THEORY AND INTERPRETATION OF LOW-ANGLE

X-RAY DIFFRACTION PATTERNS OF COLLAGEN.

by

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I. (1) Scope of these researches.

It has for long been known that the fibrous protein collagen produces a distinctive X-ray diffraction pattern (e.g. Harwood and Connell, 1934) which is very suitable as a specific test for whether a given protein is collagenous in nature or not. Only much later, however, was it discovered that the X-ray diffraction pattern of collagen contained reflections which had hitherto been unnoticed because they are at such low angles that normal diffraction equipment was unable to resolve them from each other and from the direct X-ray beam. (Clark, Parker, Scheel and Warren, 1939). This "small-angle" diffraction pattern proved to be a set of orders, twenty to thirty or more in number, of a fundamental spacing of about 640 Å, in dry collagen and about 670 Å, in the wet material (Sear, 1942); and at about the same time the basic periodic structure of collagen was photographed directly with the electron microscope (Hall, Jukes and Schmidt, 1942).

The subject of these researches is predominantly that of the small-angle pattern, although wide-angle X-ray diffraction patterns of collagen specimens have been taken for various purposes connected with the main thread of this work. It is
shown by a theoretical argument that these components of the complete
diffraction may be considered and interpreted separately.

A selection of low-angle diffraction patterns from
collagens and collagen-like materials is reproduced in Plate (1.1)
taken from a review article (Bear, 1952). Patterns like those of
figures 11 and 15 of Plate (1.1), which are typical of wet collagen,
are adequately treated by reference to a model of the collagen fibril
known as the smooth cylinder model (Bear and Bolduan, 1950). With
these patterns the major interest centres upon the relative intensi-
ties of the diffraction orders, which are related to the periodic
distribution of electron density along the collagen fibril. By a
detailed treatment of these relative intensities in the low-angle
patterns of native collagen, and collagen which has been treated with
reagents ('stains') which result in the incorporation of heavy atoms
in the structure, it is shown that much information of a unique type
may be obtained regarding the linear density distribution of the
fibril, and its chemical structure.

However, patterns like those of figures 12, 13, 14 and 16
of Plate (1.1) pose greater difficulties; they show the phenomenon
called 'fanning'; that is, the widths of the diffraction maxima tend
to increase almost linearly with the index of diffraction, and even
show, among other features, a tendency for off-meridian-axis
diffraction maxima to occur. This thesis shows that the currently
accepted theory of the fraying effect (Bear, 1951) is not satisfactory. A new, simpler and completely different theory of the phenomenon is presented which explains how the various features of the observed patterns arise.

The state of hydration of the collagen has been shown to have a remarkable affect upon the intensities of the small-angle diffraction pattern orders, whence it is deduced that the density functions of vacuo dried native collagen and of collagen under the conditions prevailing in the electron microscope, are not equivalent, contrary to the conclusions of Burge and Randall (1956). The new low-angle diffraction patterns which arise from highly dehydrated collagen have been interpreted by means of their Patterson functions. New information as to the structure of collagen and its behaviour when heated in vacuo has been thereby derived.

I. (2) Structure of collagen according to present theory.

There has been much dispute over the structure of collagen and a number of conflicting theories have been put forward to describe its various aspects. Fortunately, however, the arguments of this thesis may take their departure from ideas of the structure which are in general unexceptionable as the theory of collagen stands at present.