Exp. Eye Res. (1986) 43, 243–249

Glucose Metabolism by Human Cataracts in Culture

JOHN K. WOLFE AND LEO T. CHYLACK, JR

Howe Laboratory of Ophthalmology, Harvard Medical School and the Massachusetts Eye and Ear Infirmary, Boston, MA 02114, U.S.A.

(*Received 21 October 1985 and accepted 26 February 1986*)

Metabolism in human senile cataracts has been studied using uniformly labeled [^{14}C]glucose. Intracapsularly extracted lenses were cultured in TC-199 media with a glucose concentration of 5·5 mM. Results show that lactate production accounts for 97 % of the glucose metabolized. Under these standard incubation conditions there is negligible accumulation of α-glycerol phosphate, glucose-6-phosphate, and sorbitol. The rate of lactate production was found to be relatively uniform over a range of cataract severities which were determined from the CCRG classification. The effects of several perturbants in the medium were measured. An ATP concentration of 3 mM was found to inhibit lactate production. Labeled glucose-6-phosphate in the medium was found to produce lactate at a rate approximately one half that of glucose. Elevated glucose concentration resulted in a slight decrease in lactate production and, in some lenses, production of a small amount of sorbitol. Overall, the glycolytic pathway appears to be functioning normally and without regard for cortical and nuclear opacification.

Key words: metabolism; cataract; CCRG; glucose.

1. Introduction

Glucose metabolism provides the energy for the maintenance of lens transparency. The ATP produced regulates the intralenticular ionic environment to favor protein solubilization. In addition, glucose provides a defense against oxidative damage through the generation of hexose monophosphate-derived NADPH.

While the activities of the glycolytic enzymes and the levels of metabolites of glucose have been studied extensively, there is no definitive evidence that a metabolic deficiency causes human senile cataract. Excess sorbitol production has been shown to be a principal etiologic stress in galactosemic and diabetic cataracts in rats. However, the levels of sorbitol in human cataracts have been shown to be approximately the same as those in clear lenses (Chylack, Henriques and Tung, 1979), and the levels of aldose reductase (which reduces glucose to sorbitol) in human lenses are so low that sorbitol accumulation is not likely to be as osmotically stressful as it is in 'sugar cataracts' in animal lenses (Jedziniak et al., 1981).

In animal lenses, activation of the pentose shunt is part of the response to oxidative stress (Giblin, McCready and Reddy, 1982). NMR studies of glucose-stressed animal lenses have shown an increase in both sorbitol (Gonzalez, Barnett, Aguayo, Cheng and Chylack, 1984) and α-glycerol phosphate (α-GP) (Gonzalez, Barnett, Cheng and Chylack, 1984). However, changes in glucose metabolism in human senile cataracts have not been documented.

Incubation with [^{14}C]glucose and subsequent separation of labeled metabolites have been established as sensitive methods for the determination of changes in the distribution and rates of production of metabolites (Wolfe, Gillis and Chylack, 1985).

Please send reprint requests to Dr John K. Wolfe, Howe Laboratory of Ophthalmology, 243 Charles Street, Boston, MA 02114.

0014–4835/86/080243+07 $03.00/0

By using uniformly labeled glucose and accounting for 100% of the label, all of the pathways of glucose metabolism can be measured simultaneously. Using variations of this technique, this paper is concerned with three questions.

(1) What are the general characteristics of the distribution of glucose among metabolites in cataractous lenses?

(2) Are there alterations in glucose metabolism which can be associated with cataract severity?

(3) How do perturbations of the incubation medium affect the metabolism within the lens?

2. Materials and Methods

Lenses

Lenses were obtained during the course of routine intracapsular cataract extraction from the operating rooms at Massachusetts Eye & Ear Infirmary. They were photographed and classified according to Cooperative Cataract Research Group (CCRG) procedures (Chylack, Lee, Tung and Cheng, 1983). Classifications of cortical opacities (CXA, CXE, and CXP)* were converted to a volume per cent opacity using elliptical geometry. Each quadrant of cortical opacity in the equator (CXE) is 18·5% of the total cortical volume. Similarly, each quadrant of anterior cortex (CXA) is 4·3% of the volume and each quadrant of posterior cortex (CXP) is 2·1% of the volume. The nuclear opacity classifications (values of N) range from 0 to 4, where 0 indicates a clear nucleus and 4 indicates the most opaque nucleus. Metabolic data was correlated with the volume fraction of the cortex which was opaque for a given level of nuclear opacification. This approach does have its drawbacks, but the classification has become standardized and elliptical geometry provides the best estimate of opacity volume from the classification data.

Incubations

Intact lenses were incubated as follows. Each lens was pre-incubated for 17 hr under standard conditions: TC-199 medium with ^{14}C uniformly labeled 5·5 mM glucose. The medium was then changed and the incubation continued for another 24 hr under either standard or perturbed conditions. The perturbed conditions were set up as follows:

(1) elevated ATP: standard conditions including [^{14}C]glucose with the addition of ATP to a concentration of 3 mM;

(2) [^{14}C]glucose-6-phosphate ([^{14}C]G6P): standard conditions except that 5·5 mM [^{14}C]G6P was used in place of 5·5 mM [^{14}C]glucose;

(3) elevated glucose: standard conditions except that 16·5 mM [^{14}C]glucose was used in place of 5·5 mM [^{14}C]glucose.

In all conditions, labeled intermediary metabolites were measured. At the end of the second incubation, the lens was homogenized in 2 ml of 0·4 M $HClO_4$. The media samples were mixed with one half volume of 0·4 M $HClO_4$. For each lens, then, three samples were analysed separately: the pre-incubation medium, the second incubation medium, and the lens homogenate. Analysis of the rate of labeled lactate production in the pre-incubation medium is an index of the metabolic activity of the lens under standard conditions. Addition of the rates of production of labeled intermediates in the second incubation medium plus the homogenized lens gives the total rate under perturbed conditions. In the case of lactate, the ratio of the total perturbed rate to the pre-incubation rate gives a normalized lactate production.

*Classification of human senile cataracts follows the American Cooperative Cataract Research Group protocol. CXA, CXE, and CXP refer to cortical anterior, equator, and posterior respectively. N refers to the degree of nuclear opacification.

HPLC

Labeled glucose metabolites in the perchloric acid extracts were separated by HPLC as previously described (Wolfe et al., 1985). A Bio Rad HPX-87 column was run at 85°C using 0·005 M sulfuric acid as an eluant at 0·3 ml min^{-1}. Peaks in the effluent were quantified with a Berthold LB 504 Radioactivity monitor connected to a Spectra Physics 8000 data system. This system permits detection of 1 nmol of an intermediate in our incubation system.

Data reduction was done using Fortran 77 programs written for the UNIX operating system on a VAX 11/780. The HPLC data system outputs the relative distribution of radioactivity in the sample. The initial concentration of glucose in the media was known and its specific radioactivity was 0·45 μCi $\mu mole^{-1}$. The quantity of unlabeled glucose in the lens before incubation was estimated to be less than 5 % of the total glucose based on the levels found after incubation. This quantity was not considered in these calculations. The micromoles of a hexose intermediate is calculated using:

$$x = f^* [G]$$

where:

x = micromoles of the intermediate;
f = fraction of label as the intermediate;
$[G]$ = micromoles of initial glucose.

For trioses (lactate and α-glycerol phosphate) this number is multiplied by two. Total radioactivity in each sample was determined by liquid scintillation counting and then used to determine the distribution between lens and medium and to insure complete recovery of the label. The rates of lactate production and glucose consumption are referenced to the respective rates in the pre-incubation.

3. Results

Typical metabolite distribution

A standard chromatogram showing metabolites in a calf lens homogenate which can be seen under incubation conditions is shown in Fig. 1. Figure 2 is a typical chromatogram showing the ^{14}C-labeled metabolites extracted from a human cataractous lens after incubation. As with the calf lens incubations the predominant metabolic activity in human cataractous lenses is lactate production. In these human lens incubations, 100 % of the initial radioactivity was recovered in the perchloric acid extracts of the lens and media. Average lactate production in a series of 24 cataracts was 0·21 μmol hr^{-1} per lens (0·95 μmol hr^{-1} g^{-1} lens); calf lenses incubated under similar conditions produced 0·9 μmol hr^{-1} per lens (1·2 μmol hr^{-1} g^{-1} lens). It is also noteworthy that cataracts, under these conditions, accumulate no sorbitol, no α-glycerol phosphate, and no glucose-6-phosphate. Calf lenses typically produce 5 % of the label as sorbitol, 4 % of the label as glucose-6-phosphate, and negligible amounts of α-glycerol phosphate (unpub. data). The metabolism in human cataracts appears to be directed solely to lactate, and hence ATP, production.

In order to compare the effects of perturbations on a series of lenses it was necessary to develop a reference value for the metabolic status of each cataractous lens. A 17-hr pre-incubation and determination of [^{14}C]lactate in the medium were used for this purpose. Perturbation results are normalized, then, by comparing two rates of lactate production: the μmol per hr for the first 17 hr (which considers only that found in the media) and the μmol per hr for the next 24 hr (which includes both that in the lens and that in the media). Since the amount in the lens makes up a small fraction of the total, we would expect this number to be approximately 1 for lenses incubated under standard conditions. In a series of five lenses treated this way as controls, the

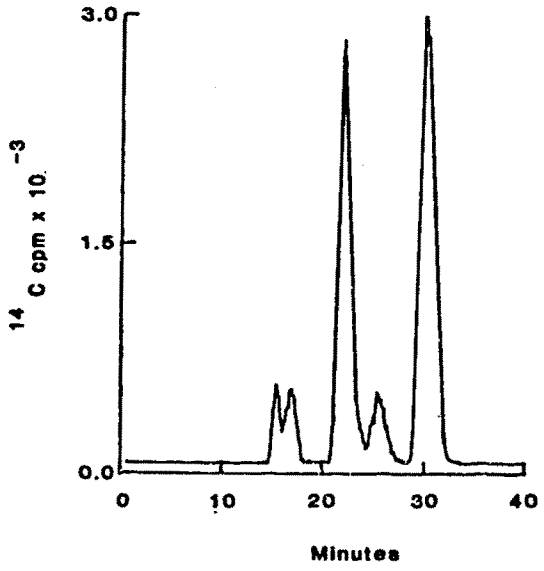

FIG. 1. Standard HPLC separation of glucose metabolites. Peaks are glucose-6-phosphate at 16·1 min, α-glycerol phosphate at 17·5 min; glucose at 22·5 min, sorbitol at 25·8 min, and lactate at 30·4 min.

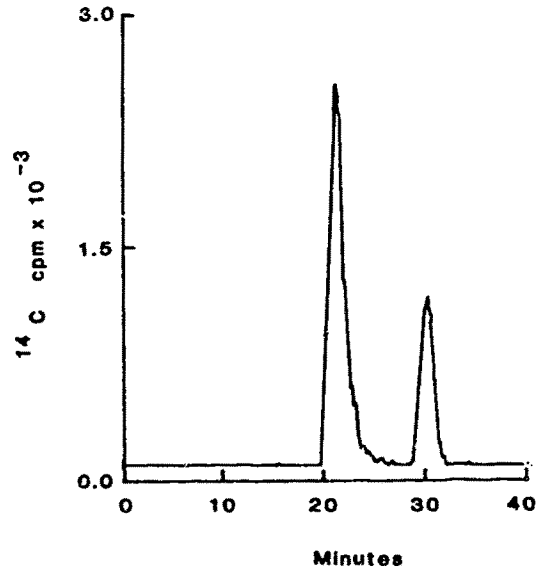

FIG. 2. HPLC separation of glucose metabolites from a human cataractous lens extract after a 24-hr incubation showing only labeled glucose and lactate within the lens. In the medium, also, the only labeled metabolites are glucose and lactate.

ratio of lactate production rate in the second incubation to that in the pre-incubation was $0{\cdot}829 \pm 0{\cdot}153$. That it is not exactly 1 is not critical, since it is used only as a reference point for the perturbed incubation conditions.

Cataract severity and metabolism

In the 24 lenses used in this study, the rate of lactate production in the pre-incubation was examined as a function of the severity of the cataract as

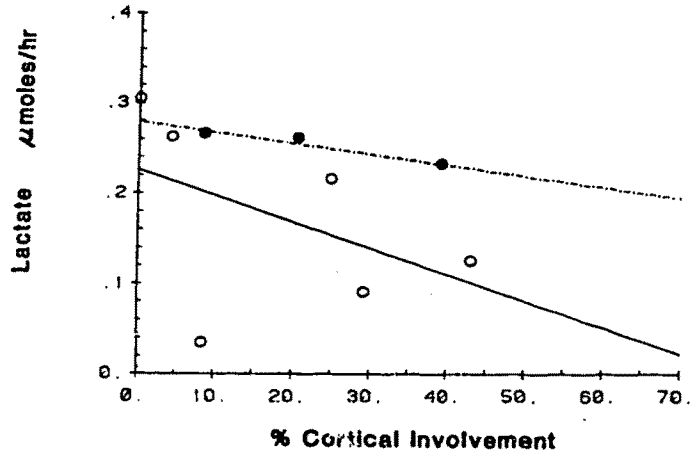

FIG. 3. Rate of lactate production as a function of the volume per cent of cortical opacification at different levels of nuclear opacification, N. N = 0 indicates a clear nucleus; N = 1 indicates a slightly opaque nucleus. The % Cx is calculated from the cortical classification (CXA, CXP, and CXE) using elliptical geometry. Each point represents one lens. Lines were calculated by the method of least squares. (●—·—●, N = 0; ○——○, N = 1.)

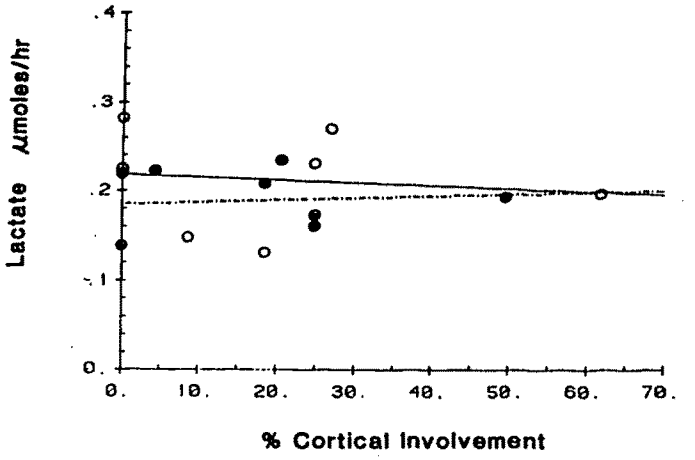

FIG. 4. Rate of lactate production as a function of the volume per cent of cortical opacification at different levels of nuclear opacification, N. N = 2 indicates a moderately opaque nucleus; N = 3 indicates the most opaque nucleus found in lenses used in this study. The % Cx is calculated from the cortical classifications (CXA, CXP, CXE) using elliptical geometry. Each point represents one lens. Lines were calculated by the method of least squares. (●—·—●, N = 2; ○——○, N = 3.)

determined by the CCRG classification. Figures 3 and 4 show the rate of lactate production as a function of the volume per cent of cortical opacification for each of four levels of nuclear opacification.

In the classification scheme the point corresponding to N = 3 and %CX = 61·9 (Fig. 4, open circles) is the most severe cataract. Yet its lactate production, 0·197 μmol hr^{-1}, is about average. The line drawn through the points in each graph is calculated by least squares. In the cases of N = 2 and N = 3 it is clear that there is little variation in the lactate rate as a function of cortical opacification. The slopes are 0·025 and −0·0205, respectively.

The calculated line for N = 1 has a slope of −0·290. However, the randomness in

TABLE I

Metabolic effects of perturbed media

Perturbant	Number*	Lactate†	S.D.‡	*P*-value§
Control	5	0·829	±0·153	
3 mM ATP	4	0·258	±0·223	< 0·01
[^{14}C]G6P‖	3	0·449	±0·104	< 0·025
16·5 mM glucose	8	0·604	±0·151	< 0·05

* Number of lenses used for each perturbing condition.
† Ratio of μmol hr^{-1} lactate produced during the incubation to the μmol hr^{-1} produced during the pre-incubation.
‡ Standard deviation of the ratio.
§ Probability that the lactate production ratio in the perturbed condition does not differ from that in the control condition.
‖ G6P, glucose-6-phosphate.

the data shown by a correlation coefficient of 0·215 and an F ratio of 1·1 suggest that there is no significant correlation of lactate production with cortical opacity at N = 1. Figure 3 with N = 0 shows for these purposes pure cortical cataracts. While the values for lactate do decrease as the %CX increases, the number of data points is not sufficient to make a significant correlation. With the 24 lenses studied, then, no correlation could be demonstrated between the rate of lactate production and either the extent of cortical opacification or the intensity of nuclear opacification.

Perturbed incubation conditions

Previous work has shown that 3 mM ATP in the medium of incubated animal lenses causes a decrease in lactate production. The data in Table I show that this effect is also true in incubated human cataracts. The normalized lactate rate is 0·258, significantly lower than that in control lenses. Inhibition of lactate production by ATP resulted in the appearance of trace amounts of G6P within the lens. Animal lenses incubated in 3 mM ATP contained relatively large quantities of G6P both within the lens and in the medium (unpubl. data).

The effect of elevated glucose concentration on metabolism is of interest because of the association between cataract and diabetes and because of possible excess sorbitol production. As shown in Table I, 16·5 mM glucose in the medium results in a slightly decreased rate of lactate production, significant at the 0·05 level. Four of eight lenses incubated in 16·5 mM glucose produced detectable sorbitol. The average quantity in these four was 38 nmol per lens after a 24-hr incubation which is less than 2% of the average glucose consumed.

4. Discussion

The data presented here indicate that lactate is the predominant metabolite of glucose in human cataracts. Further, the rates of lactate production are fairly constant through a range of opacities in the cataracts.

Incubated calf lenses contained at least some G6P which indicates some inhibition of phosphofructokinase by ATP (Wolfe et al., 1985). The lack of G6P in human cataracts suggests that the intralenticular level of ATP is too low to cause inhibition

of phosphofructokinase. This means that the lens either cannot make as much ATP as it needs or cannot keep the ATP within the cells.

The use of G6P as an energy source bypasses the regulatory enzyme, hexokinase. In a cell-free system the use of [^{14}C]G6P as an energy source would be expected to increase lactate production. However, cell membranes in general are not permeable to phosphorylated compounds (Newsholme and Start, 1973) and, therefore, a significant decrease in normalized lactate rate would be expected when intact lenses are incubated with G6P as the only energy source. The data in Table I indicate that, while normalized lactate is decreased, lactate is produced from extralenticular G6P. This may indicate increased membrane permeability to phosphorylated intermediates. The ability of cells to compartmentalize metabolites is an important part of metabolic regulation.

The studies using ATP as a perturbant suggest that ATP may be membrane-permeable to some extent. The permeability of G6P further suggests a problem with membrane integrity. Cheng, Chylack and von Saltza (1981) arrived at a similar conclusion studying ATP production in cataractous lenses.

Previous incubations of human cataracts in 35·5 mM glucose resulted in a 100% increase in the level of sorbitol (Chylack et al., 1979). The present work indicates only a slight increase in sorbitol in lenses incubated in 16·5 mM glucose. However, this is consistent with the low activity of human lens aldose reductase (Jedziniak et al., 1981).

ACKNOWLEDGMENTS

This project was supported by NIH grant EY 01276, the Cooperative Cataract Research Group Consortium (NIH grant EY 03247), and the Brigham Surgical Group Foundation.

REFERENCES

Cheng, H., Chylack, L. T., Jr and von Saltza, I. (1981). Supplementing glucose metabolism in human senile cataracts. *Invest. Ophthalmol. Vis. Sci.* **21**, 812–18.

Chylack, L. T., Jr, Henriques, H. F., III and Tung, W. H. (1979). Inhibition of sorbitol production in human lenses by an aldose reductase inhibitor. In *Documenta Ophthalmologica Proceedings Series*, Vol. 18. (Ed. Hockwin, O.). Dr W. Junk: The Hague.

Chylack, L. T., Jr, Lee, M. R., Tung, W. H. and Cheng, H. (1983). Classification of human senile cataractous change by the American Cooperative Cataract Research Group (CCRG) method. I. Instrumentation and technique. *Invest. Ophthalmol. Vis. Sci.* **24**, 424–31.

Giblin, F. J., McCready, J. P. and Reddy, V. N. (1982). The role of glutathione metabolism in the detoxification of H_2O_2 in rabbit lens. *Invest. Ophthalmol. Vis. Sci.* **22**, 330–5.

Gonzalez, R. G., Barnett, P., Cheng, H. and Chylack, L. T., Jr (1984). Altered phosphate metabolism in the intact rabbit lens under high glucose conditions and its prevention by an aldose reductase inhibitor. *Exp. Eye Res.* **39**, 553–62.

Gonzalez, R. G., Barnett, P., Aguayo, J., Cheng, H. and Chylack, L. T., Jr (1984). Direct measurement of polyol pathway activity in the ocular lens. *Diabetes* **33**, 196–9.

Jedziniak, J. A., Chylack, L. T., Jr, Cheng, H., Gillis, M. K., Kalustian, A. A. and Tung, W. H. (1981). The sorbitol pathway in the human lens: aldose reductase and polyol dehydrogenase. *Invest. Ophthalmol. Vis. Sci.* **20**, 314–26.

Newsholme, E. A. and Start, C. (1973). *Regulation in Metabolism.* John Wiley & Sons: London.

Wolfe, J. K., Gillis, M. K. and Chylack, L. T., Jr (1985). Glucose metabolism in the calf lens. *Exp. Eye Res.* **40**, 629–41.