XV-65. MOLECULAR STRUCTURE OF COLLAGEN. By W. Traub and A. Yonath, Dept. of Chemistry, and D. M. Segal, Depts. of Biophysics and Chemistry, Weizmann Institute of Science, Rehovot, Israel.

Though it has been widely accepted that collagen has a three-stranded helical structure, controversy concerning the detailed conformation and the amount and mode of interchain hydrogen bonding has continued for over a decade. In recent years several polytripeptide models for collagen have been synthesised. The X-ray diffraction patterns of some of these indicate that they have molecular structures closely similar to that of collagen.

A detailed structure analysis of the polytripeptide $(Gly\text{-}Pro\text{-}Pro)_n$ has been made by systematically computing possible conformations and testing these in terms of intramolecular and intermolecular van der Waals distances and agreement between calculated and observed X-ray intensities. These criteria have led to a unique conformation which resembles the collagen II model in its mode of interchain hydrogen bonding.

Several other polymers of the form $(Gly\text{-}Pro\text{-}X)_n$ appear to have this same conformation, whereas two polytripeptides with a $(Gly\text{-}X\text{-}Pro)_n$ sequence, $(Gly\text{-}Gly\text{-}Pro)_n$ and $(Gly\text{-}Ala\text{-}Pro)_n$, have been found to form structures which are not three-stranded. However, four polyhexapeptides, incorporating a variety of tripeptide sequences, have all been found to show collagen-like X-ray patterns. These polymers include $(Gly\text{-}Ala\text{-}Pro\text{-}Gly\text{-}Pro\text{-}Pro)_n$, $(Gly\text{-}Pro\text{-}Ala\text{-}Gly\text{-}Pro\text{-}Pro)_n$, $(Gly\text{-}Ala\text{-}Pro\text{-}Gly\text{-}Pro\text{-}Ala)_n$ and $(Gly\text{-}Ala\text{-}Ala\text{-}Gly\text{-}Pro\text{-}Pro)_n$. Detailed conformational analyses have shown that all four have the same mode of hydrogen bonding as $(Gly\text{-}Pro\text{-}Pro)_n$ and three-stranded conformations closely similar to that found for this polymer. It seems very probable that collagen itself has a conformation of the same type as has been found for this variety of model compounds, and, indeed, the intensities of the collagen X-ray pattern can be satisfactorily accounted for on this basis.

XV-66. TECHNIQUES FOR STUDYING LIVING SMOOTH MUSCLES. By J. Lowy and P.J. Vibert, Biophysics Institute, Aarhus University, Denmark, and A. Elliott, Biophysics Department, King's College, London, G.B.

New combinations of apparatus have been used to study the structure and organisation of filaments in living smooth muscles. The X-ray source was an Elliott-Automation GX3 fine-focus rotating anode generator, using a nominal focal size of 1 mm x 0.1 mm. A focal area of this size on the copper target may be loaded with about 5 times the power of a stationary anode.

The X-ray camera was a modified version of the toroidal focusing camera devised by A. Elliott. (J.Sci.Instr. 42,312.1965). The interfocal distance was increased to 60 cm, and the beam aperture stopped down to two opposed quadrants of annulus. With a specimen to film distance of 12 cm, first order resolution of more than 200Å was obtained along one axis. The beam cross-section at the specimen position was about 1.5 mm. Provision was made for independant evacuation of source-to-specimen and specimen-to-film regions. This camera had an effective speed about 5 times greater than a conventional Franks camera, with uncoated 6 cm mirrors, used at the same resolution and specimen to film distance. (Franks.Brit.J.Appl. Phys. 9,349.1958. G.F. Elliott & Worthington. J.Ultras.Res. 9,166.1963). Our camera is slower, and has less first order resolution, than either double crystal monochromator or mirror/monochromator cameras. However, the chief disadvantage of the latter is the large beam cross-section at the specimen. (H.E. Huxley and W. Brown. J.Mol.Biol. 30,383.1967). This may be up to 5 mm in one direction, and only very rarely can specimens be obtained that possess good order over such distances.

The physiological state of the live muscles mounted on the X-ray camera could be continuously monitored through connections to a tension transducer and UV recorder. Both electrical and drug stimulation were possible.

Diffraction patterns with maxima in the region 150-50Å from the live resting anterior byssus retractor muscle of the mussel Mytilus edulis can be obtained in about one hour. The actin helix was found to have an axial near-repeat of 365±10Å, and the actin-containing filaments have a liquid-like organisation, with regions of limited three-dimensional order. The pattern remains essentially unchanged when the X-ray exposure is carried out during isometric tonic contraction produced by stimulation with acetylcholine.

There are good prospects for further development of these methods for studies of living molluscan smooth muscles during phasic contraction, and of living mammalian smooth muscles in various states.

XV-67. THE STRUCTURE OF NUCLEOHISTONE. By J. F. Pardon and B. M. Richards, Searle Research Laboratories, High Wycombe, England, and M. H. F. Wilkins, M.R.C., Biophysics Unit, Kings College, University of London.

In most cells of higher organisms DNA is complexed with basic histone proteins, the latter may be acting as genetic repressors. The X-ray diffraction patterns from fibre specimens of native nucleohistone include a series of low angle diffraction rings arising from a structure with overall dimensions greater than either the DNA double helix or the individual histone molecules.

A super-helix model has been proposed to explain the diffraction pattern. Fourier transform calculations have been made for single helices and in order to explain the variation of diffraction with concentration of nucleohistone various possible conformations for the packing of helical molecules have been investigated.

Experimental studies in which:-

(a) Fibres of nucleohistone have been stretched.
(b) Histone molecules have been removed by salt dissociation.
(c) Nucleohistone has been thermally denatured.

provide evidence for an extendable structure for nucleohistone.

Complexes of DNA with fractionated histone have been formed to determine whether a particular histone fraction constrains the DNA double-helix into a super-helix. It is hoped that the diffraction patterns obtained from "reconstituted nucleohistone" will contain more information than previous patterns from native material.