Post-mortem water uptake by sheep lenses left in situ

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**Purpose:** Human lenses are generally obtained from eye bank eyes, which have been stored in the cold for extended periods. It is not known what effect the storage has on the lens. In this study, we examine the effects of post mortem time on the weights of sheep lenses left in the eye.

**Methods:** Lenses were removed from 299 ex vivo sheep eyes (200-day-old), which had been kept on ice for various times up to 76 h. Wet and dry weights were then determined. In addition, wet and dry weights were obtained from 147 414-day-old and 149 660-day-old lenses removed within one hour of death.

**Results:** After about 30 h in the eye, lens wet weights started to increase. By 76 h, the water content had increased by 25%.

**Conclusions:** Uptake of water by lenses while stored in the eye at low temperature can significantly alter lens properties. It is suggested that caution is needed when interpreting data obtained with lenses from eye bank eyes unless it can be demonstrated that there has been no water uptake.

Studies of the biometric, metabolic, optical, and physical properties of ex vivo human lenses have provided useful information on the growth and ageing of the lens, which is needed for understanding the development of presbyopia and cataract. In most such studies, it is generally assumed that the properties of the ex vivo lens closely reflect those in vivo. This is not necessarily correct.

Data collected from ex vivo lenses tends to be highly variable and this variability is usually attributed to individual differences. However, it may also reflect post mortem changes since lenses are often not removed from the eye until several days following death. There does not appear to be any specific information available but anecdotal evidence, some from as early as the nineteenth century, indicates that lenses will take up water if left in the post mortem eye [1,2]. However, this effect has not been documented.

As part of a recent study on sheep growth, it was observed that lens wet weights for animals of the same age appeared to be highly variable. Since detailed information was available on the collection and storage of the lenses and eyes, we were able to examine the effect of post-mortem storage time on lens weights. In this report, we show that lens weight increases with increasing time in the post mortem eye.

**METHODS**

Eyes were obtained from genetically similar Merino x Poll Dorset cross sheep, bred as part of the Australian Sheep CRC resource flock. The ages of the sheep were accurately known since they had been produced through artificial insemination and were born within the space of 8 days. Eyes were collected, over a 3 day period, from 150 sheep aged 200±4 days and were immediately chilled on ice. The time of death was noted, as was the time the eyes were removed. The eyes were transported to the laboratory at the end of the second collection day and remained on ice until the lenses were removed, up to 2 days later. Lenses were weighed within 2 min of removal from the eye and placed in 5% formalin for 2 weeks. The fixed lenses were then dried at 80 °C until constant weight was achieved, usually around 4 days. Lenses from another 147 sheep, aged 414±4 days, were removed and weighed within 1 h of death, then dried. Eyes were also collected from 149 sheep aged 660±4 days. For these, the lens was removed from one eye within 1 h and weighed. The other whole eye was placed in 5% formalin and the lenses were removed after 2 weeks and dried, as before.

**RESULTS & DISCUSSION**

Average lens wet and dry weights from the 200-day-old eyes, collected on two different days, are presented in Table 1. It may be seen that the dry weights of the two sets of lenses were identical, as would be expected for animals of the same age and genotype. However, the lenses stored in the eye for 2-3 days had significantly higher average wet weights and lower dry weight/wet weight ratios than those stored for 1-2 days. From the recorded times of eye and lens removal, it was possible to determine the time for which the lenses had been left in the post-mortem eye at 0 °C. This varied from 32-76 h.

It was not possible to obtain data for lenses of the same age, collected immediately after death. However, the dry weight/wet weight ratios, 0.339 and 0.343, respectively, were available for 414 and 660 day old lenses, collected within 1 h of death (Table 1). Since this ratio increases with age, as a result of cell compression during the continuous growth of the lens [3], we extrapolated backwards to obtain an estimate of the ratio for 200-day-old lenses. This yielded a value of 0.334.
TABLE 1. WET AND DRY WEIGHTS OF SHEEP LENSES STORED IN THE POST MORTEM EYE

<table>
<thead>
<tr>
<th>Storage time (h)</th>
<th>n</th>
<th>Age (days)</th>
<th>Wet weight (mg)</th>
<th>Dry weight (mg)</th>
<th>Weight ratio (dry:wet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>33-58</td>
<td>75</td>
<td>200</td>
<td>833 (41)</td>
<td>265 (15)</td>
<td>0.321 (0.009)</td>
</tr>
<tr>
<td>56-76</td>
<td>75</td>
<td>200</td>
<td>894 (55)</td>
<td>264 (14)</td>
<td>0.300 (0.011)</td>
</tr>
<tr>
<td>1</td>
<td>147</td>
<td>414</td>
<td>1031 (54)</td>
<td>248 (16)</td>
<td>0.338 (0.007)</td>
</tr>
<tr>
<td>1</td>
<td>149</td>
<td>660</td>
<td>1218 (66)</td>
<td>419 (22)</td>
<td>0.344 (0.017)</td>
</tr>
</tbody>
</table>

Lenses were removed and weighed at various times after the enucleated sheep eyes were placed on ice. They were then fixed in 5% glutaraldehyde for 2 weeks before being dried at 80 °C until constant weight was attained. The table shows that the ratio of dry weight to wet weight, for the 200-day-old lenses, decreases with increasing time in the eye, indicative of water uptake. Standard deviations are listed in parentheses.

The amount of water per gram of lens dry weight was calculated from the dry/wet weight ratios. The data for the 200-day-old lenses are plotted in Figure 1 as a function of post mortem time left in the eye. Also shown is the water content at 1 h, calculated from the extrapolated ratio. This value (1.99 g/g dry weight) is very similar to the 1.95 obtained for 414-day-old lenses.

It may be seen that the water content increases markedly during storage, from about 2 ml/g to about 2.5 ml/g after 76 h storage, a 25% increase. It would appear that there is little water uptake until about 30 h post mortem but, thereafter, the increase is rapid. When expressed on a wet weight basis, the increase corresponds to an uptake of 30-40 μl of water by a 200-300 mg lens.

Most likely, this uptake of water can be attributed to the lowering of lens temperature. Maintenance of hydration levels involves a number of mechanisms, including energy requiring ion pumps such as the epithelial Na⁺,K⁺-ATPase. Lowering the temperature of the lens would decrease the activity of these pumps and the pathways generating the ATP required. This, in turn, would allow ions and water to accumulate. Our data suggest that lens ATP stores probably become depleted around 30 h after cooling.

It may reasonably be assumed that similar water uptake will take place in the lenses from other species, including humans, left in the eye for extended periods at low temperatures. This offers a plausible explanation for the large variations in wet weights (e.g., 220 mg to 270 mg for 60-70-year-old human lenses) reported in the literature [1,2,4-7]. It could be argued that the observed variability of human lens weights simply reflects their genetic diversity. However, there is no evidence to suggest that such genetic variability exists and anecdotal evidence [1,2] is consistent with the uptake of water.

Our observations indicate that caution is needed when interpreting data obtained with lenses, which have been stored in the eye for extended periods. These could have taken up significant amounts of water, raising the possibility that some of the parameters being measured may be affected. Unfortunately, this is probably the case for most lenses obtained from eye bank eyes that have usually been stored for several days. Where feasible, lenses should be weighed and not used for whole lens studies if they have obviously taken up water. It has been argued that the high lens weights observed in one study must be correct since the eyes had been stored and handled “under ideal conditions by the eye bank” [7]. While the storage conditions used in eye banks may be ideal for preserving human corneas, it is unlikely they will have much effect on the lens.

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REFERENCES


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