THE FIBRILLAR ORGANIZATION OF COLLAGEN IN CONNECTIVE TISSUE

A thesis presented in partial fulfilment
of the requirements for the degree of
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DEDICATION

This thesis is dedicated to my wife - Wendy

and our children

Michael, Kim, Kirsten and Hadley.

ABSTRACT

Although certain aspects of connective tissue structure have been studied in considerable detail, comparatively little effort has been devoted to studying one of the largest structural units present in most tissues - the collagen fibril. In this thesis electron microscope observations have been made on the transverse dimensions of fibrils from tissues as diverse as cornea, skin and tendon. Collagen fibril diameter distributions have been measured for such tissues from a wide range of animals - predominantly mammals, but also fish, amphibians, reptiles and birds - at varying stages of development. These data have allowed the growth of collagen fibrils to be studied quantitatively and their size distributions to be related to their mechanical attributes. Diseased tissues or tissues containing anomalous fibril diameter distributions have also been studied and, where possible, the data have been related to the altered mechanical properties of the tissue and to its mode of growth and development. In a coordinated study with other research workers, the content of the individual glycosaminoglycans in a tissue have been shown to be related to the mass-average diameters of the collagen fibrils in those tissues. These results provide a basis for understanding the feedback mechanism by which fibril size distributions may be modified in line with changing mechanical needs and indicate the fundamental steps in the growth and development of fibrils.

In addition to these studies, two other specific problems were addressed. In the first, the ultrastructure of a specialized connective tissue - the cornea - was studied in detail. By maintaining precise experimental protocols and measurement procedures it was shown, contrary to the previous data of others, that the

collagen fibrils in mammals, birds, reptiles, amphibians and cartilaginous fish were similar to one another but significantly different to the corneal stromal fibrils of the bony fish. Further studies, which indicated that the fibrils were constant in diameter across the width of the stroma, clarified previous results which had indicated a gradual change in diameter with varying depth in the stroma. An age-related study of fibril diameters in the cornea was also undertaken. The second problem investigated was the degree of shrinkage introduced during the preparative procedures for electron microscopy. In collaborative studies with others, X-ray and electron microscope observations were made on the same tissue in hydrated and dehydrated states respectively. Analyses of these data indicated that significant lateral shrinkage does indeed occur in fibrils from foetal or immature tissues as well as in mature tissues containing only small diameter fibrils. Throughout the thesis possible sources of artefact introduced by the technique of electron microscopy have been considered and the data interpreted conservatively.

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In my earlier years at DSIR Keith Williamson introduced me to, and gave me sound guidance in, the principles and techniques of electron microscopy. Subsequently my Director, Ray Bailey, gave me the encouragement and provided the impetus for me to embark on this present course of study.

Throughout this thesis the experimental results obtained by electron microscopy have, where possible, been related to biochemical and X-ray diffraction data kindly made available to me by Barbara Brodsky, Eric Eikenberry, Michael Flint, Gerry Gillard and Isabel Williams. Gary Thomas and Bob Fletcher supplied me with histogram plotting and population deconvolution programs and together provided me with oft-needed statistical advice. Doug Hopcroft has been responsible for the excellent maintenance of the electron microscopes and Ray Bennett printed the micrographs. I was assisted by many friends in typing the manuscript but it was June Tipoki who bore the lion's share of this chore.

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