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NUTRITIONAL FACTORS IN CATARACT[1]

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CONTENTS

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INTRODUCTION

The eye is the organ that enables animals to perceive the world by converting light into electrical signals. The lens is the part of the eye that focuses light on the retina and thus assures that near and distant objects can be seen with equal clarity. To fulfill this function the lens must be transparent. The high density of lens structural proteins and their spatial arrangement in a repeating lattice produces a body with a nearly uniform refractive index and thus transparency. Events that cause loss of order and induce abrupt fluctuations in refractive index are the physical bases for increased light scattering and loss of transparency (4, 7, 91). Perturbations capable of producing opacities include formation of protein aggregates and regional changes in water content. When the opacific changes are sufficient to cause significant loss of visual acuity, the subject is said to have a cataract.

The chief sources of cataract prevalence data for the United States are the Framingham Eye Study (66) and the National Health and Nutrition Examination Survey (NHANES) (124). In the first of these studies, senile cataract was found in approximately 5% of persons at ages 52–64, 18% at ages 65–74, and 46% at ages 75–85. The NHANES data essentially agree with these figures. Further study of these results by investigators at the National Eye Institute determined that the prevalence of cataract was 3–4 times higher in diabetics than in nondiabetics under 65 years of age (44), thus confirming a long-standing clinical impression on this question.

LENS ANATOMY AND PHYSIOLOGY

The ocular lens is an encapsulated organ without blood vessels or nerves. In a sense, it is in a state of organ culture in the eye, in that it is bathed by the aqueous humor on the anterior surface and by the vitreous humor on the posterior surface. The capsule is composed of complex polysaccharides and collagen elaborated by epithelial cells. The lens epithelium is a single layer of cells found only on the anterior undersurface of the capsule. At the equatorial region the epithelial cells elongate and transform into fiber cells, a process that continues throughout the life of the organism. As new fiber cells are formed, older cells are compressed into the center of the lens and form a nucleus and cortex. This differentiation process is characterized by loss of cellular organelles such as mitochondria and nuclei and the synthesis of structural proteins called crystallins.

Three major crystallin families—alpha, beta, and gamma—constitute approximately 90% of the proteins of the mammalian lens. The alpha and beta polypeptides exist in polymeric assemblies, whereas the gamma crystallins remain monomeric. Modern techniques of molecular biology have revealed that crystallins are products of gene duplication and divergent evolution from

ancestors of different function. Alpha crystallins possess a sequence similarity with the small heat shock proteins of *Drosophila*. Beta and gamma crystallins appear to be related to a bacterial spore coat calcium-binding protein. Other crystallins found in the lenses of various species seem to have arisen from common cellular enzymes. These fascinating relationships have been recently reviewed in depth (92, 132).

Although the majority of the lens mass is represented by the fiber cells and their content of structural crystallins, the lens epithelium occupies a position of special importance because it is critical to the growth, development, and maintenance of the interior cells. This single layer of cells covering the anterior surface is the major permeability barrier and the site of various transport systems. Because the lens is avascular, the interior cells depend largely on the epithelial layer to maintain a constant supply of nutrients and suitable electrolyte balance and to pay the necessary metabolic cost of this enterprise. Similarly, the capsule-epithelium is constantly exposed to hydrogen peroxide and other toxic molecules and plays the primary role in defending the interior cells from such hazards. Although most studies on lens metabolite concentrations and enzymic specific activity have reported data on whole lens, in fact a steep gradient exists from the surface toward the interior in substances such as ATP and the antioxidant glutathione (49, 58). In addition, enzymes such as glutathione reductase, catalase, superoxide dismutase, Na,K-ATPase, aldose reductase, and hexokinase are concentrated in this crucial perimeter (59). Given this set of circumstances, the vitality and integrity of the lens epithelium are clearly of paramount importance in the maintenance of the transparency of the organ. This view was supported by Karim et al (67), who found that posterior subcapsular cataract, cortical cataract, and mixed cataract all correlate with significant morphologic changes in epithelial cells.

PATHOGENESIS OF CATARACT

Although the exact cause of cataract remains obscure, two different, fundamental processes that provoke loss of transparency appear to be involved: osmotic damage and protein aggregation. Osmotic effects are observed in the lens cortex, whereas protein aggregation occurs primarily in the lens nucleus. The cortical osmotic type of cataract, which involves the more recently formed fibers, may result from defects in cation transport processes, increases in membrane permeability, or accumulation of osmolytes, and the net increase of sodium chloride and water can culminate in rupture of fiber cell membranes. When osmotic imbalance occurs, water vacuoles or clefts appear and create a nonuniform area of index of refraction and thus increased light scattering. Duncan & Bushell (42, 43) suggested that membrane malfunctions are wholly or partly to blame for approximately 70% of all cataracts.

Protein aggregation occurs by means of both covalent and noncovalent forces and can take place in the absence of changes in hydration or electrolyte content. The resultant loss of organization is followed by increased light scattering and nuclear sclerosis.

Oxidation may cause or accompany cortical and nuclear cataract. Hydrogen peroxide is present (20–30 μM) in the aqueous humor (93) and can give rise to superoxide anion, singlet oxygen, and hydroxyl radicals. Oxidants that elude the various defensive barriers can attack components of epithelial and fiber cell membranes and enzymes engaged in energy production or the maintenance of electrolyte balance, with an eventual loss in the ability of these cells to maintain homeostasis. Activated forms of oxygen produced within the lens by near-ultraviolet radiation (1) or oxidation of sugars (62) may oxidize lens crystallins and thereby promote intermolecular crosslinking and the formation of protein aggregates. That oxidation is an important mechanism of cataractogenesis is generally conceded (112).

Several other chemical modifications of lens proteins may also contribute to the emergence of opacities. Nonenzymatic glycosylation of the epsilon amino group of lysine can produce crosslinking and unfolding through the formation of complex Maillard products; this process may be significant in diabetic cataract (119). Cyanate, produced spontaneously from urea, can react with crystallins to produce carbamylated adducts, causing conformational shifts and increased susceptibility to oxidation (6). Harding (55) has suggested that severe episodes of diarrhea and heatstroke with consequent dehydration and uremia might generate levels of cyanate sufficient to inflict cataract-promoting injury upon the lens. The virtual absence of protein synthetic capacity by fully differentiated lens fiber cells means that adduct formation or loss of protein through proteolysis will not be subject to replacement or repair.

The initiation and evolution of aging-related (senile) cataract are multifactorial, a fact reflected in the diversity of appearance and physicochemical characteristics of the various clinical forms of opacities. Although no unifying general theory can predict the course or rate of cataractogenesis, risk factors can be listed that can interact to produce light-scattering defects in lens structure. In this review, we examine nutrients linked to the generation of this malady. The most recent in-depth review on this topic was published in 1980 (14).

ANIMAL STUDIES

Carbohydrates

Glucose readily enters lens epithelial and underlying fiber cells, where it is phosphorylated by hexokinase and metabolized by the familiar pathways of

glycolysis and the citric acid cycle. In the event of an abnormal elevation of blood glucose, as occurs in diabetes, excess glucose is reduced by NADPH-dependent aldose reductase to sorbitol. This sugar alcohol tends to accumulate, since it is poorly diffusible and its rate of removal by sorbitol dehydrogenase is much slower than its rate of synthesis. An osmotic disequilibrium is thus created that leads to a net influx of water, swelling, increased membrane leakiness, and cation imbalance. Morphologically, the lens progresses through a vacuolar stage (hydropic lens fibers) followed by cortical opacities and ultimately a dense nuclear cataract. Cataracts can be produced even more rapidly by diets rich in galactose, since galactitol is more rapidly produced and less quickly metabolized in the lens than sorbitol. The development of aldose reductase inhibitors has been of great value in unraveling the pathogenesis of "sugar cataracts" and in providing potentially valuable therapeutic agents to slow or prevent diabetic cataract. Several reviews provide a detailed accounting of the biochemistry and history of the polyol-induced cataract (30, 71, 72).

Many investigators have employed either high-galactose diets or the experimental induction of diabetes with alloxan or streptozotocin as models for investigations into the role of nutrition in cataractogenesis. In general, diets rich in antioxidant chemicals, riboflavin, protein, or fats have delayed the rate of appearance of the end-stage cataract. Few such studies, however, have used slit lamp or histologic evaluations of the lens. Early lesions of cortical cataract might easily be overlooked if only the unaided eye is used in determining the opacific state. Yet these cortical blemishes may seriously hinder visual acuity. Studies on the protective impact of nutrients should not rely on the end-stage nuclear opacity as the sole evaluation criterion in this model.

Infantile hypoglycemia is also associated with cataract. The juvenile lens is highly dependent on glucose as its energy substrate. Studies on young rat lenses have shown that incubation in a medium containing <2.0 mM glucose for 20 hours leads to swelling, loss of ion balance, depletion of ATP, and the appearance of a thin lamellar opacity around the anterior and posterior surfaces (29). After 48 hours of glucose deprivation, a nuclear cataract forms. Hexokinase, the rate-limiting enzyme of glycolysis, is unstable at body temperature in the absence of its substrates glucose and ATP. Its loss precludes rapid recovery of energy supplies upon restoration of glucose supply. This experimental cataract bears a striking resemblance to the neonatal, ketotic, and idiopathic types of hypoglycemic cataract seen in children. As the lens matures, it develops the capacity to meet its energy needs more efficiently via aerobic metabolism of amino acids and becomes resistant to hypoglycemic stress.

Riboflavin

An association of experimental riboflavin deficiency with cataract was first suggested in 1928 when weanling rats were fed diets free of "B complex" (104). Subsequent studies showed that only "vitamin G" was effective in preventing the lenticular lesions in such animals (35, 36). Cataract incidence varied widely, ranging from 0% (51) to 45% (106) to 85% (3), as riboflavin deficits were imposed in the presence of adequate supplies of the other vitamins. Confirmation of the cataractogenic potential of a riboflavin deficiency was also obtained in the young of other species such as the pig (81), cat (48), salmon (54), and trout (94).

The rationale for expecting riboflavin to be necessary for the maintenance of lens transparency lies in the fact that one of its biologically active derivatives is the cofactor flavin adenine dinucleotide (FAD) for the enzyme glutathione reductase (GR). This enzyme is necessary for the maintenance of the cellular pool of reduced glutathione, which serves as a reductant to convert hydrogen peroxide to water and to prevent inappropriate formation of protein disulfides. The rate of depletion of lens GR to a critical level was examined by Srivastava & Beutler (115, 116). When weanling rats were fed an otherwise complete riboflavin-deficient diet for 21 days, erythrocyte GR activity fell to 60% of normal as compared with a 25% decline in the lens, but there was no change in lens total thiol or reduced glutathione. Furthermore the researchers did not observe cataract in animals maintained for as long as 16 weeks on the deficient diet. When the riboflavin deficit coincided with an excess (68%) of dietary galactose, however, 80% of the test animals but only 10% of the riboflavin-sufficient animals displayed cataract after 18 days. Clearly, the impact of diminished dietary supply of riboflavin on lens transparency is subject to numerous interactions including absorption of the vitamin, efficiency of conversion to the active derivative, turnover of GR and cofactor in the lens, and the level of peroxidative challenge.

Vitamin E

Descriptions of the syndrome of uncomplicated dietary deficiency of vitamin E in animals emphasize detrimental effects on the central nervous system, reproductive failure, myopathy, and hematopoietic disorders (111) but do not include cataract except under the circumstance of prenatal deprivation. Ferguson et al (45, 46) noted a "cloudiness" in the central portion of the lenses of embryos from eggs laid by turkey hens fed an all-vegetable protein diet without an added supply of vitamin E. Jensen & McGinnis (65) confirmed the existence of cataracts in hatched poults and estimated the incidence to be 5%. Chaves et al (27) reported a 30–35% incidence of cataract and microophthalmia in the progeny of female rats fed a vegetable protein–based diet during gestation and lactation. Bunce et al (15) confirmed these results with the same

diet and observed that supplements of either L-tryptophan or vitamin E allowed normal eye development. Bunce & Hess (16) also fed a 12.4% protein diet using L-amino acids as the sole nitrogen source and varied either tryptophan, vitamin E, or both. When the diet was low in vitamin E alone (0.1 mg/100g diet) nuclear cataracts occurred at weaning in 7 of 111 progeny from 13 dams. When both tryptophan and vitamin E were suboptimal, the incidence of nuclear opacities rose to 42 of 126 pups from 19 dams. The same level of L-tryptophan (0.075 g/100 g diet) in the presence of adequate vitamin E had no effect on the lens, although it reduced weaning weight by 40%. These results suggest a role for vitamin E in lens development that may be quite independent of its redox chemistry.

The most widely accepted physiologic function of vitamin E is that of a lipid-soluble, chain-breaking antioxidant. Such action can prevent oxidant injury to polyunsaturated fatty acids and thiol-rich protein constituents of cellular membranes and the cytoskeleton. Many studies have been performed in which whole animals or animal tissues have been exposed to oxidants or oxidant-promoting circumstances and the presence of vitamin E (or other antioxidants) has produced an amelioration of toxic effects. The lens has not been excluded from such trials. Varma et al (127), for example, maintained rat lenses in a fluid medium exposed to high light intensity for 21 hours and measured the appearance of an oxidation marker product malondialdehyde (MDA). The presence of 10^{-7}M α-tocopherol prevented the fivefold increase in MDA seen in controls. Charalampous & Hegsted (24) reported that either omission of dietary fat (a source of potential free radicals) or inclusion of 0.2% of α-tocopherol delayed the time of cataract appearance in alloxan diabetic rats from 52 days to 72–75 days without altering the level of glycosuria. Trevithick et al (123) exposed rat lenses to either normal (5.6 mM) or highly elevated (56 mM) glucose for 48 hours and found that addition of 2.4 μM α-tocopherol acetate protected the lenses against swelling and deterioration without alteration of sugar uptake or conversion to sorbitol. They also reported that daily injections of 1 g α-tocopherol/kg body weight prevented the cataracts arising from streptozotocin-induced diabetes in rats (102). A level of 5 g vitamin E/kg diet was ineffective in preventing cataract in rats fed a diet containing 50% galactose (32). Supplementation of 0.4% butylated hydroxytoluene, an artificial antioxidant, delayed cataract appearance from 23 days to 38 days (114). Bhuyan & Bhuyan (11) reduced lens damage in rabbits treated with aminotriazol by simultaneous administration of vitamin E.

Fundamental to the evaluation of the role of α-tocopherol as an in vivo antioxidant is the analysis of its concentration in ocular tissues at various levels of dietary intake. Stephens et al (118) used microdissection to obtain samples of appropriate size and measured vitamin E by a gas chromato-

graphic–mass spectrometric technique capable of detecting less than 1 pg/g tissue. The basal diet was the AIN-76 diet, which contains approximately 50 mg vitamin E/kg diet. Supplemented and depleted variants were prepared to contain 3000 or 0 mg vitamin E/kg, respectively. After 102 days, whole lenses from animals fed the basal diet contained between 1 and 2 μg vitamin E/g dry wt, and lens along with cornea, vitreous, and sclera showed "only minor change" in vitamin E content by addition or deletion of the vitamin for this period. By contrast, rod outer segments (ROS) and retinal pigment epithelium (RPE), tissues with high content of unsaturated lipids and oxygen, contained approximately 160 μg vitamin E/g dry wt in animals fed the control diet, compared with 500–800 μg/g dry wt or 15–20 μg/g dry wt in those on supplemented or depleted rations. According to these results, the lens contains little endogenous vitamin E and is resistant to change in concentrations over a wide range of intakes.

Vitamin C

Ascorbic acid accumulates in the lens of most mammals in excess of 30- to 35-fold over plasma levels because of poor diffusibility of the predominantly reduced intracellular product. It absorbs ultraviolet light and is an efficient, water-soluble reductant that reacts with oxygen, hydrogen peroxide, and free radicals of oxygen. Cataracts have not been observed in scorbutic animals, including humans, although the short time until death may not allow sufficient time for emergence of this lesion. Kosegarten & Maher (74) found that scorbutic guinea pigs fed a high-galactose diet developed cataracts more rapidly than ascorbate-supplemented littermates. Dietary ascorbate supplements also slowed the onset of dinitrophenol-induced cataracts (86) and glucocorticosteroid-induced cataracts (85). Varma et al (128) reported that ascorbate supplements protected against the loss of cation pump efficiency in rat lenses exposed to light in vitro. Blondin et al (12) observed that lens damage following postmortem exposure to ultraviolet light was much less in guinea pigs previously provided abundant dietary ascorbate than in those provided limited intakes. Ascorbate reduces the tocopheryl radical and may therefore be necessary to its endogenous regeneration (84).

Ascorbate is also a candidate for contributing to lens deterioration. Glycosylation (nonenzymatic covalent bond formation between sugars and protein amino groups) may be of importance in the formation of diabetic cataract (119). Aldoses (including ascorbate) can undergo fragmentation during oxidation with the production of highly reactive enediol products. Several groups (5, 47, 62, 88, 89, 103) have studied this phenomenon in model systems by incubating lens proteins with concentrations of ascorbate similar to those encountered in the lens. They observed protein alterations judged to be highly similar to those that occur with aging in human and bovine

lenses. In comparing lenses from five mice that had been fed a diet containing 8.3% ascorbic acid from the age of 7 weeks to 1 year with lenses from age-matched control rats fed standard laboratory chow, however, Bensch et al (5) found no significant differences.

Amino Acids

Cataract has been recorded as an outcome of the restriction of the intake of some but not all of the indispensible amino acids. The first such report, by Curtis, Hauge, & Kraybill (33), described in 1932 "a white opaqueness of the eye and lens" in tests of various proteins as sole sources of nitrogen for young rats. The proteins employed were known to have a low content of tryptophan. Subsequent studies have verified the cataractogenic potential of a tryptophan deficiency in rats and guinea pigs (40, 130). The minimum requirement for L-tryptophan for normal growth by the weanling rat is 0.14% (133). In weanling rats, cataracts occurred within 3 weeks on a semipurified diet containing no tryptophan. Inclusion of L-tryptophan at a level of 0.05% caused cataract to appear within 7–9 weeks in 80% of the test animals (19). A further increase to 0.09% left the lenses clear after 10 weeks, although histologic abnormalities appeared (77). Delayed delivery of L-tryptophan several hours after a meal of a low-tryptophan diet was ineffective in preventing cataract (105), but replacement of the deficient diet with a complete diet led to the resumption of normal fiber growth and the migration of the subcapsular opacific zones toward the center of the lens (58).

Hall et al (52, 53) performed a systematic comparison of the cataractogenic potential of several other indispensible amino acids relative to tryptophan. Using a test diet providing three times the minimum requirement of each amino acid save the one under study, they demonstrated a substantial degree of lens damage, including dense nuclear opacities within three weeks in the complete absence of dietary tryptophan, phenylalanine, or histidine. Frank cataract was absent when the diets lacked leucine, isoleucine, valine, threonine, lysine, or methionine, but various morphologic abnormalities appeared such as haziness, separation of the superficial fibers, widening of the sutures, and a refractile line separating cortex and nucleus. Omission of arginine had no effect. In studies of histidine deprivation in the kitten, Quam et al (95) observed cataracts of both eyes in two of nine female kittens fed diets containing either 2.0 or 2.5 g histidine/kg diet for 128 days (the recommended level is 3 g/kg diet). Although methionine deficiency did not cause frank cataract in the rat studies conducted by Hall et al (52), it was reported to have a marked cataractogenic effect in fingerling salmonids. Poston et al (94) found a 90% incidence of grossly discernible bilateral cataracts after 12 weeks in Atlantic salmon fed a commercial soy isolate as the sole source of dietary protein. This diet restricted growth to less than one

fourth of normal. Supplementation of this diet with 0.9% D,L-methionine restored growth to near maximum and completely prevented cataract.

While amino acid imbalances clearly produce lens injury sufficient to culminate in cataract, lens transparency generally seems to be maintained in the face of a low intake of total dietary protein. In the studies by Hall cited above (53), simultaneous omission of the entire group of indispensable amino acids produced some morphologic defects but no obvious cataract. McLaren (78) maintained 147 Wistar rats from weaning into advanced maturity on diets containing only 2 or 4% protein and did not observe a single cataract despite severely retarded growth. In a similar study, Bagchi (2) designed a low-protein, high-carbohydrate diet to simulate the proportions present in human populations in which protein-calorie malnutrition is prevalent. This diet consisted of maize starch, 92%; casein, 3%; salt mixture, 3%; and yeast, 2%. Rats fed this diet for up to 350 days postweaning were reduced in size but active and displayed no lens abnormalities upon either slit lamp or histologic evaluation. Kauffman & Norton (68) divided 31 weanling pigs into four groups and fed them diets containing either 0, 5, 10, or 12–16% protein derived from mixtures of soybean meal and corn for 146 days. They noted no gross lens abnormalities. Moreover, the relative nitrogen content of the lens did not diminish even on the 0% protein intake, whereas brain nitrogen declined to 90% of control, and heart, liver, and muscle nitrogen were only 10–25% of normal. Tenacious defense of lens nitrogen levels in these circumstances undoubtedly arises from the lack of active turnover of the bulk of lens crystallin proteins.

Vainisi et al (125) observed that 12 wolf pups (7 litters) removed from their mother and fed a commercial canine milk–replacement produce developed posterior subcapsular opacities. Supplementation of the commercial diet with either arginine or lactose prevented cataract in littermates but supplements of vitamin C, L-tryptophan, methionine, or corn oil were ineffective. They concluded that these opacities were an expression of arginine deficiency and explained the lactose benefit as enhanced arginine absorption. In the previously cited studies by Hall (52), arginine was the one amino acid that failed to provoke either cataract or morphologic abnormalities upon its omission from the diet of weanling rats. The wolf pup cataracts may in fact have been an outcome of neonatal hypoglycemia, and the arginine supplement may have served as a glucose precursor rather than as a participant in protein synthesis.

Calcium

Increased membrane leakiness and swelling occurs in lenses maintained in media lacking calcium (37, 38, 56, 80, 122, 129). Clinically, cataract has been well documented as being associated with hypocalcemic tetany (13). Delamere & Paterson (39) have presented a thorough review of the bio-

chemistry and physiology of hypocalcemic cataract. The production of nutritional hypocalcemia with resultant cataract was demonstrated in rabbits by Swan & Salit (120) and in rats by Chang et al (21, 22, 23) and Chen et al (28) in 1941 using diets containing <0.005% calcium. No tests have examined the effect of prolonged consumption of diets less severely limited in calcium.

Zinc

In recent years, cataracts have appeared in significant numbers of trout and salmon reared in hatcheries in the United States, Canada, Iceland, Great Britain, Africa, and Japan. In several instances, zinc supplementation alone successfully prevented cataract and improved growth under hatchery conditions (70, 98). Ketola (70), for example, observed 75–85% cataract in fingerling trout after 16–38 weeks of consumption of a practical ration containing by analysis 60 ppm of zinc. A supplement of 150 ppm of zinc completely relieved this condition. Practical salmonid diets generally include fish meals and phytate-containing plant meals. The emergence of the cataract problem coincided with increased use of calcium-rich fish meals obtained from filet removal during processing. Diminished availability of dietary zinc in diets simultaneously abundant in calcium and phytate was first described in the condition of parakeratosis in swine; it has been intensively studied in mammalian species without mention of cataract, nor have lens opacities been noted in numerous studies of acute and chronic zinc deficiency produced with otherwise balanced diets. There are, however, reports of cataracts in children with acrodermatitis enteropathica, a rare hereditary abnormality of zinc metabolism (20, 96). The biochemical functions of zinc necessary to the development and preservation of salmonid lens transparency have not been defined.

Selenium

The recognition of the essentiality of selenium for the function of the enzyme glutathione peroxidase and the importance of oxidant defense for the maintenance of lens transparency suggested that cataract might be one outcome of selenium deficiency. Lawrence et al (75) examined this possibility in rats maintained on a chromium- and methionine-supplemented Torula yeast diet (20 ppb Se) for 9 months and in their offspring bred at 7.5 months and fed the low-selenium diet for 6–9 months postweaning (75). Selenium concentration in the lens was reduced by 10-fold, and glutathione peroxidase activity declined to 15% of controls but no lens opacities were detected. Cataract did appear, however, in the second and third generations (20%, 8 of 39 pups, and 40%, 4 of 10 pups, respectively). Sprinkler et al (113) also used a Torula yeast diet (18 ppb Se) but without the chromium-methionine supplement. They reported a progressive lens deterioration culminating in a completely

white lens in brown rats after 220 days. In a further study, they reported severe cataract in four second-generation rats. The severity of these changes was relieved but not eliminated by supplements of chromium, vitamin E, or 100 ppb of selenium (131). Selenium deficiency apparently must be of great magnitude before the lens is placed at risk.

Selenium excess, on the other hand, poses a grave risk for cataract when administered subcutaneously or intraperitoneally as selenite to rats younger than 18–20 days (90, 108). A single dose of 20–30 μmoles/kg body weight results in virtually 100% bilateral nuclear cataracts within 3–4 days postinjection. The first 24 hours is marked by evidence of metabolic distress, including a 70% decline in lens glutathione, a 15% decline in lens ATP, and a two- to three-fold increase in glycerol-3-PO_4 (17, 57). These changes are followed during the next 24–48 hours by a rise in lens calcium to three- to five-fold over normal (18, 107). Opacities emerge coincident with the Ca-stimulated attack of calpain II upon lens crystallins (34). The specific cause of the loss of calcium homeostasis has not been identified, but the loss is thought to be a consequence of oxidative injury. Huang et al (61) showed that coadministration of butylated hydroxyanisole, a lipophilic antioxidant, greatly diminished calcium increase and the incidence of cataract. Why the lens of the postweaning rat is so much more resistant to the cataractogenic potential of selenite is unclear, but the resistance may be related to the appearance of an endogenous calpain inhibitor. The effect of chronic dietary selenium excess on lens development in utero and on age-related cataract deserves further study.

Caloric Restriction

Caloric restriction, if started early in life and continued until death, increases average and maximal survival times of mice and rats. Moreover, the longer life spans are associated with more youthful physiologic and immunologic responses. Leveille et al (76) investigated the effect of caloric restriction on biochemical parameters typical of aging in the rodent lens. Female mice derived from naturally long-lived strains were weaned at 21 and 24 days, individually caged, and randomly assigned to one of two diet groups. Control mice received 85 kcal/wk, whereas the restricted group had access to only 50 kcal/wk. The restricted diet was enriched in protein, vitamins, and minerals to match the intake of the animals fed the control ration. The aging marker selected for the lens was the rate of disappearance of the γ-crystallins, the smallest of the water-soluble structural proteins and the richest in thiols. This loss is believed to arise as a consequence of spontaneous oxidation. Comparison of the proportions of lens proteins at 2, 11, and 30 months showed caloric restriction to be consistently associated with a significant deceleration in the rate of loss of the γ-crystallin band.

Taylor et al (121) recently concluded similar studies with the addition of assessing the rate of cataract appearance as a function of dietary calories. The Emory mouse is a relevant and useful model for human senile cataracts because: (*a*) a large percentage of the Emory mouse population develops cataracts spontaneously; (*b*) the cataracts occur in adults rather than juveniles; and (*c*) the transition from clear to opacific lens occurs in association with a decline in the ratio of soluble protein to total protein. Feeding Emory mice a diet restricted by 21% in total calories from weaning to age 12 months depressed the incidence of cataract from 85% (n=29) to 41% (n=34). Since the protein profiles in mice with similar grades of cataract were similar, the authors concluded that caloric restriction slowed rather than altered the inherent nature of those reactions involved in cataractogenesis.

HUMAN STUDIES

Studies with experimental animals permit the demonstration of causal relationships with effective control of confounding variables. Surveys of human populations lack such precision but are necessary to suggest associations that may prove to have a causal link. The results of recent studies with human subjects are presented below.

Riboflavin

The common procedure for assessment of riboflavin is to measure activity of the FAD-dependent enzyme glutathione reductase (GR) in red blood cells in the presence and absence of added cofactor. Using this approach, Glatzle et al (50) found 32% of 124 elderly subjects deficient in riboflavin. Skalka & Prchal (109, 110), however, found no association between riboflavin status and cataract in a study of patients in a nursing home in Alabama; van Veelen et al (126) came to the same conclusion in a study of 156 healthy elderly persons living at home in the Netherlands. Bhat (8) examined 37 patients with mature cataract and 16 control subjects at an eye clinic in India. Both groups displayed evidence of inadequate tissue levels of thiamine and pyridoxine. The cataract group showed greatly increased GR activity in the presence of added FAD, but the basal activity level was not depressed relative to controls.

The demonstration of depleted GR activity in opaque lenses would strengthen the argument for a causal link. Several investigators have attempted such comparisons. Srivastava et al (117) found no significant difference in enzyme activity between the epithelia of normal and cataractous lenses. Three other groups reported lower GR activity in lenses with cortical cataract but no decrease in lenses with nuclear cataract (87, 97, 101). A recent study (60) measured GR activity in freshly excised human lens epithelium

after cataract surgery and in control eye-bank lens epithelia. Of 32 epithelia obtained from cataractous lenses, 14 showed no measurable GR activity. Activity was observed in 8 of these 14 upon addition of FAD, implying that the apo-enzyme was functional but that endogenous FAD was absent. Another group of 8 displayed some native GR activity that increased significantly upon addition of FAD. The other 10 samples from cataractous lenses showed normal levels of native activity and no stimulation after addition of FAD. Five of 10 eye-bank control lenses also showed significant stimulation in GR activity in the presence of an FAD supplement.

Opacities have been described in association with myxedema, but the incidence was only 4.5% in a 400-patient sample (13). The lenticular changes were relatively mild in that they were visible only by slit lamp microscopy and seldom interfered with vision. Thyroid hormone regulates riboflavin metabolism by increasing the activities of flavokinase and FAD-pyrophosphorylase, the enzymes responsible for the conversion of riboflavin to FAD (41, 99). Endogenous GR is depressed in red blood cells of hypothyroid adults (31) and elevated five- to ten-fold in epithelia of cataractous lenses removed from adults taking daily supplements of synthetic thyroid hormone (synthyroid) (60). Given the relative frequency of hypothyroidism, further information on its effect on riboflavin metabolism, lens GR activity, and senile cataract would be useful.

Multiple Nutrients

Jacques et al (63, 64) surveyed 77 subjects with cataract in at least one lens and 35 age-matched controls seen at the Brigham and Women's Hospital Clinic in Boston between January 1981 and December 1985. The subjects were between the ages of 40 and 70 years. Nutritional status was determined by blood analysis. Substances measured included plasma levels of carotenoids, vitamins A, D, E, and C; erythrocyte levels of enzymes requiring cofactors derived from thiamin, riboflavin, and vitamin B_6; and serum levels of magnesium, copper, selenium, and zinc. These workers also evaluated the activity of three erythrocyte enzymes critical to oxidant defense: glutathione peroxidase, superoxide dismutase, and catalase. For statistical evaluation, they grouped subjects into quintiles and estimated odds ratios (OR) for all cataract, cortical cataract, or subcapsular cataract for the middle three quintiles combined and the highest quintile relative to the lowest quintile. The investigators then adjusted the ORs for age, sex, race, and self-reported history of diabetes. The results were reported in two papers: one evaluated nutritional status (64); the other looked at antioxidant status (63). In the first (64), subjects with cataract had lower levels of vitamin D than control subjects. Because cataract occurrence is positively linked to sunlight exposure, this result was the opposite of that expected. The authors suggested that

once cataract is present, persons might tend to avoid the sunlight due to visual impairment or increased sensitivity. Estimated ORs indicated a substantial decrease in risk of cataract associated with moderate and high carotenoid levels. β-Carotene has good radical-trapping properties at low partial pressures of oxygen such as are found in the lens. No statistically significant relationships were detected between cataract and the nutritional status of the patients with regard to vitamins A, E, riboflavin, or thiamin. Either high vitamin B_6 status or low plasma vitamin C level seemed to be associated with a modest increase in risk of subcapsular cataract. Among the minerals, high blood selenium level appeared linked to cataract; the link was significant at the 10% level but not at the 5% level of probability.

In the second report (63), the erythrocyte oxidant enzymes, either individually or in combination, did not appear to differ between subjects with or without cataract. Subjects with high blood levels of at least two of the three vitamins (vitamin E, vitamin C, or carotenoids), however, appeared to be at reduced risk of cataract compared with subjects in the lowest quintile for these vitamins ($p<0.05$). An inherent and critical difficulty with this study is that it attempted to assess nutrient status at a single point in time after the pathology was already visible. The absence of a nutrient history is a vital omission if one is attempting to determine a causal link, especially in a disease such as cataract that develops over such a long period of time. Robertson et al (100) used an interview technique in an attempt to obtain such a history. They interviewed 152 patients with cataract and an equal number of age- and sex-matched cataract-free subjects to determine the subjects' consumption of supplementary vitamins C and E. The cataract-free subjects were more likely to have consumed regular supplements of either vitamin ($p<0.05$ and $p<0.004$, respectively) than were control subjects.

The clinical experience of ophthalmologists in Africa, China, India, and Pakistan has led them to conclude that senile cataract is more common and occurs at an earlier age in the indigenous populations of these regions than in Western nations (79). Chatterjee et al (25, 26) provided an important documentation of this impression. They examined almost 20,000 people living in the dry plains region of Punjab. Age-grouped prevalence was as follows: 30–39 years, 2.6%; 40–49 years, 9.0%; 50–59 years, 22.5%; and 60+ years, 31.4%. The age-standardized prevalence in the population as a whole appears to be nearly three times that found in the United States.

The India-United States Case-Control Study Group (83) recently concluded a hospital-based study of 1441 patients with age-related cataract and 549 control subjects. The study evaluated associations between types of cataract and a number of physiologic, behavioral, environmental, and biochemical variables. Although investigators with small numbers of subjects are tempted to collapse the subject pool into simple cataract vs noncataract groupings,

statistical modelling suggested that this method was not accurate and led these workers to employ the categories nuclear, cortical, posterior subcapsular, mixed, and noncataractous controls. A questionnaire was used to estimate dietary intake of eight nutrients. Since persons with a high intake of one nutrient tended to have a high intake of all nutrients, dietary protein was chosen as the nutrient representative of the plane of nutrition. Higher levels of protein intake were protective ($p=0.02$) for posterior subcapsular, nuclear, and mixed types of cataract but not cortical cataracts. Chatterjee et al (26) previously reported an association between cataract and low frequency of current use of protein foods in the Punjab area of India. A battery of biochemical tests was performed on a subset (1135/1990) of the study population. The only significant associations of the single variables were higher risk of combined posterior subcapsular-cortical cataracts with high serum copper levels, higher risk of combined posterior-nuclear cataract with higher plasma ascorbate levels, and lesser risk of combined posterior subcapsular-nuclear cataract with higher blood hemoglobin values. Previous Indian studies with much fewer subjects found no associations between cataracts and plasma zinc or copper (9) and thiamine, pyridoxine, or vitamin E (8, 10).

Mohan et al (83) also calculated three antioxidant indexes. One index, based on levels of red blood cell glutathione peroxidase and glucose-6-phosphate dehydrogenase and plasma ascorbic acid and vitamin E, appeared to be protective for posterior subcapsular cataracts and combined posterior subcapsular-nuclear cataracts. No associations appeared with indexes based on glutathione peroxidase and ascorbic acid or glutathione peroxidase, ascorbic acid, and vitamin E. A decreased risk for all pure cataract types was found among persons with larger amounts of lifetime cloud cover at place of residence, a crude index of exposure to sunlight or ultraviolet light. In contrast to the study of Minassian (82) in a rural area of India, however, no association was found between self-reported previous severe diarrheal disease and cataracts.

SUMMARY AND CONCLUSIONS

Age-related cataract is a condition characterized by multiple mechanisms and multiple risk factors. The mechanisms that bring about a loss in transparency include oxidation, osmotic stress, and chemical adduct formation. Risk factors for cataract include diabetes, radiation (ultraviolet B, x-ray), certain pharmaceutical substances, certain nutritional states, and possibly acute episodes of dehydration. Interaction occurs between and among mechanistic factors and risk factors. Thus nutrition must be considered as one part of a tapestry of intertwined events and responses.

Certain experimental models for nutritional cataract have been useful for

study of the cataractogenic process but are probably not important factors in the human disease. Little current evidence supports significant roles in human senile cataract for imbalances of tryptophan or other amino acids, deficiencies of calcium or selenium, or excessive intake of selenium. Overconsumption of galactose is likely to be hazardous only in subjects with genetic inability to metabolize this sugar. Vitamins with antioxidant potential (riboflavin, vitamin E, vitamin C, carotenoids) deserve further research scrutiny to ascertain their significance in cataract etiology. Excessive caloric intake needs to receive added emphasis as a factor contributing to cataract. Diabetes increases the likelihood of cataract three- to four-fold. Obesity, defined as more than 20% overweight, is considered a major risk factor for non-insulin-dependent, or type II, diabetes (69, 73). Weight control can be recommended as a prudent, safe, economic, and effective means of lowering risk probability for diabetes and the associated complication of cataract.

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