. 1(*b*). In the gap and overlap regions the space is occupied uniformly by the available molecular strands; in the crystalline model of Woodhead-Galloway (1977) the strands occupy lattice points; here it might be suggested that the arrangement in the gap and overlap region is liquid-like. The model introduced by Woodhead-Galloway & Machin (1976*a*,*b*) is particularly useful in testing this hypothesis in that the structure depends only on the packing fraction, η, and the overlap and gap regions have structures which depend on packing fractions in the ratio 5:4. It seems very probable that a model of this latter kind is appropriate for the structure of the needles of elastoidin (Woodhead-Galloway & Knight, 1977; Woodhead-Galloway, Hukins, Knight, Machin & Weiss, 1978).

The data collected for rat-tail tendon, however, support a model which is more of a compromise between the theory of Woodhead-Galloway (1977) and the one just outlined. There are a number of reasons for thinking that this might be the case, none of them very strong, but, taken together, suggestive. First, there is the question of how the short-range order may be reconciled with the relatively sharp Bragg reflections which indicate that the crystalline region may be as large as 100–150 nm across. Second, the theoretical curve for the diffuse scatter (Fig. 2) based on an equatorial projection recovers the experimental curve very well – slightly better than the curves calculated for elastoidin. Third, the strong off-equatorial Bragg reflections fall at the edge of the 'fan' of diffuse scatter. What these points suggest is that the lateral disorder is largely confined to the gap region where the density is low and where the molecular segments are tilted irregularly.

Thus the diffuse intensity would be given by

$$I(k') \sim f(k')^2 \, S(k') \, k'^{-1}, \qquad (5.1)$$
$$\text{gap}$$

on the equator. However, it has already been shown (Fig. 2) that an expression of this sort does fit the experimental density trace and, furthermore, that the value of η which allows a good fit is close to 0·65. This is an interesting value; as we have seen, a simple regular cyclinder model seems to give quite a good basis for intensity calculation. The packing fraction for a tetragonal array of regular cylinders is $\pi/4$. Then, if, as conjectured by Woodhead-Galloway (1977), this is the molecular arrangement in the overlap region, the corresponding packing fraction in the gap is $\pi/5 = 0{\cdot}628$ – differing from the value estimated from the diffuse scatter by only 3% – adding more weight to the idea of gap regions in which the arrangement of molecules is less regular than in the overlap regions.

The gap and overlap regions are less clearly differentiated in elastoidin (Woodhead-Galloway & Knight, 1977), probably by virtue of the material's low density. Tilting in the gap and overlap regions may well be more similar, and the observed intensity contributed roughly

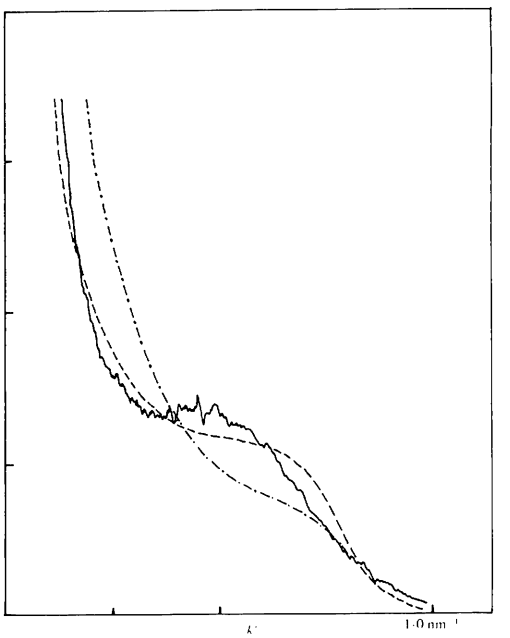


Fig. 5. Comparison of strict crystallographic model of § 3 (– – –) with the suggestion of § 5 (–·–·–·) and curve (1) of Fig. 3(*a*) (——). Packing fraction used in the calculation of § 3 and for overlap calculation was 0·51 (consequently that for gap-region calculation = 0·8 × 0·51 = 0·41). In the calculation of § 5, the gap region was presumed to be 0·6*D* and the overlap region, 0·4*D*. The degree of tilting in gap and overlap was presumed to be the same. The problem of layer lines with different intensity distributions met in § 3 is not met here since intensity arises only from layer lines l = multiples of five and is the same function of k' on all (at least to a first approximation).

equally by the gap and overlap regions. Fig. 5 shows intensity calculated for the two models of §§ 3 and 5 and compared with the experimental intensity for elastoidin. The comparison is intended to be illustrative only, although a reasonable fit between theory and experiment has been attained for the latter model for a value of the molecular diameter of 1·2 nm and a value of packing fraction in the overlap region of $\eta = 0{\cdot}51$. The degree of tilting in the gap and overlap regions is presumed to be the same so that the intensity is given by

$$I(k') \sim \left[x S(k')_{\text{gap}} + (1-x) S(k')_{\text{overlap}} \right] f(k')^2 k'^{-1} \qquad (5.2)$$

where x is the fraction of the D period occupied by the gap region, known to be about 0·6. The intensity calculated for the model of § 3 is based on the same packing fraction, 0·51, and includes contributions from the same layer lines as the calculation done for rat-tail tendon. It appears that the random-stagger model is the inferior of the two.

6. Discussion and conclusions

(i) On the whole, the paper supports the contention of Woodhead-Galloway & Machin (1976*a*,*b*) that the chief reason for diffuse near-equatorial intensity is a two-dimensional liquid-like irregularity in the disposition of single molecules.

This explanation is, of course, very much at variance with that of, say, Miller & Wray (1971) and Miller & Parry (1973), who considered the intensity profile to be that of a five-stranded microfibril (Smith, 1968). In this they followed an earlier argument of Atkins (1967), who felt that the (similar) intensity distribution in the diffraction pattern of the silk of *Apis melifera* suggested a four-stranded coiled coil. It seems that a combination of lateral irregularity with some disorderly tilting or shearing offers an adequate explanation for the diffuse intensity (of both materials).

(ii) The calculations reported here are not sufficiently refined for this conclusion to be drawn without reservation, but sufficient agreement with experiment has been demonstrated for them to be regarded seriously.

(iii) Of the two models explored in the paper, the second where the available space is filled uniformly by the molecular segments is clearly favoured by the available data. It is important that a model can be found relatively easily which complements that suggested by Woodhead-Galloway (1977) for the Bragg reflections seen in the pattern given by rat-tail tendon.

(iv) It is disappointing that the question of detailed axial relations among the molecules cannot be answered by an appeal to X-ray data if the conclusions of the paper are correct.

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