Cold Spring Harbor Symposia on Quantitative Biology

SOME RECENT DEVELOPMENTS IN THE X-RAY STUDY OF PROTEINS AND RELATED STRUCTURES

W. T. Astbury and Florence O. Bell

Cold Spring Harb Symp Quant Biol 1938 6: 109-121
Access the most recent version at doi:10.1101/SQB.1938.006.01.013

SOME RECENT DEVELOPMENTS IN THE X-RAY STUDY OF PROTEINS AND RELATED STRUCTURES

W. T. Astbury and Florence O. Bell

This paper falls naturally into three sections, for the reason that over the last few months we have been interested in three main topics; and on the principle that almost anything about proteins is interesting, even at a protein symposium, there is no very great difficulty about either offering or accepting them together.

I. X-rays and the Stoichiochemistry of Proteins

A troublesome aspect of protein structure which has always been at the back of one's mind, and is now very much to the forefront, has to do with the stoichiometrical relations, or otherwise, between the constituent amino acid residues. Experimentally, the problem has proved so full of obstacles that until quite recently only the vaguest ideas emerged, for the available chemical analyses were both contradictory and incomplete. Vickery, Block (1) and others had demonstrated certain fairly constant ratios between the proportions of the basic residues, for instance, but it was not until the work of Bergmann and Niemann (2) that the stoichiometric outlines of protein structure became in any way recognisable. It is true, of course, that the specificity, the stepwise molecular weights, the X-ray diffraction patterns, etc., of proteins make it inconceivable that there should be anything seriously haphazard in their architecture; yet all the same, there was this apparent analytical dilemma.

From a consideration of their new data supplemented by results of others workers, Bergmann and Niemann conclude that both the total number of amino acid residues and also the numbers of residues of the various types are expressible in the form 2^n3^m, and on this basis the minimum molecular weights of chicken egg albumin, cattle haemoglobin, cattle fibrin, and silk fibroin are found to correspond to 288, 2×288, 2×288 and 9×288 residues, respectively. Now broadly speaking, the proteins fall, or seem to fall, into two principal types, the fibrous and the non-fibrous, which is roughly the same as the not-visibly crystalline and the visibly crystalline. Both types are undoubtedly, in the fundamental sense, based on polypeptide chains, but whereas X-ray (3) and physico-chemical studies hardly leave room for doubt that the former are essentially greatly elongated chain-groups, similar studies of the latter (4), and examination with the ultracentrifuge (5) in particular, make it just as certain that their molecules are large, and often effectively roundish, bodies[1] with, what is more important still, weights which are approximate multiples of some unit of the order of 17,000 to 18,000.

The earlier results with the ultracentrifuge left it an open question whether the multiple molecular weights of the globular proteins are really exact multiples of a unit *weight*; *a priori* it seemed unlikely, but in any case it is clear now that they are very probably multiples of, or are derived from, a unit *structure,* and the rather rough agreement between the weights within any one group is an expression of the fact that though the amino acid distribution is variable, the average weight per residue is always of the same order, namely, about 120. Each member of the 36,000 group, for example—the most familiar group of the globular proteins, containing egg albumin, pepsin, insulin, Bence Jones protein, etc.—must therefore be built up of some 300 residues.

The experimental link between the fibrous and the globular proteins is provided by X-ray studies of the elastic fibrous proteins, keratin and myosin (8), and of the mechanism of protein denaturation (9). Two points stand out: (*1*) that keratin and myosin in their normal, or α-, state are constructed of polypeptide chains thrown into a linear series of regular folds (10), leading to the suggestion (11) that the globular proteins are three-dimensional generalisations of such folds; and (*2*) that solutions of denatured globular proteins can be spun into artificial fibrous proteins which give, on stretching, X-ray photographs like those given by stretched keratin and myosin (12), implying that the act of denaturing a globular protein involves the unfolding of polypeptide chains from some specific folded configuration. For these and other reasons it is a fair conclusion that the stereochemistry of proteins is that of polypeptide chains *and their states of folding,* and that the linear fold first discovered in the fibrous protein keratin is only a prototype of the generalised folds of the globular proteins.

It is possible to give a quantitative interpretation of the X-ray photographs and elastic properties of keratin by postulating that the intramolecular folds of the α-form which are opened on stretching are hexagonal rings (10), and since this theory was first put forward it has been

[1] The term "globular proteins", which I believe I originated (6), seems now to have passed into use when referring to the macro-crystalline proteins; but because the molecules of many of them—the tobacco mosaic virus, for instance—are by no means spherical, but rather rod-shaped, perhaps a better term, proposed by Ostwald (7), would be "corpuscular proteins".

strengthened by the suggestion of Frank (13) that the opening of such rings might involve a lactam-lactim transformation; other possibilities are the opening of a hydrogen bond (14), or an enol-keto transformation (15).

Wrinch has made a systematic geometrical study of the types of molecule which may conceivably be built up by continuously folding the polypeptide chain in hexagonal rings of the lactam form (16)—the kind of ring originally proposed for the linear sequence of folds in α-keratin—and has developed in this way her "cyclol theory" of protein structure. Two deductions from this theory which are of immediate consequence for the present discussion are (*1*) that in general the molecular weights will increase in step-wise fashion, just as has been observed with the ultracentrifuge and other means; and (*2*) that in particular there is a closed globular structure (the C_2 cyclol) which requires 288 amino acid residues and would therefore correspond in weight to Svedberg's 36,000 group (17).

The point which now strikes the X-ray analyst with regard to the findings of Bergmann and Niemann is not simply that they have arrived at an experimental stoichiometrical basis for the results of Svedberg and his collaborators, but that chemical analysis places not only two undoubtedly globular proteins, egg albumin and haemoglobin, but also two undoubtedly fibrous proteins, fibrin and fibroin, in one and the same scheme of multiple molecular weights. Whatever the inner structure of the egg albumin and haemoglobin molecules, the X-ray photographs of fibrin and fibroin, when viewed in the light of other X-ray data on the fibrous proteins, can hardly be interpreted except in terms of polypeptide chains in the extended, or β-, configuration. Here then we seem to be debarred from invoking *directly* the idea of step-wise molecular weights defined by the geometry of closed globular systems or their aggregates, and all that we may infer for the moment is that there must be some common factor in the method of synthesis of all proteins, whether fibrous or globular—that, in fact, there is no real difference between the two types.

Various lines of X-ray and related evidence have been leading to a similar conclusion over the last few years (18): such, for example, are the high equatorial spacings in the fibrous proteins keratin, myosin, feather keratin, collagen (19), and fibrin (20), and the general analogy between the fibre photograph of feather keratin and that given by the tobacco mosaic virus (21). The X-ray diffraction diagram of feather keratin (and who can doubt, on examining it, that the protein molecule is a beautiful pattern? There must obviously be a stoichiometric basis for such regularity)—is full of instructive features, more, unfortunately, than we have as yet been able to decipher completely. The evidence is overwhelming that feather keratin is a long polypeptide chain system in a slightly constricted β-state, yet in a sense—and similar remarks apply to collagen—it is a globular protein too, of which the units must be massive bundles of chains, rod-like bodies, like the units of the tobacco mosaic virus. Indeed, many things point now to an analogy between the virus units, which like the diamond, are both molecules and crystals, and the long units from which the fibrous proteins are constructed. In the latter also it may be that the molecules are built on such a grand scale, and of such internal regularity of structure, that it is immaterial whether we speak of them as molecules or as crystals.

In view of these considerations it is natural to enquire whether X-ray studies can contribute anything to the actual stoichiometric bridge between the fibrous and the globular proteins, apart from confirming the molecular sizes and weights of certain of the latter (4). In the case of keratin, a highly resistant and insoluble fibrous protein not amenable to the more familiar methods of molecular weight determination, they can quite definitely, and can supplement the Bergmann-Niemann argument by offering a means of calculating the average residue weight *directly* (18); for there is no way of knowing this quantity directly by chemical means, owing to incomplete analyses. For β-keratin the average residue weight can be calculated from the density (1.3)[2] and the X-ray measurements of the average residue dimensions: 9.7 A for the side-chain spacing, 4.65 A for the backbone spacing, and 3.33 A for the length in the direction of the main-chain. These figures give an average residue weight of

$$\frac{9.7 \times 4.65 \times 3.33 \times 1.3}{1.65}\ ,^{3}$$

or about 118, corresponding to approximately 0.85 gram-residues per 100 gm. of keratin. The number 0.85, when divided by values of 2^n3^m, should give, according to the Bergmann-Niemann theory, the numbers of gram-residues of the various acids in 100 gm. of keratin. The following table shows the results of the calculations for wool keratin:—

[2] For an estimate of the density of β-keratin we are indebted to Mr. H. J. Woods.

[3] 1.65×10^{-24} gm. is the mass of the hydrogen atom.

Acid	*Frequency*	*Gm.-res. in 100 gm. wool*	
		Calc.	*Obs.*
Glutamic	8 (2^3)	0.106	0.103
Arginine	16 (2^4)	0.053	0.059
Aspartic	16 (2^4)	0.053	0.054
Tyrosine	32 (2^5)	0.027	0.027
Lysine	48 ($2^4.3$)	0.018	0.019
Tryptophane	96 ($2^5.3$)	0.008_9	0.009
Histidine	192 ($2^6.3$)	0.004_5	0.004
Amide-N	9 (3^2)	0.094	0.098
Cystine[4]	8 (2^3)	0.106	Mean value
Methionine	192 ($2^6.3$)	0.004_5	Mean value

Estimates have been published of the proportions of other amino acids than are quoted in the above table, but they are probably unreliable. Those quoted are now considered to be reliable[5], when account is taken of the well known acid-combining power of wool, for instance, and other factors.[6]

It will be seen from the Table that the measure of agreement is quite good, and though it is difficult yet to prove that only powers of 2 and 3 are involved, there is already sufficient evidence to warrant the belief that keratin also will be found to conform to the common stoichiometric plan. The first group of data in the Table requires a molecule containing a minumum of 192 residues, including 17 basic residues. The molecular weight is thus at least $192 \times 118 \approx 22{,}600$, and the equivalent weight comes to about 1,330, which agrees reasonably well with the estimate (about 1,250) given by acid absorption (28). Approximately one ninth of the residues are in the form of acid amides, and should it be that these amides mark out a strict pattern at such a frequency, that would mean a minimum number of residues of 2×288, corresponding to haemoglobin and fibrin.

The widely varying sulphur content of the keratins seems to be a real difficulty at the moment, for the cystine values do not fall into a number of groups, each conforming with the theory, as might be expected, though the mean estimates, both for methionine and cystine, conform roughly. It might be argued that there is a mixture of proteins each obeying the proposed law, but in that case any number, whether belonging to the 2^n3^m series or not, could be claimed to fit the theory. It may be, though, that this actually is the correct general solution, and that the problem is like that of alloys and mixed crystals; the law would then apply to amino acid "sites", and would be directly apparent in ideal or limiting cases only.

The conclusion then, taking everything into consideration, is that we have good reasons for the belief that ultimately the fibrous and the globular proteins will prove to be at least closely related, if not actually similar, in structure. A satisfactory scheme must include such greatly different molecular shapes as the fully-extended β-proteins and effectively spherical proteins such as insulin. The lessons of the intra-molecular transformation of keratin and myosin and of the production of artificial fibrous proteins from denatured globular proteins show at once how the whole field may be covered under the concept of the folded polypeptide chain, but we are still left with the question of a common law of multiple molecular weights applying both to β- and to round proteins. The problem reminds us of the old nursery difficulty, "Which came first, the chicken or the egg?" Either all proteins are, or have been, fibrous at some stage of their synthesis; or all are, or have been, globular. Really, all that we can say with safety yet is that they must have some common factor in their method of synthesis, though perhaps the balance of X-ray and related evidence at the moment rather suggests that in some way all proteins are fundamentally fibrous. The densities of the globular proteins, for instance, are all very much the same as those of the demonstrably fibrous proteins[7].

By an unfolding process akin to denaturation[8], however, or by a re-arrangement of the peptide linkages of globular molecules followed by secondary condensation (30), it should be possible to adapt to extended chains the concept of step-wise molecular weights determined initially by closed groups. The latter mechanism, in particular, gives a very reasonable explanation of an important characteristic of the X-ray diffraction diagram of feather keratin; for the feather diagram suggests a super-pattern imposed on a skeleton of extended polypeptide chains (31).

[4] Calculated as half-cystine residues.

[5] For advice on chemical points we are indebted to Dr. J. B. Speakman and Prof. A. C. Chibnall.

[6] The actual experimental yields (gm.) of the various acids on hydrolysing 100 gm. of wool are:—glutamic acid, 15.27 (Cotswold wool) (22); aspartic acid, 7.27 (Cotswold wool) (22); amide nitrogen, 1.37 (Cotswold wool) (22); arginine, 10.2 (23); lysine, 2.8 (23); histidine, 0.66 (24); tyrosine, 4.8 (23, 25); tryptophane, 1.8 (23); methionine, 0.44-0.67 (26); cystine, about 3½ p.c. sulfur, as a mean of wide variations (27).

[7] As an example of this Dr. H. B. Bull has kindly supplied us with the following densities of egg albumin measured in hydrogen at 25°C.:— native, 1.350 $\pm$ 0.008; surface denatured (coag.), 1.399 $\pm$ 0.005; heat denatured (coag.), 1.347 $\pm$ 0.004.

[8] As was suggested in a contribution to an earlier Symposium of this series (29).

II. An X-ray Study of Thymonucleic Acid and some of its Compounds with Proteins

The interplay of proteins and nucleic acids must constitute some of the basic, indispensable reactions of the life processes, and our understanding of either is incomplete without a knowledge of both. We are beginning to learn something about the shapes and patterns of the proteins, but the exploration of the stereochemistry of the nucleic acids is still only at its beginning. The time is now ripe to try to correlate their main structural features with those of the proteins, and the following preliminary X-ray study (32) holds out, we feel, a promise of success.

A few years ago cursive X-ray examination of a small specimen of sodium thymonucleate[9] gave an imperfect fibre photograph with a strongly-marked period along the fibre axis of about 3.5 A, but the observation was not followed up till recently, when the opportunity presented itself of continuing the investigation with material prepared in the Department of Chemistry of the Caroline Institute, Stockholm,[10] by the method of Hammarsten and Bang (33). The viscosity and double refraction of flow of this material in aqueous solution were the subject of a preliminary communication by Signer, Caspersson and Hammarsten (34).

Sodium thymonucleate is soluble in water in all proportions, forming, even at low concentrations, viscous solutions which can be spun into fibrous threads. The optical properties of the solid fibres have been investigated by Schmidt (35), who showed that they are strongly negative, while the solutions show streaming birefringence which is also negative (34). The hydrodynamic properties and examination with the ultracentrifuge indicate that the units in solution are particles about 300 times as long as they are thick, and of weight between 600,000 and 1,000,000 (34).

Films of sodium thymonucleate were made by quickly drying a pool of the sol on a glass plate, and stripping it off after moistening with 70 p.c. aqueous alcohol. Strips about 2 mm. wide were then stretched by about 250 p.c. in the same liquid. The drying on a surface causes the elongated units to lie approximately parallel to the surface, and the subsequent stretching swings them round to parallelism. An X-ray photograph with the beam perpendicular to the direction of stretching and parallel to surface of the film is shown in Fig. 1. As a "fibre photograph" it is still rather imperfect because of incomplete parallelism of the elongated units, but nevertheless it gives at once some valuable information regarding their structure.

[9] Kindly provided by Prof. W. J. Schmidt of Giessen.

[10] We are very much indebted to Prof. E. Hammarsten and Dr. T. Caspersson both for preparing for us the experimental material and for biochemical data.

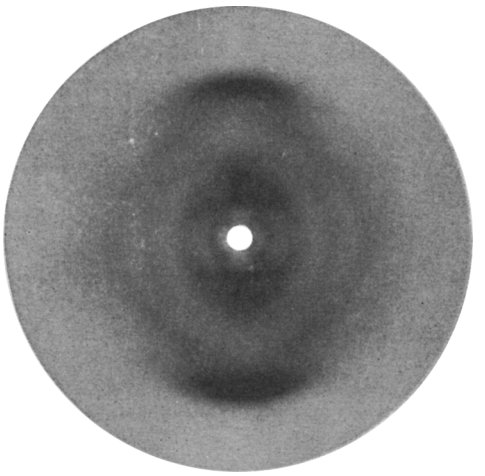

Fig. 1 X-ray fibre photograph of oriented sodium thymonucleate. (Direction of stretching — the fibre axis — vertical. Distance from specimen to photographic film = 4 cm. Cu $K\alpha$ rays.)

In the first place, there is the strongly-marked period along the fibre axis of 3.3_4 A. The natural conclusion is that this spacing is that of a close succession of flat or flattish nucleotides standing out perpendicular to the long axis of the molecule to form a relatively rigid structure which would be strongly optically negative and show streaming double refraction of the same character. Such a structure may be represented very diagrammatically by Fig. 2, right, in which each disc stands for

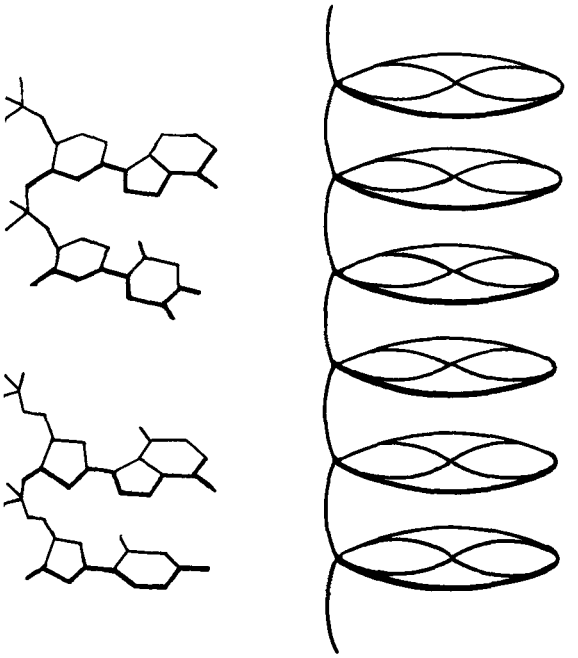

Fig. 2. Diagrammatic representation of the column of nucleotides constituting the unit of thymonucleic acid. Each nucleotide is spaced at 3.3_4 A from the next.

a nucleotide. Each nucleotide is made up of a purine or pyrimidine base linked to a sugar ring, and successive nucleotides are linked through phosphoric acid. Alternative formulae for a pair of (purine and pyrimidine) nucleotides are shown in Fig. 2, left, (36). Roughly, the idea is that of a very tall column of discs with a linking rod down one side.

At first sight the distance 3.3_4 A between successive nucleotides might seem improbably small, but the flat molecules of ascorbic acid (37) are piled together at an even smaller distance (3.16 A); and in any case it is confirmed by the high density, which is 1.62 - 1.63 gm./cc. for the dry substance, as compared with 1.74 gm./cc. for ascorbic acid. Using known interatomic and intermolecular distances, a plausible estimate of the effective dimensions of a purine nucleotide is something of the order of 15×7.5 A, while the average weight of a sodium nucleotide is about 330. The distance apart of successive nucleotides should therefore be roughly

$$\frac{1.65 \times 330}{15 \times 7.5 \times 1.62} \approx 3.$$

The photograph shown in Fig. 1 has received no more than a preliminary analysis, but the principal side-spacing is about 16.2 A. If now, as the properties of the solutions suggest, the molecule is some 300 times as long as it is thick, the molecular weight should be of the order of

$$\frac{16.2 \times 300 \times 330}{3.34}$$

or, say, half a million, which agrees reasonably well with the estimate quoted above given by the ultracentrifuge.

The combined evidence, then, is strongly in favour of a structure like that illustrated in Fig. 2, the unit in solution being the column-like molecule itself. The actual order of sequence of the nucleotides in the column is still unknown, but already the X-ray data raise the question of what interpretation should be attached to the present verdict of analytical chemistry, that only four different nucleotides are used, for the true period along the long axis seems to be at least seventeen times the thickness of a nucleotide. The four nucleotides can therefore hardly follow one another always in the same order.

The significance of these findings for chromosome structure and behaviour will be obvious, for we cannot fail to be struck by the fact that the spacing of successive nucleotides is almost exactly equal to *the spacing of successive side-chains in a fully-extended polypeptide chain* (see above). It is difficult to believe that the agreement is no more than a coincidence: rather it is a stimulating thought that probably the interplay of proteins and nucleic acids in the chromosomes is largely based on this very circumstance. In the mitotic cycle there is a rhythm also in the way nucleic acid makes its appearance in the chromomeres (38), and we can well imagine that some critical stage in mitosis, involving elongation of the protein chains, is realised under the influence of the linear period of the interacting nucleotides.

The idea is equivalent to saying that the molecule of thymonucleic acid fits so perfectly on the side-chain pattern of a fully-extended polypeptide chain that interaction should take place almost without any steric hindrance whatsoever; most easily between the basic side-chains and the phosphoric acid groups, but presumably, too, between the acid side-chains and the basic groups of the nucleotides. Furthermore, the products of combination should also be fibrous (33), like the two original constituents.

We were afforded an opportunity of carrying out a decisive test of this hypothesis through the kindness of Dr. Albert Fischer, of the Carlsberg Foundation, Copenhagen, who suggested that we should examine, among other things, the compounds formed by thymonucleic acid with clupein and edestin. Following his instructions, they were prepared as described below.

The clupein thymonucleate was formed as an elastic membrane at the interface when about 1 cc. of a viscous sodium thymonucleate solution at the bottom of a centrifuge tube was covered by a similar volume of clupein solution. After an hour or so the membrane was removed and well washed with distilled water.

The most effective way of orienting the molecules was found to be by rolling out the membrane, which was relatively tough, into a thin ribbon by means of a glass rod. An X-ray photograph (Fig. 3) was then taken with the beam parallel to the surface and perpendicular to the direction of rolling. The result was a "fibre photograph" *remarkably like that of sodium thymonucleate*—so very like, in fact, that at first sight the two photographs seem to be the same.

Actually, they differ in the reflections near the centre, and though again the clupein thymonucleate photograph has not yet been analysed, it seems clear from its general appearance that the "resemblance with a difference" is to be interpreted simply in terms of a fixation of the clupein chain down one side of the thymonucleate column. This would leave both the prominent period along the fibre axis, and also one of the principal side dimensions, practically unchanged, while increasing the other principal side dimension, just as the

Fig. 3. X-ray "fibre photograph" of oriented clupein thymonucleate. (Direction of stretching — the fibre axis, — vertical. Distance from specimen to photographic film = 4 cm. Cu K α rays).

transition from Fig. 2 to Fig. 3 suggests. It is known that the clupein molecule is an open peptide chain of up to 28 residues, of which as many as 20 are arginine residues (39), so this interpretation fits in perfectly with interaction between the arginine side-chains and the phosphoric acid groups to give a composite columnar molecule, which should still be optically negative. And the preparation of clupein thymonucleate just described *is* optically negative with respect to the long axis of the molecule, just as are the giant salivary chromosomes with respect to their length (40).

The chief components of the chromosomes are apparently compounds of protamines and nucleic acids. We have thus a first experimental indication of the direction of the protein chains in the chromosomes, namely, along their length, and therefore a reasonable molecular basis for the linear sequence of genes demonstrated by the cytologists. Knowing what we know now from X-ray and related studies of the fibrous proteins, how they are built from long polypeptide chains with linear patterns drawn to a grand scale, how these chains can contract and take up different configurations by intramolecular folding, how the chain-groups are penetrated by, and their side-chains react with, smaller co-operating molecules, and finally how they can combine so readily with nucleic acid molecules and still maintain the fibrous configuration, it is but natural to assume, as a first working hypothesis at least, that they form the long scroll on which is written the pattern of life (41). No other molecules satisfy so many requirements.

Edestin thymonucleate was prepared by adding a solution of crystalline edestin in N/100 HCl to a viscous solution of sodium thymonucleate and stirring with a glass rod; a fibrous mass adhered to the rod which could be stripped off and washed. When the fibres were suitably oriented and mounted, again an undoubted X-ray "fibre photograph" was obtained, but this time the diagram did not seem to resemble either that of sodium thymonucleate or that of stretched fibres of denatured edestin (42). However, further work is necessary to improve on these merely preliminary results, the important point for the present being that it is possible to obtain true fibrous products by combining thymonucleic acid not only with fibrous proteins such as clupein, but also with globular proteins such as edestin. The globular molecules must unfold during the course of the reaction, and presumably become fully extended under the influence of the period of 3.34 A along the thymonucleic acid molecules. The experiment thus lends support to the suggestion put forward above, that some stage in mitosis, involving elongation of the protein chains, may be realised simply by virtue of the controlling period of the nucleotides.

Other nucleic acids and polynucleotides and their compounds with proteins are now being studied and will be reported on in due course. The other chief nucleic acid, that of yeast, gives only thin solutions and a non-fibrous product on drying. Its X-ray photograph—only a ring photo has been obtained so far—is different from that of thymonucleic acid and still remains to be interpreted.

Optical and X-ray Examination and Direct Measurement of Built-up Protein Multilayers[11]

Blodgett, Langmuir and co-workers (43) have described how piles of protein monolayers may be built up on a chromium-plated metal slide by successive vertical movements of the latter through a monolayer spread on a liquid substrate. Following this technique, we have built up from recrystallised egg albumin films composed of up to 1764 monolayers (44).

A single monolayer of barium stearate was formed first on the slide by one upward movement through a monolayer of stearic acid spread on a 0.0001 molar solution of barium carbonate, using castor oil as a compressing substance. The protein was then laid down on top of the barium stearate by beginning with a downward movement through a mono-layer of egg albumin spread on a 0.003 molar buffer solution of pH as near as possible to the isoelectric point of the protein (4.7), and containing also 1 millimole of zinc sulphate per litre. After the succeeding upward

[11] This investigation is being carried out in collaboration with Prof. E. Gorter and Dr. J. van Ormondt, of the Hospital for Children's Diseases, Leiden (44).

movement the slide had collected two monolayers and was left to dry for a few minutes, whereupon the operations were repeated, and so on.

Our first apparatus was operated by hand, but a later design, which will be described in detail elsewhere, was completely automatic and enclosed. The movements of the slide were controlled by a cam, a counter registering the number of monolayers deposited, and the film was dried for four minutes in a slow stream of nitrogen after each upward movement.

X-ray examination of even the thickest films *in situ* gave only disappointing results, because of the overwhelming effect of reflections from the metal slide itself; but fortunately it was found that the thicker films could be stripped off the metal base without any great difficulty, and the way was thus opened up to all sorts of experiments.

The first significant observation on the detached films was that they tend to tear parallel to the direction in which the slide was moved through the liquid surface, suggesting a fibrous structure oriented in that direction. The next was that, unlike built-up films of barium stearate, for example (45), they are birefringent when viewed perpendicular to the surface, and the slow vibration is also parallel to the direction of movement of the slide. This again points to a fibrous structure, for the natural protein fibres are always birefringent with the slow vibration parallel to the fibre axis. The most striking observation, however, was that our first thicker films, made with the open hand-operated apparatus, show numerous boat-shaped holes, like negative tactoids, again all pointing parallel to the direction of movement of the slide, and framed by regions of much higher birefringence still. Fig. 4 is a photo-micrograph between crossed Nicols of a detached film of 1764 monolayers at 45° to the extinction position. It will be noted how all the "boats" are parallel to the edge of the film, which is parallel to the direction of movement of the slide, how the film as a whole is birefringent, and how the "boats" are framed by regions of much higher birefringence. The birefringence of the film as a whole, as judged by means of colour plates, is of the order of 0.007, while round the periphery of the holes it is probably as high as about 0.06.

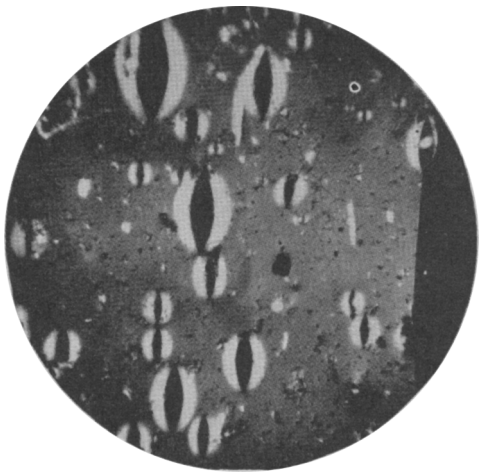

Fig. 4. Photo-micrograph between crossed Nicols, and at 45° to the extinction position, of a detached film built up from 1764 monolayers of egg albumin.

A preliminary optical examination of the 1764 film[12] was carried out with a view to placing also the fastest and intermediate vibrations, and the indications were that the fastest vibration lies in the plane of the film and perpendicular to the direction of movement of the slide, while the intermediate vibration lies normal to the plane of the film. The mechanical and optical properties of the film, and the shape and orientation of the holes, therefore all combine in the verdict that the built-up multilayers must consist of polypeptide chains preferentially oriented along the direction of movement of the slide, with their side-chains normal, or roughly normal, to the surface. A peculiarly satisfying argument in favour of this interpretation lies in the actual numerical value of the birefringence round the periphery of the holes, which is at least as high as that of silk fibroin (0.05).[13] Since there is apparently no thickening of the film round the holes comparable with the great increase of birefringence, the latter must be due to more perfect parallelism of the chains. The chains then, when viewed perpendicular to the surface of the film, must be analogous to fibroin chains, a distinguishing feature of which is that they are almost devoid of all but the shortest side-chains: in other words, the birefringence of the built-up multilayers when viewed perpendicular to their surface must arise from polypeptide chains *and their "backbone linkage"* (46), which leaves the side-chains themselves pointing normal to the surface.

By far the most perfect of our films, that of 1450 monolayers, was built up with the improved apparatus. Its optical properties were similar to those made by less careful means, but the boat-shaped holes were absent. It offered a piece of evidence, though, as to its fibrous nature perhaps even more convincing than that of the holes—a fringe along its lower edge of very delicate, but very real, fibres once more parallel to the direction of movement of the slide. It was fibrous not only in the molecular sense, but also in the macroscopic sense.

[12] In this connection, it is proposed to carry out a systematic optical examination of the 1450 film, which is very much more perfect (see below).

[13] For this measurement of the birefringence of natural silk we are indebted to Mr. H. J. Woods.

By dint of repeated folding, stripped ribbons of film were formed into flat, correctly oriented pads, and examined by X-rays. Photographs were taken with the beam perpendicular to the surface, parallel to the surface and perpendicular to the direction of movement of the slide, and parallel both to the surface and to the direction of movement of the slide. The results confirm directly the indications of the optical properties, and establish without doubt that built-up multilayers of egg albumin consist of polypeptide chains lying roughly parallel to the direction of movement of the slide, with their side-chains roughly perpendicular to the surface. Fig. 5 is the X-ray photograph taken with the beam parallel both to the surface and to the direction of movement of the slide.

Fig. 5 X-ray photograph of a pad of stripped multilayers of egg albumin taken with the beam parallel both to the surface of the layers and to the direction of movement of the slide. (Layers vertical. Distance from specimen to photographic film = 4 cm. Cu K α rays.)

It shows at a glance how the side-chain reflection is concentrated about the equator, while the backbone reflection is concentrated about the meridian; which means, since the plane of the layers is vertical and we are looking along the mean direction of the polypeptide chains, that the side-chain spacing lies roughly perpendicular, and the backbone spacing, roughly parallel, to the surface. The side-chain spacing is approximately 9.5 A and the backbone spacing 4.6 A, agreeing closely enough with values given by natural and artificial protein fibres in the β-form. As a matter of fact, from X-ray data given by the natural fibrous proteins, the deduction was made several years ago (46, 47) that the structure of protein monolayers must be such that the side-chains stand normal to the surface, and here at last we have a direct and quantitative proof.

From the X-ray photographs it follows also that the dried films are quite imperfect in the crystallographic sense—the preferential orientation is no more than approximate—whatever they were at the moment of laying down; and they are not composed of globular protein, in the sense of the original egg albumin molecule. The cohesive forces evoked by drying seem to have brought about aggregation at the expense of orientation, as one would rather expect. The main-chains of successive monolayers will not in general be parallel, and their side-chains will neither fit nor match, with the result that chain-bundles are formed which are only a compromise.

The simplest description of the X-ray photographs obtained up to the time of writing is that they are analogous to those of keratin that has been squeezed laterally in steam (48): this treatment not only brings about the α-β transformation, but also orients the side-chains perpendicular to the plane of flattening. Some of the film photographs show more reflections than those of keratin, and there is hope that with better orientation of the main-chains it may be found possible to determine the amino acid period.

The appearance of boat-shaped holes in the earlier films was a very fortunate accident—an anisotropic accident, so to speak, of which the cause raises a point of some interest. Either they were produced during the process of drying on the metal plate, or they had formed while the monolayer was still on the liquid substrate. It was observed that in general they did not penetrate through the whole thickness of the built-up film, and the most probable explanation of their origin is that they were produced by oil-contaminated specks of dust. Oil-contaminated particles would split a fibrous protein monolayer, and the holes would naturally be drawn out in the direction of movement of the metal slide; furthermore, as successive monolayers were superposed on the slide, the splitting effect arising from the contamination of a single particle, aided by the tendency of parallel chain-molecules to orient others parallel to themselves, might well be transmitted through many neighbouring monolayers.

In any case, one deduction stands out of quite fundamental importance, and that is that since the up-and-down movement of the slide through the monolayer on the liquid substrate has resulted in the deposition of polypeptide chains roughly parallel to the direction of movement, we have here the familiar process of spinning long-chain molecules (12), not into a filament, but into a ribbon: *and the chains must pre-exist on the surface of the substrate*. In egg albumin monolayers there cannot be groups closed more tightly than can be opened and oriented by the movement of the slide. The original globular molecules have unfolded and have therefore, in this sense, become denatured, aggregation being afterwards brought

about by drying on the metal slide. The whole process is the two-dimensional analogue of spinning artificial fibrous proteins from globular proteins denatured and dissolved in strong urea (12). And we know that orientation must have taken place on the surface of the liquid substrate, because if denaturation had taken place only on drying on the slide, the result would have been not an X-ray photograph showing preferential orientation, but simply a ring photograph.

Finally we have succeeded in measuring the thickness per monolayer by direct mechanical means. Our first method was to insert the film under one of the feet of a small three-legged interferometer specially constructed for the purpose,[14] thus altering the angle of an air-wedge included between two pieces of optically flat glass. The upper piece of glass was attached to a balanced metal frame supported by the three legs, and the lower formed the actual table on which rested the film and the three feet (Fig. 6). The three feet marked out an isoceles triangle of

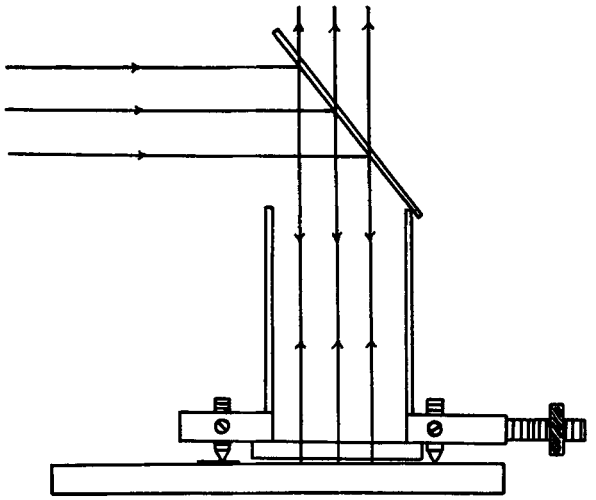

Fig. 6. Small interferometer for direct measurement of the thickness of built-up protein multilayers.

height about 2 cm., the film was inserted under the foot at the apex, and the system was balanced about the line forming its base. Approximate balance was achieved in the design itself of the apparatus, final balance, so that the apical foot pressed on the film as lightly as possible, being attained with the aid of a small balancing screw. The measurement consisted in counting with a travelling microscope the number of interference bands (sodium light) first without and then with the film inserted under the apical foot.

In this way, and it should be noticed that *no optical properties of the film itself are invoked,* we have measured the thicknesses of films composed of 600, 800, 1000, 1450, and 1764 monolayers, respectively, and the results all agree in fixing the thickness per monolayer at about 9.5 A, with a probable error of the mean of about 0.2 A. This value agrees perfectly with the side-chain spacing given by the X-ray photographs.

The second method of direct measurement was simply by means of a screw micrometer. A single film was too thin to measure in this way, but ribbons of film were repeatedly folded so as to build up pads of various thicknesses. The accuracy of measurement was not high, admittedly, but it was sufficiently good to point once more to a thickness per monolayer of about 10 A. And anyway, it was a sheer delight to be able for the first time to measure, if only approximately, the thickness of a protein chain by so ridiculous a means!

[14] For collaboration in constructing this interferometer we are greatly indebted to Mr. H. J. Woods.

REFERENCES

1. Cf. R. J. Block, Yale J. Biol. and Med., *9*, 445 (1937).
2. M. Bergmann and C. Niemann, J. Biol. Chem., *115*, 77 (1936); *118*, 301 (1937); Science, *86*, 187 (1937).
3. Cf. W. T. Astbury, Compt. rend. trav. Lab. Carlsberg, *22*, 45 (1938).
4. J. D. Bernal and D. Crowfoot, Nature, *133*, 794 (1934); D. Crowfoot, Nature, *135*, 591 (1935); D. Crowfoot, I. Fankuchen, J. D. Bernal and M. Perutz, Nature, *141*, 521 (1938).
5. See, for example, T. Svedberg, Nature, *139*, 1051 (1937).
6. W. T. Astbury and R. Lomax, Nature, *133*, 795 (1934). See also, for instance, Nature, *137*, 803 (1936); Chem. Weekbl., *33*, 778 (1936).
7. W. Ostwald, Kolloid-Beiheften (1935).
8. For summary, see W. T. Astbury, Compt. rend. trav. Lab. Carlsberg, *22*, 45 (1938).
9. W. T. Astbury and R. Lomax, J. Chem. Soc., 846 (1935); W. T. Astbury, S. Dickinson and K. Bailey, Biochem. J., *29*, 2351 (1935); W. T. Astbury, Nature, *137*, 803 (1936).
10. W. T. Astbury and H. J. Woods, Nature, *126*, 913 (1930); Phil. Trans. Roy. Soc., A. *232*, 333 (1933); W. T. Astbury and S. Dickinson, Nature *135*, 95, 765 (1935).
11. W. T. Astbury and R. Lomax, Nature, *133*, 795 (1934).
12. W. T. Astbury, S. Dickinson and K. Bailey, Biochem. J., *29*, 2351 (1935).
13. See W. T. Astbury, J. Text. Inst., *27*, P282 (1936); Chem. Weekbl., *33*, 778 (1936); F. C. Frank, Nature, *138*, 242 (1936).
14. D. M. Wrinch and D. J. Lloyd, Nature, *138*, 758 (1936); M. L. Huggins, Nature, *139*, 550 (1937).
15. W. T. Astbury and D. M. Wrinch, Nature, *139*, 798 (1937).
16. See references in D. M. Wrinch, Phil. Mag., *25*, 705 (1938).
17. D. M. Wrinch (1936).
18. W. T. Astbury, Nature, *140*, 968 (1937).
19. W. T. Astbury and A. Street, Phil. Trans. Roy. Soc. London, A, *230*, 75 (1931); W. T. Astbury and T. C. Marwick, Nature, *130*, 309 (1932); W. T. Astbury, Trans. Faraday Soc., *29*, 193

(1933); Kolloid-Z., *69*, 340 (1934); Chem. Weekbl., *33*, 778 (1936); G. L. Clark *et al.*, J. Am. Chem. Soc., *57*, 1509 (1935); R. B. Corey and R. W. G. Wyckoff, J. Biol. Chem., *114*, 407 (1936).
20. W. T. Astbury and S. Dickinson, unpublished results.
21. W. T. Astbury, Nature, *140*, 968 (1937); Compt. rend. trav. Lab. Carlsberg, *22*, 45 (1938).
22. J. B. Speakman and F. Townend, Nature, *139*, 411 (1937).
23. H. R. Marston, Council of Scientific and Industrial Research, Commonwealth of Australia, Bulletin 38 (1928).
24. H. B. Vickery and R. J. Block, J. Biol. Chem., *86*, 107 (1930).
25. C. S. Whewell, Ph. D. Thesis (University of Leeds, 1935).
26. J. Barritt, Biochem. J., *28*, 1 (1934).
27. For summaries and references, see J. B. Speakman, Jubilee Issue of J. Soc. Dyers and Colourists, 34 (1934); J. Barritt and A. T. King, J. Text. Inst., *24*, T119 (1933); S. G. Barker, "Wool Quality" (1931).
28. J. B. Speakman and E. Stott, Trans. Faraday Soc., *30*, 539 (1934); *31*, 1425 (1935).
29. W. T. Astbury, Cold Spring Harbor Symp. Quant. Biol., *2*, 15 (1934).
30. W. T. Astbury, S. Dickinson and K. Bailey, Biochem. J., *29*, 2351 (1935).
31. W. T. Astbury and T. C. Marwick, Nature, *130*, 309 (1932).
32. W. T. Astbury and F. O. Bell, Nature, *141*, 747 (1938).
33. E. Hammarsten, Biochem. Z., *144*, 383 (1924).
34. R. Signer, T. Caspersson and E. Hammarsten, Nature, *141*, 122 (1938).
35. W. J. Schmidt, "Die Doppelbrechung von Karyoplasma, Zytoplasma und Metaplasma" (Berlin, 1937).
36. P. A. Levene and L. W. Bass, "Nucleic Acids" (New York, 1931).
37. E. G. Cox, Nature, *130*, 205 (1932); E. G. Cox and T. H. Goodwin, J. Chem. Soc., 769 (1936).
38. T. Caspersson, Skand. Arch. Physiol., *73* (1936), Supplement No. 8.
39. K. Linderstrøm-Lang, Trans. Faraday Soc., *31*, 324 (1935); K. Linderstrøm-Lang and K. E. Rasmussen, Compt. rend. trav. Lab. Carlsberg, *20*, 1 (1935).
40. W. J. Schmidt, Naturwiss., *25*, 506 (1937).
41. Cf. D. M. Wrinch, Protoplasma, *25*, 550 (1936).
42. W. T. Astbury, S. Dickinson and K. Bailey, Biochem. J., *29*, 2351 (1935).
43. K. M. Blodgett, J. Am. Chem. Soc., *57*, 1007 (1935); I. Langmuir, V. J. Schaefer and D. M. Wrinch, Science, *85*, 76 (1937).
44. W. T. Astbury, F. O. Bell, E. Gorter and J. van Ormondt, Nature, *142*, 33 (1938).
45. K. M. Blodgett and I. Langmuir, Phys. Rev., *51*, 964 (1937).
46. W. T. Astbury, Trans. Faraday Soc., *29*, 193 (1933).
47. W. T. Astbury, Trans. Faraday Soc., *29*, 217 (1933). See also papers by Gorter, Rideal and their co-workers.
48. W. T. Astbury and W. A. Sisson, Proc. Roy. Soc. London, A, *150*, 533 (1935).

DISCUSSION

Dr. Danielli: Is it possible that the force you exert in removing the film from a surface sufficiently to line up the chains is sufficient to undo ring formations in the same way as they are broken in stretching a hair?

Dr. Astbury: The processes may be closely analogous, and I think we are all agreed that these ring formations must be easy to undo. There may be something of the nature of the lactam link which is undone. Whatever it is, we are agreed that the protein must be initially folded in some way which is easily undone.

Dr. Neurath: It would be interesting to extend X-ray investigations to those proteins which, according to our present knowledge, are apparently very asymmetrical in shape. We may be able to determine by this means whether or not these non-fibrous proteins have also the specific polypeptide configuration, typical for the fibrous proteins. Another case which occurs to me is that of reduced serum albumin. Burk has found that if serum albumin is reduced the protein solution becomes very viscous and shows thixotropic behaviour. I suggest that the reduced serum albumin be investigated, because this might be one of the bridges between the structure of globular and that of fibrous proteins.

Dr. Astbury: I shall be indebted to those present for filling the gaps in our paper by discussion of the various possible shapes of the globular proteins. I believe the discussion to be as important as the paper itself. It seems quite certain that even though the errors may be a matter of two or three hundred per cent, an enormous amount of leeway is left, and there are still all sorts of shapes associated with roughly constant molecular weights.

Dr. Neurath: Your hypothesis of the egg albumin molecule consisting of four layers separated by a distance of 10 A could hold for a spherical molecule. I wonder whether your idea could be applied equally well to a molecule having a ratio of the axes of 3:1, as seems to be the case for egg albumin.

Dr. Astbury: Once you postulate such a structure, everything depends on the area of the discs. If you take them as equal to $4\pi r^2$, the cylindrical molecule so produced will be effectively spherical in solution.

Dr. Mudd: Sevag in my laboratory has been getting out nucleic acids this year from streptococcal cells, and while the work is rather young, it has become apparent that the nucleic acids possess immunological activity. The possibility has been indicated that there are two kinds of specificity manifest in the two nucleic acids derived from the same streptococcal strain. This, I believe, is an entirely new observation; at least I know of nothing in the literature to indicate that nucleic acids in themselves possess specific serological reactivity. I find your diagram of the

structure of nucleic acid exceedingly reassuring, because certain chemists who have discussed this data from the point of view of analytical chemistry have questioned whether there is enough variation in composition of nucleic acids to give the possibility for serological specificity, but from your diagram it is obvious that there is more than enough variation possible. We know that changes in configuration and spatial relations of the same components do give an adequate basis for serological specificity. The rotation of the H and OH groups about an asymmetric carbon atom may give differences detectable serologically. So it seems apparent that these piles of nucleotide units, by slight changes in the order in which nucleotides occur, or possibly by other changes in configuration, give us an adequate basis for specificity. It is evident from your data that the possibility exists, and from our data it has been experimentally shown, we believe, that serological specificity exists in this class of compounds.

Dr. Astbury: There is one point I forgot to mention which bears directly on this question. You know that the thymonucleic acid is said to be a tetranucleotide. We have not worked this out completely, but it is clear from the photographs that the true period along the nucleotide column is at least 17 times the thickness of a nucleotide. So the nucleotides do not follow each other always in the same order, and this gives you another chance of great variation.

Dr. White: In connection with serological specificity, there is a report to the effect that one can demonstrate immunological differences in keratins prepared from various species after they have been made soluble by the method of reduction. I wonder whether there is any evidence from X-ray analysis which enables you to pick up a structural difference in keratins prepared from the different species.

Dr. Astbury: The differences are small, but the X-ray photographs of the keratins are not absolutely alike. Perhaps in the future we shall be able to measure these differences quantitatively.

Dr. Fruton: What is the significance, from a chemical point of view, of the value of 9.5 A obtained for the thickness of the monolayer, in view of the fact that the side groups jutting out from the main polypeptide chains vary greatly in length?

Dr. Astbury: The structure of keratin and myosin, so far as I can see from a number of years of X-ray examination, is not that of a polypeptide chain but a polypeptide grid. The side-chains all lie approximately in the plane of the grid, and there are many different kinds of cross-linkage. The side-chain spacing (9.5 A) measured by X-rays is the *average* distance of separation of the main-chains in the direction of the side-chains.

Dr. Steinhardt: Do I understand that you subscribe to Linderstrøm-Lang's views on the way in which polypeptide chains can be elaborated from the higher condensed structure? Do you think that in denaturation there is actual breakage of the pre-existing folded chains and recreation of new chains across the breakages?

Dr. Astbury: There are two ways in which you can get a fibrous protein from a globular protein: one is by unfolding and the second is by secondary condensation. I put forward the idea of the "jumping cracker" transformation to explain the second process, but I do not think it is generally true. I feel that the most general method is unfolding.

Dr. Steinhardt: I think it can be shown in the case of hemoglobin that the mechanism is likely to be unfolding rather than the production of new straight-chains by breaks at the folds which are in contact, because it is possible to produce a dissociation of some of the condensed structures prior to denaturation by treatment with substances similar to urea. The specific chemical properties are not changed in the primary dissociated product, but there is no doubt that further treatment causes further changes and the eventual elaboration of polypeptide chains.

Dr. Waddington: With regard to chromosomes, you pointed out that the spacing in nucleic acid is similar to the fully extended polypeptide chain spacing. Is it not the case that you find the nucleic acid attached to the chromosome at a time when the chromosome is contracted? The only time we know definitely that the nucleic acid is in the chromosome is when the chromosome is still contracting. Is anything known about any contracted states of the fibres of thymonucleic acid? Further, one knows that the nucleic acid is not present uniformly throughout the whole chromosome but is concentrated in certain regions, the darkly-staining bands or chromomeres. Is anything known about what difference there might be between the proteins in the parts of the chromosome to which nucleic acid is attached and in the parts which remain free of nucleotide acid?

Dr. Astbury: It is not quite clear whether the thymonucleic acid is free thymonucleic acid or not. If you can imagine that when you get strong absorption bands the thymonucleic acid is actually liberated, that fits in nicely. The free protein could then contract. In the fully extended form you would not get obvious thymonucleic acid.

Dr. Stern: Going back to the point on globular and fibrous proteins, I should like to mention some experiments by White and myself.

When we reduce insulin at pH 2 with thioglycolic acid, the curve of liberation of the SH groups flattens out at a point where about 3 SH groups are liberated in something like 150 minutes. When we follow the viscosity of the system the viscosity stays constant during this time and increases rapidly afterwards. In about 200 minutes the system solidifies to a stiff gel. The working hypothesis I have formed to account for this phenomenon is that if we start with globular insulin we might think that the reducing agent will attack at first SS links which are exposed. We know that as long as there are only one or two of these links broken the protein is essentially in the native state. When the viscosity goes up the protein shows symptoms of denaturation. This may be due to a rupture of SS groups which are important cross-links within the molecule. This process would then cause an unfolding of the chain and that would account for the high viscosity. So it may be that the bridging link between the globular and fibrous proteins is the reduced, denatured globular protein.

Dr. Astbury: This experiment of yours is analogous to the action of strong urea on the seed globulins. The viscosity gradually increases till you can spin it like cellulose and obtain X-ray photographs like those of stretched keratin.

Mr. Myers: I have some experimental evidence which may be of interest here. I have taken the globular protein, egg albumin, and denatured it by various reagents, some organic bases and also acetic acid, and have found thixotropy to appear after a certain length of time. In this case I did not use pure albumin (15 p.c. egg white, 70 p.c. water, 15 p.c. pyridine). As I shook it up and mixed the ingredients there was no particular increase in viscosity at first but after 15 minutes it was very great and set to a gel. If one shakes the gel, it will become fluid and flow quite readily, and all one has to do is to allow it to stand a half minute and it will go back to the original gel state. I do not know the interpretation of this, but it must be due to an unfolding of this globular protein, with the new formation of some kind of linkage, perhaps a hydrogen bond.

Dr. Astbury: The X-ray photographs of your preparation will be those of unfolded polypeptide chains in the β-form. Your preparation is rather more viscous than ordinary denatured egg albumin. The viscosity of denatured egg albumin is not very great as compared with the native egg albumin, and the inference is that the albumin molecule on denaturation gives shorter chains than the globulins, and the X-ray photographs show the same thing.

Dr. Harris: We get just the opposite type of mechanism reported by Stern. When we start to reduce wool, we obtain a viscous solution which rapidly becomes less and less viscous until it approaches the viscosity of water. This appears to be an example of transformation of a fibrous protein to a globular one.

Dr. Neurath: When Bull and I investigated the properties of surface-denatured egg albumin we found it to be completely insoluble in all solvents except sodium hydroxide and, when dissolved in sodium hydroxide, the solution becomes thixotropic, in exactly the same manner as was shown by Myers. I feel that this behavior is strongly associated with the fibrous structure of denatured egg albumin.

Dr. Bull: I should like to point out that asymmetry is not a necessary condition for thixotropic behavior. For example Solenhoff slate has symmetrical particles, but forms thixotropic suspensions.

Dr. Astbury: The clays have been shown by X-rays to be layer structures.

Dr. Bull: The particle itself is symmetrical, as shown under the microscope.

Dr. Steinhardt: There is a good deal of evidence that unfolding takes place at some stage in the process of denaturation, but it is well to remember that denaturation may occur in more than one way. I do not think it can occur in an infinite number of ways. Pepsin inactivation is a case in point. When denatured by alkali and then precipitated by acid, it is very easily brought into solution at pH 5.7 in the presence of 0.1 molar salt, but the protein denatured by mechanical shaking is insoluble under the same conditions. Both products are highly inhomogeneous as to molecular size, but they are reproducible and well-characterized. On the other hand, alkaline denaturation without subsequent acid precipitation gives a homogeneous product at or near this pH.

Dr. Astbury: That goes to prove that we must make up our minds that denaturation may involve many steps, and I propose that the denatured state should be defined as that in which the polypeptide chains are completely unfolded.

Dr. Steinhardt: There appear to be trigger mechanisms which bring about definite successive stages in the unfolding. There seem to be only a few demonstrably different denatured products from a given protein. They may constitute a progressive series, as you suggest.

Dr. Mathieu: I think it may be useful to point out the fundamental difference between Wrinch's ideas and Astbury's model of a globular protein. Astbury thinks that in a globular unit the main chains fold *in parallel planes perpendicular to the side chains*. Let us call "chain planes" these planes containing the folded main chains. Saying that the chains are all parallel in one unit does not mean that in the building of a crystal all the

"globular" molecules are necessarily parallel. Wrinch supposes that *the chain planes can be the faces of a polyhedron.* Nobody can know which of these two ideas will be kept in the future, but all the arguments put forward by Langmuir to emphasize the advantages of the polyhedral three-dimensional structure are debatable.

(1) Bergmann and Niemann's analysis, as well as the molecular weights previously found by Svedberg, are to be considered as the consequences of *periodicities* in the structure of protein molecules; but there is *nothing in these numbers to say what the molecular periodicities are.*

(2) If by denaturation of a protein, we mean the rupture of a "cyclol fabric", we cannot help but wonder what happens when a polyhedral structure is broken. It is then reduced to its faces, more or less torn into pieces; as a consequence, we can consider only complete degeneration, that is to say, only one stage in the denaturation process. It is much easier to think of *a progressive unfolding and tightening up of the chains* from a globular state to a complete denatured state.

(3) The idea of folding and unfolding the chains easily may be one of the most fruitful in the future, in order to explain some of the specific characters of the different kinds of proteins. There may be some day a new chapter in the chemistry of proteins similar to the one opened the last few years by Ruzicka in the chemistry of the polyterpenes. The relations between carotenoids and sterols are now easily understood by the foldings of the $-C=C-$ chains of the carotenoids. With their various compositions, with their side chains linking the chain planes together more or less tightly, with the possibility of reversible hydrolysis of the peptidic linkages now proved to be possible, with the folds of the main chains in parallel planes, the protein molecules are not a *rigid fabric.* With such a model as the one proposed by Astbury, *and based on a great number of experiments,* the crystallography and chemistry of proteins can be developed to fit all the biological requirements.