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Interaction Properties of Elongate Protein Macromolecules with Particular Reference to Collagen (Tropocollagen)*

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ORGANIZATION and the interaction of organized structures at all levels of complexity are of cardinal significance in life processes. This organization may be manifested within the protein or other biomolecule or may depend upon the interaction of many individual entities.

For purposes of biophysical and biochemical investigation of such interaction of organized systems, it is profitable to deal with relatively simple systems, preferably with pure substances. Such systems are available in materials which are primarily one-dimensional (fibrous), two-dimensional (membranous), or three-dimensional (crystalline). The present paper is concerned exclusively with the properties of highly elongate, fibrous macromolecular systems. In a study of the forces between such highly organized fibrous systems lies rich opportunity for fundamental biophysical research.

Many substances of crucial importance in cells and tissues occur as very thin (10 to 30 A), highly elongate (1000 to 5000 A) particles. Proteins (such as myosin, paramyosin, actin, fibrinogen-fibrin, collagen, and the nerve-axon protein), nucleic acids (DNA and RNA), and polysaccharides (such as cellulose, hyaluronic acid, chondroitin sulfate) are examples. Many of these substances are themselves polymers (as the protein macromolecules are polymers of many amino-acid residues) but, as monomers, these elongate macromolecules polymerize end-to-end and laterally to form fibrous structures.

These macromolecular systems lend themselves to detailed physicochemical, crystallographic, and electron-optical study. Polyelectrolyte theory may be applied fruitfully to them. In many cases, energy is coupled with the macromolecular system by interaction with "energy-rich" substances such as adenosine triphosphate (ATP), but the mechanism by which this available energy is caused to do work (mechanical, chemical, electrical, or osmotic) is still poorly understood.

SOME TYPICAL BIOLOGICAL SYSTEMS IN WHICH FUNCTION DEPENDS UPON SPECIFIC INTERACTION OF ELONGATE MACROMOLECULES

It will help to identify the types of biophysical problems involved by mentioning a few typical examples.

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1. Functions Involving Mechanical Properties

In this category, mention is made of but three types that may serve to illustrate fundamental problems: fibrous systems designed to afford high tensile strength; those which produce tension or undergo contraction; and those which, by forming gelled clots, occlude regions such as blood vessels and prevent bleeding.

An excellent example of a fibrous system that achieves high tensile strength (*ca* 100 000 lb/in.^{2}) by a lateral bonding of macromolecular polymers are the structures such as skin, tendon, and other forms of connective tissue involving the protein collagen. The macromolecules are synthesized within fibroblast cells, find their way into the intercellular tissue spaces, and eventually aggregate at the appropriate place and time to form fibers. The mechanism of fibrogenesis may be very complex, involving processes of activation and homeostatic control of such processes so as to facilitate fibrogenesis when needed (as in wound repair) and to prevent excessive fibrinogenesis (such as occurs in aging and in certain pathological processes as in atherosclerosis, and in certain rheumatoid and so-called "collagen diseases").

The second mechanical function, that of contraction or tension production, poses a problem as to whether the shortening occurs essentially as an intramolecular process of superfolding of polypeptide chains, or is rather an intermolecular process involving a rapid and reversible change in the affinity or interaction between two or more species of fibrous proteins leading to a shortening of the fibrous system without substantial change in the helical configuration of the intramolecular chains characteristic of the native macromolecules.

Another rather striking problem is well illustrated in the embryogenesis of fibrous structures such as striated muscle, in which the axial repeat (sarcomere length) is so precise as to give several orders of diffraction with visible light. This production of supermacromolecular patterns well may involve the specific aggregation of several species of macromolecules, each having lengths of the order of several thousands of Ångström units. Perhaps some day it will be possible to produce such super-repeating patterns by interacting several kinds of fibrous macromolecules (protein, nucleic acid, or polysaccharide) under appropriate conditions *in vitro*.

The third type of mechanical function mentioned in the foregoing is that of the clotting of fibrous protein,

as in the transformation of the soluble fibrinogen in the blood into the insoluble fibrin of blood clot. This involves an enzymatic activation of the soluble protein monomers by means of an enzyme (itself activated by a series of interdependent processes) to produce "intermediate polymers" several thousand Ångström units long which then polymerize spontaneously to form the clot. The enzymatic activation itself is controlled by a highly complex system of kinases and antikinases by means of which a high degree of homeostatic control of this vital process is achieved.

2. Functions Involving Enzyme Action

As was emphasized by Engelhardt and Ljubimova,[1] large macromolecules may function enzymatically. This may occur either because the macromolecule as a whole acts like an enzyme or because a portion of the molecule is enzymatically active. Myosin, with which Engelhardt and Ljubimova were concerned,[1] was found to exert enzymatic action in splitting ATP. It is known now that only one component ("heavy meromyosin") is enzymatically active as ATPase. It remains to be seen how many other kinds of elongate macromolecules will be found to have enzymatic properties. It may be mentioned that, when an enzymatic group forms part of a large macromolecular complex, the configuration of the macromolecules or smaller molecules in the environment, for steric reasons, may strongly influence the availability of the enzymatic site. Such regulatory action well may be involved in muscle contraction.

3. Functions Involving the Maintenance of a Specific Linear Sequence of Chemical Groups as in Genetic Determiners

It is believed that in the linear sequence of nucleotide residues in the double helix of DNA is to be found the coding responsible for transmitting genetic information. The DNA occurs as highly elongate macromolecules extractable from the chromosomes by mild methods (such as by treatment with hydrogen bond breakers). It seems probable that the ability of such macromolecules to exert their specific controlling influence during development and differentiation must depend importantly upon their interaction with other constituents of the chromosomes such as histones, protamines, and protein macromolecules, and perhaps also with other nucleic-acid macromolecules.

4. Other Functions

Engelhardt[2] suggested that macromolecules may perform other types of work such as osmotic and electrical. These possibilities have not yet been explored thoroughly. The function of certain fibrous proteins, such as the axoplasmic protein of nerve, remains completely unknown.

Perhaps one may mention in this connection also the action postulated by Weiss[3] as important in determining the ordering of cells into typical tissues by means of the interaction of specific types of macromolecules at the surfaces of the cells that form the tissues. Presumably because of a complementarity or ordered type of interaction of the surface molecules, the cells are caused to aggregate in patterns characteristic of each tissue.

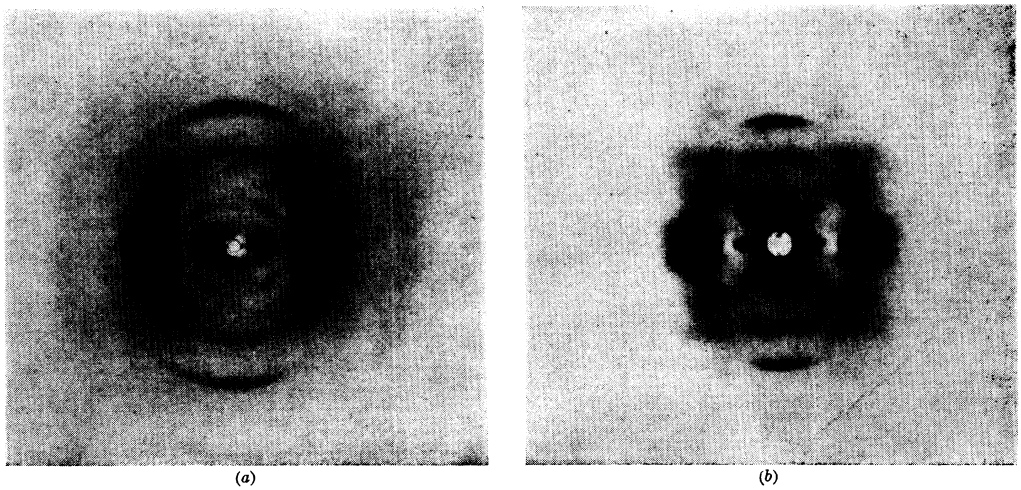
From this very brief description, it is obvious that crucial biological functions depend importantly upon the ways in which highly specifically structured macromolecules interact with one another and upon the manner in which environmental conditions change or regulate this interaction. It is the purpose of this paper to illustrate this specificity of interaction by a consideration of the properties of one particular type of macromolecule, collagen, chosen because of its specially favorable chemical and structural properties. Like many other biological macromolecules, collagen is very asymmetric (*ca* 14×2800 A). It has, however, the valuable property that, in its aggregation patterns, it forms fibrous structures characterized by highly specific band patterns as seen in the electron microscope. By analysis of these band patterns, it is possible to deduce the type of macromolecular interaction responsible for each characteristic pattern. From such studies, valuable lessons are learned that have direct application to other types of macromolecular systems in which no band patterns exist to serve as guides.

BIOPHYSICAL AND BIOCHEMICAL PROPERTIES OF COLLAGEN

In contradistinction to most proteins, collagen (or perhaps more correctly the collagen class) possesses characteristic structural and chemical properties which permit its definitive identification. For present purposes, it is necessary only to sketch those properties necessary for an understanding of the internal structure, and the chemical properties of the collagen macromolecule. For recent excellent surveys of these properties, see Gustavson,[4] Highberger,[5] the CIOMS Symposium on Connective Tissue,[6] and that on Gelatin and Glue Research.[7]

Collagen occurs in dense fibrous tissue of high tensile strength, as in tendons, or less tightly woven tissue fabrics, as in skin, or in more sparse distribution as in loose connective tissue. The fibrous protein occurs in various hierarchies of fiber size, including the following: *fibers*, visible macroscopically or microscopically and having diameter of the order of micra; *fibrils* with widths of the order of a few hundred to several thousand Ångström units, observable in the dark-field microscope and resolvable in the electron microscope; the *protofibrils* which originally were defined as constituting "The unit columnar arrays which, when associated laterally, form the collagen fibril";[8] and the *collagen* (or "tropocollagen") *macromolecules* which constitute the monomeric units of the protofibrillar polymer.

For x-ray diffraction and for chemical-analytical



(a) (b)

FIG. 1. Large-angle x-ray diffraction patterns of collagen from rat-tail tendon.[10] (a) Unstretched; (b) stretched 8% [from J. T. Randall, J. Soc. Leather Trades' Chemists 38, 362 (1954)].

studies, gross macroscopic fibers or whole tissues are used. For electron-microscopic investigation, the fibrous category of interest is the fibril which manifests a detailed and characteristic band pattern which, as is brought out below, results from a specific pattern of aggregation of the elongate native collagen macromolecules.

The collagen class of proteins, as Astbury[9] referred to them, is uniquely characterized by its amino-acid composition, its x-ray diffraction pattern, and its banded appearance in the electron microscope. These characteristics may be described briefly as follows.

The collagenous proteins differ from other proteins in that they contain the amino acids hydroxyproline and hydroxylysine. In mammalian collagen, about one-third of the amino-acid residues are glycine. Proline and hydroxyproline together make up almost another third, leaving approximately one-third for other amino-acid types. From a determination of the hydroxyproline and glycine content of a given preparation, one can make a good estimate of the collagen content.

Perhaps the most distinctive characteristic of collagen is its large-angle x-ray diffraction pattern (Fig. 1) which reflects the internal organization of the collagen macromolecule and is, therefore, characteristic of this class of proteins. Astbury[9] early called attention to the 2.86-A meridional reflection, which he considered to represent the length of the amino-acid residue, along the fiber axis, in a coiled polypeptide chain, and to the equatorial reflections at 10 to 15 A (depending upon the degree of hydration) which he attributed to the separation between main chains. With more refined technique, it has been possible to obtain far more reflections in the pattern and to achieve a higher degree of orientation by stretching fresh tendon. From such patterns, it has been possible for several groups of workers to agree that the diffractions are interpreted best in terms of a macromolecule containing three chains coiled in helical fashion about each other to form a coiled coil (see particularly the papers of Crick and Rich).[11–13] The proposed triple-stranded structure is shown schematically in Fig. 2.

It has been proposed also that there are only two types of three-stranded helical models of collagen structure, based on the so-called structure I and structure II, derived from a consideration of polyglycine, and compatible with the x-ray, infrared, analytical, and physicochemical data. In these models, the axial repeat occurs at 28.6 A. In collagen II, to agree best with the diffraction data, the OH groups of the hydroxyproline residues extend radially from the three chains, making it possible to form hydrogen bonds with CO groups of adjacent three-stranded macromolecules. In the collagen I structure, the hydrogen bonds from hydroxyproline are directed internally, bonding the three chains intramolecularly. The hydroxyproline content appears to be determinative of the denaturation temperature, which is a measure of the energy needed to disrupt the internal organization of the macromolecule. This fact tends to support the collagen-II type of structure. Rich (p. 50) has suggested that one type of structure might be convertible into the other and that this may result from application of stress to the fiber.

Although there is fairly general agreement that the collagen macromolecule is a three-stranded helix, it is not certain that the macromolecule is thus constructed over its entire extent. Gallop[14] suggests that as much as 30% may have a different configuration.

Treatment of soluble collagens with hydrogen-bond breakers like urea, or by heating, causes denaturation with the liberation of the constituent chains to form "parent gelatin." From the original macromolecule, having a weight of 360 000, there is formed, according to Doty and Nishihara,[15] one chain with a weight of

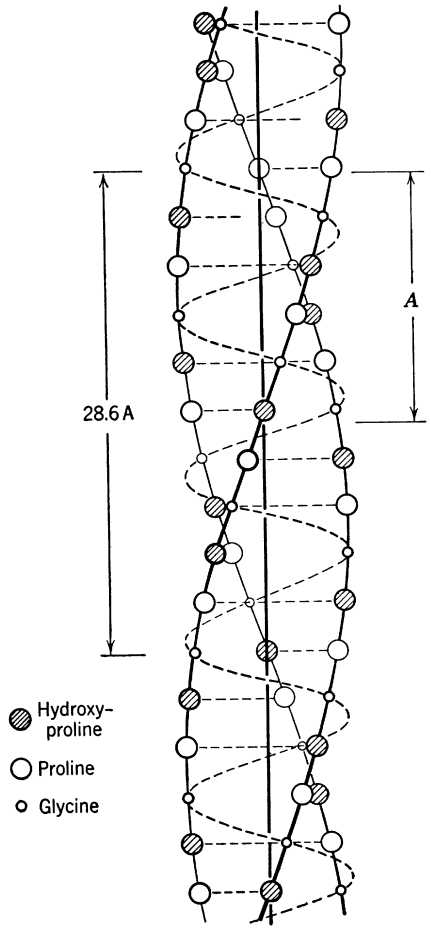


FIG. 2. Three-stranded helical structure of collagen macromolecules (courtesy A. Rich).

120 000 and another with a weight of 240 000. These authors believe that an alkali-labile ester bond links two chains of weight 120 000 to form the heavier chain obtained from denatured collagen. This is to be compared with the corresponding values of Orekhovitch and Shpikiter.[16]

In addition to the large-angle x-ray pattern, arising from the internal, presumably three-stranded, structure of the macromolecule, collagen also manifests a well-developed small-angle x-ray pattern (Fig. 3) consisting of many (*ca* 50) orders of a large axial repeat which Bear[18,19] showed to be 640 A in air-dried fibers and nearer to 700 A in moist fibers. Although all native collagen fibers from a wide variety of sources showed this axial repeat, its significance in terms of molecular structure was not obvious. The simplest early interpretation was that it represents the molecular length of the collagen molecules, an assumption that seemed to gain support from the fact that a similar axial repeat was observed in the band pattern observed in electron micrographs. Bear and Morgan[20] attempted to relate the positions of the intraperiod bonds observed electron optically with the characteristic intensities of the various orders of the small-angle x-ray pattern. As is shown in the following, the collagen macromolecule probably has a length of four times the 700-A period, i.e., about 2800 A.

The band pattern observed in high-resolution electron micrographs of teased collagen fibrils stained with phosphotungstic acid (PTA), or other heteropolyacid, is uniquely characteristic of collagen (see Fig. 4). This axial pattern repeats at about 700 A and contains a number of bands and interbands of characteristic density and position. It was suggested by Bear[21] that the bands represent regions of relative disorder due to the interaction of side chains of relatively large size while the interbands represent regions of relative order due to the interaction of the smaller side chains which are found in considerable abundance in collagen. Another interpretation of band structure depends upon the characteristic interaction of groups such as the guanidino groups of the arginine side chains with PTA, as suggested by Kühn *et al.*[22] It is noted from Figs. 4 and 5 that the band pattern—i.e., the intraperiod positions and the relative densities of the bands—is a polarized, asymmetric pattern. The significance of this pattern was discovered only after it became possible to take the native fibrils apart into their constituent macromolecules and to cause these to re-aggregate in characteristic and new band patterns. These results may now be described briefly.

FORMATION OF ORDERED AGGREGATION STATES OF COLLAGEN BY PRECIPITATION FROM SOLUTION

A formidable difficulty in the characterization of the collagen molecules lay in the relative insolubility of collagen fibers. However, certain types of collagen, such as in rat-tail tendon and in the fish swim bladder, are

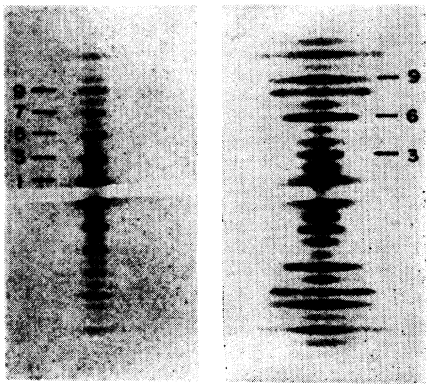


FIG. 3. Small-angle x-ray diffraction patterns of kangaroo-tail tendon collagen; (a) moist preparation; (b) after brief exposure to water and drying under tension.[17] Layer line indices are indicated [from R. S. Bear, O. E. A. Bolduan, and T. P. Salo, J. Am. Leather Chemists' Assoc. 46, 107 (1951)].

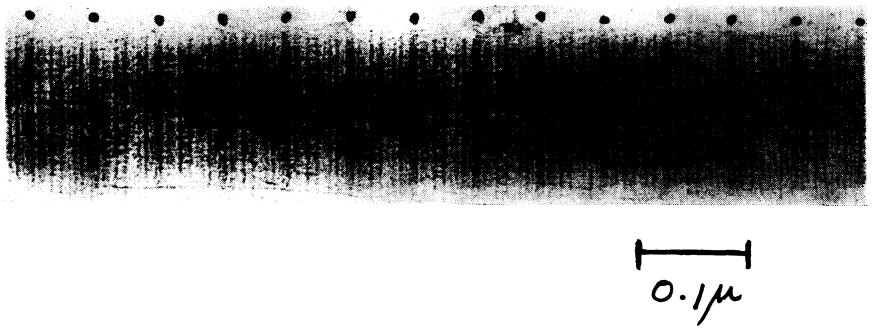


FIG. 4. Electron micrograph of calfskin collagen reconstituted from solution, stained with phosphotungstic acid. Band pattern is of the native type. Axial repeats marked (courtesy A. J. Hodge).

soluble in dilute acid. From the classical early work of Zachariades, Nageotte, Fauré-Fremiet, Wyckoff, and Corey, and others, it is known that, by appropriate adjustment of the *p*H and ionic strength of such acid solutions, the collagen can be precipitated reversibly in fibrous form. Examined in the electron microscope after staining with PTA, the reprecipitated fibrils were found to possess structure, the type of which depends upon the conditions of precipitation. With increasing ionic strength, the band pattern may be that characteristic of native fibrils (period ≅700 A), it may be about one-third this value, or the precipitate may have tactoidal appearance, showing no bands at all. These different forms can be produced reversibly from acid solutions of highly purified collagen; presumably the different ordered states depend only upon the collagen and require no additional organic material.

When certain types of extracts are made from connective tissue or when certain organic substances, particularly highly negatively charged substances, are added to the collagen solutions and the conditions are adjusted appropriately, a new modification is found which manifests an axial repeat or identity period about four times that of normal collagen (i.e., about 2600 to 3000 A) and which, therefore, are called "long-spacing" types. Two such forms, called "fibrous long-spacing" (FLS) and "segment long-spacing" (SLS) are shown diagrammatically in Fig. 5. The FLS modification is produced routinely by addition of α-1 acid glycoprotein to an acetic-acid solution of collagen, followed by dialysis against water. The SLS modification is produced routinely by addition of ATP to the acid solution of collagen; the precipitate forms directly without further adjustment of conditions.

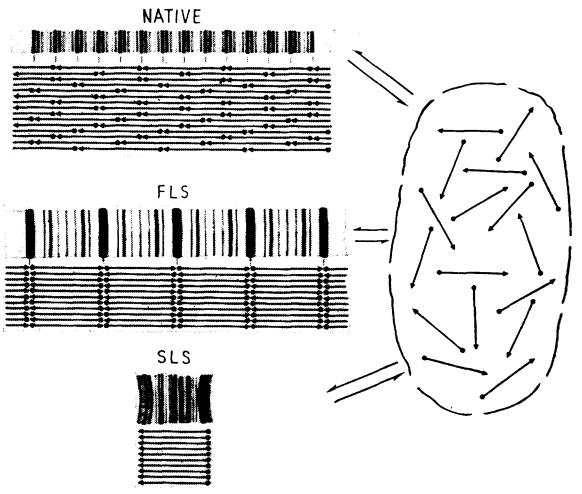


FIG. 5. Diagrammatic illustration of patterns of aggregation of tropocollagen macromolecules in native, FLS, and SLS types. Polarization of macromolecules indicated by arrow.

It is noted that the FLS type has a symmetrical, nonpolarized band structure while the SLS has an asymmetrical, polarized pattern of banding.

Each of the five band patterns described may be produced reversibly from an acid solution of collagen. The particular patterns produced depend for their specificity upon the collagen rather than upon the other substances added or conditions imposed; rather these substances and conditions serve to evoke the structure inherently characteristic of the collagen itself.

MACROMOLECULAR MONOMER OF COLLAGEN—THE "TROPOCOLLAGEN" HYPOTHESIS

The structures described in the foregoing, discovered in collaboration with Gross and Highberger, were interpreted as follows (see summaries of this work by Schmitt, Gross, and Highberger;[23] Schmitt;[24] Gross;[25] and Highberger.[5] It is assumed that the long-spacing (about 2800 A) represents the length of the native collagen macromolecule which has a three-stranded helical internal structure, as deduced from the large-angle x-ray pattern (see the foregoing). The long, thin macromolecules were given the term "tropocollagen" (TC), because they are capable of "turning into" or forming the native collagen structure, and also to distinguish them from various other collagen fractions (such as procollagen) previously described.

The TC macromolecules are assumed to be essentially identical in structure and composition and to be themselves polarized in the sense of the linear sequence of amino-acid residues in the constituent intramolecular strands.† This is indicated by the arrows on the TC

† The unit of native collagen structure is referred to as a macromolecule rather than as a molecule because it appears to be composed of several covalent polypeptide chains bonded together by hydrogen bonds.

FIG. 6. Electron micrograph of tropocollagen macromolecules prepared by the method of Hall[28,29] (courtesy C. E. Hall).

macromolecules in Fig. 5. The hypothesis assumes that the various types of ordered patterns of TC aggregation occur by virtue of relatively stable bonding between terminal groups on the side chains of laterally adjacent macromolecules. Each type of ordered aggregation type represents a particular pattern of interacting side chains. In the SLS form, it is assumed that the TC macromolecules are essentially in register with respect to their ends and are "pointing" all in the same direction; i.e., they are in parallel array. The SLS pattern, therefore, provides a molecular "fingerprint" of the sequence of amino-acid residue types along the TC macromolecule—information not deducible by examination of the band pattern of native fibrils. The FLS is assumed to be formed by an antiparallel packing of TC in which the macromolecular ends are approximately in register (see Fig. 5).

Since the axial repeating pattern of native fibrils, both as seen in electron micrographs and as measured in the small-angle x-ray pattern, is about a quarter of that of the length of the TC macromolecules, it was assumed that the latter are arranged in parallel array but are displaced in the axial direction by one-quarter of a length in adjacent macromolecules (see Fig. 5). A specific suggestion somewhat along the same line has been proposed by Tomlin and Worthington.[26]

This concept of the structure and properties of the native macromolecule of collagen was deduced from the electron-optical observations of the various ordered aggregation types observed. The hypothesis received confirmation from the physicochemical studies by Boedtker and Doty[27] performed on solutions which were highly monodisperse with respect to the monomer macromolecules (achieved by centrifuging out the larger aggregates). These data indicated that the macromolecules behave like rigid rods with dimensions about 14×2800 A and molecular weight about 360 000. Previous estimates of other workers about particle sizes were considerably greater, probably because their preparations were heterodisperse, containing polymers of collagen as well as the monomers.

Finally, the tropocollagen macromolecules were visualized directly in the electron microscope by a method developed by Hall.[28] This consists in depositing the molecules upon the atomically smooth surface of freshly cleaned mica by spraying a very dilute solution of the protein. After drying, this surface is shadowed by evaporation of platinum at a small angle. The metalized layer then is backed with a thin collodion supporting film, stripped from the mica, and examined in the electron microscope at high resolution. From such electron micrographs (see Fig. 6), Hall[29] found the fibrous particles to be about 15 A in width but their lengths to be somewhat smaller than had been predicted by the physicochemical data of Boedtker and Doty. Subsequently, with improved technique, Hall and Doty[30] obtained a weight-average length of 2820 A, in good agreement with the physicochemical data and with the lengths determined in this laboratory on the same solutions used by Hall and Doty by conversion to the FLS modifications and measurement of the axial period (average value was 2700 A).

The problem of the nature of the precursor of fibrous collagen in the fibrils of connective tissue has been the subject of much investigation. Orekhovitch *et al.*[31] suggested that the fraction soluble in citrate buffer ($\mu \cong 0.2$, $p\mathrm{H} \cong 3.5$) is such a precursor and, therefore, gave the material the name "procollagen." However, from turnover studies or the incorporation of C^{14}-labeled glycine, Harkness *et al.*[32] suggested that the precursor is to be found in the material soluble in slightly alkaline buffer, with a much shorter half-life than citrate-soluble collagen. This conclusion was confirmed by Jackson[33] using other methods. The possibility that tropocollagen macromolecules soluble in neutral salt solutions (Gross, Highberger, and Schmitt,[34]) may be the precursor of fibrous collagen has been discussed in some detail by Gross.[35] Orekhovitch and Shpikiter[16] have concluded that procollagen and tropocollagen are, in fact, identical. The possibility that collagen, as synthesized in the fibroblasts, requires activation before it is capable of being incorporated into fibrous tissue has been much investigated, but the details of the process remain to be disclosed.

FRAGMENTATION OF TROPOCOLLAGEN MACROMOLECULES BY SONIC IRRADIATION

The discovery by Nishihara and Doty[36] that sonic irradiation of tropocollagen rapidly reduces the viscosity without substantial reduction in optical rotation suggested that the irradiation fragments the macromolecules into shorter pieces, actually into halves and quarters, which retain the triple-chain helical structure characteristic of the native macromolecules. This possibility was confirmed by Hodge and Schmitt[37] by electron-microscopic examination of irradiated collagen. The loci along the macromolecules which undergo scission could be determined with considerable precision

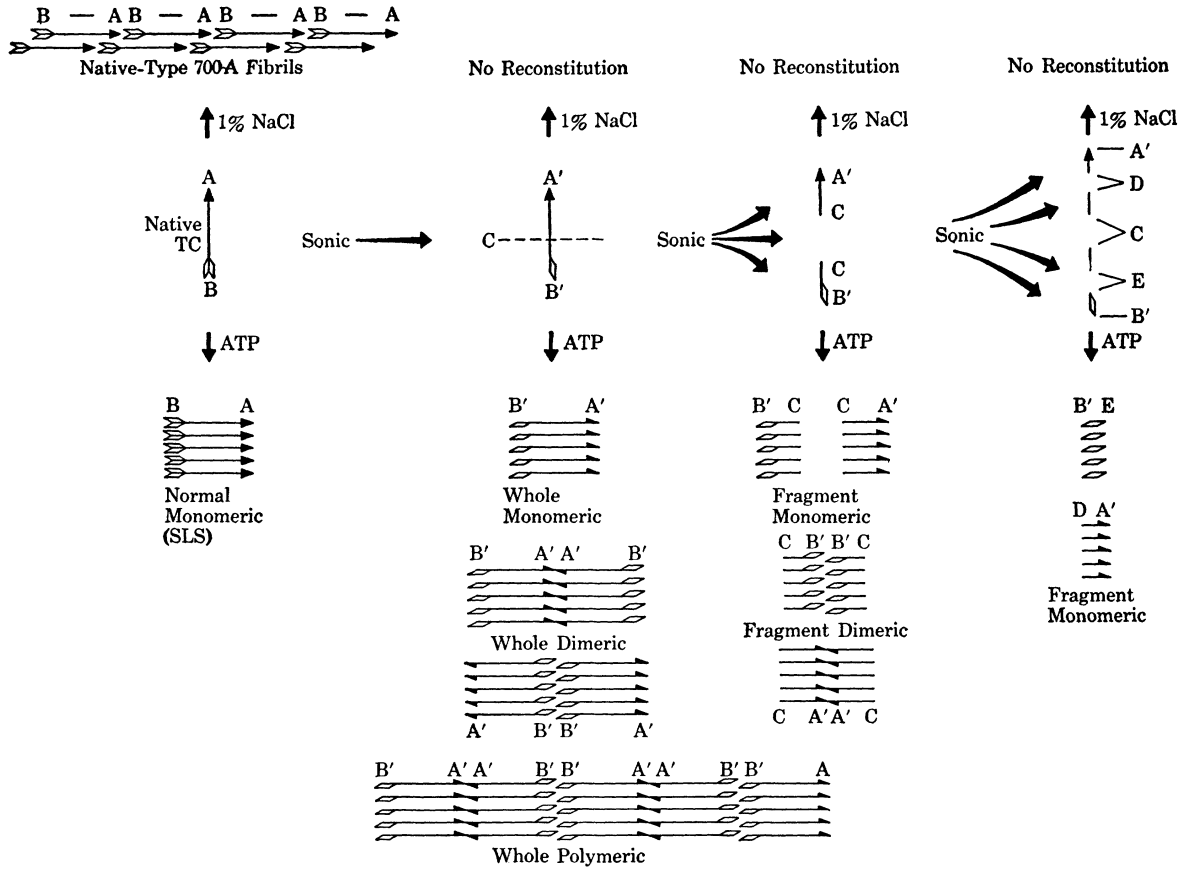


FIG. 7. Diagrammatic illustration of the chief effects of sonic irradiation on the tropocollagen macromolecules [from A. J. Hodge and F. O. Schmitt, Proc. Natl. Acad. Sci. U. S. 44, 418 (1958)].

by reference to the band patterns of the SLS-type aggregates produced by the addition of ATP to the acid solutions after irradiation.

It was discovered that sonic irradiation produces profound effects in addition to that of scission of the macromolecules into smaller fragments. The most striking of these is an alteration of "end regions," produced by relatively short periods of irradiation, without change in the length of the macromolecules. The results are shown schematically in Fig. 7, wherein the native TC macromolecules are represented by an arrow with A and B ends, indicative of the asymmetric distribution of amino acid residues reflected in the SLS type of aggregation pattern. It is thus possible to tell at a glance which is the A and B end of any particular SLS; in addition to the specific band pattern at each end, the polarization of the TC is shown at once by the position of the broad, slightly off-center interband (labeled *F-G* by Schmitt, Gross, and Highberger[23]). As was indicated earlier, the formation of the native type (700-A axial repeat) involves the formation of protofibrils that are actually linear polymers of TC by end-to-end interaction of the A-B type; lateral aggregation of such protofibrils occurs in a manner such that adjacent protofibrils are displaced axially with respect to one another by a quarter of a macromolecular length (*ca* 700 A). It is this A-B type of interaction of macromolecular ends that is first affected by sonic irradiation. In the diagrammatic representation, the altered ends are designated A′ and B′.

Following irradiation, sufficient to prevent the formation of native-type fibrils (tested for by dialysis *vs*

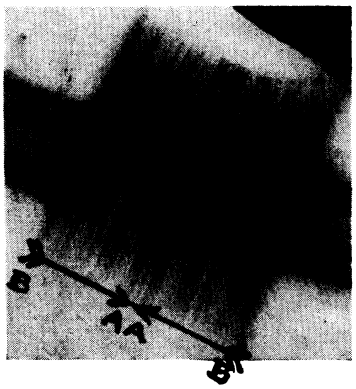


FIG. 8. Dimeric aggregation form of tropocollagen macromolecules of the type

B′—A′—A′—B′.

Produced by sonic irradiation of calfskin collagen for 20 min [from A. J. Hodge and F. O. Schmitt, Proc. Natl. Acad. Sci. U. S. 44, 418 (1958)].

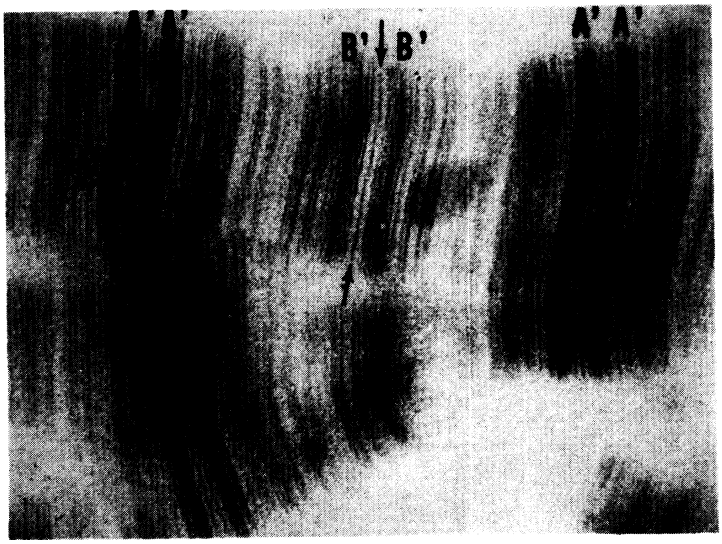


FIG. 9. Whole polymeric aggregation types of SLS aggregates from a solution of calfskin collagen treated with sonic irradiation for 240 min. Locus of A′ and B′ ends of macromolecules labeled. Arrow points to dense band at the junction between macromolecules at the B′ end [from A. J. Hodge and F. O. Schmitt, Proc. Natl. Acad. Sci. U. S. 44, 418 (1958)].

1% NaCl), i.e., by alteration of macromolecular ends, two pronounced changes are found in the ATP precipitates: (1) an increased side-to-side interaction, producing a highly exaggerated lateral aggregation into long ribbons of SLS forms; and (2) a progressive increase in the amount of A′—A′ and B′—B′ types of interaction. As a result, the formation of dimeric and polymeric forms is favored (see Figs. 8 and 9). With longer irradiation, the macromolecules are fragmented, the locus of the scission being indicated by the band pattern of the fragments.

It is noteworthy that end-to-end polymerization of scission products never involves ends produced by the fragmentation (such as those designated C, D, or E in Fig. 8). Apparently, the original ends of macromolecules are different from those produced by sonic scission. From the high density of bands (i.e., regions which combine preferentially with phosphotungstic acid) in end regions of SLS, it seems clear that certain amino-acid side chains (possibly the guanidino groups of arginine, as suggested by Kühn, Grassmann, and Hofmann,[22] or the ε-amino groups of lysine) may be concentrated in the end regions of the macromolecule.

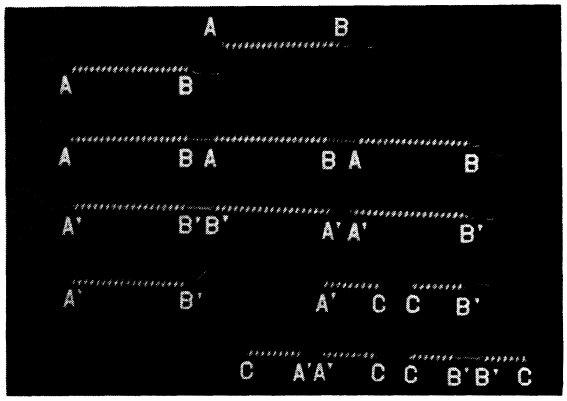


FIG. 10. Diagrammatic illustration of suggested end-to-end interaction of normal collagen macromolecules by formation of mutual coiling of terminal chains (at A-B junctions). Abnormal A′—A′ and B′—B′ junctions in sonic irradiated preparations. Scission of chains at C illustrates effect of longer periods of sonic irradiation. Note that no C—C linkage occurs [from A. J. Hodge and F. O. Schmitt, Proc. Natl. Acad. Sci. U. S. 44, 418 (1958)].

Because of the special properties of the end regions in end-to-end polymerization, it is important to obtain evidence concerning the structure in these regions. Clues are afforded by a study of the band fine structure in A′—A′ and B′—B′ linkages in the dimeric and polymeric forms. As shown in Fig. 9, the first bands at the A′ ends are separated by a region, about 100 A long, which is a typical interband (i.e., shows no dense band, hence presumably contains relatively few side chains reacting with the phosphotungstic-acid "stain"). In the case of the B′—B′ junctions, however, the separation between the first bands is about 180 A, and a darkly staining band occurs in the middle of the junctional region.

This behavior is highly suggestive concerning the nature of macromolecular ends and of end-to-end polymerization of macromolecules as follows: (1) chain appendages may occur at both ends of the native TC macromolecule; (2) these appendages may have lengths of about 100 A and 200 A at the A and B ends, respectively; (3) the amino-acid composition of the terminal chain appendages resembles typical interband regions—i.e., lacking concentrations of basic amino-acid side chains thought to characterize the band regions (except for a portion of the B end as mentioned in the foregoing).

Such considerations led Hodge and Schmitt[37] to suggest that normal end-to-end polymerization of TC monomers may involve a specific type of coiling of the terminal chains at A and B ends about each other to form a highly ordered, possibly helical, structure (see

Fig. 10). The "coiling energy" of such interaction in fact may be represented by the difference between the thermal shrinkage and denaturation temperatures (found by Doty and Nishihara[15] to be constant and equal to 29°C in three different types of collagen). It seems probable that such end-chain interaction involves primarily rather weak, hydrogen bonding. This would be consistent with the findings of Gross[25] that thermal gelation of salt solutions of collagen are inhibited by hydrogen-bond breakers, such as urea. In addition to such weak bonds, more stable bonds may be formed between terminal chains in the collagens of certain kinds of connective tissue, such as those which resist acid solution.

DISCUSSION

It is apparent from the behavior of TC macromolecules already described that the interaction between these macromolecules and the various types of ordered structures that result from such interaction is determined by two important factors:

(1) The specific properties and reaction potentialities that are built into the macromolecules by virtue of the linear sequence of amino-acid residues in the covalent chains, the number of chains in the macromolecule and the specific type of coiling of these chains.

(2) The chemical environment of the macromolecules which may evoke one or another of the various types of interaction patterns made possible by virtue of the internal structure of the macromolecules.

A few fibrous proteins other than collagen have been investigated along similar lines and results consistent with the foregoing conclusions obtained. Thus, Hodge (p. 409) found paramyosin to be a long (*ca* 1400 A) macromolecule capable of forming an FLS type of structure when packed in antiparallel array, though in the native fiber giving rise to a 145-A repeat or a larger period five times this long. Tropomyosin also appears to behave somewhat similarly (Hodge[38]). Also, the repeat pattern in fibrin fibrils is considerably less than the length of the fibrinogen molecules (Hawn and Porter[39] and Hall[40]), and it has been suggested that the latter are staggered with respect to molecular ends (Ferry *et al.*[41]).

Glimcher, Hodge, and Schmitt[42] presented evidence in support of the concept that the initiation of mineralization involves the nucleation of inorganic crystals by a precise juxtaposition of groups in the organic matrix to form specific stereochemical arrays. In the nucleation of hydroxyapatite in calcification, only when the purified TC macromolecules were precipitated as native-type fibrils (640-A repeat) did the system induce the formation of hydroxyapatite crystals when exposed to otherwise stable solutions of calcium and phosphate ions. If the TC macromolecules were allowed to assume other types of aggregation patterns, no nucleation occurred. In the most general case, an example is seen here of how specific macromolecular interaction serves to govern very fundamental processes, such as that of the deposition of inorganic material of specific crystalline form.

If macromolecules also possess enzymatically active sites, it is obvious that even more-complex interaction behavior may occur, particularly if products of the enzyme reaction themselves strongly influence the behavior of one or more macromolecular species in the system (e.g., ATPase in myosin).

Crucial clues to many vital biological processes, such as those briefly mentioned at the beginning of this paper, eventually may be found if such biophysical and biochemical properties of elongate macromolecules are kept in mind.

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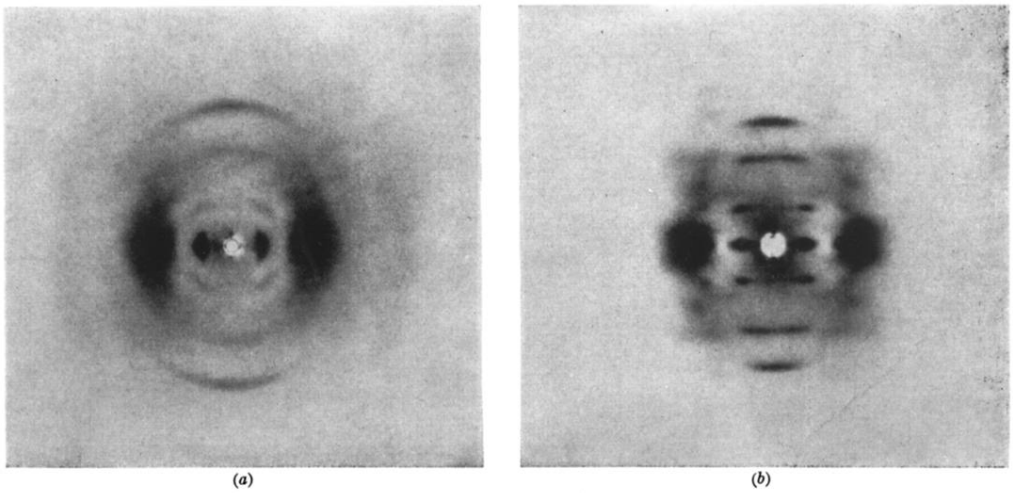


(*a*) (*b*)

FIG. 1. Large-angle x-ray diffraction patterns of collagen from rat-tail tendon.[10] (a) Unstretched; (b) stretched 8% [from J. T. Randall, J. Soc. Leather Trades' Chemists **38**, 362 (1954)].

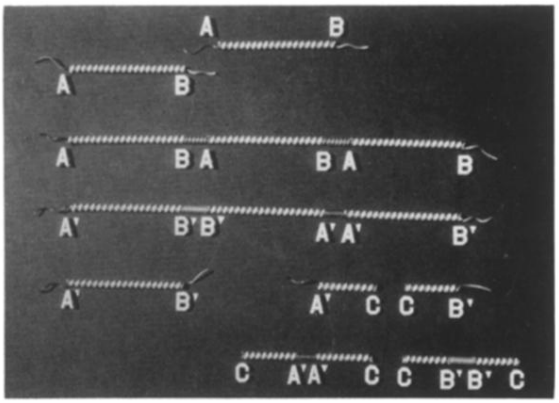
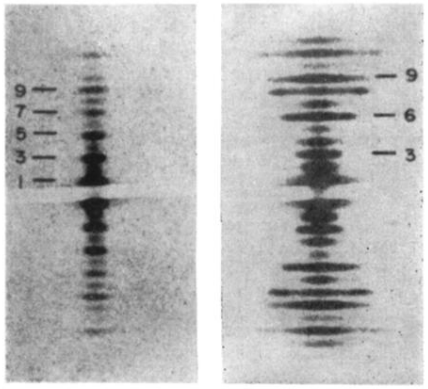


FIG. 10. Diagrammatic illustration of suggested end-to-end interaction of normal collagen macromolecules by formation of mutual coiling of terminal chains (at A-B junctions). Abnormal A′—A′ and B′—B′ junctions in sonic irradiated preparations. Scission of chains at C illustrates effect of longer periods of sonic irradiation. Note that no C—C linkage occurs [from A. J. Hodge and F. O. Schmitt, Proc. Natl. Acad. Sci. U. S. **44**, 418 (1958)].



(a) (b)

FIG. 3. Small-angle x-ray diffraction patterns of kangaroo-tail tendon collagen; (a) moist preparation; (b) after brief exposure to water and drying under tension.[17] Layer line indices are indicated [from R. S. Bear, O. E. A. Bolduan, and T. P. Salo, J. Am. Leather Chemists' Assoc. **46**, 107 (1951)].

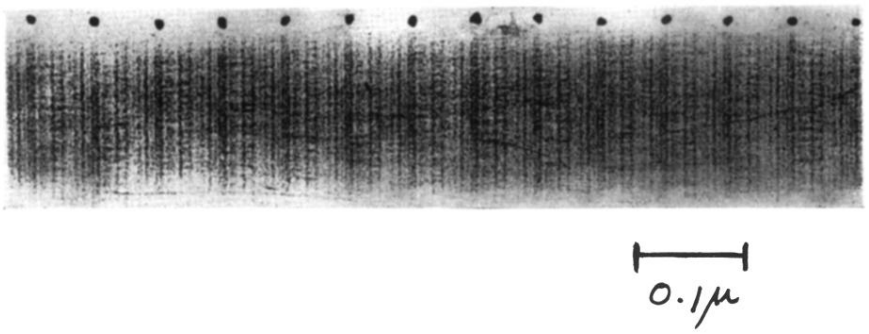


FIG. 4. Electron micrograph of calfskin collagen reconstituted from solution, stained with phosphotungstic acid. Band pattern is of the native type. Axial repeats marked (courtesy A. J. Hodge).

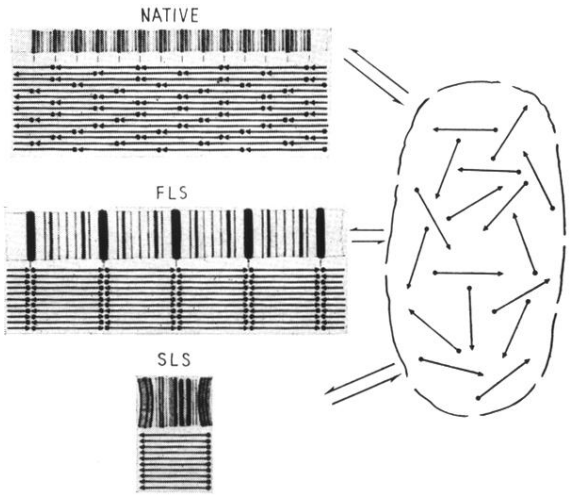


FIG. 5. Diagrammatic illustration of patterns of aggregation of tropocollagen macromolecules in native, FLS, and SLS types. Polarization of macromolecules indicated by arrow.

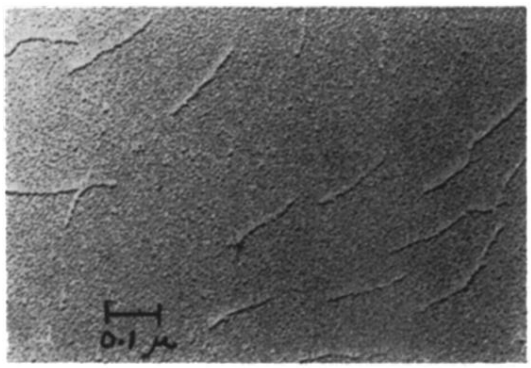


FIG. 6. Electron micrograph of tropocollagen macromolecules prepared by the method of Hall[28,29] (courtesy C. E. Hall).

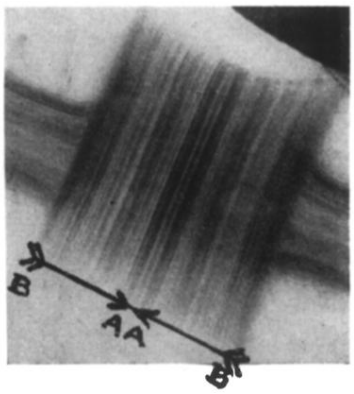


FIG. 8. Dimeric aggregation form of tropocollagen macromolecules of the type

$$B'-A'-A'-B'.$$

Produced by sonic irradiation of calfskin collagen for 20 min [from A. J. Hodge and F. O. Schmitt, Proc. Natl. Acad. Sci. U. S. **44**, 418 (1958)].

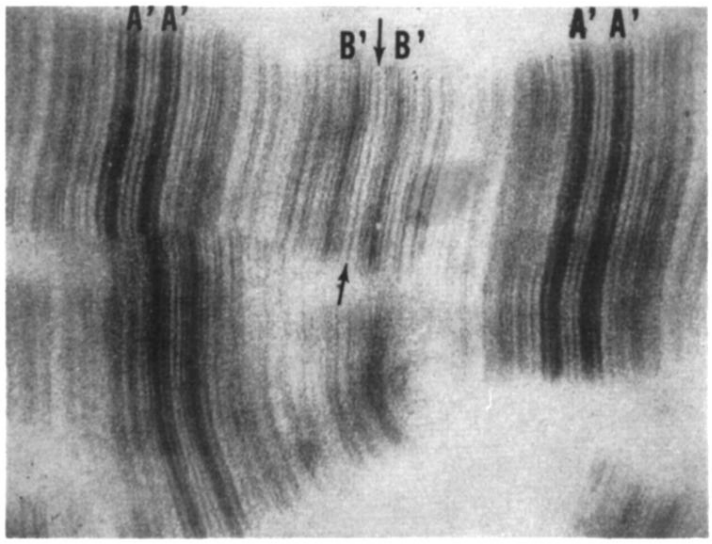


FIG. 9. Whole polymeric aggregation types of SLS aggregates from a solution of calfskin collagen treated with sonic irradiation for 240 min. Locus of A′ and B′ ends of macromolecules labeled. Arrow points to dense band at the junction between macromolecules at the B′ end [from A. J. Hodge and F. O. Schmitt, Proc. Natl. Acad. Sci. U. S. **44**, 418 (1958)].