CCLXXIX. THE X-RAY INTERPRETATION OF DENATURATION AND THE STRUCTURE OF THE SEED GLOBULINS.

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The X-ray diffraction photographs usually obtained from apparently non-fibrous proteins have so much in common with one another and with the photographs given by certain natural protein fibres when disoriented that the inference seems clear that all proteins at some stage of their existence are fibrous in the molecular sense [Astbury, 1933; 1934, 1, 2]. Recently [Astbury and Lomax, 1934, 1, 2; 1935] this concept has been expressed as a generalised interpretation of denaturation in the conclusion that the two more stable and insoluble states of protein structure, the fibrous and the denatured, are based on fundamentally similar modes of molecular arrangement; that, in fact, the denatured state[2] is essentially a fibrous state inasmuch as it always consists of peptide chains, often fully extended, and aggregated after coagulation in parallel bundles, as in fibroin [Meyer and Mark, 1928], β-keratin [Astbury and Street, 1931; Astbury and Woods, 1933], β-myosin [Astbury and Dickinson, 1935], fibrin [Katz and De Rooy, 1933] *etc.* It was found that heat-denaturation of the albumins, for instance, merely makes the X-ray photograph more like that of a random arrangement of fibres of β-keratin (stretched hair, horn, *etc.*; *cf.* Plate V, Figs. 1 and 3). β-Keratin is built from almost fully-extended polypeptide chains linked side-to-side, firstly by combinations between their side-chains ("side-chain linkage"), and secondly in a direction at right angles [Astbury and Sisson, 1935], through attractions between the =CO and =NH groups of neighbouring main-chains ("backbone linkage"). The three-dimensional structure is that of a pile of polypeptide "grids", the average distance apart of the main-chains in the plane of each grid being about 9·8 Å. ("side-chain spacing") and the distance between the grids being 4·65 Å. ("backbone spacing"). These two spacings correspond respectively to the two reflections, 001 and 200, seen on the equator of Fig. 4: they are the two principal side-spacings of the β-keratin crystallites, whilst the reflection 020 (spacing about 3·4 Å.) gives the average length of an amino-acid residue in the direction of the main-chains. Fig. 4 is a "fibre photograph" of crystallites all pointing roughly in the same direction (the fibre-axis) and taken with the X-ray beam perpendicular to this direction; but when the crystallites are

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[2] The seed globulins, at least, appear to pass through an intermediate fibrous state before reaching complete denaturation. It is possibly better therefore to reserve the word "denatured" for the final fibrous state analogous to β-keratin, and to describe intermediate states as "degenerate" (see below).

disoriented the result is very like (though not strictly identical with) Fig. 1, which is an X-ray view along the fibre-axis of stretched horn.

The disoriented photograph of β-keratin thus appears mainly as two more prominent rings, the inner corresponding to the side-chain spacing and the outer to the backbone spacing, and a less prominent ring arising mostly from the "diagonal reflection" 210 (spacing about 3·7 Å.) but partly also from the "meridian reflection" 020 (spacing about 3·4 Å); and this is just the kind of photograph that is typical of a coagulated denatured protein [Astbury and Lomax, 1934, 1, 2; 1935], as is shown clearly by reference to Fig. 3, the photograph of boiled egg-white dried in the air.[1] Practically all proteins so far examined by X-rays under ordinary conditions give either a photograph of a similar kind or one structurally related to it [*cf.* also Katz, 1934], and it is a reasonable enough inference that most proteins as commonly available for X-ray examination are either degenerate or completely denatured.

Svedberg's [1930] derivation with the ultracentrifuge of the molecular weight and globular form of many proteins in solution suggests such an inference, but the question has recently been settled crystallographically by the work of Bernal and Crowfoot [1934] and Crowfoot [1935] on the structure of unaltered crystalline pepsin and insulin. Large crystal spacings have also been observed by Clark and Corrigan [1932], Fankuchen [1934] and Wyckoff and Corey [1935], but Bernal and Crowfoot have been able to carry the X-ray analysis sufficiently far to show geometrically that the molecules in both crystalline pepsin and insulin are indeed very much of the shape (roughly spherical) and weight (34,500) deduced by Svedberg [1931] and his collaborators [Sjögren and Svedberg, 1931; Philpot and Eriksson-Quensel, 1933]. Even single crystals of pepsin,[2] however, give the usual powder photograph of the type just described, once they have been allowed to dry [Astbury and Lomax, 1934, 1]. Obviously then, most so-called crystalline proteins are only pseudomorphs after degeneration or denaturation: the molecules tend towards "cannibalism", so to speak, and their complex structure is frequently so precariously balanced, because it is so complex and so rich in charged centres, that they may have to be protected even from one another. This is the reason why protein crystals, for stability, generally require such a high water content, or to be kept in some specific mother-liquor. On dehydration or raising the temperature, for instance, one can imagine active side-chains reaching out to their neighbours, upsetting the balance of the whole system and leaving finally only the débris of the original special configuration. X-rays indicate that this débris, whether produced by disintegration or by unidirectional rearrangement, consists simply of peptide chains.

So far the argument has been indirect: it has been based on the study of protein "powder photographs" in relation to the "fibre photographs" of macroscopic fibres such as β-keratin, which is now known beyond reasonable doubt to be constructed out of parallel bundles of fully-extended polypeptide chains. Clearly then, if it is sound, it should be possible to make (probably elastic) macroscopic fibres even out of a "globular" protein, once it has been denatured. If polypeptide chains are there, but in random orientation, it should be possible to draw them into parallel alignment and so to obtain, not merely a powder photograph resembling that of disoriented β-keratin (Fig. 1)—which, after all, still does not constitute an incontrovertible proof of similarity, in spite

[1] An almost identical photograph is given by serum albumin, denatured and coagulated by gently heating an aqueous solution [Astbury and Lomax, 1935].

[2] From the same source as those of Philpot and Eriksson-Quensel and of Bernal and Crowfoot [Northrop, 1930].

of the strong support afforded by hydration studies [Astbury and Lomax, 1935]—but a genuine fibre photograph analogous to that of oriented β-keratin (Fig. 4). Such an experiment, if successful, would provide a wholly satisfactory demonstration for the protein under examination—and thence, by implication, for proteins in general—that the denatured state is indeed a fibrous state arising out of the liberation or spontaneous generation of peptide chains.

Denatured fibres and films.

The vegetable globulins edestin [Svedberg and Stamm, 1929], excelsin and amandin [Svedberg and Sjögren, 1930], legumin [Sjögren and Svedberg, 1930] and pomelin [Krejci and Svedberg, 1934] have been examined with the ultracentrifuge and found to constitute a group of molecular weight about $6 \times 34{,}500$ and molecular shape not far removed from the spherical. Edestin is thus a typical "globular" protein, very definitely crystalline, which should serve admirably for the X-ray test of denaturation just proposed.

In the first experiments edestin was denatured by dissolving in aqueous urea, and fibres were prepared by squirting the viscous solution so formed (8% protein and 43·5% urea) into water, or water containing 0·5% NaCl, at various temperatures up to about 55°. The fibres were then stretched in water or in the salt solution at temperatures up to about 70°. All showed striking, largely reversible, long-range elasticity, the extensibility increasing with the temperature of stretching up to something of the order of 700% at 70°. Those squirted and stretched at ordinary temperatures could be extended in water to just over twice their initial length and quickly recovered almost completely when released, whilst others stretched at higher temperatures extended further but showed only imperfect recovery. X-ray examination of these various types of fibre was hardly as profitable as had been hoped, but at least it drew attention once more to the part played by temperature in the alignment process (*cf.* rubber) and emphasised the point that denaturation does not necessarily proceed by a single stage (see below). At least two kinds of incipient fibre photograph were obtained, but nothing so definite as those shown in Figs. 4 and 5. The experiments were not for the time being carried further, partly owing to the tedium of preparing parallel fibre bundles sufficiently large for X-ray examination and partly on account of the simplicity with which it was found that films could be made.

Denatured edestin films were made by two methods, similar in principle to those used for the preparation of myosin films [Astbury and Dickinson, 1935]. In the first the urea solution mentioned above was poured evenly over a glass slide which was then gently inserted into water until the protein was deposited as a thin layer. This was then left in water for about half an hour, allowed to dry, and the process repeated several times in order to build up a deposit of suitable thickness, which was finally re-wetted and peeled off. Several superposed thicknesses were then compressed between pieces of plate glass, and the resulting film was allowed to become almost dry and cut into strips.

In the second method an aqueous solution containing 4% protein and 10% $CaCl_2$ was poured on to a glass plate, allowed to dry, washed for about 10 min. and drained. As before, the process was repeated several times and the accumulated layer peeled off wet and washed overnight in running water. A number of superposed layers were then compressed between plate glass to about one third their initial thickness and cut into strips.

The urea films gave extensions of 200–250% at about 40° and contracted when released in water to a residual extension of the order of 100%, whilst one calcium chloride film gave an extension of 280% with a similar residual extension

on release. On the whole, the calcium chloride films gave better-defined X-ray photographs, the urea films, like the corresponding fibres, sometimes showing besides traces of a second fibre photograph. Fig. 2 is a "powder photograph" of unstretched denatured edestin prepared from urea solution (*cf.* Figs. 1 and 3), and Fig. 5 is a "fibre photograph" of the film from calcium chloride solution which was stretched to an extension of 280% (*cf.* Fig. 4). There can be no doubt of the success of the experiment and of the configurational analogy between stretched denatured edestin and β-keratin. The side-chain spacing for denatured edestin is about 10 Å., the backbone spacing about 4·5 Å., the diagonal spacing about 3·7 Å., and the meridian spacing about 3·3 Å. (*cf.* β-keratin, above).

Preliminary experiments of a similar nature were carried out with excelsin also, but so far it has not been found possible to obtain a photograph strictly comparable with Figs. 4 and 5. Both elastic fibres and films were prepared as described above, but only imperfect orientation was attained even in a film (made from calcium chloride solution) stretched by 330%. The photographs obtained however were certainly crude "fibre photographs" of peptide chains, with the side-chain reflection and the backbone reflection both converging on the equator as in Figs. 4 and 5; but there was always present another reflection of spacing about 6 Å. A photograph of this type had already been found with edestin fibres spun from urea solution and stretched by 270%, and a similar photograph was obtained again later with a stretched film of denatured egg albumin prepared from urea solution (see below); but pending further investigation it will be more convenient at the present stage to suspend judgment as to its more exact interpretation.

The most interesting result was obtained with the X-ray examination of "poached" films of egg-white. Svedberg [1930] showed that the egg albumin molecule in the ultracentrifuge is practically spherical and of weight 34,500, and in view of the strong resemblance between Figs. 2 and 3 an enquiry into the effect of stretching the denatured protein was obviously indicated. Films of egg-white were poached for about a quarter of an hour at the bottom of a small covered beaker standing in boiling water, allowed to dry and then cut into strips. Some of these strips, if not re-wetted too much, were found to stretch to twice their initial length or more, and to give when stretched X-ray photographs such as that shown in Fig. 6. The striking—and, it must be confessed, unexpected—feature of this photograph is that the backbone reflection lies, not on the equator, but on the meridian. The side-chain reflection lies on the equator, as in Figs. 4 and 5, but in the original negative there can be seen also a vague outer equatorial reflection apparently corresponding to the amino-acid spacing; that is to say, 020 and 200 have changed places. The interpretation of this interchange between backbone and amino-acid spacing must mean that the peptide chains in denatured egg-white are relatively so short that the average length of the crystallites in the direction of the main-chains is shorter than their thickness in the direction of the backbone spacing; in other words, whereas the chain-bundles in β-keratin and denatured edestin are much longer than they are thick, those in denatured egg-white are actually shorter than they are thick. Fig. 7 illustrates the point. *A* represents, purely diagrammatically, a crystallite formed by the parallel alignment of long peptide chains, whilst *B* represents a corresponding crystallite built from short chains. In both the amino-acid spacing is in the direction of the main-chains of course and the latter are linked side-to-side by side-chain linkages (shown) and backbone linkage (not shown) at right angles. The chain-bundles so formed are thinnest in the direction of the side-chains [Astbury and Sisson, 1935] and lie normally along the natural fibre-axis or the axis of stretching.

If however it should happen that the main-chains are shorter than the width of the bundles in the direction of the backbone spacing (the side-chain dimension is apparently always short), the act of stretching will tend now to bring the backbone spacing parallel with the axis of extension and leave on the equator not merely the side-chain reflection, which is usual, but also the reflection corresponding to the length of the amino-acid residues. This is what happens when denatured egg-white is stretched: but the difference between chain-length and the thickness of the crystallites in the direction of the backbone spacing cannot be very great, otherwise short arcs or spots would be obtained instead of the comparatively long arcs shown in Fig. 6.

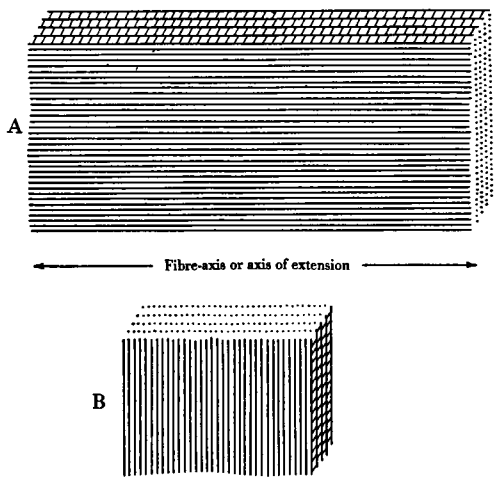


Fig. 7. Two ways of orienting crystallites (parallel bundles) of peptide chains by stretching denatured protein. *A*, long chains (keratin, denatured edestin *etc.*); *B*, short chains (denatured egg-white).

Films of denatured egg albumin were also made from urea solution (5% dried protein and 44% urea). The solution was spread on glass, allowed to evaporate, and the urea crystals washed out, after which several superposed strips were pressed between pieces of plate glass. Strips of the resulting film were stretched in 0·5% aqueous NaCl at 40°, but extensions of the order of 70% only were realised before rupture. The X-ray photograph of this urea film appeared to be of the type mentioned above as having been obtained with edestin fibres made from urea solution and excelsin films made from calcium chloride solution; that is to say, it showed not only the side-chain and backbone reflections, but also another reflection of spacing about 6 Å. Furthermore, orientation was shown by the side-chain reflection only, indicating that the length of the main-chains was more nearly equal to the thickness of the crystallites in the direction of the backbone spacing than was the case with the films of poached egg-white; in fact, one may think of the crystallites in the urea egg albumin films as more of the nature of square than oblong tablets.

It will be convenient to resume the discussion of the structure of the above-described denatured fibres and films after a short account of the results obtained with single excelsin crystals. One further point however may be noted here, *viz.* that they were all shown to be insoluble in water or 5% aqueous sodium chloride.

Crystalline excelsin.

The detailed X-ray investigation of crystalline excelsin will be reported later, but the unique results even now available have such an important bearing on the theme of the present paper that a brief preliminary account is offered at once, though only on the understanding that no finality is claimed at the moment.

Rapid dialysis of excelsin solutions gave microscopic hexagonal plates, whilst crystallisation from alcohol-water-sodium chloride gave rhombohedra. Very slow dialysis at 25° of a solution containing 2% protein and 10% NaCl gave however unusually large plates, up to some 2 mm. across, of a variety of shapes (mostly pentagonal) apparently derived from the hexagonal by inequalities of growth and truncation by rhombohedral and different prism faces. Owing to the demands of other work at the time these large excelsin crystals were unavoidably—but fortunately, as it happens—stored for some weeks in water.

For the first trial a crystal was lodged on the top of a short glass capillary tube the lower end of which dipped into a tiny trough of water, and the whole arrangement, which undoubtedly kept most of the crystal wet, was mounted on the X-ray spectrometer. The crystal was then photographed with the basal plane oscillating 5° on either side of the direction of the X-ray beam. The result was a composite photograph showing not only large crystal spacings like those given by crystalline pepsin and insulin [Bernal and Crowfoot, 1934; Crowfoot, 1935], but also an unmistakable fibre pattern. A similar composite photograph, not quite so sharp, was obtained also from a similarly oriented crystal dried in the air and mounted in the ordinary way. Furthermore it was demonstrated that it was unnecessary to oscillate the crystal in order to obtain the fibre diagram, as is usual in the X-ray examination of natural fibres. With the X-ray beam perpendicular to the basal plane another set of large crystal spacings was obtained and quite a different fibre diagram, very obviously multiple this time and symmetrical about a triad axis and three planes of symmetry. Finally intermediate fibre patterns were obtained with intermediate positions of the crystal.

The detailed analysis of these composite excelsin photographs will probably take some considerable time, but already it is clear that the fibre pattern corresponds fairly closely to the powder photographs of tobacco-seed globulin and squash-seed globulin (and probably edestin) described recently [Astbury and Lomax, 1935]. It is definitely not a fibre pattern corresponding to that of fully-denatured edestin (Fig. 5). At the moment it appears that the original crystal structure is symmetrical about a triad axis perpendicular to three dyad axes, and that it degenerates into six sets of fibrous crystallites lying approximately parallel with the basal plane, with the side-chain spacing (11·4 Å. wet) also roughly parallel and the backbone spacing (4·55 Å.) roughly perpendicular to this plane. Even after this metamorphosis followed by drying in the air the crystals retain their sharp outlines and remain perfectly transparent and isotropic along the triad axis. Drying the crystals in the air was found to reduce their water content from 39·2% to 11·8% of the crystal weight (or from 64·4% to 13·3% of the protein weight). Their solubility in 5% aqueous NaCl at 25° was found to be 14 mg. of protein per 100 ml., though undenatured excelsin forms thick opalescent solutions.

Preliminary attempts have been made to obtain corresponding results from edestin crystals, but hitherto the latter have yielded little more than the backbone spacing in random orientation. Edestin crystals are cubic however and the presence of four triad axes instead of only one might be expected to lead to more or less random degeneration.

Discussion.

Three main questions raised by the present communication invite a few concluding remarks: (1) that of successive stages of degeneration or denaturation; (2) that of the apparent striking difference in chain-length between denatured egg-albumin and edestin; (3) that of the structure of the unaltered seed globulins and the actual mechanism of their degeneration.

(1) The present experiments show directly that the seed globulins pass through at least one intermediate stage before final denaturation. This intermediate stage is fibrous but uncommonly well crystalline for a fibrous protein, in which respect it resembles feather keratin [Astbury, 1934, 1, 2]. It seems to correspond to the *proteans*, edestan, excelsan *etc.*, described by Osborne [1924, Chap. VIII], who determined the amounts of such products generated during contact with water or dilute acids. In this connection it should be noted that the excelsin crystals examined by X-rays were also found to have almost lost their solubility in salt solution (see above); they would perhaps be more correctly described therefore, not as excelsin, but as excelsan.[1]

Other X-ray results quoted above also suggest the existence of successive stages of degeneration or denaturation: excelsin itself for instance, presumably denatured and at any rate quite insoluble and in the form of parallel chain-bundles, has not yet been brought to give a fibre pattern of the strict β-keratin type; and both edestin and egg albumin, though it is true that each gave such a photograph finally,[2] have been shown to give a slightly different fibre pattern, like that of excelsin, in fact, under certain conditions of denaturation. In view of these findings, if it should turn out that the β-keratin type of pattern is indeed a sound general criterion for the last stage of denaturation, then it may be found convenient to adopt the proposal put forward at the beginning of this paper, that the term "denatured" be reserved for this final state and the term "degenerate" for intermediate states.

It should perhaps be emphasised that though a coagulated, fully-denatured protein is probably always configurationally analogous to β-keratin in the sense that it consists of bundles of parallel peptide chains, the converse is by no means true. A protein may be built out of parallel polypeptide chains, *e.g.* myosin [Weber, 1933; Astbury and Dickinson, 1935], yet still retain its solubility and other characteristic properties. Such proteins may be said to be "configurationally disposed" towards denaturation: once the chains are given the chance of more intimate lateral contact, by drying for instance, the system denatures with extreme readiness. The properties of myosin illustrate this point well, and even gelatin becomes progressively more insoluble on long standing.

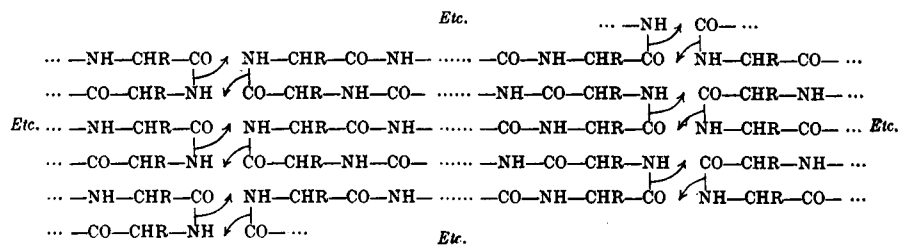
[1] One curious point must however be mentioned, *viz.* that the specimens of tobacco-seed globulin and squash-seed globulin whose X-ray powder photographs most closely correspond to the fibre diagram given by the degenerate excelsin crystals are still largely soluble in salt solution, whereas the edestin specimen (only a minute amount was available for test) is apparently not. But it would be profitless at this stage to discuss the significance of such an isolated observation: it is obviously necessary to study a much wider range of specimens before attempting any definite conclusions as to the interrelations between degeneration, solubility and X-ray diffraction pattern; and in any case it is clear enough from other results reported above and elsewhere that there are important variations among the seed globulins, whether degenerate or not.

[2] In spite of its unfamiliar lay-out the egg albumin photograph (Fig. 6) may be described for present purposes as of the β-keratin type, though exact correspondence cannot be considered to have been proved till better resolution has been attained through more perfect orientation of the crystallites.

(2) It was found that solutions of edestin in 20% aqueous calcium chloride gave only brittle, non-elastic fibres when squirted into water. The globulin under these conditions is not denatured, and as might be expected, is now incapable of producing true fibres in the molecular sense. Solutions in urea however, as already described, do give genuine fibres which are beautifully elastic when wet, for this solvent denatures the globulin. These urea solutions can be very viscous, and the development of such a property obviously corresponds to the increase in viscosity reported by Loughlin and Lewis [1932], for example, when egg albumin is denatured, the explanation of which is now clear. In the light of the present X-ray observations the denaturation of a "globular" protein such as egg albumin or edestin must almost certainly be accompanied by an increase in viscosity and, moreover, may open up the possibility of spinning true (probably elastic) fibres. As we have seen, this twofold prediction is fully confirmed in the case of the seed globulins, but it is only half justified in the case of egg albumin. Solutions of egg albumin in urea are comparatively thin and give when squirted into water only the poorest fibres which break almost at a touch. Films are more coherent and elastic but still do not show anything like the extensibility and elastic range of denatured edestin films.

This behaviour is just what one might infer from the X-ray photographs. Viscosity, extensibility and diffraction pattern all agree therefore in suggesting that the peptide chains in denatured egg albumin are relatively short, and clearly there is here a most promising line of attack on the structure of the unaltered globular proteins. It would hardly be justifiable at the moment to discuss exactly how, but it seems more than likely that the greater solubility of the albumins as compared with the globulins will ultimately be traced to the difference in chain-length revealed by X-ray examination of the denatured state.

(3) A reasonable inference from the experiments described above is that the unaltered structure of the seed globulins must be fairly closely related to the fibrous configuration of the proteans, otherwise one would hardly expect the degeneration of excelsin to cause so little disturbance of the external form and internal symmetry of the original crystal structure. Perhaps a factor contributing to this minimum of disturbance is the sixfold nature of the seed globulin molecules; for according to Svedberg and his collaborators they are made up of stable groups of six protein units of weight 34,500, which might conceivably be already joined by primary valencies. But whether this be so or not, a simple rearrangement of valency bonds consistent with existing experimental data to produce straight from folded chains may be illustrated diagrammatically thus:



Bernal and Crowfoot [1934] recently suggested a ring-chain polymerisation analogous to the formation of polyoxymethylene from trioxymethylene in order to explain the production of peptide chains when the globular protein pepsin degenerates [Astbury and Lomax, 1934, 1, 2], and Bernal [1934] has expressed the

opinion that long peptide chains do not pre-exist in the albumins and globulins at all; but experimental evidence at the moment is inconclusive, and some such transformation of a "gridiron" structure as that given above might very well harmonise the chain aspect of proteins with the polymerisation mechanism proposed by Bernal. The excelsin results reported here do certainly appear to support the polymerisation idea in a most striking fashion, yet it seems improbable that all degeneration of globular proteins so develops, and the difference in chain-length between denatured egg-albumin and denatured edestin may be due, in part at least, to the non-occurrence of polymerisation when the former is produced.

However that may be, it may now fairly be claimed that the link has been found between the long-chain proteins typical of natural fibres and the globular proteins investigated by Svedberg. Globular edestin has been transformed into elastic threads of what may now appropriately be called β-edestin, globular excelsin has been photographed in the actual process of polymeric degeneration into an intermediate fibrous form, and egg albumin itself, almost the prototype of globular proteins, has also been shown to be changed by denaturation into peptide chain-bundles similar to, but shorter than, those found in denatured edestin. The significance of these results in the study of fibre-building in the living organism may be very great indeed. Above all they strengthen the hypothesis that such fibre-building is no other than a kind of controlled and directed manifestation of the familiar "laboratory" degeneration of proteins, and that the X-ray photograph of feather keratin, for instance, contains not only the pattern of extended polypeptide chains, but also the dimensional impression left by the smaller units from which they were originally constructed [Astbury, 1934, 1, 2].

Needless to say, it is intended to examine shortly excelsin crystals which have not been allowed to stand long in water, but continued progress in the elucidation of the inner structure of the globular proteins must depend much on being able to catch intermediate forms such as the proteans. It cannot be overemphasised that the keratin-like β-form appears to be in general only the last product of successive stages of degeneration.

Summary.

1. The X-ray interpretation of protein denaturation suggests that it always involves the liberation or generation of peptide chains which aggregate on coagulation into parallel bundles like those found in the structure of β-keratin and similar fibres.

2. For the globular protein edestin this has been confirmed directly by the preparation of denatured elastic threads and films which give, on extension, an X-ray photograph analogous to that given by stretched animal hairs *etc.*

3. Denatured egg-white, when stretched, gives also an X-ray photograph of a similar type, though of an orientation which can be explained only if the peptide chains are much shorter than those found in β-keratin and denatured edestin.

4. Crystalline excelsin has been photographed in process of symmetrical degeneration *in situ* into an intermediate fibrous form provisionally identified with excelsan.

5. The results support the polymeric theory of the formation of fibrous proteins and provide a link between the structure of the natural fibres and that of the globular proteins.

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DESCRIPTION OF FIGURES IN PLATE V.

Fig. 1. X-ray photograph of stretched horn (55% extension) looking along the fibre-axis (effectively disoriented β-keratin).

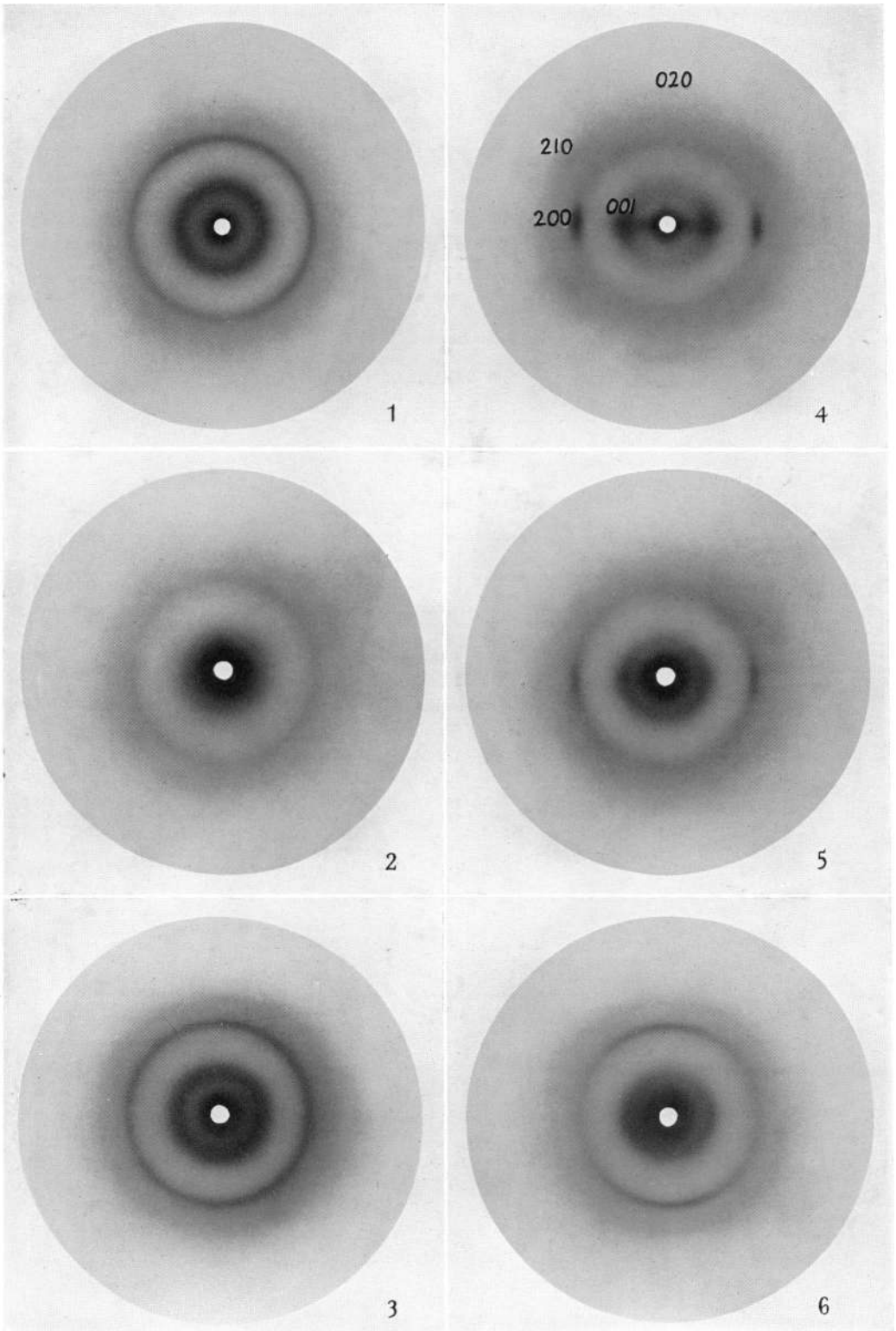
Fig. 2. X-ray photograph of edestin precipitated from urea solution (disoriented denatured (or β-) edestin).

Fig. 3. X-ray photograph of "poached" egg-white (disoriented denatured (or β-) egg albumin).

Fig. 4. X-ray photograph of stretched horn taken perpendicular to the fibre-axis (oriented β-keratin: fibre-axis vertical).

Fig. 5. X-ray photograph of edestin film (prepared by evaporation of $CaCl_2$ solution) stretched by 280% (oriented denatured (or β-) edestin: axis of extension vertical).

Fig. 6. X-ray photograph of "poached" egg-white stretched by 100% (partially oriented denatured (or β-) egg-albumin: axis of extension vertical).



Figs. 1–6.