CHEMISTRY AND THERAPY OF COLLAGEN DISEASES

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FOREWORD

Our Living Chemistry Series was conceived by Editor and Publisher to advance the newer knowledge of chemical medicine in the cause of clinical practice. The interdependence of chemistry and medicine is so great that physicians are turning to chemistry, and chemists to medicine in order to understand the underlying basis of life processes in health and disease. Once chemical truths, proofs, and convictions become sound foundations for clinical phenomena, key hybrid investigators clarify the bewildering panorama of biochemical progress for application in everyday practice, stimulation of experimental research, and extension of postgraduate instruction. Each of our monographs thus unravels the chemical mechanisms and clinical management of many diseases that have remained relatively static in the minds of medical men for three thousand years. Our new Series is charged with the *nisus élan* of chemical wisdom, supreme in choice of international authors, optimal in standards of chemical scholarship, provocative in imagination for experimental research, comprehensive in discussions of scientific medicine, and authoritative in chemical perspective of human disorders.

Dr. Neustadt of Louisville and Dr. Rotstein of New York correlate the chemistry of unrelated hereditary and acquired diseases with underlying unifying mechanisms by virtue of the common injury to connective tissue or ground substance and the characteristic deposit of abnormal substances in collagen fibers. The principal lesions appear early in collagen tissues: in the synovia and periarticular tissues of the joints; in the fibrous septs of the myocardium and skeletal muscles; in the endocardium and in blood vessels; and in the skin and subcutis producing the pathological cycle of necrosis → collagenolysis → antibody → antibody injury → necrosis. Systemic connective tissue diseases is a more accurate designation for this group of chronic disturbances of recurring

fever, dysglobulinemia, plasma-cytosis, and raised sedimentation rate with widely differing clinical features. But names are much more persistent than the functions upon which they were originally bestowed, hence we perpetuate the original collagen concept and bow to usage. The used key is always brighter. Yet words are the stumbling blocks in the way of truth; you cannot think rightly until you think of the phenomena rather than of the words that misrepresent them.

The chemical basis for this unsuspected classification was demonstrated two decades ago as a fibrinoid physicochemical change arising from the disintegration of the collagen fibers of connective tissue which assumes the tinctorial characteristics of fibrin. The nature and origin of fibrinoid degeneration remains controversial though freed from association with fibrin of blood clotting. Fibrinoid is considered a new substance formed by the combination of the colloid residue and dissociation products of suppressed collagen synthesis in the region of rheumatic nodules, with other tissue and plasma components. It is comparable to the microscopic lesions in allergic diseases in which fibrinoid degeneration of collagen is the most conspicuous tissue change, thus giving rise to the current belief that collagen diseases are related pathogenetically and, therefore, produce allergic manifestations. Actually, the fibrinoid complex reveals significant differences in each type of disorder, markedly affected by the abnormal immune response to unknown antigens.

The authors describe clearly and concisely the chemical anatomy of the connective tissue, the chemistry of its components, and the physicochemical changes induced by various physiologic, pharmacologic, and traumatic stimuli as the experimental basis for the relationship between connective tissue and collagen diseases. It represents a heuristic concept of intercellular pathology based on scientific knowledge which is insufficient to prove its high probability. Nevertheless, the clinician can now think in terms of chemical reactivity and the chemist of cellular structure in relation to metabolic function despite the disparaging beginnings of two centuries ago. Von Haller (1757) decried the lowly role of tela cellulosa as body packing material. Virchow (1850) evolved his views on cellular pathology from connective tissue studies without

altering that concept. But, Schultze (1860) considered intercellular substance derived from cells. Schade (1923) pictured connective tissue as a highly integrated colloid system. Klinge (1933) attributed rheumatic fever to bacterial hyperergy of connective tissue. Klemperer (1942) introduced the concept of collagen diseases to integrate intercellular pathology. The clinical dogmas of the quiet past are totally inadequate to the stormy present. We must disenthrall ourselves, think anew, and act anew as clinical explorations of connective tissues unravel the altered structure and function of the massive molecules that regulate the pivotal cells in collagen diseases.

"This is not the end. It is not even the beginning of the end,
But it is perhaps, the end of the beginning."

I. NEWTON KUGELMASS, M.D., PH.D., SC.D., *Editor*

PREFACE

The designation "diffuse collagen disease" was first introduced by Klemperer, Pollack and Baehr (1942) to indicate that the connective tissue was the site of a widespread pathologic process. The involvement of the tissues is characterized by certain common microscopic changes. Although the diseases relegated to this category exhibit several similar clinical features, there is no definitive evidence that the histopathologic features arise from a common etiologic or pathogenic mechanism. Progress in elucidating the mechanisms of these diseases has been slow and, until recently, the attention of clinicians and investigators was directed chiefly to morphologic and pathophysiologic changes. However, interest has shifted to the biochemical approach in recent years. This book represents an attempt to describe the current important biochemical contributions related to the pathology and therapy of "collagen" diseases. General clinical and pathologic descriptions have not been included in this volume, since they are readily available elsewhere.

The mechanisms of the connective tissue diseases would be more easily unraveled, if we understood the biochemical processes underlying the composition, biosynthesis, and metabolism of the components of normal connective tissue. The ideal approach in attempting to fulfill the implication of this book's title would be an orderly presentation, describing successively: the normal physiochemistry and metabolism of connective tissue constituents; the significant biochemical alterations which may produce the clinical manifestations of a "collagen" disorder (rheumatoid arthritis, systemic lupus erythematosus, periarteritis nodosa, dermatomyositis or diffuse scleroderma). Unfortunately, such an orderly sequence is not possible. Much of the information which has evolved seems to be isolated bits and facts frequently lacking both a beginning and end. The many "blank" spaces, which interrupt the "story," remain to be filled in through further investigation.

I have not attempted to include all of the contributions on

this subject, but rather to sift the vast accumulated literature and select the evidence and data which appear most likely to be significant to the pathogenic processes. It is, of course, not always possible to predict the potential worthiness of a basic experiment which, although seemingly unrelated at the time, may be the stepping stone to a major contribution. Highly speculative and untested concepts have been omitted. Older concepts which have been substantially modified, or shown to be untenable, are also omitted. Whenever possible, emphasis is placed on the biochemical factors which may be related to the clinical aspects of a disease. Any known influences of regulatory factors such as hormones, enzymes, or other therapeutic agents on the chemical processes in connective tissue are described. Many new techniques and procedures have been developed in immuno-histochemistry and applied to studies of both healthy and diseased connective tissue. Although details of complex methodology are not included, further information on the investigative tools can be found by consulting the articles listed in the references.

Since it is not yet possible to correlate the biochemical disturbances and the therapeutic approach, therapy for these disorders remains empiric. A section on therapy has been included, however, following a description of the current biochemical aspects of each disease. I am hopeful that sufficient information has been provided to aid the clinician in selecting and applying the available therapeutic agents to full advantage. Some of the subject material in these sections overlaps, but I have tried to reduce duplication to a minimum. When my convictions regarding the value or application of therapeutic agents vary with those of others, I have tried to present a reasonable account of the contrary opinions. Greater knowledge and understanding of the altered biochemistry may eventually lead to more effective therapy, but today exciting developments in steroid chemistry with the introduction of new, useful analogues are inestimable contributions to the therapy of rheumatic disorders. In addition to their clinical value, the discovery of these remarkable drugs stimulated and renewed enthusiasm in the further study of connective tissue diseases. A short glossary has been appended to the text to minimize any confusion in terminology and nomenclature.

D.H.N.

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I want to thank Dr. I. Newton Kugelmass for extending the invitation to write this volume. I am especially grateful to Dr. Jerome Rotstein, my collaborator, for his invaluable contributions, including the chapter on biochemistry of connective tissue and on agammaglobulinemia associated with connective tissue diseases.

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DAVID H. NEUSTADT, M.D.

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Chapter 1

THE BIOCHEMISTRY OF CONNECTIVE TISSUE

The connective tissue of the body is a chemically complex system which unites and supports the specialized organs. This system provides the milieu in which the life of the organism takes place. It is composed of three morphological components: cells, fibers and ground substance or matrix. The cellular component is comprised of fibroblasts (and analogous variants, chondroblasts and osteoblasts), mesenchyme cells, histiocytes, mast cells, lymphocytes, plasma cells, and pigment cells.[1] There are three different types of fibers; collagen, reticulin and elastin.[2] The matrix is composed of protein-polysaccharide complexes, non-collagen protein and carbohydrates. The character and thickness of the connective tissue differ throughout the body. It is very thin in the lungs; it is thicker in the liver and kidney; in the heart it forms a major portion of the organ; and in the bone, cartilage and tendon it composes the entire tissue.[4] In most varieties of connective tissue, the water component constitutes over 70 per cent of the weight of the tissue. The solutes, including electrolytes, are exchanged in the fluid phase. The manner in which the fluid phase is bound in connective tissue is not known.[5]

CYTOLOGICAL COMPONENTS

The fibroblasts secrete processes which interlace to form a network. The spaces of the network are filled with the gel-like matrix. The histiocytes, mast cells and mononuclear cells enter the matrix from the blood.[1] The proportion of cellular elements to the other constituents varies in the different types of connective tissue. In the intestine and uterus, the connective tissue is extremely cellular whereas in the tendons and ligaments there are few cells and an abundance of fibrous elements.[2]

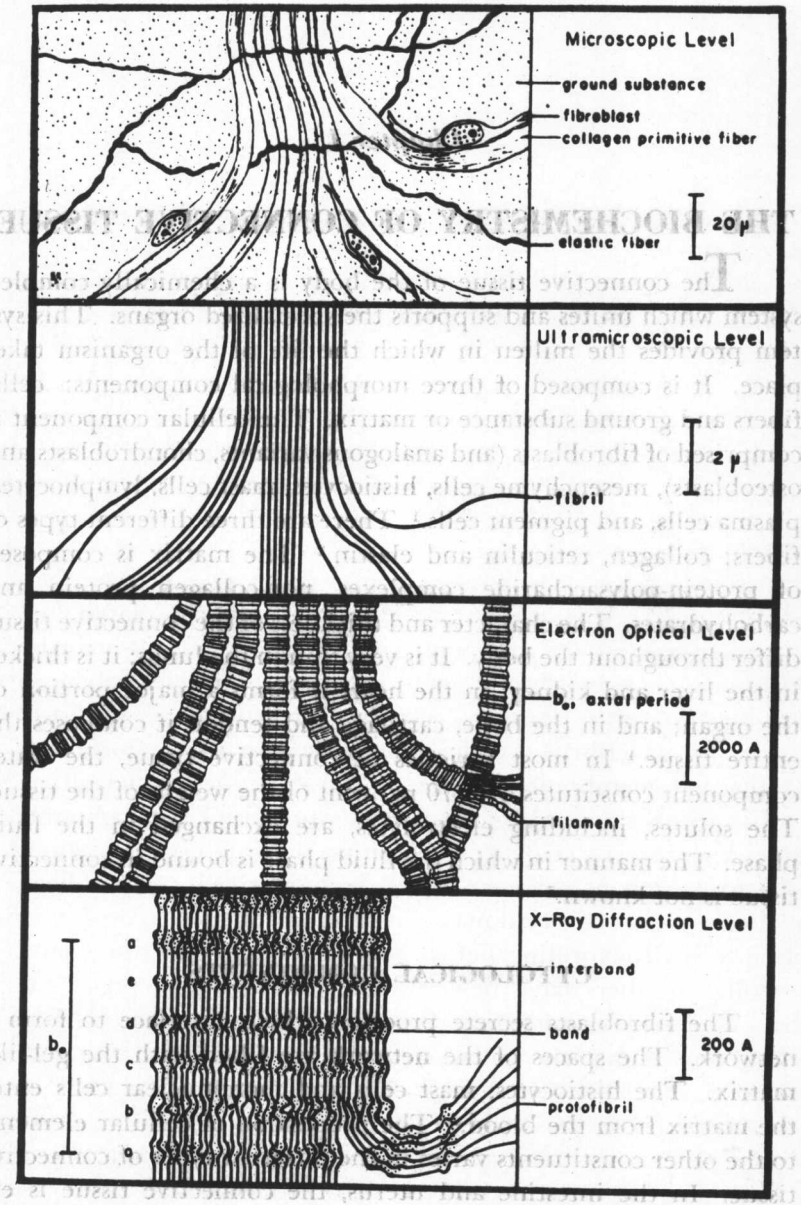

Fig. 1. a) Microscopic composition of connective tissue. b) c) and d) show the structural elements of the collagen fiber in steps of increasing magnification. (From R. S. Baer, Advances in Protein Chemistry.)

The mast cells, first described in 1877 by Paul Ehrlich, have been recently subjected to detailed study. These cells, characterized by cytoplasmic granules which display metachromasia with stains such as toluidine blue, may be encountered throughout connective tissue, but are especially numerous in the walls of blood vessels and the adjacent tissue. Although it seems established that mast cells contain heparin[6] and histamine,[7] their function in normal and disease states remains speculative. Evidence for other specialized functions of these cells in humans, such as the production of hyaluronic acid and 5-hydroxytryptamine (serotonin), is still controversial.[8,9]

FIBROUS COMPONENTS

The morphogenesis of the fibers of connective tissue has not been established.

Collagen

Certain evidence indicates that the collagen fibers are synthesized within the fibroblast and extruded into the matrix.[10,11] It has also been stated that the basic collagen protein may be produced intracellularly and then aggregate extracellularly into fibrils and then into fibers.[12] However, a recent report indicates that the collagen fibrils may be formed outside of the fibroblasts near the cell membrane.[13] None of these studies is definitive. Ascorbic acid is required for maximum synthesis of large amounts of collagen. Present evidence concerning the role of this vitamin in the formation of collagen is still inconclusive.[14] It has been suggested that ascorbic acid may be necessary for the conversion of proline to hydroxyproline before synthesis of the peptide chain.

Collagen fibers have been studied chiefly by roentgen ray diffraction, electron microscopy and biochemical analysis. These methods reveal that the collagen fibrils vary in length, ranging from 2000 Å to 3000 Å.[15] All vertebrate collagen is characterized by regularly occurring light and dark bands or striations from 300 Å to 800 Å wide, with an average width of 640 Å (Fig. 2).[16-18] Embryonal collagen has an average band pattern of 210 Å.[19] The precise structure of the bands is not known. One widely held theory relates the bands to the distribution of amino-acid sequences.[20]

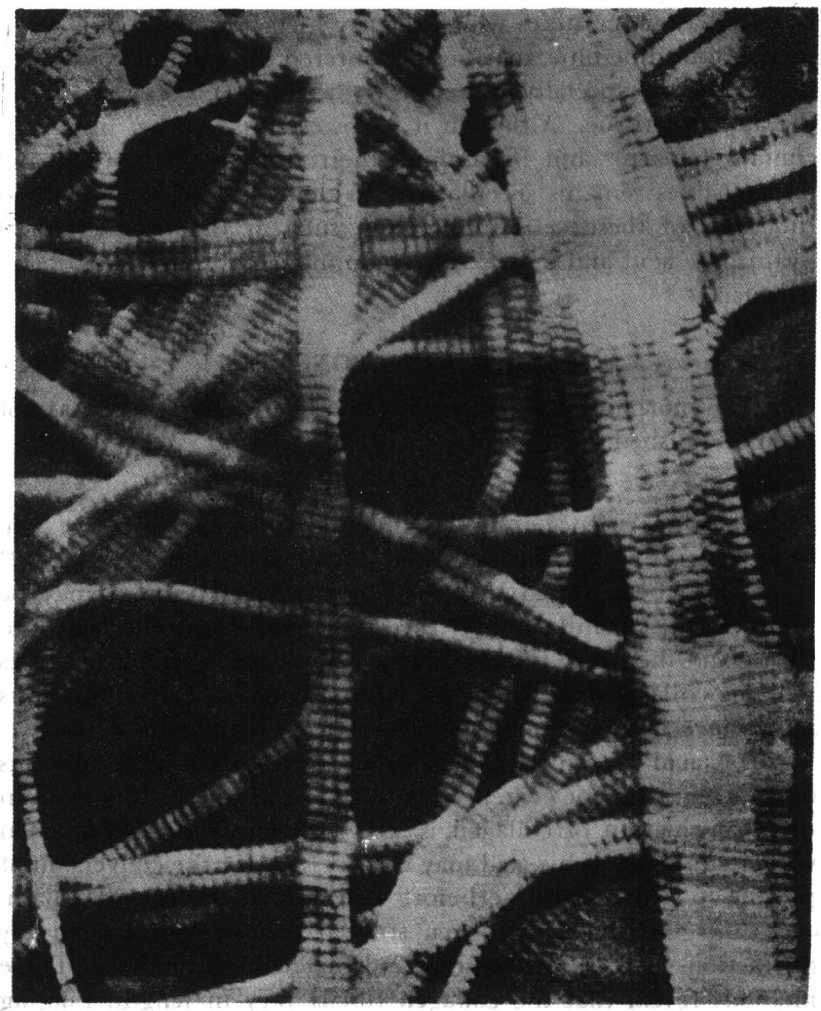

Fig. 2. Electron micrograph of collagen fibrils. Note the evenly spaced periodic bands about 640 angstrom units apart. (Courtesy of Jerome Gross, M.D.)