



Bar = 20  $\mu$ m.

A, Clark's fixative, Feulgen stain. Low background and bright nuclei, but loss of regular matrix and nuclear morphology.

B, NBF fixative, Feulgen stain, transverse section. Nuclei discernible but background is moderate. In rotation it was apparent that most of the nuclei were truncated by the top and bottom slices of the confocal stack.

C, as for B but in longitudinal section. Nuclei discernible, background moderate. Matrix and nuclear morphology maintained. Nuclei contained entirely within confocal stack.

D, Clark's fixative, Feulgen stain, transverse section. Tendon matrix brightly stained, nuclei impossible to discern.

E, NBF fixative, Feulgen stain, longitudinal section. Tendon matrix brightly stained, nuclei impossible to discern. Tendon matrix well maintained.

F, PFA fixative, propidium iodide stain, RNase included. Nuclei clearly demarcated from dark background, arranged in columns. Most nuclei contained entirely within confocal stack. This was the histochemical protocol used for the experimental work.

**Figure 2.1** Histochemical Method Development