Structure and Function of Connective and Skeletal Tissue

G. R. Tristram

STRUCTURE and FUNCTION of CONNECTIVE and SKELETAL TISSUE

Proceedings of an Advanced Study Institute
Organized under the Auspices of N.A.T.O.
St. Andrews
15–25 June 1964

Scientific Committee

S. Fitton Jackson R. D. Harkness
S. M. Partridge G. R. Tristram

LONDON
BUTTERWORTHS
1965

Suggested U.D.C. number: 576·72
Suggested additional numbers: 611–018·2
612–014·2

Printed in Northern Ireland at
The Universities Press, Belfast

FOREWORD

The Advanced Study Institute on 'Structure and Function of Connective and Skeletal Tissue' was held under the auspices of N.A.T.O. at St. Andrews, Scotland, from 15th to 25th June, 1964. Knowledge of the structure and function of connective tissue has for long been a vague no man's land in biology, but in the last decade striking advances have been made both in the structure and chemistry of the macromolecular components, and in our understanding of the architecture of cells and the extracellular matrix. These advances have, in their turn, thrown a new light on those problems of morphogenesis and the interactions of cells with their environment which are presently of such vital interest to medical men.

In planning the meetings the scientific committee strove to provide an unfolding story, from detailed investigations of the chemistry and physics of the fibres and ground substance to final discussion of the implications of the new chemical and biological knowledge for medicine. The result was certainly quite the longest international meeting that had ever been held on connective tissue, but we believe that the sessions achieved their aim, and the aim of the sponsors, in displaying a panoramic picture of the field as it exists today.

We feel that the participants will wish to join us in thanking the Scientific Affairs Division of the North Atlantic Treaty Organisation for their faith in the value of international meetings as a means of organizing and disseminating advances in scientific knowledge, and for their more than generous support of this St. Andrews Study Institute. We particularly wish to thank Dr. Bryan Coleby of N.A.T.O. for his kindness and understanding of our difficulties and his helpful advice. The arrangements at St. Andrews were the responsibility of Dr. G. R. Tristram, his staff and colleagues; we offer our thanks to the following members of his committee, without whose generously given help the conference could never have been brought into being: Mrs. R. M. McAllister (who acted as secretary to the Study Institute), Dr. S. Bayne, Dr. G. Goodlad, Dr. D. Thirkell and the following students: Pamela Brunt, James Daglish, John Hunter, Fiona Lumsden, Linda Mollison, Rosemary Roger, Margaret Swain, David Steer, Robin Strang, Timothy Suffolk and David Worship. All those who attended the Study Institute owe a great debt of gratitude to them, to the Master of St. Salvator's College for his hospitality and to the Domestic Bursar for her excellent arrangements.

INDEX TO SPEAKERS

PARTICIPANTS OF STUDY INSTITUTE

ALEXANDER, W. R. M.	Rheumatic Diseases Unit, Northern General Hospital, Edinburgh, Scotland.
ANDERSEN, SVEND OLAV	Zoophysiological Laboratory B, University of Copenhagen, Juliane Maries Vej 36, Copenhagen Ø, Denmark.
ANSETH, ARVID	Department of Ophthalmology, University Hospital, Lund, Sweden.
BALL, J.	Rheumatism Research Centre, Clinical Sciences Building, University of Manchester, York Place, Manchester 13, England.
BARNES, M. J.	Low Temperature Research Station, Cambridge, England.
BAZIN, SUZANNE	Institut Pasteur, Annexe de Garches, Seine-et-Oise, France.
BELL, G. H.	Department of Physiology, Queen's College, Dundee, Scotland.
BENSUSAN, HOWARD B.	Department of Biochemistry, Western Reserve University School of Medicine, Cleveland, Ohio, U.S.A.
BERLEPSCH, K.	Im. Esterl: 20 Riehen. Switzerland.
BLUMENFELD, OLGA O.	Department of Biochemistry and Unit for Research in Ageing, Albert Einstein College of Medicine, Yeshiva University, New York 61, U.S.A.
BOWES, J.	British Leather Manufacturers' Research Association, Milton Park, Egham, England.
BURGE, R. E.	Physics Department, Queen Elizabeth College, University of London, London, W.8, England.
BYWATERS, E. C. L.	Rheumatism Research Centre, Canadian Red Cross, Memorial Hospital, Taplow, Maidenhead, England.
CANDLISH, J. K.	Department of Biochemistry, Bute Medical Buildings, University of St. Andrews, Scotland.
CASTELLANI, A.	Istituto di Chimica Biologica, Università di Pavia, Via T. Taramelli, 1, Pavia, Italy.
CESSI, C. CESSI, FRANCA SERAFINI	Istituto di Patologica Generale, Università degli Studi di Cagliari, Via Purcell, 4, Cagliari, Sardinia, Italy, Formerly of Università di Bologna.
COLEBY, B.	NATO Division de Affaires Scientifique, Place du Marechal De Lattre de Tassigny, Paris XVI, France.
COMTE, P.	Institut Pasteur, Annexe de Garches, Seine-et-Oise, France.
COURTS, A.	The Gelatine and Glue Research Association, 2a Dalmeny Avenue, Holloway, London, N.7, England.

DELAUNAY, ALBERT	Institut Pasteur, Annexe de Garches, Seine-et-Oise, France.
DI FERRANTE, N.	Department of Physiology, University of Cincinnati College of Medicine, Cincinnati 19, Ohio, U.S.A.
DINGLE, J. T.	Strangeways Research Laboratory, Cambridge, England.
DODSON, J. W.	Strangeways Research Laboratory, Cambridge, England.
DORFMAN, A.	La Rabida Sanitarium, University of Chicago, Chicago 49, Illinois, U.S.A.
DOUGLAS, D. M.	Department of Surgery, Queen's College, Dundee, Scotland.
DUTHIE, J. J. R.	Rheumatic Diseases Unit, Northern General Hospital, Edinburgh 5, Scotland.
EASTOE, J. E.	Department of Dental Science, Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.2, England.
ELSDEN, D. F.	Low Temperature Research Station, Downing Street, Cambridge, England.
ENGEL, JÜRGEN	Max-Planck-Institut für Eiweiss- und Lederforschung, 8 München 15, Germany, and The Weizman Institute of Science, Rehovoth, Israel.
FESSLER, J. H.	Gordon G. Alles, Laboratory for Molecular Biology, Division of Biology, California Institute of Technology, Pasadena, Calif., U.S.A.
FITTON JACKSON, SYLVIA	Strangeways Research Laboratory, Cambridge, England.
FLEISCH, H.	Laboratorium für Experimentelle Chirurgie, Schweizerisches Forschungsinstitut, Davos, Switzerland.
FRANZBLAU, C.	Department of Biochemistry, Boston University, College of Medicine, Boston, Mass., U.S.A.
GALLOP, P. M.	Department of Biochemistry and the Unit for Research in Ageing, Albert Einstein College of Medicine, Yeshiva University, New York, U.S.A.
GARDELL, SVEN	Department of Physiological Chemistry, University of Lund, Lund, Sweden.
GIBSON, T.	Department of Surgery, The Western Infirmary, Glasgow, Scotland.
GILES, B. G.	Unilever Research Laboratory, Colworth House, Sharnbrook, England.
GILLMAN, THEODORE	Department of Experimental Pathology, Institute of Animal Physiology, Babraham, Cambridge, England.
GLIMCHER, MELVIN J.	Department of Orthopaedic Surgery, Massachussets General Hospital, Boston, 14, Mass., U.S.A.
GLYNN, E.	Rheumatism Research Centre, Canadian Red Cross Hospital, Taplow, Maidenhead, England.
GOTTE, L.	Istituto di Istologia-Embriologia, Università di Padova, Via A. Gabelli, Padova, Italy.

GOULD, BERNARD S.	Biology Department, Massachusetts Institute of Technology, Cambridge, Mass., U.S.A.
GRASSMANN, W.	Max-Planck-Institut für Eiweiss- und Lederforschung, 8 München 15, Schillerstr. 46, Germany.
GREEN, HOWARD	Department of Pathology, New York University School of Medicine, 550 First Avenue, New York 16, U.S.A.
GROSS, JEROME	Lovett Memorial Group, Massachusetts General Hospital, Boston 14, Mass., U.S.A.
GUSTAVSON, K. H.	Drottning Kristinas Vag. 45, Stockholm, Sweden.
HALL, D. A.	Department of Medicine, University of Leeds, General Infirmary, Leeds, England.
HANNIG, KURT	Max-Planck-Institut für Eiweiss- und Lederforschung, 8 München 15, Schillerstr. 46, Germany.
HARKNESS, MARGARET L. R.	Department of Physiology, University College, University of London, Gower Street, London, W.C.1., England.
HARKNESS, R. D.	Department of Physiology, University College, University of London, Gower Street, London, W.C.1., England.
HEKKELMAN, J. W.	Laboratorium voor Celbiologie en Histologie, Universiteit de Leiden, Academisch Ziekenhuis, Rijnsburgerweg 10, Leiden, Holland.
HERRING, G. M.	M.R.C. Bone Seeking Isotopes Research Unit, Churchill Hospital, Headington, Oxford, England.
HODGE, A. J.	Gordon A. Alles Laboratory for Molecular Biology, California Institute of Technology, Pasadena, Calif., U.S.A.
HÖRMANN, HELMUT	Max-Planck-Institut für Eiweiss- und Lederforschung, 8 München 15, Schillerstr. 46, Germany.
HOUCK, J. C.	Biochemical Research Laboratory, Children's Hospital, Washington 9, D.C., U.S.A.
HUNTER, JOHN	Department of Biochemistry, St. Salvator's College, University of St. Andrews, St. Andrews, Scotland.
JACKSON, D. S.	Department of Surgery, University of Oregon Medical School, 3181 S.W. Sam Jackson Park Road, Portland, Oregon, U.S.A.
JAMES, D. W.	Department of Anatomy, University College, University of London, Gower Street, London, W.C.1, England.
KENEDI, R. M.	Department of Bio-engineering, University of Strathclyde, Glasgow, Scotland.
KENT, P. W.	Department of Biochemistry, University of Oxford, Oxford, England.
KODICEK, E.	Dunn Nutritional Laboratory, University of Cambridge, Cambridge, England.
KÜHN, KLAUS	Max-Planck-Institut für Eiweiss- und Lederforschung, 8 München 15, Germany.
KULONEN, E.	Department of Medical Chemistry, University of Turku, Turku 3, Finland.
LAPIERE, C.	Institut de Médécin Hôpital de Baviere, Liège, Belgium.

LAURENT, T. C.	Institute of Medical Chemistry, University of Uppsala, Uppsala, Sweden.
LINDNER, J.	Pathologisches Institut, Universitäts-Krankenhaus Eppendorf, 2 Hamburg 20, Martinistrasse 52, Germany.
LLOYD, A. G.	Department of Biochemistry, University College, University of Wales, Cardiff, Wales.
LOEVEN, W. A.	Department of Chemistry and Physics, Nederlands Instituut voor Praevertieve Geneeskunde, Wassenaarseweg 56, Leiden, Holland.
MANDL, INES	Department of Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University, New York 32, U.S.A.
MATHEWS, MARTIN B.	La Rabida Sanitarium-University of Chicago, Chicago 49, Illinois, U.S.A.
MCCALLUM, S.	Strangeways Research Laboratory, Cambridge, England.
MELCHER, A. H.	Department of Dental Science, Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.2, England.
MESTER, L.	Institut de Chimie des Substances Naturelles, Centre National de la Recherche Scientifique, Gif-sur-Yvette, Paris, France.
METCALFE, J. C.	Department of Colloid Science, University of Cambridge, Free School Lane, Cambridge, England.
MEYER, KARL	Department of Medicine, College of Physicians and Surgeons, Columbia University, New York 32, U.S.A.
MUIR, HELEN H.	Medical Unit, St. Mary's Hospital, London, W.2. England.
NICHOLS, GEORGE, JR.	Department of Medicine, Harvard Medical School, Boston 15, Mass, U.S.A.
O'DELL, D. S.	Strangeways Research Laboratory, Cambridge, England.
OPLATKA, A.	Polymer Department, Weizmann Institute of Science, Rehovoth, Israel.
ORLOFF, S.	Laboratoire de Médecine Expérimentale, Foundation Médicale Reine Elisabeth, Université Libre de Bruxelles, Bruxelles, Belgium.
OWEN, MAUREEN	M.R.C. Bone Isotopes Research Unit, Churchill Hospital, Headington, Oxford, England.
PALFREY, A. J.	Department of Anatomy, St. Thomas's Hospital Medical School, London S.E.1., England.
PARTRIDGE, S. M.	Low Temperature Research Station, Downing Street, Cambridge, England.
PICARD, JACQUES	Centre de l'Athérosclérose, Hôpital Saint-Antoine, Paris, XII^{e}, France.
PIEZ, KARL A.	National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland, U.S.A.
REED, R.	Procter Department of Food and Leather Science, University of Leeds, Leeds, England.
RICHELLE, J.	Institut de Thérapeutique Expérimentale, Université of Liège, 32 Boulevard de la Constitution, Liège, Belgium.

concerns the insolubility and dimensional stability of the connective tissue as a whole, since it is this body component which carries the sole responsibility for the maintenance of shape and form. As with elastin, collagen also forms increasing numbers of cross-links as the newly laid-down structures mature, and since both proteins share the same environment and are embedded in the same milieu, it is probable that similar basic reactions govern the processes leading to increasing polymerization with age.

The ground substance itself appears more complex as more is known about it, and discussions emphasized that the environment in which the fibres are laid down can no longer be regarded as a structureless gel. Much of the newer data obtained with components of the ground substance are summarized in Chapter 3. Based, in the main, on the early chemical work of Meyer and his colleagues, important advances have been made in an understanding of the molecular structure of the various acid mucopolysaccharides; and the nature of the attachment of the polysaccharide chains to protein forms a major part of recent work. A further element of complexity is introduced by the recent finding of large amounts of non-collagenous protein in the ground substance and the effect of this on water-holding capacity is beginning to attract experimental investigation.

Although interaction phenomena between different intercellular macromolecules are frequently referred to in subsequent chapters their significance within the living tissue still too often remains obscure and a unifying theory or new ways of approaching the problem appear to be required. The varying proportions of the different constituents in tissues must be related, at least in part, to the gross mechanical properties of the tissues; too frequently the extracellular constituents have been rather generally thought of as forming a dull if necessary framework, but their importance in general metabolic processes is now becoming apparent, as is their function in the process of mineralization.

Many papers in the second part of the book are devoted to the biology of the tissues. Recent results on various cellular activities are presented, and include studies on the synthesis of various intercellular constituents and the action of degradative enzymes. But only a few pieces of the jigsaw are yet in our hands; Paul Weiss has stressed the complexity of the problems that confront us when we try to establish the sequence of events that occur during growth. That the immunological properties of defined chemical species, and of the tissue as a whole, are only now beginning to yield to systematic investigation is apparent from the papers in Chapters 10 and 12; such studies, it would be reasonable to guess, will ultimately prove of major importance in many diseases affecting connective tissue.

We hope this book will prove useful both to those who attended the meeting, and the many more who inevitably could not, as a précis of recent knowledge and as a guide to the literature. We would like to take this opportunity to thank all the authors for their co-operation. Any royalties arising from this book will be used to found a small sum to enable workers interested in this subject in other lands to visit the United Kingdom to find out what we are doing and to tell us what they have done.

INTRODUCTION

Much of the subject matter presented at the Study Institute on the Structure and Function of Connective and Skeletal Tissue is recorded in this book, and is concerned primarily with the most recent studies.

A high proportion of the papers is devoted to the structural chemistry and interrelationships of connective tissue components. Perhaps the most significant breakthrough in the study of the molecular structure of collagen occurred in 1953 when Randall and his colleagues showed that a much better high-angle x-ray diffraction pattern of the fibres could be obtained if they were stretched by about 10 per cent of their length; the result made plain the helical character of the molecule. Subsequently, in 1954–55, triple-chain helical models were proposed, first by Ramachandran's school in Madras, and, almost simultaneously, by Rich and Crick in Cambridge, and by Randall's group in London. The proposed structure consisted essentially of three left-handed helical chains held together by the formation of a right-handed helix about a common central axis. Ramachandran's proposal differed from the others in that stability was satisfied by two interchain hydrogen bonds per 3 residue repeat; the other models provided for a single hydrogen bond in the same repeating unit. Further refinement of the structure has included thermodynamic analysis and recent results strongly support the idea that most of the molecule conforms to Ramachandran's model. Much of our knowledge of the arrangement of the amino acid residues within the molecule is due to the intensive work of Grassmann's school at Munich, while the pioneer work of Schmitt and his colleagues in Cambridge, Mass., led to the idea of the tropocollagen particle, which may be considered to represent the collagen macromolecule, and demonstrated how such particles associated together in a regular fashion to form fibrous structures of a higher order of size. Much data from recent investigations of the structure of the helical molecular units and their possible arrangement in the fibrils are given in Chapter 1.

The constitution, biosynthesis and enzyme reactions of elastin are discussed in Chapter 2. Small amounts of elastin accompany collagen in almost all connective tissues, but in the walls of the larger blood vessels and in other situations which require repeated elastic deformation, the mechanically strong inextensible fibres of collagen are partly replaced by structures formed from elastin. In insects another protein, resilin, takes the place of elastin in similar situations. Both proteins are typical elastomers, and like rubber they owe their properties to a remarkable absence of lateral interaction between chains and the presence of firm chemical cross links at intervals along the chains. The chemical nature of the cross-links is now known for both proteins and the surprising fact emerges that in the two proteins entirely different amino acid side chains are used to connect the link. In both cases the precursor protein, synthesized by the cell, must be a soluble substance capable of diffusing to the growing surface of the insoluble polymerized structure. Indeed, one of the most fundamental problems

WEIS-FOGH, TORKEL	Zoophysiological Laboratory B, University of Copenhagen, Juliane Maries Vej 36, Copenhagen Ö, Denmark.
WEISS, PAUL	Graduate School of Biomedical Sciences, Texas Medical Centre, University of Texas, Houston 25, Texas, U.S.A.; Formerly of The Rockefeller Institute, New York 21, U.S.A.
WEISSMANN, GERALD	Department of Medicine, New York University School of Medicine, 550 First Avenue, New York 16, U.S.A.
WESTERBORN, OLLÉ	Department of Pathology, University of Uppsala, Uppsala, Sweden.
WILLMER, E. N.	The Physiological Laboratory, University of Cambridge, Downing Street, Cambridge, England.
WOESSNER, J. F. JR.	Laboratory for Cardiovascular Research, Howard Hughes Medical Institute and University of Miami School of Medicine, Miami, Florida, U.S.A.
WOOD, G. C.	Department of Pharmacy, University of Strathclyde, Glasgow, Scotland.
WORRALL, JEAN	Department of Biochemistry, Bute Medical Buildings, University of St. Andrews, Scotland.
WRIGHT, V.	Department of Medicine, General Infirmary, Leeds, England.
WYNSTON, L. K.	Max-Planck-Institut für Eiweiss- und Lederforschung, 8 München 15, Schillerstr. 46, Germany.

ROBERT, L.	Laboratoire de Biochemie, Centre de Recherches Allergiques et Immunologiques de l'Institute National d'Hygiene, Paris XIVe, France.
RODÉN, LENNART	La Rabida Sanitarium, University of Chicago, Chicago 49, Illinois, U.S.A.
ROBERTSON, WM. VAN B.	Department of Pediatrics, Stanford University School of Medicine, 300 Pasteur Drive, Palo Alto, California, U.S.A.
RUDALL, K. M.	The Astbury Department of Biophysics, University of Leeds, Leeds, England.
SCHILLER, SARA	Department of Experimental Pathology and Biochemistry, New York Medical College, Flower and Fifth Avenue Hospitals, Fifth Avenue at 106 St., New York, U.S.A.
SCHUBERT, MAXWELL	Department of Medicine and the Study Group for Rheumatic Diseases, New York University School of Medicine, 550 First Avenue, New York 16, U.S.A.
SEIFTER, S.	Department of Biochemistry and the Unit for Research in Ageing, Albert Einstein College of Medicine, Yeshiva University, New York 61, U.S.A.
SERAFINI-FRACASSINI, A.	Istituto di Istologie di Embriologia, Università di Padova, Via A. Gabelli, Padova, Italy.
SINCLAIR, R.	Chemical Biology Unit, Department of Zoology, University of Edinburgh, Edinburgh, Scotland.
SLEDGE, CLEMENT B.	Strangeways Research Laboratory, Cambridge, England.
SNELLMAN, OLLE	Radiumhemmet Forskningslaboratorium, Karolinska Sjukhuset, Karolinska Institut, Stockholm 60, Sweden.
STAINSBY, G.	Procter Department of Food and Leather Science, University of Leeds, Leeds, England.
STEVEN, F. S.	Department of Biochemistry, Bute Medical Buildings, University of St. Andrews, Scotland.
SZIRMAI, J. A.	Research Laboratories, Department of Rheumatology, University Hospital, Leiden, Holland.
THOMAS, J.	Low Temperature Research Station, Downing Street, Cambridge, England.
TRISTRAM, G. R.	Department of Biochemistry, Bute Medical Buildings, University of St. Andrews, Scotland.
VEIS, ARTHUR	Department of Biochemistry, Northwestern University School of Medicine, Chicago 11, Illinois, U.S.A.
WADDINGTON, C. H.	Department of Animal Genetics, University of Edinburgh, West Mains Road, Edinburgh, Scotland.
WARD, A.	Department of Leather and Food Sciences, University of Leeds 2, England.
WEIDMANN, S. M.	Biological Research Unit, Dental School and Hospital, Blundell Street, University of Leeds, Leeds, England.

CONTENTS