Report on the thesis entitled "Studies on Collagen" submitted by G. K. Ambady for the Degree of Master of Science

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Among the fibrous proteins, collagen, which is the chief component of the connective tissues, is of great biological interest, and its structure has therefore formed the subject of intensive study by x-ray crystallographers. Among the major steps in the advance towards the elucidation of its structure may be mentioned the following.

(1) The wide angle x-ray diffraction pattern, which is presumably due to the sub-unit of the collagen molecule, is found to be practically independent of the source from which the specimen of collagen has been obtained. Apart from demonstrating the distinctiveness of this unit, this observation also implies a certain chemical stability of this unit. Since the wide angle diffraction pattern can be studied conveniently with the usual cameras, this was naturally the earliest to be studied.

(2) The occurrence of a long period of about 640A along the length of the fibre, has been observed under the electron microscope, and has also been verified in x-ray diffraction. Unlike the spacings in the sub-unit which produce the wide angle pattern, and which remain constant, the long spacing is variable and sensitive to various factors. Since the angle of diffraction corresponding to this long spacing is about 8 minutes of arc, it requires a specially large camera for its study, and a long collimating slit system for the incident x-rays. Such a large camera naturally requires to be evacuated in order to minimize the absorption of the x-rays in their passage through the camera. Even so one has to use Geiger Muller counters for studying the diffraction.

(3) The third among the important steps was the theoretical realization that in order to be able to build these long cells and to endow them with the observed symmetry elements, the small sub-units referred to, which have themselves no element of symmetry, cannot be arranged linearly, and there should be a rotation associated with the translation along the axis. A repetition of this operation would obviously lead to a helical structure. Indeed several alternative helical structures have been proposed.

(4) From various considerations the amide group could reasonably be regarded as planar in its structure.

(5) A side observation, namely that when treated with alkaline salts the wide angle pattern shows several new spots, which originally were attributed to the formation of an additive compound, has since become quite significant. The significance arises from a very convincing demonstration made in the present thesis that the new spots can definitely be identified as due to oriented crystal deposits, whose orientations are determined by the collagen fibre lattice, but which do not form an integral part of the fibre structure, as would be implied by the formation of an additive compound. The candidate has done extensive studies in collagen treated with various salts in alkaline media and he finds many of them get deposited crystallographically in definite orientations. The candidate gives a long list of about a dozen crystals that are deposited this way, and the extra diffraction spots that appear in wide angle diffraction of such treated collagen are shown to be identical with those of the respective single crystals suitably oriented. Indeed the orientation observed gives a clue to the occurence of a diffraction maximum corresponding to a spacing of about 28 to 30 A^{o}. This must obviously be a repeat pattern in collagen fibre itself. It further enables the candidate to obtain the precise magnitude of this spacing

spacing since this distance must be very close to an integral multiple of the lattice spacing of the deposited crystal in this direction.

The precise determination of this spacing has further implications. From extensive studies made on stretched collagens, the candidate is able to conclude that the repeat pattern corresponds to a helix with ten residues in three turns, that is, making three full turns during a translation of about 29.4 A°, which covers ten residues. A further consideration shows that the structure is not that of a simple helix but resembles more a coiled coil. The major helix has a diameter of about 5 A°, and is wound in a direction opposite to that of the minor helix.

The wide angle patterns of both wet and dry collagens have also been studied by the candidate extensively, specifically with the view of locating the water molecules, which he finds sandwiched between the collagen rods.

The thesis is a useful contribution to a very difficult subject, and irrespective of the ultimate validity of the details of the structure proposed for collagen, it is an important step in the analysis The candidate shows commendable critical judgment in his handling of the extensive and considerably divergent earlier views on the subject, and in the discussion of his own extensive data. The thesis, in my opinion, is "highly commendable".