3-FG as Substrate for Investigating Flux through the Polyol Pathway in Dog Lens by $^{19}$ F-NMR Spectroscopy

Martin J. Lizak,1 E. Filippo Secchi,1 Jung Wha Lee,1 Sanai Sato,1 Eri Kubo,2 Yoshio Akagi,2 and Peter F. Kador1

**PURPOSE.** To investigate flux through the polyol pathway in the dog lens by $^{19}$ F-nuclear magnetic resonance ($^{19}$F-NMR) spectroscopy, using 3-fluoro-3-deoxy-D-glucose (3-FG) as a substrate.

**METHODS.** 3-FG metabolism was monitored by $^{19}$F-NMR analysis. Dog lenses were incubated in Dulbecco's modified Eagle's medium containing 10 mM 3-FG. Enzymatic reductase and dehydrogenase activities were spectrophotometrically determined, whereas the analyses of 3-FG metabolites were conducted by $^{19}$F-NMR analysis. Aldose reductase (AR) was immunohistochemically localized in dog lens with antibodies raised against dog kidney AR.

**RESULTS.** $^{19}$F-NMR spectra indicate that incubation of purified dog lenses AR with 3-FG results in the formation of 3-fluoro-3-deoxy-D-sorbitol (3-FS) and that incubation of dog liver sorbitol dehydrogenase (SDH) with 3-FS results in the formation of 3-fluoro-3-deoxy-3-fructose (3-FF). This confirms that 3-FG is metabolized to 3-FF by the polyol pathway enzymes. The affinity ($K_m$) of AR for 3-FG is ~20-fold better than that for D-glucose, whereas the $K_m$ of SDH for 3-FS was fourfold less than for D-sorbitol. 3-FG in cultured dog lenses is metabolized primarily to 3-FS; however, small amounts of 3-FF and 3-fluoro-3-deoxy-D-glucuronate (3-FGA) are also formed. 3-FS formation was reduced by the AR inhibitor AL 1576, and 3-FF formation was eliminated by the SDH inhibitor CP-166,572. In dog lens epithelial cells cultured with 3-FG, only 3-FS is formed. Similarly, only 3-FS is formed when lens capsule containing primarily epithelial lens contaminated with superficial epithelial cells was incubated in 3-FG. Similar incubation of the remaining cortex resulted primarily in the formation of 3-FS and 3-FGA. This enzymatic distribution was confirmed by spectrophotometric activity analysis and the immunohistochemical localization of AR.

**CONCLUSIONS.** The data confirm that flux through the polyol pathway primarily results in sorbitol accumulation. The absence of fructose and gluconic acid from cultured lens epithelium suggests that the epithelial cells primarily contain AR, whereas differentiated fiber cells also contain SDH and glucose dehydrogenase. (Invest Ophthalmol Vis Sci. 1998;39:2688-2695)

Aldose reductase (EC 1.1.1.21) together with sorbitol dehydrogenase (EC 1.1.1.19) forms the polyol (sorbitol) pathway where glucose is metabolized to fructose through the sugar alcohol sorbitol. Because glucose is rapidly phosphorylated by hexokinase, glucose metabolism through this pathway is minimal under normal physiological conditions. However, with hyperglycemia resulting from diabetes, the flux of glucose through the polyol pathway is significantly increased. This results in the abnormal accumulation of sorbitol, which has been linked to the onset of various diabetic complications.1-3

The importance of increased sorbitol accumulation in sugar cataract formation has been experimentally established through the use of animal models and through prevention studies with aldose reductase inhibitors. For example, the onset and severity of sugar cataract formation directly correlate with lenticular levels of aldose reductase. The lenses of the rat, degu (Octodon degus), and Mongolian gerbil contain a high level of aldose reductase and quickly develop sugar cataracts, whereas mice do not develop sugar cataracts even under prolonged diabetic or galactosemic conditions because their lenses contain extremely low levels of aldose reductase. In contrast, transgenic mice in which the human aldose reductase gene is overexpressed in their lenses, quickly develop sugar cataracts.6-7 Prevention studies indicate that sugar cataract formation is inhibited by the administration of aldose reductase inhibitors.4,8-14

Recently, it has also been proposed that redox changes initiate or contribute to the pathogenesis of diabetic complications. Known as the pseudohypoxia1,5 and/or reduced redox state hypothesis,1,5,10 this concept emphasizes that the alteration of the NAD+ /NADH ratio by the increased oxidation of sorbitol to fructose rather than the accumulation of excess sorbitol is important in initiating the diabetic complication. Because to date no direct methods have been available for measuring flux through the polyol pathway, inhibitor studies have been used primarily to probe the relative importance of aldose reductase versus sorbitol dehydrogenase in initiating diabetic complications. Aldose reductase inhibitors, which directly inhibit the aldose reductase catalyzed sorbitol formation and indirectly inhibit sorbitol dehydrogenase by reducing the...
availability of its sorbitol substrate, has been clearly shown to be beneficial in ameliorating diabetic complications. In contrast, the effect of inhibiting sorbitol dehydrogenase has been mixed. A complicating factor in clarifying the relative importance of these two enzymes in diabetic complications comes from our inability to directly measure the flux of glucose through the polyol pathway in intact tissues.

Nuclear magnetic resonance (NMR) spectroscopy represents a promising powerful method for simultaneously evaluating the reduction of glucose and the oxidation of sorbitol through the polyol pathway in intact tissues. The use of 19F-NMR spectroscopy to monitor fluorinated glucose metabolism was first reported by Nakada et al. Fluorine has a spin sensitivity similar to that of hydrogen, and its background signal is negligible because endogenous fluorine is not present in most tissues. Metabolites of fluoroglucose possess chemical shifts that are distinct and easily identifiable. Using 19F-NMR spectroscopy, 2-fluoro-2-deoxy-β-glucose has been used to investigate glucose metabolism in the rat brain, and 3-fluoro-3-deoxy-β-glucose (3-FG) has been used to monitor aldose reductase activity. Subsequently, Karino et al. reported that 3-FG is metabolized to 3-fluoro-3-deoxy-β-sorbitol (3-FS), 3-fluoro-3-deoxy-β-fructose (3-FF), and 3-fluoro-3-deoxy-β-gluconic acid (3-FGA) in dog lenses. Unlike 2-fluoro-2-deoxy-β-glucose, 3-FG is not phosphorylated by hexose kinase, and phosphorylated metabolites have not been observed. Here, we report the feasibility of using 3-FG to measure flux through the polyol pathway.

MATERIALS AND METHODS
Reagents and Chemicals
Unless otherwise stated, all chemicals used in this study were of reagent grade. Reagents for column chromatography were purchased from Pharmacia-LKB Biotechnology (Uppsala, Sweden). 3-FG and 3-FS were obtained from Omicron Biochemicals (South Bend, IN). Media and media supplements for lens and cell cultures were obtained from Biofluids (Rockville, MD). The aldose reductase inhibitor AL 1576 (2,4-difluorospirofluorene-zino-[2-hydroxymethyl]pyrimidine) was obtained from Pfizer Central Research (Groton, CT).

Beagle eyes were obtained from Marshall Farms USA (North Rose, NY). Eyes used in this study were immediately enucleated after the animal's death and stored on ice for overnight delivery. All eyes were used within 24 hours of enucleation. Frozen dog liver was purchased from Pel Freeze (Rogers, AK).

Enzyme Preparation
Aldose reductase was purified from dog lens by a series of chromatographic procedures as previously reported. Sorbitol dehydrogenase was partially purified from dog liver by gel chromatography. Dog liver was homogenized with 20 mM phosphate buffer (3 ml), pH 7.5, containing 7 mM 2-mercaptoethanol. After centrifugation at 10,000g for 10 minutes, the supernatant was applied to a column of Sephacryl S-300 (HiPrep 16/60; Pharmacia-LKB). The column was developed with the same phosphate buffer at the flow rate of 1 ml/min, and the eluent was collected into 1-ml fractions. The fraction containing the highest activity of sorbitol dehydrogenase was used for the kinetic and incubation studies, with 3-FS as the substrate.

Determination of Reductase and Dehydrogenase Activities
Aldose reductase activity was monitored by following the spectrophotometric decrease of NADPH at 340 nm for 4 minutes. The reaction mixture contained 0.3 mM NADPH, 100 mM α-xylose substrate, 0.1 M phosphate buffer, pH 6.2, and 2 to 3 μM enzyme. Sorbitol dehydrogenase activity was monitored by following increases of NADH at 340 nm with 50 mM of α-sorbitol as substrate as described by Maret and Auld. Each 1.0-ml cuvette contained equal units of enzyme, 0.10 M glycine/NaOH buffer, pH 10, 0.3 mM NADH with/without 10 mM substrate, and inhibitor. Glucose dehydrogenase activity was assayed at pH 10 by monitoring increases of NADH at 340 nm with 100 mM α-D-glucose as a substrate. One enzyme unit was defined as the amount of enzyme consuming or producing 1 μM of either NADPH or NADH per minute under assay conditions. Kinetic calculations were conducted using the binkin2 public procedure of the Prophet computer program (Bolt, Berak, and Newman, Cambridge MA). Protein concentrations were determined according to the method of Bradford using bovine serum albumin as the standard.

Immunohistochemical Staining
Immunohistochemical studies with antibodies against dog kidney aldose reductase were conducted as previously described. Briefly, excised dog lenses were washed with graded (10%, 15%, 20%) sucrose solutions in 0.1 M phosphate-buffered sulfate (PBS), frozen, and sectioned (ca. 15 μm). The sections were then divided into two groups. One group of sections was incubated for 2 hours with PBS containing 2% normal goat serum followed by an overnight incubation at 4°C with anti-serum against dog kidney aldose reductase (1:4000 dilution in PBS containing 0.3% Triton X-100) raised in goat. The second group of sections, which served as control, was also incubated for 2 hours with PBS containing 2% normal goat serum followed by an overnight incubation at 4°C with normal goat serum in place of the primary antibody. Both groups of sections were then incubated at 22°C for 2 hours with a 1:400 dilution of linked serum (Miles Laboratories, Elkhart, IN) followed by a 2-hour exposure at 22°C to a 1:800 dilution of peroxidase-antiperoxidase complex (Miles Laboratories). The antibody complex was visualized with a solution of 3,3'-diaminobenzidine and hydrogen peroxide.

In Vitro Incubation of Aldose Reductase with 3-FG
Purified dog lens aldose reductase (0.2 U with 5 mM 3-FG) was incubated at 25°C for 5 hours with a reaction mixture consisting of 10 mM NADPH, 7 mM 2-mercaptoethanol, and 1 mM 3-FG in 100 mM phosphate buffer, pH 7.5 (1 ml total volume). The reaction was stopped by rapid freezing of the reaction tube in dry ice, and the mixture was stored at −20°C for 19F-NMR analysis.

In Vitro Incubation of Sorbitol Dehydrogenase with 3-FS
Partially purified dog liver sorbitol dehydrogenase (ca. 0.2 units with 3-FS) was incubated at 25°C for 5 hours in a mixture
FIGURE 1. $^{19}$F-Nuclear magnetic resonance spectra of dog lens incubated in 10 mM 3-fluoro-3-deoxy-D-glucose without (A) or with (B) the aldose reductase inhibitor AL 1576 and without (C) or with (D) the sorbitol dehydrogenase inhibitor CP-166,572. (A) and (B) are the result of 24-hour incubations, whereas (C) and (D) are the result of 72-hour incubations. Peaks a, b, and c indicate $\beta$-3-fluoro-3-deoxy-D-glucose ($\beta$-3-FG), $\alpha$-3-FG, and 3-fluoro-3-deoxy-D-sorbitol (3-FS), respectively, and peaks d and e indicate 3-FGA and 3-fluoro-3-deoxy-D-fructose, respectively.

containing 3 mM NAD$^+$ and 3-FS in 100 mM phosphate buffer, pH 7.5 (1 ml total volume). The reaction was stopped by rapid freezing of the reaction tube in dry ice, and the mixture was stored at $-20^\circ$C for $^{19}$F-NMR analysis.

Whole Dissected Lens Cultures

The dog eye was dissected at the equatorial region into two halves. The lens was isolated by carefully cutting under a dissecting microscope Zinn's zonules with Castroviejo scissors. The removed lens was either incubated intact or further dissected to remove the lens capsule containing adhering epithelial cells from the remaining lens by carefully peeling the capsule from the lens under the dissecting microscope. Either the intact lens or dissected lens and lens capsule were separately placed for 24 hours (unless otherwise indicated) in 4 ml Dulbecco's modified Eagle's medium (DMEM) at 37°C under a 5% CO$_2$ atmosphere supplemented with 2 mM glutamine, 50 $\mu$g/ml gentamicin, and 25 mM HEPES and containing 10 mM 3-FG. At the end of the incubation, the lens was homogenized in the incubation medium by sonication (Sonicator XL, Misonix, Farmingdale, NY) and stored at $-20^\circ$C for $^{19}$F-NMR analysis.

Lens Epithelial Cell Cultures

The isolated lens capsules containing epithelial cells obtained as described above were cut into small pieces with two blades and cultured in DMEM supplemented with 20% fetal calf serum, 2 mM glutamine, 50 $\mu$g/ml gentamicin, and 25 mM HEPES. When the cells reached confluence at passage 4 or 5, the medium was replaced with DMEM containing 2 mM 3-FG and cultured for 4 more days. After incubation, the cells were harvested by incubation with 0.5 mM EDTA in PBS, washed to isolate cells, suspended in deionized water, and sonicated (two 30-second bursts, with a 30-second interval between them). The sonicated mixture and culture media were collected and stored separately at $-20^\circ$C for subsequent $^{19}$F-NMR analysis.

$^{19}$F-NMR Analysis

$^{19}$F-NMR spectroscopy was conducted on a General Electric 4.7-T Omega spectrometer as described by Berkowitz et al.$^{25}$ $^{19}$F-NMR spectra were calculated from free induction decays that were acquired using a one pulse experiment with proton decoupling. In most cases each spectrum was the result of 3600 acquisitions with a 2-second repetition time. Chemical shifts, relative to trifluoroacetic acid, were established for each metabolite. A reference standard containing 100 mM 3-FG was run before each experiment to reestablish the chemical shift scale. Metabolite concentrations were established from peak integrals and tabulated.
FIGURE 2. $^{19}$F-Nuclear magnetic resonance spectra obtained from dog lens aldose reductase incubated with 1 mM 3-fluoro-3-deoxy-D-glucose (3-FG) for 5 hours in the absence (A) or presence (B) of the aldose reductase inhibitor AL 1576. Peaks a, b, and c indicate β-3-FG, α-3-FG, and 3-fluoro-3-deoxy-α-sorbitol, respectively.

RESULTS

Intact dog lenses were incubated for 24 to 72 hours in DMEM containing 10 mM 3-FG to obtain adequate levels of metabolites for detection. After incubation, the lenses were homogenized in the incubation medium to ensure no loss of label would occur, and the entire mixture was analyzed by $^{19}$F-NMR spectroscopy. In addition to the α- and β-anomers of 3-FG, three new peaks corresponding to the formation of 3-FS, 3-FF, and 3-FGA can be observed (Figs. 1A, 1C). 3-FS is the major metabolite, followed by 3-FGA and a small amount of 3-FF. These spectra are consistent with those previously reported by Karino et al. and indicate that 3-FG is metabolized to 3-FF through the polyol pathway or oxidized to 3-FGA through the gluconic acid pathway. Although absolute quantitation of metabolites is difficult because of variations in lens mass, metabolite ratios can be used to compare different lenses. For each lens, the metabolite peaks were integrated, and the concentrations obtained were expressed as the ratios of 3-FS to 3-FGA and of 3-FS to 3-FF. For whole lenses incubated for 24 hours with 10 mM 3-FG, the average ratio of 3-FS to 3-FGA was 3.7 ± 0.2, and the ratio of 3-FS to 3-FF was 15.2 ± 0.3 (n = 8). Similar incubation in the presence of 10 μM of the aldose reductase inhibitor AL 1576 dramatically reduced the formation of the

FIGURE 3. $^{19}$F-Nuclear magnetic resonance spectra obtained from dog liver sorbitol dehydrogenase incubated with 1 mM 3-fluoro-3-deoxy-α-sorbitol (3-FS) for 5 hours in the absence (B) or presence (C) of CP-166,572. Panel (A) illustrates the spectrum of the starting material. Peaks d and e indicate 3-FS and 3-fluoro-3-deoxy-α-fructose, respectively.
Aldose reductase

Substrate          $K_m$ (mM)  Relative Activity (%)

α-Glyceraldehyde   0.31 ± 0.04  100
β-Glucose          177 ± 15   2.1
3-Fluoro-3-deoxy-β-glucose  9.3  21.8
Sorbitol dehydrogenase
α-Sorbitol         3.4 ± 0.4   100
3-Fluoro-3-deoxy-α-sorbitol 12.5 ± 3.6  54.3 ± 4.9

* Relative activity was expressed as the percentage of the activity at either 10 mM concentration compared with that with α-glyceraldehyde or 50 mM concentration compared with that with α-sorbitol as substrate.

3-FS peak and slightly increased the formation of 3-FGA to give a 3-FS-to-3-FGA ratio of 0.128 ± 0.008 (n = 2; Fig. 1B). The reduction of the 3-FS peak also resulted in a concomitant reduction of the 3-FF peak. 3-FF formation was also eliminated by incubation of the lenses in medium containing 10 μM of the sorbitol dehydrogenase inhibitor CP-166,572 (Fig. 1D).

To confirm that the conversion of 3-FG to 3-FS observed in the whole lens was catalyzed by aldose reductase, purified dog lens aldose reductase was incubated with 1 mM 3-FG for 5 hours in the presence of NADPH. 19F-NMR analysis of the incubation medium indicated the appearance of a peak corresponding to 3-FS (Fig. 2), which was reduced by 73% by the addition of 1 μM of the aldose reductase inhibitor AL 1576 to the incubation medium. No formation of 3-FS was observed in the absence of aldose reductase. Similarly, the enzymatic conversion of 3-FS to 3-FF was confirmed by the 5-hour incubation of dog liver sorbitol dehydrogenase with 1 mM 3-FS in the presence of NAD+. 19F-NMR analysis of the incubation media indicated the formation of a small peak corresponding to 3-FF (Fig. 3) whose formation in similar experiments was inhibited by the addition of 1 μM of the sorbitol dehydrogenase inhibitor CP-166,572. No 3-FF formation occurred in the absence of sorbitol dehydrogenase.

Because clearly 3-FG and 3-FS are metabolized by aldose reductase and sorbitol dehydrogenase, respectively, a comparison of the relative affinity ($K_m$) of these enzymes for the fluorinated versus natural substrates was conducted. As summarized in Table 1, the $K_m$ of dog lens aldose reductase for 3-FG is approximately 20-fold better than that of β-glucose, and the relative activity observed with 10 mM 3-FG is ca. 10-fold higher than that with β-glucose. Sorbitol dehydrogenase displayed a slightly lower affinity for 3-FS than for α-sorbitol. The $K_m$ for 3-FS (12.5 mM) was ca. 4-fold larger than that of α-sorbitol (3.4 mM), and the relative activity with 3-FS at the assay condition of 50 mM decreased to almost half of that with α-sorbitol as substrate.

The lens epithelium, which forms an anterior subcapsular monolayer that extends to the equator, is metabolically the most active region of the lens. Therefore, the metabolism of 3-FG was also investigated in in vitro cultured dog lens epithelial cells. DMEM containing 2 mM 3-FG was added to confluent cultures of cells at passage 4 or 5. After 4 days of incubation, the 3-FG-containing medium was removed, and the harvested cells and medium were analyzed separately by 19F-NMR. As illustrated in Figure 4, only a single peak corresponding to 3-FS was observed in the isolated cells, whereas the medium contained peaks corresponding to 3-FG and 3-FS. The addition of 10 μM AL 1576 eliminated the formation of 3-FS. The lack of fluorodeoxyglucose peaks indicates that 3-FG entering into cells is rapidly metabolized by aldose reductase to 3-FS and that the intracellular accumulation of 3-FS apparently results in permeability changes that lead to leak-out into the medium.

The absence of 3-FF and 3-FGA from epithelial cells and culture medium suggests that sorbitol dehydrogenase and glucose dehydrogenase are absent or markedly reduced in lens epithelial cells. To investigate this possibility, lens capsules containing primarily epithelial cells with some superficial fiber cell contamination were carefully dissected from dog lenses, and the capsule and remaining lens were incubated separately for 24 hours in medium containing 10 mM 3-FG. These separate fractions were then homogenized in their respective incubation medium to ensure no loss of label would occur in the subsequent analyses. 19F-NMR spectra of the decapsulated lens

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_m$ (mM)</th>
<th>Relative Activity (%)</th>
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<tr>
<td>Aldose reductase</td>
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<tr>
<td>α-Glyceraldehyde</td>
<td>0.31 ± 0.04</td>
<td>100</td>
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* Relative activity was expressed as the percentage of the activity at either 10 mM concentration compared with that with α-glyceraldehyde or 50 mM concentration compared with that with α-sorbitol as substrate.

FIGURE 4. 19F-Nuclear magnetic resonance spectra obtained from dog lens epithelial cells cultured in 2 mM 3-fluoro-3-deoxy-α-glucose for 4 days in the absence (A) and presence (B) of 10 μM AL 1576. Peak d indicates 3-fluoro-3-deoxy-α-sorbitol.

TABLE 1. Kinetic Properties of Dog Lens Aldose Reductase and Dog Liver Sorbitol Dehydrogenase with 3-Fluoro-3-Deoxy Substrates

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* Relative activity was expressed as the percentage of the activity at either 10 mM concentration compared with that with α-glyceraldehyde or 50 mM concentration compared with that with α-sorbitol as substrate.
Aglucose, respectively, as substrates and appropriate cofactors. The observed distribution, summarized in Figure 6, indicates that the capsular/epithelium fraction contains primarily aldose reductase activity, whereas sorbitol dehydrogenase and glucose dehydrogenase activities both were present in the remaining decapsulated lens composed of cortical lens fibers and nucleus. The enzymatic distribution of aldose reductase activity is supported by the immunohistochemical localization of aldose reductase in dog lens using antibodies against dog kidney aldose reductase (Fig. 7). Strongest staining was observed along the single epithelial layer, indicating that aldose reductase is localized in the lens epithelial cells. Staining to a lesser extent was also observed in the differentiating epithelial cells at the bow region of the peripheral cortex. This staining decreased with progression from the superficial to the deeper cortex. This is consistent with the results obtained in the rat and in the human lens. 29

DISCUSSION

Hyperglycemia associated with diabetes increases the flux of glucose into the polyol pathway, and this can result in the intracellular accumulation of the intermediate sugar alcohol sorbitol in cells possessing the polyol pathway. In seminal studies conducted in the lens, the increased intracellular accumulation of sorbitol was shown to initiate osmotic imbalances, which initiated a cascade of biochemical changes that lead to cataract formation. Based on the efficacy of aldose reductase inhibitors in animal studies, worldwide efforts to develop specific aldose reductase inhibitors have been made. This concept has recently been challenged by a proposal that redox changes associated with altered NAD+/NADH ratios resulting from an increased metabolism of sorbitol to fructose by sorbitol dehydrogenase initiate diabetic complications. Clarifying the importance of sorbitol dehydrogenase versus aldose reductase inhibition has been difficult because results with sorbitol dehydrogenase inhibitors have been mixed 17-22 and because aldose reductase inhibitors also indirectly reduce sorbitol de-
hydrogenase activity. Measurement of flux through the polyol pathway by \( ^{19}F \)-NMR spectroscopy is an attractive alternative method for clarifying the relative importance of aldose reductase and sorbitol dehydrogenase in the initiation of diabetic complications.

Because intact normal human lenses are difficult to obtain, dog lenses were used for the present study. The dog lens contains levels of aldose reductase that are much lower than those of the rat, and on a specific activity basis its levels of aldose reductase are similar to those of human lenses.\(^{13}\) Sugar cataract formation in the dog has also been reported to be more similar to that of humans.\(^{13}\) This makes the dog lens a reasonable model for investigating polyol pathway-linked glucose utilization.

Karino et al.\(^{26}\) demonstrated that 3-FG is a sensitive probe that can be used to evaluate aldose reductase in dog lens. The aldose reductase observed through the linear, time-dependent formation of 3-FS closely correlated with standard spectrophotometric measurements of aldose reductase activity, and this activity was inhibited by aldose reductase inhibitors. The present study confirms this observation and demonstrates that the formation of 3-FS from 3-FG only occurs in the presence of enzyme. The present study also demonstrates that 3-FS is metabolized to 3-FF by sorbitol dehydrogenase. Importantly, this conversion, although small, can occur at pH 7.5. Sorbitol dehydrogenase not only catalyzes the conversion of sorbitol to fructose but also the reverse reaction, with the direction of the metabolic conversion being pH dependent.\(^{32}\) Measurement of dehydrogenase activity in the conversion of sorbitol to fructose indicates that this reaction occurs at a pH optimum of 10. Physiological pH favors the conversion of fructose to sorbitol. Kinetic studies conducted at the pH optima of aldose reductase and sorbitol dehydrogenase indicate that the relative conversion of 3-FG to 3-FS is 10-fold faster than that of glucose to sorbitol, whereas the conversion of 3-FS to 3-FF is 0.5-fold less than sorbitol to fructose. Because the overall metabolism of 3-FG through the polyol pathway favors the accumulation of sorbitol, this sugar analogue cannot be used to directly measure the actual amounts of sorbitol and fructose formed. However, the rates of formation of the fluorinated metabolites can, in principal, be related to the rates of formation of their nonfluorinated counterparts. Although the reaction of 3-FS to 3-FF is slightly less that that of its nonfluorinated counterpart, the levels of these metabolites can still be detected and adequately measured. 3-FG clearly can be used for assessing relative enzyme activities.

3-FG is not used by hexokinase as a substrate, and 3-FF is not used by either fructokinase or ketohexokinase. This minimizes the effects of complex metabolic changes induced by hyperglycemia, and allows one to focus directly on the polyol pathway. By recognizing the differences in the metabolism between glucose and 3-FG and between sorbitol and 3-FS, the use of 3-FG can be a powerful probe for assessing the flux through the polyol pathway and for determining the relative distribution of polyol pathway enzyme activities in tissues.

Examination of 3-FG metabolism in the intact dog lens indicates that 3-FG is converted primarily to 3-FS, and to a lesser extent to 3-FF and 3-FGA, whereas metabolism of 3-FG in cultured dog lens epithelial cells is limited to the formation of 3-FF. The apparent absence of sorbitol dehydrogenase and glucose dehydrogenase from epithelial cells was supported by the subsequent analysis of carefully dissected capsules containing primarily epithelial cells and the remaining decapsulated lens. This observed distribution of reductase and dehydrogenase activities has been confirmed by standard spectroscopic analyses of enzyme activities.\(^{33-35}\)

In human and rat lenses, aldose reductase is primarily localized in the epithelial cells and superficial cortical fiber cells. The distribution of aldose reductase in dog lenses appears to be similar. The concentration of aldose reductase in the epithelium and superficial cortical fiber cells coupled with the relative absence of sorbitol dehydrogenase favor the localized accumulation of sorbitol in this area. Fine peripheral vacuoles form primarily in this superficial region, and histologic studies indicate that this is preceded by cell edema and aberrant intracellular vacuole formation in the lens epithelium. These localized osmotic changes are also supported by magnetic resonance imaging studies.\(^{32,36}\) The data confirm that flux through the polyol pathway primarily results in sorbitol accumulation and support the importance of sugar alcohol accumulation in the onset of sugar cataracts.

References


20. Cameron NE, Cotter MA, Basso M, Hohman TC. Comparison of the effects of inhibitors of aldose reductase and sorbitol dehydroge-


