Collagen

Proceedings of a Symposium sponsored by the

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Foreword

The Central Leather Research Institute has been actively engaged for a long time in research on the biological, chemical, physical, and technological aspects of collagen. It is fortunate to have as its neighbor the Department of Physics of the University of Madras, under Professor G. N. Ramachandran, who is very active in the structural field of collagen. The neighbor on the other side for the Institute is the Cancer Research Institute. A happy collaboration exists between these institutions.

In the past various organizations have sponsored symposia on subjects which included collagen either by virtue of its belonging to a particular class of substances such as glue or gelatin or because of its importance in biochemical or medical research. However, the need was felt for having a symposium which would consider all the various aspects of collagen research, viz., structural, biochemical, medical, physical, chemical, and technological, the object being to bring together scientists and technologists in this field along with those belonging to other disciplines of science and technology, so that knowledge in this sphere of human endeavor might be enriched by mutual contacts.

An organizing committee, therefore, was constituted by the Director of the Central Leather Research Institute, consisting of Professor G. N. Ramachandran, Chairman; Dr. N. Ramanathan, Convener; Drs. Y. Nayudamma, S. M. Bose, V. S. Padmanabhan, and V. Sasisekharan, Members; and Messrs. N. S. Mani, K. V. Karunakaran, and C. B. Nair, Members. The papers were arranged to be presented and discussed in three sections, viz. (1) Structural studies, (2) Medical and Biochemical studies, and (3) Physical, Chemical, and Technological studies. It was realized, of course, that on such a subject as collagen, a strict classification into divisions was not possible and was needed only for convenience of presentation.

The symposium was inauguruated by Professor M. S. Thacker, Director-General, Scientific and Industrial Research (India), and Secretary, Ministry of Scientific Research and Cultural Affairs, Govern-

ment of India, who stressed the importance of industrialists utilizing the results of research conducted in the laboratories. Dr. A. Lakshmanaswami Mudaliar, the distinguished Vice-Chancellor of the University of Madras, presided over the inaugural function. Dr. Mudaliar indicated the importance of research on collagen to various fields of human activity and, in particular, to the medical profession. Professor G. N. Ramachandran delivered the Conference Lecture which also served as an introduction to the Symposium. The three sessions, which followed, were presided over by Professors S. Ramaseshan, Indian Institute of Science, Bangalore; B. S. Gould, Massachusetts Institute of Technology, Cambridge; and P. M. Gallop, Albert Einstein College of Medicine, New York. Popular lectures on scientific research, in general, and on the science and technology of collagen, in particular, were delivered by Professors Gould and Gallop.

It is gratifying to record here the very willing response of the authors of the papers, spread over ten countries, who readily agreed to contribute to the symposium and participated in the discussions by correspondence. It is appropriate to extend our thanks to them and especially to Professors B. S. Gould and P. M. Gallop, who came from a long distance at the expenditure of considerable time and effort to actively participate in the Symposium.

Our thanks particularly are due to the publishers of this volume for their ready willingness to publish the proceedings of the Symposium and for their cooperation in many ways.

Y. NAYUDAMMA

Director
Central Leather Research Institute
Madras, India

Contributors

A. M. Accary, *Kodak Pathé Society, Vincennes, France*
B. K. Bachhawat, *Christian Medical College and Hospital, Vellore, India*
J. Baló, *Medical University, Budapest, Hungary*
I. Banga, *Medical University, Budapest, Hungary*
B. C. Basu, *Central Leather Research Institute, Madras, India*
S. M. Bose. *Central Leather Research Institute, Madras, India*
J. W. Czerkawski, *University of Leeds, Leeds, England*
C. Deasy, *University of Cincinnati, Cincinnati, Ohio, U. S. A.*
A. R. Downie, *Courtaulds Ltd. Research Laboratory, Maidenhead, Berks., England*
N. E. Dweltz, *University of Madras, Madras, India*
H. R. Elden, *Howard Hughes Medical Institute, Miami, Florida, U. S. A.*
M. J. Ellis, *Leather Industries Research Institute, Grahamstown, South Africa*
S. R. Evelyn, *Leather Industries Research Institute, Grahamstown, South Africa*
P. M. Gallop, *Albert Einstein College of Medicine, Yeshiva University, Bronx, New York, U. S. A.*
Bernard S. Gould, *Massachusetts Institute of Technology, Cambridge, Massachusetts, U. S. A.*
Wolfgang Grassmann, *Max-Planck-Institut für Eiweiss- und Lederforschung, München, Germany*
A. Guha, *Christian Medical College and Hospital, Vellore, India*
K. H. Gustavson, *Garverinaringens Forskningsinstitut, Stockholm, Sweden*
D. A. Hall, *University of Leeds, Leeds, England*
Helmut Hörmann, *Max-Planck-Institut für Eiweiss- und Lederforschung, München, Germany*

MAURICE L. HUGGINS, *Stanford Research Institute, Menlo Park, California, U. S. A.*

K. THOMAS JOSEPH, *Central Leather Research Institute, Madras, India*

V. R. KHANOLKAR, *Indian Cancer Research Centre, Bombay, India*

O. KRATKY, *Institut für physikalische Chemie der Universität Graz, Graz, Austria*

E. KUHNKE, *Physiologisches Institut der Universität, Bonn, Germany*

O. KUNINA, *U. S. S. R. Academy of Medical Sciences, Moscow, U. S. S. R.*

B. R. LAKSHMANAN, *University of Madras, Madras, India*

M. LAUER, *Institut für physikalische Chemie der Universität Graz, Graz, Austria*

GEORG MANNER, *Massachusetts Institute of Technology, Cambridge, Massachusetts, U. S. A.*

V. MAZUROV, *U. S. S. R. Academy of Medical Sciences, Moscow, U. S. S. R.*

Y. NAYUDAMMA, *Central Leather Research Institute, Madras, India*

TH. NEMETSCHEK, *Bergbau-Forschungs GmbH, Forschungsinstitut des Steinkohlenbergbauvereins, Essen, Germany*

ARNOLD NORDWIG, *Max-Planck-Institut für Eiweiss-und Lederforschung, München, Germany*

V. OREKHOVITCH, *U. S. S. R. Academy of Medical Sciences, Moscow, U. S. S. R.*

T. N. PATTABIRAMAN, *Christian Medical College and Hospital, Vellore, India*

J. POURADIER, *Kodak Pathé Society, Vincennes, France*

G. N. RAMACHANDRAN, *University of Madras, Madras, India*

L. K. RAMACHANDRAN, *University Biochemical Laboratory, Madras, India*

C. RAMAKRISHNAN, *University of Madras, Madras, India*

N. RAMANATHAN, *Central Research Leather Institute, Madras, India*

A. A. RANDALL, *Courtaulds Ltd. Research Laboratory, Maidenhead, Berks., England*

N. VENKATESWARA RAO, *Loyola College, Madras, India*

M. RATZENHOFER, *Institut für physikalische Chemie der Universität Graz, Graz, Austria*

R. REED, *The University, Leeds, United Kingdom*

V. SASISEKHARAN, *University of Madras, Madras, India*

H. SEIFERT, *Mineralogisch-Petrologisches Institut, Westfälische Wilhelms-Universitat Münster/Westf., Germany*

S. SEIFTER, *Albert Einstein College of Medicine, Yeshiva University, Bronx, New York, U. S. A.*

A. SEKORA, *Institut für physikalische Chemie der Universität Graz, Graz, Austria*

V. SHPIKITER, *U. S. S. R. Academy of Medical Sciences, Moscow, U. S. S. R.*

SATYAVATI M. SIRSAT, *Indian Cancer Research Centre, Bombay, India*

S. SRIRAMACHARI, *All India Institute of Mental Health, Bangalore, India*

G. STAINSBY, *The University, Leeds, United Kingdom*

R. L. SYKES, *Leather Industries Research Institute, Grahamstown, South Africa*

D. SZABÓ, *Medical University, Budapest, Hungary*

Y. T. THATHACHARI, *University of Madras, Madras, India*

S. VENKATARAMAN, *University of Madras, Madras, India*

LEE P. WITNAUER, *Eastern Regional Research Laboratory, Philadelphia, Pennsylvania, U. S. A.*

L. M. YEDDANAPALLI, *Loyola College, Madras, India*

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Structure of Fibrous Proteins and Polypeptides*

G. N. RAMACHANDRAN, *Department of Physics, University of Madras, Madras, India*

To the Father of Modern Indian Science—Professor C. V. Raman

1. INTRODUCTION

The purpose of this paper is mainly to provide a background for the papers presented from the author's laboratory on the structure of collagen and of feather keratin and on the stereochemical aspects of polypeptide structures. We shall attempt to give a bird's eye view of the present state of knowledge regarding the molecular structure of fibrous proteins and polypeptides. Also, during the course of the studies mentioned above, the author had an opportunity of becoming closely acquainted with various problems connected with the interpretation of the x-ray patterns of fiber structures. Some of these do not appear to have been fully dealt with before and, therefore, these aspects will also be discussed in a general way in this paper. Since detailed references to the literature are given in the other papers from this laboratory, they will be omitted here. However, a short bibliography of recent publications is appended to the paper.[1] It is hoped that this paper will serve as an introduction to structural studies for the non-specialist.

As is well known, proteins and polypeptides are high polymers, whose monomeric unit is the amino acid residue —CHR—CO—NH— (also called the amide residue or the peptide residue), in which the side group R at the α-carbon atom may be one of some twenty different kinds in proteins. In this paper, we shall use the term polypeptide to refer to those polymers of this type in which the R groups are alike and the number of residues is large, say more than 20. If

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more than one type of residue occurs, it will be called a mixed polypeptide. In these compounds, the number of residues which occurs in a single chain does not materially affect its properties, so long as it is not too small, for the chains often aggregate together to form fibers. On the other hand, there are polypeptides with a comparatively small number of residues, say 8 or 10, and as many as four or five different types, some of which are biologically very active, e.g., oxytocin or antibiotics like gramicidin.* These do not form fibrous structures, but form crystals, and their analogs in proteins are the crystalline proteins, such as insulin or the protein of tobacco mosaic virus, which have a specific sequence of amino acid residues and a definite number of these. We shall not have much to say about these, but will be dealing mostly with fibrous proteins and polypeptides.

A special feature of the molecular chains in polypeptides and proteins, as distinct from those which occur in some other high polymers, is that they do not contain branches, but form a single chain, which, in most cases, is an open chain.† It is, however, possible to have strong chemical links via —S—S— bridges between two different chains, or two residues in the same chain. Otherwise, the configuration of the chains is decided essentially by the secondary valence bonds which occur between various groups in the main chain and the side chains. In fact, the distinction between crystalline and fibrous proteins lies essentially in their physical state of aggregation rather than in their chemical nature. In the crystalline form, the "molecules" of the protein take up a specific folded configuration and these are then arranged in a lattice array. When denatured, this regularity is lost; in fact, the x-ray patterns of all denatured proteins appear very similar whether they were originally crystalline or fibrous. This is because the protein molecule is a long chain of peptide residues in either case and when the structure does not have a specific configuration, the chains assume random configurations and so there is no evidence of the original regularity in the denatured state. It is,

* It appears desirable to use the term "peptide" to describe such a compound with a definite comparatively small molecular weight and to use the term "polypeptide" to describe polymerization products of amino acids with indefinite molecular weight.

† It has been reported that in some proteins like collagen and feather keratin, no end groups have been detected and conclusions have sometimes been made from this that the peptide chain is a closed one in these proteins. The experimental evidence in these cases would appear to require re-evaluation.

therefore, not at all surprising that the biological specificity of crystalline proteins is lost on denaturation. On the other hand, no phenomenon like denaturation has been reported with small biologically active peptides, which is obviously due to the fact that in such small molecules the range of stereochemically permitted configurations is rather limited and anything like a random configuration cannot occur.

Coming now to fibrous proteins, we shall consider the different types of structures observed in them in the next section and then discuss the corresponding structures observed in polypeptides in Section 3. In Section 4, we shall discuss the stereochemical aspects of these structures. Problems connected with the interpretation of x-ray diffraction patterns will be considered in Section 5. In order to make the arguments intelligible to the non-specialist, most of the details will be omitted and only the principal features of each structure will be discussed. A general fact to be remembered is that protein structures are essentially determined by the occurrence of hydrogen bonds. Of these, again, it is the hydrogen bond which links the NH's in the backbone of various residues to the carbonyl oxygens of other residues that fix the nature of the "fold" or "coiling" or any such specific configuration of the polypeptide chain which occurs in a structure.

2. TYPES OF STRUCTURES OBSERVED IN FIBROUS PROTEINS

Even before polypeptides were prepared artificially and studied, the structures found with fibrous proteins had been classified into distinct types, mainly on the basis of the x-ray diffraction patterns given by them. The pioneering work in this field is due to Astbury who classified the naturally occurring fibrous proteins into two classes (Table I): (*a*) the K.M.E.F. (keratin-myosin-epidermin-fibrinogen) group, and (*b*) the Collagen group. All proteins of the former group gave, in their natural state, x-ray patterns very similar to one another (Fig. 1*a*), while collagen from different sources always gave the same pattern (Fig. 2*a*). Feather keratin gives a very rich pattern (Fig. 2*c*) quite different from ordinary keratin of wool, while elastin (Fig. 2*g*) gives a rather poor pattern. Of these, Astbury was inclined to classify the latter in the same group as collagen, while he considered

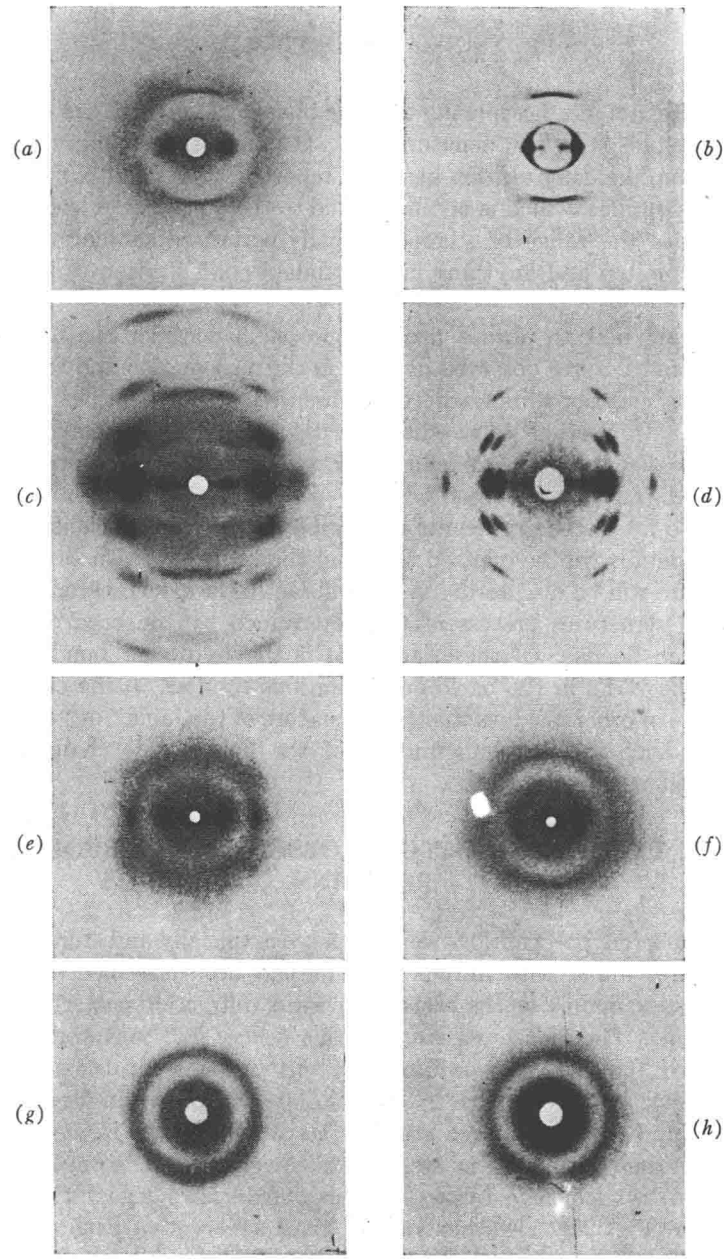

Fig. 1. X-ray diffraction patterns of proteins. (*a*) α-Keratin. (*b*) Copolymer of phenylalanine and leucine, α form. (*c*) Silk (*Bombyx mori*), extended β structure. (*d*) Poly-L-alanine, β form. (*e*) β-Keratin. (*f*) Supercontracted. (*g*) Native (crystalline) serum mercaptalbumin. (*h*) Same, denatured. Photographs (*e*) and (*f*) are reproduced from Ref. 1*d* and photographs (*b*) and (*d*) from Ref. 1*c*.