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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(*Communicated by Sir* William Bragg, *O.M., F.R.S.*)

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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).

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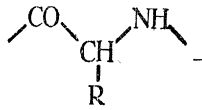
but a definite elastic elongation and contraction of the keratin complex itself. It has been proposed, I, to call the two forms of keratin thus revealed by X-ray analysis α-keratin and β-keratin, the former being the shorter, normal form.

Putting aside for the moment the question of the analysis of the normal fibre photograph (α), it is to be noticed at once that the photograph of stretched hair (β) 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 β-keratin is most probably also based on fully-extended polypeptide chains, while α-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 α-amino-acid residues, each of the general formula  — 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, electro-valent 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.

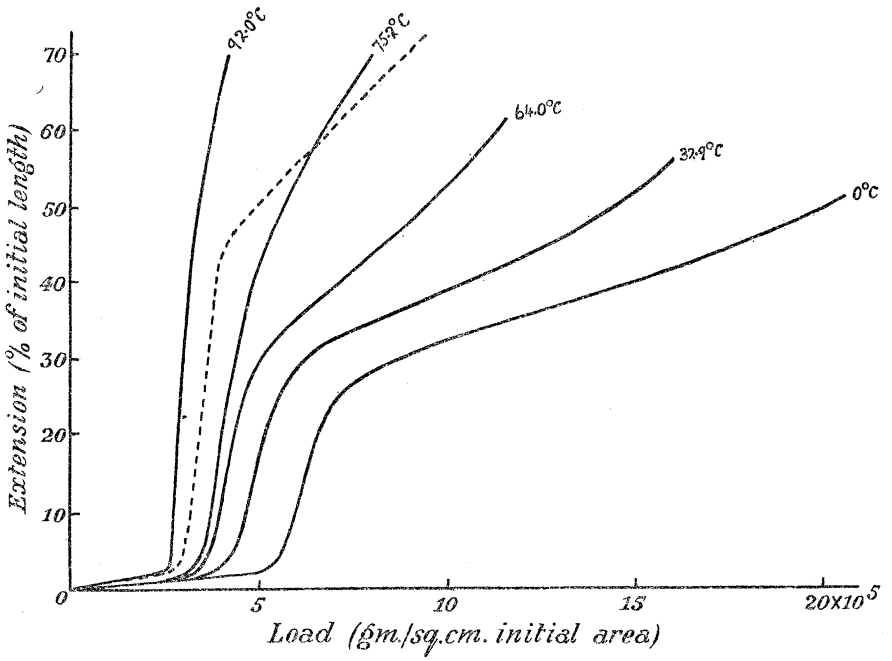


FIG. 1.—Load/extension curves for Cotswold wool in water at increasing temperatures (after SPEAKMAN). (For dotted curve, see footnote, p. 356.)

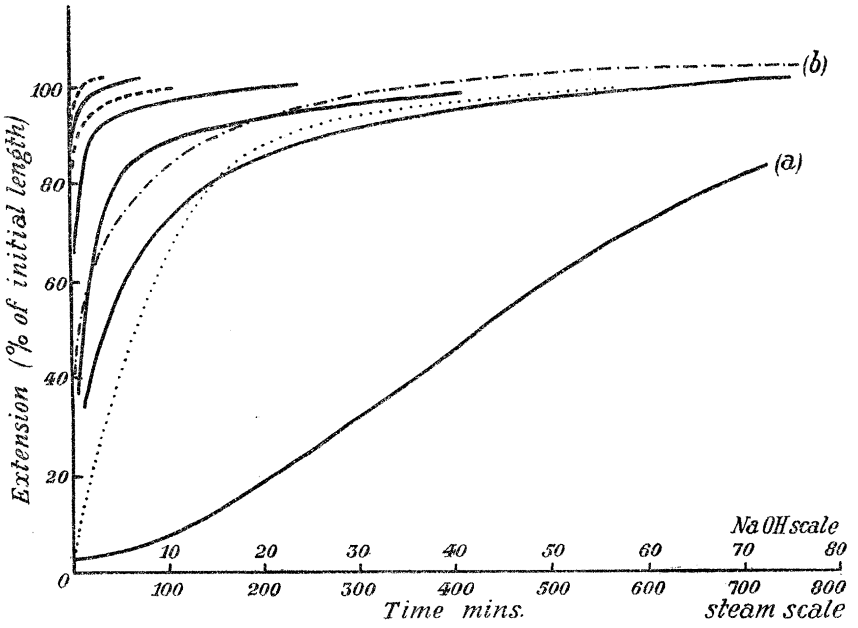


FIG. 2.—Time/extension curves for human hair and Cotswold wool under constant load in steam and in a 1% aqueous solution of NaOH. (The fibre (*a*), on account of its slow rate of extension, is referred to a time scale *half* that for the remaining steam curves.)

—— Cotswold wool in steam ; — — — human hair in steam.
— • — Cotswold wool in NaOH ; . . . . . human hair in NaOH.

loaded with a suitable small weight, placed in a steam chamber or a vessel containing the caustic soda solution, and its extension followed by means of a travelling microscope. Whatever the load, and therefore whatever the rate of extension, since the only difference between the various curves lies in the fact that the more heavily loaded fibres stretch more quickly, the limiting extension at rupture was always roughly the same. (The fibre which gave the curve marked (*a*) broke at an extension just over 80% ; this was probably due to some irregularity in diameter, aggravated by incipient decomposition during the prolonged time (1500 minutes) for which it was necessary to keep the fibre in the steam chamber under the small load used on this occasion.) The wool fibre which gave the curve (*b*) extended as far as 104% in 90 minutes without breaking.

The changes brought about in the load/extension curve of hair by steam or water at higher temperatures are for the most part permanent ; the curve which is characteristic of the normal fibre in water at ordinary temperatures, and which leads to rupture at extensions between about 50% and 70%,* can never be recovered by any subsequent treatment. It is clear that there are features in the structure of the normal hair fibre which interfere with the attainment of the maximum extension of which it is capable. This maximum extension is reached only when the restraining influence is removed by the action of reagents such as steam and caustic soda solutions. Such reagents also temporarily or permanently destroy the power of elastic recovery. In the ordinary way—that is, when stretched in water at ordinary temperatures—hair always recovers its initial unstretched length if left in the water in the absence of tension ; it shows perfect elasticity of form ; but when it has been stretched in steam, it is temporarily " set," for there remains little tendency to contract *in cold water*, even in the absence of all tension. The " set," however, is not in general permanent, even though in the wool textile industries it is commonly described as " permanent set " ; only through the prolonged action of steam on the stretched fibre does it attain any degree of permanency. " Set " produced by caustic soda solutions or by steam acting for only a few minutes is simply a temporary check on the mechanism of elastic recovery which may be reversed by removing all tension and leaving the stretched fibre in the steam or caustic soda solution. It is found then that contraction takes place, not merely to the initial, unstretched length, *but to a length considerably shorter*. The original zero has lost its significance ; the fibre is in a new and " freer " molecular state. The action of steam on stretched hair is therefore not solely a question of inhibiting the mechanism of elastic recovery ; it results also in a wiping-out of those molecular linkages which interfere with the process of extension in cold water. The appearance of the phenomenon of " super-contraction " just described is perhaps the most striking manifestation of this change, but what we may call a permanent record is afforded once more by the load/extension curve, for a fibre that has been stretched in steam or caustic soda and

* See footnote §, p. 335.

then quickly contracted, can now be stretched *in cold water* up to the limit of extensibility, that is, to a length which is about twice the unstretched length of the untreated fibre, *and shows complete elastic recovery also in cold water.*

Fig. 3 shows a "generalized" load/extension curve so obtained. The Cotswold wool fibre was first stretched under constant load (the actual fibre used was that for which the time/extension curve is given above) in a 1% aqueous solution of caustic soda to an extension of 104%. On removing the load the fibre then contracted to a length 5% shorter than its original unstretched length, whereupon it was washed in running water for 24 hours to remove all traces of caustic soda. It was then stretched carefully in cold water in stages of 15% up to 90%, between each stage being allowed to recover its new unstretched length, so as to be quite certain that it

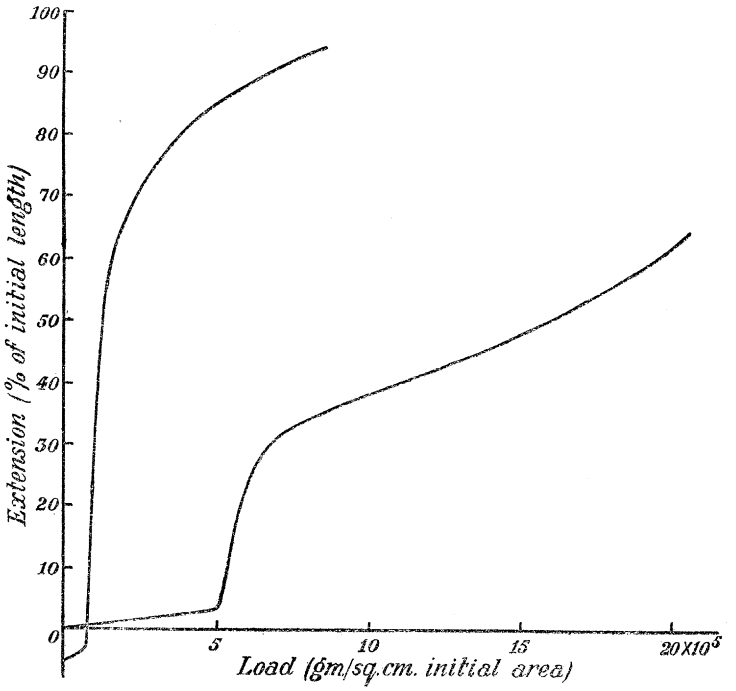


FIG. 3.—The "generalized" load/extension curve of Cotswold wool in cold water as compared with the "restricted" curve before treatment with NaOH in the stretched state.

had now really attained elasticity of form over the full range of its extensibility. The final load/extension curve after this process is the one shown to the left in fig. 3. For comparison the "restricted" load/extension curve for a normal untreated fibre is also shown.

When hair, held stretched, is exposed to steam for periods exceeding some thirty minutes, the phenomenon of "super-contraction" disappears. The "loosening" action is outweighed by the "setting" action and a permanent elongation ensues—an elongation that cannot be removed by the continued action of steam on the released fibre, and which increases with the time of steaming up to a limiting value depending

on the extension at which the steaming took place. This permanent elongation is the " true permanent set " which is a consequence of the building up of new, irreversible linkages which are strong enough to resist the contractile forces and hold the fibre in the stretched state. " Temporary set," as it may be called, can be produced either by caustic soda solutions or by short exposures to the action of steam, but true permanent set cannot be produced by the action of caustic soda alone.

Summary of X-ray Results.

The course of the intra-molecular transformation of the keratin complex as it takes place in normal hair has already been described with the aid of the appropriate X-ray photographs, I. Chiefly as an aid to description, an attempt has also been made to refer both the α- and β-photographs to suitable crystallographic axes; but we need not insist on these, since there are definite indications, more recently confirmed by X-ray investigations of feather keratin, and of muscle,*† that the three-dimensional pattern formed by a system of protein chains is in general much larger than what at first sight appears. The relatively small unit of pattern of which *the more obvious features* of the X-ray photograph are an expression should strictly be described as a pseudo-pattern, a pattern within a pattern. For instance, the molecular pattern along the fibre axis in α-keratin repeats most clearly at a distance of about 5·1 A; but there is no doubt that the true pattern is defined by a multiple, and probably a considerable multiple, of this, just as the prominent period (about 3·4 A) of β-keratin is really only the average length per residue of the amino-acid residues from which the stretched pattern is constructed.

The spacing of the strong arc on the meridian of the α-photograph was originally given as 5·15 A; but we have now been able to show that this is a maximum value arising out of the fact that it is almost impossible to build up a bundle of straight, parallel hairs without stretching them slightly. The true " unstretched " spacing is about 5·06 A, as determined from small pieces of porcupine quill, which were, of course, rigid enough to be self-supporting when held at one end only, and which had been previously soaked in water to ensure that all strains had been eliminated. On stretching the quill in the stretching-frame already described, I, the spacing was found to increase continuously and reversibly from 5·06 A up to a maximum of 5·15 A; that is, over a range of some 2%, *corresponding to the* HOOKE'S *law region of the load/extension curve.* No further spacing change is recorded until the onset of the intra-molecular transformation, when there is a discontinuous change from 5·15 A to about 3·4 A (see below).

In view of the difficulty of recording with any degree of certainty small spacing-changes in fibre photographs, it may be worth while here to describe the apparatus

* ASTBURY and MARWICK, ' Nature,' vol. 130, p. 309 (1932).

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

(fig. 4) used to demonstrate for the first time what happens in the HOOKE's law region when mammalian hairs are stretched. In the first place, it is necessary to use, not films, but plates, on account of the dimensional changes, and the consequent possibility of buckling, in the former when the temperature or atmospheric humidity varies. (Perhaps these changes, which can be serious in precision measurements, have been more than once overlooked.) The front of the plate-holder, which is normally closed by black paper or aluminium foil, F—the latter is better in the investigation of biological structures—is covered by a sheet of brass, B, with the central area cut away to form

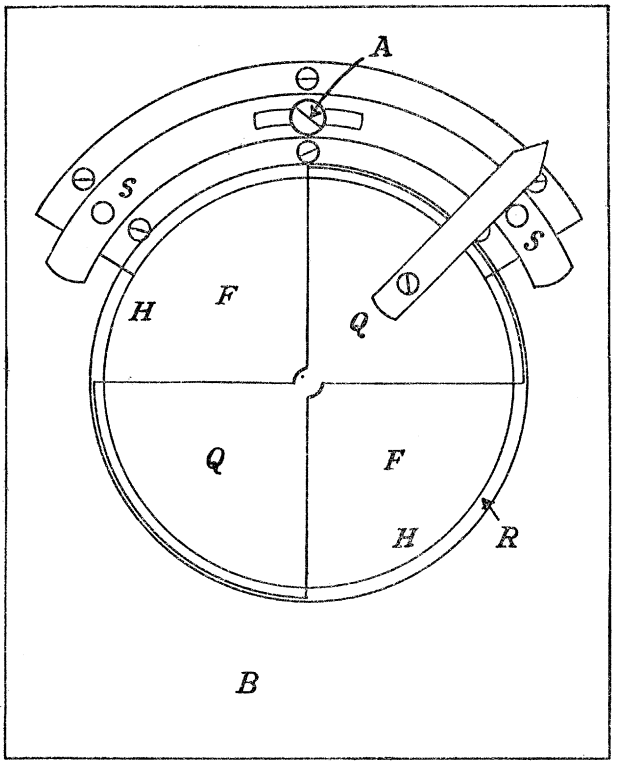


FIG. 4.—Apparatus for recording small changes of spacing in equatorial and meridian reflections simultaneously.

a large circular hole, H, with just a small internal rim, R, to support the centrosymmetrical brass quadrants, Q, which rotate inside the hole. The quadrants must be carefully cut with edges at 90° passing through the centre of rotation, so that when one of the edges is rotated through a right angle, both the equatorial and meridian "spots" are accurately bisected. By this means two photographs can be taken on one plate, each photograph occupying a pair of opposite quadrants. The only source of error lies then in the adjustment of the specimen being photographed; to eliminate this the stretching-frame, or other equipment, must be mounted on the spectrograph so that its position is defined by "stops," or some such device. The position of the quadrants, as they rotate in the hole in the main brass plate, is also limited by "stops," S, to ensure that the quadrants can be turned easily through exactly a right angle

without causing the least disturbance of the plate-holder. The preliminary adjustment of the orientation of the quadrants with respect to the specimen so that the " spots " are accurately bisected can be carried out with the aid of a trial photograph and the slotted screw, A.

Fig. 19, Plate 8, shows a typical " quadrant photograph " of the stretching of porcupine quill in the HOOKE's law region. It will be seen that in the quadrants *a* and *c* the meridian arcs are slightly nearer the centre than in the quadrants *b* and *d*. The displacement corresponds to an increase of spacing of some 2%. (The quill was actually stretched by about 4% to ensure that the HOOKE's law limit, which is not sharply defined in the load/extension curve, had really been attained.) No doubt there is a similar change in the spacing of the two diffuse equatorial spots (9·8 A), but it is obviously difficult to demonstrate this without the aid of a photometer, which was not available.

After the HOOKE's law region has been overpassed, there are no further marked changes in the X-ray photograph till extensions of about 20% are reached, when the onset of the transformation into the β-photograph first becomes perceptible. The replacement of the α-photograph then proceeds steadily with increasing extensions up to breaking ; with slow extensions in cold water this occurs at about 70%, at which point there is little to be seen of the original α-photograph. The effect of stretching in steam is two-fold : in the first place, it gives rise to a clearing-up of the β-photograph, whereby at a given extension the development of the stretched form is better defined ; while in the second place, at extensions above perhaps about 50%, it causes certain of the X-ray reflections of the β-form to " spread along the hyperbolæ," a crystallographic phenomenon associated with a spacing disturbance in a specific direction only. Under the influence of steam, as already described, the fibre can be stretched much farther than in cold water, but beyond an additional clarification and intensification of the β-photograph, no further X-ray change is to be observed (not considering for the moment the " spreading " phenomenon).

Hairs which have been stretched in steam or steamed in the stretched state, though they may still retain the capacity for contraction in steam or even develop the power of super-contraction (see above), lose the property of regenerating the α-photograph when returned to the contracted state. There are indications that for quick stretchings and contractions in steam to extensions no higher than about 25%, the photographic reversibility is not entirely destroyed, but in general we may say that X-rays show that from the very beginning the effect of steam is partly of an irreversible nature. *But this effect does not occur with caustic soda solutions ;* with the aid of such solutions extensions of the order of 100% may readily be realized and " temporary set " produced ; but if the fibre is afterwards allowed to contract in the same solution, the photograph of the α-form always returns.

It does not seem to matter in what medium, or at what speed, the fibres are stretched ; whatever the form of the load/extension curve, the onset of the intramolecular trans-

formation becomes apparent in the X-ray photographs always at roughly the same order of extension, 20%, and thereafter proceeds as described. It is clear that the progressive changes take place simply as a function of the percentage extension. A striking departure from this sequence will be discussed below, when it will be shown how the β-form can also be produced *at constant length* by making use of the differential contraction of the various parts of the keratin complex! This phenomenon appears to be closely related to the sharpening of the β-photograph on the first application of steam to the stretched fibres.

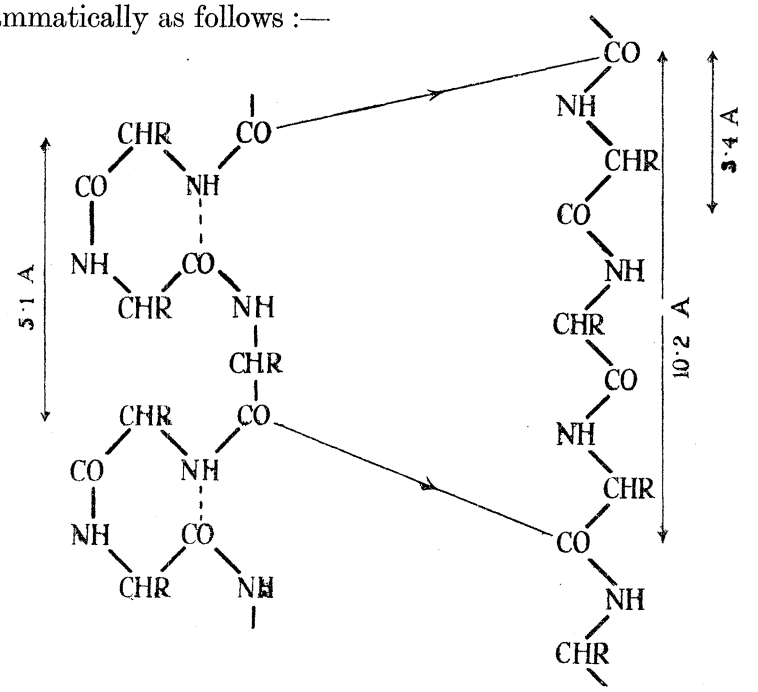
General Interpretation.

When the first paper of this series, I, was written, the true "generalized" form of the load/extension curve, the phenomenon of super-contraction, and the complete elastic reversibility of the modified keratin complex *over a very great range* had not been discovered. It was thought that the reversible changes in the X-ray photograph were simply a record of a molecular elongation of about 30%, an elongation associated with the sudden steep rise of the normal ("restricted") load/extension curve which was afterwards followed by some undefined stretching process not involving any further photographic change. The particular quantitative implication of this hypothesis must now be abandoned, but it is worth while mentioning again the chief arguments leading up to it. These were (i) the fact that the readily distinguishable reflections in the β-photograph lie on hyperbolæ corresponding to a period along the fibre-axis of about 6·7 A, which is some 30% greater than the spacing (5·1 A) of the prominent meridian arc of the α-photograph; (ii) the fact that the well-marked "shoulder" of the normal load/extension curve and the first clear appearance of the β-photograph occur both in the neighbourhood of extensions of 20–30%; and (iii) the fact that permanent damage of the fibre structure, as measured by SPEAKMAN,* is inappreciable for quick extensions of less than 30%. For these reasons it was argued that the rapid rise of the load/extension curve, accompanied by a minimum of permanent internal damage, is an expression of the rapid and easy unfolding of molecular chains followed by re-crystallization in a phase of which the pattern along the chains is 30% longer; but it is clear now that such a conception is far too limited in its outlook to explain *the whole* of the elastic properties. Argument (i) is invalidated by the realization that the true unit of pattern is probably very large and that there is a simple *general* explanation of the occurrence of both the period 6·7 A and that of half this spacing (see below), while argument (ii) obviously cannot hold now that it is shown that there is a variety of "restricted" load/extension curves but only one "generalized" curve, even though the progress of the intramolecular transformation, as revealed by the X-ray photographs, is very much the same in all cases. Argument (iii) also now takes on a more general explanation, as will be seen in the following sections.

The general idea of the sudden, and at first rapid, unfolding of molecular chains which are normally folded still holds good, of course; but in the light of wide additional

* Footnote §, p. 335.

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 β-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 β-keratin and fibroin is sound, the characteristic meridian spacing of β-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 α-form of hair, we have to decide on a method of folding these chains *which will satisfy both the quantitative requirements of the α-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 α- and β-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 β-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 α-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 α-form (5·1 A) and of the decrease of resistance of the β-form, as compared with the α-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 β-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 β-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 β-form in the stretched state and preclude once and for all the possibility of ever regaining the normal α-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 μ 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.

at extensions approaching 50% they once more show signs of an increased rate of extension faintly recalling the region of very rapid extension which immediately follows the HOOKE'S law region and precedes the " shoulder " (see the right-hand curve in fig. 3). We have here the first indications of what we may call the various parts or " phases " which together make up the keratin complex of the normal hair fibre. There is first a phase which is capable of being stretched without any aid from the internal lubricating action of water molecules ; this yields extensions of no more than 30% and brings the intramolecular transformation up to the point where the α-photograph is just beginning to be replaced by the β-photograph. Without the help of a photometer it is not possible to affirm definitely that the α-photograph does not change at all over the first stages of extension—indeed this is improbable—but it is true to say that in the absence of water only the merest beginnings of the transformation can be detected by X-ray methods. There is thus, then, a phase of the keratin complex which does not materially contribute to the more " crystalline " features of the X-ray photograph, and which can be stretched in the dry state. Water, however, strongly facilitates extension even of this " freest " phase, as the load/extension curves show clearly, and, moreover, is absolutely essential for complete recovery to the original length when the tension is removed (see below).

As the water content of the fibre increases from absolute dryness to saturation (33% of the dry weight),* the whole load/extension curve is foreshortened with regard to the axis of loads, so that the limiting HOOKE'S law load, which marks the onset of the intramolecular transformation, is for a wet fibre no more than about one-quarter of that required for a perfectly dry fibre. The capacity for extension also increases, as already pointed out, and the X-ray photographs show a steady progress in the transformation from extensions of 20% upwards. There is thus a second phase of the keratin complex which resists transformation in the absence of water, and which must be the chief basis of that part of the fibre substance which gives rise to the " crystalline " diffraction effects in the X-ray photographs. There is no sharp distinction between these two phases, the dry and the wet, which are transformed in the normal (restricted) load/extension curve ; as the water content is gradually increased we pass indistinguishably from one to the other. It will be convenient in what follows to refer to them as " the phases K_1 and K_2."

There can be no doubt whatever that the transformation of the phase K_2 takes place only by virtue of intra-crystalline (" intra-micellar " †) swelling, even though the bulk of the adsorbed water, the " regain " of the textile industries, which gives rise to an increase of fibre-diameter of 17·5% from perfect dryness to complete wetness,*‡§ has no effect on the X-ray photographs. A certain fraction of the adsorbed

* SPEAKMAN, ' J. Soc. Chem. Ind.,' vol. 49, p. 209T (1930).

† See, *inter alia*, KATZ, ' Trans. Faraday Soc.,' vol. 29, p. 279 (1933).

‡ HIRST, " Wool Industries Research Association Publication No. 17 " (1924).

§ SPEAKMAN, ' Trans. Faraday Soc.,' vol. 25, p. 92 (1929).

water does, however, penetrate the chain bundles to produce a slight change in side-spacing,* and the process by which it is " bound " by the protein complex must be intimately related to the considerable " heat of wetting " of dry wool† and to the negligible initial effect of water-content on the rigidity.‡ But it must not be inferred from this that there is some subtle distinction between crystalline and amorphous keratin, or between micelles and inter-micellar substance. Water reacts with the whole of the keratin complex, and what is meant by a " micelle " depends entirely on the swelling agent and the previous history of the fibre.* We shall return to this question again later.

In water at ordinary temperatures the phase K_2 does not function much beyond 50% when the rate of extension is fairly rapid. Table I will serve to give an idea of its range of action. Beyond 50% still another phase, K_3, comes into play, as is shown both by the new rise in the load/extension curve and the action of steam (see below). The phase K_3 offers almost complete resistance to quick transformation in water at ordinary temperatures, but is " freed " in caustic soda solutions and in hot water or steam.

TABLE I.—The extensibility of Cotswold wool for quick rates of extension in water at 18° C.

No. of fibre.	Region of fibre.	Breaking extension (%).	Time of extension. (Secs.)
1	Root	60	14
	Root-middle	57·5	20
	Middle	57	20
2	Root	61	20
3	,,	57	12
4	,,	60	15
	Middle	58	15
5	Root	61·5	15
	Middle	53	20
6	Root	62	15
	Root-middle	61	30
	Middle	65	12
	Middle-tip	58	15
7	Root	57·5	20
	Root-middle	57·5	20
8	Root	51	12

When a hair is held stretched in the presence of water or water-vapour it tends to lose both its tension and its speed of elastic recovery. This phenomenon has apparently long been known in the wool industries, but its first systematic investigation is due

* ASTBURY, footnote †, p. 339.

† HEDGES, ' Trans. Faraday Soc.,' vol. 22, p. 178 (1926).

‡ SPEAKMAN, ' Trans. Faraday Soc.,' vol. 25, p. 92 (1929).

to SPEAKMAN,* who applied to it the term " plasticity." With SPEAKMAN'S assent, we shall now give up the word " plasticity " and try to show in this paper how all the phenomena associated with loss of tension and speed of recovery fall quite naturally into the general elastic scheme of the keratin complex. Briefly, they arise out of the action of water (and other reagents) on the stretched fibre, that is, on β-keratin, as it is generated in a naturally strained state by the extension of α-keratin. The effects are non-existent in a stretched hair kept perfectly dry, but if it is held stretched in the presence of moisture, the tension decays with the time at a speed and to an extent increasing with the water-content and the percentage extension, and depending also on the particular type of hair under examination.* There is evidently an internal " loosening," a breakdown of strained bonds both within and between the various phases of the complex, which relieves the tension and makes the fibre easier to stretch. On this account, if we stretch a hair slowly in water and at intervals hold it stretched to allow the tension to decay, it is possible to attain extensions of the order of 70% even at ordinary temperatures. The restrictions between the phase K_3 and the two preceding phases are thus partially eliminated, as is seen at once from a re-examination of the load/extension curve. The fibre is now easier to stretch right from the beginning of the extension, for the limiting HOOKE'S law load is smaller (though still operating at an extension of about 2%) and the " shoulder " of the curve is higher and less accentuated. There is a limit to the process, however, at ordinary temperatures, and the curve approximates more and more to a final position on the left which marks the stage at which the internal loosening is a maximum at these temperatures. It is still a " restricted " load/extension curve, because the remainder of the phase K_3 still resists a transformation that would otherwise bring the final extension up to the neighbourhood of 100%.

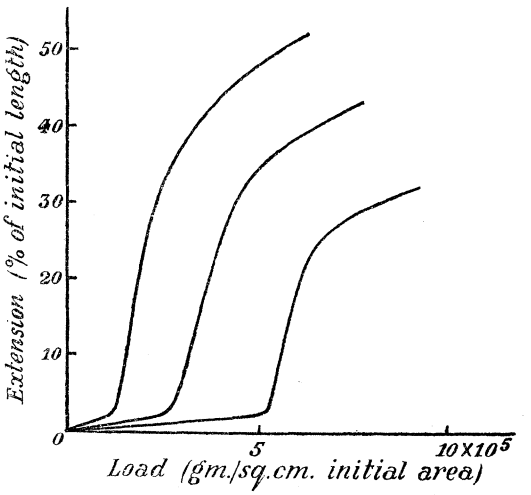


FIG. 5. — Less " restricted " load/extension curves of Cotswold wool obtained after repeated stretching and holding stretched in water at ordinary temperatures.

Fig. 5 shows how the normal load/extension curve of Cotswold wool is modified—becomes less " restricted "—under the effect of repeated stretching and holding stretched in water at ordinary temperatures. The curve nearest the axis of extension is the limiting one that can be obtained by this method. The limiting HOOKE'S law load, the load required to initiate the intramolecular transformation, is reduced to about one-fifth.

* Footnote §, p. 335.

The process of tension-decay and the " freeing " of the load/extension curve does not markedly alter the progress of the transformation as revealed by the X-ray photographs. We must conclude that *the keratin phases are both in series and in parallel*, in series because their respective transformations follow one another in a definite order and at roughly fixed intervals of extension, and in parallel because they so obviously restrict one another and are so closely inter-related that it is impossible to transform one without involving, to some extent, another. To a first approximation we may say that decay of tension produces no clear-cut changes in the X-ray photograph for a given extension. The intramolecular changes taking place can be no more than modification of certain side-chain linkages between neighbouring main-chains.* Why such changes are not immediately discernible in the X-ray photographs will be gone into more fully below. For the moment, it will suffice to draw attention once more to the fact that they do become quite obvious when steam acts on hair stretched to more than 50%, and that the " spreading " of the spots so produced is perhaps just visible after the prolonged action of water even at ordinary temperatures.

Hair that has been held stretched in cold water so as to suffer loss of tension does not lose its ultimate power of elastic recovery; it manifests, with increasing loss of tension, only an increasing loss of *speed* in regaining its initial length when the external stretching force is removed. For instance, in one series of experiments, a Cotswold wool fibre that at first snapped back almost instantaneously when released in water immediately after an extension, took 1600 minutes to recover in water at room temperature from an extension of 50·7% to one of 5·7%, after it had been held stretched in water at the higher extension for 214 days. This behaviour, in view of what has been said above about the loosening action of water on the strained side-chains, seems paradoxical, but a little consideration shows that it is not really so, if we postulate that the breakdown of the strained side-chain linkages is followed automatically by the formation of new linkages. It is then easy to understand why the process of elastic recovery is impeded by the changes which take place during the decay of tension. That this explanation is the correct one is shown very strikingly by simply re-stretching the " relaxed "† fibre. It is found then that it once more recovers its original length quickly when the stretching force is removed, and, in fact, recovers more and more rapidly as the re-stretching process is repeated. Both recovery in water and re-stretching, therefore, have the effect of reversing or destroying the new-formed side-linkages, with the final result that the fibre is left permanently easier to stretch, but with its speed of elastic recovery more or less unimpaired. Fig. 6 and Table II show

* There is just a suggestion that the *prolonged* action of water on keratin results in some hydrolysis of the main-chains (ASTBURY and WOODS, ' Nature,' vol. 127, p. 663 (1931)), but this point requires further investigation and will be reported on later.

† In order to avoid the repeated use of an adjectival clause, it seems convenient to adopt the adjective " relaxed " to describe fibres which have suffered decay of tension through being held stretched in water or other reagents.

the complete data obtained during typical experiments on the above-mentioned Cotswold wool fibre which had been held stretched at an extension of 50·7% for 214 days in water at ordinary temperatures.

TABLE II.—Recovery in water of Cotswold wool fibre after prolonged decay of tension in water at ordinary temperatures.

S_1. *Stretched* in water to 50·7% of initial dry length and held stretched for 214 days. *Released* in water. Recovery (all measurements hereafter are of the fibre in the wet state) :—

Time (minutes)	0	300	1600
Remaining extension (percentage of initial dry length)	50·7	9·9	5·7

S_2. Re-stretched in water and released immediately. Recovery :—

Time	0	0·5	10	125
Remaining extension	50·7	10·0	5·9	4·75

S_3. Stretched again in water and released immediately. Recovery :—

Time	0	0·5	1	2	5	10	160
Remaining extension	50·7	6·6	5·9	5·4	5·0	4·7	3·1

S_4. Stretched again and released immediately. Recovery :—

Time	0	0·5	2	5	15	200
Remaining extension	50·7	4·7	3·8	3·5	3·3	2·6

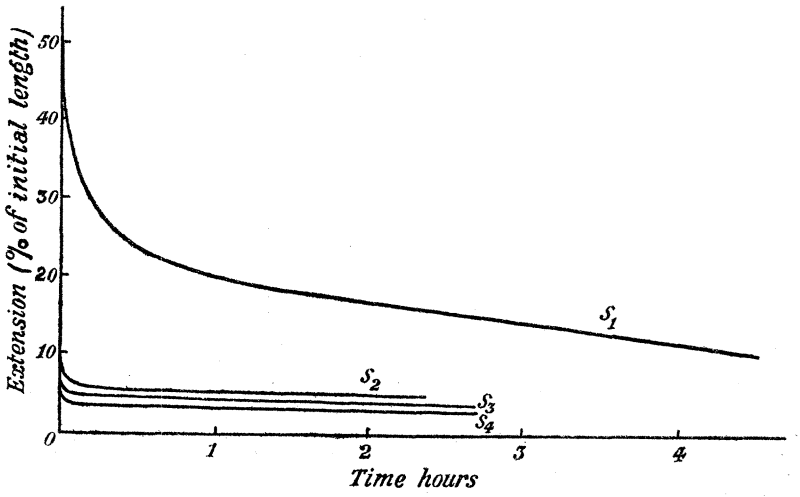


FIG. 6.—Recovery in water of a Cotswold wool fibre after prolonged decay of tension and successive re-stretchings.

Another, and more rapid, way of reversing the linkages which impede elastic recovery after prolonged decay of tension is by the use of steam or water at higher temperatures. The slow contraction of a "relaxed" hair in cold water is accelerated enormously if the temperature of the water is raised, while in steam it contracts almost

instantaneously. And this, after all, is what we should expect in the general interpretation here put forward. *The action of water molecules in the breakdown of strained side-linkages and the building-up of new linkages is in the first instance reversible.*

The phenomenon of " super-contraction " also now makes what is logically its first appearance in the interpretation. Though its true nature was first demonstrated, in the experiments here described, through a study of the action of steam on normal stretched hairs, it soon became clear that a sufficiently " relaxed " hair should show the effect, too, to a small extent at least. This prediction was verified by steaming various relaxed hairs in the absence of all tension, some after recovering their initial length, and some while still remaining partially extended. In every case contraction occurred below the initial unstretched length. As an example we may quote the case of a Cotswold wool fibre which had been held stretched in water at room temperature at an extension of 49·4% of its initial dry length for a period of 218 days. On removing the external stretching force this fibre took well over a day in water at a similar temperature to recover its initial unstretched length. After the recovery it was steamed for $3\frac{1}{2}$ hours, whereupon it was found, when dry, to have taken up a length only 88·9% of its initial, dry, unstretched length. From experiments such as this we must conclude that in hairs that have become easier to stretch through the action of water on the stretched state, the protein chains of keratin are partly " freed " ; they have become endowed with the power of contracting, given the appropriate conditions, not merely to the unstretched length defined by the original α-keratin, but to a length definitely shorter than this.

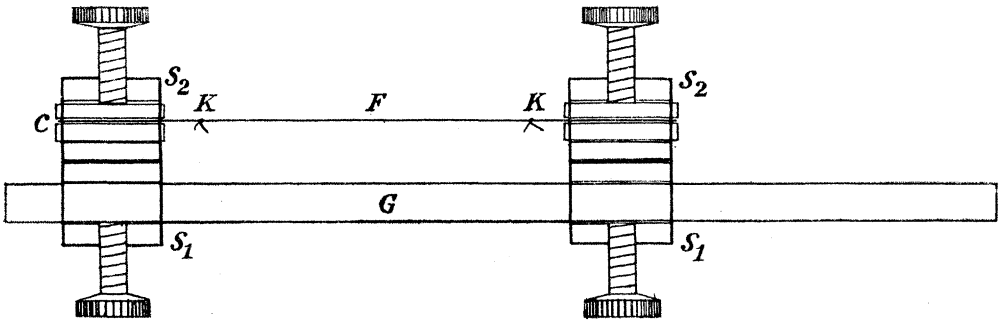


FIG. 7.—Apparatus for measuring the rate of contraction of stretched hairs under zero load.

In subsequent sections we shall describe in detail the condition of " set," both temporary and permanent, that may be produced in stretched hairs by the action of steam and other reagents ; but it is necessary now, without further delay, to emphasize this fundamental point, that *the phenomena associated with loss of tension and speed of elastic recovery in water at ordinary temperatures are the first manifestations of those phenomena of temporary and permanent set which find their strongest expression under the action of steam.*

Fig. 7 shows the device found most convenient for following, by means of a travelling microscope, the rate of contraction of stretched hairs under zero load. It is made

from glass or metal rod and ordinary electrical " binding screws," sawn into two halves S_1 and S_2, which are then soldered together in parallel position. One half, S_1, thus acts as a slide, while the other half, S_2, serves to hold and clamp the two halves of a small metal cylinder, C, split longitudinally and faced with fragments of a fine velvet. The clamps themselves cannot be relied upon to hold a stretched hair without some risk of slipping taking place, and the actual measurements are made of the distance between two external knots, K, each made by wrapping a wool fibre twice round the fibre, F, under examination, and tying it tightly. Such knots were found to remain firm over long periods and throughout the various treatments to which the fibre was submitted, since swelling in water and other reagents results only in making them tighter. By means of collars of the " Meccano " type sliding on the rod, G, " stops " can be introduced to define the required extensions, and the whole apparatus, being small and light, is easily suspended in a steam chamber, etc. The test-fibre is of course slackened when free contraction is desired, while for the act of measurement it is drawn just taut, a process which is found to introduce errors of no more than one-quarter per cent.

It may be considered as fairly certain that an intramolecular transformation of elongation taking place in a solid biological structure is bound to cause internal damage to some extent simply through departures from strict homogeneity even in one and the same component or " phase " of the structure. We may expect, in addition to the " inter-phase " breakdown discussed above, a certain amount of " intra-phase " breakdown, which may or may not be reversible. SPEAKMAN* has measured the *decrease in work* required to stretch (fairly quickly) a wool fibre a second time in water and has found that this quantity increases with the percentage extension, at first rather slowly and then, at extensions of the order of 30%, much more rapidly. The first series of changes we can ascribe mainly to restrictions acting between the phases K_1 and K_2, while the second must be due to the impedance caused by the non-extensibility of the phase K_3 in water at ordinary temperatures. If the once-stretched fibre, after recovering its original length, is allowed to rest in water for a day, the reduction in work required to stretch the fibre a second time is much less marked,* though still, as might be expected from the point of view here brought forward, showing an abrupt increase in the region of 30% extension. It is possible to interpret this effect by postulating a certain amount of reversibility in the breakdown phenomena, but it is also possible, since the experimental quantity observed is not breakdown directly, but reduction in work required to stretch a second time, to think of it as arising, partially at least, from " reverse strains " which tend to keep a fibre, once stretched, in the stretched state. Such strains, without involving actual internal rupture, would make it easier to stretch a second time and might conceivably be " cured " by allowing the unstretched fibre to rest in cold water.

SPEAKMAN has furthermore shown that a hair stretched *slowly* in water (or held

* Footnote §, p. 335.

stretched in water), even over the smallest extensions above the HOOKE's law region, cannot be cured by resting in water, and is permanently easier to re-stretch. The interpretation of this result lies in the theory of side-chain breakdown when under strain in the presence of water, as explained above and to be developed further below. The effect, which holds for all phases of the keratin complex, is clearly a function of the strain, water-content, and time, and may be thought of most simply as including that of direct internal rupture by quick extension.

The Nature of the Elastic Phases.—The complete statement of available evidence that must be taken into consideration with regard to the identification of the three phases, K_1, K_2, and K_3, into which it has been found necessary to subdivide the keratin complex of the hair fibre includes not only what has already been set out above, but also the experimental and theoretical material of all subsequent sections of this paper. In spite of this it would seem to be more convenient to bring forward even at this stage the main conclusions on this all-important point, so that the proposed underlying interpretation may always be kept in mind and an appreciation of the extensive data thereby considerably facilitated.

The most obvious feature of the three phases is their increasing power of resistance, either to mechanical extension or to chemical change, and from this limited point of view it is most tempting to suppose that they correspond merely to outer and inner " shells " of chain-bundles or " micelles "; that is to say, to regions of increasing organization or crystallinity as we proceed from the outskirts of any chain-bundle into its interior. Such an idea would satisfy well enough the fundamental postulate from the experimental facts, that the phases must be both in series and in parallel, but would be difficult to harmonize with certain phenomena which must be accounted for in any plausible working hypothesis. The chief of these are: (i) that the load/extension curves for all sorts of animal hairs do not show any striking quantitative variation from one type of fibre to another, and (ii) that the most " crystalline " phase appears to be K_2, the second in order of ease of extension and general resistance to chemical attack. Without the use of a photometer we cannot yet be quite certain on this second count, but at the moment of writing it would appear that the greatest change in the X-ray photographs takes place in the middle range of the full transformation; that is, in the range which we have ascribed to the phase K_2. Point (i) is perhaps more decisive, since the concept of the three phases as being based on gradations in the organization of chain-bundles is effectively equivalent to a theory of mixtures, sub-microscopic mixtures which would hardly be expected to remain substantially identical, both in properties and arrangement, as we pass from one type of hair to another. It seems clear that we must look for some well-defined structural arrangement which does actually remain fairly constant in mammalian hairs as a class. There is no need to look far, for we have such an arrangement in the familiar cellular structure of the hair fibre.

Mammalian hairs are built of elongated cigar- or spindle-shaped cells some microns

thick and about 110 microns long.* Taken on the average, there does not appear to be any remarkable variation in either the shape or size of these cells as we pass from one type of hair to another, so that if we are able to incorporate their properties into the elastic theory, we dispose at once of point (i) above. We shall, in fact, postulate forthwith that the elastic complex of the hair fibre arises out of the properties of the individual biological cells from which it is built and the way they are fitted together, in accordance with the scheme :—

(K_1) inter-cellular keratin,
(K_2) cell-wall keratin,
and (K_3) intra-cellular keratin.

It is interesting to note that SPEAKMAN† has also attempted to explain the elastic properties of the wool fibre in terms of its constituent biological units. He suggested that " the elastic properties of the fibre as a whole are those of the single cell, which is assumed to consist of an elastic cell wall, enclosing a fibrillar structure whose interstices are filled with a viscous medium," and that " once this critical extension (HOOKE's law) is exceeded, rapid extension commences and takes place chiefly by rotation of fibrillæ. The rate of extension is determined by the viscosity of the medium within the cell, and the elastic constants of the cell framework." The main object of the present paper is, of course, to offer an actual molecular interpretation of the elastic mechanism, but apart from this, it will be seen that our conclusions regarding the elastic contributions of the various parts of the fibre depart seriously from those of SPEAKMAN. But it is too early at this stage to be dogmatic, especially with respect to such a complicated structure as that of the hair fibre, and we wish merely to suggest that the explanation here put forward accords most with the available experimental facts : in particular it should be noted that there is no X-ray evidence at all that the observed elongations of the stretched fibre correspond to a rotation of fibrillæ. Neither from the X-ray photographs nor from optical examination under the microscope do we find any indication of an angular dispersion sufficient to account *quantitatively* for the features of the load-extension curve, and even if such an angular dispersion existed, we must conclude from the properties of cellulose and silk fibres that it cannot function as the molecular basis of true, long-range, elastic properties.‡ Furthermore we now know directly from X-ray examination that extension of the hair fibre is accompanied by a reversible, intramolecular transformation.

We know that when the hair cells are first formed at the bottom of the follicle§ they are not elongated in the direction of the fibre-axis, but are grouped together in masses

* *Cf.* GABRIEL, ' J. Text. Inst.,' vol. 23, p. T171 (1932).

† Footnote §, p. 335.

‡ *Cf.* ASTBURY and WOODS, ' J. Text. Inst.,' vol. 23, p. T17 (1932).

§ For information on the early growth of the hair fibre we are indebted to Dr. A. B. WILDMAN, formerly of Leeds University, now of Victoria College, Wellington, New Zealand.

of contiguous polyhedra which from mathematical considerations are most probably, in the ideal form that is to say, rhombic dodecahedra.* Higher up in the follicle and in the body of the fibre itself these cells are found to be very much elongated in the direction of the fibre-axis—all to lie nearly parallel to the fibre-axis—and it is not possible to define clearly their individual outlines. But we may suppose that, once proliferation has ceased, there is no bodily lateral displacement of the cells with respect to one another,† that superposed polyhedral faces remain in superposition, and that elongation proceeds, either by lateral compression or unidirectional growth or both, so as to give rise ultimately to a set of polyhedra each with one direction very much greater than the others; in other words, we may regard the long, thin cells of the mature fibre as distorted polyhedra, or as based on distorted polyhedra, which are fused together by means of a *continuous* intercellular medium. There seems to be sufficient general histological evidence for the existence of such a medium, and there is no doubt that for hair itself this medium, which we have identified with the phase K_1, is the least resistant to chemical attack, as is shown by a number of experiments in which the fibre is readily disintegrated into its constituent cells by a process of chemical or bacterial "retting."

Fig. 8 shows a diagrammatic scheme for the distribution and interaction of the three elastic phases of the hair fibre. It is based on idealized rhombic dodecahedra elongated along a tetrad axis, but the precise form is immaterial to the argument that the phase K_1 effectively forms a continuous sheet between each layer of cells, and that all three phases act both in series and in parallel. Furthermore, it must be emphasized that the whole treatment in this paper is based on the hypothesis, which appears to be consistent with the known facts, that *there is no sharp demarcation between the phases, and that, fundamentally, they must all be considered as "keratin," though keratin slightly variable mainly through certain side-chain modifications.* From this point of view we must regard the intercellular keratin, K_1, as being deficient in certain of the chemical cross-linkages which bridge the main chains (such as the cystine bridge—see below), the cell-wall keratin, K_2, as being most crystalline or most "organized" (as one might expect from other X-ray investigations of cell-wall structure‡), and the intracellular keratin, K_3, as being physico-chemically the most resistant of all three.

An ideal load/extension curve for three such transformable phases, in series only and each perfectly homogeneous, would have something of the form of the thick line OABCDEF in fig. 9, in which the small HOOKE's law extensions are neglected, and the steps are roughly of the scale observed experimentally in hair. The actual load/extension curve does not, of course, follow this ideal path, but is continuously displaced

* *Cf.* THOMPSON, "Growth and Form," Camb. Univ. Press (1917).

† *Cf.* PRIESTLEY, 'New Phytologist,' vol. 29, p. 112 (1930).

‡ *Cf.* investigations of the cell-wall of cellulose fibres, and that of *Valonia ventricosa* (ASTBURY, MARWICK, and BERNAL, 'Proc. Roy. Soc.,' B, vol. 109, p. 443 (1932)).

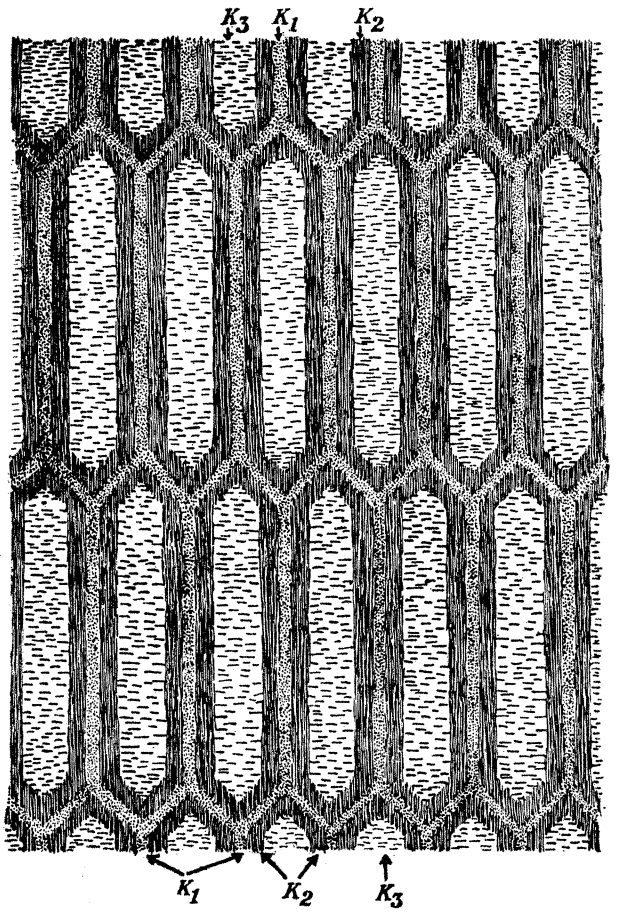


FIG. 8.—Suggested scheme for the three elastic phases of the hair fibre, – – – intercellular keratin (K_1), cell-wall keratin (K_2), and intracellular keratin (K_3).

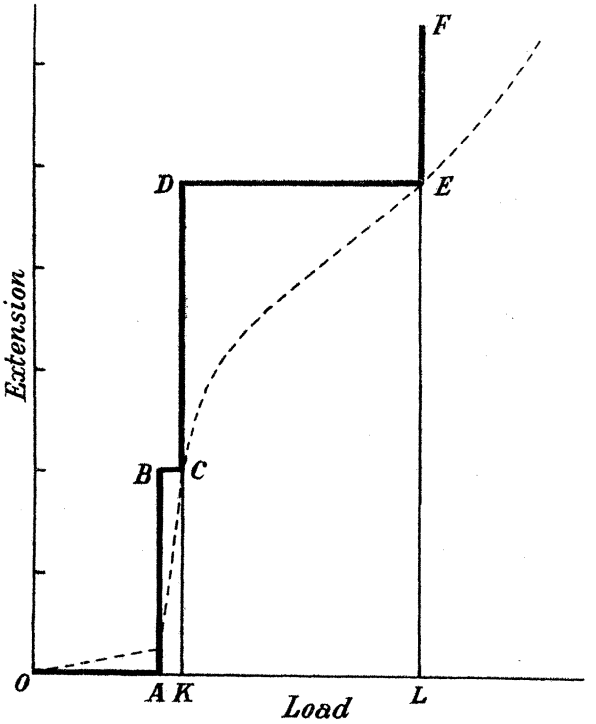


FIG. 9.—Showing the evolution of the three-phase load/extension curve of the hair fibre.

to the right on account of non-homogeneity, a non-homogeneity which is of two kinds, intra-phase and inter-phase, and which causes increasing impedance as the transformation proceeds. OA, OK, and OL represent the limiting HOOKE'S law loads which are necessary to initiate the transformation in each of the three phases. It seems clear that OA and OK must be considered to be almost equal, if we are to explain the forms of the various available load/extension curves, the observed HOOKE'S law changes in the X-ray photographs, fig. 19, Plate 8, and, in fact, the general experimental conclusion that the first two phases, K_1 and K_2, are very closely inter-related. In a system such as that shown in fig. 8 we may fairly argue that K_1 will transform with comparative ease, just as experiment shows, but that, as the strains of extension spread down between the cells, internal damage will gradually increase and in such a manner as to show more or less sharp discontinuities as the *effective* boundaries of the phases are reached. These boundaries also mark roughly the limits of extension when the fibre is dry and when it is wetted with cold water, but they are to all intents and purposes eliminated by the action of caustic alkalis and of steam (see below). The raising of the " shoulder," shown in fig. 5, of the normal, restricted load/extension curve after the fibre has been held stretched in water must be ascribed to the hydrolytic breakdown of the side-chain restrictions both between K_1 and K_2 and between $(K_1 + K_2)$ and K_3, but chiefly the latter, as is clear from a consideration of fig. 9, which shows also how there is really an infinite variety of " restricted " load/extension curves, each of which is determined by the rate of extension* and the nature of the medium in which the stretching is carried out. The actual observable differences between the load/extension curves of the various types of hair must depend on the precise composition and relative properties of the three phases; that is, *on the shape and packing of the biological cells.* It is proposed to investigate more fully this last-mentioned aspect of the problem and report on it later. Fig. 20, Plate 8, is a photomicrograph of the tip of a human hair in process of disintegration into the constituent cells.

The Action of Caustic Soda.—All the experiments on the action of caustic soda were carried out with one per cent. aqueous solutions at room temperatures. The technique of obtaining the " generalized " load/extension curve with the aid of sodium hydroxide has already been described above in connection with fig. 3. Such a generalized curve as that given in the figure is typical of the type of hair under examination—it is reproducible on repeated extension—and corresponds to a state of the fibre in which the phases of the transformation follow each other in the normal series order, but without the restrictions arising out of the original physico-chemical differences between the phases and their association in an arrangement which is both in series and in parallel. *To a first approximation we may consider the generalized load/extension of hair as that of a single phase transformation* comparable with that of rubber (see below). The region associated with K_1 and K_2 is now practically vertical throughout (compare fig. 9),

* A particularly good example is afforded by the dotted curve of fig. 1, which was obtained on a recording extensometer with Cotswold wool stretched in boiling water at a rate of 50% in 16 secs.

but as we draw towards the end of the transformation of K_3, intra-phase restrictions due to the increasing ratio of the transformed to untransformed phase cause the curve to bend over more and more towards parallelism with the axis of load. In the generalized state hair has perfect elasticity of form, even in cold water, over the full range of its extensibility.

The X-ray examination of generalized wool or hair reveals no obvious fundamental change either in the features of the individual photographs or in the progress of the transformation. At the higher extensions there occurs the " spreading " of certain reflections along the hyperbolæ, but the effect does not seem to be so pronounced as when produced by the action of steam, and we shall discuss the phenomenon more fully in that connection. The main conclusion, therefore, is that *the transformation sequence is independent of the form of the load/extension curve*, which is merely an expression of intra- and inter-phase impedance continuously variable between the limits defined by the two typical curves of fig. 3. The generalized curve shown in this figure is thus strictly not the graph of a single-phase transformation (though very nearly so as pointed out above), but once more that of the transformation of the three phases, K_1, K_2, and K_3, following one another in the original order under almost equal HOOKE'S law loads (in terms of fig. 9, OA $\lessapprox$ OK $\lessapprox$ OL).

Figs. 21 and 22, Plate 8, show X-ray photographs of α- and β-keratin, respectively, in the generalized state produced by the technique described. It will be seen that they are essentially similar to corresponding photographs of wool or hair in a restricted or semi-restricted state, I.

The load/extension curves shown in fig. 10 constitute an interesting epitome of the main effects of caustic soda solutions on the elastic properties of hair. OA is a normal (restricted) load/extension curve of human hair in water at ordinary temperatures. When the fibre was held stretched in the water for half a minute at an extension corresponding to the point A, the tension fell (along the path AA′) to that given by the point A′, after which it was allowed to contract to its original unstretched length and placed in 1% aqueous caustic soda for fifteen minutes. A second load/extension curve, OB, was then taken with the hair immersed in the caustic soda solution and the decay of tension once more observed over half a minute (BB′). *Immediately after* recovery to zero extension a third load/extension curve was taken (of the same hair, and still immersed in the caustic soda solution); this final curve is OC. The rate of extension for all these curves was about 50% per minute, and they show clearly that *the process of generalizing the elastic properties of hair by means of caustic soda rests for the most part on changes taking place in the fibre in the stretched state.* The reactions involved, to judge by the X-ray photographs, are, like those of steam (see below), side-chain reactions only.

The curve OD* was obtained by the slow, discontinuous extension of a human hair

* A similar curve has been obtained by SPEAKMAN for wool stretched in formic acid (' Proc. Roy. Soc.,' A, vol. 131, p. 187 (1932)).

at the rate of 4% every half-minute. (This procedure was necessitated by the mechanical limitations of the extensometer available,* the plunger of which had a minimum speed too high for the purposes of this experiment.) It will be seen that it is closely related to fig. 9, and it should be noticed that, since the fibre had not been stretched previously, the HOOKE'S law limit is considerably greater than that of the curve OC, and that the point S, which marks the beginning of the transformation of the phase K_3, is again found at an extension of about 45%, just as in the normal load/extension curve in water at ordinary temperatures. The action of caustic soda during the slow extension of a hair which has not previously been stretched is thus to reduce the loads corresponding to the various stages of a more rapid extension.

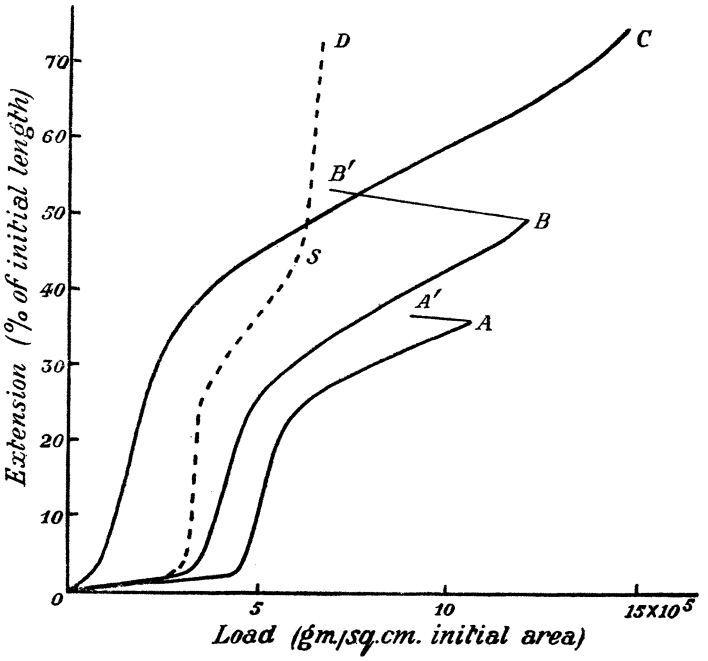


FIG. 10.—The effect of caustic soda solutions on the elastic properties of human hair. (For details, see text.)

A full understanding of the action of caustic soda solutions on the elastic properties of hair is so bound up with that of the nature of the three elastic phases that it is proposed now to defer further argument till a later report on the latter problem. At the moment we know enough to be sure that this action is a function of a number of variables embracing the previous history of the fibre, the rate and limits of extension, the concentration and temperature of the solution, and the constitution and properties of the elastic phases, but in addition to what has been put forward above there are still several interesting points which are perhaps not yet quite ripe for discussion.

The action of hot water and steam.—The effect of water of increasing temperatures on the load/extension curve of Cotswold wool is shown in fig. 1 and has already been

* DENHAM and LONSDALE, 'J. Sci. Instr.,' vol. 5, p. 348 (1928).

discussed to some extent. It will be seen that increasing temperature tends to "generalize" the load/extension curve after the manner of caustic soda at ordinary temperatures. As before, the process involved is one of decay of tension and permanent obliteration of the discontinuities characteristic of the three phases, and once more it seems clear that we are dealing with side-chain transformations only which are functions of both temperature and time. At higher extensions these changes are revealed in the X-ray photographs by the "spreading" of certain spots already mentioned, an effect which shows at its best in photographs of steam-stretched fibres (fig. 23, Plate 8, human hair stretched in steam to 100% extension). The phenomenon, which will be dealt with crystallographically below, is one of first-rate importance from the point of view of the general theory of protein structure, because it shows that *the dimensional disturbance in the keratin crystallites under the action of water, steam, and other reagents, is confined to a single direction only, that of the amino-acid side-chains.** The disturbance first makes itself evident at extensions exceeding 50–60%; that is to say, soon after the onset of the transformation of the phase K_3, from which it seems a reasonable conclusion that it arises or is considerably intensified—in the transformed crystalline phase, K_2, at any rate—through the rapid rise in stress in the transformed, and therefore inextensible, K_2 during the progress of the transformation of the final phase, K_3. It is therefore a direct consequence of the fact that K_3 cannot be completely transformed into its β-modification without the aid of steam or caustic soda solutions.

The super-contraction of hair.—It was observed by SPEAKMAN† that aqueous solutions of caustic soda and of sodium sulphide can not only relieve the "set" of a steam-stretched wool fibre, but can also cause the fibre to contract to a length which is definitely shorter than the original. The explanation of this phenomenon at the time of its discovery was not apparent, but it is now clear from the X-ray interpretation of hair structure given here that it is a particular manifestation of a general effect to which we have ventured to attach the term "super-contraction," to distinguish it from the property of a normal stretched hair, when wetted, of returning exactly to its original unstretched length. The general phenomenon of super-contraction is to be traced to side-chain modification or breakdown when β-keratin is subjected to the action of water or other hydrolytic reagents, a process which leaves the long polypeptide chains in a "freer" state endowed with a greater power of contraction than that possessed by the normal β-keratin. It has already been shown above that super-contraction can be demonstrated even after the action of water at ordinary temperatures, and that it is a necessary consequence of decay of tension, however produced, provided secondary effects do not interfere (see below). Perhaps the simplest way of showing it is to stretch a wool fibre slowly in 1% aqueous caustic soda solution to

* Footnote †, p. 339.

† 'J. Soc. Chem. Ind.,' vol. 50, p. 1T (1931).

an extension of, say, 80 or 90%, and then to remove the stretching force, while still leaving the fibre in the solution. It is found then that the fibre quickly contracts to a length which may be as much as 10% shorter than the original (see details given above for the preparation of a hair in the " generalized " state). A more systematic demonstration is illustrated by figs. 11 and 12, in which each fibre was stretched in cold water to an extension of 50% and then exposed to the action of steam or hot water for the times given by the abscissæ. During such an exposure there is a rapid fall of tension—a greatly accelerated manifestation of the phenomenon which occurs even at ordinary temperatures (see above)—due to side-chain changes in the strained β-keratin, and the molecular chains are freed to such an extent that, on removing the

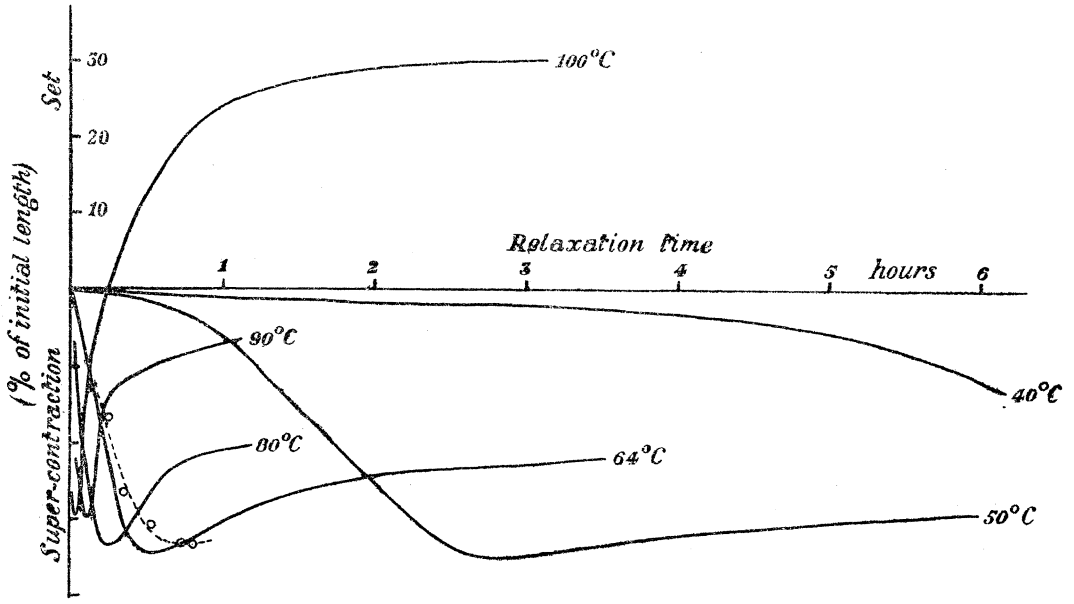


FIG. 11.—The super-contraction of Cotswold wool in steam after being held stretched at 50% extension in steam or hot water for the times given by the abscissæ. (These times must be multiplied by three for the 40° curve. For the significance of the dotted curve, see text.)

stretching force and placing the fibre in a steam chamber, a contraction of as much as 30% below the initial unstretched length is readily obtained. In every case included in the curves given the relaxed fibre was left in the steam chamber until no more contraction was observed. This procedure was consistent, but to a certain extent arbitrary, since what we are trying to estimate is the amount of " loosening " in the stretched keratin complex caused by exposure to hydrolytic action for a given time at a given temperature, a treatment which temporarily destroys its power of spontaneous contraction in water at ordinary temperatures. We have therefore subsequently to expose the treated fibre, after the stretching force has been removed, to the action of aqueous caustic soda, water at higher temperatures, or steam, so that the new side-chain combinations which, as we have seen above in the section on the influence of

humidity at ordinary temperatures, are in the first instance reversible, may also be broken down to allow the contractile forces to have full play. It must be emphasized that after the decay of tension of a stretched hair in caustic soda, hot water, or steam, the original unstretched length loses its significance*—it becomes merely a point on the contraction curve—because the side-chain junctions are so modified that the minimum equilibrium length is now shorter than the original.

Each of the curves of figs. 11 and 12 shows a maximum super-contraction effect of about 30%. The course of each is such that the super-contraction first increases with the "relaxation time" (the time of exposure in the stretched state to hot water or steam) and then, more slowly, decreases. The increase of super-contraction with

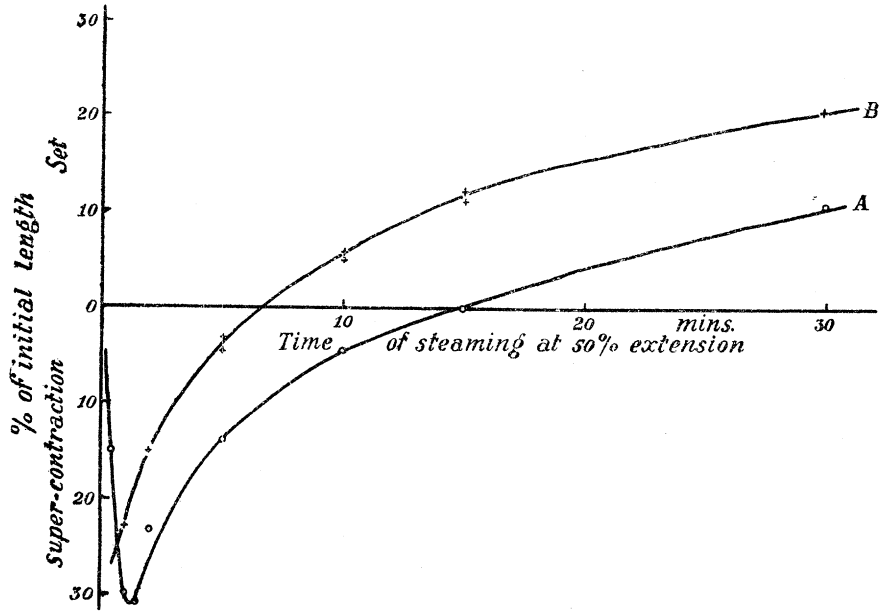


FIG. 12.—The effect of irradiation with ultra-violet light on the super-contraction of Cotswold wool. The fibres were steamed at 50% extension for the times shown, and then contracted in steam. (A, normal fibres; B, irradiated fibres.)

relaxation time is clearly due to increase of side-chain breakdown with time of exposure, but side-by-side with this process there proceeds still another phenomenon, that of side-chain re-combination. We shall discuss this in detail in the next section; it will be sufficient here to point it out as the cause of the rise in the right-hand portion of each curve. In steam, the relaxation time required to produce the maximum super-contraction is quite short, a matter of two minutes only; this is shown more clearly in fig. 12, curve A, which is an enlargement of the 100° curve of fig. 11. Relaxation in water at lower temperatures requires a correspondingly longer time to produce maximum super-contraction, while the extent of side-chain re-combination is also continuously decreased as the relaxation temperature falls. As we have seen above,

* ASTBURY and WOODS, 'Nature,' vol. 126, p. 913 (1930).

the changes are still perceptible even at ordinary temperatures, though in this case the relaxation time required is of the order of months.

For the curves of fig. 11, each fibre was placed in the steam chamber while still at an extension of 50% ; the uncertainty with regard to the effect measured lies therefore in the fact that for a short time—the time required to contract in steam to zero extension, which varies from about a minute after relaxation at 100° C. to a few seconds after relaxation at 50° C.—the fibre suffers additional relaxation in steam whatever the relaxation temperature under investigation. The uncertainty thus becomes less as the relaxation temperature is lowered, but when the latter is at or near 100° C., the curves show definitely that the minimum is raised by additional exposure of the fibre to steam while still in the stretched state, because of the marked side-chain recombination that occurs at high temperatures (see below). Other experiments were carried out in which the relaxed fibres were placed in the steam chamber only after they had first been caused to contract to within a few per cent. of their initial lengths by leaving them in water at the relaxation temperature. At 64° C. the time required for this operation varied from about 15 seconds after a relaxation time of 15 minutes to about $2\frac{1}{2}$ minutes after a relaxation time of 50 minutes, so that again there is a similar uncertainty in the effect being measured. The results, however, plotted on the dotted curve of fig. 11 bear out the argument just given and are in close enough relation to the rest to show that the principles involved are always essentially the same.

As would be expected from the fact that super-contraction arises out of side-chain modification in β-keratin, the maximum observable effect increases with the percentage extension at which the relaxation is carried out. Fig. 13, which has been prepared to show the relation between a number of phenomena, includes a curve showing the increase of super-contraction in steam as the relaxation extension is increased. (For this curve, each fibre of Cotswold wool was stretched in cold water to the extension given by the ordinates, exposed to the action of steam for 2 minutes at this extension, and then steamed back, with the stretching force removed, as far as it would go.) It is remarkable how the changes in the direction of the curve correspond to those which are found in various " restricted " load/extension curves, and which we have ascribed above to the onset of the intramolecular transformation in the three elastic phases. (The two load/extension curves given in the figure are adapted from the normal restricted load/extension curve of Cotswold wool in water at ordinary temperatures (see fig. 3), and the load/extension curve of human hair stretched slowly in 1% aqueous caustic soda solution (see fig. 10). The HOOKE'S law region in both curves has been omitted.) The fact that we have to leave out the HOOKE'S law region of the load/extension curves in order to bring out the closeness of the parallel is evidence of the strongest kind that *both super-contraction and permanent set are associated, not with the α-keratin of the normal unstretched hair fibre, but with the transformation of α-keratin into β-keratin by the act of stretching beyond the* HOOKE'S *law region ;* while the correspondence between the super-

contraction curves, the permanent set curves, and the restricted load/extension curves affords still another powerful argument in favour of the three-phase theory put forward above. Horizontal lines have been drawn in the figure to mark the *effective* boundaries between the three phases (at roughly 20% and 45% extension, *cf.* fig. 9), and it will be seen that there can be no doubt that the existence of these phases is one of the basic facts in the study of hair structure.

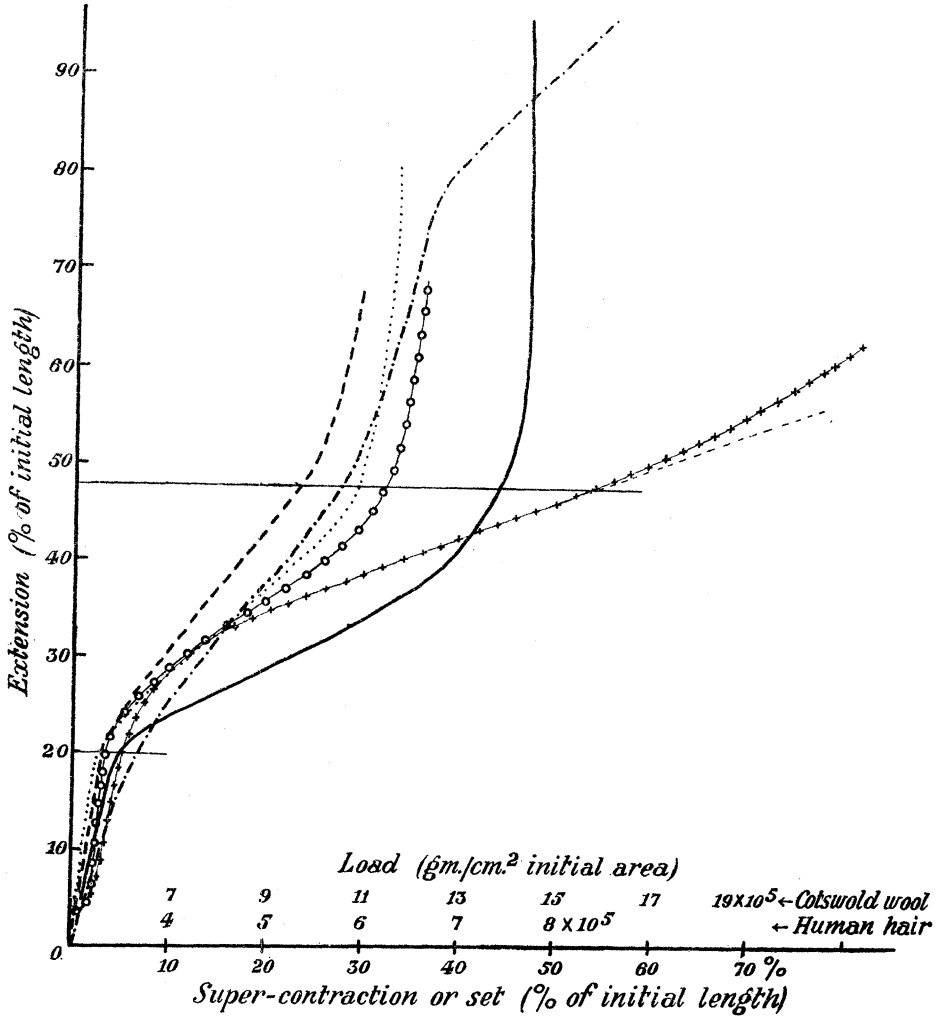


FIG. 13.—Showing the relation between the phases of the load/extension curve and the development of super-contraction and permanent set.

— — — Permanent set (human hair); . . . . super-contraction with steam only (Cotswold wool); — · — Permanent set (Cotswold wool); —— super-contraction with NaOH and steam (Cotswold wool); ○ Load/extension curve for human hair in NaOH; + Load/extension curve for Cotswold wool in water.

The experimental results plotted in fig. 13 may be summarized most briefly by the statement that *the super-contraction increases slowly with the extension when the fibre is easy to stretch, but more quickly when the fibre is harder to stretch;* in other words, it is in

some way a function of the stress generated by the restrictions between the elastic phases during the process of extension. We shall not in the present paper go further into this problem, but shall confine ourselves at this stage to pointing out that the generalization just formulated is in agreement with, and forms one of the strongest arguments for, the theory that decay of tension, and temporary and permanent " set," are all consequences of side-chain modification in strained β-keratin.

Fig. 13 shows also a curve of super-contraction brought about by the action first of caustic soda and then of steam. For this curve each fibre, while held stretched at the extension given, was immersed for 10 minutes in 1% aqueous caustic soda solution, and then allowed to contract to zero extension in the same solution ; it was then washed for 24 hours in running water and afterwards placed in the steam chamber to develop super-contraction. It will be seen that the general run of the curve is similar to that found when steam only is used, but that the maximum super-contractions obtained are greater ; with steam alone they ultimately attain rather more than 30%, but with caustic soda and steam they reach a value of over 40%. We shall refer to the numerical significance of this result below, when we discuss the crystallographic interpretation of the structure of the keratin complex.

It will be convenient to deal with the X-ray findings on super-contraction in the following section.

The " setting " of hair.—It has already been pointed out several times above that the process of side-chain modification or breakdown to which we have given the name " relaxation " is accompanied by a fall not only in tension, but also in speed of elastic recovery on account of a rebuilding of side-chain linkages which is in the first place reversible. In the present section we shall proceed to develop this concept so as to include the whole of the phenomena of " temporary " and " permanent " set.*

The higher the temperature of the water in which a hair is stretched or held stretched, the more rapidly is its tension dissipated and its speed of elastic recovery in cold water diminished and ultimately destroyed. Early investigations in this field are due to HARRISON† and SHORTER,‡ but the first systematic examination was carried out by SPEAKMAN,§ who concluded that the " set " of wool produced by water below about 90° C. is not really permanent, but is reversible in hot water to an extent which depends on the temperature of the hot water, the time and temperature of setting, and the percentage extension of the fibre. The same author showed that " set " can also be reversed by cold solutions of caustic soda and of sodium sulphide (SPEAKMAN, *loc. cit.*,

* Before the X-ray interpretation of hair structure such a unification of ideas had not been attained, though the general phenomenon of stress dissipation by means of hot water or steam is well known in the wool industries under the name " permanent set." In the light of what is now known, we have found it unavoidable to introduce a more comprehensive nomenclature.

† ' Proc. Roy. Soc.,' A, vol. 94, p. 460 (1918).

‡ ' Trans. Faraday Soc.,' vol. 20, p. 228 (1924).

§ ' Trans. Faraday Soc.,' vol. 25, p. 169 (1929).

1931). We are now in a position to extend the scope of these observations still further. The results plotted in fig. 11 show that *wool must be held stretched in steam for at least twenty minutes* before any true positive† set can be realized; the "set" produced by hot water or by short exposures to steam is destroyed by subsequent exposure to steam in the absence of tension and is finally replaced by contraction below the initial unstretched length.* We shall thus reserve the term "permanent set" for positive set which cannot be reduced even by the prolonged action of steam in the absence of tension, while to that "set" which is permanent merely in water at room temperature we shall apply the term "temporary set." Temporary set can be removed by the action of aqueous caustic soda at ordinary temperatures, by steam, or by hot water at a temperature depending on the time and temperature of setting and the percentage extension of the fibre. The set produced by holding a stretched hair in aqueous caustic soda and afterwards washing it for several hours in running water is also only temporary set; it can be rapidly removed by re-immersion in the caustic soda solution or by exposure to

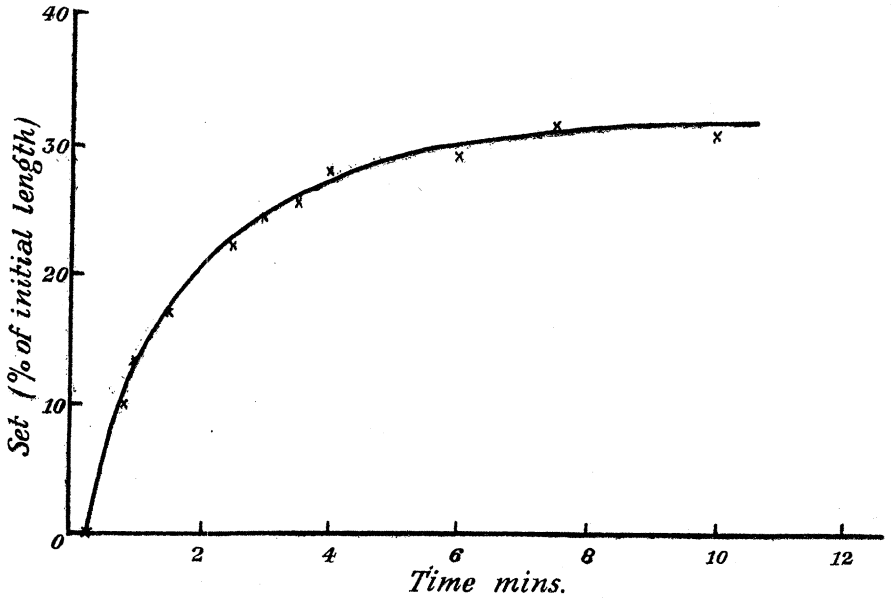


FIG. 14.—The development of temporary set with time of exposure to steam at a constant extension of 38·5% (Cotswold wool).

steam. *The phenomenon of super-contraction is a consequence of the reversibility of the side-chain linkages which are the cause of temporary set; under the action of steam these linkages are in time either replaced or reinforced by junctions of an irreversible nature which preclude super-contraction and give rise to the true permanent set.*

Fig. 14 illustrates the rate of development of temporary set in stretched wool fibres

* The exact time varies somewhat with the type of animal hair under investigation, partly on account of variations in molecular constitution, and partly on account of differences in fibre diameter.

† That is, positive with respect to the *initial* unstretched length. As pointed out above, this length loses its significance after "relaxation." With respect to the *minimum* (super-contracted) length, fig. 11 reveals that permanent set develops even in hot water, as is also shown by X-rays.

exposed to steam. For this curve each fibre was stretched in cold water to an extension of 38·5% and then steamed in the stretched state for the times shown; the temporary set was measured as the remaining elongation when the fibre was afterwards placed in cold water in the absence of tension and finally dried at room humidity. It should be noticed that there is an "induction period" of several seconds which is probably conditioned by the time of penetration of the fibre by the steam, and that the maximum set is always less than the extension at which the setting was carried out.

Fig. 15 shows the relation between the temporary set of human hair in steam and the extension at which the setting was carried out. Each fibre was stretched in cold water to the extension given, exposed to steam for 2 or 5 or 60 minutes (curves (*a*), (*b*), and (*c*)

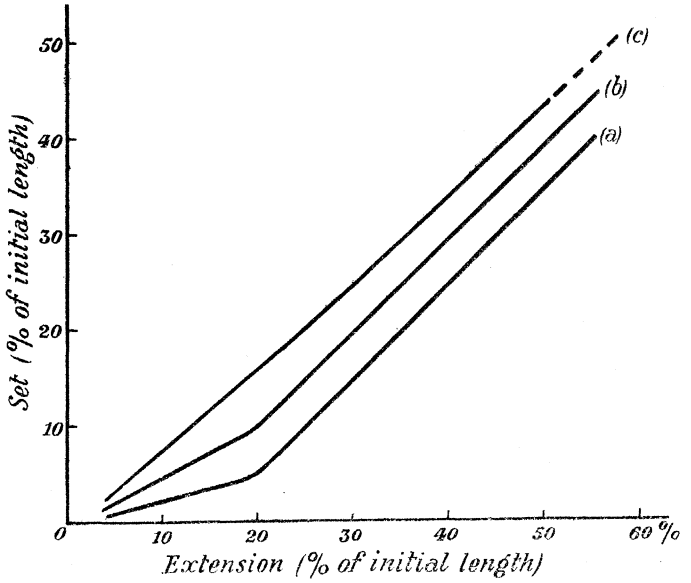


FIG. 15.—The relation between temporary set in steam and the extension at which the setting was carried out (human hair). (The dotted line indicates the continuation of an approximately linear relationship up to the maximum possible extension.)

respectively), and then placed in cold water without tension; as before, the remaining elongation after drying at room humidity was measured as the temporary set. It is clear from this figure how the amount of temporary set realized falls short of the extension at which the setting was carried out. Curve (*c*) is approximately the limiting curve obtained after prolonged steaming under tension and corresponds to the maximum temporary set of the keratin that has been transformed. Like curves (*a*) and (*b*), it appears to intersect the axis of extensions at about 2%, from which we must conclude that, possibly as a consequence of the HOOKE'S law region, there is an inevitable recovery of at least this amount. In actual practice the recovery is still more—it may amount to something like 7% for an extension of 50%—because the measurements are complicated by the occurrence of longitudinal swelling, which varies from about 1% in a normal wet hair to about 5% in a hair held stretched at 50% extension in steam (see

below). We can correct for this either by subtracting the longitudinal swellings from the extensions given by the abscissæ, or by measuring the set fibre while still in the wet state, a procedure which shows that the true recovery of the fibre substance itself is only of the order of 2%, or, in other words, *after prolonged steaming under tension, practically the whole of the transformed keratin is temporarily set with respect to water at ordinary temperatures, though in actual practice the contraction observed can be of the order of* 13% *of the extension at which the steaming was carried out.*

Curves (*a*) and (*b*), corresponding to two and five minutes steaming respectively, show in the first place that even temporary set is incomplete after short exposures to steam, and in the second place, that *there is a distinct lag in the setting of the first phase*, K_1. This is finally overcome by prolonged steaming (curve (*c*)), but there can be no doubt of its existence in the early stages of setting.

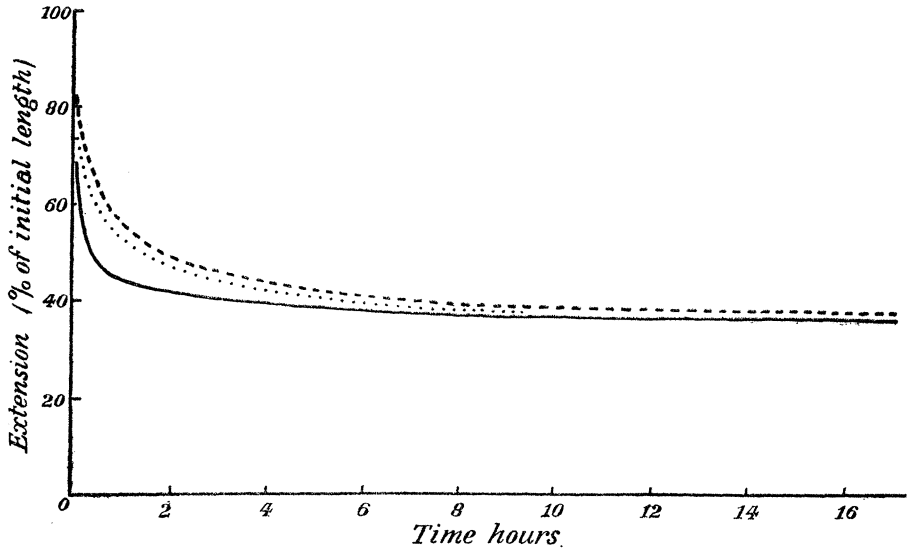


FIG. 16.—Recovery of wool and hair in steam after steaming for 8 hours in the stretched state. The remaining elongation is the true permanent set.

— — — human hair from 84% ; . . . . merino wool from 80% ; —— Cotswold wool from 81%.

The true permanent set induced by the prolonged steaming of a stretched hair can be measured after prolonged re-steaming in the absence of tension. The curves of fig. 12 and the 100° curve of fig. 11 show clearly how the permanent linkages built up by the action of steam soon overcome the effect of super-contraction and lead to permanent elongation, while fig. 16 illustrates the process of incomplete recovery during re-steaming. For fig. 16 each fibre was steamed for 8 hours at an extension of about 80% (see data on figure), and then re-steamed in the absence of tension. After about 16 hours re-steaming contraction had almost ceased, leaving a true permanent set of roughly 40%.

Just as for super-contraction, permanent set in steam does not increase uniformly with the percentage extension at which the setting was carried out, but is once more

controlled by the properties of the three elastic phases and the proportions of each that have been transformed into the β-state, as is demonstrated in a striking manner by the relevant curves of fig. 13. These show that permanent set is inconsiderable up to extensions of about 20% (phase K_1), but afterwards increases more rapidly up to extensions of about 50% (phase K_2), then remains fairly constant until the final stages of extension (phase K_3), when it once more increases, but never to an extent sufficient to involve the whole of the keratin substance. It would thus appear that *true permanent set is for the most part associated with the middle phase*, K_2, that the first phase, K_1, is relatively insusceptible, and that even in K_3, also, the change is only partial. For the permanent set curves given in fig. 13 it may be noted that each human hair was " set " for 6 hours and re-steamed for 6 hours, while it was found sufficient to set Cotswold wool for $3\frac{1}{2}$ hours only before re-steaming for 6 hours. The results show that the maximum permanent set that can be realized from extensions in water at ordinary temperatures is of the order of only 35%, while the maximum set for complete extension (100%) is still no more than about 60%.

With regard to X-ray examination of the effects of super-contraction and of temporary and permanent set, the conclusion reached is just what one might reasonably expect from the experiments described above (see fig. 11), that, in fact, the hotter the water in which the stretched hair is " set," the shorter the time required to destroy the reversibility of the intramolecular transformation. After only a few minutes' exposure of the stretched fibre to steam the β-photograph completely loses its power of reverting to the α-form, even though super-contraction can still be demonstrated ; but it requires several hours' treatment of a stretched hair with water at 60° C., for instance, before the β-photograph is " set " ; even after treatment for half an hour at this temperature the α-photograph reappears more or less unchanged when the fibre is caused to contract. The X-ray photographs thus offer the clearest evidence of the onset of permanent set, evidence, moreover, which is of the nature of " advance news," since the crystalline part of the fibre substance is fixed in the β-form (as revealed by X-rays) long before the fibre as a whole has acquired its maximum set. The setting of the crystalline part alone, however, is insufficient to hold up permanently the contraction (in steam or hot water) of the main body of the fibre. Fig. 23, Plate 8, illustrating the " spreading of the spots " caused by the uni-directional attack of steam on the keratin crystallites, is a typical X-ray photograph of steam-set hair, while figs. 21, 22, Plate 8, X-ray photographs of wool and human hair " generalized " by treatment (in the stretched state) with caustic soda, serve also to illustrate the point that the side-chain changes accompanying temporary or permanent set, though clearly detectable photographically, are inadequate to destroy the main outlines of the keratin chain system.*

* It is perhaps hardly necessary to point out that the interpretation given here of the principles underlying temporary and permanent set in wool applies equally well to the " permanent wave " now fashionable in human hair.

It may appear somewhat surprising that the β-photograph remains "set" after exposing the stretched fibre to steam even for a few minutes only, and that subsequently the α-photograph does not return during the process of either contraction or super-contraction; but this cannot but be due to the fact that the well-crystallized part of the fibre substance, the part from which the X-ray "crystal" photographs arise, constitutes a relatively small fraction of the whole, as is evident from the long X-ray exposures required. The molecular chains in this well-crystallized part lie parallel and in the closest possible juxtaposition and are therefore in a condition most favourable for the formation of new side-linkages; they will naturally succumb first to the process of "setting." The change does not, however, lead immediately to widespread permanent set, because the three elastic phases, K_1, K_2 (the most crystalline phase), and K_3 (see fig. 8), are both in series and in parallel, so that the super-contraction effect in the less-crystalline keratin is bound to predominate for a time. (Compare fig. 15, which shows how the setting of K_1 lags behind that of K_2.) The conflict between the contraction of the less-crystalline and the setting of the more-crystalline keratin is best revealed in X-ray photographs of hair stretched and then contracted in steam. Throughout both the extension and contraction of *normal* hair, the X-ray photographs, whether α or β, show parallel alignment of the keratin crystallites; in either direction the intramolecular transformation proceeds so smoothly as not to interfere seriously with the crystalline orientation; but whereas extension in steam maintains the parallelism of the crystallites, subsequent contraction in steam causes the "spots" of the "set" β-photographs to spread out into arcs. We have thus the interesting observation that if we take an X-ray photograph of hair stretched, say, to 70% extension, then proceed to stretch it farther in steam and finally bring it back again in steam to 70% extension and take another photograph, we obtain a "spot photograph" going up but an "arc photograph" coming down; that is to say, the parallelism of the crystallites which held during extension is destroyed during contraction, and to an extent revealed by the amount by which the spots are drawn out into arcs. It does not follow from this that contraction in steam is simply and solely due to loss of orientation in originally parallel crystallites; the angular dispersion in the arcs is much too small to account quantitatively for the observed contractions. The inference seems to be sound that we must think of the contractile mechanism of the hair fibre as being, in the first place, effectively continuous and in series throughout its length,* and in the second place as associated with regions which overlap one another, after the manner suggested by fig. 8.

When super-contraction is brought about without setting of the β-photograph, for

* In this connection it is worth mentioning an experiment in which wool fibres, stretched and then contracted in steam, were photographed under the microscope. It was found that at any stage of contraction a photomicrograph of a certain area of the fibre scale-sheath could be superposed exactly on a photomicrograph of the same area at the corresponding stage of extension.

instance, by means of dilute caustic soda, there is again a loss of crystalline orientation—this time in the α-photograph—when the super-contracted length falls markedly short of the original unstretched length, and once more the loss of orientation is quite insufficient to account quantitatively for the observed contraction. Moreover, the α-photograph does not pass over into still another new form; except for loss of orientation, it preserves its identity. These two facts, the maintenance of the α-photograph during super-contraction and the non-quantitative loss of orientation, thus afford additional strong evidence that the complete elastic mechanism of hair consists of parts which lie both in series and in parallel. Whether the fibre is in a state of super-contraction or of "set," it still retains its elasticity wholly or in part.*

Perhaps the most striking demonstration of simultaneous super-contraction and set in different parts of the same fibre is shown by the following experiment. Cotswold wool was stretched to 50% extension, treated at this extension with water at about 60° C. for half an hour, allowed to contract to its original length in the same hot water, and then photographed by X-rays; as expected, the photograph was in the α-form. The fibres were then held so as to prevent further contraction and steamed for about an hour. Under such treatment, if not held at constant length, they would have developed super-contraction (see the corresponding curve of fig. 11); but because they could not contract as a whole, certain parts must have contracted so powerfully as to stretch other parts in series with them, for a second X-ray photograph showed the presence of both α- and β-keratin. *We have here a method of producing steam-set β-keratin without any elongation of the fibre as a whole.* This experiment gives also an explanation of an observation made early in the studies described here, that X-ray photographs of hair steamed at a given (intermediate) extension always show more β-keratin and less α-keratin than corresponding photographs of unsteamed hair† (see above). Here again it seems clear that the super-contraction in certain regions of the fibre substance overpowers that in other regions which are more crystalline than and in series with the former, thus leading, at constant length, to an increase in the ratio of β- to α-keratin as revealed by the X-ray photographs.‡

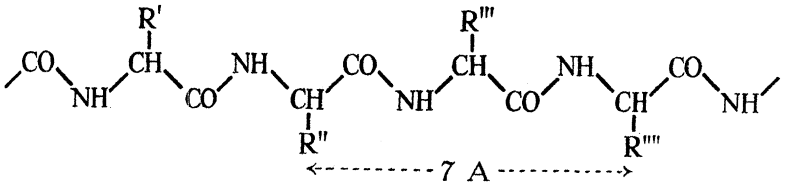
* As shown above, after super-contraction in caustic soda hair is elastic *in cold water* over a range of well over 100% of its initial length. After super-contraction or setting in steam, the full range is to a certain extent curtailed by the new permanent linkages of the side-chains.

† Compare the *initial* action on stretched wool of dilute aqueous solutions of sodium sulphide, I.

‡ [*Note added in proof, October 10th*, 1933.—The essential validity of the theory given here of the nature of relaxation, super-contraction, and set in animal hairs has now received striking confirmation by the isolation of the separate curves of super-contraction and set (Woods, 'Nature,' vol. 132, p. 709, (1933)). As explained above, the curves given in figs. 11 and 12 are really composite, in that they arise from the simultaneous operation of the two processes in conflict. The new curves show (*a*) the development of super-contraction unimpeded by set, and (*b*) the development of set from the stage of complete super-contraction. It is noteworthy, in view of the estimate made on p. 387 below, that the maximum super-contraction revealed by curve (*a*) is of the order of 45% (Cotswold wool).]

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 (α-keratin) and one in a fully-extended state (β-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 Kα 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 β-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 :—



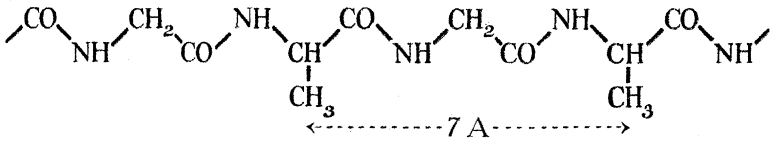
the side-chains project alternately on opposite sides of the main-chain. (This point will be clearer from the models discussed below.) From an examination of all the available evidence, we have to conclude that the submicroscopic crystallites of biological structures are simply bundles, of varying degrees 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 β-keratin is, like that of α-keratin (see above), slightly variable according to previous treatment and the state of tension. The mean value of the spacing of the (020) arc 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 ($a = 9{\cdot}68$ A., $b = 7{\cdot}0$ A., $c = 8{\cdot}80$ A., $\beta = 75°\ 50'$) 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 β-keratin, I, is most conveniently referred to an orthogonal cell of dimensions, $a = 9{\cdot}3$ A., $b = 6{\cdot}7$–$6{\cdot}8$ A., and $c = 9{\cdot}8$ 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 α-photograph is transformed to the β-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 α- and β-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.

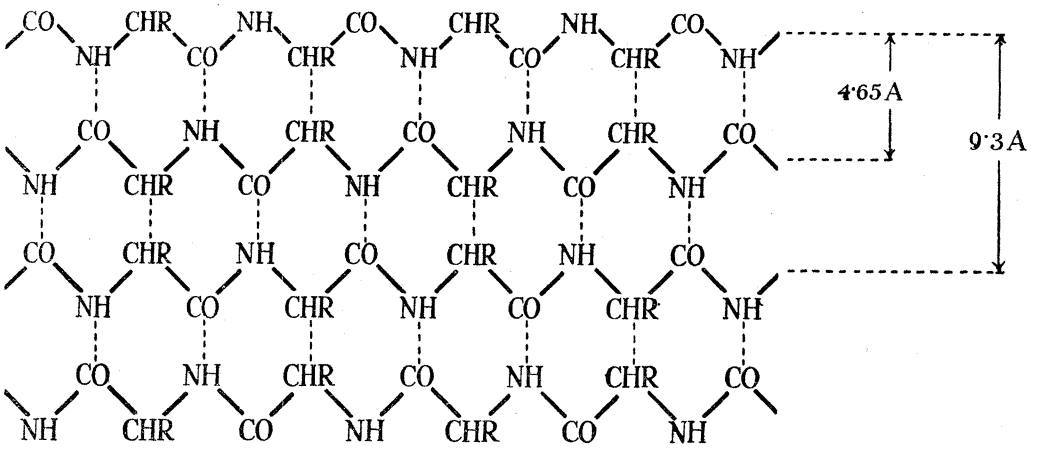
† Much the greatest proportion of the amino-acid mixture obtained by the hydrolysis of silk fibroin is glycine and alanine, but it is not certain that the X-ray photograph is incompatible with arrangements other than strict alternation of the residues of these two acids.

‡ '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 (= NH) and (= CO) groups, * † ‡, whereby the chains are grouped in pairs, thus :—



Such an arrangement accounts readily for the fact that the a-dimension of the simplest orthogonal cell given above is not 4·65 A., but 9·3 A., represented on the equator by an intense second order (200).

The strongest evidence that the equatorial spacing, 9·8 A. (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 α and β, 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 A., 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 β-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 A., *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

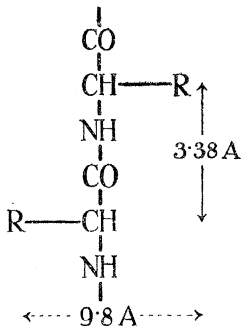
* Footnote *, p. 348.

† Footnote †, p. 339.

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

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

in the extended chains of β-keratin : they are 3·38 A., 4·65 A., and 9·8 A., in accordance with the scheme :—



the remaining dimension, the thickness of the " backbone," being normal to the plane of the paper. If this scheme is sound, it should agree, among other things, with the observed density of hair, 1·3 gm./cc.* (From measurements of the cross-section of stretched hair, the density of β-keratin must be practically the same.) The weights of the amino-acids that have been obtained to date by the hydrolysis of 100 gm. of wool†‡ are shown in Table III, which gives also the weights of the corresponding residues and the number of gram-residues of each per 100 gm. of wool.

The weighted mean residue-weight is thus :—

$$\frac{\Sigma(W/M) \,.\, R}{\Sigma\,(W/M)} = \frac{65{\cdot}5}{0{\cdot}568} \approx 115,$$

and therefore the average number of residues associated with the volume ($3{\cdot}38 \times 4{\cdot}65 \times 9{\cdot}8$) A^3 is

$$\frac{3{\cdot}38 \times 4{\cdot}65 \times 9{\cdot}8 \times 1{\cdot}30}{115 \times 1{\cdot}65} = 1{\cdot}06 \approx 1.$$

In view of the shortcomings of the chemical analysis, this result is not as conclusive as might be desired, but it carries strong conviction when considered in combination with all the other arguments presented here. Furthermore, it may be extended to predict the density of proteins in general and the probable weight per unit area of mono-molecular protein films on water. For both of these calculations we shall not be far wrong if we take 120 as the average residue-weight of the amino-acids, and ($3\frac{1}{2} \times 4\frac{1}{2} \times 9\frac{1}{2}$) A^3 as the average volume associated with each residue.§ These values give for the average density of proteins in general :—

$$\frac{120 \times 1{\cdot}65}{3\frac{1}{2} \times 4\frac{1}{2} \times 9\frac{1}{2}} \approx 1{\cdot}3,$$

* KING, ' J. Text. Inst.,' vol. 17, p. T53 (1926).

† BARKER, " Wool : a Study of the Fibre " (Publication of the Empire Marketing Board, 1929).

‡ SPEAKMAN and HIRST, ' Trans. Faraday Soc.,' vol. 29, p. 148 (1933).

§ Footnote †, p. 339.

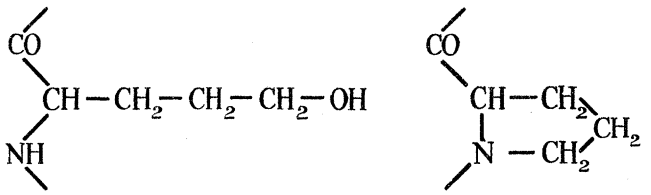
which is more or less what is found by experiment. With regard to mono-molecular protein films, HUGHES* argues that when these are compressed into the "gelatinous" state the side-chains are forced to lie normal to the surface so as to permit the main-chains to approach as closely as possible. In these circumstances we have an average weight of ($120 \times 1{\cdot}65 \times 10^{-24}$) gm. associated with a surface area of ($3\frac{1}{2} \times 4\frac{1}{2} \times 10^{-16}$) sq. cm., whence the weight per unit area is

$$\frac{120 \times 1{\cdot}65 \times 10^{-24}}{3\frac{1}{2} \times 4\frac{1}{2} \times 10^{-16}} = 1{\cdot}26 \times 10^{-7} \text{ gm.}$$

TABLE III.—The Hydrolytic Products of 100 gm. of Wool.

Amino-acid.	(W.) Wt. from 100 gm. of wool.	(M.) Molecular weight.	(R.) Residue weight.	(WR./M.) Wt. of residues.	(W./M.) No. of gm. residues.
Glycine	0·6	75	57	0·5	0·008
Histidine	0·6	155	137	0·5	0·004
Tryptophane	1·8	204	186	1·6	0·009
Aspartic acid	2·3	133	115	2·0	0·017
Valine	2·8	117	99	2·4	0·024
Lysine	2·8	146	128	2·5	0·019
Serine	2·9	105	87	2·4	0·028
Alanine	4·4	89	71	3·5	0·050
Proline	4·4	115	115*	4·4	0·038
Tyrosine	4·8	181	163	4·3	0·027
Arginine	10·2	174	156	9·1	0·059
Leucine	11·5	131	113	9·9	0·088
Glutamic acid	12·9	147	129	11·3	0·088
(Cystine)/2†	13·1	120	102	11·1	0·109
			Total	65·5	0·568

* The proline residue in the chain can conceivably be either



(*a*) or (*b*)

For the purposes of this calculation the former has been assumed, though it does not seriously alter the numerical result if we take the latter formula instead.

† Assuming that each cystine molecule is shared between two neighbouring main-chains (see text).

For egg albumin, glutenin, and gliadin, HUGHES finds about ($1{\cdot}4 \times 10^{-7}$) gm. per sq. cm. (roughly the same as the earlier value, 1×10^{-7} gm. per sq. cm., found by GORTER and GRENDEL†), which is in very satisfactory agreement with prediction.

* 'Trans. Faraday oc.,' vol. 29, p. 211 (1933); HUGHES and RIDEAL, 'Proc. Roy. Soc.,' A, vol. 137, p. 62 (1932).

† 'Proc. Acad. Sci. Amst.,' vol. 32, p. 770 (1929).

An atomic model of part of the β-keratin system of extended main-chains linked by side-chains is shown in fig. 26D, Plate 9, which is a view in the direction [100]. No significance must be attached to the particular side-chains used in the model, except so far as they are of different lengths, in order to illustrate the next step in the argument, that even in β-keratin the main-chains are to be considered parallel only as regards their general direction along the length of the fibre. The continuous variation in length and linkage of the side-chains will distort the main-chains and give rise to what we may term the " primary fold "—" primary," because such a fold can occur in *any* fibrous protein as a specific stereo-chemical feature which may not be destroyed by the application of tension. Moreover, since *any distortion of the main-chains implies a contraction*, the primary fold alone will reduce the average length of an amino-acid residue to something shorter than the 3·5 A. found in silk fibroin, in which, on account of the insignificance of the side-chains, — H and — CH_3, the primary fold is negligible. For β-keratin we may conclude that the primary fold reduces the average length of an amino-acid residue to 3·38 A.

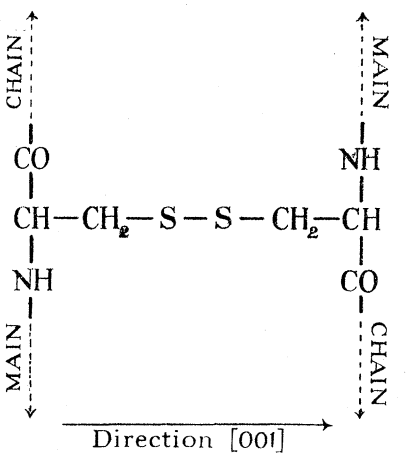
The gross fold in the main-chains which appears when β-keratin contracts to α-keratin we may term the " secondary fold " ; its orientation follows from the fact already pointed out, that the " side-chain spacing," 9·8 A., is found in both the α-and the β-photograph, though the latter shows in addition the " backbone spacing," 4·65 A. It seems clear, therefore, that *the secondary fold is formed in a plane transverse to the side-chains*, the general direction of which is kept more or less unchanged during the transformation. The most reasonable stereo-chemical change to satisfy the *quantitative* requirements of the transformation, viz., an intramolecular period of 5·1 A. in the folded chains of α-keratin, an average residue length of 3·4 A. in the extended chains of β-keratin, and a resultant increase in fibre length of approximately 100%, has been given above in the section on General Interpretation. To the remarks there found we may now add that the plane of the pseudo-diketopiperazine rings must lie transverse to the side-chains.

With regard to the source in the α-photograph of the strong spot of spacing 4·65 A. which appears on the equator of the β-photograph, we have to assume, since the fibre density is approximately constant, that this spacing is derived from one of roughly twice this amount on the equator of the α-photograph, a spacing which is a measure of the effective width of the secondary fold in its own plane (001). The α-photograph has already been discussed in detail, I, but it is so poor in interpretable diffraction data that we can only say that both equatorial spots, the small diffuse spot near the centre of mean spacing about 27 A., and the (probably complex) spot of spacing 9·8 A., could conceivably supply the spacing required.

Fig. 26 (A) and (B), Plate 9, shows an atomic model of part of the α-keratin system of folded main-chains linked by side-chains, (A) being a view in the direction [001] and (B) in the direction [100]. Again no significance must be attached to the particular side-chains used (they are of equal lengths in this model and correspond, as a matter of

fact, to cystine "rungs"—see below). Fig. 26 (C), Plate 9, shows the same mode pulled out into the β-form and viewed in the direction [100]; it is the counterpart of fig. 26 (D), in which the side-chains have been made of different lengths. These models, in addition to illustrating the arguments brought forward above, serve also, since the β-models were derived simply by extension of the corresponding α-models, to bring out another important point, that *the transformation of α-keratin to β-keratin does not necessarily involve the rupture of direct cross-linkages.* All that appears to happen when one model is transformed to the other is a sequence of oscillations about various bonds, though undoubtedly (see below) departure from the α-form must give rise to a system of very complex stresses and strains. We have here an interesting demonstration of how it becomes possible for hair to preserve its elastic properties after innumerable extensions and contractions, and how it is that the X-ray photographs retain their definition throughout.

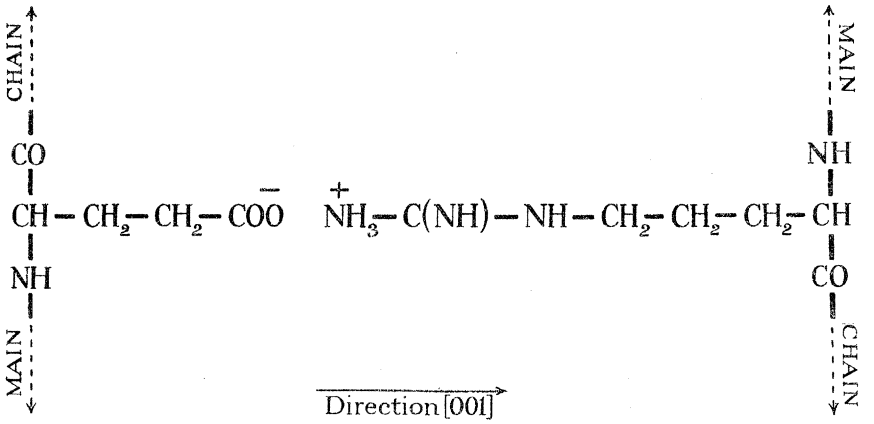
It seems likely that the most permanent source of lateral cohesion in the keratin complex is to be found in cross-linkage by cystine, the "double" amino-acid so characteristic of the keratins which appears to be responsible for all or most of the observed sulphur content,* thus :—



Speakman and Hirst, *loc. cit.*, have brought forward strong reasons for the presence also of cross-linkages of a salt-like nature between acidic side-chains such as those of glutamic and aspartic acids, and basic side-chains such as those of arginine, lysine, and histidine. It is noteworthy that the amounts of these two types of side-chains are roughly equivalent in keratin, and Speakman's work on the changes in elastic properties brought about by treatment with acids, alkalis, etc., together with his studies of acid adsorption, diazotization, and swelling, undoubtedly point to the existence of inter-

* Barritt and King, 'J. Text. Inst.,' vol. 17, p. T386 (1926); vol. 20, pp. T151, T159 (1929); Barritt and Rimington, 'Biochem, J.,' vol. 25, p. 1072 (1931); Rimington, 'Biochem. J.,' vol. 23, pp. 41, 726 (1929); 'J. Soc. Chem. Ind.,' vol. 49, p. 139T (1930).

chain salt-linkages. A typical linkage of this kind (between glutamic acid and arginine) is :—



SPEAKMAN has also suggested* the possible existence of an oxygen linkage derived from such hydroxy-acids as serine, proline, and tyrosine.

Bearing these considerations in mind, it is possible to divide the residues found in hair tentatively into five main groups, thus : (1) basic, (2) acidic chemically equivalent to the basic, (3) hydroxy (? condensing to form an ether-linkage), (4) inactive (comprising residues with alkyl-groups, acids combined with amide-nitrogen, and the non-basic tryptophane), and (5) " halved " cystine residues. Table IV is drawn up on the basis of this scheme with the aid of the analytical results given in Table III.

TABLE IV.—Scheme for the Grouping of the Amino-acid Residues in Keratin.

Basic.—Arginine 59, lysine 19, histidine 4 (= 82*).
Acidic.—Glutamic acid 88, aspartic acid 17 (= 105 = 82 + 23†).
Hydroxy.—Serine 28, proline 38, tyrosine 27 (= 93).
Inactive.—Amide-acids† 23, tryptophane 9, glycine 8, alanine 50, valine 24, leucine 88 (= 202).
(*Cystine*)/2.—109.

* These would react with 82 cc. of N.HCl ; actually, SPEAKMAN and HIRST *loc. cit.* find 80 cc.

† Following SPEAKMAN and HIRST, 82 are allotted as chemically equivalent to the 82 bases above, leaving 23 for combination with amide-nitrogen, though, as a matter of fact, even more are required to account for all the amide-nitrogen found.

We can thus derive the following series of ratios, in successive degrees of approximation :—

Basic.	Acidic.	Hydroxy.	Inactive.	(Cystine)/2.
82	82	93	202	109

* " The Swelling of Proteins," pp. 95–109 (Conference of the Int. Soc. Leather Trades' Chem., 1933).

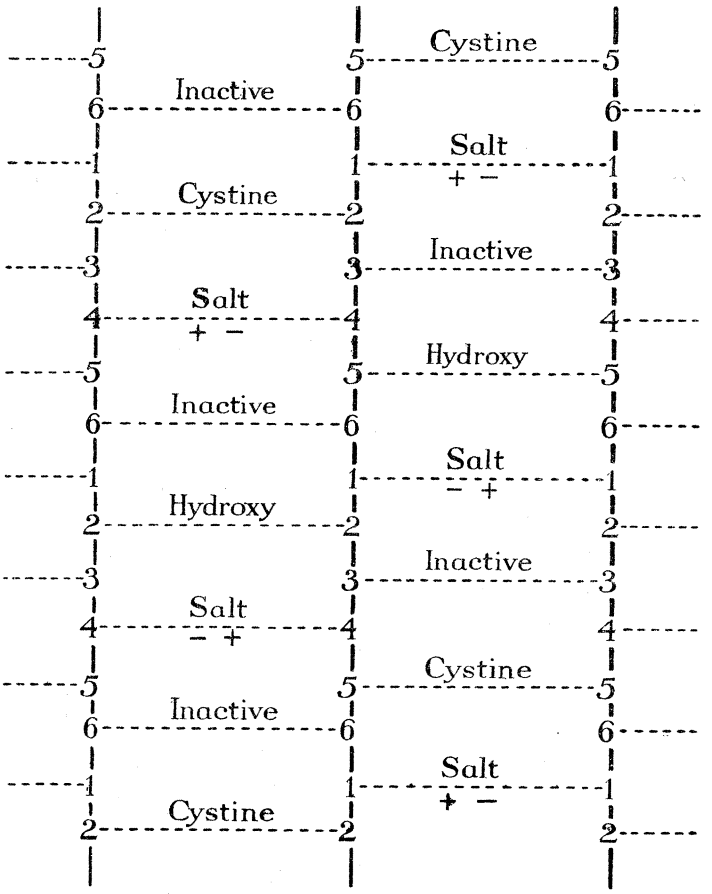
or (contained in 48 residues, *i.e.*, 8 groups of 6 each) :—

Basic.	Acidic.	Hydroxy.	Inactive.	(Cystine)/2.
7	7	8	17	9

or (contained in each group of 6) :—

1	1	1	2	1

The aim underlying this process of successive approximations is to try to gain some idea of the constitution of the pattern in the secondary fold of α-keratin. By X-rays this pattern contains at least two units each of length 5·1 A., I, and the conclusion arrived at here is that each of these units comprises a linked pseudo-diketopiperazine ring built out of a succession of three residues (see the models and the proposed transformation formulæ given above). A rough average constitution of the cross-linked chain system may therefore be somewhat as follows :—



On the assumption that the side-chains are fully extended and lie approximately at right angles to the main-chains it is possible, from known inter-atomic and inter-molecular distances and the relative proportions of the residues given in Table III, to form an estimate of the average distance of separation of the main-chains in the direction

[001] of the side-chains. It is unnecessary to give the details of the calculation, but the result comes out to be about 11 A. From the X-ray photographs, if our present views are sound, the actual distance is 9·8 A., a reasonably satisfactory agreement when we remember that it is highly improbable that *all* the side-chains are fully extended, or even at right angles to the main-chains. For want of data we cannot as yet enter into a discussion of the latter possibility, but it cannot be overlooked; similarly, it is not at all unlikely that *there may be linkages between side-chains of one and the same main-chain.**

The model of the keratin complex developed above resolves itself into a system of *polypeptide grids* constructed out of parallel main-chains, functioning as the sides of ladders of which the rungs are supplied by the side-chains all lying more or less parallel to one crystallographic plane (100). In this plane the type of cross-linkage is variable, but it seems fairly certain that at least there are co-valent bonds represented by cystine and electro-valent bonds represented by salt-linkages; the side-chain cohesion must therefore be considerable, and it is not at all surprising that the keratins are so resistant to chemical attack, especially in the α-form, where the secondary fold must give rise to still further intramolecular saturation. The grids themselves are held together by intermolecular forces probably of the kind postulated above to account for the occurrence of the spacing 4·65 A., and the multiple molecular weights of proteins.†

The reflection (200), corresponding to the spacing 4·65 A., is the strongest in the β-photograph, and it is quite clear from the models of fig. 26, Plate 9, why this should be so, for the polypeptide grids, containing the main-chains and side-chains, lie in the planes giving rise to this reflection. The main-chains lie also, in both the α- and the β-form, in planes parallel to (001), so that a strong reflection from this plane occurs too, and persists, as already pointed out, in spite of the intramolecular transformation. The occurrence of the meridian reflection (020), at 3·4 A., may also be predicted from the models, since the side-chains are distributed along the main-chains so as to lie in planes of this spacing, though the fact that they project on alternate sides of the main-chains leads to an " identity period " of 6·8 A.

The Intramolecular Elastic Mechanism.

The intramolecular long-range elasticity of keratin is so striking as to invite the closest study, for the principles underlying it, or principles sufficiently analogous, must lie also at the bottom of numerous other dimensional changes in biological structures, notably those occurring in muscle. On approaching the problem it is natural to consider once more the elastic behaviour of silk fibroin, which constitutes probably the simplest protein fibre available. X-rays show why silk fibroin has no long-range elasticity; it is, in fact, because its long-chain molecules are normally in a fully-extended state com-

* Footnote †, p. 339.

† Footnote *, p. 348.

parable to that found in hair only when under extension; but the question arises immediately as to whether it is possible to *contract* the fibroin molecule into some folded state comparable to that found in normal, unstretched hair. Now it is known that silk fibres, under the action of acids within certain narrow limits of concentration, contract spontaneously,* and so an X-ray examination of this effect was carried out. Photographs were taken of silk threads of "electrical tram"† which had been treated for four minutes with 8·475 N. HCl, but though the contraction recorded was 27·3%, there was no fundamental departure from the normal fibroin photograph (see fig. 24, Plate 8), but only a loss of crystallite orientation. The experiment shows, therefore, that at least the crystalline constituent of silk fibres cannot be contracted by the treatment described, but, of course, this does not rule out the inter-crystalline material, which is possibly richer in the amino-acid residues other than those of glycine and alanine.‡ All that can be said from X-rays at the moment is that this inter-crystalline material is apparently disturbed so as to cause loss of orientation in the crystalline micelles and consequent loss of length; but whether the inter-crystalline material suffers some sort of contractile intramolecular transformation cannot be decided. The conclusion with regard to the fibroin crystallites, however, seems clear, that they are not so transformed; neither do the fibres acquire hair-like elasticity, showing on re-extension simply a recovery of crystallite orientation.

We have thus no evidence from fibroin that the protein "backbone"—or, more strictly, the protein "backbone" studded with short and inactive side-chains—is endowed with inherent contractile powers sufficiently strong to overcome, even in the presence of water or steam, the accumulated forces of inter-chain cohesion, and we are led to infer that the long-range elasticity of hair is a consequence of the linkages and interactions of the keratin side-chains. The model of the keratin complex developed above helps us to see at a glance how this may come about if only for the reason that the polypeptide "grids" must take up an equilibrium form depending on the nature and distribution of the cross-links and the external force applied in the direction of the main-chains. From actual manipulation of the atomic models shown in fig. 26, Plate 9, it seems clear that the essential features of the grids can be preserved throughout successive extensions and contractions, but it is also clear that *there will be a definite equilibrium configuration of the main-chains for any particular distribution of the cross-links;* in normal hair (α-keratin) this equilibrium configuration is obviously given by the secondary fold, which reduces the length taken up by the main-chains to approximately one-half of the possible maximum. From this point of view the elastic properties of the grid-like units of keratin are in no way different in principle from those of the simpler molecules;

* Farrell, 'J. Soc. Dys. Col., Bradford,' vol. 21, p. 70 (1905).

† For this "electrical tram" and the details of the treatment described, we are indebted to Dr. W. S. Denham, Director of the British Silk Research Association.

‡ Footnote †, p. 334.

the latter, too, are susceptible to distortion within the limits imposed by inter-bond angles, electrostatic attractions, rotation about bonds, etc., but in keratin the possibilities are so enormously enhanced by the length and mobility of both main-chains and side-chains that we appear at first sight to have an entirely new phenomenon.

The general stability of the grids we may ascribe to the cystine " rungs," with the possibility of some other type of co-valent linkage not yet defined; but the effect of these in determining the equilibrium configuration of the main-chains must also be controlled by olhete 'ıss permanent, types of linkage, notably the salt-linkage postulated by SPEAKMAN. In particular, we may expect the salt-linkage to function not only in drawing the chains together laterally, but also in tending to shorten their effective length. From the tentative scheme of cross-links given above it becomes apparent how attractions between the electro-positive (basic) side-chains and the electro-negative (acidic) side-chains can operate both laterally and longitudinally, just as has already been proposed by MEYER to explain the contractile mechanism of muscle.*† This concept serves also as the basis of SPEAKMAN'S study of the amount of work required to stretch a wool fibre by 30% as a function of the amount of acid (HCl) adsorbed‡; he found that the work required decreases linearly as the amount of acid adsorbed increases. For a detailed discussion of these experiments reference should be made to the original paper‡; it will be sufficient here to quote two of the main conclusions, that " each molecule of hydrochloric acid combined with wool therefore contributes a definite quantum to the total reduction in the resistance to extension observed in strongly acid solutions," and " in acid solution, the ionization of the glutamic and aspartic acids in the side link is depressed, and the attraction between the peptide chains diminished in proportion to the number of acid groups displaced from combination; alkali functions in a similar manner and both reagents facilitate fibre extension on this account." SPEAKMAN'S assumption, however, that " molecular rearrangement (that is, by intramolecular transformation) is impossible unless the side linkages are capable of free rotation about the long peptide chain," perhaps becomes unnecessary in view of the actual experiments with atomic models described above, though this, of course, does not invalidate his main conclusions just quoted. That the contractile power of hair does not depend uniquely on the presence of *amino*-groups follows from SPEAKMAN'S recent observation§ that diazotized hair still retains this power.

A full account has already been given above of the profound influence of water molecules on the extensibility of hair; reference must now be made to experiments of a complementary character which show that *the contractile power of stretched hair is also a function of its degree of hydration.* Fig. 17 (*a*) shows a series of time/contraction curves for Cotswold wool fibres stretched first in water to an extension of 50%, then dried for about

* ' Biochem. Z.,' vol. 214, p. 253 (1929).

† Footnote †, p. 334.

‡ Footnote ‡, p. 374.

§ Footnote *, p. 378.

20 minutes at room humidity, left for a further $3\frac{1}{2}$ hours to attain humidity equilibrium over dilute sulphuric acid in a closed chamber at constant temperature (22·2° C.), and finally allowed to contract freely in the same chamber. (A departure from this procedure was made in the experiments at 0% and 10% R.H., 3 days and 1 day, respectively, being allowed for the fibres to attain humidity equilibrium.) It will be

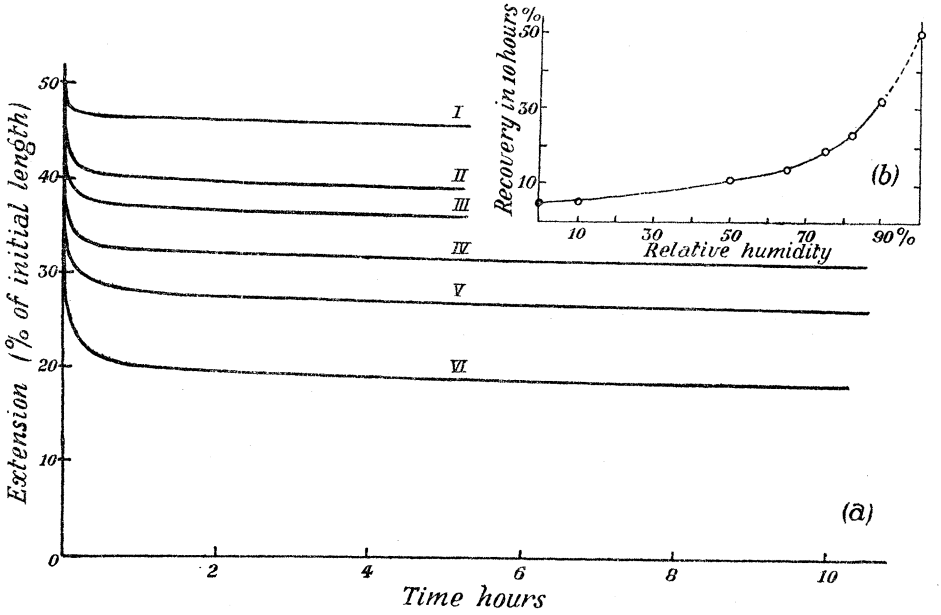


FIG. 17 (*a*).—Time/contraction curves of Cotswold wool fibres stretched to 50% extension and allowed to contract freely at constant humidity. I—10% R.H., II—50% R.H., III—65% R.H., IV—75% R.H., V—82·5% R.H., VI—90% R.H. (*b*).—The recovery after 10 hours of the fibres of fig. 17 (*a*).

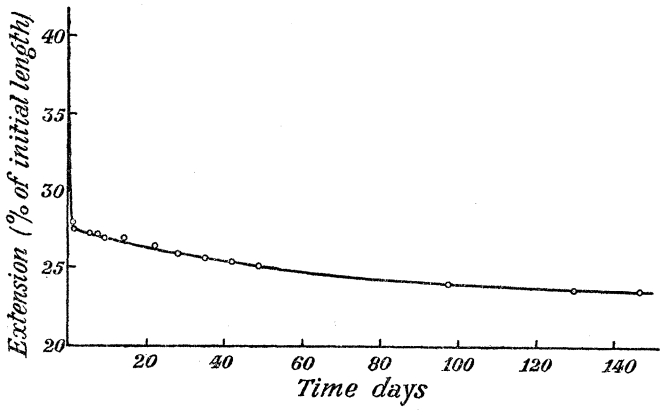


FIG. 18.—Time/contraction curve of Cotswold wool at 75% R.H.

seen that at any given humidity a stretched wool fibre tends asymptotically to assume a contracted length which is in general greater than its unstretched length, but which approaches more and more to this length as the humidity increases. The elastic recovery of a perfectly dry fibre is insignificant.

A more prolonged test of the phenomenon is illustrated graphically by fig. 18, which

is a time/contraction curve of Cotswold wool, stretched and brought to 75% R.H. as just described, and then allowed to contract freely for 140 days at this humidity and room temperature. The results show that even after such a long period the fibre had contracted only about half-way, with little or no promise of ever contracting completely at this humidity; yet when afterwards placed in water it contracted the remaining half in a few minutes.

The interpretation of this effect of incomplete contraction through lack of water appears to rest on the existence of a continuous range of molecular aggregates ("micelles") of varying degrees of stability under hydration. In the absence of water the extended chains of β-keratin cohere so strongly by virtue of their own numerous points of attraction that the contractile tendency is practically eliminated; but on hydration of the various polar groups located chiefly in the side-chains* the lateral cohesion is diminished and the grid system contracts spontaneously to the α-configuration. (A similar effect can be brought about in stretched rubber simply as a consequence of thermal vibrations†‡; in fact, most of the effects of hydration on the elastic properties of hair are paralleled by the effects of heat on the elastic properties of rubber.) We may thus suppose that at the water concentration associated with any given relative humidity, for only a certain fraction of the chain aggregates—a fraction determined by their size distribution and variations of organization—is there a reasonable probability of *simultaneous* hydration of those groups all of which must be "freed" before complete contraction can take place. (In this connection it is interesting to note that the amount of water taken up by wool at complete saturation corresponds almost exactly to an average of two molecules per amino-acid residue; for, as we have seen above, the average weight of a residue is about 115, while SPEAKMAN§ has shown that Cotswold wool at saturation takes up an amount of water equivalent to 31·9% of its dry weight, whence, if n be the number of water molecules per residue, we have

$$n = \frac{115 \times 31{\cdot}9}{18 \times 100} = 2{\cdot}04 \simeq 2.)$$

The concept of crystals or micelles in a great variety of conditions has also been put forward by Katz|| in outlining the main features of "permutoid" swelling. As an example he cites the formation of natron-cellulose by the action of concentrated caustic soda on cellulose, where the amount of the latter transformed increases with the concentration of the caustic soda. The combination of dry β-keratin and water as a prelude to re-transformation into α-keratin would appear to be quite analogous. The progress

* The question of protein hydration and its influence on swelling, cohesion, etc., has recently received a valuable discussion by JORDAN LLOYD and PHILLIPS, footnotes pp. 373, 378.

† HOCK, 'Kolloid. Z.,' vol. 35, p. 40 (1924).

‡ ASTBURY and WOODS, footnote, p. 353.

§ 'Trans. Faraday Soc.,' vol. 25, p. 92 (1929).

|| Footnote †, p. 345.

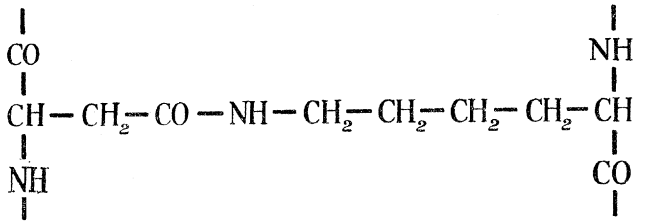
of the change is illustrated graphically by the curve of fig. 17 (*b*), which shows the recovery from 50% extension in 10 hours at various humidities.

The elastic recovery of stretched hair at various humidities does not, however, depend solely on such considerations ; it is further complicated by the side-chain re-distribution and re-combination which accompanies the decay of tension when the strained keratin complex is exposed to the action of water (see below). Experiments in this field have already been described in an earlier section, where it was seen how the speed of elastic recovery which falls simultaneously with the tension is partially restored when the act of recovery is complete and still further improved by additional subsequent extensions and contractions. As there explained, the effect is due to the dissipation of the temporary new junctions formed in the stretched keratin, and similar experiments carried out in the present connection serve again to illustrate the point. For instance, a Cotswold wool fibre was stretched in water to 51% extension and held stretched at this extension in water for 24 hours ; it was then dried and brought to 85% R.H. as described above and allowed to contract freely in the closed chamber, when the recovery followed fairly closely *the curve for* 50% *R.H.* given in fig. 17 (*a*). The same fibre, after recovering to an extension of 38·8% at 85% R.H., was allowed to recover the remainder in water, after which it was once more stretched quickly in water and allowed to recover at once ; its recovery to within 2% of its original length was almost instantaneous. It was then stretched a third time to 52% extension, dried and brought to 85% R.H. as before, and again allowed to contract freely at this humidity. This time the recovery curve lay *between the* 75% *R.H. and* 82·5% *R.H.* curves of fig. 17 (*a*)—it would probably fit an 80% R.H. curve. Such tests show clearly that the curves of figs. 17 and 18 are really composite, because the very act of leaving a stretched hair in the presence of water vapour involves intramolecular readjustment, with the result that the curves for higher humidities indicate smaller recoveries than they would were it possible to isolate the pure re-transformation effect. This circumstance does not, however, invalidate the main conclusion to be derived from the experiments just described, that the re-transformation of β- to α-keratin in the body of a hair fibre can proceed to completion only at sufficient water concentration.

The " lubricating " action of water on the linkages of keratin whereby both extension and recovery are very much facilitated is the natural forerunner also of the phenomena of tension-decay, super-contraction, and temporary and permanent " set." Under the combined influence of applied stress and the molecular field of the water molecule the cross-linkages of the strained polypeptide grids tend to re-arrange themselves in new equilibrium configurations ; at a rate depending on the extension, the temperature, and the water concentration, the tension falls continuously, but more and more slowly as the re-distribution proceeds, while at the same time new linkages are built up to retard the process of contraction when the external force is removed. The eventual outcome of all this, as the intensity of the factors just mentioned increases, is a " setting " of the grids in an elongated form which may or may not be permanent under the action

of reagents or temperature continued after the external force has been withdrawn. For " setting " by moderately hot water or by dilute caustic soda the new grid configuration, as we have seen above, is not permanent either to caustic soda, steam, or even hot water given sufficient time; but with hotter water or steam first the crystalline β-keratin of the X-ray photographs and then ultimately a considerable fraction of the whole fibre substance becomes permanently fixed in the extended form.

It may be that the simple re-formation of salt linkages in other positions between other acidic and basic side-chains is sufficient, when the process has proceeded far enough, to account not merely for temporary set, but for true permanent set also, though the available experimental evidence seems rather against this hypothesis. It would hardly seem likely that salt linkages which succumb so readily during the action of steam or caustic soda on stretched keratin will show such resistance to further prolonged action of steam or caustic soda as is shown by permanently set hair. One possible explanation of true permanent set, early considered in the researches described here, is that it is due to the formation of lateral peptide linkages; for example (between the side-chains of aspartic acid and lysine)—



but the question is still *sub judice*. In any case, it is clear that the amino-groups are in some way involved in the reaction, now that SPEAKMAN (*loc. cit.*, p. 382) has shown that the tendency to permanent set of stretched hair under the action of steam can be partially or completely eliminated by diazotization.

The phenomenon of super-contraction fits well into the scheme proposed here for the elastic mechanism of keratin, for it is now seen that like positive " set " it is only another manifestation of the basic principle that the equilibrium configuration of the main-chains is a function of the nature and distribution of the side-chains. When the polypeptide grids are under stress, the first action of water molecules or alkalis, especially at higher temperatures, is to relieve this stress by way of hydrolytic reactions with the cross-linkages, chiefly, though probably not uniquely, the salt linkages postulated by SPEAKMAN. This loosening action is followed then by a re-formation of junctions which leads ultimately to permanent set, but between the two limits *there must be an intermediate, labile, stage where enhanced powers of contraction may be looked for.* As we have seen above, this prediction is strikingly justified by experiment, for when, for instance, the action of steam on stretched hair is restricted to two minutes only,* the fibre can be

* Compare also the action of X-rays followed by that of steam, a combination which causes super-contraction *without* the aid of the stresses due to extension (see below).

made to contract to two-thirds of its original length if the steaming be continued when the stretching force is removed. Under the joint influence of water and thermal vibrations, and with certain cross-linkages " freed," the main-chains now crumple more than ever and various side-chains are brought into even closer proximity. As to this latter process, it is tempting to suppose that it originates in and is controlled by the longitudinal attractions between basic and acidic side-chains mentioned above, which one might reasonably expect to have fuller play when once any lateral linkages have been weakened or broken down. Actually this concept is found to be in very fair quantitative agreement with experiment, when we refer to the tentative side-chain scheme given on p. 379. In this scheme which, of course, is based on available chemical analyses, each basic side-chain is separated from the next acidic side-chain on the same side of the main-chain by an average distance equal to the length of six amino-acid residues; that is to say, by $6 \times 3{\cdot}4 = 20{\cdot}4$ A. in β-keratin; and the closest we can expect the side-chains to approach is of the order of $4\frac{1}{2}$–5 A. (*cf.* 4·65 A. for the main-chains). This means that we may expect a maximum contraction of the β-form to between about one-quarter and one-fifth; that is to say, to some 50–60% below the original unstretched length, a result which agrees well enough with the curves of fig. 13 when we remember that, owing to the conflict between " set " and super-contraction when stretched hair is subjected to the action of steam, the maximum super-contraction is probably never realized by this method.

We may conclude this section with a few remarks on an interesting comparison between the elastic recovery of hair and that of india rubber, and on the relation between the elastic properties of the three keratin " phases." With regard to the former it is natural to enquire why rubber gives only an " amorphous " X-ray photograph when in the unstretched state, while hair gives a " crystalline " fibre-photograph whether stretched or unstretched. The answer seems to lie in the grid-like structure of keratin as opposed to the " single-chain " structure of rubber. When the extended chains of the latter collapse into the contracted form, they do so in quite an irregular manner because there are no permanent inter-chain linkages, but only VAN DER WAALS' attractions; but when the extended chains of β-keratin contract to α-keratin the chemical cross-linkages preserve the polypeptide grids intact, the only apparent change being the formation of secondary folds in planes transverse to the grids. There is thus every reason to expect the crystallites to be preserved also, with a characteristic fibre-photograph and all the other various manifestations of fibre structure, just as experiment shows.

Throughout this paper we have assumed—and existing experimental data appear to justify this assumption—that there is fundamentally only one keratin as the basic fibre substance of mammalian hairs, but that, in the hair itself, there are three main variations on the primary theme, the three " phases," K_1, K_2, and K_3, which we have associated respectively with inter-cellular keratin, cell-wall keratin, and intra-cellular keratin. We have also pointed out that there is no sharp demarcation between these three phases, that one gives place to another almost imperceptibly, and that after the

process of " generalization " (by stretching in dilute caustic soda, for example) they behave so similarly that the maximum extension and recovery can be demonstrated repeatedly even in cold water, while the load/extension curve takes up a limiting position and form characteristic of a single-phase transformation (see fig. 3). The interpretation of these phenomena in terms of the present molecular model does not appear at the moment to offer any peculiar difficulty, since nothing in the study of hair stands out more than the fact of its variability and the way its properties can be modified without destroying its identity as hair. The minimum essential of hair-substance, the common factor of all hairs as revealed by their common X-ray photograph, we must associate with the more permanent features of the keratin grid, the co-valent skeleton network of main-chains and " rungs." The latter must involve the cystine content at least, but probably there are other co-valent cross-links besides, while alternating with such stronger links, something after the manner of the rough scheme sketched out above, we must find the less permanent junctions, notably those of an electro-valent character, which are capable of a certain degree of re-distribution without seriously affecting the cohesion and identity of the structure as a whole. In a molecular framework of this kind, especially since the true pattern along the chains is undoubtedly a multiple of that given by the main features of the X-ray diffraction diagram, it will be seen that there is ample room for secondary variations such as distinguish the three keratin phases, different types of hair, or even different parts of the same hair; and if the proposed interpretation is sound, the intramolecular differences between any of the forms of keratin discussed here, the progressively resistant phases of normal hair, " relaxed " hair, " generalized " hair, " temporarily set " hair, " permanently set " hair, or " super-contracted " hair, are all of the same general character, depending in the last resort on variations in detail in a common plan. The common plan is that of a durable but deformable grid; the details involve the proportions and stability of certain of its joints.

Miscellaneous.

There are a few other points which have received preliminary examination during the present investigations, and which would appear to call for some mention even at this stage.

Radiation Effects. (*a*) *The Action of Ultra-violet Light.*—The effect of irradiation with ultra-violet light at ordinary humidity on the elastic properties of wool fibres is shown most strikingly by a marked displacement of the curve of super-contraction and " set." Fig. 12 shows two corresponding curves, one (A) for the root-ends of normal Cotswold wool fibres and the other (B) for similar fibres first irradiated for 4 hours at a distance of 15 cm. from a mercury vapour lamp. In both the fibres were stretched in water to an extension of 50%, steamed at this extension for the times stated, and then re-steamed without restraint until no more contraction was observed. It will be seen that in this form of experiment *the action of ultra-violet light is to decrease the time of*

steaming under extension required to produce a given super-contraction or set. By analogy with the action of alkalis and that of X-rays described below, it seems clear that this acceleration is simply the result of an increase in that side-chain modification or breakdown which has been shown above always to precede super-contraction and set. It is thus apparent that by purely photochemical means changes can be brought about in the *unstretched* keratin complex similar or analogous to those caused by the action of water or alkalis on the stretched complex. For the time of irradiation quoted the *unstretched* fibres did not show spontaneous super-contraction in steam, as do those which have been subjected to long exposure to X-rays (see below), but it is not unreasonable to expect such an effect after sufficiently intense irradiation.*

A test was made to see whether the presence of water is essential for ultra-violet light to be effective in the manner just described. Cotswold wool contained in a quartz tube was dried over phosphorus pentoxide and irradiated as before, but there was still a definite displacement of the super-contraction curve. A stricter comparison, however, was postponed for the time being.

(*b*) *The Action of X-rays.*—By hanging bundles of wool or hair directly in front of the window of an X-ray tube (running at about 40,000 volts) it was found possible to bring about changes similar to those caused by ultra-violet light, but much more pronounced. After some 6 hours' irradiation with the full beam of a Shearer tube there was still no spontaneous super-contraction of the unstretched fibres in steam, but there was a displacement of the curve of super-contraction farther to the left even than that shown in fig. 12; for instance, after 10 minutes' steaming in the stretched state (50% extension, as before) the "sets" taken up by five fibres examined were +13·2%, +9·7% +9·8%, +14·5%, and +10·7%, respectively, as compared with +5% in fibres irradiated with ultra-violet light, and —4·5% in normal fibres (see fig. 12).

After about 12 hours' irradiation with the same X-ray tube still other changes manifested themselves, for it was no longer found possible to stretch the fibres to an extension of 50% in cold water (every one of a dozen fibres examined broke round about 40% extension), and there appeared the striking new property of spontaneous super-contraction in steam *without previous treatment in the stretched state.* Fibres of this batch, for example, when exposed for 1 hour to the action of steam showed a mean contraction of 28% below their initial unstretched length. We have here a "laboratory mutation" of hair analogous to those mutations recently produced by the action of X-rays on the chromosomes of the living germ cell. What it means, of course, in terms of the interpretation of the keratin complex set out above, is that the energy of X-rays can in time bring about intramolecular changes in unstretched keratin equivalent to those brought about by water and alkalis on the complex only when it is in a state of stress due to extension. It is possible that water aids the action of X-rays too, though probably

* All the points briefly discussed in this section are being submitted to further detailed examination and will be reported on later.

not essential to it, for two similar samples of wool, one dried over phosphorus pentoxide for several weeks and the other saturated over water, irradiated for 40 hours near the window of an X-ray tube did not contract equally when afterwards exposed to steam. After 2 hours' steaming the moist fibres showed a mean super-contraction of about 27%, while the dry fibres showed only 17%.

With still longer exposures to X-rays, super-contractions in steam approaching 40% were realized, but such intensely irradiated fibres showed an extensibility in cold water of less than 30%, though, rather unexpectedly, it was observed that this apparent degeneration could be cured simply by steaming the fibres while held at their normal length. After this treatment they could be stretched in cold water by as much as 80%! They also showed spontaneous super-contraction *in cold water*; for example, one fibre irradiated for 16 hours and then steamed at zero extension for 10 minutes subsequently contracted in water by 13·7% (dry), while another, steamed without restraint for the same time, contracted in the steam by 24%.

All these interesting effects are now being investigated more fully. One of the most promising lines of enquiry concerns the possible disruptive action of X-rays on the linkages of the main-chains. There is good reason to believe that such an action takes place, for an X-ray photograph of a piece of porcupine quill which had been exposed to X-rays for many days showed a definite "fuzziness" of the meridian arc. It is hoped that photometric study of this broadening will permit the suspected photochemical breakdown to be followed quantitatively.

(*c*) *The Action of Light and Potassium Dichromate.*—A number of wool fibres were soaked in a saturated solution of potassium dichromate and exposed to sunlight for half an hour. By analogy with the well-known action of light and potassium dichromate on gelatine it was considered possible that there might be some action on keratin also. The effect observed, on subsequently stretching the fibres to 50% in water, steaming for various times at this extension, and resteaming without restraint till no more contraction took place, was a *delay* in the setting action of steam—just the opposite to what was observed with ultra-violet light. Without further tests it is not yet clear whether light is essential in this experiment; at the moment, the results would appear to be analogous to those obtained by SPEAKMAN (*loc. cit.*, p. 382) with de-aminated hair. The acidic dichromate apparently reacts with the amino-groups so as to prevent their participation in the process of permanent set. Support is given to this explanation by the fact that wool dyed with eosin also showed a similar behaviour.*

Variations along the Fibre Length.—In a series of super-contraction tests of the roots, middles, and tips of Cotswold wool fibres marked variations were found in proceeding from one end to the other. The super-contraction curve for normal roots is shown in

* [*Note added in proof, October 10th*, 1933.—Further work (WOODS, 'Nature,' vol. 132, p. 709, 1933), has now shown that Cotswold wool fibres, after immersion for only ten minutes in a saturated solution of potassium dichromate, give rise to a well-defined curve of super-contraction, but have lost almost entirely the property of being set by steam in the elongated form.]

fig. 12 (A), the mean super-contraction for 10 minutes' steaming at 50% extension being 4·5%. For the middles and tips the corresponding figures were found to be 6·7% and 13·9%, respectively; that is to say, those parts of the fibre further removed from the roots showed *less* tendency to permanent set, just as do de-aminated fibres and fibres treated with potassium dichromate or eosin. It is tempting to suppose that this variation is an example of a phenomenon apparently well known in hairdressing circles, that it is difficult to set hair in "permanent waves" during pregnancy. With the lambing season at the beginning of the year and shearing in summer we might expect the root-ends of the fibres to be most immune from such an effect; but in the absence of any definite information as to the source of the particular wool examined this is no more than a conjecture, and it may be that further investigation will reveal this type of variation as a normal characteristic of hairs in general.

Longitudinal Swelling.—The question of the mechanism of the longitudinal swelling of hairs in water and other reagents is one by no means free from difficulty,* though it is generally assumed that the swelling is an inverse measure of the average length of the chain-bundles, and therefore indirectly of the chains themselves. In normal hair it is of the order of 1% from dryness to saturation with water, but after various treatments it can be considerably greater than this figure.† It seems, therefore, worth while, pending further consideration of the matter, to put on record here a few of the observations made during these researches.

In the first place, it is clear that the action of water on stretched hair is to increase the longitudinal swelling, quite slowly at ordinary temperatures, but quickly at the temperature of steam. The fibres discussed above as having suffered pronounced decay of tension after being held stretched at about 50% extension for many days in cold water showed something like double their normal longitudinal swelling, and a certain "fuzziness" in the meridian arc of an X-ray photograph certainly strengthens the idea that some hydrolysis of the main-chains had taken place.‡ Another Cotswold wool fibre, stretched to 51·1% in cold water and steamed for 2 minutes at this extension, recovered to an extension of 43·4% after 1¼ hours in cold water and then to 39% at room humidity; that is, showed a longitudinal swelling of 4·4%,§ which was increased to 5·4% on re-steaming to produce a super-contraction of 19·1%. Similarly, fibres stretched in steam to higher extensions gave correspondingly higher swellings, sometimes a figure of 9% being attained. Stretching in dilute caustic soda also increases the swelling.

As would be expected from what has been said above, irradiation by X-rays causes increased longitudinal swelling too, especially if steam be applied afterwards. For instance, a Cotswold wool fibre which initially showed a swelling from room humidity

* Footnote ‡, p. 353.

† Compare, for example, the action of dilute aqueous sodium sulphide, I, which results in a longitudinal swelling of the order of 50%.

‡ Footnote *, p. 348.

§ These swellings are expressed as percentages of the *initial* lengths of the fibres.

to saturation of 0·7% gave after irradiation for 16 hours 3·3%, which was increased to 7·7% on steaming for 10 minutes at zero extension; while another, which had been steamed without restraint to produce a super-contraction of 37% developed a longitudinal swelling of as much as 10%.

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Summary.

(1) From the examination of several hundred X-ray photographs of mammalian hairs, spines, etc., under a great variety of conditions, and comparison with the results of numerous collateral studies, the following conclusions have been drawn concerning the molecular structure and elastic properties of the keratin complex, which is the basis of all such epidermal growths.

(2) The X-ray fibre photograph of stretched hair (β-keratin) is analogous to that of natural silk (fibroin), whether stretched or unstretched. Stretched hair is therefore built of extended polypeptide chains, while unstretched hair (α-keratin) must consist of the same chains in a folded state, so that the elastic mechanism is that of a reversible intramolecular transformation.

(3) By means of "quadrant photographs" it is shown that the molecular complex of α-keratin stretches reversibly by about 2% before the onset of the main transformation.

(4) The limiting elastic extensibility of all mammalian hairs is about 100% of their initial, unstretched length. By combining this fact with the X-ray data exact quantitative agreement is found by assigning to α-keratin intramolecular folds of the nature of linked pseudo-diketopiperazine rings which open up on extension to produce the normal zigzag protein chain.

(5) These folds ("secondary folds") are transverse to the general direction of the side-chains.

(6) The side-chains of β-keratin are roughly coplanar and serve to unite neighbouring main-chains by a variety of cross-linkages, including both co-valent and electro-valent bonds. This leaves still another fold (the "primary fold") in the main-chains even in the extended configuration and reduces the average length of an amino-acid residue to rather less than is found in fibroin.

(7) The structure of β-keratin is one of flat polypeptide " grids " adhering by virtue of attractions between (=CO) and (=NH) groups of the main-chains of neighbouring grids. The normal equilibrium form of these grids involves folds (the " secondary folds ") transverse to the grids. The general intramolecular elastic mechanism of keratin thus falls into line with that of simpler molecules, though the usual possibilities are enormously enhanced by the mobility of both main-chains and side-chains and by longitudinal attractions between electro-positive and electro-negative centres in the latter.

(8) The available chemical analyses of keratin are in close agreement with the concept of an average volume per amino-acid residue of $3{\cdot}38 \times 4{\cdot}65 \times 9{\cdot}8$ A., as deduced from the β-photograph, the first dimension being the average length of a residue, the second the thickness transverse to the side-chains, and the third the average width in the plane of the side-chains. It is shown how these quantities may be used to calculate the average density of all proteins and the average thickness of mono-molecular protein films.

(9) An approximate tentative scheme for the distribution of the keratin side-chains is proposed.

(10) The elastic properties of hair may be referred to three main variations on the keratin theme, three " phases," K_1, K_2, and K_3, which have been associated with inter-cellular keratin, cell-wall keratin, and intra-cellular keratin. These phases, in the order given, show increasing powers of resistance to extension and the action of reagents, and function elastically both in series and in parallel.

(11) It is shown how, by the progressive action on keratin under stress of water at increasing temperatures, or by the action of dilute caustic soda at ordinary temperatures, the special side-chain differences between the three phases may be successively eliminated, so as to lead finally to a constant, " generalized " load/extension curve as the limit of a continuous series of " restricted " curves commencing with that of normal hair.

(12) It is shown directly by X-rays that water adsorbed by keratin fibres penetrates not only between chain-bundles but also into their interior, and that during the hydrolytic modification of β-keratin by steam or dilute alkalis spacing disturbances occur in the direction of the side-chains.

(13) These side-chain disturbances are the intramolecular basis of the decay of tension which occurs when hair is held stretched in water, and which is the logical beginning of the series of changes which culminate in the phenomena of temporary and permanent " set."

(14) Temporary and permanent " set " arise from the re-distribution of side-chain linkages, chiefly, but probably not uniquely, those of an electro-valent character, following on hydrolytic modification of the keratin grid when in a state of impressed deformation. It is thus simply a manifestation of the principle that the equilibrium form of the grid is a function of the nature and distribution of its linkages.

(15) Intermediate between the stages of side-chain breakdown and side-chain re-combination there is a labile stage characterized by enhanced powers of contraction (to

as much as 45% below the normal unstretched length). This new phenomenon (" super-contraction "), together with the subsequent " setting " process in hot water and steam has been studied in quantitative detail.

(16) It is found that the observed limit of super-contraction can be explained quantitatively if it is defined by the closest approach of acidic and basic side-chains.

(17) The elastic recovery of β-keratin to α-keratin is a function of its degree of hydration, but is further complicated by the side-chain re-distribution which occurs when water acts on the strained complex. The adsorption of water by hair appears to have the features of a " permutoid " reaction.

(18) It is found that irradiation of unstretched keratin with ultra-violet light or X-rays causes intramolecular changes analogous to those caused by the action of water, etc., on stretched keratin, with the result that both super-contraction and " set " are accelerated. Unstretched hairs after sufficient irradiation with X-rays contract spontaneously in steam.

(19) The action of $K_2Cr_2O_7$ and of eosin is to retard the process of " set " in steam.

(20) Continuous variation in super-contraction may be found from one end of a wool fibre to the other.

(21) The reaction of water, etc., with stretched keratin or irradiation of unstretched keratin by X-rays causes increased longitudinal swelling.

DESCRIPTION OF PLATES.

PLATE 8.

FIG. 19.—" Quadrant photograph " showing the initial reversible stretching of α-keratin before the intramolecular transformation into β-keratin (porcupine quill).

FIG. 20.—Photomicrograph showing cortical cells at the tip of a diseased hair.

FIG. 21.—" Generalized " α-keratin (Cotswold wool).

FIG. 22.—" Generalized " β-keratin (human hair).

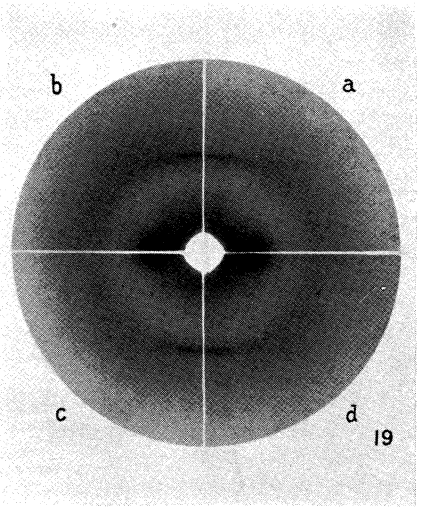
FIG. 23.—Human hair stretched in steam to twice its initial length. The " spreading " of certain spots along the hyperbolæ shows that there is a spacing disturbance in the direction [001] only.

FIG. 24.—Fibre photograph of natural silk (fibroin) (Cu Kα rays, cylindrical camera).

FIG. 25.—Fibre photograph of human hair at an extension of 100% (*cf.* fig. 24). (Cu Kα rays.)

PLATE 9.

FIG. 26.—Atomic models illustrating the grid-like structure of the keratin complex. (A) α-keratin viewed in the direction [001] to show the " secondary fold." (B) The same viewed in the direction [100] (equal cross-links). (C) The same pulled out into the β-form and viewed in the direction [100]. (D) β-keratin with unequal cross-links viewed in the direction [100] to show the " primary fold."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d
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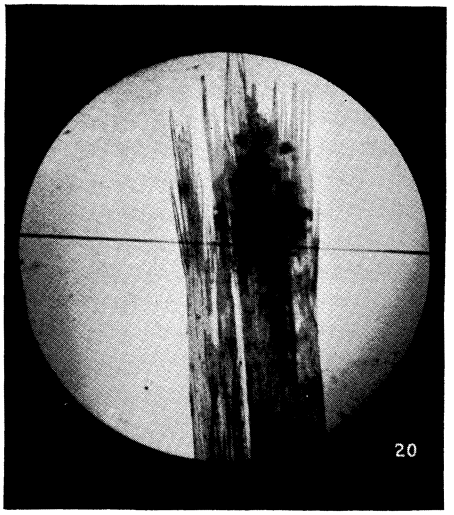
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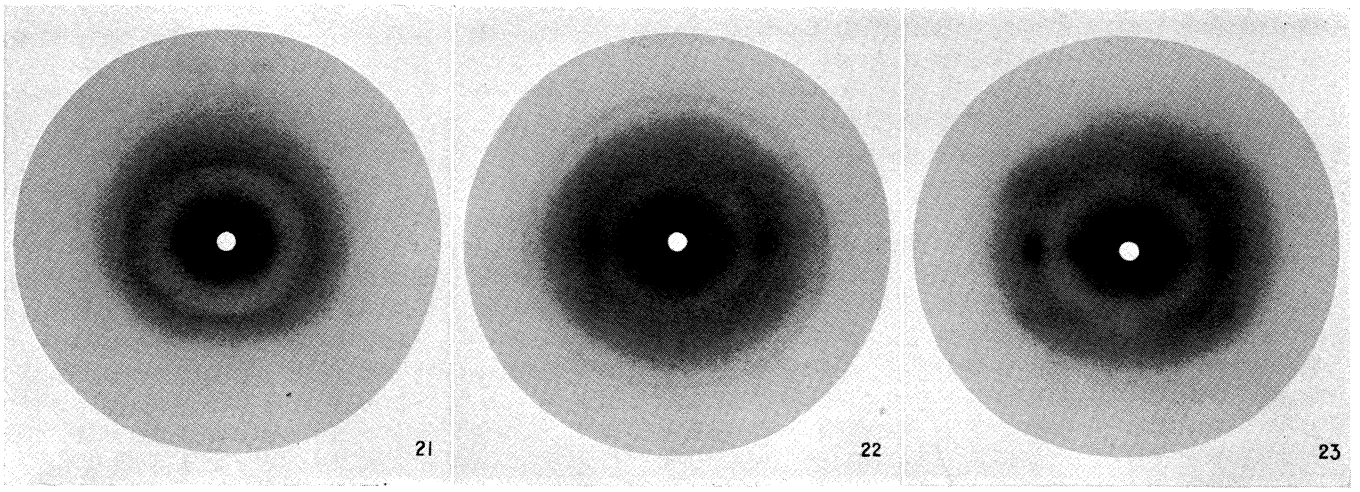
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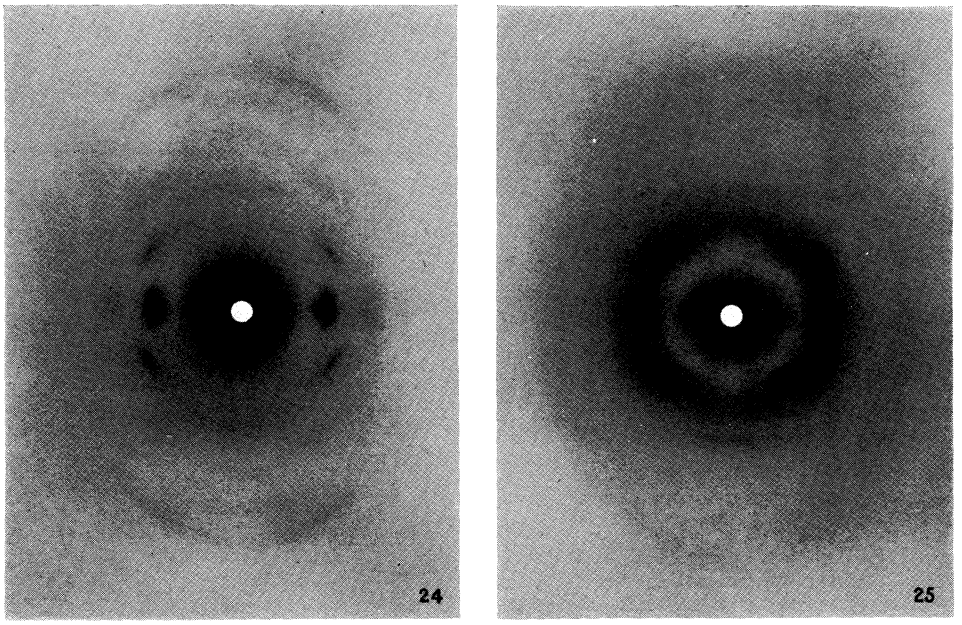
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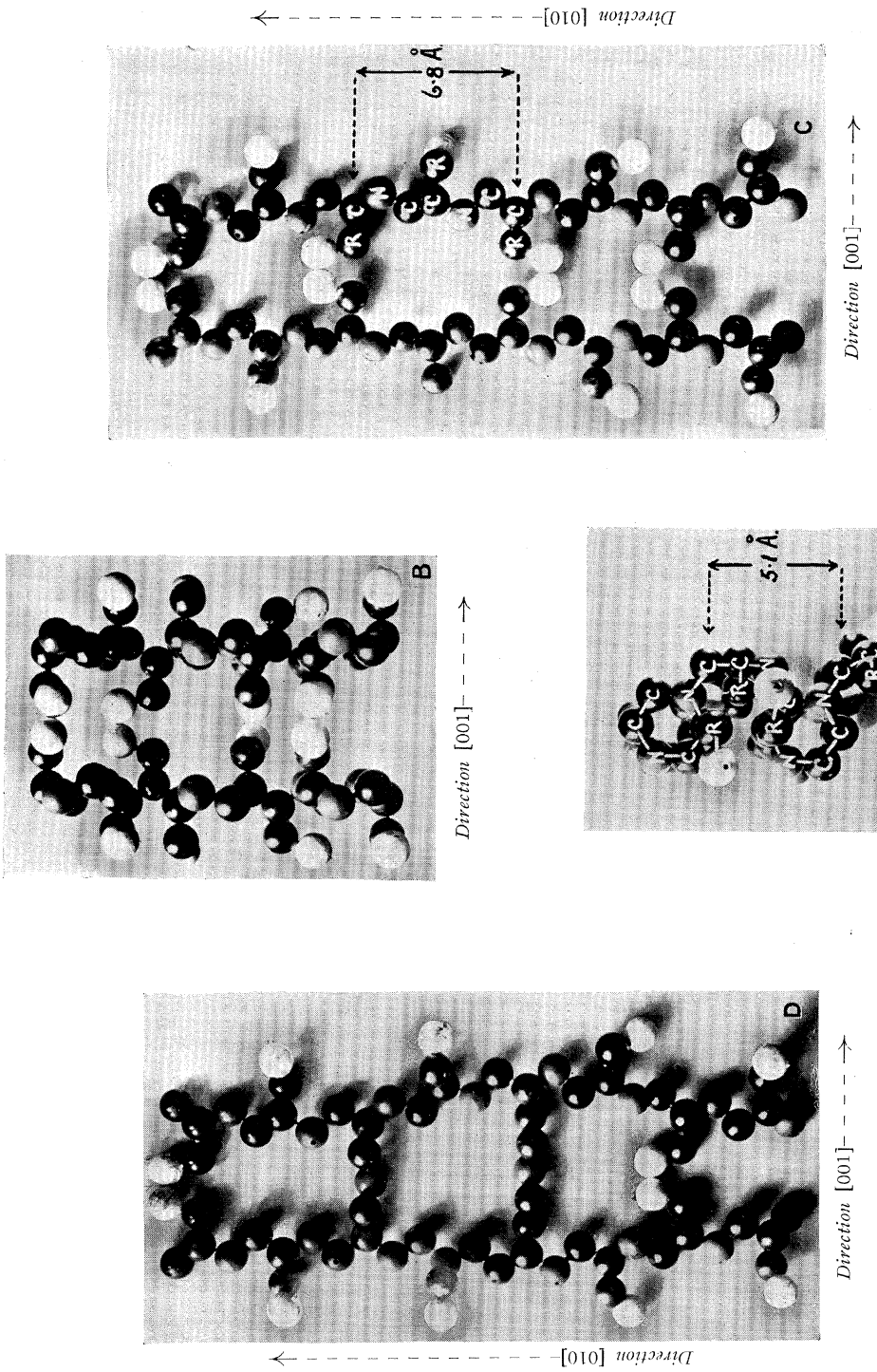
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6·8 Å.
C
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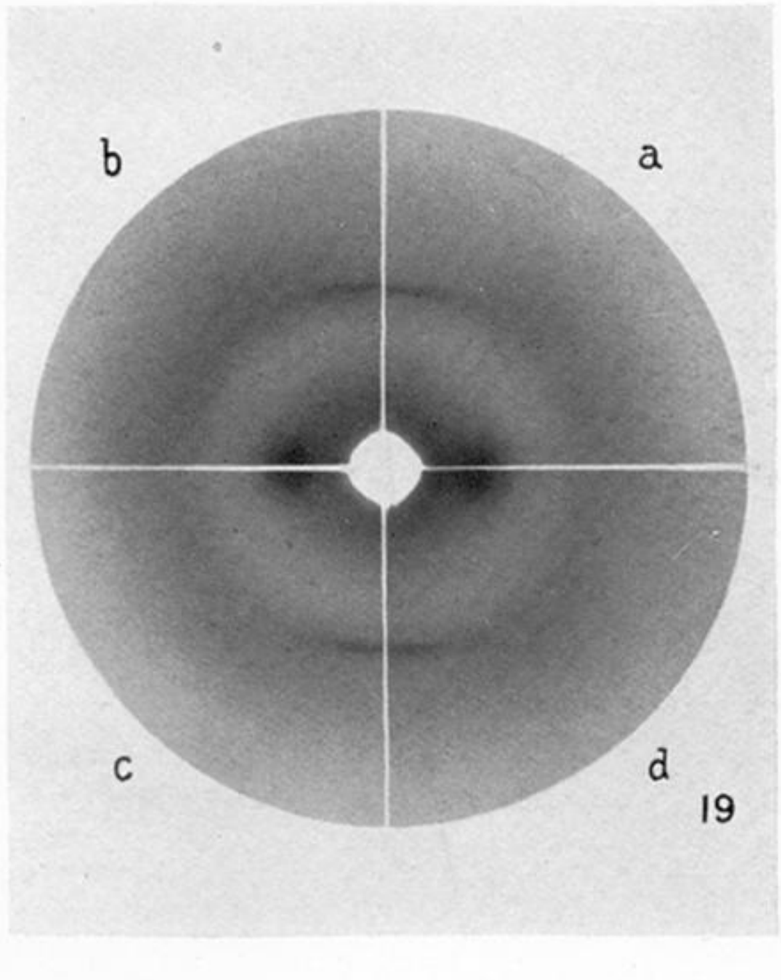
Direction [001]
Direction [010]

Fig. 26.



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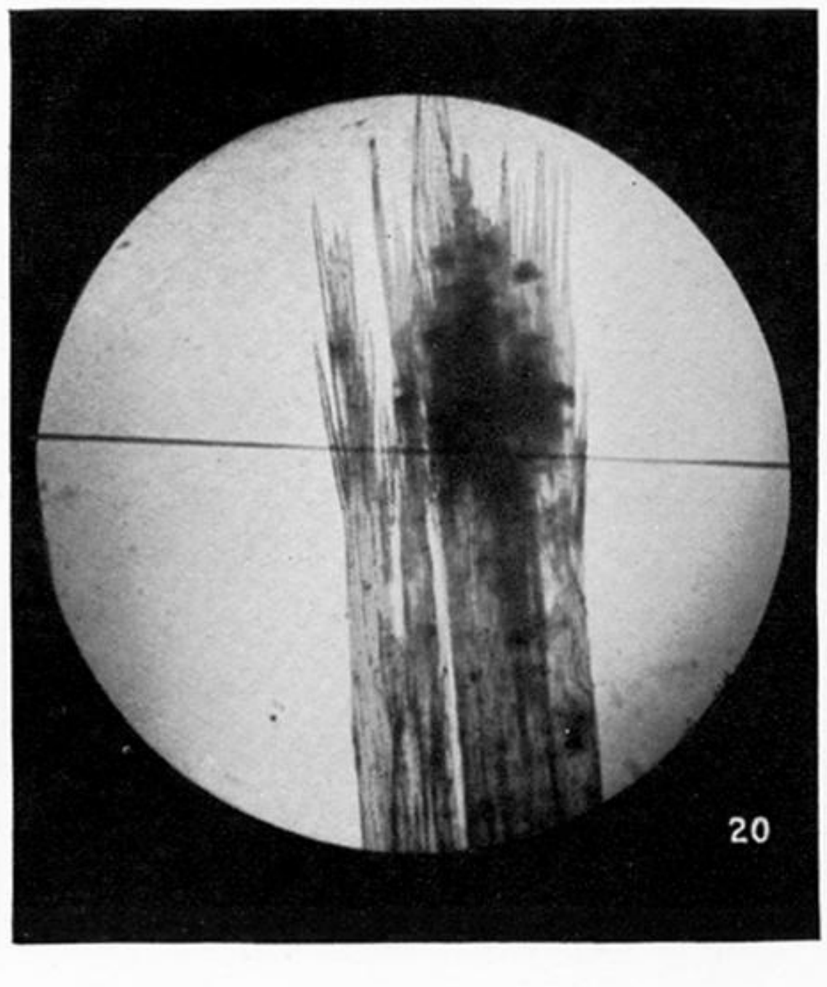

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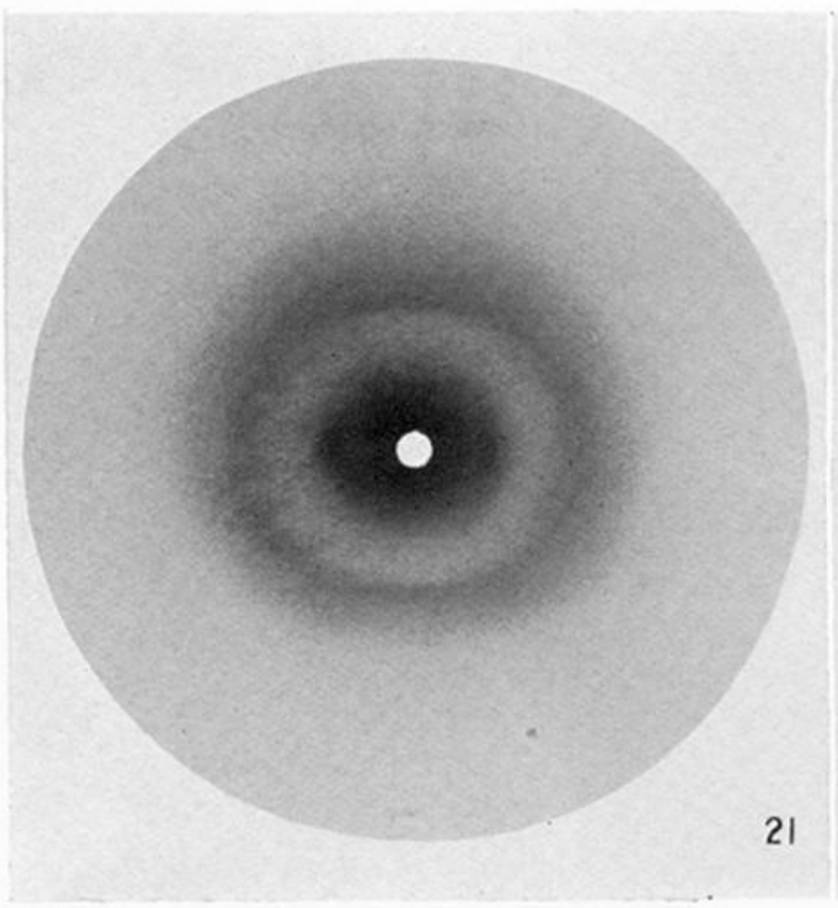

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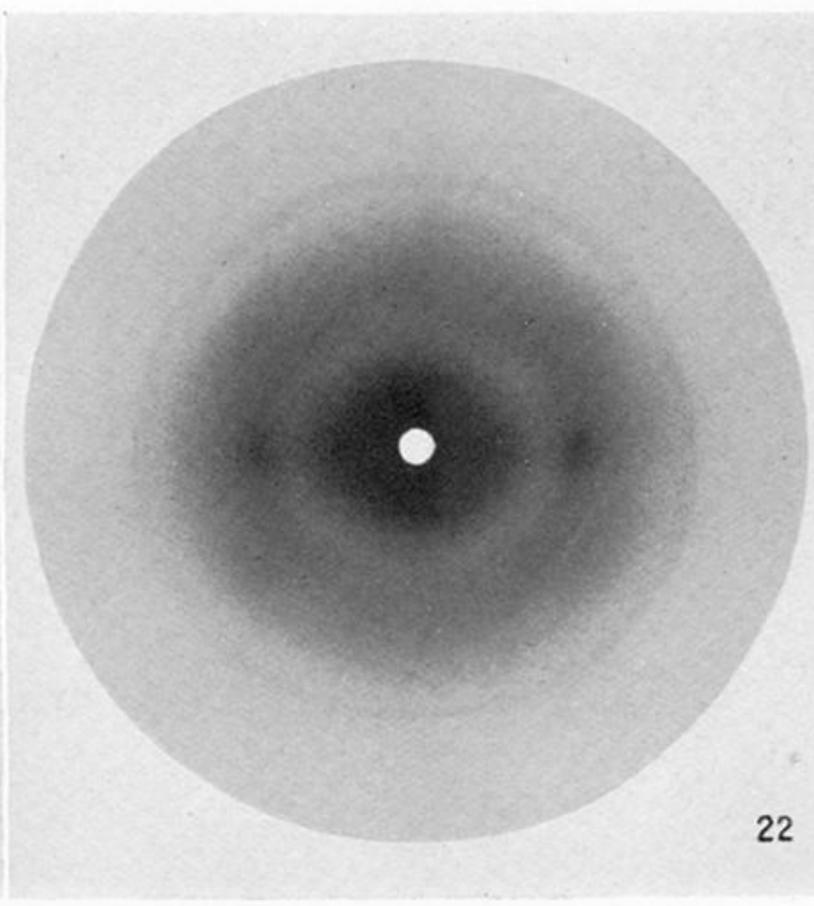

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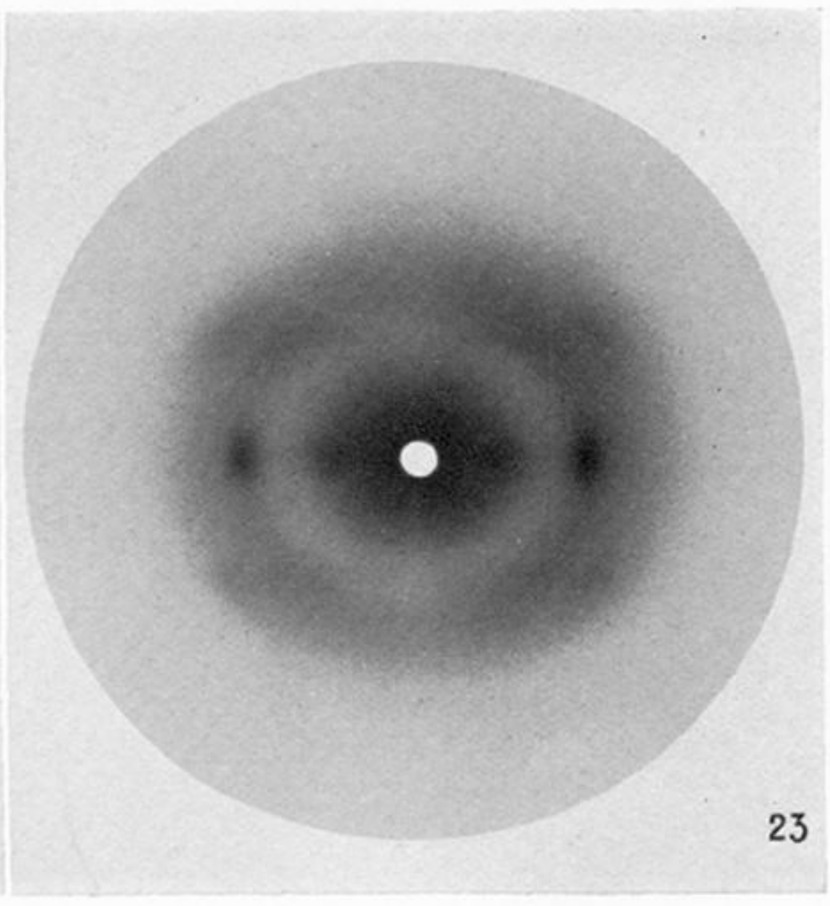

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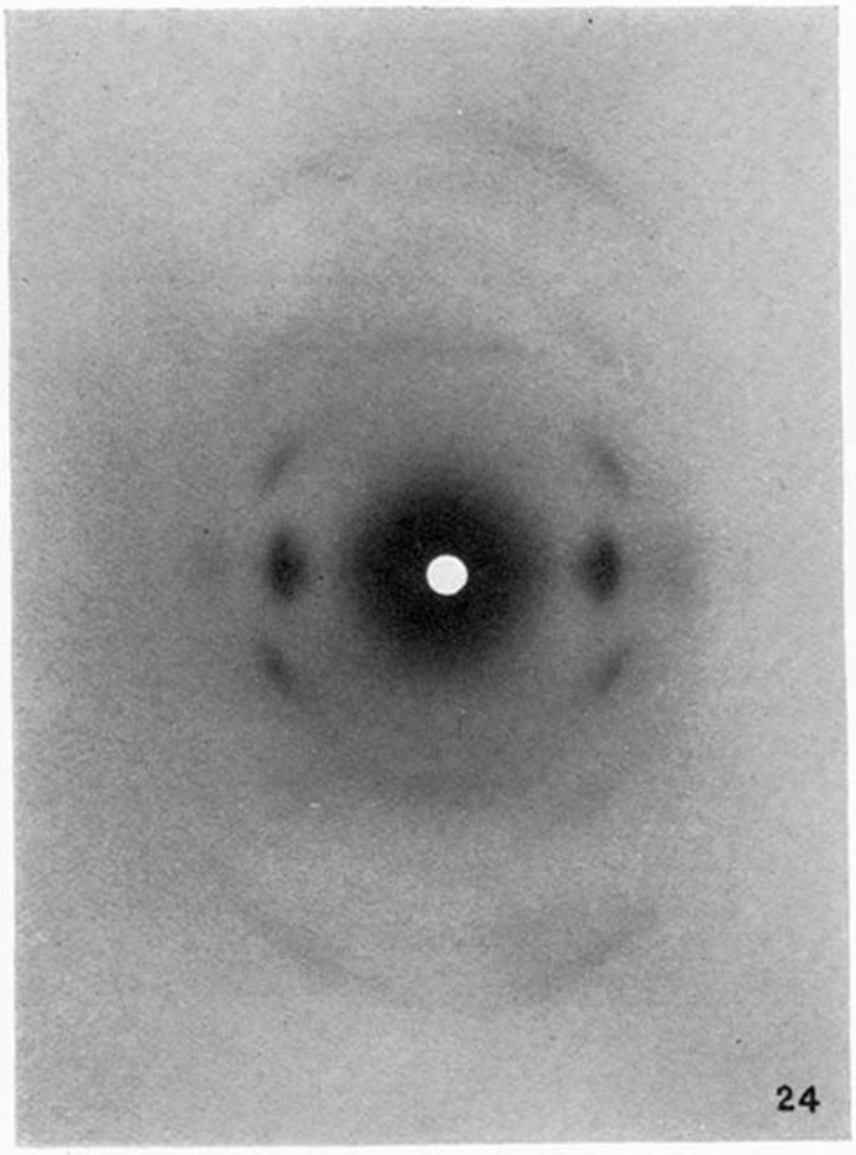

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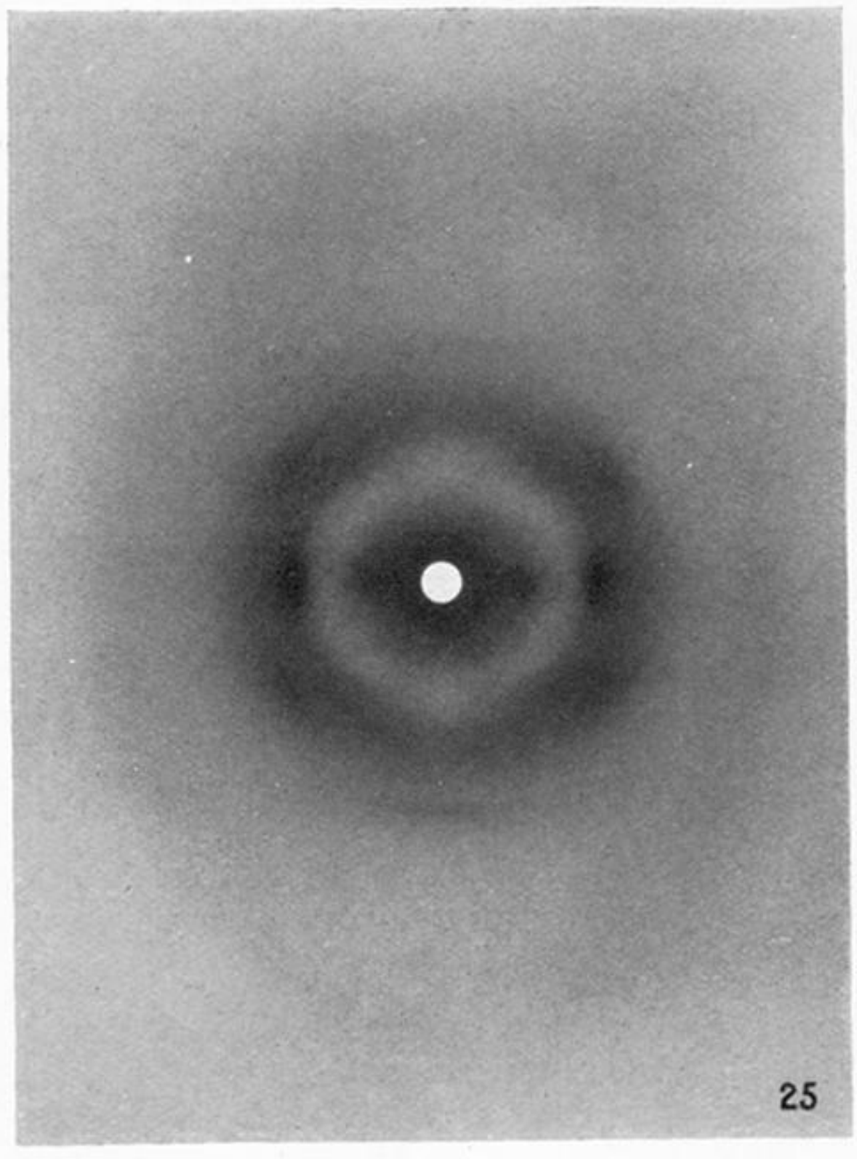

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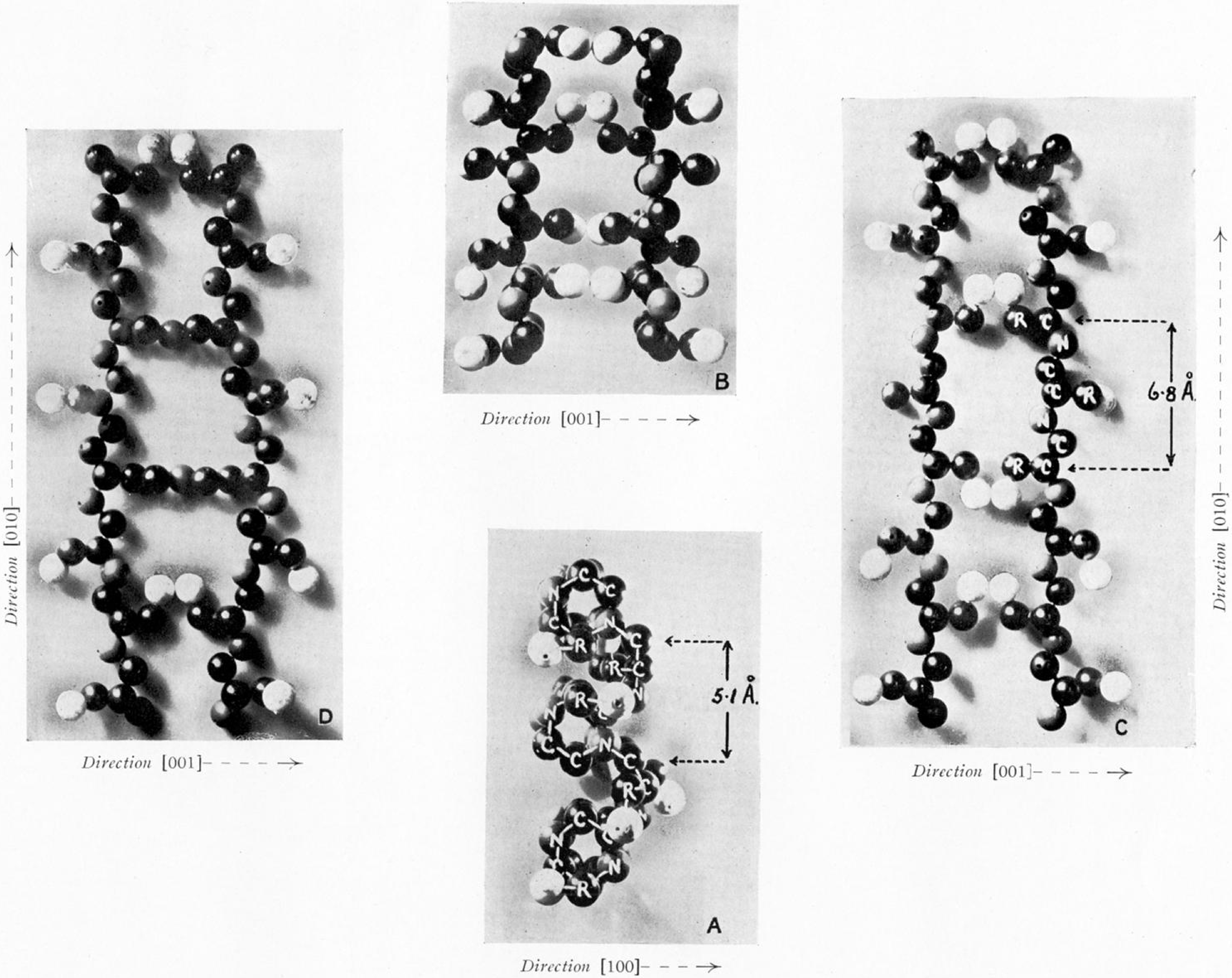
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Fig. 26.