Biochem. J. (1972) **129**, 507–509
Printed in Great Britain

The Role of Respiration in the Energy Metabolism of the Bovine Lens

By P. TRAYHURN and RUTH VAN HEYNINGEN
Nuffield Laboratory of Ophthalmology, University of Oxford, Walton Street, Oxford OX2 6AW, U.K.

(*Received* 21 *June* 1972)

The lens is avascular, and depends for its O_2 on the small amount dissolved in the aqueous humour (see van Heyningen, 1969; Kuck, 1970). The role of respiration in the production of energy by the lens is uncertain. Mitochondria are almost entirely confined to the monolayer of epithelial cells at the anterior surface (Wanko & Gavin, 1958; Cohen, 1965). There have been a number of reports on the effect of anaerobic conditions on various aspects of lens metabolism *in vitro*. The most comprehensive was that by Kinoshita *et al.* (1961), who found that absence of O_2 had no effect on the Na^+, K^+ and '7min-acid-hydrolysable phosphate' concentrations, nor on the incorporation of [^{14}C]arginine into protein, when calf lenses were incubated in the presence of glucose. Only by using a glucose-free medium could a role for O_2 in the production of energy be shown. Other workers have found that the active accumulation of amino acids by calf and rabbit lens is unaffected or slightly diminished by incubation under N_2 in the presence of glucose (Kern, 1962; Kinsey & Reddy, 1963; Reddy, 1970). A Pasteur effect has been observed for calf lens (Kern, 1962) and rabbit lens (van Heyningen, 1965).

While studying the metabolism of amino acids by the adult bovine lens, we observed that protein synthesis was inhibited by anoxia (P. Trayhurn & R. van Heyningen, unpublished work). This prompted us to reappraise the extent to which the lens is dependent on respiration for its energy and to find which metabolic processes are most affected by anoxia. The effect of anoxia on the Na^+ and ATP concentrations, the formation of lactate and the uptake and incorporation into protein of tracer amounts of a radioactive amino acid were therefore investigated in the presence and in the absence of glucose. Adult bovine lenses (wet wt. 1.9–2.1g) were used.

Materials and methods

Each lens was incubated for 22h at 35°C in a culture tube (Merriam & Kinsey, 1950) containing 15ml of a modified Krebs–Ringer phosphate medium, pH7.4, with antibiotics (Trayhurn & van Heyningen, 1971). Glucose, when required, was at a concentration of 10mM. Tyrosine was used as a tracer amino acid; it is not metabolized by the bovine lens other than by incorporation into protein (P. Trayhurn & R. van Heyningen, unpublished work). L-[U-^{14}C]Tyrosine (The Radiochemical Centre, Amersham, Bucks., U.K.) (3μCi; specific radioactivity 513mCi/mmol) was added to 15ml of medium. The radiochemical purity was found to be >99%.

The lenses were incubated under four different conditions: with and without glucose in air, and with and without glucose in N_2. When N_2 (containing <5p.p.m. of O_2) was used, the culture tubes were gassed for the first hour. Lenses were usually incubated as pairs from one animal, under two different conditions.

After 22h each lens was removed and gently blotted dry. It was immediately frozen in liquid N_2, and placed in a weighed centrifuge tube containing 9.0ml of ice-cold 0.6M-$HClO_4$. The tube was reweighed, the lens was crushed and the tube was then left at room temperature for 15min before being centrifuged. ATP was at once determined in the supernatant, by using a test combination from Boehringer Corp. (London) Ltd., London W.5, U.K. This is a modification of the method of Adam (1963). Radioactivity was measured in the supernatant after neutralization with K_2CO_3 and also in the precipitated protein. Counting equipment and other methods were as described by Waley (1964) and van Heyningen (1965). Na^+ was measured in trichloroacetic acid extracts by flame photometry (Trayhurn & van Heyningen, 1971). The medium was assayed for lactate by the method of Barker & Summerson (1941), as modified by Hullin & Noble (1953).

Lens water was taken to be 64.5% of the wet weight (Amoore *et al.*, 1959), and protein 33.3% of the wet weight. The bovine lens contains only traces of fats and glycogen (see van Heyningen, 1969).

Results and discussion

Effect of anoxia in the presence of glucose. The results are shown in Table 1 (columns I and II). In the absence of O_2 the Na^+ concentration was increased by 8%; this slight increase was statistically significant ($P<0.05$), on the basis of an unpaired Student's t test.

Incorporation of [U-^{14}C]tyrosine was decreased by anoxia to 47% of its value in air. This was apparently not due to an inhibition of transport of the amino acid into the lens, for the radioactivity of the $HClO_4$-soluble fraction was increased by 24% under

N_2, although the total radioactivity in the lens ($HClO_4$-soluble plus protein) was slightly decreased (85% of the aerobic value). Inhibition of the incorporation of a radioactive amino acid into protein by anoxia has been observed in several tissues (see Jefferson *et al.*, 1971), whereas the transport of amino acids appears to be insensitive or less sensitive to anoxia (Riggs & Walker, 1963; Jefferson *et al.*, 1971).

Extrusion of Na^+ and incorporation of amino acids into protein are both processes that use energy. The small increase in Na^+ and the large decrease in labelled protein under anoxia are presumably due to the fall in concentration of ATP, to 68% of that in air. The method of ATP analysis is not specific, since GTP, ITP and UTP are also measured (Adam, 1963), but ATP accounts for about 80% of nucleoside triphosphates in the bovine lens (Klethi & Mandel, 1965).

The observed decrease in ATP concentration occurred even though the amount of lactate formed (and therefore the amount of glucose used) was increased to 148% of that formed aerobically. This suggests that normally at least 33% of the energy is derived from aerobic metabolism. This value may well be an underestimate, since the concentration of ATP was not maintained in spite of increased glycolysis. Various workers have made assessments of the contribution of aerobic metabolism to energy production in the lens of various species, ranging from 'slight' to 33% of the total energy (see van Heyningen, 1969; Kuck, 1970). In a number of other tissues an increase in glycolysis under N_2 is unable to compensate for the loss of aerobically produced ATP, but transport of cations is scarcely affected (van Rossum *et al.*, 1971).

Metabolism in the absence of glucose. Table 1 (columns III and IV) shows that the lens can obtain a substantial amount of energy from the oxidation of endogenous substrates. There seems to be little difference between the exclusive use of either anaerobic glycolysis (column II) or the oxidation of endogenous substrates (column III) in the ability of the lens to maintain its metabolic integrity. There is no significant difference ($P>0.05$) in the concentration of ATP or in the amount of [U-^{14}C]tyrosine incorporated into protein. Although the amount of labelled tyrosine in the $HClO_4$ extract was significantly less in column III, the total radioactivity in the lens was not significantly different.

The main difference lies in the Na^+ concentration. Incubation in air without glucose (column III) results in a Na^+ concentration of 26.3 μmol/g of lens water, a value 38% higher than that found after anaerobic incubation in the presence of glucose (column II). Thus, although the lens incubated in air without added substrate has as high a concentration of ATP and as great an incorporation of tyrosine into protein as the lens incubated anaerobically with glucose, it is less able to maintain its Na^+ concentration.

Table 1. *Incubation of the bovine lens, in air and in N_2, with and without added glucose: concentrations of Na^+ and ATP, uptake and incorporation into protein of [U-^{14}C]tyrosine and formation of lactate*

Results are expressed as means ± S.D. with numbers of observations in parentheses. All the radioactivities are adjusted to an initial radioactivity in the medium of 6000 c.p.s./15 ml. The P values were obtained from unpaired Student's t tests: * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Incubation conditions ...	I Glucose, air	II Glucose, N_2	100×II/I	III No glucose, air	100×III/II	IV No glucose, N_2
Na^+ (μmol/g of lens water)	17.7±1.0 (8)	19.1±1.2 (8)	108*	26.3±3.9 (6)	138**	59.2±5.4 (6)
[^{14}C]Tyrosine in protein (c.p.s./g of lens)	601±80 (7)	284±54 (7)	47***	282±54 (7)	99	26±5 (3)
[^{14}C]Tyrosine in $HClO_4$-soluble material (c.p.s./g of lens)	540±55 (7)	671±37 (7)	124***	573±61 (7)	85*	—
Total radioactivity (c.p.s./g of lens)	1141±96 (7)	955±83 (7)	85**	855±111 (7)	90	—
ATP (μmol/g of lens)	1.61±0.04 (7)	1.09±0.11 (6)	68***	1.05±0.08 (7)	96	0.17±0.04 (7)
Lactate formed (μmol/g of lens in 22h)	20.2±1.3 (8)	29.9±1.6 (6)	148***	—	—	—

This suggests that the cation transport of the lens depends more on ATP derived from glycolysis than on that derived from respiration.

As expected, anoxia has a drastic effect in the absence of glucose. The lens swells and the Na^+ concentration rises to nearly 60 μmol/g of lens water (Trayhurn & van Heyningen, 1971). The concentration of ATP is very low and there is negligible incorporation of [U-^{14}C]tyrosine into protein (Table 1, column IV).

Conclusions. The influence of O_2 on the metabolism of the adult bovine lens is greater than that reported for the calf lens. Kinoshita *et al.* (1961) found that anoxia had no effect on the calf lens incubated in the presence of glucose, whereas anoxia has a considerable effect on the adult bovine lens (Table 1, columns I and II).

Although the cells that contain mitochondria constitute a minute proportion of the whole tissue mass, respiration has a substantial role to play in the metabolism of the bovine lens. In the presence of glucose at least 33% of the energy may be derived from respiration.

We thank Dr. D. H. Williamson of the Metabolic Research Laboratory, Radcliffe Infirmary, Oxford, for helpful criticism, Dr. H. G. Epstein and Mr. C. Hahn of the Nuffield Department of Anaesthetics, Radcliffe Infirmary, Oxford, for the use of their computer, and the slaughterhousemen of A. G. Hedges (Abingdon) Ltd. for their co-operation.

Adam, H. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 539–543, Academic Press, London and New York

Amoore, J. E., Bartley, W. & van Heyningen, R. (1959) *Biochem. J.* **72**, 126–136

Barker, S. B. & Summerson, W. H. (1941) *J. Biol. Chem.* **138**, 535–554

Cohen, A. I. (1965) *Invest. Ophthalmol.* **4**, 433–446

Hullin, R. P. & Noble, R. L. (1953) *Biochem. J.* **55**, 289–291

Jefferson, L. S., Wolpert, E. B., Giger, K. E. & Morgan, H. E. (1971) *J. Biol. Chem.* **246**, 2171–2178

Kern, H. L. (1962) *Invest. Ophthalmol.* **1**, 368–376

Kinoshita, J. H., Kern, H. L. & Merola, L. O. (1961) *Biochim. Biophys. Acta* **47**, 458–466

Kinsey, V. E. & Reddy, D. V. N. (1963) *Invest. Ophthalmol.* **2**, 229–236

Klethi, J. & Mandel, P. (1965) *Nature (London)* **205**, 1114–1115

Kuck, J. F. R., Jr. (1970) in *Biochemistry of the Eye* (Graymore, C. N., ed.), pp. 262–277, Academic Press, London and New York

Merriam, F. C. & Kinsey, V. E. (1950) *A.M.A. Arch. Ophthalmol.* **43**, 969–988

Reddy, D. V. N. (1970) *Invest. Ophthalmol.* **9**, 206–219

Riggs, T. R. & Walker, L. M. (1963) *J. Biol. Chem.* **238**, 2663–2668

Trayhurn, P. & van Heyningen, R. (1971) *Exp. Eye Res.* **12**, 315–327

van Heyningen, R. (1965) *Biochem. J.* **96**, 419–431

van Heyningen, R. (1969) in *The Eye*, 2nd. edn. (Davson, H., ed.), vol. 1, pp. 387–400, Academic Press, London and New York

van Rossum, G. D. V., Gosalvez, M., Galeotti, T. & Morris, H. P. (1971) *Biochim. Biophys. Acta* **245**, 263–276

Waley, S. G. (1964) *Biochem. J.* **91**, 576–583

Wanko, T. & Gavin, M. A. (1958) *A.M.A. Arch. Ophthalmol.* **60**, 868–879