STUDIES ON A MONOMER

OF FEATHER KERATIN

by

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Abstract

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Submitted to the Department of Biology on September 10, 1954 in partial fulfillment of the requirements for the Ph.D. degree.

Results derived from physical-chemical and x-ray diffraction analyses are used to determine certain physical characteristics of the feather keratin molecule as a step in the elucidation of the feather keratin structure.

Methods are given for the solubilization of feather by reductive and/or oxidative techniques. Physical chemical studies are described for oxidized preparations of both the monomer and dimer. The molecular weight found for the monomer, $M = 9,300 \pm 700$, is in agreement with the results of Woodin (1954). The molecular weight of the dimer is approximately 19,300.

Correlation between molecular dimensions obtained from sedimentation-diffusion studies and from the results of x-ray analysis of reconstituted samples lead to the conclusion that the most probable molecular shape is a rectangular parallelipiped 95 A by 34 A by 4.65 A.

It is deduced that each molecule contains three β -polypeptide chains or their equivalent, arranged side by side in the side-chain direction.

A model for the keratin structure which provides one possible means of satisfying the gross aspects of available data is presented. Its limitations are pointed out and some suggestions are made concerning types of research which show promise of providing further information from which more specific models may be derived.

Thesis Supervisor: Richard S. Bear
Title: Professor of Biophysical Chemistry

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I. Introduction

A. X-Ray Diffraction Studies

The complex x-ray diffraction pattern of feather keratin, which is illustrated in Figure 1.1, was first described by Marwick (1931). Astbury and Marwick (1932) reported that the meridional diffractions indicated a fiber axis period of at least 24.8 A. with sub-periods of 6.2 A and 3.1 A. From a comparison of the 3.1 A spacing with the slightly larger spacings observed with stretched wool and with silk, they suggested that the feather structure was composed of similarly extended, parallel chains. On the basis of the wide-angle pattern, feather has been classified by Astbury (1938) as among the β -proteins of the k.m.e.f. group.

Corey and Wyckoff (1936) presented data on the diffraction pattern but made no deductions concerning the structure involved. Bear (1944) described the small-angle and intermediate-angle scattering of feather showing the true fiber axis period to be 95 A and indicating the prominence of a large lateral dimension: 34 A.

Pauling and Corey (1951a) proposed a structure called the pleated sheet for β -keratin. It consisted of a hydrogen bonded layer of similarly oriented and extended polypeptide chains. The projected length per residue in this configuration, 3.07 A, coincides with the corresponding dimension obtained from the x-ray diffraction pattern of feather. To construct a model for feather, however, the authors (1951b) found it necessary to separate layers of these pleated sheets with double layers of α -helices in order to account for the observed 34 A equatorial spacing. In 1953, stating that the x-ray pattern of
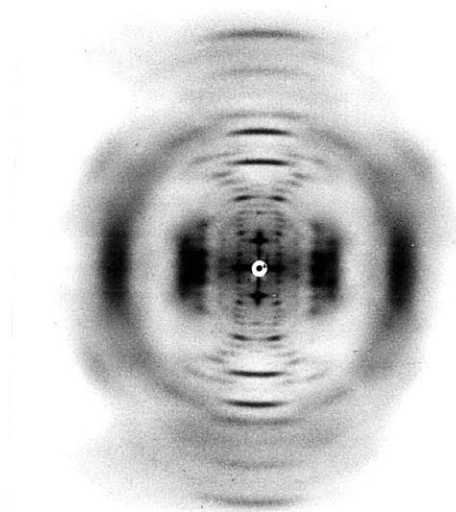
Fig. 1.1. The x-ray diffraction pattern
of feather rachis keratin.

feather may be a superimposition of α- and β -keratin patterns, they suggested that the α -keratin portion of the structure may exist in certain cable configurations.

The inclusion of α -keratin in the structure is probably based on studies of the infrared absorption spectrum of feather. Ambrose and Elliot (1951), in an analysis of the infrared dichroism of the N-H deformation and C=O stretching modes observed in swan quill, conclude that there is a component of α-protein present. Whether or not this is a keratin is not known.

Rudall (1947) concludes on the basis of x-ray diffraction studies that α -keratin structure is essentially absent in the barbs, rachis, calamus and medulla of the feather, all of which are produced by the stratum intermedium of the follicle. The stratum corneum, and stratum intermedium which contribute to the outer and inner surfaces of the feather, respectively, do produce α -keratin. It is assumed that in the infrared studies of Ambrose and Elliot, portions of these layers were not included in the 1μ sections prepared for examination.

To investigate the nature of the large structural element of feather and, with the question of the possible globular nature of the fibrous protein in mind, Bear and Rugo (1951) approached the problem in quite a different manner. They were able to draw certain conclusions concerning the large scale structure by examining the effect upon the diffraction pattern of cautious degradation procedures. A number of treatments, including principally the effects of heat and moisture, reduced the diffraction pattern to that of a simple two-dimensional net. In Figure 1.2, taken from their paper, is illustrated the arrangement of
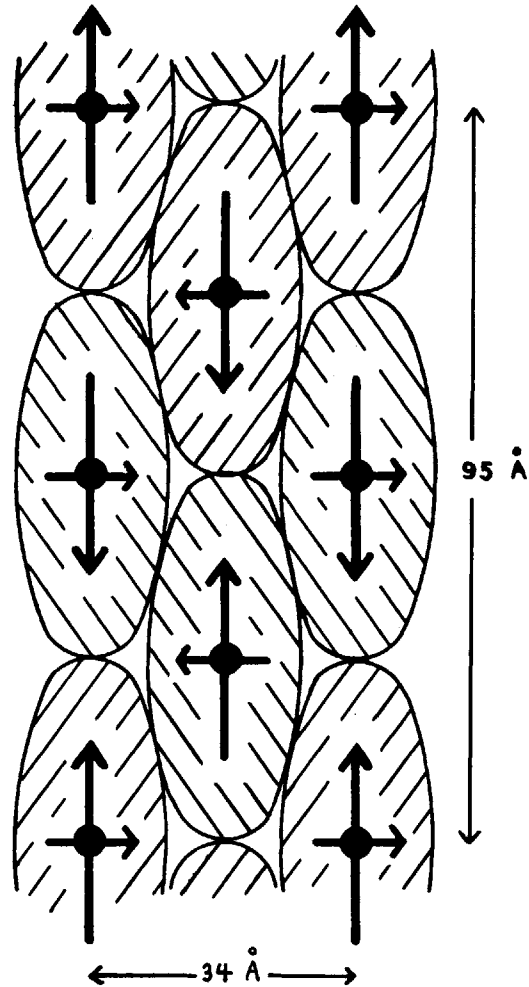
Figure 1.2. The Bear-Rugo representation of the
feather keratin unit cell. The solid circles
represent the pseudo-nodes of the basic net. The
arrows and cross-hatching indicate a possible
means of arranging identical material about each
node in such a way as to produce a true unit cell.
(Taken from Bear and Rugo, 1951)

the net. The arrows which indicate the relative orientation of material about the nodes also fulfill the requirement of patterns of native feather that the true unit cell be twice that of the simplest net cell derived from the heat moisture treatments. For those diffractions which are not ideal for the net, two possible explanations are given. They may be taken as evidence of a third lattice translation of the structure, or as the result of orientation of material about the nodes in the particular fashion shown. There are indications of lateral displacement of some row line diffractions in the patterns of untreated material which would support the first hypothesis but the evidence was believed not to require a third large cell dimension.

B. Solubilization of the keratins

The characteristic insolubility of the keratins is due primarily to their high cystine content (8.7%, Block 1951) and the consequent high frequency of disulfide linkages. Goddard and Michaelis (1934) working with wool were able to dissolve considerable amounts by the use of reducing agents at high pH. Jones and Meecham (1943), in a comparative study of the solubility of various types of keratin, demonstrated the necessity of having both disulfide and hydrogen-bond-breaking reagents present. They used primarily urea, formamide or high pH conditions in conjunction with such reducing agents as thioglycol, sodium bisulfite, and sodium sulfide, and obtained yields as high as 80 to 85% in some cases. They noted that feather keratin was more readily dispersed and less stable in sodium sulfide solution than were the keratins of hoof, hog hair, and wool.

Alexander, Hudson and Fox (1950) studied the effect of various oxidizing agents on the solubility of wool and Alexander and Earland have described (1952) a number of oxidative methods for the treatment of wool and related keratins.

Sedimentation and diffusion studies have been carried out on wool and skin keratin by Mercer and Olofsson (1951a,b) who used urea bisulfite solutions, and by Olofsson (1951) on wool with a variety of methods including oxidation of the disulfide bonds with peracetic acid. Two principle types of molecules were produced. By means of partial reduction at pH 8 a polydisperse system of particle weight (M) 80-90,000, length (l) 150 A, diameter (d) 12.8 A, was obtained. Material oxidized with peracetic acid was similar. Complete reduction at pH 12 resulted in molecules of M = 9,500, l = 165 A, d = 10.8 A. The latter appeared to make up the bulk of the wool mass. The former was limited to about 10% of the wool.

C. Previous Physical Chemical Studies on Feather Keratin

Ward, High, and Lundgren (1946) reported the first studies on feather keratin in solution. Using a variety of physical chemical tools they investigated a soluble keratin-detergent complex. Of these results , only the particle weight corrected for combination with detergent is of direct importance here, but the contribution of detergent to particle shape could not be estimated. From osmotic pressure and sedimentation-diffusion methods they estimated the particle weight to be 34,000 to 40,000. Their preparations were polydisperse, however, and the significance of this figure remains in some doubt.

During the course of the investigation reported here, Woodin

(1954) published the result of a similar study carried out with chicken

feather keratin. Using a urea-bisulfite system as his solvent he studied

the keratin, both in the reduced form (SH-keratin) and the oxidized

form (cysteic acid keratin) by sedimentation velocity, osmotic prdssure,

light scattering, intrinsic viscosity, and electrophoretic methods.

His results are consistent with the idea of a single component system of

molecular weight, M=10,000. From an intrinsic viscosity of 0.15 he ob-

tained, assuming a prolate spheroidal shape and without correcting for

hydration, an axial ratio of 13.2. No molecular dimensions were calculated.

Woodin was able to dissolve 80 to 85% of the feather with his reagent

system, and the material was electrophoretically homogenous.

Thesis objectives

The present study was undertaken in the hope of being able to

relate small-angle diffraction phenomena to specific molecular entities.

Specifically, this involves the following steps.

1. The preparation of solutions of feather keratin suitable for

study by the physical chemical techniques customarily applied to proteins.

Thus the method of dissolving the feather must be sufficiently mild so as

to preclude intramolecular degradation and the resulting solutions of

protein must be stable under the conditions of experimentation.

2. The characterization of the dissolved material with respect to

homogeneity, size and shape.

3. The reconstitution of soluble keratin and x-ray diffraction

examination of the reconstituted material. This step serves to test the

chemical techniques used in solubilization with respect to degradation of the feather protein, and to identify the soluble material with that giving rise to the x-ray diffraction pattern of the native feather.

4. The use of results from the above to propose certain hypothesis concerning the structure of feather keratin, especially in relation to its diffraction characteristics at small and intermediate angles.

II. Preparation of Material

White turkey feathers served as the source of keratin through-out this investigation. The feathers were first washed with water to remove all loose debris, and, after drying, were extracted with benzene in a soxhlet apparatus for two days. The extracted material was dried and cut into small pieces prior to solubilization.

Since the solution studies were intended to be used in conjunc-tion with x-ray diffraction results, initial experiments were carried out on a part of the feather giving a resonably well-oriented diffraction pattern, the quill tip. The quill tips were scraped free of outer dis-oriented layers, in addition to being extracted with benzene. Since the sedimentation patterns of soluble keratin obtained from the quill tip and those of material extracted from the remainder of the feather were, however, not demonstrably different, whole feathers were used. The methods used in the dissolving of keratin to be described below are, in general, variations on techniques previously reported for wool and other keratins.

Method 1-a consists of breaking the disulfide bonds by re-duction followed by dispersion of the keratin at high pH. In practice one suspends about two grams of dry feather in 100 ml. of water containing 2% thioglycol by volume. After about two days the solution is adjusted to pH 11 with NH_4OH and the suspension stirred for two hours. For more rapid and complete solution the material may be homogenized after the initial swelling has taken place. After centrifugation the supernatant is recovered and the residue is extracted with fresh solvent. The bulk

of the protein in the combined supernates is precipitated by adjustment to pH 4 with dilute HCl. Although the yield of protein may be increased somewhat by the use of salting out procedures, this was not done. The precipitation is to be regarded essentially as a fractionation step in which small peptide fragments are left behind. A yield of approximately 65% is obtained by this procedure.

Ultracentrifugation experiments have been carried out on preparations of reduced keratin of this sort redissolved in the same solvent system, but the necessity of keeping the reducing agent present and of maintaining a high pH to prevent reaggregation makes the solution unsuitable for diffusion and viscosity studies. The solution is not stable for more than a few days even at refrigerator temperatures. Apparently as a result of the loss of some thioglycol, the pH rises markedly and some gel formation takes place.

Method 1-b overcomes these difficulties by converting the protein to a more stable and workable form. The technique which has been used here is the oxidation of the -SH groups to $-SO_3H$ groups: i.e., the conversion of cysteine to cysteic acid, through reaction with peracetic acid. The peracetic acid was prepared after the method of Greenspan (1947), modified for the use of 30% H_2O_2 instead of a 90% preparation. Prior to use the peracetic acid solution was brought to the desired pH by the addition of concentrated NaOH in the cold. Oxidation was usually carried out between pH 4 and pH 7, since the reagent is highly unstable under alkaline conditions. A washed precipitate of keratin produced by method 1-a is oxidized for several hours and the cysteic acid keratin,

now water soluble, is thoroughly dialyzed against running tap water and finally distilled water to remove all of the remaining oxidizing reagent. Lyophylization of the water solution results in a stable preparation which may be dissolved easily in a suitable buffer for study.

As a matter of convenience in further discussions of products obtained from feather and in accordance with the results of physical-chemical studies to be described later, material of the size of that obtained by methods 1-a and 1-b will be referred to as the feather keratin monomer.

Method 2 provides an alternate means of preparation in which the oxidation is carried out directly on the cleaned feather. Depending upon the conditions of oxidation, however, this method may lead to a variety of products. The important experimental variables are the pH and time of oxidation, and appear to be related to the ability of the feather to swell under the conditions chosen. If oxidation is carried out at pH 4 marked swelling of the protein is observed. Considerably less swelling occurs at pH 5 and it is nearly absent at pH 6. The effect of pH on the type of product obtained appears as increasing proportions of more highly aggregated material with increasing pH over this range. At a given pH increased time of oxidation tends to convert the keratin more and more into a product corresponding to that produced by method 1-b; i.e., the monomer.

If the oxidized feather is thoroughly extracted with water, that portion which has been converted to the monomer is removed. If this extract is lyophylized and redissolved in pH 7 phosphate buffer, sedimentation patterns such as are shown in Figure 2.1-I are obtained. Results obtained by pH 11 extraction of the residue from the water extractions are of the types illustrated in Figure 2.1-II,III. Figure 2.1-III

shows the sedimentation pattern of material oxidized at pH 6 for two

hours. Figure 2.1-II was obtained from material oxidized at the same

pH but for 24 hours. Patterns similar to II are obtained from material

oxidized at pH 4 or pH 5 for shorter periods. Preparations of the type

illustrated in II will, as a matter of convenience, be referred to below

as "dimer." The justification for this will be presented later in the

section on experimental results.

The standard technique used for the preparation of the dimer

was as follows: oxidation at pH 5 for 8 to 10 hours, followed by thorough

extraction of the monomer with distilled water. The residue of the above

extraction was then suspended in pH 11 NH_4OH, centrifuged, and the

material in the supernatant precipitated by acidification to pH 4 with

dilute HCl. The precipitate was redissolved in dilute NaOH, thoroughly

dialyzed against water and lyophylized.

Insofar as the study of the low molecular weight product is

concerned, experiments to be reported here have all been carried out

on the reduction-oxidation product of method 1-b. Use of this material

avoids possible complications due to the presence of more highly ag-

gregated products. Moreover, during this type of extraction procedure

samples of reduced keratin may be removed for use in reconstitution experi-

ments. The reduced keratin is of more value in such studies than is the

oxidized product, since the sulfhydryl groups of the former are able to

reform disulfide bonds. Some physical chemical studies have also been

carried out on the dimer obtained by oxidation at pH 5.

SEDIMENTATION PATTERNS
OF OXIDIZED PREPARATIONS
OF FEATHER KERATIN

Figure 2.1

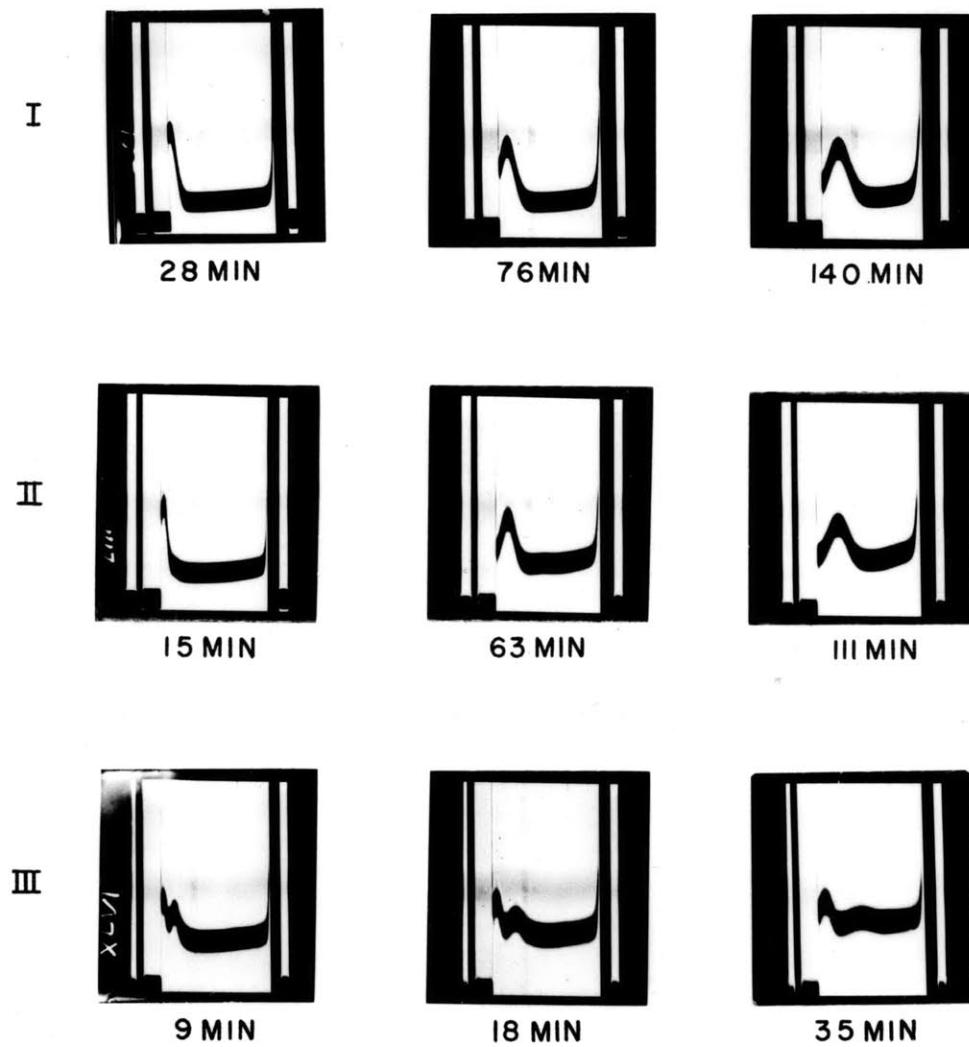In all studies on solutions of both dimer and monomer the lyophylized protein preparations were dissolved in a phosphate buffer of pH 7.0 and ionic strength 0.15. The ionic strength of the phosphate was 0.05, the remainder being made up with NaCl. Prior to use solutions were dialyzed against buffer for one to two days at 5° C. and centrifuged for at least two hours in a Spinco preparative ultracentrifuge at 40,000 rpm.

III. Experimental Techniques and Results

A. Sedimentation velocity

Sedimentation studies were carried out using a Spinco Model E Ultracentrifuge. Preliminary studies on the methods of preparation of material were done with cells of standard type at 60,000 rpm. In the case on the monomer, however, the values of the sedimentation constant as a function of concentration were determined using a cell of the type described by Kegeles (1952)[*], in which an artificial boundary may be produced by a layering of buffer over protein solution. In Figure 3.1 are illustrated the patterns obtained from the monomer with the two types of cells. The sedimentation rate of the dimer was determined at only one concentration using the standard cell. Rotor temperatures were measured during the course of the experiments by the method of Waugh and Yphantis (1952).

Sedimentation Constant of Monomer

The sedimentation constant of the monomer was calculated after the method of Cecil and Ogston (1948), in which $\Sigma \Delta x$ is plotted against $\Sigma \Delta t'$. Δx is the distance moved by the peak between successive exposures and (3.1) $\Delta t' = \bar{x} \Delta t \ \eta_{20}/\eta_T \ \omega^2$. $\bar{x}$ is the average distance of the peak from the center of rotation, and η_{20}/η_T the viscosity correction for temperature, during the time interval Δt. ω is the angular velocity in radians/second.

This method of analysis includes the principle variable quantities and when plotted gives a straight line whose slope is the sedimentation

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[*] Kindly provided by Mr. Francois Lamy, Biology Department, M.I.T.

FEATHER KERATIN MONOMER
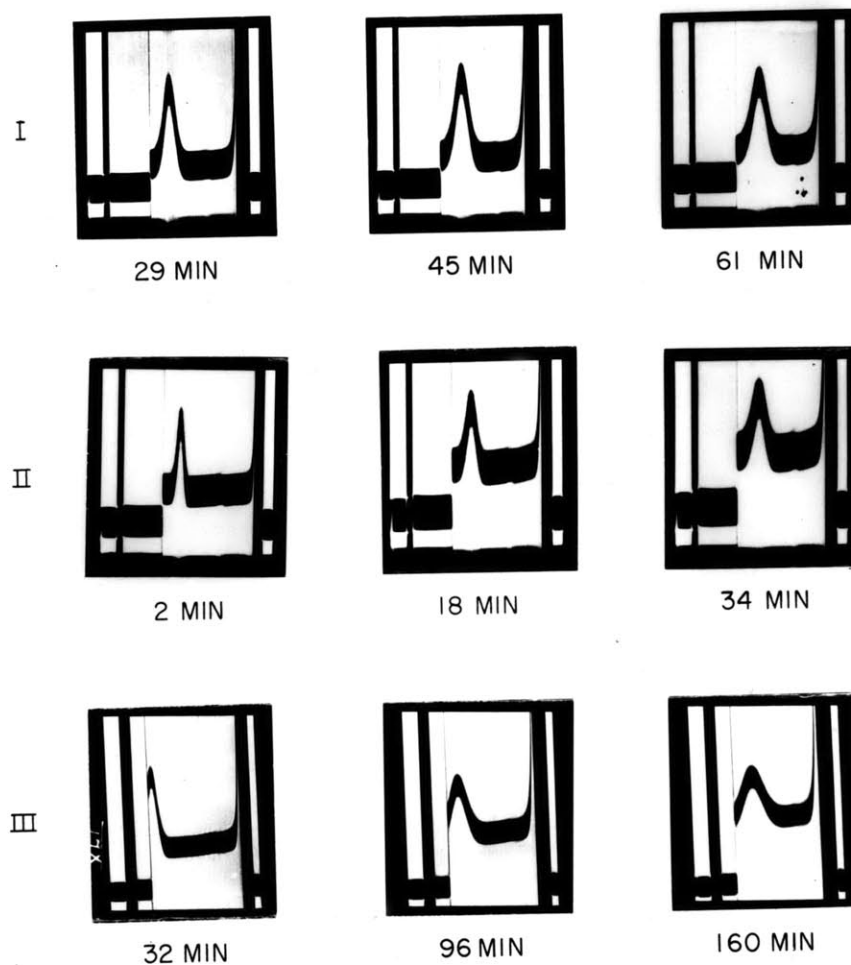SEDIMENTATION PATTERNS

Figure 3.1. I and II are obtained with the artificial
boundary cell of Kegeles (1952), at concentration of
1.6% and 0.53% protein. III illustrates an extended
run in the standard ultracentrifuge cell. Time in min-
utes after the centrifuge reached full speed is given.

constant corrected for viscosity effects due to temperature alone. The slope is determined by the method of least squares.

To correct the sedimentation constant to water at 20° C the coefficient given by equation 3.2 was used.

$$C = \frac{\eta^s}{\eta^o} \frac{(1 - \bar{V}_{20}\rho_{20})}{(1 - \bar{V}_T\rho_T^s)} \qquad 3.2$$

η^s and η^o are respectively the viscosities of solvent and water at 20° C. ρ^o_{20} and ρ^s_T are respectively the densities of water at 20° C and of the solvent at the temperature of the run. $\bar{V}$ is the partial specific volume of the protein which is assumed to remain constant over the temperature range involved. For temperatures of 26 to 27° C the correction amounts to approximately 1.07.

Sedimentation runs at protein concentrations of 1.6%, 0.8%, 0.53%, and 0.15% were calculated. The results have been plotted in Figure 3.2. The extrapolated value of the sedimentation constant, S^o_{20}, was found to be 1.06 Svedberg units. $C \rightarrow 0$

Sedimentation constant of dimer

The sedimentation constant of the dimer was measured at a concentration of 0.7%. Calculation by the above method gave the value $S^o_{20} = 1.30$ Svedberg units.

Accuracy of sedimentation analysis of monomer

The standard deviation of the sedimentation constant determined at each concentration was calculated by the usual statistical methods. For the concentrations 1.6% and 0.53% the deviation is of the order of 0.5% of S. For C = 0.8% the deviation was 2% and for C = 0.15% about 5%. The extrapolated value of the sedimentation constant is believed to be
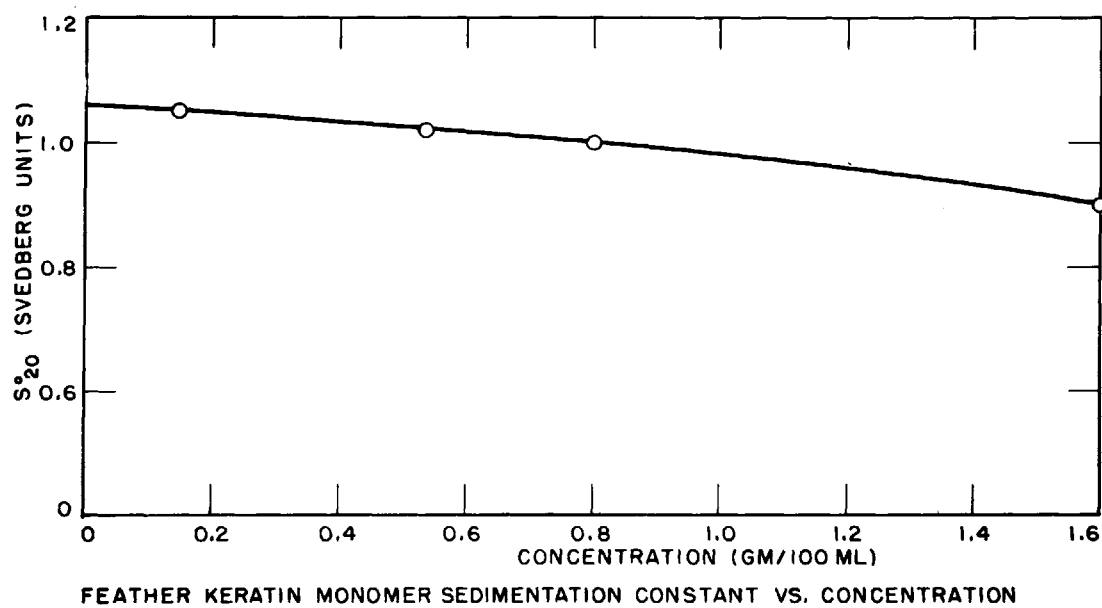
FEATHER KERATIN MONOMER SEDIMENTATION CONSTANT VS. CONCENTRATION

Figure 3.2

accurate to within about 3%.

B. Diffusion

Diffusion runs were carried out in a Pearson electrophoresis diffusion apparatus equipped with a Longsworth schlieren scanning optical system. The cell of 11 ml. capacity was of a standard type supplied by the Pyrocell Manufacturing Company. Mechanical compensation was used. Three runs on the keratin monomer were made at different protein concentrations, the temperature in each case being 20° C. Selected patterns from each of the three runs are shown in Figure 3.3. One run was made with the dimer under the same conditions. In addition the diffusion constant of the monomer was calculated from the peak spreading during the course of an ultracentrifugation. The determination was made using the artificial boundary cell, since the entire peak is never observed with this material when the standard cells are used.

Diffusion constant of monomer

Diffusion constants were calculated by the maximum ordinate area method. The photographic records of each run were projected with an enlarger and the peaks were traced. Areas were measured with a compensating planimeter.

A plot of Y^2H^2/A^2 vs. time in seconds, where Y is the reciprocal of the over-all magnification, A the area of the magnified peak and H the height of the magnified peak, leads to a straight line whose slope is equal to $4\pi D$, where D is the diffusion constant. Since the experiments were carried out at 20° C, the only correction necessary to provide D_{20}^o was that for the viscosity of the solvent which is given by equation 3.3.
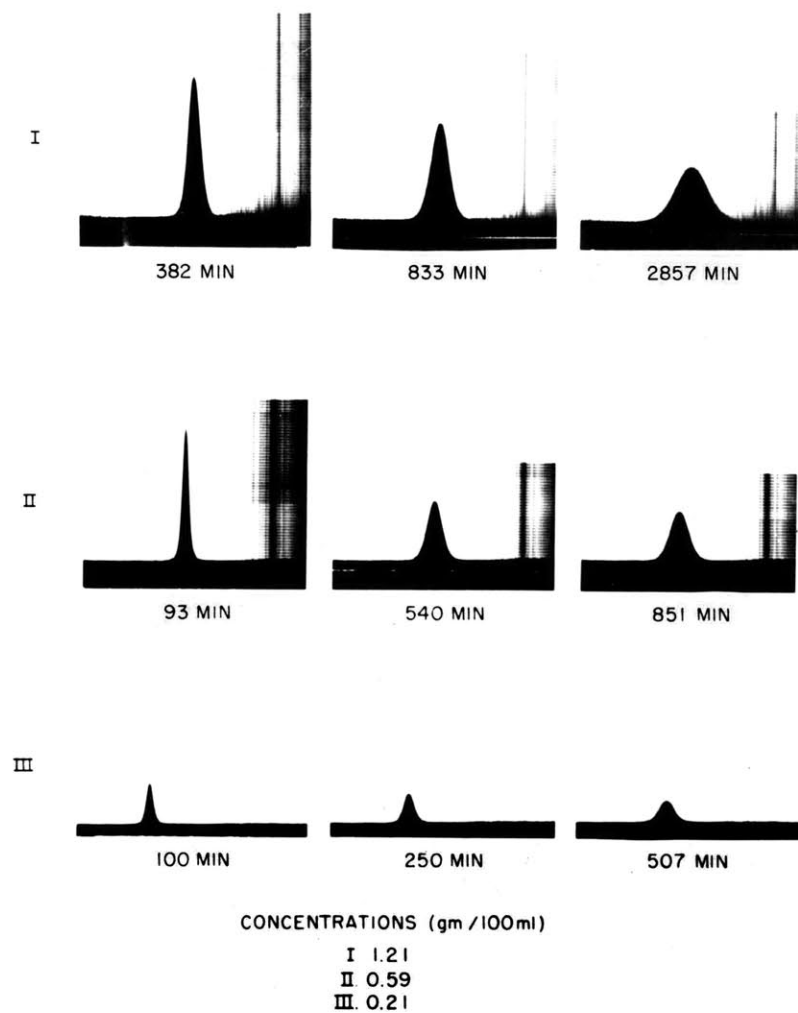
FEATHER KERATIN MONOMER
DIFFUSION PATTERNS

I

382 MIN   833 MIN   2857 MIN

II

93 MIN   540 MIN   851 MIN

III

100 MIN   250 MIN   507 MIN

CONCENTRATIONS (gm /100ml)
I 1.21
II 0.59
III 0.21

Figure 3.3

$$D_{20}^{o} = \frac{\eta^{s}}{\eta^{o}} D_{20}^{s} \qquad 3.3$$

η^{s} and η^{o} are the viscosities of solvent and water respectively at 20° C.

In Figure 3.4 are presented the results of the diffusion runs on the monomer

at concentrations of 1.31%, 0.59%, and 0.21%. $D_{20}^{o}{}_{c\to 0}$ determined by extra-

polation by the method of least squares was 10.02×10^{-7} cm^2/sec. A

value of $D_{20}^{o} = 8.4 \times 10^{-7}$ cm^2/sec. was calculated by the maximum ordinate-

area method from the sedimenting peak of an 0.71% solution of monomer.

The lower boundary of the peak was estimated by joining the portions of

the base line on either side of the peak.

Diffusion constant of dimer

The diffusion constant of a preparation of dimer, calculated

in the above fashion, was $D_{20}^{o} = 5.95 \times 10^{-7}$ cm^2/sec.

Accuracy of diffusion results

An estimation of the error in the determination of the diffusion

constant cannot be made as exactly as in the case of the sedimentation

constant. The difficulty arises from the fact that certain of the steps

cannot easily be analyzed statistically. The method of determining the

area of the peaks requires a decision on the part of the investigator

as to the proper location of the sides and base line with respect to

the blackening of the photographic plate. The usual procedure is to

estimate the line of half density but this itself is a function of the

exposure to some extent. In measurements of runs made at high protein

concentration the total area is sufficiently large so that the percentage

error may be relatively small. At low concentrations, however, the peaks
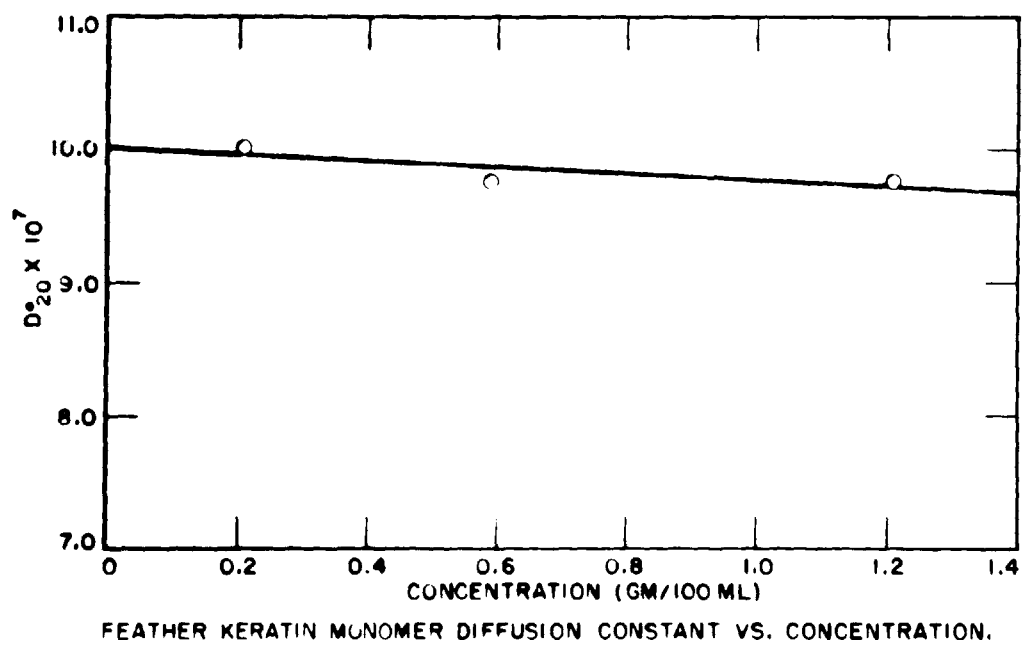
are small and the error may become appreciable.

FEATHER KERATIN MONOMER DIFFUSION CONSTANT VS. CONCENTRATION.

Figure 3.4

From the data presented here it may be seen that the diffusion

constants obtained for the monomer vary only slightly with concentra-

tion, there being only a three percent difference between the lowest

and highest concentrations. This is a reasonable result for a protein

of such low molecular weight and it is believed that the error in the

extrapolated value is not greater than five percent.

C. Viscosity Studies

Viscosity determinations were carried out on preparations of the

keratin monomer which had been dialysed against buffer for one to two

days at 5° C and cleaned in a Spinco preparative ultracentrifuge at

40,000 rpm for about 3 hours. The buffer solutions with which dilu-

tions were made were cleaned by passage through a fine sintered-glass

filter. Flow times were reproducible to 0.3 seconds.

Viscometers of both the Ubbelohde[*] and Ostwald-Fenske type were

used. A viscometer of the Ostwald-Fenske type which had been modified

by the substitution of the spiral capillary for the usual straight one

was used in addition to the standard types at low concentrations. The

flow time for this viscometer was approximately 14 minutes with water

at 20° C. For all the other viscometers, including the Ubbelohde model,

flow times were between 270 and 300 seconds under the same conditions.

In the case of the Ostwald-Fenske viscometers, dilutions were

made in small flasks and 5 ml. samples were pipetted into the visco-

meters. The results obtained showed a non-linear drop in reduced

viscosity, η_{sp}/C, with decreasing protein concentration. At all

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[*] Kindly made available by Dr. Paul Doty of Harvard University.

concentrations studied the reduced viscosity was found to be lower than values obtained at corresponding concentrations with the Ubbelohde viscometer. With the Ubbelohde viscometer in which dilutions were made directly by addition of buffer to the viscometer the results obtained were linear with only a slight concentration dependence over a concentration range of about 1.5 to 0.3 %. Since the material studied is of low molecular weight and moderate assymmetry (as determined by sedimentation - diffusion results and described in a later section) and since the velocity gradients of the two types of viscometers were about the same, gradient dependence was ruled out as a reason for the difference in results.

The shape of the η_{sp}/C vs. C curves obtained with the Ostwald-Fenske viscometers is of the type which would be obtained if there were a loss of a constant amount of protein at each concentration. The effect of such a loss would become increasingly evident at lower and lower concentrations. The amount of "lost" protein necessary to explain the effect would be in excess of 3 mg. in 5 ml. of solution or upwards of 25% at the lower concentrations. The effect, therefore, cannot be explained on this basis.

An examination of the effect of surface tension shows that it would provide an effect opposite in nature to that observed. The reduced viscosity would increase with decreasing protein concentration.

If the effect observed with the Ostwald-Fenske is real it means that a decrease in axial ratio is taking place with decrease in concentration. This would best be interpreted as a dissociation of the molecules. There is no indication in studies by other techniques that

this is true. Figure 3.2, for example, shows that the sedimentation constant has a slight <u>negative</u> concentration dependence.

Although the results obtained with the Ubbelohde viscometer are linear and nearly constant with concentration, they must also be considered suspect. The solution being investigated flows down the wall of the bulb below the capillary, and the flow behavior of buffer solution might be expected to differ from that of a surface active protein solution. Surface tension effects might immobilize the layer at the solution-air interface, resulting in a change in the flow velocity in addition to that caused by the change in viscosity with protein concentration.

Because the results obtained with the Ubbelohde viscometer are of the type usually obtained with protein solutions, however, and since no sensible extrapolation to zero concentration could be made with the results of the Ostwald-Fenske viscometer, the data obtained with the former will be used here. In Figure 3.5 the reduced viscosity η_{sp}/C = η_{rel} -1/C, is plotted against C, the protein concentration in grams per 100 ml. of solution. Extrapolation of the curve to zero concentration by the method of least squares leads to an intrinsic viscosity of 0.135.

D. Partial Specific Volume

Measurements were made at $26°$ C in a 22 ml. pycnometer, weighed to 0.1 mg. at four protein concentrations. Equation 3.4 was used to calculate partial specific volume.

$$V = \frac{1}{\rho} \left[1 - (1-W_1)(2.303) \frac{d \log M}{dW_1} \right] \qquad 3.4$$
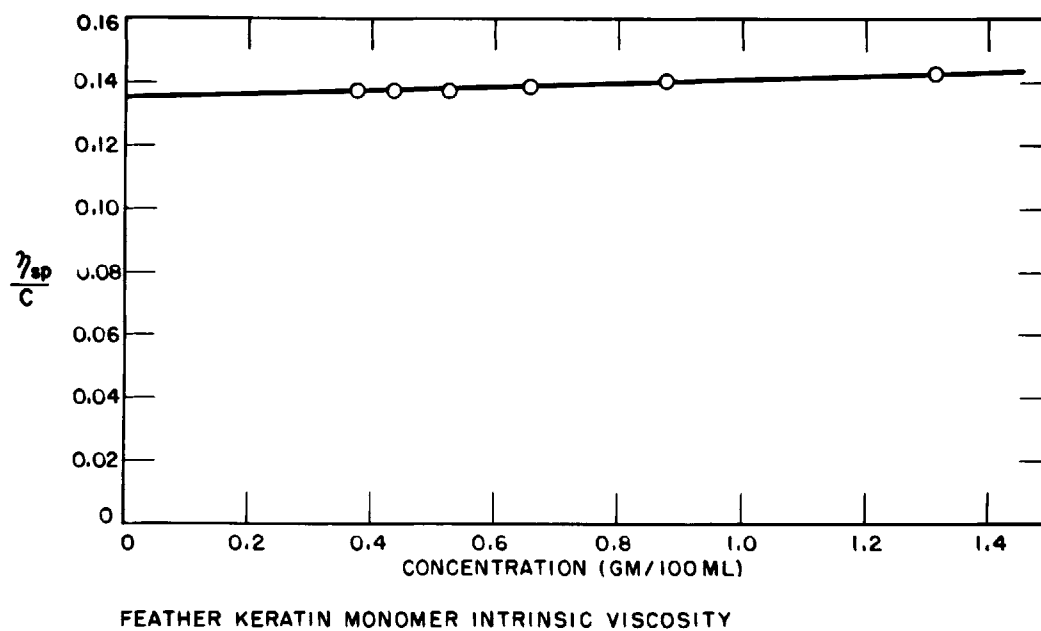
FEATHER KERATIN MONOMER INTRINSIC VISCOSITY

Figure 3.5

W_1 is the weight fraction of protein present, ρ is the solution density, and M is the weight of protein solution in the pycnometer. From the least squares value of the slope of a plot of (d log M/d W_1) and the proper values for the other terms, a partial specific volume, $\overline{V}$ = 0.725, was calculated.

E. Determination of concentration

Concentration determinations were carried out by a method involving determination of dry weights. Five ml. each of a series of dilutions from stock solution were pipetted into small weighing bottles. The solutions were dried at $95°$ C until the liquid phase had disappeared. The material was then dried at $105°$ C in a vacuum oven until constant weight was achieved. This took approximately two days. A plot of dry weight vs. relative protein concentration then gave a straight line whose slope as determined by the method of least squares was equal to the concentration of stock solution times .05 in grams per 100 ml. of solution. The standard deviations were below 1% for all such concentration determinations.

F. Calculation of size and shape of monomer

Sedimentation-diffusion data

The molecular weight was calculated by means of equation 3.5 from extrapolated values of the sedimentation and diffusion constants.

$$M = \frac{R \, T \, S \times 10^{-13}}{D(1-\overline{V}\rho)}$$

The value obtained was M = 9,300.

From equation 3.6 the frictional ratio of the monomer was found to be 1.53.

$$\frac{f}{f_o} = \left(\frac{1 - \overline{V}\rho}{D_o^2\ S\overline{V}} \times 10^{13}\right)^{\frac{1}{3}} \times 10^{-8} \qquad\qquad 3.6$$

Tables of the frictional ratio as a function of the axial ratio of the particle derived from Perrin's equation are given by Edsall (1943). Use of f/f_o = 1.53 leads to an axial ratio a/b = 9.74 if the particle is a prolate spheroid, or b/a = 11.87 if the particle is an oblate spheroid. These results were calculated neglecting the hydration of the protein. Oncley (1941) has shown that the frictional ratio may be divided into two factors, one a function of shape and one a function of hydration as in equation 3.7.

$$\frac{f}{f_o} = \frac{f}{f_e} \times \frac{f_e}{f_o} \qquad\qquad 3.7$$

The hydration factor has been shown by Kraemer (1940) to be given by:

$$\frac{f}{f_e} = \frac{WV_2 + V_1}{V_1} \qquad\qquad 3.8$$

where W is the hydration fraction of the protein in grams of water per gram proteins, and V_2 and V_1 are the partial specific volumes of protein and water, respectively. Assuming 30% hydration, a value deemed reasonable by Edsall (1943) f/f_e was found to be 1.12 and from equation 3.7, f_e/f_o, the shape factor, became 1.37. Axial ratios assuming 30% hydration were then found to be a/b = 6.83 in the prolate case, and in the oblate case b/a = 7.83. Since the volume per protein molecule may be calculated using the relation given in equation 3.9.

$$V = \frac{4}{3}\pi b^2 a = \frac{M\overline{V}}{N} \qquad\qquad 3.9$$

one may estimate the dimensions of the spheroids representing the prolate and oblate cases. For a/b = 6.83, M = 9300 and $\bar{V}$ = 0.725, b was found to be 7.33 A or 2b = 14.7 A, and 2a = 100.1 A. For b/a = 7.83, and the other quantities remaining the same, 2b = 55.1 A and 2a = 7.04 A.

Intrinsic viscosity data

From the value of intrinsic viscosity $[\eta]$ = 0.135 one may calculate the viscosity increment, ν , using equation 3.10 (Edsall, 1943).

$$\nu = \frac{[\eta] 100}{\bar{V}} \qquad 3.10$$

A value of ν = 18.6 was obtained assuming no hydration. The hydration correction is obtained by dividing ν by $(1 + W/\bar{V}\rho)$, where W is the hydration fraction in grams of water per gram protein. Here ρ is the density of solvent and $\bar{V}$ is the partial specific volume. An assumption of 30% hydration resulted in a corrected viscosity increment ν = 13.2. A table of values relating the viscosity increment to axial ratios for spheroids using the equation of Simha is given by Edsall (1943). For zero hydration, ν = 18.6 gave values of a/b = 12.3 and b/a = 25.6 for the prolate and oblate cases respectively. Assuming 30% hydration the values obtained were a/b = 9.77 and b/a = 17.6.

For random coils, Flory and Fox (1950) have related the molecular weight and intrinsic viscosity to the root mean square separation between the ends of the molecule. From equation 3.11 this effective length may be calculated. A value of 85 A° was found for the keratin monomer

$$M[\eta] = 2.1 \times 10^{21} \left(\bar{r^2}\right)^{3/2} \qquad 3.11$$

Scheraga and Mandelkern (1953) have recently presented a method for the determination of particle dimensions which makes use of sedimentation,

diffusion, and intrinsic viscosity data. S_{20}^o and D_{20}^o are used to cal-
$$c^{20} \to 0 \quad c^{20} \to 0$$

culate the anhydrous molecular weight, as has been done above. One may

then solve for a quantity β in terms of M, S_{20}^o and $[\eta]$, or M, D_{20}^o
$$c^{20} \to 0 \qquad c^{20} \to 0$$

and $[\eta]$ as is shown in equations 3.12a and b.

$$\beta = \frac{(N) \, [\eta]^{1/3} \, S \times 10^{-13}}{M^{2/3}(1-V)} \qquad\qquad 3.12a$$

$$\beta = \frac{(D) \, [\eta]^{1/3} \, M^{1/3}}{k \, T} \qquad\qquad 3.12b$$

Insertion of the values derived above gave $\beta = 2.67 \times 10^6$. From the table

of β as a function of axial ratio given by the authors the particle is

described as a prolate spheroid of axial ratio 22. β is an extremely

insensitive function of the axial ratio, varying from a value of 2.12

for the spherical case to 2.97×10^6 for a/b = 50. If the particle is

oblate and b/a 300, β must lie between 2.12 and 2.15×10^6. To obtain

the effective hydrodynamic volume, V_e, of the particle one uses the value

for axial ratio derived from β to determine the viscosity increment, ν,

described above from Simha's equation. For a/b = 22, $\nu = 48.6$. This

value is inserted into equation 3.13 along with the other appropriate

quantities.

$$[\eta] = \frac{N \, \nu \, V_e}{100 \, M} \qquad\qquad 3.13$$

$V_e = 0.430 \times 10^{-20} \, cm^3$. $\frac{M \bar{V}}{N}$, the classical anhydrous volume per molecule,

is $1.12 \times 10^{-20} \, cm^3$ In order to obtain agreement a negative hydration

must be assumed. Sherage and Mandelkern interpret such a result as indi-

cating that the molecule is free draining, and that the solid ellipsoidal

molecule assumed in the classical treatment is incorrect.

The dimension calculated from the effective volume and axial ratio given by the Sherage-Mandelkern treatment are 158 A by 7.2 A.

Since the results obtained are such sensitive functions of the experimental data, it is of interest to examine the limiting cases. Taking as the limits for the experimental data the following:
S = 1.03 to 1.09 Svedberg units; D = 9.5 to 10.5 x 10^{-7} cm^2/sec; and $[\eta]$ = 0.13 to 0.14; β varies between 2.58 and 2.77 x 10^6 corresponding to prolate spheroids of axial ratios 17 and 29 respectively.

G. Calculation of size and shape of dimer

Insertion of the values of S_{20}^o and D_{20}^o determined for the dimer into equation 3.5 and use of the value for partial specific volume derived with the monomer led to a value of M = 19,300. Equation 3.6 gave a value for the frictional ratio f/f_o = 2.02 which from the Perrin equation led to axial ratios of a/b = 21 and b/a = 30 for the prolate and oblate cases respectively. Assuming a value for the hydration of 30% as in the case of the monomer, equation 3.8 gave f/f_e = 1.12. The shape factor obtained was f_e/f_o = 1.81 which led to axial ratios of a/b = 15.5 and b/a = 20.95. Dimensions of the dimer were calculated from equation 3.9. For the prolate spheroid 2a = 220 A and 2b = 14.2 A, while for the oblate spheroid 2a = 4.66 A and 2b = 97.6 A.

H. Reconstitution of solubilized keratin

Reconstitution experiments were carried out both on SH-keratin and cysteic acid keratin. In the former case, examination was made of the diffraction patterns of material precipitated under various conditions. Best results were obtained when the pH was lowered gradually, as by dialysis. Reformation of the disulfide linkages was accomplished by

Table 3.1

Summary of Physical-Chemical Results
for Feather Keratin Monomer

S^o_{20} C--O	Sedimentation constant	1.06 ±0.03 Svedberg units
D^o_{20} C--O	Diffusion constant	10.02 ±0.5 x 10^{-7} cm^2/sec.
$\bar{V}$	Partial specified volume	0.725
M	Molecular weight	9,300 ±700
f/f_o	Frictional ratio, zero hydration	1.53
a/b	Axial ratio, prolate assumption	9.74 (zero hydration) 6.83 (30% hydration)
b/a	Axial ratio, oblate assumption	11.87 (zero hydration) 7.83 (30% hydration)
$[\eta]$	Intrinsic viscosity	0.135
ν	Viscosity increment	18.6 (zero hydration) 13.2 (30% hydration)
a/b	Axial ratio, prolate assumption, from viscosity increment	12.3 (zero hydration) 9.77 (30% hydration)
b/a	Axial ratio, oblate assumption, from viscosity increment	25.6 (zero hydration) 17.6 (30% hydration)
$(r^2)^{\frac{1}{2}}$	Effective length, random coil treatment	85 A
β	Scherage-Mandelkern factor	2.67 x 10^6
a/b	Axial ratio from β	22

the removal of thioglycol by thorough washing and air oxidation.

The most severe problem in these experiments was the difficulty in obtaining well oriented specimens. This was best accomplished by two procedures which gave approximately equivalent results. One involved rolling wet precipitates on hardened filter paper with a glass slide. As the specimen approached dryness the rolling was completed between two glass slides. Alternatively, small quantities of wet precipitate were pressed flat and dried between two siliconed glass slides. A pack of such flattened material could then be made in which there was orientation in the plane of the film surfaces. Orientation of the specimen so that the flat surfaces were parallel to the x-ray beam allowed meridional spacings to be distinguished from those belonging on the equator.

Figure 3.6a is the x-ray diffraction pattern of a specimen of the latter type. On it may be detected meridional diffractions corresponding to the 4th, 6th, *15th* and 17th orders of the 95 A fiber period. On the original pattern the 3.1 A meridional arc is also visible. On the equator of the pattern shown can be seen the 34 A equatorial spot. The diffractions corresponding to the backbone and side chain spacings appear as halos with intensification in the region of the equator. Spacings determined from the observed diffractions are given in Table 3.2.

TABLE 3.2

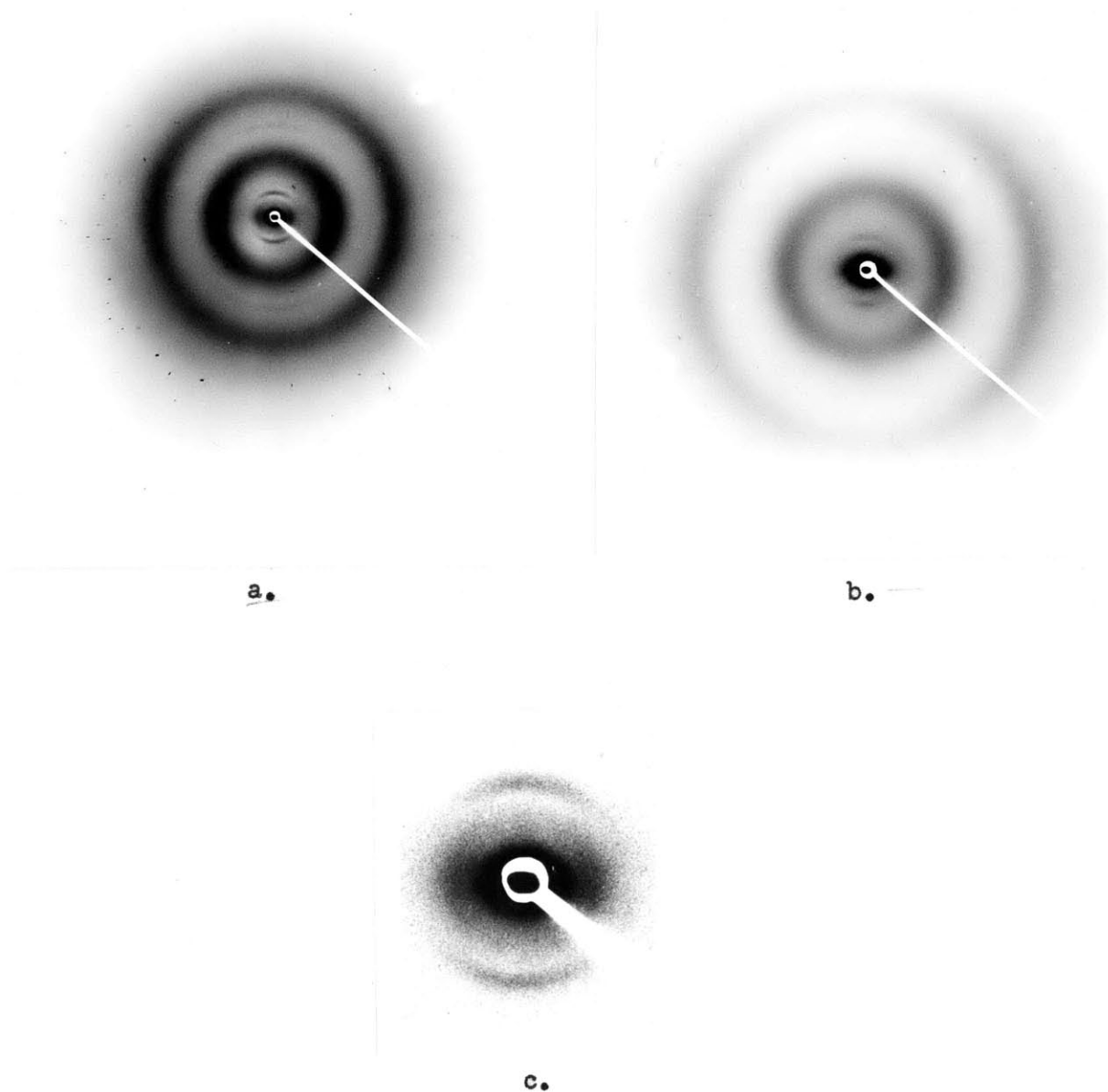|	d (Å)	Order of 95 Å period
Meridional Diffractions	22.1	4
	16.1	6
	6.20	15
	5.40	17
	3.05	--
Equatorial Diffractions	33.5	
	9.48	
	4.63	

a.

b.

c.

Figure 3.6. X-ray pattern of reconstituted feather
keratin monomer. The pattern obtained from recon-
stituted SH-keratin is shown in a. That of oxidized
material is shown in b. Fig. c is an enlargement of
the central portion of Fig. a.

The great solubility of cysteic acid keratin prohibited the making of precipitated specimens from it, since the salts remaining from a precipitation procedure could not be washed from them. A method of making thin films was therefore used. In this case the films were prepared by drying solutions of protein in distilled water on siliconed glass slides. Film packs were made and oriented as described above with the film surfaces parallel to the x-ray beam. Figure 3.6b is the x-ray diffraction pattern obtained from such a specimen of cysteic acid keratin. The specimen-to-film distance used in this case was 7 cm, in order to show more clearly the effect of oxidation on the diffractions close to the center of the pattern. Instead of the discreet 34 A diffraction seen on the equator of Figure 3.6a there is only a diffuse distribution of intensity. The 4th and 15th orders of the fiber axis period are present (although the latter is not evident in the reproduction) as are the backbone and side chain reflections.

I. Supplementary x-ray diffraction studies

Rugo (1950) described the production of a narrow rectangle of diffuse scattering which overlay the diffractions in a region approximately defined by the third layer lines and fourth row lines of the feather keratin pattern by treatment of feather with water and certain small organic molecules, notably n-propanol.

The rectangle is interpreted here as the result of diffraction by small groups of evenly spaced polypeptide chains of the β-type. Because of the extended nature of the chains one may consider as an approximation the case of sets of parallel rows of point atoms lying in a plane, the

separation between rows in each set being a constant: $\underline{a}$. If such an array is rotated about an axis parallel to the rows so that the orientation of sets resembles that of a fiber, the interference function is given by equation 3.14 (James, 1950).

$$I_0(r) = N^2 \left\{ N_1 + 2 \sum_{n=1}^{n=N_1-1} (N_1 - n) \, J_0 \, (2\pi \, nar) \right\} \qquad 3.14$$

On the zero layer line (the equator of a fiber pattern) $r = 2 \sin \theta/\lambda$. N is the number of point atoms per row and N_1 is the number of rows per set. Separate sets are considered to be diffracting individually.

To calculate $\underline{a}$ it is convenient to make use of the first minimum of the interference function along the equator. On the diffraction pattern of feather this occurs at $2 \sin \theta/\lambda = 0.066$.

The first minimum of the interference function $I_0(r)$, as a function of $(2\pi nar)$ was determined for the three cases where $N_1 = 2, 3,$ and 4. By comgining these results with the experimental information the chain separations were found to be 9.5 A, 11 A, and 12 A respectively. One may then calculate the widths of the sets of chains obtaining, in the same order, 19 A, 33 A, and 48 A.

Similarly, the intensity distribution of this rectangular area along the meridian has been taken to represent the diffraction of a single chain of length L, which may be calculated as follows assuming the chain to be a structureless rod.

The shape factor is given by:

$$S_n = F_n \, F^*_n$$

which is the square of the structure factor of the rod. Along the meridian F is given by:

$$F = \int_0^L e^{\frac{2\pi i\, y\, y^*}{\lambda}}\, dy = \frac{\lambda}{2\pi i\, y^*}\left(e^{\frac{2\pi i\, L\, y^*}{\lambda}} - 1\right)$$

Multiplying the expression for F by its complex conjugate F^* one obtains:

$$S = \frac{\sin^2\left(\pi L\, y^*/\lambda\right)}{\left(\pi\, y^*/\lambda\right)}$$

This function has its first minimum at $(\pi L\, y^*/\lambda) = \pi$, and since experimentally the minimum is found at $y^*/\lambda = 2\sin\theta/\lambda = 0.032$ to 0.023 L may be calculated to be between 33 A and 43 A. Experimentally it is much more difficult to locate the meridional minimum than that on the equator.

IV. Discussion

A comparison of the results obtained here with those reported by
Woodin (1954) shows excellent agreement for the value of S_{20}^o. They
$c \rightarrow 0$
differ by only 1%.

Intrinsic viscosity results agree less well. The value obtained here,
$[\eta] = 0.135$, is somewhat lower than the value $[\eta] = 0.15$ reported by Woodin.
This is believed to be the result of his use of solvents containing urea
which, as the author points out, may bind to the keratin. Pasynskii and
Chernyak (1950) reported a value of 0.08 grams of urea per gram of
keratin for the binding on wool. If the same value holds here for feather
keratin, Woodin was actually measuring the specific viscosity $(\eta_{rel} - 1)$
of a urea-protein complex and using the concentration of protein alone
to calculate the reduced viscosity η_{sp}/C. If his data is corrected by
assuming that the complex concentration was 1.08 times that of the pro-
tein the resulting value for the intrinsic viscosity of the complex
$[\eta] = 0.138$ more nearly approximates the value obtained here for the
protein.

The molecular weight obtained from sedimentation-diffusion studies:
$M = 9300 \pm 700$ compares reasonably well with Woodin's molecular weight
from light scattering: $M = 11,000 \pm 1,000$; and number average molecular
weight from osmotic pressure studies: $M = 9,000 \pm 990$.

Sedimentation patterns of solutions of the feather keratin monomer
show a single peak of normal behavior with no evidence of assymmetry over
periods of up to four hours of centrifugation at 60,000 rpm. This is
not to be taken as evidence of complete homogeneity but does indicate
that no species greatly different in weight is present unless it is one

which, because of a differing diffusion constant, fortuitously has the same sedimentation constant as the monomer. Better evidence for homogeneity is obtained by comparison of the diffusion constant obtained under the influence of a strong centrifugal field with that obtained under conditions where external influences are negligible. At a concentration of 0.71%, $D_{20}^o = 8.4 \times 10^{-7}$ cm^2/sec. was obtained in the ultracentrifuge while $D_{20}^o = 9.76 \times 10^{-7}$ cm^2/sec. was obtained in the diffusion apparatus. If an inhomogeneity exists one expects the centrifugal result to be considerably higher than the other, while here it is found to be somewhat lower. The lower result is in all probability due to a subjective error in the measurement of peak height and area. One must estimate on the Schlieren pattern the proper boundary of the peak and the correct position of the base line. Since the area reduced to cell dimensions is small the error may become appreciable. It is considered here that the result is strong evidence for homogeneity.

This conclusion is supported by the closeness of the number average and weight average molecular weights obtained by Woodin which have been described above, and by the fact that his electrophoresis results provide no evidence for more than one component.

For these reasons, and since on x-ray diffraction patterns of reconstituted feather keratin the 4th, 6th, 15th, and 17th orders of the 95 A fiber period, the 3.1 A meridional arc and the 34 A equatorial spot are visible, it is concluded that the monomer isolated here is in fact the material giving rise to the complex pattern of native feather. The meridional diffractions found are all of intramolecular dimension but the 34 A equatorial diffraction represents a lateral packing parameter of the

molecules. Lack of registration of further diffractions is taken to be
the result of the inability with present reconstitution techniques of
gaining anything near the high degree of order in packing as is found
in native feathers. Apparently lateral aggregation packs the molecules
side to side in reasonable order but they appear to be quite out of
register axially. The pattern obtained from oxidized monomer is quite
like that of the reduced material, but here the 34 A spot is missing and
the pattern is evidently wholly intramolecular. This is not unexpected
since the cysteic acid side chains present considerably larger groups to
prevent the closeness of packing and in addition are unable to bind the
molecules together through covalent linkages. The presence of the 24 A
meridional diffraction, which is obtained even with some of the rather
poorly organized samples of reconstituted material, suggests that it is
directly related to the location of the relatively heavy sulfur atoms;
i.e. that they tend to divide the fiber axially into quarters.

From the physical-chemical experiments results were derived dealing
with the dimensions of the monomer. Comparison of axial ratios derived
from techniques such as sedimentation-diffusion and intrinsic viscosity
are commonly used to judge whether the oblate or the prolate spheroidal
assumption is better. From Table 3.1 a closer correspondence is noted
for the prolate case. In addition, even with the wide limits of error
assumed, the Scherage-Mandelkern factor β , describes the molecule as
prolate. The dimensions derived for this shape from sedimentation-
diffusion data: length = 100 A, width 15 A must now be related to the
actual molecular shape which is in all probability not spheroidal. One
may safely conclude that the 100 A dimension corresponds to the length
of the unit cell derived from diffraction studies. The width of the

spheroid approximates one half of the unit cell width but this interpretation is not definite since the 15 A dimension is an average of the true width and thickness of the molecule. Some possible conclusions as to the true shape of the molecule will be discussed after the presentation of other pertinent information.

Although the studies carried out here are of principal importance with respect to low angle diffraction and order of large size, some information may be derived concerning the smaller structural entities of the molecules.

Since the normal x-ray diffraction pattern of feather is that of a α-protein, the 3.1 A meridional arc has been taken as the axial projection per amino acid residue. For a molecule of approximately 10,000 molecular weight, of length 95 A, it can be shown that each molecule must be composed of three chains of amino acid residues or the equivalent. To illustrate: The number of residues of 3.1 A projection which will fit into a 95 A period is 95/3.1 = 31. The average residue weight in feather keratin, calculated from the analyses of Graham, Waitkoff, and Hier (1949) is approximately 116. Thus the molecular weight of a 95 A chain is 116 x 31 = 3596, and for three such chains is 10,800 which is good agreement with the figure obtained from solution studies.

Additional information may be gained from an examination of the reconstitution results. Keeping in mind that the appearance of the meridional diffractions indicate very poor axial register of the recombined molecules, the appearance of the 34 A equatorial spot suggests that this is actually a true lateral dimension of the molecules. If, alter-

natively, the molecular width corresponded to half of the x-ray unit cell width, one would expect a corresponding diffraction to appear at 17 A. One would obtain the 34 A diffraction in this case only if the molecules were packed quite precisely in such a way that the true lateral repeat distance would be twice the width per molecule. The patterns obtained offer no indication that this is true.

The results obtained with the dimer, which it should be remembered have not been derived with extrapolated values of the sedimentation and diffusion constants, indicate a longitudinal covalent hookup of pairs of monomer through cystine. The length of the spheroidal model of the dimer is about twice that found for the monomer while the width remains approximately constant. The conversion of dimer to monomer by standard reductive procedures indicates that its original formation is due to a differential susceptibility to oxidation of disulfide bonds at various loci on the molecule. Whether this is due to a difference in the reactivity of the various bonds or to differences in the ability of the oxidizing agent to penetrate to the bonds is not known.

One may conclude from the above discussion that the keratin molecule is composed of three β-polypeptide chains, each of which may be considered as a rectangular parallelipiped of approximately 10 A, (the side chain spacing), by 4.65 A, (the backbone spacing) by 95 A, (the fiber axis period). The evidence does not specify absolutely the relationship these chains bear to one another. Thus they may be arranged laterally into a sheet, or into a prismatic structure with two chains in one plane and the third stacked on top of the pair, or all three may be arranged in a layer perpendicular to the side chain direction. From the appear-

ance of the 34 A equatorial spot in diffraction patterns of reconstituted

material, however, it has been concluded that the first of these, the

lateral arrangement of chain in a sheet, is probably correct. The re-

sults derived in section III-I from the rectangular diffraction shape

exhibited by feather under certain conditions are consistent with this

interpretation. It is with this assumption in mind that the model to be

proposed below has been derived.

In summary, the conditions which the model must fit are the

following:

1. The molecule is to be described by the unit cell dimensions of

95 A by 34 A and the backbone spacing of 4.65 A.

2. The molecule must be able to accommodate three β -polypeptide

chains.

3. The structure, including the packing of molecules, must fit

the requirements imposed by the net of Bear and Rugo (1951) and the

diffraction effects exhibited by native feather.

4. The structure must make allowance for both lateral and longitudinal

disulfide linkages between neighboring molecules. The importance of the

former is shown by the interference with lateral packing caused by the

cysteic acid side chains in preparations of oxidized material. The

necessity for longitudinal linkages is indicated by the monomer-dimer

relationship.

5. There must be six atoms of sulfur per molecule. This require-

ment is imposed by the results of Woodin (1954).

Condition 2. is already satisfied by the planar molecule described

by condition 1. One finds it more difficult, however, to reconcile the

requirement of four nodes per unit cell prescribed by the Bear-Rugo

net with the six atoms of sulfur per molecule found by Woodin. The

nodes, while not truly equivalent in the crystallographic sense (i.e.

the true unit cell is twice that required by the nodes) must nevertheless

carry nearly equal weight after the heat-moisture treatment. Thus,

they may be expected here to represent nearly equivalent amounts of

sulfur. Possible resolution of the problem lies in the fact that the

net (see Figure 1.2) may actually be the projection of several layers

of material. On this basis the type of model illustrated in Figure 4.1

is presented. Figure 4.1a is a diagrammatic sketch of an individual

molecule showing the location of sulfur atoms and polypeptide chains.

In order to obtain the net from this molecule, successive layers must

be arranged relative to one another as indicated in Figure 4.1b. Within

each layer the molecules are fitted directly side to side and end to

end in a rectangular arrangement. Molecules in successive layers are

displaced laterally one half of the unit cell and axially one fourth of

the unit cell, and are rotated 180° about out the fiber axis as indicated

by arrow. After four layers the structure repeats so an analysis of

contributions to nodes by the four layers will indicate if the structure

is suitable. If the nodes are taken to represent the sums of projected

disulfide bonds one finds that each node represents three such pairs

of sulfur atoms. The nodes are thus equivalent. The lack of true

equivalence shown by the pattern of native feather must then be due to

the way in which material is distributed about the nodes.

It might be argued that the structure would give diffraction evidence

of a spacing corresponding to the repetitive sets of four layers. The

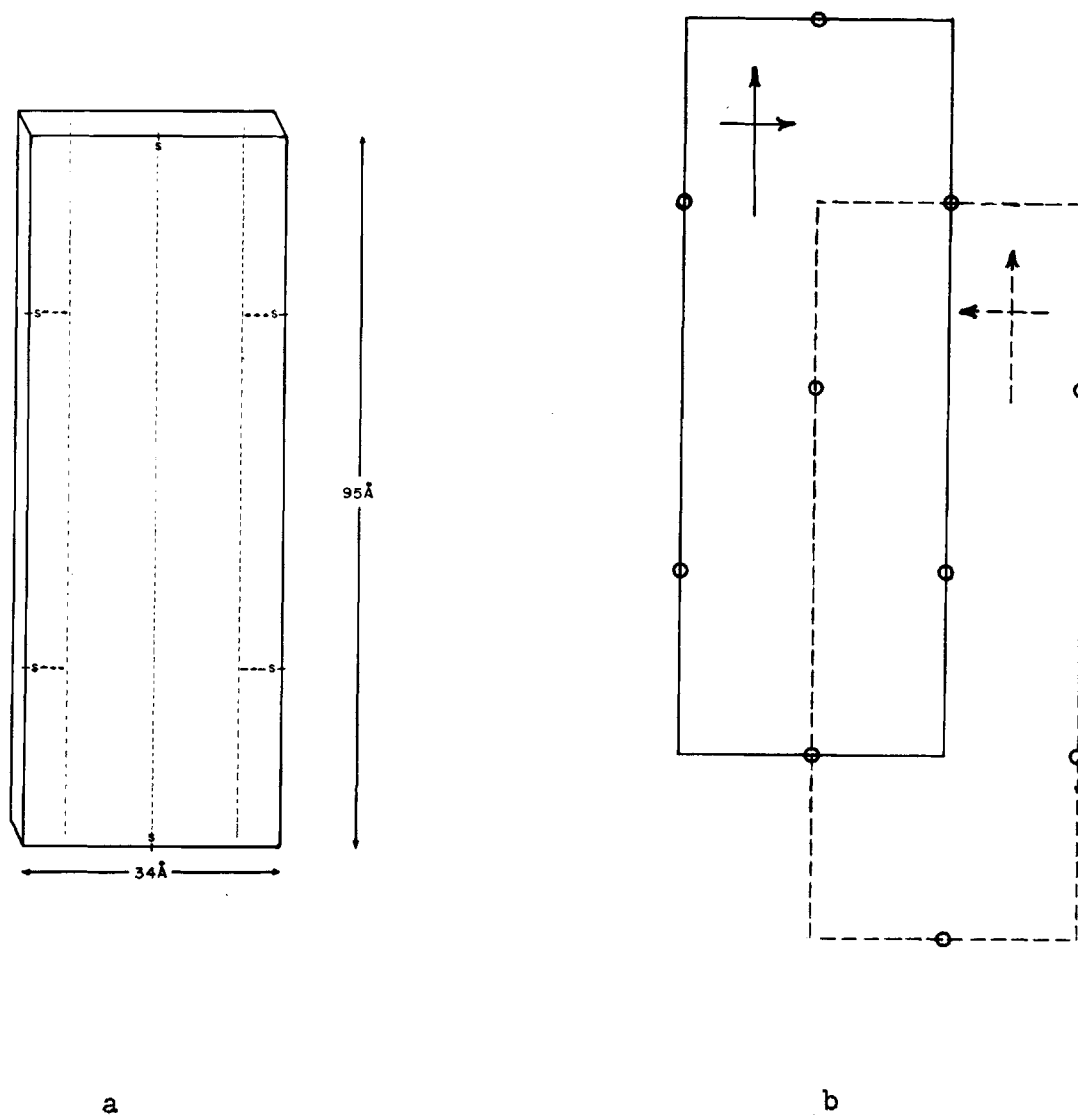a   b

Figure 4.1.   Proposed model for the large scale structure of
feather keratin.   In a is presented a diagrammatic sketch of the
molecule with position of sulfur atoms indicated by S and poly-
peptide chains of dotted lines.   In b, the relative orientation
of molecules in adjacent layers is illustrated.   The circles are
comparable to the nodes of Figure 1.2.

intensity of such diffractions would be expected to be small, however, because of the similarity of the layers involved. The absence of such a diffraction on the pattern of native feather, therefore, does not rule out the model.

The structure above does not provide the only possible arrangement of sulfur. Even the limiting case in which all the sulfur is grouped about one locus on the molecule can be made to give the net pattern by the proper arrangement of succeeding layers. This particular case may be considered as trivial because of the impossibility of successfully bonding the molecules together to form a fiber of feather keratin, but some of the intermediate cases must be considered as being as probable as that illustrated in Figure 4.1.

The findings of Ambrose and Elliot (1951) from infrared studies that there is an α-protein component present in the feather structure does not seriously affect any of the arguments presented above. Since the detailed structure of the polypeptide chains is not known, one may speculate that some portions of it may give rise to infrared modes cor-responding to those found with α-keratin. If, for example, as may well be the case, what has been described as a group of three chains is in reality one continuous chain folded back upon itself every 95 A, the structure at the folds might somehow be related to the α-type of struc-ture. It would be of considerable interest in this connection, however, to examine samples of reconstituted keratin for these infra-red character-istics.

Further studies on the dimer would probably be of help in providing some of the additional information which is needed to solve the feather

keratin structure. A study, for example, of the number of free SH-groups
formed when the dimer is converted to monomer by reduction would help
to determine the location of some of the cystine on the molecule. End
group analysis should be able to discriminate between a structure com-
posed of three separate chains and one in which there is but one chain
which is folded back upon itself.

The information derived from such techniques as these taken with
the results provided above will limit considerably the number of struc-
tures will have to be examined in detail for correspondence with x-ray
diffraction data. Bearing in mind also the limitations imposed by the
stereochemical properties of the amino acid residues which make up the
molecule, more detailed structures may be proposed and tested by, for
example, optical transform techniques for identity with the structure
of native feather keratin.

V. Summary

1. Methods are given for the preparation of solutions of both monomeric and dimeric forms of feather keratin. The former has been prepared in both the reduced (SH-keratin) and oxidized (cysteic acid-keratin) forms. The dimer has been obtained in the cysteic acid form by fractionation of oxidized preparations of feather. It may be converted into dimer by the further splitting of disulfide bonds.

2. Physical-chemical studies on the cysteic acid monomer describe it as a particle of molecular weight $M = 9,300 \pm 700$, a value which is in agreement with the results of Woodin (1954). Its shape may be approximated by a prolate spheroid of major axis equal to 100 Å and minor axis equal to 15 Å. The molecular weight of the dimer was found to be 19,300. Its length is approximately twice that of the monomer, the diameters being about the same in the two cases. The dimer is shown to consist of two monomeric units linked end to end through one or more disulfide bonds.

3. X-ray diffraction patterns of reconstituted samples of both oxidized and reduced material were made. The diffractions observed indicated that the monomer is the unit which gives rise to the complex diffraction pattern of native feather. Since the prominent equatorial spacing of 34 Å was observed with the reduced material, even under conditions where axial alignment of the molecules was quite poor, it was concluded that this diffraction describes directly one dimension of the feather molecule. The absence of this diffraction in patterns from oxidized keratin points up the importance of disulfide bonds in the reconstitution process. The large cysteic acid side chains on the oxidized molecules prevent the proper packing relationship.

4. It has been deduced from the results obtained that the molecule is best described as a rectangular parellelipiped 95 A by 34 A by 4.65 A, composed of three parallel β-polypeptide chains lying in a plane coincident with their side chains. It is pointed out, however, that a single chain folded back every 95 A in such a way that it occupies in the main the position of the group of three chains is possible.

5. A tentative model structure has been proposed which is in agreement with the results obtained here and the gross aspects of the x-ray diffraction data. The limitations of the model are discussed and some of the alternative possibilities are pointed out.

6. The applications of methods which show promise of aiding in the final solution of the feather keratin structure have been briefly discussed.

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