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FACTORS AFFECTING THE FORMATION OF SUGAR ALCOHOLS IN OCULAR LENS

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SUMMARY

1. The enzymic reactions which influence the accumulation of sugar alcohols in lens were studied. The hexose monophosphate shunt mechanism appears closely linked to the aldose reductase (Polyol: NADP oxidoreductase, EC 1.1.1.21) reaction. It was revealed that as the concentration of glucose in the incubating medium was elevated a linear increase in the oxidation of C-1 of glucose was accompanied by an increase in the production of sorbitol. Galactose also appeared capable of stimulating the reoxidation of TPNH formed in the oxidation of C-1 of glucose. The stimulation of the hexose monophosphate shunt mechanism by the aldose reductase reaction was demonstrated in lenses from rabbits, normal rats and rats fed galactose.

2. The formation of sorbitol is dependent upon a competition between aldose reductase and hexokinase (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1) for glucose. The unusally high Michaelis constant observed for aldose reductase indicates that normally glucose is phosphorylated and only a small fraction is converted to sorbitol. However, since the concentration of hexokinase is low in lens, a slight elevation of glucose is sufficient to stimulate sorbitol production.

3. Unlike sorbitol, dulcitol, is not attacked by polyol dehydrogenase in lens. Other properties of polyol dehydrogenase (Polyol: NAD oxidoreductase) and aldose reductase were studied in the intact lens and in homogenates.

INTRODUCTION

The enzymes of the sorbitol pathway in the lens, first demonstrated by VAN HEYNINGEN[1], may play an important role in the production of sugar cataracts[2-4]. Evidence has been presented indicating that the accumulation of sugar alcohol formed in the sorbitol pathway creates a hypertonic condition within the lens fibers which results in an increase in water. The osmotic effect could conceivably account for the hydropic lens fibers observed in the early phase of these cortical cataracts.

In this report the properties of the enzymes of the sorbitol pathway and the various factors which influence sugar alcohol formation are presented. The two enzymes involved in the metabolism of sugar alcohols are aldose reductase and polyol dehydrogenase. Aldose reductase catalyzes the reduction of a number of aldoses to their sugar alcohol form in the presence of TPNH. Polyol dehydrogenase

with DPN^+ as the coenzyme catalyzes the oxidation of certain sugar alcohols to their corresponding keto sugars. Since the availability of TPNH is essential for sugar alcohol formation the interaction between the pathway in which TPNH is generated, the hexose monophosphate shunt mechanism, and aldose reductase was also studied.

METHODS

The incubation procedures employing the organ-culture technique and radioactive methods have been previously described[5–7]. The incubating medium was composed of 135 mM Na^+, 5.5 mM K^+, 0.6 mM Mg^{2+}, 110 mM Cl^-, 30 mM HCO_3^-, 0.6 mM SO_4^{2-}, 0.6 mM phosphate and equilibrated with CO_2 – air (5:95). In the incubation of either calf or rabbit lens, a mixture of 9 ml of the above medium and 1 ml of a solution with a tonicity of 300 mM containing the substrate and/or NaCl was used. $CaCl_2$ concentration of 2.2 mM was employed for calf lens and 1.0 mM for rabbit and rat lenses.

For the separation of the sugars, 12 separately incubated calf lenses were pooled and deproteinized with 0.3 N $Ba(OH)_2$ and 5 % $ZnSO_4$. The lens filtrate was deionized by passage through a column of Amberlite IR-120 and a column of Amberlite IR-4B (see ref. 8) and then fractionated on a Dowex-1 borate column of KHYM AND ZILL[9]. The borate was converted to methyl borate by treatment with methanol and removed *in vacuo*[10]. The isolated sugar was measured by an appropriate color test, carrier added and the specific activity determined. The specific activity of the sugars were determined as the osazone derivatives or by the wet combustion method[11], while the specific activity of the sugar alcohol was determined by the wet combustion method.

Hexitol was determined by the procedure of WEST AND RAPOPORT[12] as modified by FAULKNER[13], keto hexose by the method of ROE[14], and total hexose by the anthrone reaction[15]. Sugars and sugar alcohols were identified by the paper chromatographic procedures employed by VAN HEYNINGEN[16].

The procedures employed for feeding rats a diet rich in galactose or glucose have been described[2].

RESULTS AND DISCUSSION

Conversion of glucose to sorbitol and fructose

To study the conversion of glucose to fructose through the sorbitol pathway [$^{14}C_6$]glucose was used as the substrate and the various sugar derivatives which accumulated in the lens during incubation were isolated on a Dowex-1 borate column (Table I). The identification of the fraction isolated was further confirmed by paper chromatography. Since only a small amount of free sugar is normally found in the lens, it was necessary to pool 12 lenses, incubated separately, to allow for adequate analyses. After isolation the specific activities of each of the sugars and the sugar alcohol were determined. As shown in Table II, the specific activities of glucose, sorbitol and fructose, in all 3 experiments were essentially identical. Apparently the three related sugar derivatives equilibrated rapidly and the results are consistent with the view that this occurs via the sorbitol pathway. Inositol is of interest because it is found in high concentrations in lens and is similar to sorbitol but is a cyclic rather than a linear sugar alcohol. The results revealed that inositol recovered after incu-

bation had an extremely low specific activity. This suggests that it is not synthesized directly from glucose as is sorbitol but probably arises from a series of more complicated reactions. In addition it most likely has a low rate of turnover.

Evidence for the reversal of the reactions of the sorbitol pathway, that is the conversion of fructose through sorbitol and to glucose was obtained by incubating calf lens with [$^{14}C_6$]fructose. The specific activities of fructose and sorbitol isolated after incubation were identical but that of glucose was about 35 % lower. Since ^{14}C was found in the glucose isolated, it appears that fructose may be transformed to glucose through the sorbitol pathway, although the possibility exists that the transformation may have occurred through the phosphorylated derivatives of the Embden–Meyerhof pathway followed by a phosphatase reaction.

TABLE I

CHROMATOGRAPHY OF SUGARS AND SUGAR DERIVATIVES ON DOWEX-1 BORATE COLUMN

$K_2B_4O_7$	*Volume of eluting fluid required for recovery of sugar*			
	Inositol	*Fructose*	*Sorbitol*	*Glucose*
0.015 M	0.3–0.5 l	1–1.6 l	2–2.5 l	
0.03 M*				2.6–2.75 l

* Immediately after the recovery of sorbitol, the eluting fluid was increased to 0.03 M.

TABLE II

THE CONVERSION OF GLUCOSE TO SORBITOL AND FRUCTOSE IN CALF LENS

Calf lenses were incubated for 24 h with labeled substrate (10 mM) in 10 ml of balanced salt solution.

Substrate	*Initial specific activity (counts/min/μmole)*	*Final specific activity (counts/min/μmole)*			
		Glucose	*Sorbitol*	*Fructose*	*Inositol*
[$^{14}C_6$]Glucose	1050	717	820	753	—
	1050	740	765	630	83
	1050	783	763	753	65
[$^{14}C_6$]Fructose	950	365	535	590	23
	950	352	586	670	75

In both the glucose and fructose experiments the initial specific activity was higher than the final, suggesting that a dilution of hexoses from an endogenous source had occurred. The average hexose content of 12 calf lenses was 1.2 μmoles/g of lens. Of this amount 58 % was fructose and 42 % was presumably glucose. The amount of sorbitol ranged from 1 to 2 μmoles/g of calf lens. Thus the endogenous sugars and sugar alcohol contribute to dilute the [^{14}C]glucose taken up by the lens but the quantities are not sufficient to account entirely for the dilution observed when the total consumption of glucose is considered. In the fructose experiment the specific activity of glucose was lower than that of sorbitol or fructose. This suggests the possibility that dilution occurred at the glucose level. The source of the dilution of glucose may have been glycogen, the presence of which in rat lens was reported by KLETHI AND MANDEL[17].

The conversion of glucose to fructose was also studied in crude extracts of lens. We confirmed the observation of VAN HEYNINGEN[1,16] that incubating glucose and TPNH with dialyzed lens extract results in a product identified by paper chromatography as sorbitol. Similarly by paper chromatography we found that in lens extract galactose was converted to dulcitol and xylose to xylitol. The incubation of sorbitol and DPN in the presence of lens extract led to the formation of fructose.

These reactions can be conveniently followed spectrophotometrically by the rate of oxidation or reduction of the appropriate forms of pyridine nucleotides. Extracts of calf, rabbit, rat, cat and human lens all possessed activities of the enzymes, aldose reductase and polyol dehydrogenase. Aldose reductase activity in rabbit lens was much higher than in calf lens so the rabbit lens was used as a source of this enzyme. On the other hand polyol dehydrogenase activity was higher in the calf lens so extracts of this source were used for studies of the second reaction of the sorbitol pathway.

To obtain some information on the substrate specificity of aldose reductase and polyol dehydrogenase, the relative activities and Michaelis constants, K_m's, of a few substrates were determined in crude lens extracts. It is obvious that a more complete and detailed study on a purified preparation of each enzyme is essential but it may prove valuable to determine how the enzymes behave in the presence of competing enzymes and other proteins. Information obtained under these conditions may be more directly applicable to the interpretation of what occurs in normal lens as well as lens from diabetic animals or animals fed a diet rich in galactose or xylose. For these reasons, we studied aldose reductase at the more physiological pH (7.4) even though the pH optimum of this enzyme is near 6.5. Most enzymes studies, dealing with relative reaction rates of different substrates, are usually conducted at a substrate concentration which saturates the enzyme. However, we used substrates in concentration which more nearly represents that encountered in the lens of a diabetic animal or of an animal fed galactose or xylose. At the concentration used the enzyme was not saturated with respect to glucose or galactose since the K_m's for these substrates are very high. Inspection of the data in Table III reveals that of the three sugars in question xylose is the best substrate for aldose reductase as shown by the lowest K_m and the highest relative activity. On this basis, of the three sugars studied, glucose is the least preferred substrate for aldose reductase. The K_m of glucose is extremely high, $3.3 \cdot 10^{-2}$ M, indicating that an unusually high concentration is needed to saturate the enzyme. As far as the substrate specificity is concerned galactose appears intermediate between xylose and glucose. As given in Table III the relative activity of galactose was 1.7 while that of glucose was 1 in rabbit lens. In cat and rat lens the relative activity for galactose was 1.9 and 2 respectively with glucose as 1. It thus appears that at a concentration of sugars of 10 mM, the rate of formation of dulcitol relative to sorbitol is about 2:1. As evidenced by the values for K_m and relative activity the best substrate appears to be glyceraldehyde. It would appear that aldose reductase in lens extract can act on a number of different aldoses indicating an enzyme with a broad substrate specificity. The K_m's obtained compared favorably with those obtained by HERS with a preparation of aldose reductase from seminal vesicles[18].

The important consideration concerning the substrate specificity of polyol dehydrogenase is, that of the three sugar alcohols dulcitol is not significantly utilized

by this enzyme (Table IV). Extracts of lens from calf, cat, rat and man all gave the same result, that dulcitol is not a suitable substrate for polyol dehydrogenase. In contrast, xylitol and sorbitol appear to be readily oxidized in lens extract.

The pH optimum curve for aldose reductase appears to be on the acid side of 7 near pH 6.5. The reverse of this reaction, the reduction of TPN^+ with sorbitol, appeared to have a broad pH optimum curve and was much more sluggish. It is known that the equilibrium of this reaction lies in favor of sorbitol formation[18].

TABLE III

SUBSTRATE SPECIFICITY OF RABBIT-LENS ALDOSE REDUCTASE

The K_m values were calculated from Lineweaver–Burk plots. The incubation medium contained 0.3 μmole of TPNH, 200 μmoles of phosphate buffer (pH 7.4) and 30 mg of lens in a final volume of 3.0 ml. The enzyme preparation was a dialyzed homogenate of rabbit lens. Relative activity was determined with substrate concentrations of 10 mM.

Substrate	*Relative activity*	K_m
D-Glucose	1	$3.3 \cdot 10^{-2}$ M
D-Galactose	1.7	$2.2 \cdot 10^{-2}$ M
D-Xylose	3.8	$1.4 \cdot 10^{-3}$ M
DL-Glyceraldehyde	8.3	$2.4 \cdot 10^{-4}$ M

TABLE IV

SUBSTRATE SPECIFICITY OF CALF-LENS POLYOL DEHYDROGENASE

The incubation medium contained 0.3 μmole of DPN^+, 240 μmoles of phosphate buffer (pH 7.4) and 100 mg of calf lens in a final volume of 3 ml. The enzyme preparation was a dialyzed homogenate of calf lens. The K_m values were calculated from Lineweaver–Burk plots. The relative activity was determined with substrate concentrations of 10 mM.

Substrate	*Relative activity*	K_m
Sorbitol	1	$5.2 \cdot 10^{-3}$ M
Xylitol	1.2	$1.7 \cdot 10^{-3}$ M
Dulcitol	0	—

The pH optimum curve for polyol dehydrogenase of calf lens indicated that the peak of activity was near pH 8.4 in the conversion of sorbitol to fructose. The reverse reaction, that of the conversion of fructose to sorbitol, had a pH optimum of 6.0.

The effect of pH on these enzymes is of important consideration when acidosis as observed in diabetic animals occurs. A lowered pH would accelerate the aldose reductase reaction as well as the conversion of fructose to sorbitol. Thus acidosis would favor the production of sorbitol both from glucose and fructose.

In experimental sugar cataracts it has been known that the younger animals are more susceptible to develop cataracts than the older animals. We wondered whether there was any correlation between changes in the aldose reductase activity of the lens and the aging of the animal. Our results indicated that there is no apparent change in aldose reductase activity with age. The lens from a 100-g rat had the same aldose reductase activity as the lens from a 300-g rat. In the calf lens we could not detect any significant difference in aldose reductase activity between the cortex and the nucleus.

Hexose monophosphate shunt mechanism

The production of sorbitol is dependent upon the availability of TPNH. Since TPNH is formed in the hexose monophosphate shunt mechanism the possible interaction between aldose reductase and the shunt mechanism was explored. From the K_m of lens aldose reductase it appeared that the production of sorbitol from glucose would be accelerated by increasing the concentration of glucose. If coupling of aldose reductase and the shunt dehydrogenase occurs in lens the increase in sorbitol pro-

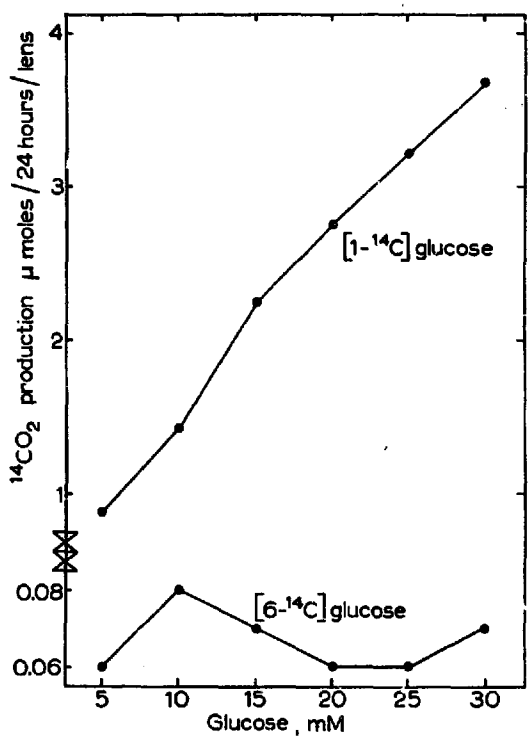

Fig. 1. The effect of concentration on glucose oxidation. Description given in Table V.

duction should be paralleled by an increase in C-1 oxidation of glucose. That such a relationship exists is shown in the results summarized in Fig. 1 and in Table V. As the concentration of glucose was increased in the medium an increase in the rate of oxidation of C-1 but not of C-6 glucose was observed. As shown in Fig. 1 the increase in the rate of oxidation of C-1 glucose was almost linear in rabbit lens as the concentration of glucose was increased from 5 to 30 mM in the medium. In this range of concen-

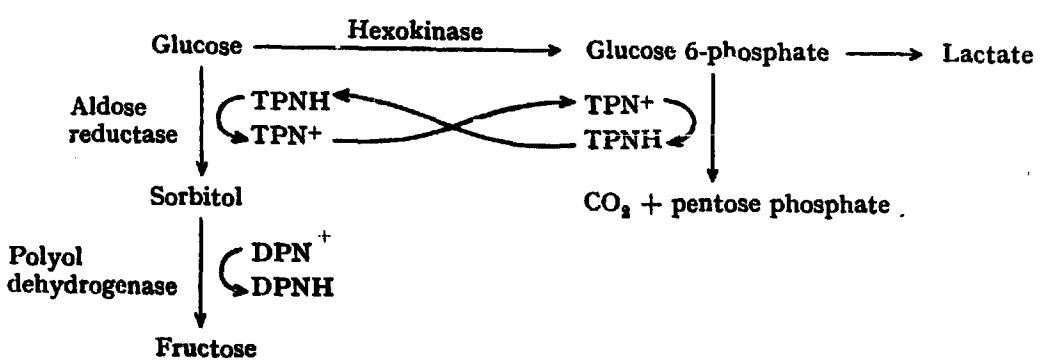

Fig. 2. Reactions affecting sugar alcohol formation.

trations about 1–4 μmoles of glucose were oxidatively decarboxylated at the C-1 position during a 24-h incubation, whereas the amount of C-6 atom of glucose oxidized remained constant at approx. 0.07 μmole of CO_2. Consistent with the fact that the C-6 oxidation did not increase was the finding that the production of lactate did not change with increasing levels of glucose in the medium. Apparently the hexokinase reaction, the rate-limiting step in lens glycolysis, is saturated at 5 mM of glucose so that further increase in sugar is not accompanied by an increase in the activities

TABLE V

THE EFFECT OF GLUCOSE CONCENTRATION ON THE SORBITOL AND FRUCTOSE ACCUMULATION AND TPNH PRODUCTION IN RABBIT LENS

Rabbit lens (175 mg) was incubated for 24 h with varying concentrations of labeled or normal glucose in 10 ml of medium. Additional NaCl was used when needed to maintain a constant tonicity. The results are averages of at least 3 experiments. The amount of TPNH formed was estimated from the $^{14}CO_2$ recovered from [1-^{14}C]glucose after correcting for the amount from [6-^{14}C]glucose, and from the initial specific activity. In rabbit lens the specific activity of glucose after incubation was within 10% of the initial specific activity.

Glucose concentration (μmoles/ml)	*Accumulation in lens (μmoles)*		*Sorbitol + fructose*	*TPNH formed (μmoles)*
	Sorbitol	*Fructose*		
5	0.4	0.1	0.5	1.6
10	1.4	0.4	1.8	2.6
15	2.6	0.5	3.1	4.4
20	3.8	0.5	4.3	5.6
25	4.0	0.6	4.6	6.2
30	5.6	0.6	6.2	7.2

of the Embden–Meyerhof or of the tricarboxylic acid cycle. Perhaps, only in lens where the hexokinase is at such low activity is it possible to demonstrate the increase in the oxidation of C-1 of glucose. The excess glucose is available to accelerate the aldose reductase reaction. HERS[18] has shown that the oxidation of C-1 of glucose in liver was stimulated by addition of glucuronolactone or glucosone, substrates for aldose reductase.

It was of interest to determine if a direct stoichiometry could be established between the amount of sorbitol formed and the amount of TPNH generated. In the shunt mechanism the oxidation of C-1 of glucose is the result of 2 reactions producing TPNH, thus the amount of TPNH formed can be approximated as twice the value of C-1 oxidized after correcting for the small amount of C-6 oxidized. Since some of the sorbitol formed is converted to fructose, the total amount of glucose reduced by aldose reductase is the sum of sorbitol and fructose recovered. As shown in Table V, with increasing levels of glucose, the production of sorbitol paralleled the increase in C-1 oxidation of glucose. The total amount of glucose reduced (sorbitol + fructose) appeared to be somewhat less than the available TPNH. This can be explained by other pathways reoxidizing TPNH or by some loss of fructose and sorbitol by further metabolism or by diffusion out into the medium. The determination of small amounts of these substances in the medium was impractical because of the presence of high levels of glucose.

The relationship between the aldose reductase reaction and the shunt mechanism was also demonstrated to exist in the rat lens (Table VI). Increasing the availability of

glucose stimulated the rate of oxidation of C-1 of glucose and the production of sorbitol.

The acceleration of the shunt mechanism by stimulating the aldose reductase reaction was also demonstrated by incubating rabbit lens in high levels of galactose. In this study the concentration of [1-^{14}C]-or [6-^{14}C]glucose was kept at 5 mM in the medium of the control lens while the contralateral lens was incubated in the same medium but with added galactose (30 mM). The presence of galactose stimulated the

TABLE VI

THE EFFECT OF GLUCOSE CONCENTRATION ON THE SORBITOL AND FRUCTOSE ACCUMULATION AND [1-^{14}C]GLUCOSE OXIDATION IN RAT LENS

Two rat lenses were incubated for 24 h in 5 ml of medium with varying concentrations of glucose. The specific activity of [1-^{14}C]glucose was 1600 counts/min/μmole. Additional NaCl was used when needed to maintain the same tonicity in all 3 cases. The results are reported as μmoles/24 h/2 lenses and are averages of 4 separate experiments.

Glucose concentration (mM)	*Sorbitol (μmoles)*	*Fructose (μmoles)*	*$^{14}CO_2$ recovered (μmoles)*
15	0.7	0.1	0.45
30	1.3	0.1	0.90
60	2.0	0.2	1.40

C-1 oxidation of glucose by almost 4-fold but did not affect the oxidation of C-6 of glucose or the production of lactate (Table VII). Our previous studies indicated that in lens the rate-limiting step in the shunt mechanism is in the reoxidation of TPNH. Increasing the rate of reoxidation of TPNH by adding an electron acceptor such as pyruvate[19] or oxidized glutathione[6] accelerated the C-1 oxidation of glucose. Since galactose and glucose produced a greater stimulation of the oxidation of C-1 of glucose in lens they appear to be much more effective electron acceptors of TPNH than either pyruvate or oxidized glutathione. In the galactose-stimulated lens the amount of C-1 oxidized through the shunt mechanism could be almost entirely accounted for by the increase in dulcitol. The accumulation of dulcitol in these rabbit lenses appeared to be 6.4 μmoles (7.0–0.4), while the TPNH formed during this period was 7.0 μmoles (2 × (3.6–0.1)). One is left with the impression that the production of dulcitol probably diverts TPNH away from the mechanism which normally reoxidizes it. It appears that an appreciable amount, if not all, of the TPNH formed in the shunt mechanism is consumed by the reduction of galactose to dulcitol.

TABLE VII

THE EFFECT OF GALACTOSE ON THE OXIDATION OF C-1 OF GLUCOSE IN RABBIT LENS

Young rabbit lenses were incubated for 25 h in a medium containing either C-1 or C-6 labeled glucose (5 mM) with and without galactose (30 mM). In the incubations without galactose, the tonicity of the medium was maintained by additional NaCl. The values were obtained from 3 separate experiments of lenses incubated in the same medium as those of the radioactive experiments. All values are corrected to a weight of 175 mg lens.

Galactose in medium	*Glucose-carbon to CO_2*		*Hexitol (μmoles)*	*Lactate (μmoles)*
	C-1 (μmoles)	*C-6 (μmoles)*		
–	3.0	0.08	7.0	30.4
+	1.0	0.07	0.4	29.3

The studies *in vitro* presented here seem at first to be in conflict with those reported by LERMAN[20] who found that in the lens of a galactose-fed rat the rate of C-1 oxidation of glucose is severely depressed. From our observations one might anticipate the rate of C-1 oxidation of glucose to be higher in the galactose lens than in the normal lens. In the studies by LERMAN[20] the oxidation of glucose was studied in a medium free from galactose, so the conditions were not ideal to evaluate changes in the rate of oxidation that takes place *in situ*. For this reason the effect of feeding a high galactose diet to rats on the rate of oxidation of glucose in their lenses was reinvestigated.

TABLE VIII

OXIDATION OF [1-^{14}C]GLUCOSE IN LENSES FROM GALACTOSE AND GLUCOSE-FED RATS

A lens from a rat fed galactose or glucose was incubated for 24 h in 5 ml of medium containing [1-^{14}C]glucose. The specific activity of the glucose was 3600 counts/min/μmole. The results of 20 determinations are given as the mean ± standard error of the mean.

Diet	*Galactose in medium*	*$^{14}CO_2$ recovered (counts/min/lens)*
Glucose	—	573 ± 17
	+	2296 ± 73
Galactose	—	477 ± 11
	+	1609 ± 65

The rats were kept on the galactose diet until the first sign of opacities (5-6 days). The lenses were removed, one was placed in a medium containing 5 mM of [1-^{14}C]-glucose and the other lens in the same medium with added galactose (30 mM). The lenses from the glucose-fed rats serving as controls were treated in an identical manner. The rate of oxidation of C-1 of glucose in the lenses of the galactose-fed rats was slightly lower (16 %) than in the control lenses when tested in a medium without galactose (Table VIII). The addition of galactose to the medium produced a marked stimulation in the rate of C-1 oxidation of glucose in the lenses from both the glucose and galactose fed rats. The rates observed in the lenses from the galactose fed rats were appreciably lower (30 %).

The results seem to suggest that *in situ* where an abnormal level of galactose is available in the aqueous humor, the rate of oxidation of C-1 of glucose is probably much higher in the lens of the galactose-fed rat than in the lens of the normal rat. Any deleterious effect resulting from the alteration of the shunt mechanism would have to be attributed to the diversion of TPNH away from the normal reoxidizing mechanism or to the accumulation of abnormal products of metabolism. The actual capacity of the shunt mechanism, as indicated by the data on the C-1 oxidation in presence of added galactose (Table VII), is decreased in the lens of the galactose-fed rat. This is probably due to the lowered activity of the glucose 6-phosphate dehydrogenase[20,21]. It seems the lens counteracts against the accumulation of sugar alcohol or products formed in the shunt by suppressing the activity of glucose 6-phosphate dehydrogenase by some mechanism.

Hexokinase and aldose reductase

In Fig. 2 the various factors which influence the sorbitol pathway are summarized. Sorbitol production is influenced by the competition for glucose between hexokinase

and aldose reductase. Whether glucose is phosphorylated or reduced would depend to a great extent on the Michaelis-Menten constants of the competing enzymes. The K_m for lens hexokinase has been reported by GREEN AND SOLOMON[22] to be 10^{-4} M. In good agreement with this, we have found a value for the glucokinase in rabbit lens of $3 \cdot 10^{-4}$ M. As shown in Table III the K_m of aldose reductase of rabbit lens with glucose as the substrate is $3 \cdot 10^{-2}$ M. The high K_m of aldose reductase indicates that a relatively high concentration of glucose is required before it is reduced to sorbitol at an appreciable rate. Qualitatively it thus appears that the affinity of hexokinase for glucose is much higher than that of aldose reductase. This suggests that if the cofactors are not limiting, glucose would be preferentially phosphorylated rather than be reduced to sorbitol. Consequently under normal conditions in the lens, where the glucose concentration is low, most of the glucose is phosphorylated and a relatively small fraction converted to sorbitol. On the other hand when the concentration of glucose is elevated and the hexokinase becomes saturated with respect to the substrate, a greater fraction of the glucose metabolized would be converted to sorbitol. Another important factor is that hexokinase is found in low concentrations in lens, so this enzyme is probably operating at maximum activity under normal conditions.

Experimental sugar cataracts

If the accumulation of sugar alcohols and keto hexoses are directly responsible for the early changes in the 3 types of sugar cataracts the differences in the course of opacification should be related to the properties of the enzymes involved. It is fairly well established that galactose cataract in rats occurs rapidly within 5 days after the initiation of the diet. It takes about 3–5 days for the opacities to develop in the lens of a rat fed xylose but the opacities eventually disappear even though the xylose regimen is continued. In the diabetic rats, the opacities take several weeks to develop. However, microscopic examinations have revealed the appearance of hydropic lens fibers within a week after diabetes has been established.

The fact that galactose cataract is the easiest of the experimental sugar cataracts to produce is consistent with the observed properties of the enzymes of the sorbitol pathway. First of all galactose is fairly rapidly reduced to dulcitol. Moreover unlike xylitol and sorbitol, dulcitol is not attacked by polyol dehydrogenase. Since the sugar alcohols do not readily penetrate biological membranes[23,24] the conditions are favorable for the accumulation of dulcitol. This would account for the extremely high quantities of dulcitol found in the lens of galactose-fed rat[1,2]. A hypertonic condition is thus created and the natural consequence is the influx of water into the lens fibers resulting in their swelling and eventual rupture.

The rate of production of sugar alcohol from glucose appears to be slower than from either xylose or galactose. Fructose, which is formed from sorbitol, is metabolized to some extent and diffuses out of the lens more readily than the sugar alcohol. The difference in the rate of the formation of galactose and diabetic cataracts could be explained by these facts.

However, the situation which occurs in xylose cataracts is more difficult to evaluate. The opacities develop rather quickly but their transient nature is difficult to explain. VAN HEYNINGEN found two unexpected features of this type of cataract[16]. First, xylose was not found in appreciable quantities, and although xylitol accumu-

lates, xylulose was never detected in the lens of rats fed xylose. It is apparently difficult to raise the level of xylose in the circulating fluid by feeding experiments. Once xylose enters the lens it is rapidly converted to xylitol which in turn is undoubtedly converted to xylulose. Since xylulose was not found in lens of the xylose-fed rat it must be further metabolized. To account for the observed changes in opacities, it may be possible that initially the rate of xylitol formation is more rapid than its withdrawal. Once an adequate concentration is reached the oxidation to xylulose and perhaps to other products are set into motion. These possibilities will have to be further explored before any definite relationship can be established between the course of this type of cataract and the properties of the enzymes of the sorbitol pathway.

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