STUDIES BY NEGATIVE STAINING ON THE STRUCTURE OF COLLAGEN FIBRILS IN NORMAL AND LATHYRITIC RATS

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Summary. Electron microscope studies on collagen from rat-tail tendon using the negative staining technique indicate the presence of fine filaments 15--30 Å in diameter within the fibres. The banding of the fibrils was only slightly affected by aminoacetylnitrile bisulphate, but an increased amount of fine fibrous material was present in preparations from experimental animals. It is suggested that this material represents a soluble form of collagen which is known to predominate after administration of the drug. Despite the fact that this would be a chemically abnormal collagen, its structure by electron microscopy corresponds remarkably well with the form suggested from X-ray diffraction and physico-chemical studies on normal soluble collagen.

TROMANS, HORNE, GRESHAM and BAILEY (1963) reported studies made on collagen from tendon of normal chicken and adult rat-tail tendon by the negative staining technique. Filaments 15--20 Å in diameter were recorded within the fibrils which were thought to correspond to the collagen macromolecule. Previous studies by X-ray diffraction suggested that the macromolecule is a coiled-coil structure with a diameter approximately 11--15 Å across. RICH and CRICK (1961) have described in their review a number of models which may fit this data. The general view from physico-chemical studies (BOEDTKER and DOTY, 1956, DOTY and NISHARA, 1958, HALL and DOTY, 1958, HODGE and SCHMITT, 1961) is that the macromolecule is probably structurally polarised and of length approximately 2800 Å with a mean diameter of 14 Å, although this refers to measurements of collagen in solution.

Salts of amino-acetyl-nitrile (A. A. N.) or amino-proprio-nitrile (A. P. N.) are known to cause changes in the state of aggregation of collagen, giving rise to the lathyritic syndrome. The earlier work using the lathyrus factor (or aminopropionitrile fumerate) has been summarised by LEVENE and GROSS (1959). No increase was found in the amount of collagen present but the amount that could be extracted in cold normal sodium chloride from skin, bone and the aorta increased dramatically. BORLE, KARNOVSKY and NICHOLS (1959) suggested that there were changes in the water and ionic content and later that the sulphated mucopolisaccharide components also appeared to be affected (KARNOVSKY, 1959, KARNOVSKY and KARNOVSKY, 1961). However, the binding of the lathyrogen itself did not appear to affect the extractability of the collagen (ORLOFF and GROSS, 1963).

Recent work by GROSS (1963) would indicate that there is an intermolecular defect of collagen in experimental lathyrism. He found that fibrils formed in vitro from extracted collagen fail to become insoluble with increasing time.

Some electron microscopy of the lathyritic collagen fibrils has been published by VAN DEN HOOF, LEVENE and GROSS (1959) in which they reported an increased variation in the fibril diameter. In both natural and extracted collagen from nor-

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mal animals the diameter of the fibrils was usually about 500 Å with a scatter of about ±200 Å. Measurements taken from lathyritic animal preparations varied from 100 Å to about 1,000 Å with nearly uniform distribution throughout the major part of the range. The resolution of the published pictures, however, was too low to show changes in the macromolecular aggregation. It is the purpose of this paper to describe studies made at high resolution, using negative staining techniques, undertaken in an attempt to find changes at the macromolecular level in the lathyritic collagen.

**Material and Methods**

Two days after weaning, young rats were given a solution of 0.1% amino-acetyl-nitrile bisulphate to drink in place of the usual water. Food was given in powdered form since in the later stages the connective tissue of the teeth degenerates to such an extent that hard biting becomes impossible and the animals would starve. Experimental animals were kept on the drug for about 10—14 days until they began to refuse to eat. At this stage they had pigmented fur patches and showed little or no activity in response to handling. Control animals were prepared at the same time and body weight records were taken for comparison. The animals were killed with a blow to the base of the skull and pieces of small intestine were removed and fixed in osmium tetroxide; the rat-tail tendon was then removed and transferred to phosphate buffer (pH 5.8) chopped with a pair of fine scissors and homogenised in the phosphate buffer. Each preparation was then dialysed against a solution of 1% ammonium acetate for 24 hours at about 2° C, (basically the method used by Tromans et al., 1963). One part of the sample was then mixed with an equal volume of 2% potassium phosphotungstate (pH 6.2) and the remaining part with 2% dodecaphosphotungstic acid. Electron microscope specimens were prepared by depositing the material onto carbon coated E. M. grids using a platinum wire loop. The specimens were examined in a Siemens Elmiskop lb electron microscope at magnifications up to 80,000 ×. The results obtained from intestinal preparations will be described in a subsequent paper.

**Results**

Experimental animals fed 1% A. A. N.- bisulphate gained about 2 g in body weight during the experimental period but the control animals gained between 11 and 32 g. Average gain of the control animals was 16 g.

![Electron micrograph of negatively stained normal collagen from rat-tail tendon. The fibril can be subdivided into two bands, A and B with prominent light cross bands C. The repeating unit along the fibril is about 650 Å. Magnification 200,000 ×](image)