CONNECTIVE TISSUE: INTERCELLULAR MACROMOLECULES

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CONNECTIVE TISSUE: INTERCELLULAR MACROMOLECULES

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PREFACE

This volume contains the proceedings of the fourth annual symposium of the New York Heart Association. Our objectives in sponsoring these scientific meetings have been: first, to bring together the most expert, productive workers in a specific area of the life sciences; second, to present new and preferably fundamental knowledge in that area to an audience oriented towards both clinical and basic scientific medicine; and third, hopefully to catalyze the generation of new ideas and endeavors which might otherwise lie dormant indefinitely.

To generalize, it might be said that these symposia represent a limited effort to solve one of the formidable and paradoxical problems of modern life, namely, communication, and particularly communication between the creative laboratory and the dynamic clinic.

That the subjects chosen for these symposia have not, with one exception, had any direct bearing on diseases of the heart or circulation has been of little concern to the New York Heart Association. Our complacency in this regard stems from the full realization that dramatic advances in the solution of a biologic problem or the eradication of a disease are often made not by frontal, but rather by flank attack. It is hoped that at least one such advance may arise from the papers and discussions that are presented here.

CHARLES E. KOSSMANN, *President*
New York Heart Association

GENERAL INTRODUCTION

Connective tissue is a mesenchymal tissue in the adult. It is made up of four morphologic components, namely, cells, vessels, fibers, and ground substance. The proportion of these constituents varies. Ground substance is found almost everywhere, in the extracellular or intercellular space. It is a gel of varying consistency, composed of salts, water, proteins, and polysaccharides, in solution. Some of these polysaccharides, known as mucopolysaccharides, exist as protein polysaccharide complexes. The physical state of this ground substance may be related to the degree of polymerization, and the latter may be dependent on the synthesis and breakdown. The function of the ground substance, as an intercellular medium, may therefore be dependent on the metabolism of these complexes.

There are three fibers, or three types of fibers, in connective tissue: collagen, elastin, and reticulum. Much more is known of the protein collagen than about elastin and reticulum. Understanding the chemistry and metabolism of collagen is an important precursor to understanding its function.

The four main macromolecular constituents of human tissue are polysaccharides, nucleic acids, proteins, and certain lipids. These are complexes that do not dialyze through a graded dialysis tubing. The constituents of connective tissue, as outlined above, do not dialyze, and fit the crude definition of macromolecules. This, therefore, in brief, is an explanation of the terms of our title, "Connective Tissue: Intercellular Macromolecules."

It has been stated that biochemistry was begot out of chemistry by an unknown father. The unknown father must at least have been some sort of a biological creature. The function of biochemistry has been to explain the complexities of biological thought, to break down biological thought to simpler entities. The more recent advances in biochemistry have been the unusual accomplishments brought about by the contributions of, one, straight chemistry, and, two, molecular geometric visualization and interpretation of physicochemical factors, combined with biological common sense which is based on biochemical mechanisms, visualization of biological fine structure, and observation of biological phenomena.

Our symposium will try to reach these levels of accomplishment in a field of biological importance and interest. The chairman of each session has organized his program with these aims in view.

SAUL J. FARBER

Physical Chemistry of Macromolecules

CHAIRMAN: THEODORE SHEDLOVSKY

Professor in The Rockefeller Institute

INTRODUCTION

The session on the "Physical Chemistry of Macromolecules" was arranged by Dr. Theodore Shedlovsky, who was to have introduced the subject. However, a macromolecule in the form of a "flu" virus intervened.

Of course, macromolecules have been around for a great many eons, but it is only recently that we have come to recognize that they have properties rather different from other kinds of molecules. I can remember the very first book on chemistry that I came across, which gave the composition of glucose as $C_6H_{12}O_6$, and then, in the next paragraph, gave the composition of cellulose as $C_6H_{10}O_5$. But those numbers do not tell anything about the difference in the properties of glucose and cellulose.

You can use a stick of wood for planting corn, as the Aztecs did, or you can use a stick of wood for beating somebody over the head, but you cannot use a stick of glucose for those purposes, and this difference in properties is not explained by the formulas that were in that early book on chemistry.

As a matter of fact, it is only recently that the beginnings of macromolecular chemistry, as a separate branch of chemistry, came into being. Its ideas are being used in a great many aspects of science, and that is why it was felt that it is time for a symposium of this sort to bring it to your attention.

The first session of this symposium, then, is going to deal with the general background of macromolecules, how their properties are related to their structures, and how they differ from the small molecules, which I suppose might be called "micromolecules" by contrast.

MAXWELL SCHUBERT

MACROMOLECULES

ORIGIN, DEVELOPMENT, SIGNIFICANCE

H. MARK

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ABSTRACT Natural polymers such as wood, cotton, wool, starch, and rubber have been known and used for a long time even though their composition and structure were unknown. At the beginning of this century systematic efforts were started to analyze these materials, elucidate their molecular pattern, and fit them into the framework of organic chemistry. It was found that they all are characterized by the presence of chain-like molecules with very high molecular weights ranging from 10000 to several millions. It was also found that these chains can be oriented by mechanical deformations and assume a laterally ordered crystal-like supermolecular arrangement. As soon as these facts become known as systematic search for synthetic counterparts of natural polymers was started which led to the laboratory preparation and large scale production of many synthetic materials which cover a wide range of properties and applications and which are becoming an increasingly important factor in science and industry. With growing understanding and know-how it also became possible to synthesize polymers with biochemically interesting properties such as the polyelectrolytes and other biopolymers.

Natural polymers such as wood, cotton, wool, silk, lacquers, rubber, and many types of gums have been used for centuries in all kinds of practical applications. Their chemical composition and structure were unknown; improvements made in the course of time were mainly through breeding, selection of the best raw materials, and through advances in the mechanics of the manufacturing processes. A few very important though probably accidental chemical discoveries were made which affected the chemical composition and structure of certain natural polymers such as the *vulcanization* of rubber, the *mercerization* of cotton, hemp, and flax, the *tanning* of leather, and the loading of silk. They led to significant technical results and were constantly improved in an empirical fashion.

Scientific work began around 1880 on the chemical composition, structure, and morphology of cellulose, wool, silk, starch, and rubber; it was largely descriptive and qualitative but provided a very useful background for further quantitative approaches. During this period the first synthetic (or artificial) polymers were made, particularly condensation products of formaldehyde with phenol, urea, and proteins (bakelite, Pollopas and Galalit) and derivatives of cellulose (nitrate and acetate); they acquired a considerable commercial importance but their development was purely empirical and their properties were, in most cases inferior to those of the corresponding natural products. Hence they were generally considered as *substitutes* or *ersatz* and were given such names as *Kunstseide, Kunstleder,* or *Kunstostoffe*. Most of their development took place in Europe, particularly in Germany, France, and England.

The ground work for the *organic chemistry* of polymers or macromolecules was laid around 1905 in the Institute of Emil Fischer in Berlin. His work on sugars and amino acids clarified in a complete manner the composition, structure, and stereochemistry of these substances and opened the way to a stepwise synthesis of progressively larger and larger molecular species. Emil Fischer himself remained strictly in the domain of classical organic chemistry of which he was the unsurpassed master and only reached the lower limits of polymer chemistry (molecular weights between 1000 and 1500) but his coworkers pioneered in all fields of true polymer research—Freudenberg, Helferich, and Hess in the field of polysaccharides, Leuchs and Bergmann in the domain of polypeptides, and Harries and Pummerer in polyhydrocarbons, particularly rubber. At the same time Ostwald and Svedberg developed *physicochemical* methods for the investigation of colloidal systems by the measurement of *diffusion, sedimentation, viscosity,* and *turbidity* and laid the fundament for the quantitative study of polymer solutions. Finally, the basic discovery of x-ray diffraction by von Laue, Bragg, Debye, and Scherrer showed the way to apply this method for the elucidation of the fine structure of the solid state even in microcystalline materials, which eventually led to the x-ray investigation of such polymeric materials as cellulose, proteins, and rubber.

The combined application of *all available* methods, organic, physiocochemical, and physical, was systematically promoted from 1920 to 1930 and led to a systematic clarification of a number of basic facts such as: synthesis of many new polymers, determination of their average molecular weight, the behavior of their solutions, and the details of their structure in the solid state. Most results were still qualitative or semiquantitative but a wide field was opened for further research and numerous attractive problems posed themselves on various topics. The industrial application of different polymers started to develop and it became evident that many valuable properties could be obtained in great variety and at low cost. A few prominent names of this period are W. A. Carothers, K. H. Meyer, E. K. Rideal, and H. Staudinger.

During the next decade there occurred a continuation of the systematic synthesis of many new polymers in the field of fibers, plastics, and rubbers, particularly through the preparation of new monomers, the discovery of new catalytic systems, and of the principle of copolymerization. There was a *quantitative formulation* of the kinetics of polymerization processes and understanding of their individual steps. Development of the statistical thermodynamics and hydrodynamics of polymer solutions leading to a *quantitative* understanding of osmotic pressure, diffusion, sedimentation, and viscosity took place. There was also a clarification of the solid state structure of polymers: theory of rubber elasticity, transition phenomena, crystallinity —amorphous system and relaxation behavior. Some prominent names in these fields are P. J. Flory, E. Guth, L. M. Huggins, W. Kuhn, W. H. Melville, and G. V. Schulz.

After 1940 we can witness a successful extension, amplification, and refinement in all directions. Specifically many new monomers, many new powerful catalytic systems, and many new polymerization techniques were developed with refinement of the statistical treatment of macromolecules in solution and in the bulk state, development of new methods for the characterization of polymers such as light-scattering, small angle x-ray diffraction, polarized infrared absorption spectroscopy, rotatory dispersion measurements, nuclear magnetic resonance signals, differential thermal analysis or sedimentation, and diffusion in a density gradient cell. There was also clarification of the mechanism of polymerization under various conditions such as in solution, suspension, and emulsion, at high pressures, high and low temperatures, and in the form of living polymers. Enormous growth in the industrial production and practical application of all polymers took place.

The following important progress can be forecast without too much risk for the near future:

(*a*) Lowering of the selling prices of all standard plastics and rubbers into the range between 20 and 30 cents per pound with some coming down as far as 15 cents. Lowering of the selling prices of most standard staple fibers into the range between 40 and 60 cents per pound with some of them coming down as far as 25 to 30 cents.

(*b*) Synthesis of polymers which will remain flexible and supple as far down as −100°C and of others which can stand temperatures of 500°C for prolonged periods.

(*c*) Development of adhesives which will make it possible to build houses, cars, and airplanes without nails, screws, or rivets.

(*d*) Polymerization at low temperatures in the liquid or solid state permitting rapid continuous production of many polymers with convenient control of molecular weight and of high purity.

(*e*) Further improvement of the methods for the characterization of polymers, particularly NMR, flow birefringence, optical activity, and differential thermal analysis.

POLYELECTROLYTES AND THEIR BIOLOGICAL INTERACTIONS

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ABSTRACT Polyelectrolytes are water-soluble electrically charged polymers. Their properties are determined by the interplay of the electrical forces, the Brownian motion of the macromolecular chain, and intermolecular Van der Waals forces. Charged polyacids or polybases are stretched by the electrostatic forces, as evidenced by increase in solution viscosity, or by the stretching of polyelectrolyte gels. The electrical field of the polyions is neutralized by a dense atmosphere of counter-ions. The counter-ion attraction to the polyions is expressed by a reduction of the osmotic activity of the polyion—the osmotic pressure being only 15 to 20 per cent of the ideal in highly charged polyelectrolytes neutralized by monovalent counter-ions, and as low as 1 to 3 per cent of the ideal for polyvalent counter-ions. Since the ionic atmosphere is only slightly dependent on added low molecular salt, the osmotic pressure of polyelectrolyte salt mixtures is approximately equal to the sum of the osmotic pressure of polyelectrolyte and salt alone. Acidic and basic polyelectrolytes interact electrostatically with precipitation at the point of polymeric electroneutrality. At higher salt concentrations the interaction is inhibited by the screening of polymeric fixed charges. The importance of these interactions in enzymatic processes is discussed. The electrical double layer is polarizable as may be deduced from dielectric and conductometric studies. The polarizability leads to strong dipole formation in an electrical field. These macromolecular dipoles may play a role in the adsorption of polyelectrolytes on charged surfaces. The final part of the paper is devoted to interactions of polyelectrolytes with cell membranes and the gluing of cells to higher aggregates by charged biocolloids.

1. INTRODUCTION

1.1 Classical colloid chemistry profoundly influenced biological thought in the early twentieth century and then seemed to undergo an irreversible decline during the nineteen thirties. Both biochemists and biophysicists lost interest in the "World of Neglected Dimensions" (1) and turned their attention to more rewarding fields of research. It was felt that a direct study of cell components and their metabolic transformations could provide deeper insights into living processes than the physicochemical characterization of ill-defined particles with colloidal dimensions. The situation changed drastically, however, in the early thirties when polymer chemists and physicists provided a conceptual framework for the understanding of the behavior of biological macromolecules. The realization that biocolloids are chain molecules, the structure of which can be analyzed in terms of well defined patterns of chemical bonds, has bridged the gap betweeen molecular physics and chemistry and cellular biology. It has paved the way for a new colloid chemistry—the colloid chemistry of macromolecules.

In 1938 Kern (2) published a couple of papers devoted to the study of electrically charged synthetic polymers. The polyacids studied by him exhibited the normal properties of polymers in solution, such as high specific viscosity, but were in addition typical electrolytes capable of conducting electrical current and giving electrode reactions. It was soon realized that the polymeric electrolytes are suitable models for the understanding of the electrochemical behavior of biopolymers; in 1947 several laboratories launched a long range investigation of the solutions and gels of these new colloids (3–7). Fuoss proposed the suitably fitting name of "polyelectrolytes" for the group of substances which, from the molecular point of view, are polymers while their electrochemical properties bring them into the realm of electrolytes. The subject of this paper is to review simply the results of the study of polyelectrolytes based primarily on the work done in the author's laboratory, although the writer recognizes clearly the conscious and subconscious impact of the ideas and results of other workers to whom only inadequate tribute can be paid in this short review.

1.2 From the electrochemical standpoint polyelectrolytes can be classified as polyacids, polybases, or polyampholytes. Their ionizable groups may be acidic-carboxylic, sulfonic, or phosphoric groups, or they may be basic amino groups, free or substituted. The ionizable groups may be ionized to various degrees and thus behave as partial or full salts. Around the fixed ionized polymeric groups there is generally to be found an atmosphere of small ions composed primarily of *counterions* and to a smaller extent of *coions* due to low molecular weight salts which may be added to the polyelectrolyte solution.

On the other hand, for the polymer chemist, polyelectrolyte molecules may be highly polymerized, or less so, and their backbone may be linear or branched. The monomeric units composing the macromolecular chain may be arranged in random sequence or they may be built in a stereospecific array. Some of the polyelectrolytes, and notably numerous biocolloids, have a crystalline, helical, pattern and may melt to form amorphous, more or less randomly kinked macromolecules.

The notion of a polyelectrolyte has a classificatory advantage and enables us to study diverse substances within a common methodological framework.

Thus, many of the gums and resins carried by the caravans of the Far East since early antiquity owe their usefulness to their polyelectrolyte components. Many important plant products such as the pectic acids of fruit jellies and alginic acid of seaweed are typical polyelectrolytes. The important polyuronic acids from animal organisms such as chondroitin sulfuric acid, mucoitin sulfuric acid, hyaluronic acid, and heparin can be regarded as polyelectrolytes. The polyelectrolyte nature of some polyacid and polybase polypeptides endows them with inhibitory activity in enzymatic reactions; and the electrochemistry of nucleic acids is determined to a large extent by the interplay of the numerous charged groups situated on their polyelectrolytic macromolecular helix.

The interactions of natural polyelectrolytes play an important role in the inner organization of the cell and the binding of cells in the organism. It is not only the chromosomes, which can be regarded as a reaction product of a polybase with a polyacid, but the structure of many cell membranes and the intracellular reticulum can be attributed to forces operating between oppositely charged polyions. In addition, as polyelectrolytes and their interactions are responsible for many of the properties of connective tissue, it is appropriate for the present symposium to get a more general view of the physical chemistry of charged macromolecules and of their interactions.

2. SHAPE DYNAMICS OF POLYELECTROLYTE MOLECULES

2.1 As a first demonstration of the joint operation of polymeric and electrical properties within polyelectrolyte molecules, we shall consider the shape dynamics of charged polymolecules. A "geometrical" macromolecule—one in which intramolecular forces play no role, performs a very complicated set of movements around its carbon-to-carbon bonds. Thermal collisions with the medium cause the molecular chains to perform rotary movements which cause constant alterations in the shape of the macromolecule; this dynamic shape resembles the random walk of the statisticians, or the Brownian movement of a single colloid particle. In the polymeric coil the steps of the random walk are the directions of the units or segments of the polymeric chains, while the statistical average of the macromolecular dimension is proportional to the square root of the number of units.

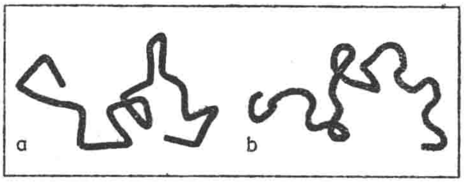
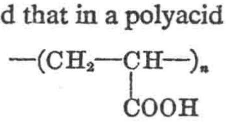


FIGURE 1 Model representation of randomly kinked macromolecules.

In a "physical" macromolecule the monomeric units of the polymer interact with each other and it is to be expected that in a polyacid of the type of polyacrylic acid



$$—(CH_2—\underset{\displaystyle COOH}{\underset{|}{C}}H—)_n$$

there will exist numerous hydrogen bonds between the carboxylic groups, which will tend to contract the molecular chain and diminish its dimensions below the statistical average. On the other hand, if some of the acidic groups be ionized, by the addition of a base such as sodium hydroxide to the solution, a strong repulsion develops between the negative carboxylate ions—COO^- which tends to stretch the macromolecule and open up the random coil. Further ionization of the acidic groups stretches the molecules to a large extent and endows parts of the chain with a cylindrical symmetry.

A suitable indicator for the changes in shape occurring upon ionization is the specific viscosity η_{sp} of polyelectrolyte solutions.[1]

Solutions of spherical macromolecules present a lower resistance to flow so that the η_{sp} of their solutions is small; on the other hand, if the molecules stretch and approach a rod-like shape the frictional dissipation of energy accompanying their flow increases and correspondingly η_{sp} reaches high values. This behavior is shown in Fig. 2 based on the measurements of Eisenberg where the specific viscosity of polymethacrylic acid is plotted against the degree of ionization.[2]

It will be observed that at all polymer concentrations, the specific viscosity changes slowly up to $\alpha = 0.15$. This slow change indicates that in this range of ionization the electrostatic repulsion is insufficient to overcome the hydrogen bonding of the carboxylic groups keeping the molecules coiled. From $\alpha = 0.15$ to $\alpha = 0.8$ there is a rapid and marked increase in η_{sp} which indicates that molecules have opened up and stretched; finally at still higher degrees of ionization there is a slight decrease in η_{sp} due to the increase in the concentration of counter-ions. Indeed, the effect of the electrostatic stretch is highest at lowest polymer and counter-ion concentration. High concentrations of counter-ions mask the repulsive effect of the counter-ions so that lower stretching effects are to be expected.

Even if the polymeric molecules have an organized helical structure, such as is found in many polypeptides, the electrostatic repulsion of charged groups may suffice to overcome the attractive forces and bring the helix to melting with subsequent stretching. This is shown in the recent measurements of Goldstein (8) on polyglutamic acid. Fig. 3 shows that up to a degree of ionization of 50 per cent, the helical structure can withstand the strong repulsion of the fixed charges. At higher α's the helix breaks down and the molecules stretch to an appreciable extent. As expected, the addition of neutral salt screens off the polymeric charges and diminishes the repulsion so that the stretching effect is lower at higher salt concentrations.[3]

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[1] Specific viscosity η_{sp} is defined as the ratio of viscosity of the solution η minus the viscosity of the solvent to that of the solvent η_o; $\eta_{sp} = \eta - \eta_o/\eta_o$. The ratio of specific viscosity and polymer concentration, at very low concentration, is known as "intrinsic viscosity" $\text{Lim}_{c\to 0}\eta_{sp}/c = [\eta]$ and it is this quantity which is directly related to polymer volume (*cf.* Flory).

[2] $$\alpha = \frac{\text{No. of ionized carboxylic groups}}{\text{Total No. of carboxylic groups}} = \frac{\nu}{P}$$

[3] A word of caution should be interjected on the viscometric measurements: the data underly-