6.1 Overall Conclusion

The cytoskeleton comprises a small proportion of the total protein in the lens, but is thought to be important in the development and maintenance of transparency. Calpain plays a role in cataract in several species (Biswas et al., 2005; Robertson et al., 2005; Inomata et al., 2002), however calpain-like cleavage sites have not been detected in human lens crystallins in vivo (Nakajima et al., 2006; Ma et al., 2005). If calpain is important in human cataract, proteolysis of proteins other than crystallin must be occurring. The cytoskeletal proteins are known calpain targets in the lens, and proteolysis of cytoskeletal proteins may play an important role in the development of cataract. The ovine lens has been used in a model for cataract development, and there is little to be found in the literature regarding cytoskeletal proteins in lenses from this species.

**Hypothesis i:** Inhibition of calpain with novel inhibitors will result in the absence of proteolytic products associated with calpain.

The research presented in Section 3 shows clearly that the cytoskeletal profile changes when lens extracts were incubated in the presence of calpain and calcium compared to extracts incubated in the presence of calpain and calcium plus inhibitor. Novel calpain inhibitors were successful in preventing the appearance of calpain proteolysis products for spectrin, vimentin and filensin, with the novel inhibitor Cat0059 the most potent of the novel calpain inhibitors investigated, inhibiting calpain-induced proteolysis at 100 nM.

**Hypothesis ii:** Lens cytoskeletal proteins in the ovine lens will display a characteristic distribution for that selected protein, and will have a similar distribution to that seen in other species.

Research presented has shown that for a selection of cytoskeletal proteins in the ovine lens there exists a characteristic pattern of expression that closely resembles that seen in other species (Section 5). Specifically, actin was found to display preferential distribution in the short sides of the fibre cells in the cortex of the lens but was absent in the nucleus, while
spectrin was found in the cortex and nucleus associated with the fibre cell membrane. Filensin was observed in the outer cortex of lens sections associated with the fibre cell membrane and cytoplasm, however the pattern of localisation was indistinct due to the abundance of filensin products in the lens. Vimentin displayed both a membrane and cytoplasmic association in the outer cortex that gradually diminished toward the lens nucleus, with membrane vimentin found only in the deeper regions of the cortex and nucleus.

Taken together, the evidence presented in this thesis shows the cytoskeletal proteins as crucial elements of the lens by way of their pervasive presence (as seen in lens sections presented in Section 5). This, coupled with evidence that lens cytoskeletal proteins are sensitive to calpain-induced proteolysis and novel calpain inhibitors inhibit this proteolysis (Section 4), suggests that these proteins may be useful targets in cataract prevention. With research suggesting calpain-induced proteolysis is crucial in the pathology of cataract (Cuerrier et al., 2006; Robertson et al., 2005; Shearer et al., 1999), the cytoskeletal proteins represent susceptible targets for that proteolysis in the lens, and inhibition of proteolysis of lens cytoskeletal proteins as has been achieved in the current study supports the approach of inhibiting calpain activity to prevent cataract development.

6.2 Future Directions

Building upon the work in this thesis, the obvious way forward would be to carry out similar work focusing on the development and characteristics of the ovine cataract, as was originally proposed. Of particular interest would be a comparison of the USF protein profile of normal and cataract lenses using the methods applied in Section 3 in this investigation. This would allow for the approximate determination of the degree of proteolysis of lens cytoskeletal proteins in the cataract lens, and this coupled with the application of cataract inhibitors directly to cataract sheep eyes, comparing the protein profile of lens USF extracts between these eyes and cataract eyes would give an indication of the applicability of these inhibitors as a treatment for cataract.

Also of interest for future investigations would be following the development of the lens during growth and cataract by immunohistochemistry. In particular, the role of calpain in normal lens development and fibre differentiation, as well as the role of cytoskeletal proteolysis in the process of cataractogenesis. The appearance of cataract damage in early
cataract lenses (see fig. 3.6.1) at the approximate boundary in the lens between the differentiating outer-fibre cells and the more mature, differentiated fibre cells might indicate the involvement of aberrant activity of protease(s) associated with normal lens fibre cell differentiation in the early stages of cataract. Immunohistochemical characterisation and localisation of calpain, similar to that completed by Ma et al. (2001), and cytoskeletal proteins in normal and cataract lenses may offer some insight into these processes in the ovine lens.
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