CONNECTIVE TISSUE

A SYMPOSIUM
organized by
THE COUNCIL FOR INTERNATIONAL ORGANIZATIONS OF MEDICAL SCIENCES
Established under the joint auspices of UNESCO *and* WHO

Edited under the direction of
R. E. TUNBRIDGE
The University of Leeds

by

MADELINE KEECH
The University of Leeds

J. F. DELAFRESNAYE
C.I.O.M.S., Paris

G. C. WOOD
The University of Leeds

BLACKWELL
SCIENTIFIC PUBLICATIONS
OXFORD

Published simultaneously in the United States of America by Charles C Thomas, Publisher, 301–327 East Lawrence Avenue, Springfield, Illinois.

Published simultaneously in Canada by The Ryerson Press, Queen Street West, Toronto 2.

FIRST PRINTED FEBRUARY 1957

PRINTED IN GREAT BRITAIN IN THE CITY OF OXFORD
AT THE ALDEN PRESS
AND BOUND BY THE KEMP HALL BINDERY, OXFORD

CONNECTIVE TISSUE SYMPOSIUM

LIST OF PARTICIPANTS

G. Asboe-Hansen	University of Copenhagen (Denmark)
W. T. Astbury	University of Leeds (U.K.)
J. Baló	University of Budapest (Hungary)
I. Banga	University of Budapest (Hungary)
Suzanne Bazin	Institut Pasteur, Garches (France)
R. S. Bear	Massachusetts Institute of Technology (U.S.A.)
Joane H. Bowes	The British Leather Manufacturers' Research Association (U.K.)
R. Consden	Canadian Red Cross Memorial Hospital (U.K.)
A. Delaunay	Institut Pasteur, Garches (France)
T. Gillman	University of Natal (Union of South Africa)
L. E. Glynn	Canadian Red Cross Memorial Hospital (U.K.)
W. Grassmann	Max-Planck-Institute for Protein and Leather Research, Munich (Germany)
J. Gross	Harvard University (U.S.A.)
K. H. Gustavson	Swedish Tanners' Research Institute, Stockholm (Sweden)
D. A. Hall	University of Leeds (U.K.)
Sylvia Fitton Jackson	King's College, London (U.K.)
D. S. Jackson	University of Manchester (U.K.)
K. Meyer	Columbia University College of Physicians and Surgeons (U.S.A.)
Helen Muir	British Postgraduate Medical School, London (U.K.)
A. Neuberger	St. Mary's Hospital Medical School, London (U.K.)
V. N. Orekhovitch	Academy of Medical Sciences of the U.S.S.R., Moscow (U.S.S.R.)
S. M. Partridge	Low Temperature Research Station Cambridge (U.K.)
R. Reed	University of Leeds (U.K.)

A. H. T. Robb-Smith	Oxford University (U.K.)
W. Schwarz	The Free University of Berlin (Germany)
O. Snellman	Uppsala University (Sweden)
B. Sylvén	Karolinska Sjukhuset, Stockholm (Sweden)
R. E. Tunbridge	University of Leeds (U.K.)
A. van den Hooff	University of Amsterdam (The Netherlands)
F. Verzár	University of Basle (Switzerland)

FOREWORD

The symposium on 'Connective Tissue' was held in London from July 22nd to 26th, 1956, under the chairmanship of Professor R. E. Tunbridge. The meeting followed the general pattern of previous multi-disciplinary symposia run by the Council. For the first time, however, scientists from Soviet Russia and Hungary accepted our invitation.

It is perhaps appropriate to mention that the C.I.O.M.S. groups some fifty international organizations devoted to the sciences basic to medicine and to the clinical branches. Its aim is to improve communication between disciplines and across national boundaries. Because of its constitution, the Council receives many suggestions for symposia; these come from the international organizations composing its membership, from national research councils or national medical societies and from individuals interested in the Council's work.

Among many topics the various aspects of the biology of connective tissue were suggested for the Council's 1956 symposium. After careful consideration, it was agreed that the meeting would centre on the biochemistry of connective tissue and on the correlations between biochemistry and structure.

The selection of participants was left to the chairman in consultation with the Council's Executive Committee. Every effort was made to make the meeting as representative as possible, both scientifically and geographically. From among the many research workers who, in various countries, are studying connective tissue, a difficult and somewhat arbitrary choice had to be made.

Official languages were English and French, but it soon appeared that English allowed communication between all participants. It is fitting to thank all those who willingly agreed to express themselves in a language which was not their own in order to help the meeting along. In this book, all papers are published in English with the exception of the one by Dr. Delaunay and Mlle Bazin but a summary in English follows the paper.

All discussions were immediately transcribed from magnetic recordings by Dr. Geoffrey Wood and Dr. Madeline Keech to whom thanks are due. The discussions are printed in précis form

at the end of each paper. Papers and discussions are to be taken together just as they were delivered and discussed. Much of the cut and thrust of debate has necessarily been lost for which we are sorry – but the irrelevant and the repetitious has been eliminated in order to present the reader with the substance of the discussion.

We hope that this monograph will be of use to all who study the biology of connective tissue.

CONTENTS

INTRODUCTION

W. T. Astbury

In nature's infinite book of secrecy
A little I can read.
Antony and Cleopatra, Act I, Scene 2.

When our Chairman kindly invited me to contribute a general introduction to this symposium and presented me at short notice with a formidable collection of summaries of papers to be contributed by the other participants, my first reaction, I confess, was to recall Sydney Smith's famous remark: 'I never read a book before reviewing it; I find it prejudices the mind.' Here now, I thought, was a beautifully analogous situation where to follow Smith's example was most strongly indicated. I have compromised though – and in the way in which I suppose I was expected to; that is to say, I have of course read the summaries, but this brief assessment of how we stand at the moment with regard to the connective tissue problem is for the most part only as I see it personally. Which means also that the bad parts are mostly mine too.

We would all agree, I believe, on the convenience of subdividing our subject, as has been done for the purposes of this symposium, under the headings: Cells, Ground Substance and Fibres; yet at the same time we all probably have a deeper tendency to think of the connective tissue system as a kind of unit – a combination that is to a first approximation self-contained and indivisible. We imagine the cells manufacturing a fundamental matrix, which then acts as site and precursor for the fibre complex that is the principal, and relatively stable, end-product of the system. And always, *ex hypothesi*, we search for common factors, common manifestations. The chief motive in fact of a meeting such as this must be, directly or indirectly, consciously or unconsciously, to inquire again what are these common features and to examine once more how far we have gone in their characterization.

Since it is still very much biogenesis and the primary synthetic steps which are the most difficult and mysterious, the procedure of least resistance in molecular biology generally, and in the connective tissue problem in particular, is to work mostly backwards from the

more permanent end-products – in this case the fibre complex of collagen, elastin, reticulin and anything else there may be, on which indeed the idea of connective tissue unity is just now most clearly focused. This feeling for a common plan underlying the fibrous end-products of the connective tissue system derives from the molecular level of X-ray diffraction analysis, which first brought about in pre-war days an astonishing simplification among the apparently enormous diversity of fibrous proteins. It was found that, in terms of polypeptide-chain types, there are in the main only two configurational schemes, one comprising the long-range elastic fibres of the keratin-myosin-epidermin-fibrinogen group (k-m-e-f group), and the other the inelastic fibres of the collagen group. The collagen group was named after its most familiar member, orthodox collagen of classical histology, but perhaps a little deceptively, because it transpired that one of the most impressive things about the group was its very wide range, including as it does numerous structures previously considered to have little or nothing to do with one another – for example, white connective tissue fibres, tendon and cartilage, the scales and fins (elastoidin) of fishes, the ichthyocol of swim-bladders, the cuticles of Annelid worms, the filaments ejected by the sea-cucumber, jelly-fish, to quote only a selection. They all (and gelatin too) give the same distinctive kind of large-angle X-ray fibre diagram, quite different from anything found with the k-m-e-f group, and this, the diffraction expression of the peculiar polypeptide-chain configuration they all have in common, still remains the only sure criterion yet discovered of their fundamental family relationship. Many of them give also the collagen small-angle X-ray diagram, corresponding in period (about 640 Å) to the bands seen in the electron microscope, but this is not an essential but is associated with the next stage of organization: the small-angle X-ray diagram is easily destroyed, and the bands seen in the electron microscope can also be altered or destroyed (there are none in any case in the fibrils of the earthworm's cuticle), but the large-angle X-ray diagram persists.

Here, beyond doubt, is one of the major designs of molecular phylogeny, comprehensive enough to take in the connective tissue fibres in its stride, so to speak – that is, potentially; for whether it does so actually, and completely, is not yet demonstrated. I myself feel that it is an eminently plausible view that the connective tissue combination should represent a sort of pocket of unity within the

greater unity; that, in other words, it should constitute a specialized subgroup of the collagen group something like the feather-keratin subgroup of the k-m-e-f group. After all, now that the reticulin fibres have been found to satisfy basic collagen-group requirements, only elastin remains to be accounted for, and even that too, these days, is at last beginning to show signs of conforming.

I think, therefore, (the odds in support of the suggestion being at least two to one), that I might very usefully devote much of the rest of this introduction to summarizing some of the findings and conclusions lately arrived at regarding that innermost question of the collagen group from which all else probably spreads out automatically. I refer to the constitution and curious polypeptide-chain configuration which gives rise to the characteristic X-ray diagram and which has offered such long resistance to attempts at elucidating it. This really is the central problem; for let me emphasize that the master plans of the fibrous proteins revealed by X-ray analysis are type-specifications, or themes, susceptible of many variations; and once we have grasped what the minimum demands of the collagen group are, we ought then to be able to proceed to correlate the various stages or ramifications, complete or incomplete, of subsequent building-up processes – 'procollagens' and 'tropocollagens' and concepts of that kind – with comparative ease, or at any rate much more confidently.

The new outlook in recent years on possible polypeptide-chain configurations is inspired, as everyone knows, by what may be called the helical interpretation of Pauling and Corey, according to which the unstretched α-form of the k-m-e-f group (and of various synthetic polypeptides), for example, is based on a helix comprising approximately 3.6 amino-acid residues per turn, each residue occupying a length in the direction of the fibre axis of about $1\frac{1}{2}$ Å. (This is only the bare bones of the k-m-e-f story; the complete explanation is an elaboration that has not yet been fully worked out.) In the β-form of the k-m-e-f group the polypeptide chains are pulled out almost straight, so that the average length of an amino-acid residue in the direction of the fibre axis is now approximately 3.33 Å, and the structure built up is very probably a certain one of the combinations of extended chains that Pauling and Corey have called 'pleated sheets'. We need not, here, consider the k-m-e-f group in any further detail, but I want to make the point that, of the two principal configurations I have just mentioned, the 3.6 α-helix (it is one of a

series of analogous helices) is held in that shape by hydrogen bonds between CO and NH groups all belonging to the same helix – they are all *intra-chain* bonds; while the β-pleated sheet is held together by hydrogen bonds linking CO and NH groups in adjacent chains – they are now all *inter-chain* bonds. What other possibilities are there that might be identified with the collagen configuration, which, as I have said, is so curiously distinct from either of these two? After the triumph of the α- and β-configurations it would seem not too difficult to advance thence on collagen and, with the aid of the elegant diffraction treatment given by Cochran, Crick and Vand (1952), to clear it up similarly, but as a matter of fact it has proved surprisingly obstinate. Ever since the early days when the collagen group was first recognized, we at Leeds have argued that the collagen chains are practically inextensible for special stereochemical reasons connected with the preponderance of proline and hydroxyproline residues; that actually they are *shorter* than in the β-configuration to the extent that the length per residue in the direction of the fibre axis has now fallen to as low as 2.86 Å; but what exactly was the nature of this constriction? That was always the question; and even with the advent of the new helical ideas, as applied by Pauling and Corey themselves, by Bear, and by Randall and co-workers, for instance, it still turned out far from easy to decide what particular form of helix or combination of helices best agreed with all the X-ray data (recently made more precise by improved diagrams at the hands of the Randall school) and supporting observations.

The break came eventually through papers by Ramachandran and Kartha (1954, 1955, 1956), Cowan and McGavin (1955), Cowan, McGavin and North (1955), Crick and Rich (1955), and Rich and Crick (1955). What it amounts to is roughly this, that Ramachandran and Kartha first proposed a structure along the desired lines, and it has seemingly been improved and rationalized, so to say, by the other authors in the light of X-ray analyses of poly-L-proline (Cowan and McGavin) and of the hitherto obscure crystallographic modification of polyglycine (Crick and Rich) that has been named polyglycine II (in polyglycine I the polypeptide chains are in an extended β-configuration). It was found that in poly-L-proline and polyglycine II the backbone configuration is the same – a helix with three residues per turn, each residue occupying a length of about 3.1 Å (though the six-fold system of

inter-chain CO. . .NH hydrogen bonds in polyglycine II is perforce absent from the polyproline chains of imino residues); and this striking result, on top of the famous meridional reflection at 2.86 Å and the well-known piece of chemical analytical information that one-third of collagen consists of glycine residues and at least another quarter of proline and hydroxyproline residues, then led irresistibly to the conclusion that the collagen structure is simply the polyglycine-polyproline scheme adapted to a constitution that is neither one thing nor the other yet includes quite a lot of both. It is based not on the straight trigonal helix but on a slight helical distortion thereof, a so-called 'coiled coil'.

One takes a triad of helices from the polyglycine-polyproline structure (there are two ways of doing this) and twists the three chains slowly round one another so that the number of residues per turn, with respect to the common axis, becomes $3\frac{1}{3}$. The period is 2·86 Å and corresponds to three turns. So at last our original supposition that 2.86 Å, the spacing of the meridional reflection so characteristic of the collagen diagram, measures the length per residue in the direction of the fibre axis, is justified.

The answer to the question I put a moment ago as to what other formal description, in contrast to the two appropriated to the α-helix and the β-pleated sheet of the k-m-e-f group, is available for the collagen group is that the collagen configuration is at heart (that is, before adaptation) a helix held in that shape by inter-chain bonds; which means that, formally again, it also falls after all into the same class as the β-configuration, which is geometrically a helix with two residues per turn. From an outside viewpoint, though, the eventual structure, that is after adaptation of the polyglycine-polyproline scheme to the requirements of the betwixt-and-between chemical constitution, could be described as belonging in a sense to the class of helices, e.g. the α-helix, held in shape by intra-chain bonds; for the inter-chain bonds linking the three initially trigonal helices are the intra-chain bonds of the compound major helix into which they are finally twisted.

The kinds of inter-chain bonds that hold the primary helical unit of the collagen structure in that shape, and indeed go further and result in the end in a triad of such helices becoming also twisted round one another to form a coiled coil, are still under debate, though obviously some must be CO. . .NH hydrogen bonds and others may well be hydrogen bonds of the type postulated by

Gustavson (our much-respected doyen of the Collagen Group!) as linking the hydroxyproline OH group with a CO group on an adjacent chain. Incidentally, we must beware of perhaps misapplying Gustavson's idea, which is a deduction from the properties of *collagens* – collagens of different hydroxyproline contents. It is concluded that the bond confers resistance to thermal shrinkage and one might expect, therefore, to solution; but gelatin is soluble in warm water yet has the same hydroxyproline content and gives the same kind of large-angle X-ray diagram as collagen – the diagram, that is, from which the coiled-coil structure has been derived; and the earthworm's cuticle gives the same kind of diagram too, though, as first shown by Singleton (1955) in our laboratory at Leeds, it has the highest known hydroxyproline content (equivalent to 14.6 per cent of the total nitrogen) combined with the very low thermal-shrinkage temperature of 32-33° C and begins to dissolve in water at about 40° C. Thus, of the two current crystallographic interpretations of Gustavson's bond: (*a*) that it links together the components of the triads, and (*b*) that it links the triads to one another, it would seem that the latter is the more plausible – without necessarily excluding the former entirely, nevertheless, because another, and contrasting, consideration not to forget is the fact that the normal large-angle diagram of collagen disappears during thermal contraction and is replaced by an 'amorphous' diagram.

Progress in sorting out the details and permissible variations within the collagen framework depends at the moment more on chemistry than on physics – this constant swinging of the research pendulum is a notable and characteristic feature of modern biomolecular studies – and keen investigations to that end are being carried out these days in more than one laboratory, investigations to determine not merely the proportions of the amino-acid residues but also their order. Already such findings as, for example, the common occurrence of the sequence -prolyl-hydroxyprolyl-glycyl- make it seem highly unlikely, because of insufficient amino residues, that the construction is uniform throughout, and it could be, therefore, that either the helices of a triad are not alike, or there are different kinds of triads or of still larger (filamentous) units, or all these things together – not to mention something else that is often a skeleton in the macromolecular X-ray cupboard: I mean the possibility that what is giving rise to the regular diffraction pattern represents only part of the structure under examination; for instance, more uniform and

crystallographically better-fitting sections of long chains that for the rest are not so adaptable, or limited groups of whole chains that are better crystallizable. Anyhow, it will be appreciated that there is plenty of scope (perhaps a little too much for present comfort) in what is now known of the molecular framework of the collagen group, including most if not all of the connective tissue fibres, to satisfy gradation and variability requirements. A great deal of difficult, painstaking work remains, to be sure, but I have the feeling that it really is all becoming more and more a question of detail than of principle. The outlines of the plan are now very nearly in focus, and I find that most inspiriting.

It follows that the main burden and responsibility of connective tissue research devolves more than ever on the procedures and results of *extraction*. The business of extraction and separation dominates biological chemistry and physics – it 'delivers the goods' – and here, in the needs of disentangling the connective tissue complex, we see a particularly fine illustration, in an exciting configurational setting too. Components are made available conforming in the first place to certain minimum structural specifications now partly recognizable, and these are then made use of, with such modifications as may be required and are permissible, in successive stages and paths of increasing organization, diversified maybe from time to time with degradational episodes also. The task is to complete the recognition of these components and their modifications and combinations by catching them on their way up or down and looking at them separately, and it is one of the most worthwhile in medical science.

And to put the aim again at its least complicated, most direct – never mind at this juncture whether too simple, too direct – it is to identify an ideal or limiting building-stone, say the tropocollagen of the F. O. Schmitt school or Orekhovitch's procollagen, or, not improbably, a precursor of both, incorporated in an ideal or limiting edifice, such as a regular aggregation of the X-ray analyst's triple coiled coils. The present incomplete and imperfect correlation between molecular form and chemical constitution is, however, symbolic and perhaps more representative of the actual state of affairs, in which the ideal plan, though governing in principle all the fibrous products, yet impresses itself to widely varying extents and, indeed, succeeds sometimes only so very indifferently as effectively to lose its identity.