

# University of Wollongong - Research Online

## Thesis Collection

Title: Regulation of tissue oxygen levels in the ocular lens

Author: Richard McNulty

Year: 2004

Repository DOI:

### Copyright Warning

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following: This work is copyright. Apart from any use permitted under the Copyright Act 1968, no part of this work may be reproduced by any process, nor may any other exclusive right be exercised, without the permission of the author. Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material.

Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

**Unless otherwise indicated, the views expressed in this thesis are those of the author and do not necessarily represent the views of the University of Wollongong.**

Research Online is the open access repository for the University of Wollongong. For further information contact the UOW Library: [research-pubs@uow.edu.au](mailto:research-pubs@uow.edu.au)

2004

## Regulation of tissue oxygen levels in the ocular lens

Richard McNulty  
*University of Wollongong*

Follow this and additional works at: <https://ro.uow.edu.au/theses>

**University of Wollongong**

**Copyright Warning**

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following: This work is copyright. Apart from any use permitted under the Copyright Act 1968, no part of this work may be reproduced by any process, nor may any other exclusive right be exercised, without the permission of the author. Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material.

Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

Unless otherwise indicated, the views expressed in this thesis are those of the author and do not necessarily represent the views of the University of Wollongong.

---

### Recommended Citation

McNulty, Richard, Regulation of tissue oxygen levels in the ocular lens, PhD, Department of Chemistry, University of Wollongong, 2004. <http://ro.uow.edu.au/theses/652>

## **NOTE**

This online version of the thesis may have different page formatting and pagination from the paper copy held in the University of Wollongong Library.

## **UNIVERSITY OF WOLLONGONG**

### **COPYRIGHT WARNING**

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site. You are reminded of the following:

Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material. Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

# **Regulation of Tissue Oxygen Levels in the Ocular Lens**

A thesis submitted in partial fulfilment of the  
requirements for the award of the degree of

Doctor of Philosophy

from

University of Wollongong

by

Dr Richard McNulty

B.A., M.B., B.S. (Syd.)

Department of Chemistry

2004

*The only use discovered of this work was that  
it may help to make a vain man humble*

-after Benjamin Franklin

## **Certification**

---

I, Richard McNulty, declare that this thesis, submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy, in the Department of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Richard McNulty

6 August 2004

## Table of Contents

---

<i>Title Page</i>	<i>i</i>
<i>Quotation</i>	<i>ii</i>
<i>Certification</i>	<i>iii</i>
<i>Table of Contents</i>	<i>iv</i>
<i>List of Figures and Tables</i>	<i>vi</i>
<i>Acknowledgements</i>	<i>xi</i>
<i>Publications</i>	<i>xii</i>
<i>Abstract</i>	<i>xiii</i>
<i>Abbreviations</i>	<i>xv</i>

## Chapter 1: General Introduction

---

1.1 Inspiration for the thesis	page 2
1.2 Physical and chemical properties of oxygen	page 2
1.3 The origins of O <sub>2</sub>	page 4
1.4 The history of O <sub>2</sub>	page 6
1.5 The roles of O <sub>2</sub>	page 10
1.5.1 Mitochondria and oxidative phosphorylation	page 11
1.5.2 Non-mitochondrial O <sub>2</sub> consumption	page 14
i. Ascorbic acid (ascorbate)	page 14
ii. Trans-plasma membrane oxidoreductase (PMOR) system	page 18
iii. Photo-oxidation	page 19

1.6 Oximetry	page 19
1.6.1 Polarography	page 20
1.6.2 Fluorescence and phosphorescence quenching	page 24
1.6.3 Hypoxia markers	page 28
1.6.4 Electron Paramagnetic Resonance (EPR)	page 30
1.7 Oxygen and the eye	page 32
1.7.1 Ocular anatomy	page 32
1.7.2 Lens physiology	page 36
1.7.3 Ocular $P_{O_2}$	page 39
1.8 Cataract	page 46

## **Chapter 2: Regulation of O<sub>2</sub> levels in the mammalian lens \_\_\_\_\_page 55**

2.1 Introduction	page 56
2.2 Materials and Methods	page 58
2.3 Results	page 67
2.4 Discussion	page 104

## **Chapter 3: Regulation of O<sub>2</sub> levels in the human lens \_\_\_\_\_page 112**

3.1 Introduction	page 113
3.2 Materials and Methods	page 114
3.3 Results	page 116
3.4 Discussion	page 130



<b>Chapter 4: Tissue O<sub>2</sub> levels in the developing chicken eye and their relation to organelle loss</b>	<b>page 134</b>
4.1 Introduction	page 135
4.2 Methods	page 138
4.3 Results	page 141
4.4 Discussion	page 145
<b>Chapter 5: Conclusions and Future Directions</b>	<b>page 149</b>
<b>Appendices</b>	<b>page 155</b>
A. Protocol for clinical study at Washington University in St Louis “The effect of pre-warmed infusion solutions on the incidence of post-vitrectomy cataract”	page 156
B. Mathematical model of lens O <sub>2</sub> consumption and diffusion	page 165
<b>References</b>	<b>page 170</b>
<b>List of Figures and Tables</b>	
<b>Figure 1.1</b> Key thinkers in the discovery of O <sub>2</sub>	page 7
<b>Figure 1.2</b> Mitochondrial morphology	page 13
<b>Figure 1.3</b> Mitochondrial oxidative phosphorylation enzymes	page 13
<b>Figure 1.4</b> Structure of ascorbic acid	page 15

<b>Figure 1.5</b> The glutathione (GSH) redox cycle and ascorbic acid	page 17
<b>Figure 1.6</b> Diagram of a Clark-style micro-electrode tip	page 21
<b>Figure 1.7</b> Polarographic standard curve	page 22
<b>Figure 1.8</b> The effect of protein concentration on electrode readings	page 23
<b>Figure 1.9</b> Graph of fluorescence lifetime decay	page 24
<b>Figure 1.10</b> The OxyLab fluorescent optode	page 27
<b>Figure 1.11</b> The metabolism of the hypoxia marker pimonidazole	page 29
<b>Figure 1.12</b> Pimonidazole staining in a squamous cell carcinoma of the human cervix	page 29
<b>Figure 1.13</b> Diagram of the human eye	page 32
<b>Figure 1.14</b> The structure of the ocular lens	page 34
<b>Figure 1.15</b> The ocular vascular supply	page 35
<b>Figure 1.16</b> $P_{O_2}$ environment of the lens	page 46
<b>Figure 1.17</b> Cataracts in the ocular lens	page 47
<b>Figure 2.1</b> Experimental apparatus for in vitro $P_{O_2}$ measurements	page 63
<b>Figure 2.2</b> Effect of protein on optode $P_{O_2}$ readings	page 67
<b>Figure 2.3</b> Optode accuracy in anoxic conditions	page 68
<b>Figure 2.4</b> Effect of measuring lens $P_{O_2}$ in the presence of an intact capsule	page 69
<b>Figure 2.5</b> Optode trace through an isolated bovine lens	page 70
<b>Figure 2.6</b> Bovine lens equilibration time	page 71
<b>Figure 2.7</b> Comparison of $P_{O_2}$ profiles in equilibrated and unequilibrated bovine lenses	page 72
<b>Figure 2.8</b> $P_{O_2}$ profiles along perpendicular axes in the bovine lens	page 73

<b>Figure 2.9</b> The effect of external $P_{O_2}$ on bovine lens $P_{O_2}$ profiles	page 74
<b>Figure 2.10</b> Bovine lens core $P_{O_2}$ as a function of external $P_{O_2}$	page 75
<b>Figure 2.11</b> $P_{O_2}$ gradients in bovine and rabbit lenses	page 77
<b>Figure 2.12</b> Respirometry trace produced by a bovine lens in atmospheric levels of $O_2$	page 78
<b>Figure 2.13</b> Effect of external $P_{O_2}$ on bovine lens $QO_2$	page 78
<b>Figure 2.14</b> Effect of temperature on bovine lens $QO_2$	page 79
<b>Figure 2.15</b> The effect of temperature on bovine lens $P_{O_2}$	page 80
<b>Figure 2.16</b> The distribution of mitochondria in the living bovine lens	page 82
<b>Figure 2.17</b> Distribution of mitochondria in the bovine lens using COX-antibody immunofluorescence	page 84
<b>Figure 2.18</b> The relationship between mitochondria and $P_{O_2}$ profiles in the bovine lens	page 85
<b>Figure 2.19</b> The role of mitochondria in bovine lens $QO_2$	page 86
<b>Figure 2.20</b> Effect of mitochondrial inhibitors on bovine lens $P_{O_2}$ profiles	page 86
<b>Figure 2.21</b> Effect of age on guinea pig lens $QO_2$	page 88
<b>Figure 2.22</b> Micrograph of anterior epithelium adherent to the bovine lens capsule	page 89
<b>Figure 2.23</b> $QO_2$ by region in the bovine lens	page 90
<b>Figure 2.24</b> $P_{O_2}$ profiles in the dissected core of the bovine lens	page 92
<b>Figure 2.25</b> Effect of an ascorbate-free diet on guinea pig weight	page 93
<b>Figure 2.26</b> Photograph of control and scorbutic guinea pig lenses	page 94
<b>Figure 2.27</b> The effect of ascorbate on $QO_2$ of the guinea pig lens	page 95
<b>Figure 2.28</b> Characteristics of ascorbate $O_2$ -consumption	page 96

<b>Figure 2.29</b> Metal-chelator inhibition studies on bovine lens core $QO_2$	page 97
<b>Figure 2.30</b> Metal-induced $O_2$ -consumption in the bovine lens	page 98
<b>Figure 2.31</b> PMOR inhibition studies on bovine lens core $QO_2$	page 99
<b>Figure 2.32</b> Effect of light on $PO_2$ profiles in the bovine lens	page 100
<b>Figure 2.33</b> The best fit of the consumption/diffusion model to $PO_2$ profiles	page 102
<b>Figure 2.34</b> The best fit of the diffusion/consumption model to $PO_2$ data of bovine lenses inhibited with azide	page 103
<b>Figure 2.35</b> The effect of mitochondrial inhibitors on calculated $O_2$ consumption time constants	page 104
<b>Figure 3.1</b> Human lens weight	page 117
<b>Figure 3.2</b> $PO_2$ profiles in a human lens pair	page 118
<b>Figure 3.3</b> Human donor lens $PO_2$ in vitro measured with a fluorescent optode	page 118
<b>Figure 3.4</b> Lens $PO_2$ profiles in three mammalian species	page 119
<b>Figure 3.5</b> The relationship between lens $QO_2$ and nucleus $PO_2$	page 120
<b>Figure 3.6</b> $QO_2$ in adult mammalian lenses	page 121
<b>Figure 3.7</b> The effect of age on human lens $QO_2$	page 121
<b>Figure 3.8</b> The effect of age on lens nucleus $PO_2$	page 122
<b>Figure 3.9</b> The effect of diabetes on $O_2$ levels	page 123
<b>Figure 3.10</b> The effect of post-mortem time on $O_2$ levels in human donor lenses	page 124
<b>Figure 3.11</b> Distribution of mitochondria in the human lens	page 126
<b>Figure 3.12</b> Depth of the mitochondria-containing cell layer in the human lens	page 127
<b>Figure 3.13</b> Mitochondrial inhibition of human lens $QO_2$	page 128

<b>Figure 3.14</b> Effect of temperature on $P_{O_2}$ in the isolated human lens	page 130
<b>Figure 4.1</b> Effect of hyperoxia on the morphometry of the embryonic chicken lens	page 138
<b>Figure 4.2</b> Intraocular $P_{O_2}$ in the embryonic chicken	page 142
<b>Figure 4.3</b> Utilisation of hypoxia markers in the embryonic chicken lens: in vitro calibration	page 143
<b>Figure 4.4</b> In vivo hypoxia in the developing chicken lens	page 144
<b>Figure A1</b> Effect of temperature on $P_{O_2}$ in the nucleus of human donor lenses	page 152
<b>Figure A2</b> Diagram of the cellular structure of the lens used for modelling	page 160
<b>Table 1.1</b> $P_{O_2}$ unit conversions	page 39
<b>Table 1.2</b> Aqueous humour $P_{O_2}$	page 40
<b>Table 1.3</b> Vitreous humour $P_{O_2}$	page 44
<b>Table 1.4</b> Cataract and the mitochondrial encephalomyopathies	page 51
<b>Table 2.1</b> Lens $P_{O_2}$	page 57
<b>Table 2.2</b> Concentration of $O_2$ in solutions	page 64
<b>Table 2.3</b> Lens $QO_2$	page 104

## Acknowledgements

---

Thanks to my family for long distance encouragement and support. During this thesis I learnt the true meaning of the phrase “the scientific community.” As such, I feel sole authorship is a generous and misleading interpretation of this PhD and I have many people to thank. Numerous colleagues have contributed in various ways to the realization of experiments: Beryl Ortwerth helped with HPLC ascorbate measurements, Richard Mathias and Huan Wang collaborated on the mathematical model, Frank Giblin, Alec Salt and Shane Hale provided guinea pigs, and David Beebe donated human lenses. Helpful advice came from Joseph Bonanno, James Dillon, Tony Hulbert, Ying Bo Shui and Abraham Spector. The Heartland Lion’s Eyebank team, especially Kelly Green-O’Neal are thanked for special effort (and cake). Thanks to the team at Trenton Processing, Illinois, for more eyeballs than I can count. Stephen Turney assisted with 2-photon microscopy. Thanks to my lab mum Peggy Winzenburger and to Anna Zandy, my favourite American.

Special thanks must go to Roger Truscott, my long suffering and long distance supervisor. And lastly, but most importantly, to Steven Bassnett, my mentor, friend and stylist, who took a gamble and ensured that the limiting factor in this work was not lack of opportunity or resources: to you, Sire, my humble thanks<sup>1</sup>.

---

<sup>1</sup>However, late night deliveries of fish and chips were somewhat lacking.

## **Publications**

---

Sections of the work described in this thesis have been reported in the following publications:

1. S Bassnett and R McNulty (2003). The effect of elevated intraocular oxygen on organelle degradation in the embryonic chicken lens. *J Exp Biol* 206, 4353-4361.
2. Richard McNulty, Huan Wang, Richard T Mathias, Beryl J Ortwerth, Roger J W Truscott and Steven Bassnett. Regulation of tissue oxygen levels in the mammalian lens. *J Physiol*, 2004 (*in press published online July 22, 2004 as 10.1113/jphysiol.2004.068619*).

## Abstract

---

Age-related nuclear cataract is a major cause of blindness and is thought to result from oxidation of key cellular components. Molecular oxygen ( $O_2$ ) is a possible oxidant in this process. The aim of this thesis was to investigate the regulation of tissue  $O_2$  levels in the ocular lens. We mapped the distribution of  $O_2$  within isolated lenses using a fluorescent optode. All lenses examined (bovine, rabbit and human) showed U-shaped  $PO_2$  profiles across both the optic and equatorial axes resulting in core hypoxia. When equilibrated in physiological conditions ( $PO_2$  environment of 36mmHg), the lens nucleus  $PO_2$  was  $1.6 \pm 0.5$  mmHg (n=6),  $12.5 \pm 2.5$  mmHg (n=6), and  $13.8 \pm 7.6$  mmHg (n=24 pairs) in the bovine, rabbit and human lens, respectively.

We examined a possible role of  $PO_2$  gradients on the development of the embryonic chicken lens. The lens contains two populations of fibre cells, differentiating fibers (DF) in the outer cortex that contain a full complement of organelles (including mitochondria), and mature fibers (MF) in the lens core that do not. Incubating chicken embryos in hyperoxic conditions resulted in larger lenses and an increase in the depth of the DF population compared to lenses from embryos raised in normoxia. This is consistent with the hypothesis that hypoxia triggers the elimination of organelles from the lens.

The consumption of  $O_2$  by the lens was region-dependent. Both respirometric measurements (performed on dissected regions of the bovine lens) and a diffusion/consumption mathematical model showed that approximately 90% of  $O_2$  consumption occurred in the cortex, the remainder occurring in the core which contains only MF. The distribution of mitochondria in the DF layer was mapped using 2-photon or confocal microscopy on living lenses treated with mitochondria-specific dyes (rhodamine



123 or Mito Tracker) and in fixed tissue by immunofluorescence using an antibody to cytochrome *c*-oxidase. The depth of the mitochondria-containing cell layer varied in different species, being 500-760  $\mu\text{m}$  in the bovine lens and 50-100  $\mu\text{m}$  in the human. The  $P\text{O}_2$  gradients in the lens extended beyond the boundaries of the mitochondria-containing DF layer, consistent with a role for non-mitochondrial  $\text{O}_2$  consumption (albeit a minor one) in the MF region in the intact lens. We therefore examined both mitochondrial and non-mitochondrial processes which may be responsible for modulating lens  $\text{O}_2$  levels. Mitochondria-inhibition studies showed that the contribution of oxidative phosphorylation to bovine lens  $\text{QO}_2$  was approximately 90%. Interestingly, mitochondria-inhibiting drugs and age had an insignificant effect on human lens  $\text{QO}_2$ . Ascorbate, found in high levels in the lens, was investigated as an  $\text{O}_2$  consumer. Bovine and human lens core  $\text{O}_2$  consumption could be induced by metals and inhibited by the metal-chelator DETAPAC, characteristics which are consistent with ascorbate-dependent  $\text{O}_2$  consumption. We found no evidence for a role for a trans-plasma membrane oxido-reductase system or photo-oxidation as non-mitochondrial  $\text{O}_2$  consumers.

The movement of  $\text{O}_2$  through the lens tissue was studied by decreasing lens  $\text{QO}_2$ . Sub-physiological temperatures resulted in a marked decrease in lens  $\text{QO}_2$  and a concomitant flooding of the lens core with  $\text{O}_2$ . The rapid rise in lens  $P\text{O}_2$  at lower temperatures may be of clinical relevance. During vitrectomy, cool,  $\text{O}_2$ -rich solutions are infused into the eye. We hypothesise that rising lens core  $\text{O}_2$  levels during vitrectomy are the cause of the strikingly high incidence of post-vitrectomy nuclear cataract. We have initiated a clinical study to examine whether the use of warmed solutions during surgery can decrease the incidence of post-vitrectomy cataract.

## Abbreviations

---

Abbreviation	Name
$\pm$	plus or minus
3D	three-dimensional
$\alpha$	Bunsen or solubility coefficient (gas volume at STP absorbed per unit volume of liquid)
AAH	artificial aqueous humour
ADP	adenosine diphosphate
ATP	adenosine triphosphate
AREDS	age-related eye disease study
ARNC	age-related nuclear cataract
BSA	bovine serum albumin
CCCP	carbonyl cyanide m-chlorophenylhydrazone
CCD	charge-coupled device
COX	cytochrome <i>c</i> -oxidase
$\Delta\Psi_m$	inner mitochondrial membrane potential
DAB	diaminobenzidine
DF	differentiating fibres
D	diopetre
DETAPAC	diethylenetriaminepentaacetic acid
$D_{O_2}$	diffusion coefficient of $O_2$
DTPA	diethylenetriaminepentaacetic acid

E	embryonic day
EDTA	ethylenediaminetetraacetic acid
EPR	electron paramagnetic resonance
fMRI	functional magnetic resonance imaging
$\lambda$	length constant for O <sub>2</sub> consumption (mm)
G	gauge
GSH	glutathione, reduced
GLUT	glucose transporter
GSSG	glutathione, oxidised
Hb	haemoglobin
hif	hypoxia inducible factor
IgG <sub>2a</sub>	immunoglobulin G <sub>2a</sub>
$K_m$	Michaelis constant
$\lambda$	length constant
Mb	myoglobin
MF	mature fibres
mmHg	millimeters of mercury ( a unit of gas tension or partial pressure)
MPA	metaphosphoric acid
mRNA	messenger ribonucleic acid
mtDNA	mitochondrial deoxyribonucleic acid
n	number of samples
NAD(P)H	nicotinamide adenine dinucleotide (phosphate), reduced

NMR	nuclear magnetic resonance
NMWL	nominal molecular weight limit
NPA	3-nitropropionic acid
OFZ	organelle-free zone
Pa	Pascal (SI unit of gas tension or partial pressure)
PC	personal computer
$P_{O_2}$	partial pressure of oxygen or oxygen tension
PBS	phosphate-buffered saline
PET	positron-emission tomography
PMOR	plasma-membrane oxido-reductase system
PMRS	plasma-membrane redox system
ppm	ppm
$QO_2$	oxygen consumption rate
ROS	reactive oxygen species
RT	real time
SD	standard deviation
STP	standard temperature and pressure (273.15 K or 0°C and 101.325 kPa, 760 mmHg or 1 atmosphere)
SVCT	sodium-dependent vitamin C-transporter
$\tau$	time constant for $O_2$ consumption (s)
TRIS	tris(hydroxymethyl)aminomethane
UV	ultra-violet

VEGF                      vascular endothelial derived growth factor

w/v                      weight/volume