# THE LENS IN DIABETES

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## SUMMARY

**This paper reviews the changes which occur in the human lens in diabetes. They include refractive changes and cataract and age-related increases in thickness, curvatures, light scattering, autofluorescence and yellowing. The incidence of cataract is greatly increased over the age of 50 years, slightly more so in women, compared with non-diabetics. Experimental models of sugar cataract provide some evidence for the mechanism of the uncommon, but morphologically distinct, juvenile form of human diabetic cataract, where an osmotic mechanism due to sugar alcohol accumulation has been thoroughly studied in diabetic or galactose-fed rats. The discrepancy between the ready accumulation of sugar alcohol in the lens in model systems and the very slow kinetics of aldose reductase (AR) has not been satisfactorily explained and suggests that the mechanism of polyol formation is not yet fully understood in mammalian systems. The activity of AR in the human lens lies mainly in the epithelium and there appears to be a marginal expectation that sufficient sorbitol accumulates in cortical lens fibres to explain the lens swelling and cataract on an osmotic basis. This is even more so in the cataracts of adult diabetics, which resemble those of age-related non-diabetic cataracts in appearance. The very low levels of sorbitol in adult diabetic lenses make an osmotic mechanism for the increased risk of cataract even less likely. Other mechanisms, including glycation and oxidative stress, are discussed. The occurrence of cataract is a predictor for increased mortality in the diabetic.**

The diabetic lens is larger than normal, disposed to refractive change and at increased risk of cataract, sometimes of a specific type. This paper discusses the factors involved.

## ANATOMY AND PHYSIOLOGY

The lens is enclosed in a collagenous capsule containing other matrix proteins and proteoglycans. A monolayer of epithelial cells is interposed between the anterior capsule and the main cellular mass of lens fibres. The lens fibres are laid down in a series of onion-skin layers, which arch over the equator to meet their opposite numbers at the lens sutures. The innermost fibres comprising the nucleus of the lens are free of organelles and show limited metabolic activity. The outer fibres comprise the cortex. The most superficial fibres of the cortex are nucleated and, like the epithelium, show the normal complement of organelles. Glucose, which enters the lens by facilitated transport,[1] is its main energy supply, although energy may also derive from amino acids.[2] The metabolism of the cortex is chiefly anaerobic, with 70% of the energy supply of the lens deriving from anaerobic glycolysis. If a lens is incubated in nutrient medium in anaerobic conditions with an adequate supply of glucose it remains transparent for a number of hours.[3,4] The metabolism of the epithelium is aerobic. New lens fibres arise by cell division in the germinative zone in the pre-equatorial region of the lens.

The epithelium and superficial cortical cells are major sites of ion pumps such as $Na^+$,$K^+$-ATPase and $Ca^{2+}$-ATPase, concerned with maintaining the water and ionic environment of the lens,[5,6] and there are also ion channels in these cells which cooperate in this regulatory function and additionally conserve cell pH.[7] Ionic equilibrium is facilitated by gap junctions present between the cells.[8] In the human lens, gap junctions occupy a relatively small fraction of the membrane, compared with that in other species. Until recently gap junctions have been thought to be represented in the lens by the major intrinsic polypeptide (MIP26), although this protein may in fact perform a volume regulatory function. The lens is protected from oxidative damage by a range of scavenger molecules located in membranes and in the cytosol. These include vitamin E within the membranes, reduced glutathione (GSH), and ascorbic acid, superoxide dismutase, catalase, taurine and beta-carotene. The distributions and concentrations available vary between species.

Thirty-three per cent of the wet weight of the human lens is protein[9,10] and over 90% of this is made up of the lens crystallins (alpha, beta and gamma) (Fig. 1). This high crystallin concentration is responsible for the high refractive index of the lens. The crystallins exhibit a short range order similar to that of glass.[11] This accounts for the high degree of transparency of the individual lens fibres.

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**Eye (1993) 7, 260–275**

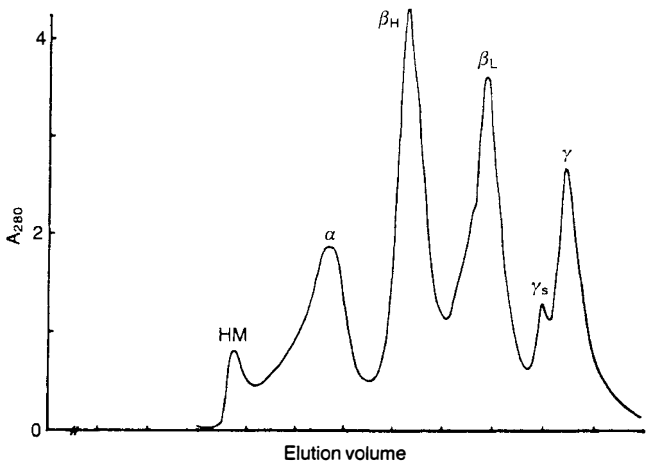


**Fig. 1.** *Gel chromatography of bovine lens proteins. Aggregated protein (HM) is followed by α, $\beta_H$, $\beta_L$, $\gamma_s$ and γ crystallins. (Beswick and Harding; from Harding[122] with permission.)*

Other factors responsible for lens transparency include the orderly arrangement of the lens fibres, the sparseness of the cellular organelles, the peripheral location of the fibre nuclei away from the optic axis, the small extracellular space (in the region of 1% of the lens volume[12]) and the narrowness of the fibre membranes. The transparency of the normal young human lens is 90% for incident light in the wavelength range 500–1000 nm.[13] Transparency decreases with age.

Disruption of fibre organisation and integrity (swelling and breakdown), expansion of the extracellular space and aggregation of lens proteins, particularly the ubiquitous crystallins, may lead to the focal increase in light scattering and degradation of the retinal image which characterise cataract. More diffuse crystallin aggregation may simply reduce light transmission by the lens in the absence of a focal lens opacity.

The lens crystallins range in size from the monomeric gamma crystallin (20 kDa) to alpha crystallin (800 kDa), which is an aggregate of subunits; beta crystallins are of intermediate mass. The alpha crystallin aggregates consist of two major polypeptides of 20 kDa, and of their phosphorylated derivatives. The polypeptides of the beta crystallins range in size from 21 to 35 kDa. The beta and gamma crystallins are highly homologous, and form one large superfamily of proteins. They are rich in thiols. The complete structures of one gamma and one beta crystallin are now known.[14,15] Unlike gamma crystallins, the alpha and beta crystallins have blocked amino-terminal residues and this is thought to have a protective function. Gamma crystallins, such as gamma II, can undergo post-translational modification by sugars such as glucose-6-phosphate.[16]

## THE LENS ZONES

In slit-lamp section the adult lens can be seen to be divided into zones of differing light-scattering properties. The first, a subcapsular, cortical zone (designated C1) is a zone which is of relatively constant width throughout life, the greater part of which is clear (C1 alpha) and the posterior border of which appears as a light-scattering zone (C1 beta). The subjacent zone is a further clear zone (C2), which increases in thickness as the lens grows in sagittal width. The relationship between C1 and C2 is interesting: as new fibres are added to the outer surface of C1, the deeper fibres of C1 beta show diminished light-scattering properties, and are incorporated into C2.[17,18] Deep to C2 is another light-scattering zone, which broadens in width with age and increases its scattering properties from about the age of 45 years. The deepest zone is again clear and is termed C4. Zones C1 and C2 comprise the superficial cortex, and C3 and C4 the deep, or perinuclear, cortex.

## LENS GROWTH

The sagittal width of the lens is about 4 mm at birth. Postnatal growth measured in the sagittal plane shows two distinct phases. The lens retains its neonatal width for the first 20 years while expanding equatorially.[19] After this time there is a steady growth in sagittal width at a rate of about 29 μm/year[20] with relatively little change in equatorial diameter. Various growth factors, including insulin-like growth factor (IGF), are found in human aqueous and vitreous[21,22] and there are receptors on the lens for insulin and IGF,[23] epidermal growth factor (EGF)[24,25] and fibroblast growth factor and (FGF).[26] An unusual feature of lens growth is that while new fibres are added at the surface, certain regions of the lens show a loss of volume, termed compaction.

## LENS CHANGES IN DIABETES[27]

The changes seen in the human lens in diabetes are summarised in Table I.

### *Lens Thickness*

Lens thickness is greater in diabetics than in normal subjects, due mainly to cortical thickening.[28,29] Other biometric changes include steepening of the front and back curvatures of the lens and shallowing of the anterior chamber.[30,31] After adjusting for age, these changes are most pronounced in insulin-dependent (type 1) diabetics, and diabetic duration exerts a powerful independent influence (Fig. 2). The annual expansion of the sagittal width of the lens is accelerated by about 70%.[30] The anterior clear zone (C1 alpha) is expanded in both type 1 and non-insulin-dependent (type 2) diabetics compared with controls. Among diabetics of both types neither age nor diabetic duration appear to play a role in this expansion. The pres-

**Table I.** Lens changes in diabetes

| |
|---|
| Lens thickness |
| Refractive change |
| Sustained |
| Transient |
| Light scattering |
| Chromophore accumulation |
| Fluorescence |
| Cataract |
| Capsular changes |
| Risk for death |

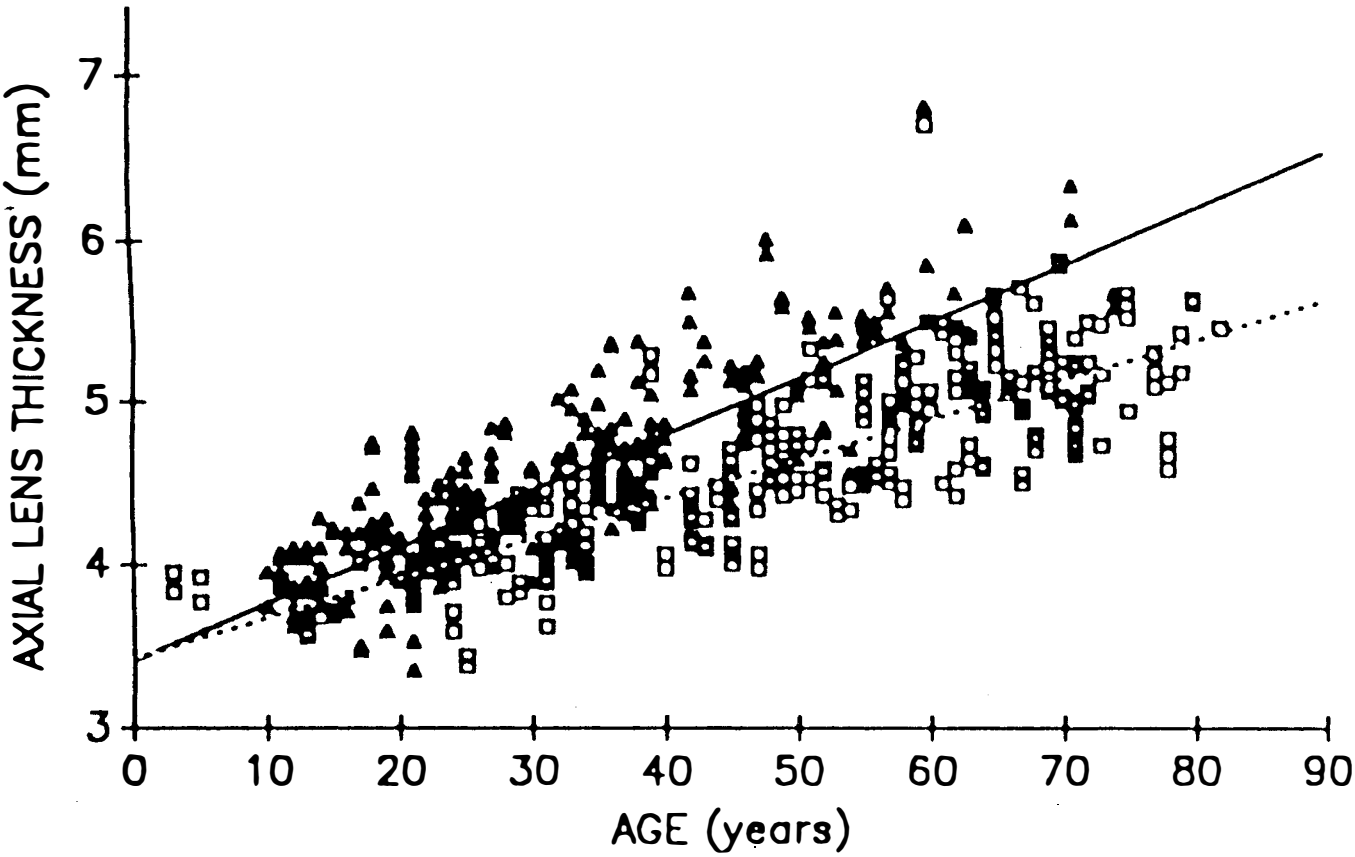


Fig. 2a.

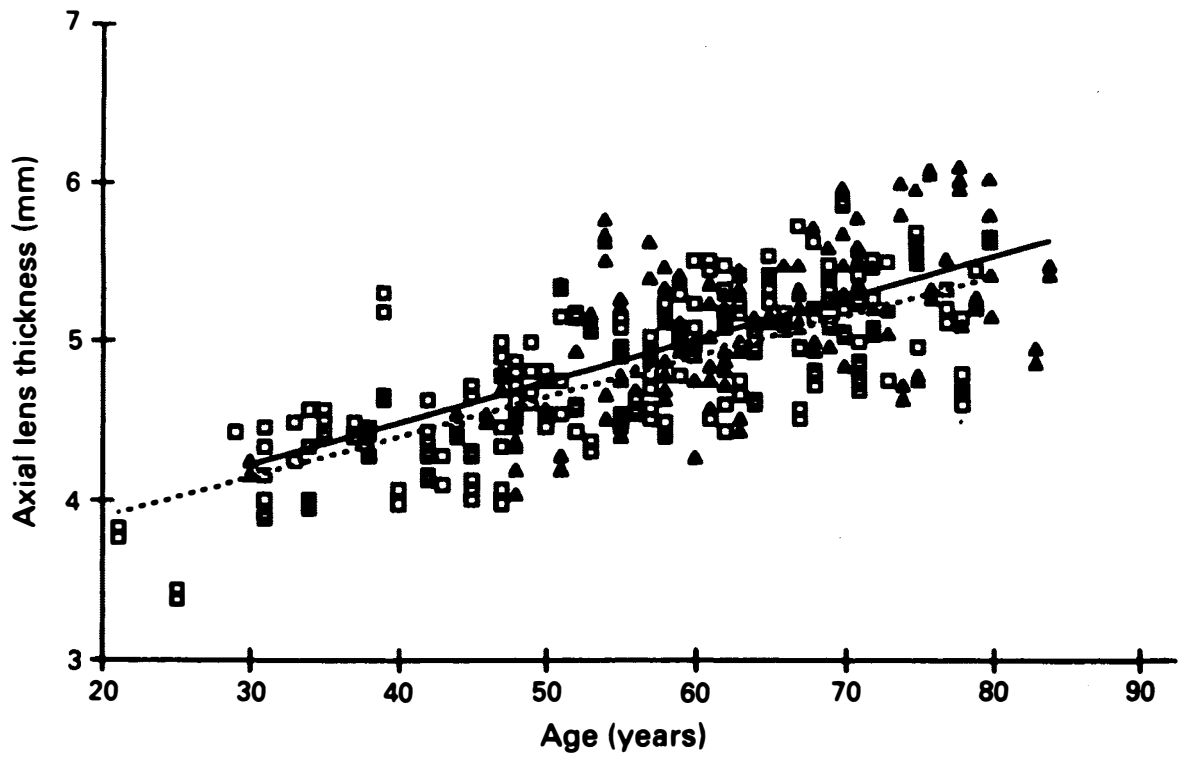


Fig. 2b.

**Fig. 2.** *Change in sagittal lens thickness with age in diabetics (triangles and continuous line) compared with controls (squares and dashed line). (a) Type 1 diabetes: plot of anteroposterior axial lens thickness derived from image analysis of Scheimpflug photographs, against age. Separate linear regression lines for diabetics and controls. (b) Type 2 diabetes: plot of anteroposterior axial lens thickness derived from image analysis of Scheimpflug photographs against age. Separate linear regression lines for diabetics and controls.*

ence of diabetic retinopathy does seem to play a role in this increase in type 1 diabetics.

Although such data may be interpreted as being due to an accelerated growth of the lens, there are alternative explanations. Thus it could be that the lens is swollen to a small degree without major or focal loss of transparency, either due to an increase in cell membrane permeability or to deficient ion pumping. Reduction in the rate of compaction would also give the impression of increased growth.

### *Refractive Changes*

There are both sustained and transient refractive changes in diabetes, with diabetics being slightly more myopic than non-diabetics.[32] Huggert[33] suggested that this should be differentiated from transient myopia. In a population of adults aged 16–66 years, Fledelius[34] found myopia to occur in 37.9% of diabetics compared with 27.5% of non-diabetics. In a further study of metabolically stable diabetics and controls 40% of the diabetics and 22% of the non-diabetics had myopia, with an onset after the age of 20 years.[35,35a] The excess of myopia was of a low degree, usually of the order of –2 dioptres. The increase in thickness[29] and in surface curvature[30,31,36] shown by diabetic lenses could explain this increased prevalence of myopia.

The diabetic eye also suffers episodes of transient refractive change which are common and are often symptomatic. Hyperglycaemia, whether due to the onset of diabetes, poor diabetic control, or a falling blood glucose due to the institution of therapy with insulin or hypoglycaemic drugs may alter refraction. Although there is some controversy, it appears that either hypermetropia or myopia may occur. Usually it is considered that myopia develops in hyperglycaemia,[37,38] and that following therapy the refraction changes back towards a less myopic, or more hypermetropic, state.[39–43] However, hypermetropia is also reported with hyperglycaemia.[44–48] Eva *et al.*[46] found hypermetropia varying between +0.75 and +3.25 dioptres.

The mechanism of transient refractive change is obscure. There is no significant acute change in lens curvature or position[45,49,50] and it is generally agreed that it is due to a change in refractive index of the lens. However, there is no knowledge of the biochemical changes which accompany these refractive events in the human lens, and any hypothesis can only be based on experimental studies.

The most obvious cause would be a change in lens hydration, which could be brought about in a number of ways. It might be argued that a subacute rise in aqueous glucose would produce overhydration by stimulating sorbitol production in the lens (see below). But an acute rise in external glucose levels, from 5.5 mM to 55.5 mM, causes dehydration of the lens *in vitro*.[51] To some extent this osmotic effect is buffered in the diabetic animal by the presence of osmotically active sorbitol in the lens in the hyperglycaemic state, since the protection which it affords can be removed by treatment with an aldose reductase inhibitor (ARI; see below).[52] The lens membranes are permeable to glucose, and it might be imagined that this transient osmotic shock would disappear once normoglycaemia had been restored. But Jacob and Duncan[53] working with amphibian lenses recorded large changes in membrane conductance in a high glucose medium, followed by lens swelling when the lens was restored to isotonic conditions; they termed this 'double osmotic shock'. This may provide a model in which water is drawn into the lens by a sustained lens osmolality while aqueous humour osmolality is falling. This argument would also apply to small molecules in high concentration in the diabetic lens, such as fructose, which would diffuse more slowly from the lens than glucose. The osmotic imbalance would then be sustained by the now greater osmotic excess of sorbitol once glucose levels had been restored. This osmotic excess would gradually diminish as synthesis of sorbitol decreased at the lower ambient level of lens glucose. If these arguments were true, then it would follow that although hyperglycaemia might be responsible for an acute dehydration of the lens, the more prolonged changes induced by either a rise or a fall in aqueous glucose would cause overhydration. An unanswered question about the refractive change is why the refractive shift may persist for up to 20 weeks after onset, and it may be relevant that in the galactosaemic rat model the recovery of biochemical changes in the lens to normal is delayed long after the restoration of lens dulcitol to control levels.[54]

As will be noted later, there are difficulties in accepting an osmotic role for the sorbitol (polyol) pathway in the human lens, and probably other mechanisms must be sought, perhaps related more directly to effects on lens membrane permeability.

One paradox in this story is the ability of a particular glycaemic shift to cause a refractive change in either direction. This would be incomprehensible if it were thought to be caused by a homogeneous change in refraction throughout the lens. However, the refractive power of the normal lens is the product of refractive gradients across the cortex and nucleus and is directly dependent upon the protein (crystallin) gradients across serial layers of the cortex, i.e. serial generations of lens fibres. There is normally a lower protein concentration and refractive index in the superficial cortex than in the nucleus.[55–62] It is thus possible to envisage a situation in which hypermetropia or myopia might ensue, for instance with overhydration, according to how the excess water was compartmentalised.

Not all those diabetics who experience a refractive change are symptomatic. Granstrom *et al.*[63] reported visual symptoms in 34% of diabetics at onset, usually of refractive origin, with a further 47% being asymptomatic. This need not be surprising, since symptoms will be dependent in part on the degree of change, in part on its direction and in part on the prior refractive status of the patient. Thus a myopic shift in a young hypermetrope might go unnoticed, since distance and near vision might remain unchanged. But a myopic shift in an emmetrope or myope would cause symptoms, and a hypermetropic shift

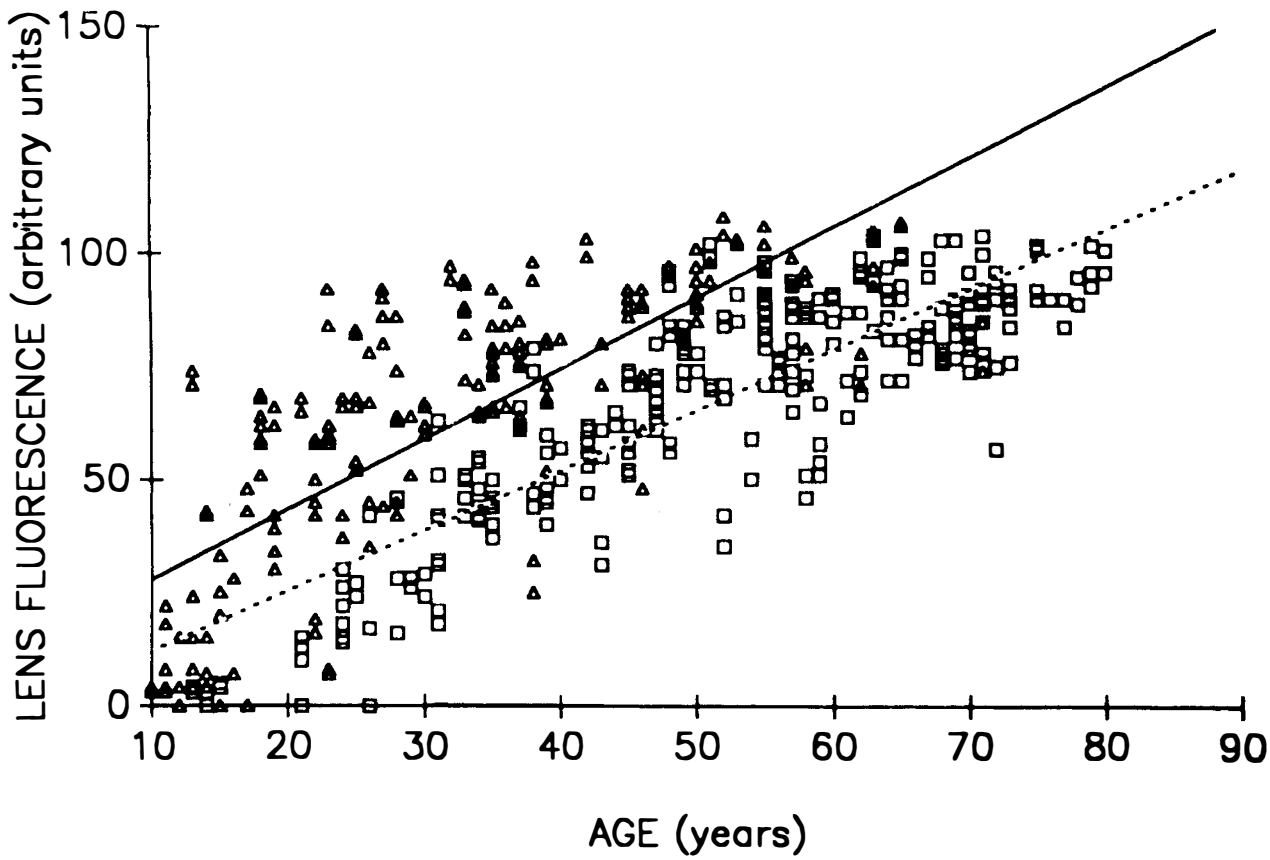


**Fig. 3.** *Plot of lens autofluorescence against age for eyes of early onset diabetics (triangles) and controls (squares). (See text for details of these populations.) Separate linear regression lines have been shown for diabetics (continuous line) and controls (dashed line). (From Sparrow et al.[31] with permission.)*

in 40-year-old hypermetrope could precipitate presbyopic symptoms.

### *Light Scattering and Lens Fluorescence*

With age the human lens becomes less transparent, more yellow,[64] and shows increasing autofluorescence.[65,66] The effect is amplified in the diabetic lens.

Weiss *et al.*[67] demonstrated an increase in light scattering by the diabetic lens using the technique of quasielastic light scattering. Changes were more marked in the presence of retinopathy than without it, and more marked still in eyes which were within 2 years of retinal laser photocoagulation. These latter effects are of interest but are unexplained. In general they reflect a change in molecular size or random movement of the lens crystallins within the fibres. Using the same technique, Bursell *et al.*[68,68a] found a reduction in protein diffusivity in diabetics correlating with levels of glycated haemoglobin (HbA1c), age at onset and diabetic duration and, also, changes in non-diabetic subjects during glucose tolerance and clamping studies which they interpreted as due to alterations in lens hydration. The absence of such an effect in their diabetic patients was attributed to a lack of 'osmotic buffering'.

A number of authors have shown an increase in green fluorescence of the lens (excitation at around 430 nm, emission at around 520 nm) in diabetics compared with non-diabetics,[68–74] particularly in young subjects (Fig. 3).[75] This increase in autofluorescence is more pronounced in type 1 than type 2 diabetics and in the former there is a powerful duration-dependent annual increase of 50% beyond that due to age in controls.[74] Fluorescence and nuclear brunescence appear to be linked.[70–74] Powerful effects of diabetes duration have been noted for lens fluorescence and brunescence which remain strongly associated after accounting for age.[74] Nuclear light scatter is also dependent on diabetes duration and is similarly associated with fluorescence.[36,74] Diabetic retinopathy has been strongly associated with increased autofluorescence in type 1 diabetics, especially in those with short duration of disease.[74] A relationship with diabetic control was suggested by an increase of 11% in autofluorescence for each 1% increase in HbA1c.[73]

The parts of the lens most affected by these phenomena are the nuclear and perinuclear regions, where there is negligible protein turnover and the long-lived proteins are vulnerable to post-translational modification. Some of the extractable pigments of the lens are fluorescent chromophores, which accumulate with age and are derived from proteins modified by glycation (Fig. 4a). Certain reducing sugars such as glucose, fructose, selected pentoses and also ascorbate[76,77] may form Schiff-base compounds with the free amino groups of the crystallins (e.g. the epsilon amino group of lysine, and to a lesser extent the terminal glycine amino group of gamma crystallin. This is followed by an Amadori rearrangement to form more stable products[78] such as fructoselysine (Fig. 4b). At

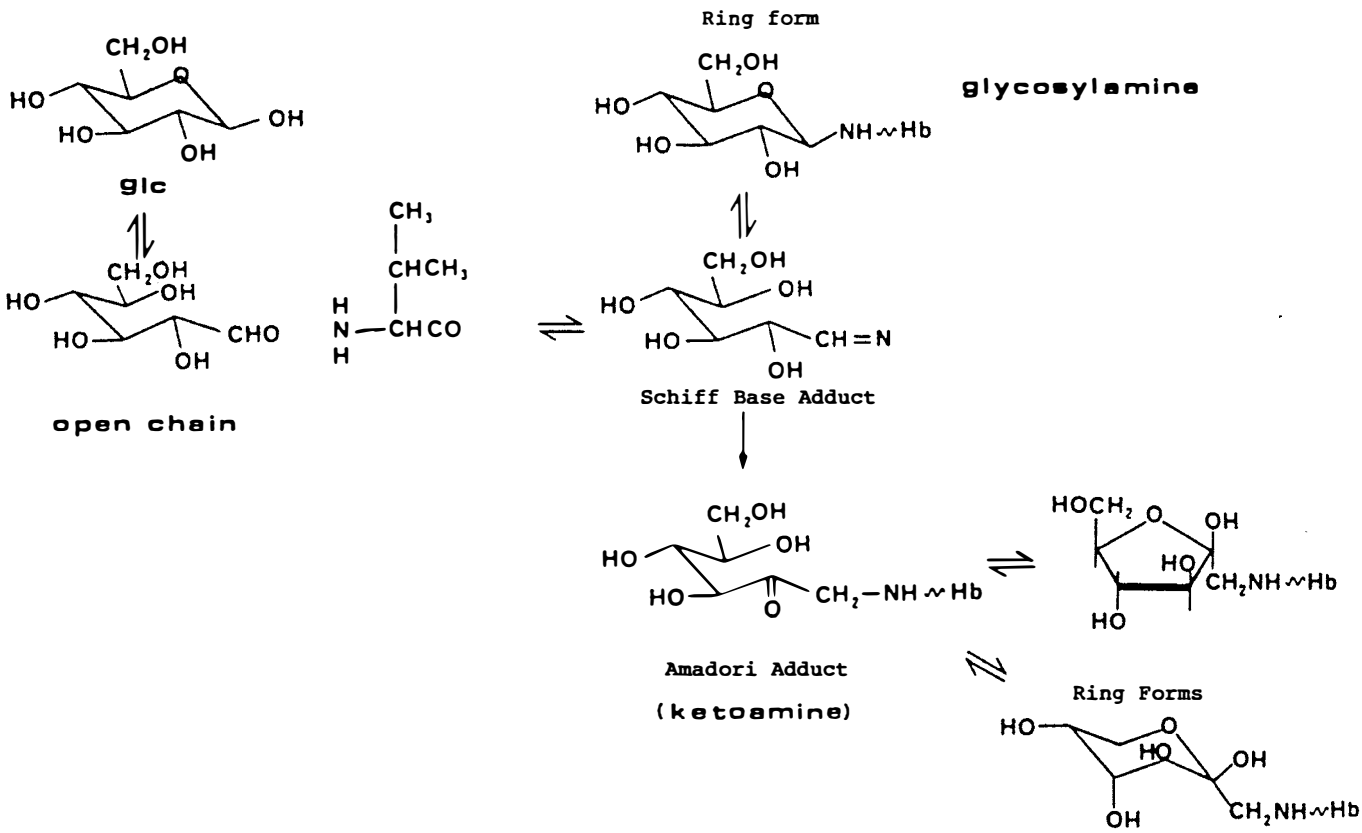


**Fig. 4a.** *Early steps in the glycation of a protein (haemoglobin).*

later stages the non-fluorescent carboxymethyl-lysine and brown fluorescent Maillard products, including pentosidine, may be formed.[79] An age-dependent increase of pentosidine has been found in the lens,[80,81] and absolute levels 2–3 times higher in the water-insoluble fraction suggests a contribution to insolubilisation.[82] In keeping with this, there is a major increase in brunescent cataract in which the degree of cross-linking in the nucleus is particularly high. In that study there was no difference in pentosidine levels between diabetic and non-diabetic lenses, although a significant increase in diabetic cataractous lenses was reported by Lyons *et al.*[83] Pentosidine can be formed by incubation of bovine crystallins with ascorbate and its oxidation products.[84] Carboxymethyl-lysine increases in the lens with age and there is a greater accumulation in the diabetic lens.[85]

### *Cataract in Diabetes*

Cataract is the major cause of blindness on a world scale, and its frequency is increased in diabetics, particularly in women.[86] It makes an important contribution to diabetic blindness.[87,88]

Although not associated with the diabetic state itself, hypoglycaemia induced by therapy may cause cataract, because glucose is the major energy source for the lens.[89,90] Also, hypoglycaemia inhibits the activity of lens hexokinase,[91] the major rate-limiting step for entry of glucose into the glycolytic pathway.[92,93]

It has long been known that diabetes or exposure to diets rich in selected sugars will induce cataract in experimental animals,[94–97] but great caution must be used in transferring such data to the human situation. There are many known species differences in lens biochemistry.[98,99] Cataract has been induced, usually in the rat, in the diabetic animal or with sugar feeding with galactose, xylose or arabinose.[4,100] A 35% or 50% galactose diet induces cataract more readily and of a more severe degree than does diabetes itself because, it has been argued, of the greater activity of aldose reductase towards such sugars.[101]

A summary of those events which precede cataract formation is given in Table II. These events are more likely to be of aetiological importance than those arising after the formation of cataract.

Cataract formation is associated with swelling and vacuole formation, and later with increased membrane permeability, with a loss of myoinositol and potassium and an influx of sodium, and later a leakage from the lens of larger molecular species including the crystallins. There is impairment of $Na^+,K^+$-ATPase activity[102] and a loss of amino acids, myoinositol,[103,104] choline and taurine due to an increase in membrane permeability or possibly a decrease in transport functions. There is also a reduction in protein synthesis.[105]

### *Aldose Reductase and the Polyol Pathway*

The mechanism which has gained most attention in the acute sugar cataract model has been one dependent on activation of the polyol (sorbitol) pathway.[98,100,101,106] The conversion of hexose (glucose; galactose) or pentose sugar (xylose) to its sugar alcohol is held responsible for

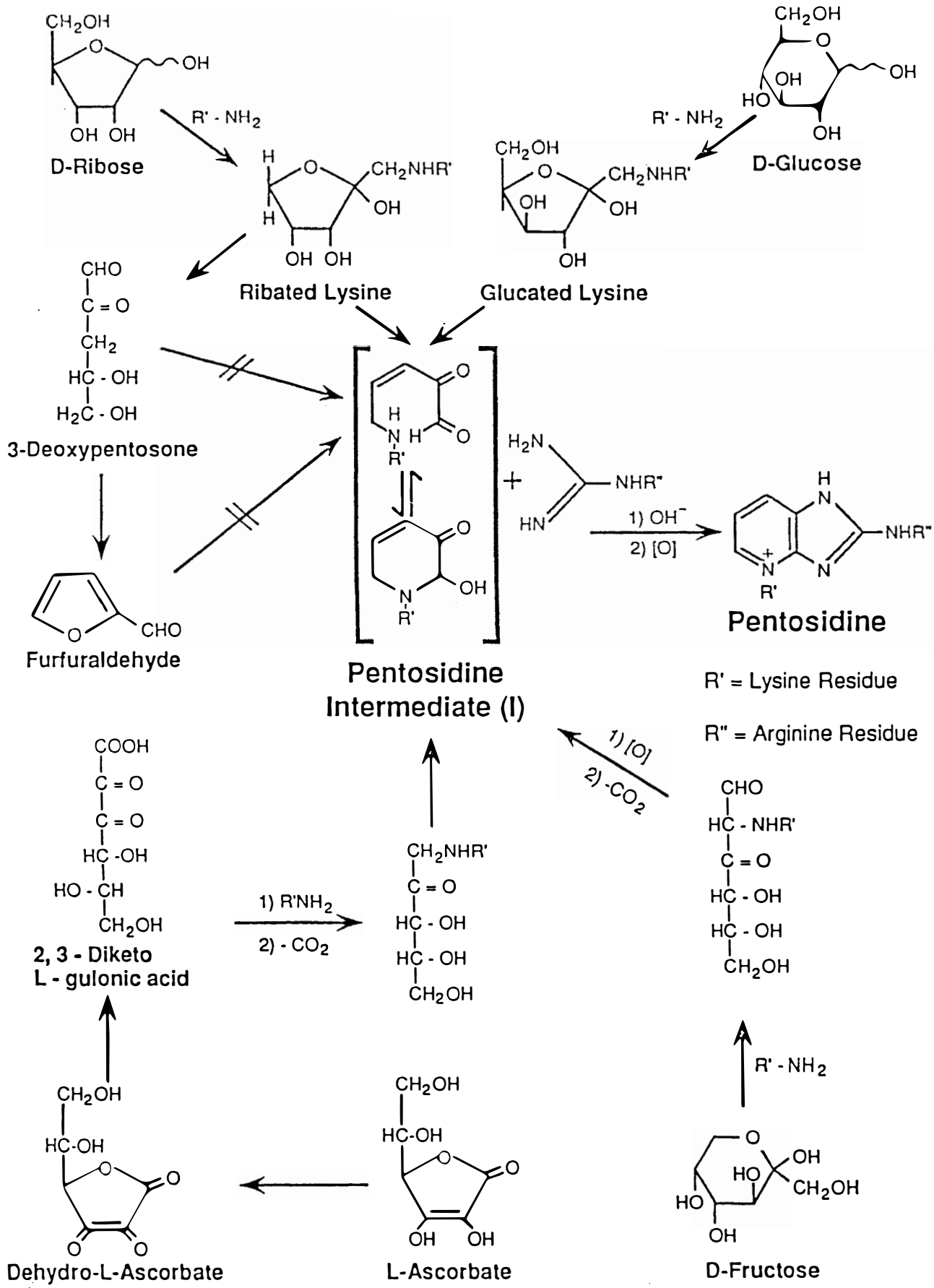


**Fig. 4b.** *Proposed mechanism of pentosidine formation from ribose, glucose, fructose and ascorbate. (After Grandhee and Monnier*[84] *with permission.)*

**Table II.** The lens in diabetes

| Increases | Decreases |
|---|---|
| *Changes preceding the first opacities* | |
| Glucose | Amino acid uptake |
| Fructose-3-phosphate | Amino acid content |
| Sorbitol-3-phosphate | Taurine content |
| Sorbitol | Glutathione |
| Fructose | NADPH |
| Mitosis | Myoinositol |
| NADP+ | Glucose-6-phosphate dehydrogenase |
| Glycated protein | 6-Phosphogluconate dehydrogenase |
| Disulphide cross-linked protein | Pentose phosphate pathway |
| | Phosphofructokinase |
| | Aldolase |
| | Choline uptake |
| | Phosphorylcholine |
| | ATP |
| | Lactic dehydrogenase |
| | Protein synthesis |
| *Changes at the time of the first opacities* | |
| Swelling and degeneration of cortical cells | |
| Covalent modification of membrane protein | |
| Calcium | |
| Leakage of gamma crystallin | |
| *Later changes* | |
| Extracellular space | Sorbitol |
| Sodium | Fructose |
| | $Na^+$,$K^+$-ATPase |
| | Potassium |

See Harding[122] for sources of data.

inducing an osmotic load within the lens, causing swelling, fibre breakdown and opacification. Reduction of sugar to sugar alcohol requires the presence of the enzyme aldose reductase, the activity of which, measured by indirect techniques, is high in the rat lens (Fig. 5). It is located in the lens epithelium and to a lesser extent in the superficial fibres.[107–109] Activity is high in the rabbit and guinea pig and higher still in the octagon degu, a small South American rodent in which sugar cataract is readily induced by mild diabetes;[110] however, it is low in the mouse (one tenth the activity in the rat), so that the diabetic mouse does not develop cataract.[111] Levels are also very low in the calf, and in the human lens.[108] Early studies of sugar cataract showed hydropic swelling of the lens fibres,[112,113] and it was subsequently demonstrated that the accumulation sorbitol in the rat lens was sufficient to account for an osmotic swelling.[114] In both diabetic and galactosaemic cataracts polyol accumulation has been related to lens hydration, especially in the epithelium and outermost fibres.[4,114–16] Studies by Kinoshita using cultured lenses showed that if osmotic swelling of the lens was prevented by creating a balancing osmotic force outside the lens, early changes such as the fall in GSH, depletion of myoinositol and amino acids, and electrolyte changes did not occur, and cataract was prevented.[101]

There are some fundamental difficulties in understanding involvement of aldose reductase in causing cataract, and particularly its role in human cataract and other diabetic complications. The activity of the enzyme is usually determined indirectly, using glyceraldehyde as a substrate and measuring the consumption of NADPH.[117] Crabbe[120] has suggested that apparent activity may be due to monosaccharide-generated peroxy radical oxidation of NADPH rather than to enzyme activity. Aldose reductase activity towards glucose and to a lesser extent galactose or xylose[99,118] is extremely low, its specificity is broad and it lacks stereospecificity. Its rate enhancement is also poor.[119,120] The behaviour of aldose reductase is thus very different from other enzymes. In keeping with this, the purified enzyme has yet to be shown to produce sorbitol in significant amounts from glucose and coenzyme without preincubation of substrate,[121,122] and intriguingly the recently published structure of the enzyme shows it to possess a hydrophobic cleft which would not readily accept a sugar.[123]

The accumulation of sugar alcohol at an early stage of experimental sugar cataract is not in question, but its source is. It should be remembered that sorbitol may also be produced from fructose, through the action of polyol dehydrogenase and the oxidation of NADH to NAD (Fig. 5).[51]

There is nonetheless some interest in those studies in which sugar cataract has occurred despite a lack of net osmotic change, or has been prevented or retarded despite the accumulation of sugar alcohol, with the implication that such cataracts do not have an osmotic basis. Of greatest interest is the report by Malone *et al.*[124] concerning diabetic rats developing cataract. The rise in sorbitol was inversely related to the level of lens taurine in such a way that there was no net change in lens osmolar activity. Treatment with the aldose reductase inhibitor (ARI) sorbinil prevented cataract and restored the sorbitol and taurine levels, but did not influence lens water. Earlier studies reported the inhibition of sugar cataract after feeding with diets rich in vitamin E[125–127] or fat,[128] including unsaturated fat.[128–131] Unsaturated fats and vitamin E have membrane protective effects, and it is surmised that a high fat diet would encourage vitamin E absorption. Partial protection has also been reported from supplementation of rat diets with vitamin C.[132] Although these studies provide evidence for an oxidative rather than an osmotic mechanism for damage, not all reports confirm them,[133] and the possi-

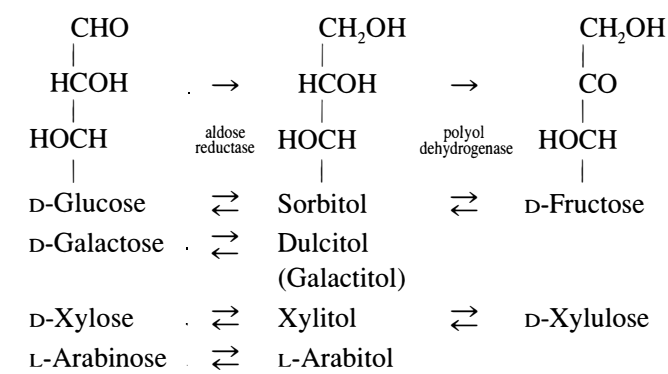


**Fig. 5.** *Sorbitol (polyol) pathway. Configuration of the first three carbon atoms of the sugars and polyols. Adapted from van Heyningen.*[100]

bility of methodological problems in some of these studies has been raised by Kador.[134]

There is, however, no doubt that, as in the lens, sugar alcohol accumulates in other tissues or cells exposed to high levels of various sugars. This includes Schwann cells of the peripheral nerve,[136–137] the pericytes of the retinal vessels[109,137] and the corneal epithelium.[138–139] Also, with the development of the ARIs[140–142,142a] it has been clearly demonstrated that both experimental galactose and diabetic cataract may be inhibited or prevented by oral or topical ARI administration, accompanied by an inhibition of sugar alcohol formation. Some ARIs (e.g. Quercitrin, Sorbinil and ICI 105552), but not all, have free-radical scavenging activity, and therefore their effect cannot universally be explained through an antioxidant activity. Sorbinil itself has an inhibitory action on polyol dehydrogenase.[108] It must be accepted that there are discrepancies in the aldose reductase story which have yet to be resolved.

A paradoxical finding made by several authors is that galactose feeding delays the onset of X-ray induced cataract in rats[143,144] and mice.[145,146] It has been suggested that this may be due to the free-radical scavenging activity of sugars and sugar alcohols.

### *Oxidative Stress*

Evidence for a role for oxidative stress in experimental cataract is mainly indirect. Like other tissues the lens is constantly exposed to oxidative events and there is some indication that this is increased in the diabetic model. There is greater evidence that the susceptibility of the lens to such stress is diminished, and some evidence for oxidative damage or for protection of the lens by antioxidants, some of which has been alluded to.

The lens is vulnerable to oxidative stress. $Na^+$, $K^+$-ATPase possesses a reactive thiol at its active site[147] and is vulnerable to such oxidation. Oxidation of lens membrane thiols increases membrane permeability.[148]

There is an early fall in lens glutathione in experimental cataract[149] which is generally thought to be due to a deficiency of the NADPH necessary for its regeneration.[51] It has been argued that this results from an excessive consumption of NADPH during the conversion of glucose to sorbitol in the polyol pathway (Fig. 5), with the implication that hexose monophosphate (pentose) shunt activity, the chief regenerative source of the reduced coenzyme, fails to restore this. Most studies indicate that pentose shunt activity in the lens is stimulated by the diabetic or galactosaemic state, with a linear increase with increasing glucose up to 60 mM, to at least 10 times the basal activity.[51] It is surprising, therefore, that this activation is not able to sustain GSH levels. It may be noted that in one study of galactose cataract the fall in GSH was not accompanied by a reduction in synthesis. Activation of the shunt occurs in several situations of oxidative stress, for instance by exposure of the lens to thiol oxidants such as hydrogen peroxide.[151] At higher levels exposure may, however, inhibit shunt activity. Since NADPH is used during the regeneration of GSH, it could be argued that a deficiency of NADPH could arise as a result of this process.

Levels of ascorbic acid are reduced in the aqueous of alloxan-diabetic animals and this would be expected to affect levels in the lens.[151] Levels of aqueous oxidised glutathione (GSSG) are raised and GSH levels are normal.[152] It should be noted, however, that lens GSH is synthesised in the lens from its precursor amino acids,[153] and therefore the implications of aqueous levels for oxidative stress in the lens itself are not clear. Lens cells are poorly permeable to GSH and more readily permeable to GSSG. It should therefore also be considered whether the increased GSSG arises from the lens itself, since GSSG is rapidly excreted from the lens. As noted earlier, however, lens GSH levels are reduced as an early event in the diabetic rat. Taurine, which is a scavenger of hypochlorite, is also found at reduced levels in the lens.[124]

Various kinds of evidence suggest that oxidative damage occurs in the lenses of diabetic and sugar-fed animals. There is a fall in protein thiols in the lens and an increase in crystallin cross-linking.[122] Treatment with the antioxidant, butylated hydroxytoluene, has inhibited cataract in some[122a,b] but not all[122c] studies.

### *Covalent Modification of Lens Proteins*

Another mechanism which is likely to be of more significance in the chronic diabetic state than in the acute diabetic model is that of glycation. The alteration of surface charge leads to an unfolding of the proteins, with exposure of buried crystallin thiols which are then susceptible to oxidation.[16] This gives rise to the formation of relatively insoluble high molecular weight (HMW) aggregates, the large size of which leads to a greater degree of light scattering.[154] A correlation between opacification and the degree of lens protein glycation has been reported in the streptozotocin-diabetic rat.[155]

The late products of glycation and their consequences to the lens were discussed earlier. There is interest in blocking the effects of these late changes with aminoguanidine and related compounds.

Granstrom *et al.*[39] have shown covalent modification of several forms of MIP26 in the diabetic rat and that the degree correlates with the severity of lens opacification. One of these, diaminoguanidine, also has ARI activity.[156]

## CATARACT IN DIABETIC PATIENTS

### *Juvenile Diabetic Cataract*

Juvenile diabetic cataract is now uncommon with the advent of effective hypoglycaemic therapy. It occurs in insulin-dependent diabetics whose onset was before the age of 30 years.[157] Rarely it is recorded in infants. The limited period over which snowflake cataract may occur (chiefly in the first two decades of life) contrasts with the extended period over which refractive change occurs (from youth into the eighth decade). It is of interest that snowflake cataract occurs at a period of life when the lens is undergoing a major physiological shape change, with negligible sagittal and major equatorial expansion. It may be that the mechanisms for refractive change and cataract

are the same, but that age-related factors such as the ability of the lens to swell may protect the older lens from cataract formation. The ability of the lens to swell decreases with age.[158]

Typical features of the cataract are subcapsular and cortical 'snowflakes', and polychromatic opacities and vacuoles. These may proceed to mature cataracts within weeks or months[159] and, rarely, may be reversible on treatment over some weeks or even within 24 hours.[160–162]

The rat sugar cataract model is an attractive model for juvenile diabetic cataract in terms of its acute development and other features. It is also relevant to human galactosaemic cataract, in which the lens is exposed to high levels of aqueous galactose. The first visible indication of galactosaemic cataract is the 'oil-droplet' change on retroillumination, due to a change in refractive index between the inner and outer parts of the lens.

It has been noted that there are difficulties in accepting a role for aldose reductase in human cataract. Even though sorbitol is found in increased amounts in the human diabetic lens[163,164] the amounts detected have been quite low, and insufficient on a lens mass basis to account for osmotic damage.[164,165] Data on a cell-to-cell basis, which would be appropriate, are not available. Although Vadot and Guibal[166] considered that there was sufficient sorbitol in young diabetic lenses to induce cataract, Lerman and Moran[167] could not demonstrate the accumulation of significant amounts in sugar-incubated lenses over the age of 20 years. There is no information about levels in juvenile diabetic cataract itself. Jedziniak *et al.*[108] found a higher aldose reductase activity in the young lens than in the adult and calculated that it was sufficient to generate a significant osmotic stress. However, these calculations referred to the lens epithelium and assumed that sorbitol was not removed. Since polyol dehydrogenase is more active than aldose reductase in the human lens, the calculated levels would be expected to be lower. Lin *et al.*[168] demonstrated accumulation of dulcitol and loss of myoinositol in 72-hour cultures of infantile human lens epithelium in a 30 mM galactose medium, associated with vacuolar changes at ultrastructural level. These changes were reversed by Sorbinil and AL1576. Similar changes have been produced in dog epithelial culture within 6 hours.[137] Lin *et al.*[168] suggest that damage in the human lens may reflect compartmentalisation of aldose reductase activity, for instance in the epithelium. If sorbitol accumulation in the epithelium (and not the fibres) were the basis of juvenile cataract then a failure of epithelial permeability or pumping functions would be a more likely cause of lens swelling and cataract than an osmotic mechanism. There is no information available as to whether an oxidative mechanism, dependent on the polyol pathway or not, is operative in juvenile diabetic cataract.

### *Cataract in Diabetic Adults*

Cataract has a greater prevalence in diabetics with a greater risk for women, and is dependent on diabetes duration. The morphology is no different from that of age-related cataract, although the frequency of some subtypes is increased.

Klein *et al.*[169] in a population study found cataract to be more prevalent in early and late onset diabetes with significant associations with age, severity of retinopathy and diuretic usage. Diabetes duration and the level of glycated haemoglobin were also associations in early onset diabetics. In a second report, cataract was found to be the second most common cause of severe visual loss in older onset diabetics.[87] Various other reports have shown an association between cataract, and diabetes duration[170] or retinopathy.[171] The frequency of cataract extraction is greater in diabetics than non-diabetics.[159,172]

Ederer *et al.*[173] found an excess risk of cataract in two population studies. The Framingham study showed a significant excess risk in the 50–64 year age group (relative risk 4.02), while the HANES study showed a relative risk of 2.97 in this age group and 1.63 in the 65–74 year age group. Both studies showed an excess prevalence of cataract in diabetics in 50–64 year age groups which disappeared at an older age. This has been attributed to the higher mortality in diabetics with cataracts. However, a case–control study in Oxford found an increased risk for cataract extraction in diabetics in the age group 50–79 years, and a small increase in risk for women relative to men (Table III).[86,174–176]

It has already been noted that the morphology of cataract in the diabetic resembles that seen in age-related cataract in the absence of diabetes. Thus the major features are nuclear cataract (increased nuclear scattering and brunescence) and cortical spoke and posterior subcapsular cataract. In the Lens Opacity Case Control study, diabetes increased the risk of posterior subcapsular, cortical and mixed forms of cataract.[177] Individual features may not have an identical aetiology, but it is likely that those metabolic changes identified in experimental cataract are relevant for the human state in varying degree. There is no relation between cataract type, and the level of either sorbitol or myoinositol in lens epithelium from patients with cataract and diabetes.[178]

It has been suggested that the increased nuclear scattering and brunescence in diabetic lenses is likely to be the result of increased glycation[179–186] and the formation of advanced glycation end-products. There is evidence for a fall in free lysine amino groups in the human diabetic lens.[187] It is also possible to induce a change in tertiary structure in alpha crystallin (bovine) incubated with glucose and glucose-6-phosphate.[188] A threefold increase in

**Table III.** Diabetes as a risk factor for cataract in different age groups and in males and females

| Subgroup | Relative risk | Confidence interval | *p* |
|---|---|---|---|
| All (50–79 years) | 5.0 | 3.4 to 7.4 | «0.001 |
| 50–59 years | 12.6 | 2.8 to 58 | <0.001 |
| 60–69 years | 5.6 | 2.7 to 11.3 | <0.001 |
| 70–79 years | 4.2 | 2.6 to 16.8 | <0.001 |
| Males | 3.4 | 2.1 to 5.7 | «0.001 |
| Females | 7.9 | 4.3 to 14.3 | «0.001 |

Data from Harding *et al.*[86]

glycation was measured in diabetics and controls by Vidal *et al.*[189] but there was no correlation with the degree of browning of the lenses measured spectrophotometrically, and they concluded that other chromophores were responsible for the browning at the relevant wavelengths. Certainly a number of other candidates have been proposed to contribute to nuclear brunescence of the non-diabetic lens,[99] but since the diabetic state is not anticipated to increase their concentration, glycation products are still the most likely candidates to explain the excess in diabetics.

Cortical cataract can be caused experimentally by agents which interfere with membrane permeability and ion water control. Oxidation of membrane thiols causes lens clouding[148] and incubation of the lens with ouabain causes lens swelling and cataract.[190] The non-diabetic, ageing human lens, free of cataract, shows an increased membrane permeability[191] which parallels the increase in optical density which occurs from about the fifth decade.[65,66] There is evidence of degradation of the lens protein MIP26 with age in non-diabetics, which could be responsible for a functional abnormality.[192] This channel protein has until recently been regarded as the gap-junctional protein, but may in fact serve as a volume-regulating channel. Disturbance of either function could increase the risk of cataract. It would be of great interest to see whether these events are accentuated in the diabetic lens. The greater thickness of the diabetic than the non-diabetic lens noted earlier could be relevant to this point, as is the change in $Na^+$,$K^+$-ATPase kinetics reported by Garner and Spector[193] with exposure to glucose-6-phosphate and a similar change noted in diabetic human lenses. Hydrogen peroxide is present in normal human aqueous, and present at raised levels in the aqueous of patients with cataract.[194] Higher levels are found in the aqueous of diabetic patients with cataract.[187] Simonelli *et al.*[195] have also shown an increase in malondialdehyde in cataractous compared with non-cataractous lenses which is greater in the cataracts of diabetic patients. Malondialdehyde is a product of lipid peroxidation of cell membranes, and is regarded as an indicator of oxidative membrane damage. These are important findings, although the methods of measurement are not entirely specific.[196]

The potential role of the sorbitol (polyol) pathway in juvenile cataract was discussed earlier. Recent studies of cultured lens epithelium from cataract patients have shown negligible or absent levels of sorbitol in the epithelium of non-diabetics. In diabetics sorbitol levels are raised in most epithelia, in proportion to the blood glucose levels, while there is an inverse relationship between blood glucose and myoinositol.[178]

It has been noted that oxidative stress may cause lens membrane damage experimentally. It may also cause damage to DNA.[197] Subcapsular cataract may be regarded as due to an aberration of lens mitosis and lens fibre differentiation, and could be the result of oxidative damage. There are no data which link this to human subcapsular cataract.

### *Other Cataract-Related Events*

A higher rate of capsular rupture reported in diabetics undergoing intracapsular[172] or extracapsular[198] extraction could be related to structural and chemical changes which are known to occur in the capsule. Thickening of the capsule has been reported in diabetic humans and animals, including the genetically determined diabetic *kk* mouse.[199] There is an increased risk for death for patients with cataract,[200] and several studies have indicated a greater risk in diabetics.[201–203] Cohen *et al.*[203] found lens opacities to be a powerful predictor of death, independent of other factors and with an odds ratio of 2.4 (95% confidence interval 1.5–3.9).

Key words: Diabetic lens: thickness, fluorescence, refraction, cataract.

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