

X. *X-Ray Studies of the Structure of Hair, Wool, and Related Fibres.*  
 II.—*The Molecular Structure and Elastic Properties of Hair Keratin.*

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[PLATES 8 AND 9.]

*Introductory Summary.*

In a previous communication\* an account was given of a preliminary exploration, chiefly by X-ray methods, of the problem of the molecular structure of animal hairs. The present paper is a natural continuation of the record, in which earlier tentative suggestions are either confirmed or rejected, and an attempt is made to lay bare the general structural principles underlying the properties of the protein, *keratin*. It will be unnecessary here to outline once more the historical development of the subject; we shall proceed at once to the main point of this introductory section, which is to give what appears to be the solution of the problem before setting out in detail the experimental facts and arguments leading up to it. Such a procedure is advisable because of the complex nature of the properties under discussion; such a long series of experiments have been involved in their elucidation, that without some sort of preliminary statement of the chief conclusions, the issue is apt to grow confused.

Briefly, the whole argument rests on the discovery† that the X-ray “fibre photograph” which appears to be common to all mammalian hairs, human hair, wool, whalebone, nails, horn, porcupine quills, etc., and which is undoubtedly the diffraction pattern of crystalline, or pseudo-crystalline, keratin, the common fibre substance of all these biological growths, is changed into a quite different fibre photograph when the hair is stretched. The change is a reversible one, recalling that previously discovered by KATZ‡ in rubber, because when the hair is returned to its initial unstretched length, the normal keratin photograph reappears. It is clear that the X-ray effects give a diffraction record of a reversible transformation involving not merely an internal slipping of the fibre substance or a rotation of “micelles” into stricter alignment,

\* ASTBURY and STREET, ‘Phil. Trans.’ A, vol. 230, p. 75 (1931); referred to later as I. Cf. also ASTBURY and WOODS, ‘Nature,’ vol. 126, p. 913 (1930).

† ASTBURY, ‘J. Soc. Chem. Ind.’ vol. 49, p. 441 (1930); ‘J. Text. Sci.’ vol. 4, p. 1 (1931).

‡ ‘Chem. Z.’ vol. 49, p. 353 (1925); ‘Naturwiss.’ vol. 13, p. 411 (1925).

but a definite elastic elongation and contraction of the keratin complex itself. It has been proposed, I, to call the two forms of keratin thus revealed by X-ray analysis  $\alpha$ -keratin and  $\beta$ -keratin, the former being the shorter, normal form.

Putting aside for the moment the question of the analysis of the normal fibre photograph ( $\alpha$ ), it is to be noticed at once that the photograph of stretched hair ( $\beta$ ) is closely analogous to that always given by the protein of natural silk\*, *fibroin*, whether unstretched or stretched, and there is every reason to believe†, both from X-ray and general physical and chemical evidence, that the fibre substance of silk is for the most part built of fully-extended polypeptide chains of the simple kind postulated by FISCHER. It follows, therefore, that  $\beta$ -keratin is most probably also based on fully-extended polypeptide chains, while  $\alpha$ -keratin must be constructed out of the same chains in some shorter, folded form. Natural silk is thus virtually non-elastic, while mammalian hairs, on account of the inherent configurational instability of the extended keratin complex, show long-range elasticity of a most valuable and instructive character.

We may picture a polypeptide chain as a long series of  $\alpha$ -amino-acid residues,

each of the general formula  $\begin{array}{c} \text{CO} \diagup \\ \text{CH} \\ \text{R} \text{ } \diagdown \\ \text{NH} \end{array}$  — a kind of molecular centipede whose

legs represent the various univalent "side-chains" denoted in the general formula by the letter R; and in a fibre such as hair, built, as X-rays show, from a system of polypeptide chains all lying more or less parallel to the fibre axis, we can see that the equilibrium form of the protein complex must be decided chiefly by the interactions of the side-chains, both of the same main-chain and neighbouring main-chains. Both the pattern formed by the crumpling or folding of the main-chains, which may or may not be seriously distorted by the interactions or actual chemical linkages, electrovalent and co-valent, between the side-chains, and also the lateral extension of the side-chains, may under favourable conditions be examined by X-ray methods; and when the results of such an examination are correlated with general physical and chemical properties, we may reasonably expect to be able to draw conclusions of a much more fundamental kind than is possible along more restricted lines of investigation. This has occurred in the analysis of the molecular structure of hair; correlation of all the available data, both X-ray and physico-chemical, has shown that we must think of it as based on parallel polypeptide chains which are linked laterally by both electro-valent and co-valent bridges, and which are normally in equilibrium in a contracted or folded form. These chains may be pulled out into the straight form

\* HERZOG and JANCKE, 'Festschrift der Kaiser Wilhelm Gesellschaft' (1921); BRILL, 'Liebig's Ann.,' vol. 434, p. 204 (1923); KRATKY, 'Z. phys. Chem.,' B, vol. 5, p. 297 (1929); KRATKY and KURIYAMA, *ibid.*, vol. 11, p. 363 (1931).

† MEYER and MARK, 'Ber. deuts. chem. Ges.,' vol. 61, p. 1932 (1928); MEYER and MARK, "Der Aufbau der hochpolymeren organischen Naturstoffe," Leipzig (1930).

by the application of tension, and they may even be contracted still further when certain lateral linkages are broken down, while they can also be "set" in the extended form by building up new lateral linkages. The elastic properties of hair are almost bewildering in their variety, but they all appear to be based on a molecular mechanism which, in its essentials, is relatively simple.

We shall first, to avoid all confusion regarding the many aspects of the properties under discussion, give a general account of the elastic phenomena and X-ray results, together with a general explanatory scheme. Thereafter it will be convenient to go into the various details as thoroughly as our present knowledge permits.

### *General Elastic Properties.*

Since the pioneer work of HARRISON,\* SHORTER,† and KARGER and SCHMID,‡ the most thorough investigation of the load/extension curve of wool, under varying conditions of humidity, temperature, and time, is due to SPEAKMAN.§ Tension/extension curves adapted from SPEAKMAN'S, for Cotswold wool at 25° C. and at humidities ranging from 0% to 100%, have already been given, I. Broadly speaking, they show that as the moisture content of wool is increased, the fibre stretches more easily and farther. In every case the curves show an initial "HOOKE'S law region," where  $dE/dT$  is small, up to extensions of about 2%, and then a region of rapid extension for the next 25% or so, which passes into a region where the rate of extension gradually decreases again (see, for example, the right-hand curve of fig. 3). Fig. 1, also after SPEAKMAN, shows how the form of this load/extension curve changes with rising temperature when the fibre is stretched in water. It will be seen that the limiting stress of the HOOKE'S law region continuously decreases as the extensibility increases and the "shoulder" of the curve becomes less obvious—in other words, raising the temperature accentuates the effect of humidity by making the fibre still more easy to stretch and enabling it to be stretched to still greater elongations.

The limiting extensibility of hair,|| when the fibres are carefully chosen for uniformity, appears to be of the order of 100%; the most they can be stretched without rupture, even in steam, is to perhaps a little more than twice their original length. Fig. 2 shows a set of time/extension curves for human hair and Cotswold wool stretched under constant load in steam and in a 1% aqueous solution of caustic soda. Each fibre was

\* HARRISON, 'Proc. Roy. Soc.,' A, vol. 94, p. 460 (1918).

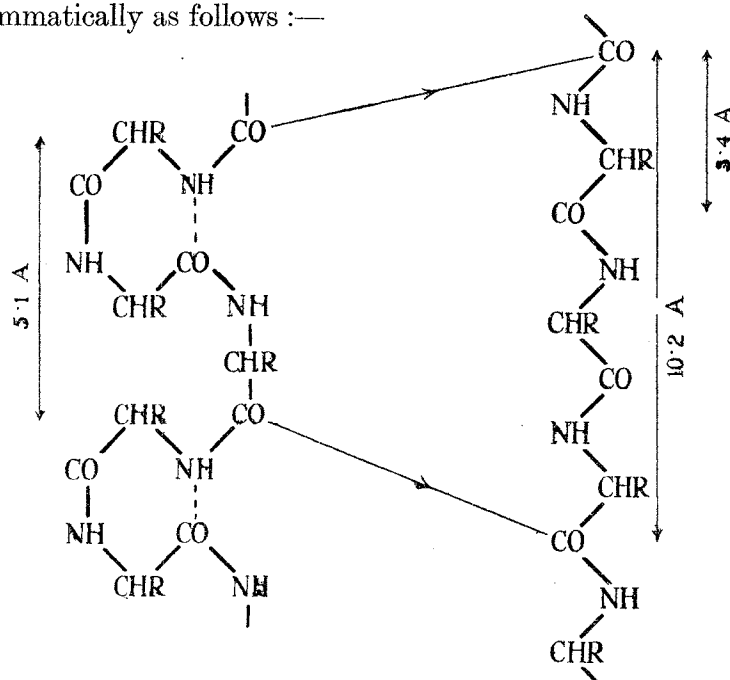
† SHORTER, 'J. Text. Inst.,' vol. 15, p. T207 (1924); 'Trans. Faraday Soc.,' vol. 20, p. 228 (1924); 'J. Soc. Dy. Col., Bradford,' vol. 41, p. 212 (1925).

‡ KARGER and SCHMID, 'Z. techn. Phys.,' vol. 6, p. 124 (1925).

§ See, *inter alia*, 'J. Text. Inst.,' vol. 17, p. T457 (1926); vol. 18, p. T431 (1927); 'Proc. Roy. Soc.,' B, vol. 103, p. 377 (1928).

|| We shall use the word "hair" for mammalian hairs in general, whatever the source of origin. When hair of a definite type is referred to, it will be named, *e.g.*, human hair, wool, etc.

knowledge of the elastic properties, we must now conclude that the whole process of extension and recovery in the hair fibre is based on a protein chain-system which, under the proper conditions, is capable of being stretched to twice or contracted to half its normal length. These (approximate) limits rest on exhaustive experimental tests of numerous actual fibres, and also find a complete quantitative interpretation in all the available X-ray data, not only of keratin itself, but of other protein fibres also.\* The true starting-point of the line of argument is the observation that *the X-ray photograph of  $\beta$ -keratin (stretched hair) is in all essentials analogous to that of the fibroin of natural silk, which is the same whether stretched or unstretched.* From every point of view we must assume that fibroin is built from *fully-extended* polypeptide chains lying closely side by side to form long, thin crystalline "bundles" or micelles, and that the effective length of each amino-acid residue in such a system is 3.5 A.† It follows therefore that if the postulated analogy between  $\beta$ -keratin and fibroin is sound, the characteristic meridian spacing of  $\beta$ -keratin, I, 3.4 A (approximately), corresponds to the average length of an amino-acid residue in the *fully-extended* keratin chains, so that to explain the occurrence of the normal  $\alpha$ -form of hair, we have to decide on a method of folding these chains *which will satisfy both the quantitative requirements of the  $\alpha$ -photograph and the 100% extension revealed by the generalized load/extension curve.* In addition, the molecular model must give, at least, a qualitative interpretation of the main physico-chemical differences between  $\alpha$ - and  $\beta$ -keratin, and also promise a basis for a quantitative treatment of the super-contraction phenomenon. The type of intramolecular transformation which best satisfies *all* these various requirements is shown diagrammatically as follows:—



\* ASTBURY, 'Trans. Faraday Soc.', vol. 29, p. 193 (1933).

† Footnotes \* and †, p. 334.

The  $\beta$ -form is thus represented by fully-extended peptide chains in which each amino-acid residue takes up, on the average, a length along the fibre-axis of 3.4 A, while the  $\alpha$ -form is represented by a series of pseudo-diketopiperazine rings which follow each other according to a pattern of length 5.1 A. The unfolding of the rings is clearly accompanied by an elongation of 100%, and the suggested pattern offers an explanation of both the characteristic meridian reflection of the  $\alpha$ -form (5.1 A) and of the decrease of resistance of the  $\beta$ -form, as compared with the  $\alpha$ -form, to the action of reagents such as steam, etc.

Only a part of the elastic properties of hair are to be interpreted by the application of this principle of intramolecular unfolding; many of its most striking characteristics are to be referred to the nature and distribution of the side-chains denoted above by the general symbol R. Though there does not appear to be any *sharp* discontinuity in the physical and chemical properties of the keratin complex as a whole, we have to recognize that both the form and the limits of the load/extension curve may be varied over a wide range simply as a result of the changes which take place in the configuration of the side-chains only. As the most convenient example of such side-chain disturbances it will serve for the present to quote the preferential attack of steam which is to be noticed in  $\beta$ -photographs for extensions of 50% and upwards, and which is undoubtedly the cause of the increased capacity for extension. Other changes which are less clear from the X-ray point of view, but which nevertheless are very obvious when examined by more familiar physico-chemical methods, are the freeing of certain side-chain restrictions so as to give rise to the phenomenon of super-contraction, and the "permanent set" of the  $\beta$ -form on prolonged steaming of the fibre in the stretched state. This latter transformation evidently involves the building-up of new side-chain linkages which fix the  $\beta$ -form in the stretched state and preclude once and for all the possibility of ever regaining the normal  $\alpha$ -photograph.

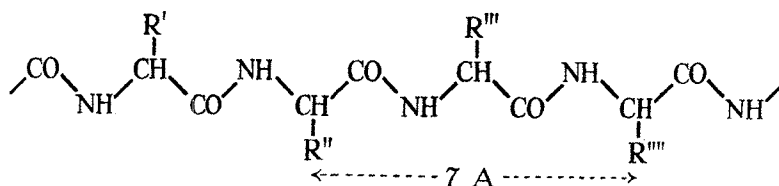
#### *Detailed Discussion of the Elastic Properties.*

*The influence of humidity at ordinary temperatures.*—From an examination of load/extension or tension/extension curves of wool already given,\* I, it appears that the main effect of varying the water-content at ordinary temperatures is to alter the scale of the load (or tension) co-ordinates; the fibre becomes progressively easier to stretch. At the same time it can be stretched farther, so that whereas it seems impossible to stretch a perfectly dry hair by more than about 30% at the very most, a wet hair may readily be stretched in one operation to something of the order of 55%. (In SPEAKMAN'S curves\* for wool of 40 $\mu$  diameter the rate of loading was 1.8 gm. per minute.) Curves obtained under these conditions of fairly rapid rate of loading at 100% R.H. are characterized by a "shoulder" commencing in the region of 20% extension, while

\* Footnote §, p. 335.

*The Structure of the Keratin Complex.*

As explained in the Introduction, the fundamental structural basis underlying the whole of these investigations is the existence of an intramolecular transformation considered to take place between a folded polypeptide chain-system ( $\alpha$ -keratin) and one in a fully-extended state ( $\beta$ -keratin) analogous to that found normally in the fibroin crystallites of natural silk. Figs. 24, 25, Plate 8, are X-ray photographs of small bundles of unstretched silk and stretched human hair, respectively, taken under similar conditions with Cu  $K\alpha$  rays in a cylindrical camera of radius 3.98 cm. (The hair was stretched in steam to an extension of about 100%.) It will be seen that there is a strong resemblance between the two photographs, in particular with regard to the repetition of pattern parallel to the fibre axis. For silk the period in this direction is about 7 A.,\* while for  $\beta$ -keratin it is rather less, something between 6.7 and 6.8 A.† (for the detailed description of the two keratin photographs, see I). It is not at all probable—for keratin at least (see above)—that lengths such as these represent the true period along a polypeptide chain-system, but are rather an expression of the fact that in the simplest formulation of the fully-extended general chain:—



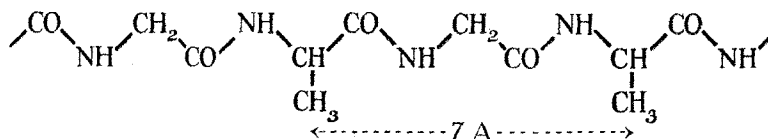
the side-chains project alternately on opposite sides of the main-chain. (This point will be clearer from the models discussed below.) From an examination of all the available evidence, we have to conclude that the submicroscopic crystallites of biological structures are simply bundles, of varying degrees of neatness, of long molecular chains which for fibres such as silk and hair all lie approximately parallel to the direction of the fibre axis‡. Unlike ordinary “laboratory” crystals, therefore, the molecule or molecular complex in a fibre crystallite is in general considerably longer than the length of the crystallographic cell in the direction of the chain axis; the chains run straight through the geometrical cells, so to speak, in such a manner that the primitive

\* Footnotes \* and †, p 334.

† Owing to an inherent lack of definition and paucity of reflections, the translations and spacings in X-ray photographs of biological subjects can rarely be measured with any great accuracy. It is not unlikely that the period along the fibre axis of  $\beta$ -keratin is, like that of  $\alpha$ -keratin (see above), slightly variable according to previous treatment and the state of tension. The mean value of the spacing of the (020) are appears to be 3.38 A.

‡ ASTBURY, “The Structure of Fibres” (‘Annual Reports of the Chem. Soc.’ for 1931, vol. 28—issued 1932).

translation along the fibre axis is given by an *intra*-molecular period, while the primitive translations transverse to this are given by the side-to-side separation of the chains. In silk fibroin the apparent cell given by the X-ray photographs ( $a = 9.68$  A.,  $b = 7.0$  A.,  $c = 8.80$  A.,  $\beta = 75^\circ 50'$ ) is associated with a weight equivalent to four glycine residues and four alanine residues,\* from which the simplest conclusion seems to be that the chains are for the most part built out of alternate glycine and alanine residues, thus† :—



and that four parallel chains constitute a *crystallographic* group. From an exhaustive consideration of all the X-ray data, KRATKY and KURIYAMA‡ have shown that the lateral separation of these chains is not less than 4.5 A. and not greater than 6.1 A., a conclusion which agrees well with what we might predict for the polypeptide given above.

The X-ray photograph of  $\beta$ -keratin, I, is most conveniently referred to an orthogonal cell of dimensions,  $a = 9.3$  A.,  $b = 6.7-6.8$  A., and  $c = 9.8$  A. (see footnote†, p. 371), of which  $b$  is the most prominent period along the molecular chains, while  $a$  and  $c$  are "side-spacings." With regard to the latter two points emerge, (i) that the equatorial "spot" nearer the centre which gives the  $c$ -spacing is preserved more or less unchanged when the  $\alpha$ -photograph is transformed to the  $\beta$ -photograph, and (ii) that the transformation calls into existence on the equator a very strong spot of spacing  $a/2$ , *i.e.*, 4.65 A. From a study of existing X-ray data on proteins§ the interpretation of these results seems clear, that, in fact, the spacing 9.8 A. common to both  $\alpha$ - and  $\beta$ -photographs arises from the lateral extension of the side-chains (the R-groups of the general formula given above), while the spacing 4.65 A.|| represents the distance of approach of the main-chains on those sides free from side-chains. The controlling factor in this closest approach of neighbouring "backbones" is most probably attraction

\* Footnotes \* and †, p. 334.

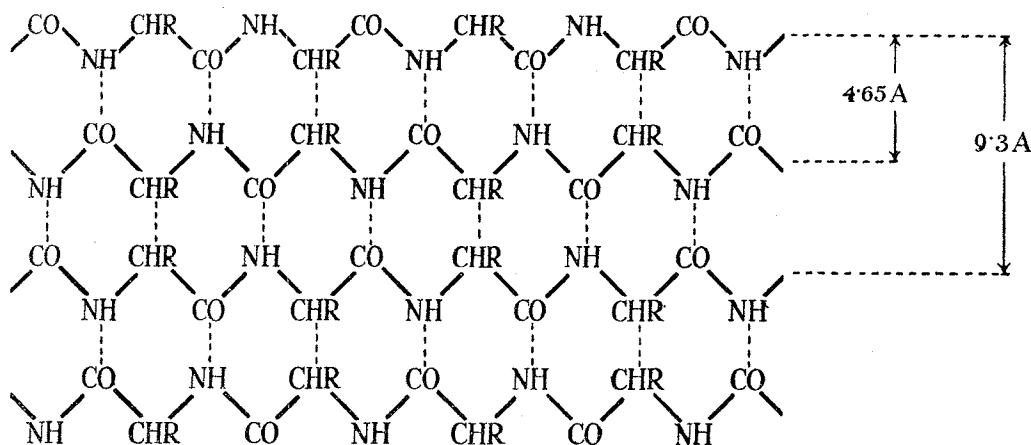
† Much the greatest proportion of the amino-acid mixture obtained by the hydrolysis of silk fibroin is glycine and alanine, but it is not certain that the X-ray photograph is incompatible with arrangements other than strict alternation of the residues of these two acids.

‡ 'Z. phys. Chem.,' B, vol. 11, p. 363 (1931).

§ Footnote †, p. 337.

|| In I attention was drawn to the fact that this spacing is practically equal to the chief spacing in the X-ray photograph of cystine, the most abundant amino-acid in hair; but in the light of subsequent evidence, we wish now to withdraw the suggestion that the two spacings have anything more than a numerical relationship.

between ( $=\text{NH}$ ) and ( $=\text{CO}$ ) groups, \* † ‡, whereby the chains are grouped in pairs; thus :—



Such an arrangement accounts readily for the fact that the  $\alpha$ -dimension of the simplest orthogonal cell given above is not 4.65 Å., but 9.3 Å., represented on the equator by an intense second order (200).

The strongest evidence that the equatorial spacing, 9.8 Å. (the reflection (001)), must be associated with the lateral extension of the side-chains comes from an X-ray study of water adsorption and the action of steam. "Quadrant photographs" (see above) of porcupine quill, both  $\alpha$  and  $\beta$ , brought first to 0% R.H. by prolonged drying over phosphorous pentoxide and then to 100% R.H., show that though the bulk of the water adsorbed by animal hairs leaves the X-ray photograph unchanged, I, some of it does actually penetrate the crystallites in such a way as to increase the spacing, 9.8 Å., by a few per cent. The action of steam, however, as already mentioned, is even more striking. Fig. 23, Plate 8, an X-ray photograph of human hair stretched in steam to twice its original length, shows a marked "spreading" of certain spots along the hyperbolæ ("smear lines"). *The only reflections in the photograph of  $\beta$ -keratin which are unaffected by the action of steam belong all to the zone [001], from which it follows that the spacing disturbance is confined to the zone-axis of this zone, i.e., to the direction of the spacing, 9.8 Å., which we have associated with the lateral extension of the side-chains.* This observation must be considered as lending valuable support, of a purely geometrical kind, to the views on protein hydration recently put forward independently by JORDAN LLOYD and PHILLIPS.§

We are thus led to the concept of the average dimensions of an amino-acid residue

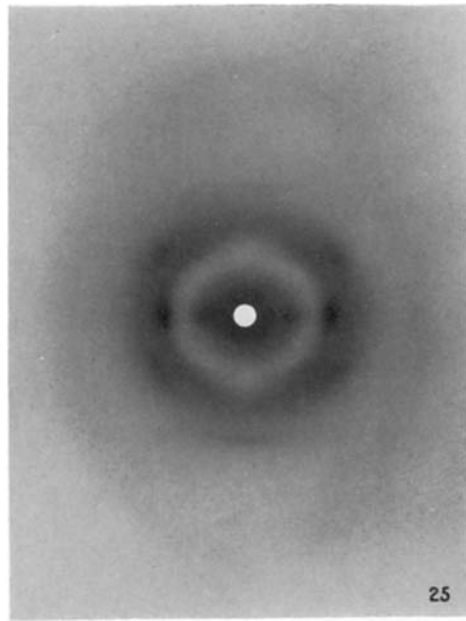
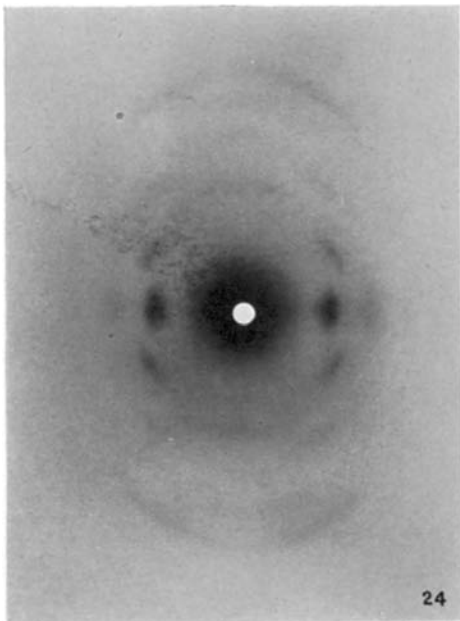
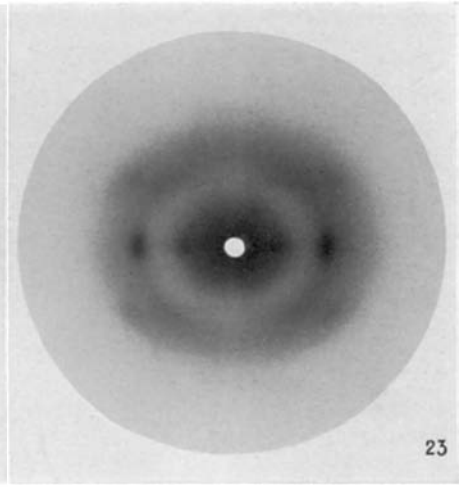
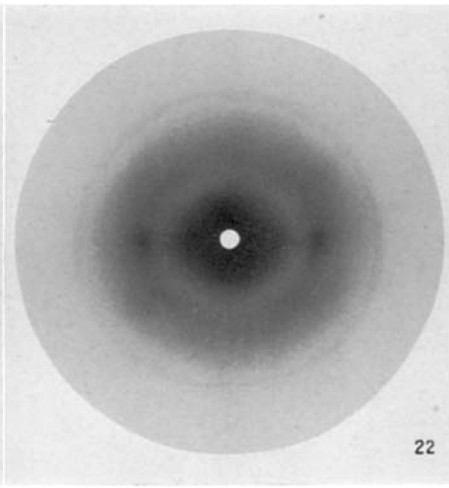
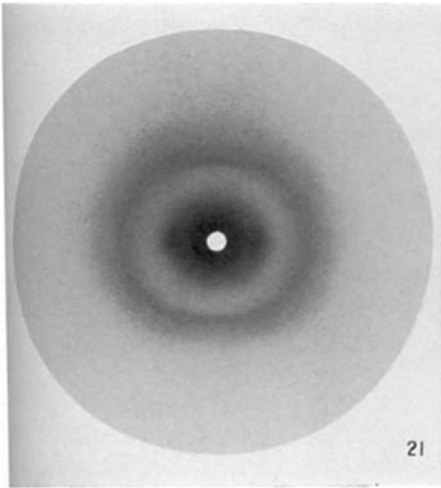
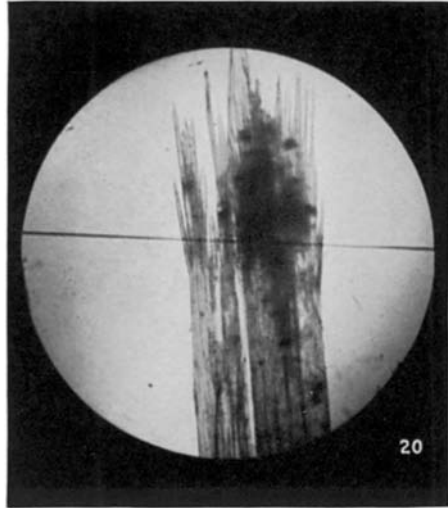
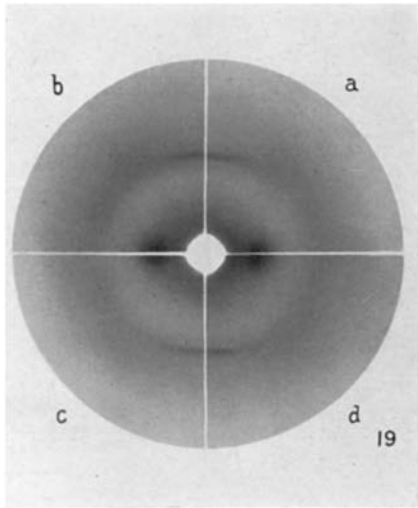
\* Footnote \*, p. 348.

† Footnote †, p. 339.

‡ LLOYD, 'J. Soc. Chem. Ind.,' vol. 51, p. T141 (1932).

§ LLOYD and PHILLIPS, 'Trans. Faraday Soc.,' vol. 29, p. 132 (1933).





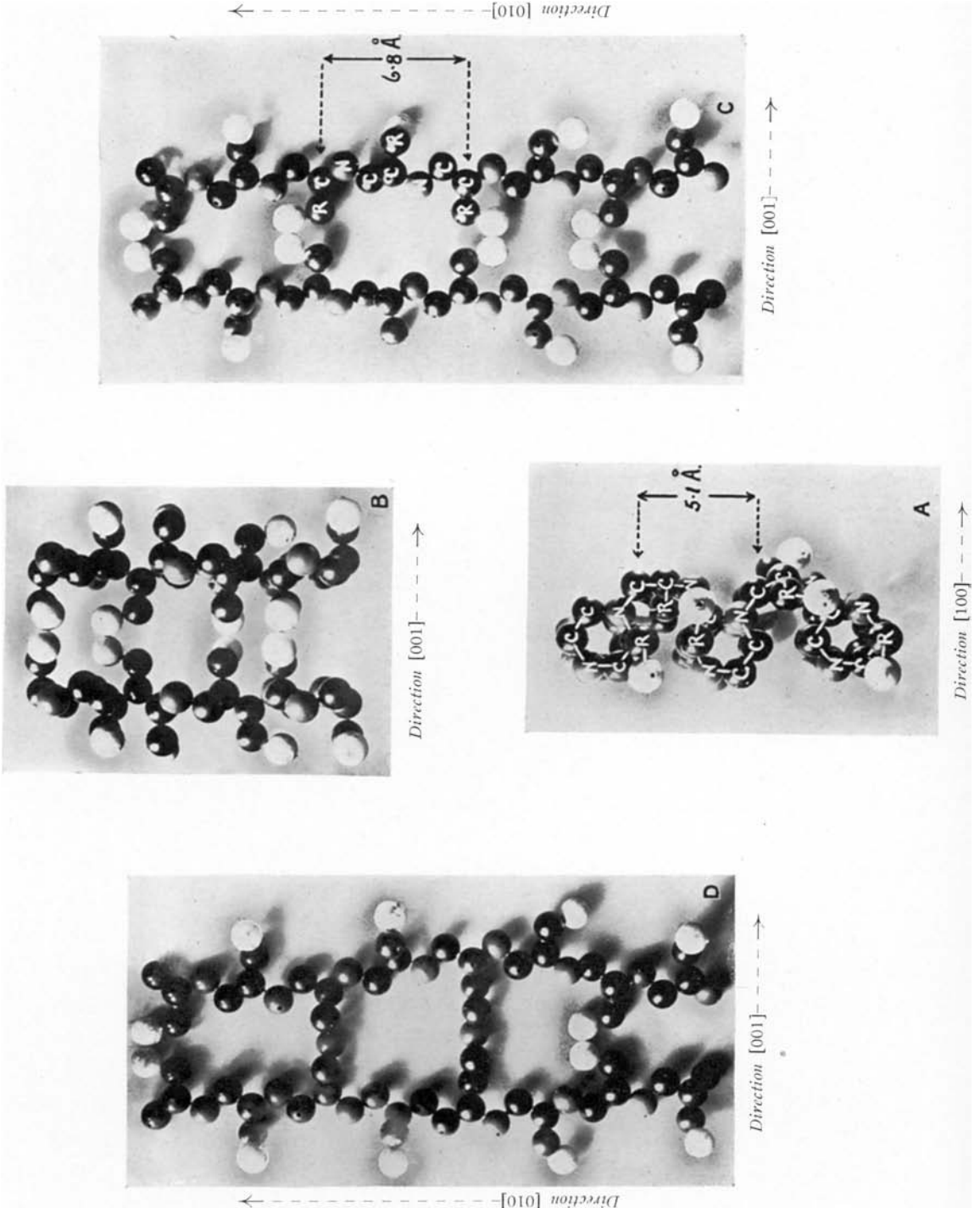


Fig. 26.