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Procollagens

A citrate-soluble fraction of collagen is assumed to form a special group of connective tissue proteins.

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Connective tissue proteins are attracting a lot of attention, and many works have been devoted to them. If we consider only procollagen, with which we are going to deal in this article, this protein alone has already been the subject of a great number of researches. The relevant communications are so numerous that, frankly speaking, we are in a predicament, finding it entirely impossible to discuss them more or less comprehensively in this report. So far, we are not in a position to make any generalizations on the basis of published works. The point is that many investigations on procollagen are in the stage of experimental development as yet, and therefore a generalization of theoretical and practical significance may be expected only in the future. To cope with this situation, we have decided to report only the results of some investigations on procollagen carried out in our laboratory and published in Soviet and foreign scientific literature.

In our researches on proteins of the skin, we found in the latter a protein fraction soluble in an acid citrate buffer solution. It appeared that the protein making up this fraction contains about 25 percent of glycine as well as about 25 percent of proline and hydroxyproline, its content of aromatic and sulfur-containing amino acids being low. These data pointed to the proximity of this protein to collagen, and, consequently, it should be considered one of the group of collagen-like proteins. We are accustomed to think that collagen, constituting the bulk of collagen fibers, is an insoluble protein. Therefore we assumed that the citrate-soluble fraction is the biological precursor of collagen, and we named it procollagen, implying thus that the soluble collagen serves to form the principal insoluble mass of collagen fibers.

A number of observations concerning the quantitative content of these proteins in various physiological and pathological states of the organism testified in favor of the conception that procollagen is the biological precursor of collagen. For instance, it is well known that, with aging of the organism, the rate of collagen accumulation in the tissues gradually decreases. It turned out that procollagen content sharply decreases with the aging of animals. In guinea pigs 10 days to 6 months old, procollagen content varies from 7 to 10 percent, whereas in aged animals (more than 8 months old) it decreases to 1 percent. Further, collagen accumulation and the formation of new collagen fibers is greatly inhibited in scurvy-affected animals. It appeared that in these animals procollagen content decreases sharply, twice as much as in the control animals. These results can be explained by the reduction in procollagen formation with aging and in scurvy; in these conditions the source of formation of new collagen fibers is exhausted. It goes without saying that these data cannot be regarded as direct proof; nevertheless, they raise our supposition about procollagen being the biological precursor of collagen to the status of probability.

The amino acid content of soluble procollagen and that of collagen insoluble under ordinary conditions have been studied repeatedly. In our respective researches, when comparing data on the amino acid content of procollagen with those quoted with regard to collagen in the well-known paper by Bowes and Kenten (*1*), we have noted a difference in the content of such amino acids as phenylalanine, histidine, proline, and hydroxyproline. The difference in the chemical composition of soluble and insoluble collagens was also discovered by a number of other investigators—for example, by Bowes, Elliot, and Moss (*2*). It might be assumed that in the process of transformation of procollagen into collagen there take place some, although small, changes in the chemical composition of the former, rendering it insoluble. We cannot, however, insist on such an assumption, since obtaining pure insoluble collagen involves certain difficulties, and one should always keep in mind the possibility of errors in interpreting such data.

It is worthy of mention that, as far back as in 1900, Zachariadés (*3*), after treating tendons of rat tails with acidified water, found a soluble collagen-like protein in the solution. In 1927–30 Nageotte (*4*) thoroughly investigated the phenomenon of the passing of tendon proteins into solution. He found various ways of isolating this protein from the solution, the protein precipitates being obtained in the form of fibers or gel. Nageotte affirmed that this protein, or collagen A, occurs only in the tendons of rat tails. Somewhat later Leplat (*5*) succeeded in proving that the above-mentioned protein, soluble in acidified water, may be found not only in the tendons but also in the skin of various animals.

Following Nageotte and Leplat, other researchers also began to study collagen A. Some of them arrived at the conclusion that collagen A and collagen are identical. It follows from our data, how-

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ever, that collagen A belongs to the procollagen group. It is true that some investigators entertain doubts about the expediency of applying the term *procollagen* to proteins which are very similar in chemical composition to collagen, and they prefer to call such proteins "soluble collagens." Others doubt whether proteins soluble in acid media are the precursors of collagens. The supposition is even expressed that the actual precursor of collagen is a fraction extracted with alkaline and neutral salt solutions. We shall revert to this matter later. Now we should like to note that the solution of these moot problems presents some difficulty. This is due to the fact that, so far, we are not in possession of sufficiently detailed data on insoluble collagen. Therefore it is difficult to tell how the transformation of soluble into insoluble protein (that is, of procollagen into collagen) takes place, whether it is accompanied by any chemical changes in procollagen, or whether only physical changes—a peculiar thickening of procollagen due to the formation of intermolecular bonds—are of significance. We prefer to use the term *procollagen* rather than *soluble collagen*. We believe that at present it would make no sense to enter into a discussion on terminology.

Methods of Obtaining Procollagens

Now we shall dwell briefly on the methods of obtaining procollagens and their occurrence. The conventional method of obtaining procollagen is the extraction of minced tissue with a citrate buffer solution (*p*H 3.5 to 4). Each 24 hours the liquid is decanted, and a new batch of the buffer solution is added. This is repeated 3 to 5 times. Each extract is filtered twice through a paper filter. The protein is isolated from the filtrate, either by dialysis against tap water or a weak alkaline buffer solution or by precipitation by addition of acetone (up to 30 percent) or sodium chloride (up to 5 percent). Precipitates obtained by dialysis usually consist of needle-like formations resembling crystals. It should be mentioned that these are not true crystals from the crystallographic point of view, since x-ray investigation has shown no periodicity in the three spatial directions. The patterns obtained were similar to that of collagen. It is noteworthy that in dialyzing the filtrate against a limited volume of a phosphate buffer solution with *p*H of about 8, we succeeded in obtaining "crystals" up to 3 millimeters in length.

We have isolated procollagens from organs and tissues of man and from those of representatives of various classes of vertebrates. Procollagen content not only differs in various species of animals but also varies greatly in different tissues of the animals of the same species. Procollagen was isolated from the skin as well as from the stomach and bladder tissues of man. We failed to discover procollagen in the tissues of blood vessels and intestines. It was isolated from the skin of cattle, a rabbit, a dog, a cat, a pike perch, a chicken, a turtle, and a frog and from the tissues of dog stomachs, chicken goiters, and pike-perch swim bladders. Procollagen was found in small amounts in the skin of a grass snake. Small quantities of this protein are also contained in cattle tendons. We failed to isolate procollagen from the tissues of some invertebrates—namely, rainworms and pond snails; obviously there is no such protein in the tissues of these animals. It is necessary to emphasize that the conditions for extracting procollagen from the tissues of various animals are different. Thus, the most suitable materials for extracting procollagen from chicken skin are buffer solutions with *p*H 3.0 to 3.5, whereas solutions with *p*H 2.0 to 3.0 are the best for extracting protein from turtle skin.

Incorporation of Labeled Compounds

A series of our investigations was devoted to the study of the incorporation of labeled compounds. These experiments were carried out with the aim of obtaining additional data in support of the assumption that procollagen is the biological precursor of collagen. With this object in view, we used glycine containing radioactive carbon in the carboxyl group. After a single injection of labeled amino acid, the animals were killed at different time intervals. Their skin was minced, and procollagen was extracted from it by treatment with a citrate buffer solution. It was shown by such investigations (the results of some of them are given in Table 1) that procollagen radioactivity during the first hours after the glycine injections rapidly increases and reaches a peak in 24 hours. Then it gradually decreases, and in 35 to 40 days its values approximate the values of collagen radioactivity which are characteristic for it during the initial period after the glycine injection. In contrast to procollagen, carbon-14 content of collagen exhibits a constant increase as a function of time after the radioactive glycine injection, and in 40 days its activity exceeds 7 to 10 times that of procollagen of the first extraction.

The characteristic change in the radioactivity of proteins obtained from the successive extractions of the skin, as a function of time, is of particular interest. Procollagens isolated from the first, the second, and the third extracts are characterized by a rapid incorporation of carbon-14 and its rather rapid elimination upon reaching the peak, whereas with proteins isolated from the subsequent extracts, such a regularity is somewhat disturbed. In this case one may observe either a certain decrease in the rate of carbon-14 elimination or an increase in the content of radioactive carbon with time, which, as noted above, is typical of collagen as well. Such a phenomenon permits us to consider procollagens of the successive extractions as transient forms of this protein, from the most metabolically active to the least active, and, ultimately, to collagen.

Indeed, if we take into consideration the fact that, by the time the greatest amounts of radioactive collagen have formed, the bulk of carbon-14 should have been eliminated from the organism and that consequently there is no sufficient source of radioactivity left, then this process of change in the radioactivities of procollagen and collagen can be pictured as follows. During the first 24 hours after the introduction of the tracer, procollagen is synthesized, chiefly at the expense of the carbon-14 of the glycine. Therefore, the protein obtained in the first extraction is very rich in radiocarbon. Protein fractions passing into solution in subsequent extractions were synthesized mainly prior to the introduction of glycine-C^{14} and therefore unlabeled proteins prevail in them.

At this stage collagen radioactivity is also low. With the passage of time the radioactive procollagen obtained in the first extraction, being transformed into collagen through intermediate stages, causes the enrichment of these proteins in radiocarbon. This transformation, as well as the continuous elimination of carbon-14 from the organism without any intake from outside, results in the fact that the synthesis of new portions of collagen is accompanied by the incorporation of a gradually diminishing quantity of radiocarbon. The formation of unlabeled protein which dilutes the formerly synthesized radioactive procollagen causes a decrease in its radioactivity; 40 days after the injection of glycine we see that the radioactivity of the

Table 1. Radioactivity of collagen and procollagens from successive extracts of skin of normal guinea pigs as a function of time elapsed since single injection of glycine-C^{14} (disintegrations per minute per 10 mg of protein).

Proteins	No. of hours after injection																		
	3	3	6	6	12	12	24	24	96	96	480	840	840	960	960	960	960	960	960
Procollagens:																			
Extract 1	71	52	119	99	150	100	157	180	97	71	64	32	18	18	7	8	6	16	18
Extract 2	62	45	114	86	110	82	119	124	75	58	59	44	37	32	39	20	12	26	38
Extract 3	40	37	79	70	84	45	84	84	52	40	52	40	41	47	41	44	27	37	40
Extract 4	50	43	60	50	64	38	64	47	42	35	44	52	53	82	71	38	33	36	41
Extract 5	35	32	65	44	60	30	64	53	53	37		55	35	87	70		30	25	33
Extract 6	27	24	32	39	38	40	48	38	32	39	40	32	31	65	44	39	20		40
Collagen	12	12	17	9	25	17	26	20	30		40	50	42	108	80	53	44	43	46

procollagen of the first extraction is 7 to 10 times as low as that of collagen. At the same time the proteins isolated from the subsequent extracts still contain a considerable amount of carbon-14, which augments with the increase of the serial number of the extraction. The probability of such a transformation process is confirmed by researches carried out by other authors [Neuberger (*6*); Neuberger, Perrone, and Slack (*7*); Perrone and Slack (*8*)], who are also inclined to explain the increase in collagen radioactivity by the accumulation of the newly synthesized, "young" collagen. At present we cannot define the nature of the difference between these transient forms of procollagen. It might be that they differ in the degree of intermolecular interaction of individual primary particles in the connective tissue.

Procollagens in Alkaline and Neutral Salt Solutions

At this point it is necessary to mention the results of the investigations of collagen-like proteins soluble in alkaline solutions and in neutral salt solutions. For instance, Harkness, Marko, Muir, and Neuberger (*9*) found that the incorporation of glycine carbon-14 in alkali-soluble collagen proceeds at a higher rate than in citrate-soluble collagen. A conclusion was drawn, on this basis, that alkali-soluble collagen is the actual precursor of collagen. In agreement with these data are Jackson's investigations (*10*) on collagen formation in connective tissue after a local injection of carrageenin. It was found in these investigations that collagen soluble in neutral salt solutions was again the first to form, and that the fraction of citrate-soluble collagen appeared later. The rate of glycine-C^{14} incorporation in the neutral soluble fraction was much higher than in the citrate-soluble fraction and in collagen, whereas it was the same for the latter two. In this connection it should be mentioned that we investigated physicochemical properties of alkali-soluble collagen and found that these two proteins are identical with respect to glycine and hydroxyproline content, the sedimentation constant, and the intrinsic viscosity value. These proteins have very similar molecular weights and particle shapes and, consequently, do not differ from each other in principle. Alkali-soluble protein is soluble in a citrate buffer solution, too, and therefore we have encountered it in the first procollagen extracts when carrying out the experiments with radioactive glycine described above. Obviously, this protein is one of the above-mentioned transient forms of procollagen. The same holds true for the fraction soluble in neutral salt solutions.

It is worth saying a few words about the protein termed "tropocollagen"—that is, the precursor of collagen. As is known, this name was given by Gross, Highberger, and Schmitt (*11*) to structural elements of the so-called FLS (fibrous long spacing) having a length of about 3000 angstroms and discovered with the aid of electron microscopy. It is just this length that is characteristic of particles of procollagen (as well as of ichthyocol, which is similar to it) in solution, according to the results of the physicochemical investigation of these proteins in the soluble state, and to the recent electron-microscopic investigations (*12*). It should be admitted, therefore, that the terms *procollagen* and *tropocollagen* refer to one and the same protein.

Molecular Weight and Particle Length

We have given much attention to the study of procollagen solutions by physicochemical methods. In the first place we were interested in the molecular weight of procollagen and in the shape of its molecules. At one time it was believed that the molecular weight of procollagen is 70,000 and its particle length is 380 angstroms, the degree of asymmetry being around 1/20. Such data were obtained by Bresler *et al.* (*13*) on the basis of studies of the sedimentation and diffusion of this protein. As was discovered later, these data are not applicable to native procollagen, since procollagen was dissolved at about 40°C—that is, under conditions where this protein is entirely denaturated and breaks up into constituent parts. Therefore the molecular weight 70,000 applies rather to the degradation products—that is, to the so-called parent gelatin.

In this connection we undertook investigations with the aim of determining the molecular weight of native procollagen. We have studied the sedimentation, diffusion, and viscosity of procollagen dissolved in a citrate buffer solution with *p*H 3.7, containing either 1 percent calcium chloride or 0.5*M* urea. The latter two substances were added in order to prevent spontaneous coagulation of procollagen on the boundary, which was frequently observed in studying diffusion when only citrate buffer solution was used as a solvent. We obtained the sedimentation constant ($s = 3.05$ to 3.25 Svedberg units), the diffusion constant ($D = 0.35$ to 0.40×10^{-7} cm²/sec), and intrinsic viscosity ($[\eta] = 16$ to 17). The respective coefficients were determined at the lowest concentrations possible and extrapolated graphically to infinite dilution. One of the determinations of the diffusion coefficient was, for instance, made at a protein concentration of about 0.02 percent by means of a polarization interferometer.

On the basis of these data we calculated the molecular weight of procollagen (about 700,000) and its particle length (approximately 6000 angstroms). The difficulties we faced in studying collagen solutions, which were caused by

the extremely high degree of asymmetry of this protein, did not permit us to regard these values as precise. As we learned later, other investigators simultaneously studied the molecular weight of procollagen and similar proteins (for example, ichthyocol). It is interesting to note that they obtained identical sedimentation constants (about 3 Svedberg units) and intrinsic viscosity values (about 15). This signified that the molecular weights and the sizes of these molecules should be approximately the same. However, greatly varying values for molecular weight were obtained. Very high values exceeding one million were obtained by the light-scattering method. Such data should be treated with some caution, for it is quite difficult to clarify solutions of these proteins from dust, the presence of which heavily distorts the results. Noda (*14*) obtained data similar to ours for soluble collagen from a rat tail ($s = 3.5$ Svedberg units; $D = 0.5 \times 10^{-7}$ cm²/sec; $M = 700{,}000$). Peng Chia-Mu and Tsao Tien-Chin (*15*) found the molecular weight of procollagen to be equal to 400,000, using the osmometry method.

In our judgment, the most thorough investigations appear to be those carried out by Boedtker and Doty (*16*) who found a convenient way to remove dust from solutions and who measured the molecular weight and the particle size of ichthyocol by the light scattering, sedimentation, viscosimetry, osmometry, and birefringence methods. The results obtained by the use of these methods were in good agreement. Boedtker and Doty found the molecular weight to be 350,000 and the particle length about 3000 angstroms. These values appear quite probable. However, the diffusion constant (0.5×10^{-7} cm²/sec) is in discord with this molecular weight. So are the data on the molecular weights and the quantitative ratio of the components resulting from the breakup of procollagen molecules under certain conditions. We shall deal with this problem later on. Meanwhile, we should like to emphasize once again that the investigation of this protein by physicochemical methods is accompanied by a good many difficulties, since procollagen solutions are far from ideal. Some errors are quite possible, but they will be corrected in the future as our knowledge of this protein is expanded.

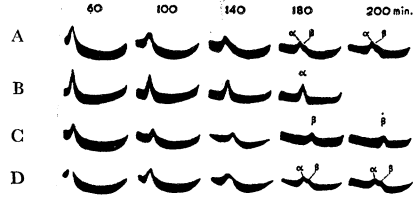

Fig. 1. Sedimentation diagrams from experiments on the isolation of the individual components of procollagen. Sedimentation was performed in a Svedberg type ultracentrifuge, at a speed of 56,000 rev/min and at a rotor temperature of about 20°C. The material was dissolved in phosphate buffer at *p*H 8, containing 10 percent of potassium thiocyanate. (*A*) Sedimentation of the initial mixture of two breakdown products of procollagen. The slower component is designated the α-component and the faster one, the β component. (*B*) Sedimentation of α-component. (*C*) Sedimentation of β-component. (*D*) Sedimentation of a mixture of α and β components in which the relative proportion of β-component was intentionally made greater than in the initial mixture.

Denaturation

Procollagen in solution retains its native state only under certain conditions. When solutions are heated, procollagen is denaturated, that is, decomposed into its components. The denaturation of procollagen is accompanied by a decrease in viscosity and optical rotation. It should be noted that the terms *native* and *denaturated,* as employed here, may seem somewhat conventional. We apply the word *native* in the sense of "nondenaturated." We do not see any reason for not using the term *denaturation,* though in the case under study this process is not accompanied by a "classical" increase in viscosity and optical rotation characteristic for the "classical" denaturation of globular proteins. By "denaturation" we imply here "the loss of specific structure of the macromolecule without chemical degradation" (*16*).

Thus, the heating of procollagen solutions is followed by a drastic decrease in viscosity and optical rotation. This process is irreversible, just as is the transformation of collagen into gelatin, and it is to some extent similar to the thermal shrinkage of collagen. The temperature threshold of such denaturation of procollagen from rat skin depends on the nature of the medium. In a citrate buffer solution with *p*H 6, it was observed at about 40°C; with *p*H 4, at about 37°C; with *p*H 2, at about 30°C. The addition of urea to the citrate buffer solution with *p*H 4 elicited a marked reduction of the threshold of denaturation (30°C at $3M$ and 23°C at $6M$). Guanidine chloride, potassium thiocyanate, calcium chloride, and so on, had the same effect. On ultracentrifuge studies of a procollagen preparation in a citrate buffer solution with *p*H $3 + 3M$ urea, which was preheated for 10 minutes at 30°C, we discovered two peaks on the sedimentation diagrams.

The pretreatment conditions and the abrupt reduction in viscosity of the solution without a considerable change in the sedimentation coefficients indicated that, in this case, there takes place the decomposition of the procollagen molecule into individual components as a result of the rupture of hydrogen and, perhaps, salt bonds. We also observed two peaks on the sedimentation diagrams for denatured ichthyocol and soluble collagen from codfish skin. This permits us to believe that such a two-component nature is a common characteristic of the structure of all the proteins of the collagen group. Such an opinion was also expressed by Doty in his letter to us. It is remarkable that the behavior of procollagen in solutions—namely, the change in its properties on denaturation—is similar to the process of denaturation of desoxyribonucleic acid in solutions. In the latter case the viscosity also decreases at a certain temperature, hydrogen bonds are also ruptured. In this respect the investigation of the process of the denaturation of procollagen and of the decomposition products of this protein is of interest not only as a solution of a particular problem but, possibly, also of more general problems pertaining to the principles of building of biologically important fibrillar formation.

Isolation of Components

Our further objective was the isolation of the above-mentioned components. Since these components resemble gelatin with respect to a number of properties, we applied some methods employed in gelatin fractionation. We attempted to carry out separation by the alcohol method, by precipitation with ammonium sulfate, and by using sodium dodecyl sulfate. But we never achieved the desired results. At best, we obtained one component with a lower content of the other, as compared with the initial mixture. At last we succeeded in working out a technique yielding satisfactory results. The technique consisted in precipitating components from their solution in $5M$ urea by adding an ammonium sulfate solution at 37°C. The faster component, designated the β-component, was the first to precipitate; then, as the concentration of ammonium sulfate increased, the

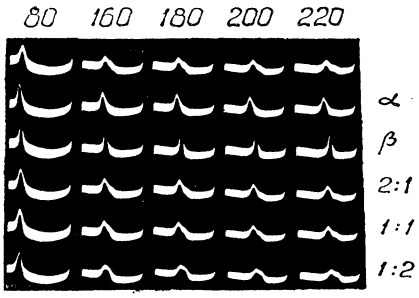

Fig. 2. Sedimentation diagrams (scale in minutes). (Top) Initial component mixture after denaturation of procollagen. (α) Isolated α-component. (β) Isolated β-component. (2:1, 1:1, and 1:2). Sedimentation diagrams of artificial mixtures of α- and β-components in weight ratios 2:1, 1:1, and 1:2, respectively.

slower component, designated the α-component, separated out. On subsequent reprecipitations, rather pure preparations (as judged by the respective sedimentation diagrams) were obtained. To prevent a possible aggregation, we examined these components in a solution containing 10 to 20 percent of potassium thiocyanate.

Figure 1 shows sedimentation diagrams. The first row represents initial mixture sedimentation; the second, α-component sedimentation; the third, β-component sedimentation; the fourth, the sedimentation of an artificial α–β component mixture. In the latter case the quantitative ratio of these components is changed as compared with the initial mixture. Figure 2 demonstrates similar sedimentation diagrams of experiments undertaken with a view to establishing the quantitative α/β weight ratio in a procollagen molecule. Here, as well as in Fig. 1, the first row corresponds to the initial component mixture after procollagen denaturation in 5*M* urea at 37°C; the second row represents the isolated α-component; the third row, the β-component; the remaining rows represent sedimentation diagrams of artificial α–β component mixtures with known weight ratios. The comparison of diagrams of the artificial mixtures with those of the initial "natural" mixture indicates that the α/β weight ratio in a procollagen molecule is 1:1.

Nature of Components

Now we should like to make a brief excursion into the nature of these components by saying a few words about the doubts that occurred to us and that may occur to others. The basic problem was whether these components are various structural elements of the procollagen molecule or whether they represent different degrees of aggregation of one and the same substance. We believe them to be various structural elements. Their release takes place under conditions that cause a rapid rupture of all the hydrogen type bonds. Under such conditions, the preservation of particle aggregates linked by hydrogen bonds is most unlikely.

We observed two components in the sedimentation of procollagen pretreated by heating and with high concentrations of thiocyanate or urea at room temperature. Thus, different treatments produce identical effects, and this points to the rupture of nonvalence bonds in all these cases. The possibility of separating the components by means of ammonium sulfate shows that they are of different nature. Finally, according to the preliminary data, hydroxyproline content of the β-component is lower than that of the α-component.

All these data indicate that in this case we are dealing with various structural elements of the procollagen molecule and not with a monomer or dimer (or polymer), or with the products of an irregular cleavage (*17*).

Conclusions

Now we shall dwell briefly on the results of studying the sedimentation and diffusion of these components. A phosphate buffer solution with *p*H 8, containing 10 percent of potassium thiocyanate, was used as a solvent in these experiments.

We have found the sedimentation constant (4 Svedberg units) and the diffusion constant (2.6×10^{-7} cm²/sec) for the α-component and the respective values for the β-component ($s = 5.7$ Svedberg units and $D = 1.6 \times 10^{-7}$ cm²/sec). These data were obtained by extrapolation to infinite dilution of protein in solution. The molecular weights calculated on the basis of these values proved to be equal to 125,000 and 290,000 for the α and β components, respectively. It should be remarked that the measurement of the diffusion coefficients has not always led to reproducible results, especially in the case of the β-component. Taking this into consideration, we are not quite sure of the precision of the molecular weights obtained. These values may be somewhat exaggerated, due to the tendency of the component particles to aggregate.

Recently we had an opportunity of getting acquainted with a paper by Doty and Nishihara read at the conference devoted to the recent advances in gelatin and glue research, held at the University of Cambridge, July 1957. In this paper some relevant data are presented. It is shown that the molecular weight of soluble collagen (or, as we call it, procollagen) of calf skin is 360,000 and its particle length is 3300 angstroms. On denaturation, it decomposes into components having molecular weights of about 120,000 and 230,000. These molecular weights are close to those obtained by us for the α and β components of procollagen from rat skin. However, there is a great difference here in the calculations of the molecular weight of the initial protein on the basis of molecular weights of the components. Indeed, Doty admits that the molecule of soluble collagen from calf skin may contain one particle of one component and one particle of the other. But if our data on the quantitative ratio of the α and β components are correct, and if the structures of the molecules of procollagens from rat and calf skin are similar, then there must be a mistake somewhere, as the molecular weight of the initial protein should be equal to about 500,000 and not to 360,000. It seems to us that the results of the experiments on the determination of the quantitative ratio of the components are sufficiently clear and convincing. It means that some or other of the molecular weight values should be checked. Such discrepancies once again emphasize the difficulties associated with study of these proteins.

The data on the sedimentation and diffusion constants of the α and β components enabled us to estimate the degree of asymmetry. Here we found a value of 1/30 for the α-component and 1/50 for the β-component. Certainly, the process of denaturation itself, as well as the presence of a large amount of thiocyanate in the solution, greatly changes the configuration of the components as compared with their state in the original molecule. Nevertheless, these data enable us to make an assumption that the α and β components in the procollagen molecule are linked side by side, so that the total length of two particles of the α-component is equal to the length of a β-component particle. The two-chain model is in better agreement with this supposition. But the two-chain structure is not in accord with conceptions that arise from x-ray structural investigations —that is, conceptions of the three-chain structure of collagen. Not being specialists in the field of structural chemistry and physics, we are not in a position to

discuss this point. So far, we do not possess a sufficient number of facts about the α and β components to advance any definite hypothesis as to their position in the structure of procollagen—that is, to speak about the latter's structure.

We hope that the further study of procollagen will enable us to answer many questions concerning the structure and transformation of this protein (*18*).

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17. The study of physicochemical and chemical properties of these components will be the subject of further investigations. These investigations continue in our country and will evidently interest other researchers. In particular, Dr. Doty and his coworkers have also undertaken such investigations.
18. Other publications in this field are the following: V. Orekhovitch, *Procollagens, Their Chemical Structure, Properties and Biological Role* (Moscow, 1952). ———and V. Shpikiter, *Biokhimiya* 20, 438 (1955); *Doklady Akad. Nauk S.S.S.R.* 101, 529 (1955); *C.I.O.M.S. Connective Tissue Symposium, London* (1956). V. Orekhovitch and L. Pavlikhina, *Voprosy Med. Khim.* 3, 195 (1957). ———, ———, V. Shpikiter, *Biokhimiya* 22, 210 (1957). V. Orekhovitch and V. Shpikiter, *Doklady Akad. Nauk S.S.S.R.* 115, 137 (1957); *Recent Advances in Gelatin and Glue Research* (Pergamon, London, 1957), p. 87. J. Nageotte and L. Guyon, *Arch. biol. (Liége)* 41, 1 (1931).

Hemoglobin Standard

This is the second and final report on a proposal to establish a certified standard for general use.

Division of Medical Sciences,
National Academy of Sciences–National Research Council

In 1953 the Hematology Study Section of the National Institutes of Health requested the Division of Medical Sciences of the National Academy of Sciences–National Research Council to explore the possibility of establishing a hemoglobin standard for general use throughout the country. In response to this request, the Division, in 1954, organized an Ad Hoc Panel for the Establishment of a Hemoglobin Standard under its Subcommittee on Blood and Related Problems.

George Cartwright (School of Medicine of the University of Utah) accepted the chairmanship of the panel. Its members were David L. Drabkin (University of Pennsylvania Graduate School of Medicine); William H. Crosby, Jr. (Walter Reed Army Institute of Research); George Brecher (National Institutes of Health); Wallace Brode (National Bureau of Standards); Israel Davidsohn (Mt. Sinai Hospital, Chicago), representing both the College of American Pathologists and the American Society of Clinical Pathologists; and A. H. Neufeld, representing the National Research Council of Canada. In 1956 John Gould succeeded Brode, and in May 1957, Bradley E. Copeland (New England Deaconess Hospital, Boston) and Donald Brown (Hackensack General Hospital) succeeded Davidsohn as representatives of the College of American Pathologists and the American Society of Clinical Pathologists, respectively. The panel was also fortunate in having the cooperation of E. J. King of the Postgraduate Medical School of London, who has given his full support to this undertaking.

Routine Methods

Most routine methods of clinical hemoglobinometry depend upon the photometric measurement of a blood sample after quantitative conversion of the hemoglobin which it contains into one or another of its derivatives. For the standardization of such a procedure there is needed a color standard which, when measured in the photometer and cuvette in routine use, will establish the relation between instrument reading and concentration of the particular hemoglobin derivative. This relation can then be used in converting instrument readings for unknown samples of blood to concentration of hemoglobin by the use of Beer's law or by construction of a calibration curve.

The problem, however, is complicated by the fact that several methods of analysis involving conversion to different forms of hemoglobin are in common use. Each of these methods should have its own standard. Since a multiplicity of standards is neither desirable nor practicable, the panel decided, early in its deliberations, to select only one method of analysis, for which a suitable direct standard would be developed. It should, however, be pointed out that this procedure does not preclude the use of the standard for the indirect calibration of another method. All that need be done is to construct a calibration curve based on the measurement of a small series of normal blood samples by the standardized method and also by the method which the analyst prefers to adopt for routine use.

In the opinion of the panel, the most significant contribution which could be made to the refinement of clinical hemoglobinometry would be the widespread adoption of a single method of analysis. Failing this, the indirect calibration of other methods with the chosen method offers the only simple photometric means for the comparison of data. The procedure, however, is subject to error if the blood sample contains significant amounts of certain of the abnormal forms of hemoglobin. For example, methemoglobin and carbon monoxide-hemoglobin are

This article was prepared by the staff of the Division of Medical Sciences, National Academy of Sciences–National Research Council. R. Keith Cannan is chairman.