The Effect of Oxidation on Sorbitol Pathway Kinetics

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SUMMARY

The rapid conversion of glucose to sorbitol by aldose reductase and the consequent hyperosmolarity of the cytoplasm has been shown to be the primary cause of the so-called "sugar" or "osmotic" cataract in many animal lenses. It is not as clear, however, that hyperosmolarity is the principal factor in the etiology of cataracts in human diabetic subjects. In fact, the comparatively low activity of aldose reductase in the human lens as compared with several animal lenses, and the osmotically insignificant levels of sorbitol pathway products (sorbitol and fructose), suggest that hyperosmolarity, per se, may not be as important a factor in human cataract formation as it is in animals.

We present evidence that the flux of glucose and sorbitol through the rat lens is markedly reduced by oxidative stress (0.1 mM H_2O_2). Sorbitol accumulation is reduced by 114%, sorbitol turnover is reduced by 78%, sorbitol production is reduced by 90%, fructose accumulation is reduced by 60%, and fructose turnover is reduced by 76% in the presence of 36 mM glucose. H_2O_2 does not affect glucose turnover, the glucose rate constant, or the ATP level significantly at 36 mM glucose, but at 5.5 mM glucose, 0.2 mM H_2O_2 leads to a rapid loss of ATP that can be prevented by 0.04 mM sorbinil, an aldose reductase inhibitor.

These results suggest that inhibition of aldose reductase by sorbinil renders rat lenses better able to cope with oxidative stress. In the absence of an aldose reductase inhibitor, elevating ambient glucose may render a lens less able to scavenge oxidants by diverting NADPH into sorbitol production. The importance of the rate of flux of glucose through the sorbitol pathway, rather than the absolute concentration of sorbitol or fructose, is stressed in considering the mechanisms underlying complications of diabetes mellitus.

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Hyperglycemic activation of the sorbitol pathway is associated with detrimental effects in the lens and other tissues. In animal lenses exposed to high ambient glucose conditions, the rapid accumulation of sugar alcohols causes swelling and opacity over a period of hours to days because cell membranes are not permeable to these compounds, which collect in the lens, causing an "osmotic stress."[1-3] The concomitant morphologic and biophysical changes are readily explained by a rapid influx of extracellular water into the lens. The sorbitol pathway is active in human nerve, and sorbitol accumulation has been verified in this tissue in diabetic subjects but in low (<1 mM) concentrations.[4,5]

Similar aldose reductase activity has been recently documented in bovine retinal and cerebral microvasculature.[6] Tissue damage under high-glucose conditions was not demonstrated in cerebral vascular endothelium, so that the role of sorbitol accumulation in damage to these tissues is uncertain.

The "osmotic stress hypothesis" does not explain chronic damage in diabetic tissues, particularly the lens. Aldose reductase (AR) from human lenses has a higher K_m for glucose than AR from other species,[7] and sorbitol accumulation in senile diabetic lenses is not high enough to account for a significant osmotic stress (0–0.4 μmol/g lens).[8] Chronic damage may be the direct result of elevated glucose levels, e.g., nonenzymatic glycosylation of enzymes and other proteins,[9] or, more likely, may be due to related metabolic derangements. Both AR and glutathione reductase (GR) require NADPH as a cofactor. This competition is illustrated in Figure 1. Both oxidation and sorbitol production cause activation of the hexose monophosphate shunt, the principal source of NADPH in the lens.[10] It is possible that an active sorbitol pathway competing for NADPH chronically hampers the ability of a tissue to scavenge oxidants, such as H_2O_2.[11] Availability of NADPH is directly affected by the rate of flux through the pathway, so that sorbitol production rather than sorbitol accumulation alone is emphasized in this mechanism in ex-

plaining deleterious effects. Inhibition of the sorbitol pathway by an AR inhibitor (sorbinil) enables the lens to recover much more rapidly and completely from an oxidative challenge.

MATERIALS AND METHODS

Materials. Lenses were obtained from 100–125-g male Sprague-Dawley albino rats. The incubation media consisted of TC199/bicarbonate buffer, as previously described,[3] which was adjusted to the desired concentrations of glucose (12, 24, or 36 mM). The osmolarity was adjusted to 295 ± 5 mosmol using sorbitol. H_2O_2, 0.1 mM, was used as an oxidant. For C-13 NMR (nuclear magnetic resonance) experiments, the media were prepared with $^{13}C_1$-glucose (Merck and Co., Rahway, New Jersey). Incubations were carried out in an environment of 95% air, 5% CO_2, and 100% humidity at 37°C.

Sorbitol, glucose, and fructose concentrations were determined by enzyme assays as previously described by Bergmeyer.[12] These assays were used to calibrate the NMR intensity data for each NMR experiment.

Nuclear magnetic resonance (NMR) spectroscopy. All experiments were carried out on a superconducting Bruker HX-270 that operated at 109.3 MHz for P-31 and 68 MHz for C-13. Natural line widths using standards were <2 Hz. The spectrometer was operated without a deuterium lock under proton-decoupled conditions in the Fourier transform mode. P-31 NMR experimental parameters were: sweep width, ±5000 Hz; RF pulse, 12 μs (57°); delay time, 0.1 s; acquisition time, 0.4096 s; total scan time, 50 min/spectrum; and 20-Hz exponential convolution, with methylene diphosphonic acid in an eccentrically placed capillary as an external reference standard. C-13 NMR parameters: sweep width, ±10,000 Hz; RF pulse, 16 μs (67°); delay time, 0.4 s; total scan time, 30 min/spectrum; and 4-Hz exponential convolution. The beta resonance of glucose was used as an internal reference standard for chemical shift. Other parameters: quadrature phase detection, 1-W proton-decoupling power (normal gating), with 4K (P-31) or 8K (C-13) data points per acquisition. Lenses were placed carefully in 10-mm NMR tubes on a Teflon vortex plug to center them in the magnetic field. A Nicolet 1080 computer was used with NTCFT software for integration, peak height analysis, and other data manipulations.

To assess normal sorbitol pathway kinetics, lenses were preincubated in media containing 12, 24, or 36 mM $^{13}C_1$-glucose for at least 24 h, and placed in an NMR tube. This was followed either by spectral acquisition over the next several hours, i.e., "saturated label" experiments, or by reincubating the lenses in medium containing unlabeled glucose, followed by spectral acquisition, i.e., "pulse-chase" experiments.

To assess changes in sorbitol pathway kinetics with the addition of an oxidative stress, lenses were preincubated in media containing 12, 24, or 36 mM $^{13}C_1$-glucose for at least 24 h. C-13 NMR spectra were accumulated every 0.5 h for 4 h to observe sorbitol and fructose accumulation rates. The lenses were then rinsed with and incubated in either labeled or unlabeled medium containing 0.1 mM H_2O_2, and spectral acquisition was continued. A similar experiment was carried out in which the onset of oxidative stress and high-glucose conditions was simultaneous. In this case, preincubation was in medium containing 5.5 mM glucose.

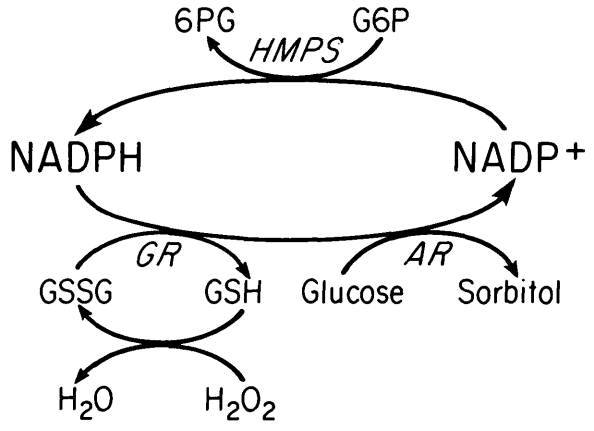

FIGURE 1. Aldose reductase, the first enzyme in the sorbitol pathway, competes with glutathione reductase for the cofactor NADPH. NADPH regeneration depends in turn on hexose monophosphate shunt activity.

P-31 NMR spectra were acquired on lenses incubated in 36 mM glucose with 0.1 mM H_2O_2 over a period of 8 h. One spectrum was acquired each hour.

The protective effect of AR inhibition on the lens was evaluated by preincubating lenses overnight in medium containing 36 mM glucose, and then incubating in medium containing both 36 mM glucose and 0.1 mM H_2O_2, either with or without the AR inhibitor, sorbinil (0.04 mM). Again, P-31 spectra were acquired over 8 h at one spectrum per hour. Peaks corresponding to sugar phosphates (mostly alpha-glycerophosphate), Pi, and the alpha-, beta-, and gamma-ATP peaks were integrated and expressed in terms of percent of total phosphorus integral. The integrals were then plotted against time. Since the beta-ATP peak is the only ATP peak that is not contributed to by other minor resonances, the fraction of beta-ATP of original was computed and plotted against time. These data were not intended to be quantitative, but rather a qualitative indication of the recovery of the lens as indexed by relative changes in ATP levels.

Kinetic analyses. Analysis of sorbitol pathway kinetics is described below. Flux rates were determined from a combination of C-13 NMR studies and specific enzymatic assays as follows. Sorbitol production from glucose (J_{gs}) is equal to sorbitol accumulation ($\Delta[S]$) added to the amount of sorbitol converted to fructose (J_{sf}), as expressed by the equation:

$$J_{gs} = \Delta[S] + J_{sf} \quad (1)$$

Fructose production from sorbitol is:

$$J_{sf} = \Delta[F] + J_{fo} \quad (2)$$

where J_{fo} is the rate of conversion of fructose to other products, and $\Delta[F]$ is the accumulation of fructose.

The reverse reactions do not occur to a significant extent.[13] Evidence that this is true stems from the observation that, after withdrawal of labeled glucose from the incubation medium, glucose essentially disappears over 3–4 h, while substantial sorbitol and fructose peaks remain. Labeled lactate continues to be produced while the sorbitol and fructose resonances decrease according to first-order kinetics. Furthermore, lenses incubated in medium containing ^{14}C-fruc-

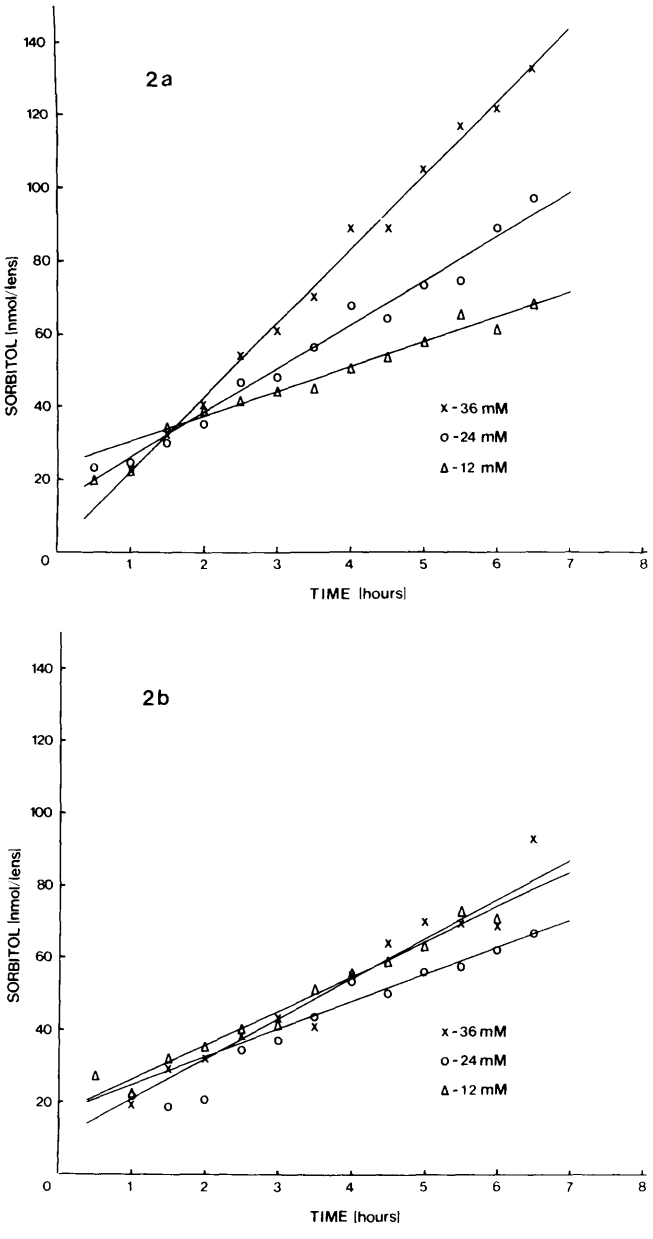

FIGURE 2. Plot (A) shows the accumulation with time of sorbitol when lenses are incubated overnight in medium containing 5.5 mM unlabeled glucose, and then placed in medium containing 12, 24, and 36 mM $^{13}C_1$-glucose immediately before beginning NMR acquisition. Each point represents the intensity of the sorbitol peak from a single 0.5-h C-13 NMR spectrum. Spectra were accumulated serially for a total of 8 h. Plot (B) shows the rates of sorbitol accumulation with the same concentrations of glucose in the incubation medium, but with the simultaneous addition of 0.1 mM H_2O_2. Note the decrease in sorbitol accumulation with the addition of oxidant so that the maximum sorbitol production rate seems to occur at 12 mM. The maximum rate without oxidation cannot be estimated, but the increase in sorbitol accumulation with increasing glucose concentration appears to be approximately linear.

tose showed only a fructose and a lactate peak by high-pressure liquid chromatography analysis, with no ^{14}C-labeled sorbitol or glucose detectable.

First, Δ[S] and Δ[F] were determined. This was accomplished by incubating lenses in $^{13}C_1$-glucose and plotting the increase in sorbitol peak intensity with time. Immediately after the acquisition of the final spectrum, the lenses were frozen in liquid nitrogen and assayed for glucose, fructose, and sorbitol (N = 6 for each experiment). The sorbitol and fructose concentrations by biochemical assay could then be equated to the sorbitol and fructose peak intensities of the final spectra. After converting from peak intensity, which is in arbitrary units, to concentration, the slope of the plots of sorbitol and fructose concentration versus time were equal to Δ[S] and Δ[F], respectively.

A set of pulse-chase experiments was performed. Once again, plots of labeled metabolite concentrations with time were made. Two methods for calibrating peak intensity in terms of metabolite concentration were compared. First, the average metabolite concentration per unit intensity from the experiments with saturated label were used. Then, using the accumulation rates, Δ[S] and Δ[F], determined in the saturated label experiments along with metabolite concentrations determined at the end of the pulse-chase experiment by enzymatic assay, metabolite concentrations at the beginning of the experiment could be extrapolated, and the initial ratio of metabolite concentration to peak intensity determined. The values obtained by these two different methods varied by 0–5%. Values determined via the second method were used to calculate the data presented, so that for each value in Tables 1 and 2, both NMR and enzymatic assay data were determined from the same set of lenses.

Labeled glucose declined to background levels 2–4 h after the beginning of the "chase." During the initial decline of the glucose label, sorbitol label stayed approximately constant. After that, the rate of disappearance of sorbitol label (M_s) is equal to label being converted to sorbitol minus label being converted to fructose, or:

$$M_s = J_{gs} \times G_r/G_i - J_{sf} \times S_r/S_i \quad (3)$$

where G_r and G_i represent the residual and initial glucose label, respectively, and S_r and S_i represent the residual and initial sorbitol label, respectively. M_s is simply the slope of the plot of ^{13}C-sorbitol peak intensity versus time, after conversion from intensity units to units of nanomoles per lens. At the disappearance of the glucose label, G_r is essentially 0, and $S_r/S_i = 1$, so that:

$$M_s = -J_{sf} \quad (4)$$

Similarly, the change in fructose label may be expressed by the equation:

$$M_f = J_{sf} \times S_r/S_i - J_{fo} \times F_r/F_i \quad (5)$$

Again, at the beginning of the experiment, S_r/S_i and $F_r/F_i = 1$. Substituting for J_{sf} gives:

$$J_{fo} = M_s - M_f \quad (6)$$

Thus, with a combination of steady-state and pulse-chase experiments, all of the flux and accumulation rates for the sorbitol pathway may be determined.

Since the accumulation of label and the flux rates derived from them (J) are linear, the reaction constants are equal to the flux rates $k_{gs} = J_{gs}$, $k_{sf} = J_{sf}$, and $k_{fo} = J_{fo}$.

Glucose utilization is a first-order process, and thus may

TABLE 1
Flux rates of sorbitol and fructose

Glucose conc.	1 Sorbitol accum. Δ[S]	2 Sorbitol turnover J_{sf} (4 + 5)	3 Sorbitol produced J_{gs} (1 + 2)	4 Fructose accum. Δ[F]	5 Fructose turnover J_{fo}
12 mM	6.2 (0.5)	1.7 (0.4)	7.9 (0.6)	0.0 (0.2)	1.7 (0.3)
$+H_2O_2$	1.1 (0.1)	3.2 (0.7)	4.4 (0.7)	−0.7 (0.1)	3.9 (0.7)
24 mM	8.0 (0.7)	7.7 (1.0)	15.7 (1.2)	−0.4 (0.1)	8.1 (1.0)
$+H_2O_2$	1.0 (0.02)	3.9 (0.2)	4.9 (0.2)	−0.4 (0.05)	4.3 (0.2)
36 mM	12.2 (0.9)	26.0 (3.4)	38.2 (3.5)	0.0 (0.3)	26.0 (3.4)
$+H_2O_2$	−1.7 (0.2)	5.7 (0.8)	4.0 (0.8)	−0.6 (0.1)	6.2 (0.8)

All units are nmol/lens/h. Values were calculated by a combination of linear regression analysis of C-13 NMR assays of living lenses accumulated every half hour and specific enzyme assays, as described in MATERIALS AND METHODS. Values in parentheses indicate standard deviation.

be described by a rate constant $k_g = \Delta(\ln[\text{glucose}])/t$. Rate constants for glucose utilization were determined by making a semilog plot of the NMR peak intensity data and using linear regression analysis to determine slope and y-intercept (all r-values >0.94). Enzymatic assays for glucose (N = 6 for each concentration) were used to calibrate the y-intercept in terms of glucose concentration, and the slope was converted to ln[glucose] in nanomoles per lens. Glucose concentrations as predicted by NMR intensities differed from assay values by <5%.

RESULTS

The effect of competition for the cofactor NADPH may be seen in Figures 2A and 2B. In each case, 10 rat lenses were incubated overnight in 5.5 mM glucose, then transferred to medium containing 12, 24, or 36 mM glucose with or without 0.1 mM H_2O_2 immediately before spectral acquisition. Sorbitol accumulation rates (Δ[S]) are recorded in Table 1. In the control lenses (Figure 2A and Table 1), sorbitol accumulation was approximately proportional to glucose concentration. When 0.1 mM H_2O_2 was added to the incubation medium, there was a dramatic decrease in sorbitol accumulation (Figure 2B and Table 1). The maximum rate of conversion of glucose to sorbitol was reduced, so that increasing glucose concentrations above 12 mM did not increase sorbitol accumulation.

Sorbitol accumulation, as demonstrated by the saturated label experiments (preincubated in 36 mM $^{13}C_1$-glucose), is shown in Figure 3. Note again the decrease in sorbitol accumulation (Δ[S]) with the addition of H_2O_2 to the incubation medium. Flux rates and rate constants were determined by linear regression analysis at the three different glucose concentrations and are recorded in Tables 1 and 2. With oxidation, the maximum rate of sorbitol production occurred at a glucose concentration of about 8 mM. With 36 mM glucose and H_2O_2 in the incubation medium, there was a net decrease in the sorbitol level (Δ[S]) in the lens, while sorbitol flux (J_{sf} or k_{sf}) was still significant. Fructose accumulation (Δ[F]) was generally close to zero at steady state, so that the turnover (J_{fo} or k_{fo}) was approximately equal to that of sorbitol. Fructose accumulation and flux data are also recorded in Table 1.

Figure 4 shows glucose, sorbitol, and fructose peak intensities plotted versus time for a typical pulse-chase experiment. For this experiment, lenses were preincubated for 24 h in medium containing 36 mM $^{13}C_1$-glucose, then transferred to medium containing 36 mM glucose and 0.1 mM H_2O_2. The concentration of labeled sorbitol remained about the same until the glucose label approached background level. It then decreased linearly for 3 h ($r > 0.99$) at a rate of 5.7 nmol/lens/h [M_s, see Eqs. (3) and (4)]. This rate reflects sorbitol to fructose flux [J_{sf}, Eq. (4)]. The fructose label also decreased linearly (M_f), implying a more rapid turnover of fructose (J_{fo}) than sorbitol, with 0.8 nmol/lens/h net decrease in fructose {(Δ[F], Eq. (2)}. At 5 h, an abrupt acceleration of both sorbitol and fructose label turnover occurred (26.0 and 4.5 nmol/lens/h, respectively). Unfortunately, sorbitol accumulation cannot be measured simultaneously because of the nature of the pulse-chase experiment, but in the analogous saturated label experiments, an abrupt increase in sorbitol production could be seen at 2–6 h. The timing and extent of the recovery of sorbitol accumulation were variable.

Glucose flux rates and rate constants were determined as previously described[14] and are compiled in Table 2. Glucose turnover was not affected greatly by oxidation. Rate constants were found to be similar to those determined for the rabbit lens.

As found previously for the rabbit lens, sorbitol accumu-

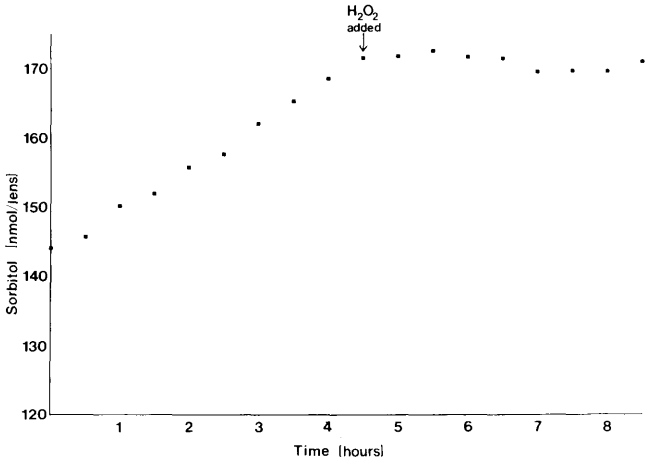

FIGURE 3. Sorbitol peak intensity versus time; the sorbitol pool remains saturated with ^{13}C-label. Sorbitol accumulation essentially stops with the addition of 0.1 mM H_2O_2 to the incubation medium. There is still a significant flux through the sorbitol pathway, however, as determined by "pulse-chase" type experiments.

TABLE 2
Glucose turnover rates

Glucose conc.	H_2O_2	Glucose turnover (nmol/lens/h)	Glucose rate constant (h^{-1})
12 mM	−	14.8 (2.4)	0.58 (0.09)
	+	19.8 (3.1)	0.39 (0.06)
24 mM	−	49.2 (1.4)	0.48 (0.01)
	+	45.5 (7.1)	0.43 (0.07)
36 mM	−	104.4 (10.2)	0.40 (0.04)
	+	88.4 (13.9)	0.38 (0.06)

Values derived from C-13 NMR and enzyme assay data as in Table 1.
Rate constants derived from linear regression of ln[glucose] versus time.

lation and flux rates were surprisingly high,[14] accounting for almost one-third of the total glucose turnover at 36 mM.

Changes in beta-ATP resonance, expressed in terms of fraction of initial resonance, are shown for various conditions in Figure 5. Similar results were obtained by plotting the ratio of beta-ATP to Pi. By plotting the change in the ratios of resonance areas, T1 attenuation effects are held constant. Note that, in general, beta-ATP resonances in lenses treated with sorbinil remained above initial values, while in those without sorbinil, it decreased initially with subsequent recovery. The most important feature of these data is that the final ATP levels were not significantly decreased, ruling out extensive irreversible damage to lens function. It is interesting that the level continued to rise in the high-glucose group with sorbinil but without oxidative stress. This was a reproducible effect.

The short-term protective effect of sorbinil against oxidation at normal glucose concentrations is shown in Figure 6. Here, rat lenses were exposed to 0.2 mM H_2O_2 with 10 paired lenses in each sample; 0.04 mM sorbinil was included in the medium of one set of lenses. It can be seen from this plot of total ATP resonance versus time that with AR inhibition, initial ATP levels were maintained, whereas without the inhibitor, ATP levels dropped sharply. It was noted that lenses treated with AR inhibitor retained clarity (based on visual inspection) and survived longer (based on retention of ATP levels with time) than did lenses without AR inhibition, although quantitative statistics were not tabulated.

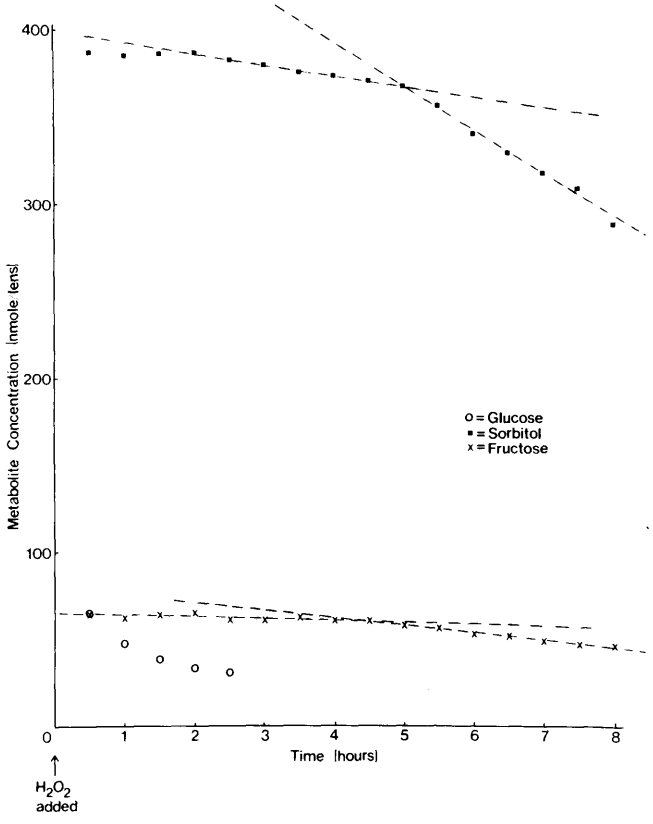

FIGURE 4. ^{13}C-metabolite peak intensity versus time in a pulse-chase type experiment. Sorbitol to fructose conversion increases by a factor of 4.5 after 5 h. The persistence of labeled glucose is responsible for the initial flat portion of the sorbitol curve. In other experiments, in which "chase" was not employed, a parallel increase in sorbitol accumulation was observed. There is clearly a resumption of sorbitol pathway activity. The reason for the abruptness of this resumption is unclear.

DISCUSSION

These data show that sorbitol production in the rat lens under high-glucose conditions is modulated by the presence of H_2O_2 in the incubation medium. The kinetics of this interaction indicate a competition for NADPH, which is a cofactor for both AR and GR. The turnover of the NADPH/NADP pool is remarkably rapid. Our data indicate a turnover time of 50 s based on sorbitol flux alone, using total NADPH/NADP levels in the rat lens as determined by Giblin and Reddy.[15] This may underestimate the turnover rate, since the cycling assay used does not discriminate between free and bound cofactors.

Higher glucose utilization at 12 mM (Table 2) with oxidation probably reflects increased demand of the hexose monophosphate shunt (HMPS) along with the lack of inhibition of

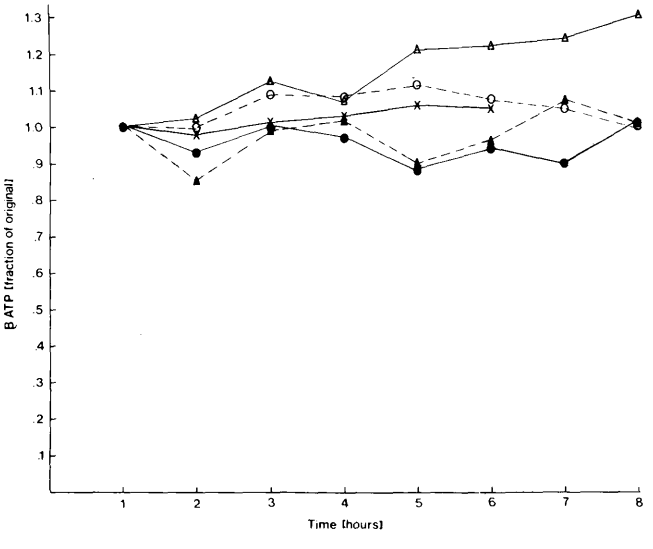

FIGURE 5. Beta-ATP resonance as determined by P-31 NMR, expressed as percent of original, and plotted against time. X = 5.5 mM glucose, without H_2O_2, without sorbinil (control); ▲ = 36 mM glucose, 0.1 mM H_2O_2, without sorbinil; ● = 5.5 mM glucose, 0.1 mM H_2O_2, without sorbinil; △ = 36 mM glucose, 0.1 mM H_2O_2, 0.04 mM sorbinil; and ○ = 5.5 mM glucose, 0.1 mM H_2O_2, 0.04 mM sorbinil. Original ATP levels appear to be reestablished under all of the experimental conditions. It is interesting that ATP levels increase overall only under high-glucose conditions with sorbinil.

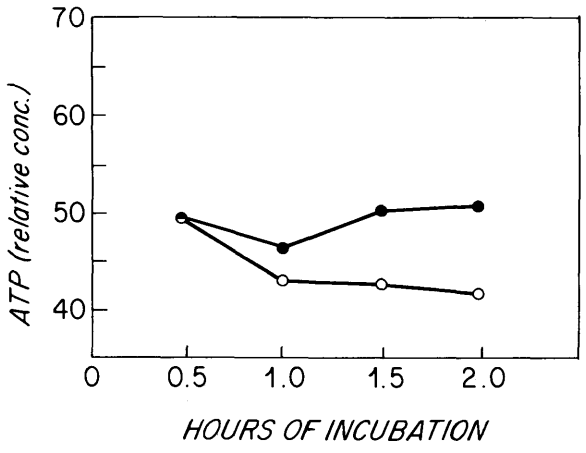

FIGURE 6. The short-term protective effect of sorbinil on ATP levels is demonstrated. Both samples were incubated in medium containing 5.5 mM glucose and 0.2 mM H_2O_2. The sample in medium containing 0.04 mM sorbinil maintained its original ATP level, while the one without an AR inhibitor showed a pronounced drop over 2 h. In this case, the percent of total phosphorus resonance of all three ATP peaks as determined by P-31 NMR was summed.

the sorbitol pathway by oxidation at this concentration of glucose. The decrease in glucose utilization under high-glucose conditions with the addition of H_2O_2 may be due to generalized damage to glycolytic enzymes, to inhibition of the sorbitol pathway, or both. It is interesting that the decrease in glucose flux under high-glucose conditions with and without oxidant is almost exactly one-half of the difference in the sorbitol pathway flux rates. This suggests that the entire difference in glucose flux may be due to the decrease in sorbitol flux with an increase in HMPS activity due to oxidation. This must be hypothesized with caution because of the possibility of damage to glycolytic enzymes with 0.1 mM H_2O_2. From these experiments, there is no evidence that glucose entry into the lens is decreased or that glycolytic flux is significantly reduced.

Since the function of AR has been shown to depend on reduced sulfhydryl groups,[16] it seemed very possible that the observed inhibition of sorbitol production was in fact due to direct oxidation of the enzyme. An attempt was made to exclude this mechanism as the principal cause of the observed changes in sorbitol accumulation. Figure 4 shows a pulse-chase experiment in which the level of labeled sorbitol versus time appears to demonstrate recovery of sorbitol pathway activity. Two distinct slopes may be fitted by linear regression, each with an r-value of >0.99. The ratio of the recovered flux rate to the initial is 4.5 to 1, and the final rates are identical to those determined for the sorbitol pathway in the absence of oxidation. In a similar experiment in which the lenses remained saturated with ^{13}C-label, recovery of sorbitol production to up to 90% of its initial rate could be seen. The abruptness of this apparent recovery is intriguing. It is probably a result of detoxification of H_2O_2 from the incubation medium and the lenses, and implies a critical level of oxidant below which the sorbitol pathway activity is switched on. The higher affinity of GR for NADPH may play a role in this phenomenon.[7,17]

The purpose of the P-31 NMR studies with 0.1 mM H_2O_2 was to show that the lens's energy metabolism was essentially intact. There was no significant difference in ATP levels over 8 h between lenses incubated in media containing 5.5 mM glucose, 5.5 mM glucose and 0.1 mM H_2O_2 with and without 0.04 mM sorbinil, and 36 mM glucose with 0.1 mM H_2O_2. The lenses incubated in 36 mM glucose with 0.1 mM H_2O_2 and 0.04 mM sorbinil showed a steady increase in ATP.

The effect of high-glucose levels on GR activity is difficult to establish directly. While depletion of GSH under high-glucose conditions has been found,[18] depletion of NADPH or of GSH under high-glucose conditions with the addition of an oxidant has not been documented. Glutathione assays under various conditions and evaluations of lens functions as protected by GSH are being carried out. However, direct assay might obscure deleterious effects resulting from localized GSH depletion, for example, at the outer epithelial membrane where H_2O_2 and glucose enter the lens and most transport takes place. Alternatively, the availability of GSH to protect lens function, e.g., the Na,K-ATPase, would probably be best assessed by functional assay of the ATPase.

The data presented here suggest that chronic, pathologic changes under high-glucose conditions are due in part to a chronic depression of protection against oxidation. Sorbitol accumulation per se is not of significance for this mechanism; rather, flux through the sorbitol pathway is. If polyol dehydrogenase activity is high, as it is in humans, there can be significant polyol pathway flux with little or no net sorbitol accumulation. The mechanism proposed is a competition for NADPH, as evidenced by rapid turnover of NADPH→NADP, the effect of glutathione pathway activation on AR kinetics, and the effect of AR inhibition on energy metabolism in lenses under various conditions.

Morrison et al.[19] found significant consumption of glucose through the sorbitol pathway in red cells (3%) at 5 mM glucose. In our study, C-13 NMR assays are not sensitive enough to document sorbitol flux at normal concentrations of glucose. This information needs to be acquired, either through more laborious enzyme assays or with the extrapolation of NMR-acquired data. The latter would require enough data points to give a reliable curve for sorbitol flux versus glucose concentration.

If AR inhibition can enhance the ability to scavenge oxidants in a tissue that possesses sorbitol pathway activity, long-term administration may prevent chronic, cumulative oxidative damage. This may also be true to a lesser extent under normoglycemic conditions, which could have profound implications as to the possible therapeutic effects of AR inhibition on the aging process.

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