THE PATHOLOGY OF SPONTANEOUS CATARACTS IN BOBWHITE QUAIL

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ABSTRACT

THE PATHOLOGY OF SPONTANEOUS CATARACTS

IN BOBWHITE QUAIL

By

Janver D. Krehbiel

This investigation was conducted to characterize the pathologic and physiologic alterations of spontaneous cataracts in Bobwhite quail. Birds 1-1/2 to 4-1/2 years of age were examined clinically and cataracts were graded on the extent of lens opacity. Cataractous lenses from these birds were then chemically analyzed for water content, sodium and potassium ions, glutathione, and soluble and insoluble proteins.

Three experiments were conducted on young quail and the incidence of cataracts was recorded for each study. The influence of diet, population density and environment was evaluated.

Morphologic changes in the cataracts of young and aged quail were examined by slit lamp, light, and electron microscopy. Microbiologic studies were also conducted on quail in both age groups to identifiy or eliminate microorganisms as etiologic agents in cataract formation.

The first change noted in cataract formation was focal degeneration in the anterior polar cortex and epithelium. There was occasional simultaneous involvement of the posterior polar cortex with epithelial

hyperplasia. Histopathologic changes were progressive and resulted in lens liquefaction with epithelial proliferation and thickening of the capsule in advanced cataracts.

Ultrastructural alterations in the central anterior epithelium of cataracts consisted of increased outfoldings of the cell membranes and early degeneration of cytoplasmic organelles.

Chemical changes of cataracts paralleled morphologic alterations and decreases were seen in the potassium ions, glutathione, total and soluble proteins. Sodium ions, water content, and insoluble protein increased with lens opacification.

Lens opacities could not be attributed to congenital causes, metabolic disturbances, or infectious microorganisms. Population density and nutrition did not affect the incidence of cataracts but environment significantly influenced cataract formation. The primary difference in the 2 environments studied was the type and duration of light exposure. Those birds exposed to continuous artificial light had greater numbers of cataracts at an earlier age than did those birds exposed to cyclic natural light.

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Janver D. Krehbiel

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INTRODUCTION

A cataract may be defined as any opacity of the lens or its capsule and, as such, has been observed in a variety of animals. In man lenticular opacity is particularly significant due to his dependence on the visual sense. In spite of years of extensive research there is still no complete, or satisfactory explanation for the mechanism of cataract formation. Experimental animals have been used to study the pathogenesis of cataracts; however, these efforts have been largely confined to mammalian species. In this study an avian species, *Colinus virginianus*, with spontaneous cataracts, was utilized as a new animal model in cataract research.

The objectives of this investigation were: (1) to attempt to establish the clinical, histological and ultrastructural detail of normal and affected lenses of Bobwhite quail; (2) to utilize various culture techniques to identify or eliminate infection as a cause of the ocular lesion; (3) to do comparative biochemical studies of normal and abnormal lenses related to weight change and change in Na and K ion content of the lens; and (4) to evaluate the influence of other factors, including nutritional deficiencies, hereditary influences, husbandry practices and environment.

LITERATURE REVIEW

Reports of spontaneous cataracts in avian species are infrequent, as is general information related to lens abnormalities in birds. Therefore, pertinent references, which characterize normal and cataractous lenses of man and other species, are included as comparative information for this study.

The Normal Lens

Embryology. Mann (1949) described the embryology of the human lens noting that it was derived entirely from surface ectoderm overlying the primitive optic vesicle. This ectodermal layer was thought to be "induced", by some unknown stimulus, to form sequentially a lens plate, vesicle and finally the epithelial organ suspended within the optic cup.

Smelser (1965) reviewed the morphologic aspects of lens differentiation which occurred during the process of induction. These studies, based largely on amphibian and bird embryos, indicated that a lens could be formed from any surface ectoderm if it came in close relation to the optic vesicle at the correct stage of development. This induction process has been extensively investigated by McKeehan (1951, 1954, 1956, 1958), Babcock (1961), Hunt (1961) and others; however, it still escapes complete explanation. Irrespective of the exact mechanism of the induction process or the species, Smelser summarized the sequential development of the lens as follows:

> "The lens is derived from surface ectodermal cells which lie directly over the optic vesicle from which they are separated by a basement membrane. These cells thicken and invaginate to form a vesicle surrounded by a basement membrane, which becomes converted into the capsule. The cells of the posterior wall of the vesicle elongate and fill the vesicle forming the primary fibers. Secondary fibers are formed from the epithelial cells at the equator and extend around the primary fibers, growing between them and the overlying capsule."

Morphology of the Adult Lens. Francois (1963) described the lens as a transparent biconvex structure, located between the iris and the anterior face of the vitreous, with fibrous attachments to the ciliary body called zonules.

Duke-Elder and Wybar (1961) divided the lens structurally into 3 parts: the capsule, epithelium and lens substance. They considered the capsule a clear homogeneous membrane which entirely surrounded the lens and varied in thickness, with the thicker portion located anteriorly. The lens epithelium was described as a single layer of cells which extended under the anterior capsule to the equator (the point where the anterior and posterior capsules meet). Epithelial cells in the central portion of the lens were considered "resting cells", while those in the peripheral or equatorial portion were called "germinal cells", from which new lens fibers formed. The lens substance consisted of 2 parts: the central nucleus (composed of old fibers) and the cortex (composed of young fibers, the least mature located nearest the capsule).

Walls (1942) considered the anatomy of the lens to be very similar in all species of mammals. Davson (1962) stated that the lens of the bird differed from that of mammals in that it contained a "lens pad" which consisted of several layers of epithelial cells at the

equator. Prince (1956) described the avian lens as spherical, and he identified the thickening of epithelial cells at the equator as the "ringwulst". He stated that the shape of the lens was intimately linked with an animal's environment and accommodative needs. Prince considered the "rungwulst" important in effecting this accommodative power and noted its presence in some amphibians and fish, as well as birds.

Ultrastructure of the Lens. Wanko and Gavin (1959) studied electron microscopic details of the various zones of the lens epithelium and lens fibers of rats, rabbits, monkeys and calves. They found the types of cellular components, and their distribution within lens fibers, similar in all species. It was noted, however, that the number and distribution of cell constituents, in all species examined, varied according to the location of the fiber within the lens. Those fibers nearest the surface contained the greatest numbers of organelles.

Jakus (1964) stated that the lens capsule, like Descemet's membrane, is a fibrous collagen-like structure. She studied the central anterior lens capsule and epithelium of the rabbit and described the epithelium as flat on 2 surfaces with complex lateral contours. Mitochondria, rough endoplasmic reticulum, and Golgi material were identified in the matrix of epithelial cells. She also described small heavy-walled vesicles, some continuous with the intercellular space, and occasional dense segments suggestive of desmosomes which obliterated the intercellular space.

Jakus (1964) also examined the anterior capsule of fowl lens and described a radial disposition of filaments similar to that observed in Descemet's membrane.

Cliffe and Waley (1961) found a paucity of organelles in the lens. Their studies revealed that nuclei, mitochondria and microsomes accounted for only 4% of the protein in calf lens, compared to 50% in liver. They suggested that the reduction of organelles was probably related to visual function and this reduction was essential for transparency of the lens.

Physiology of the Lens. Moses (1970) summarized some of the current concepts of lens metabolism. He stated that the lens did not have a high rate of metabolism and energy requirements were small. The primary need for energy was limited to maintenance of lens transparency and nourishment for the development and growth of new lens fibers. He pointed out that after birth the lens had no blood supply and therefore was dependent on the aqueous humor for transport of foodstuffs and waste products. Composition of the aqueous humor and permeability of the lens capsule were identified as the primary factors which influenced normal lens metabolism.

Field *et al.* (1937) studied the mean oxygen consumption of young rabbit lenses, and whole lenses were found to require minimal amounts. Almost the entire amount of oxygen utilized was consumed by the epithelium and cortex, while the capsule and nucleus utilized only negligible amounts.

Van Heyningen (1969) stated that lens metabolism involved the sorbitol pathway, the hexosemonophosphate shunt and anaerobic glycolysis. The latter pathway was considered most important in that 80% of lens glucose was broken down to lactic acid by this route.

History and Classification of Cataracts

In a historical review extending back some 4000 years, Duke-Elder (1969) described some of the misconceptions held about the exact cause of cataracts. In Hindu medicine, cataracts were thought to result from derangement of intraocular fluid. Writings of Hippocrates made no clear distinction between cataracts and glaucoma and Celsus incriminated a corrupt inspissated humor, located between the pupil and lens, as the cause of visual opacity. Duke-Elder (1969) also stated that much confusion arose from the erroneous belief that the lens was located in the center of the globe, and that this structure was the essential organ of sight.

Rucker (1965) stated that Francois Quarré, a French physician, was the first to conceive the idea that a cataract is an opacity of the lens. This new concept was not popular and the true nature of cataracts was not generally recognized or accepted until the early 1700's. Rucker (1965) further credited Jacques Daviel with the publication of a classic paper on the deliberate extraction of cataracts, and thus in 1753, inaugurated a revolution in ophthalmic surgery and disproved the theory of inspissated humor. Since the recognition of the correct location of the lens, numerous types and varieties of cataracts have been described.

Lerman (1964) considered the clinical characterization by biomicroscopic examination the most useful method of classifying cataracts. Duke-Elder (1969) classified cataracts by etiology, clinical type and experimental type. Francois (1963) devoted an entire book to subdivisions of congenital cataracts.

While the method of classification varied with the discipline of the writer (clinical medicine, surgery, physiology, etc.) the more

important consideration was the recognition of the different types of lens opacity.

Specific Types of Cataracts

Cataracts Induced by Injury

Mechanical trauma. Duke-Elder (1969) described some of the various etiologic factors that caused mechanical injury to the lens. He stated that, in concussion injuries, proteins were coagulated in the epithelial cells and lens fibers. Protein aggregates were then dispersed with the return of translucency, or necrosis of cells followed by the formation of cataracts. The pathogenesis of lens opacities resulting from ruptured lens capsules was considered a different process. Capsular rupture resulted in altered permeability and the loss of essential diffusible substances or entrance of aqueous humor.

Pathophysiologic change

Osmotic cataract. Kunde (1857) first demonstrated that osmotic hydration or dehydration of the lens caused opacity *in vitro* (Duke-Elder, 1969).

Bellows and Chinn (1941) produced a similar effect *in vivo* by intravenous injection of hypertonic solutions.

Kudo (1921) reported that prolonged deprivation of drinking water could also be followed by the development of cataracts.

Cold-induced cataract. Kunde (1857) described the development of cataracts in young animals that were cooled to temperatures below 10 C (Duke-Elder, 1969). The reversible opacity produced was thought to be due to a precipitation of gamma-crystallin, a protein

which possesses a large number of exposed hydrophobic groups. Lerman (1966) reported that this precipitation occurred only if the gamma-crystallin was present in higher concentration than the combined alpha- and beta-crystallin. It is for this reason that cold cataracts were observed only in young animals, since at an early age the gamma-crystallin constituted the major part of the lens proteins.

Duke-Elder (1969) stated that a high concentration of urea prevented precipitation of gamma-crystallin. He suggested that the absence of cold-precipitation in dogfish may be due to the unusually high content of urea in its aqueous humor and lens.

Heat-induced cataract. Daddi (1898) reported that the lens becomes opaque when warmed above 65 C, an effect due to an irreversible coagulation of proteins (Duke-Elder, 1969). Bedrossian (1958) stated that intense heat exposure for many years produced disc-shaped opacities in the posterior cortex of the lens. Exfoliation of the superficial lamella of the anterior capsule was also observed in glassblowers or heat cataracts.

Electric cataract. Electric cataracts were reported by Logan and Klein (1945) and, while the exact mechanism was not identified, protein coagulation was thought to be involved.

Radiation cataracts. Van Heyningen (1969) divided radiation cataracts into those produced by ionizing radiation (x-rays, beta and gamma rays, and neutrons) and lenticular changes induced by non-ionizing radiation (ultraviolet, infrared and microwaves).

Ionizing radiation cataracts in man have resulted from accidental exposure, radiation therapy and atom bomb explosions. In experimental

mammals the severity of radiation cataract was influenced by the magnitude of the dose, the age of the animal and degree of exposure of the lens.

Pirie (1959, 1961) reported that the lenses of embryonic and adult chickens did not develop cataracts after x-irradiation. Some cell damage was observed; however, the lack of cataractous response by an avian species was thought to be related to the anatomical differences between mammalian and avian lenses. Hanna and Keats (1967) suggested that cataracts may have formed in the chicken lenses but were not identified due to the short 6-month observation period. This suggestion was based on the studies of Hanna and O'Brien (1961) which revealed that dividing cells of the chick lens required about 2 years to migrate from the annular pad to the lens cortex, whereas the same migratory route in mammalian lenses (rat and mouse) took only 4 months.

Non-ionizing radiation cataracts were considered much less important than ionizing radiation cataracts. Lerman (1964) stated that ultraviolet waves, when applied to the eye, were almost all absorbed by the cornea and that the cataractogenic action was of little significance.

Cloud *et al.* (1960, 1961) reported that laboratory animals could be sensitized to ultraviolet rays with methoxsalen and phenothiazine. When these photosensitizing drugs were given to mice and guinea pigs exposed to ultraviolet rays, a high incidence of cataracts was observed, as well as some damage to the cornea and iris.

Langley *et al.* (1960) reported that lenticular lesions, following exposure of an animal to infrared radiation, occurred adjacent to burned areas of the iris. Damage produced to the lens epithelium was thought to have resulted from the absorption of infrared rays by the iris with transfer of heat to the capsule and underlying epithelium.

The injury produced by infrared rays consisted of lamellar splitting of the anterior lens capsule combined with posterior cortical opacification.

Dailey *et al.* (1952) produced cataracts in rabbit eyes with microwave diathermy. Posterior cortical cataracts were produced and were thought to have resulted from the excessive heat of the microwaves.

Nutritional Inadequacies. Van Heyningen (1969) stated that cataracts have never been proved to be caused by dietary deficiency in human infants; however, lens opacities have been induced in several experimental animals fed deficient diets.

Protein deficiency. Hall *et al.* (1948) produced cataracts in young rats fed a protein-deficient diet and McLaren (1959) observed similar opacification of the lens in protein-deficient pigs. Specific amino acids, critical to lens metabolism, have been identified by several researchers. Trotter and Day (1942), Buschke (1943), and Ferraro and Roizin (1947) all observed cataracts in rats on tryptophan-deficient diets. Von Sallmann *et al.* (1959) discovered that tryptophan cataracts were produced more easily in guinea pigs than in rats; however, histologic changes were similar in both species. Less dramatic lens opacities were observed with deficiencies of other amino acids including phenylalanine (Bowles *et al.*, 1947), histidine (Sydenstricker *et al.*, 1947), and methionine (Bagchi, 1959). Patch (1943) found that the larvae of the tiger salamander, fed on a diet deficient in cystine, developed cataracts that could be prevented by adding the amino acid to the food.

Vitamin deficiency. A deficiency of vitamins, particularly those of the B group, has resulted in the development of cataracts in experimental animals. Day *et al.* (1931) found that 94% of the rats fed a diet deficient in riboflavin developed cataracts. The first change he observed was a central opacity which later spread peripherally. These cataracts were reversed by the administration of riboflavin.

Wintrobe *et al.* (1944) found that a diet deficient in riboflavin was also cataractogenic in pigs. Cataracts in these animals appeared as wave-like opacities near the anterior and posterior poles of the lens. The deep cortex and nucleus were clear; however, fiber separation was observed in the outer cortex.

Gershoff *et al.* (1959) observed cataracts in riboflavin-deficient cats supplemented with high levels of fatty acids.

Nelson *et al.* (1955) reported that maternal deficiency of folic acid has caused cataracts and other developmental anomalies in young rats.

Vitamin E deficiency was found to be cataractogenic in turkey embryos. Ferguson *et al.* (1954, 1956) observed cataracts in turkey embryos hatched from eggs laid by turkey hens fed vitamin E-deficient diets. Histologic studies of these cataracts revealed epithelial changes in the lens accompanied by corneal alterations.

Mineral deficiency. Swann and Salit (1941) reported that rabbits placed on low-calcium diets developed lens opacities. The changes were observed during the second week of the deficiency and consisted of slits, vacuoles and dots near the equator of the lens. Tetany and reductions in serum calcium were also observed.

Cataracts Due to Abnormal Metabolism

Sugar cataracts. Mitchell and Dodge (1935) observed cataracts in rats fed diets rich in lactose. Since their original report other researchers have identified a triad of sugars which were considered potentially cataractogenic in experimental animals (Duke-Elder, 1969). The 3 sugars known to produce this effect were D-galactose, D-xylose and (in diabetic animals) D-glucose. All 3 types of cataracts were similar in appearance, and the development of lens opacities varied directly with the degree of hyperglycemia and concentration of sugar in the aqueous humor.

Galactose cataracts have been studied by many investigators in several species of experimental animals (Duke-Elder, 1969). Lerman (1959) studied galactosemic cataracts in human infants and found the enzyme galactose-1-phosphate uridyl transferase completely lacking in affected children. The absence of this enzyme was considered an inborn error in metabolism and infants so affected were unable to metabolize one of the components of lactose (galactose).

Xylose cataracts, while easily induced in young rats, have never been reported in man (Lerman, 1966).

Diabetic cataracts. Rollo described cataracts in humans suffering from diabetes mellitus as early as 1798 (Duke-Elder, 1969). Pirie and van Heyningen (1964) demonstrated high levels of sorbitol, glucose and fructose in the lenses taken from diabetics. Similar elevations have been observed in experimental animals rendered diabetic by toxic agents, such as alloxan, or after pancreatectomy. Lerman (1966) reported that metabolic abnormalities in diabetic cataracts were associated with early impairment of the hexosemonophosphate shunt.

Anaerobic glycolysis was thought not to be affected until the late stage of opacification. Van Heyningen (1969) suggested that elevated concentrations of sorbitol, glucose and fructose played a role as toxic factors in the development of cataracts in diabetes.

Natural diabetic cataracts are reported by Smythe (1958) to be a rarity in animals when compared with their incidence in man. Magrane (1965) discussed diabetes in dogs and suggested that while cataracts were observed their incidence was low and largely confined to middle-aged and older animals.

Cataracts Produced by Toxic Substances. Cataracts have been produced experimentally by the introduction of many different toxic agents. Duke-Elder (1969) grouped these cataractogenic substances as follows: (1) hydrocarbons or substituted hydrocarbons (particularly naphthalene and dinitrophenol), (2) salts of certain heavy metals (thallium, cobalt and selenium), (3) antimitotic agents (Mimosine and Myeleran), (4) enzyme-inhibitors (iodoacetate and triparanol), and (5) certain toxic drugs (cholinesterase inhibitors, morphine-like drugs and corticosteroids).

The substance 2,4,dinitrophenol (DNP) was of particular relevance to this study in that Robbins (1944) successfully produced cataracts with DNP in avian species. Experimental mammals were not susceptible to the drug; however, cataracts were easily produced in chickens and ducklings and resembled those observed in man. Van Heyningen (1969) stated that DNP uncoupled oxidative phosphorylation and suggested that the comparatively higher content of epithelial cells, in avian lenses, rendered this species more susceptible.

Congenital Cataracts. Francois (1963) reported that congenital cataracts caused 10 to 30% of congenital blindness in children. Some of the causes he associated with this type of cataract included infection, toxins, heredity, immunologic and deficiency factors and inborn metabolic errors. Similar factors were considered operative in animal species.

According to Smythe (1958) heredity was the primary factor responsible for the occasional cataracts observed in newborn cattle, sheep, horses, pigs and dogs. He also stated that in certain species of cage birds, particularly canaries, cataracts were extremely common until breeders refrained from breeding affected birds, after which the condition disappeared.

Congenital cataracts, some of which were hereditary, were also reported in rats and mice (Saunders, 1967).

Alterations in Cataract Formation

Biochemical Changes

Water. Moses (1970) stated that in most experimental cataracts there was an initial increase in lens water which disappeared gradually as the cataract matured. Total water content eventually decreased and the weight of the lens diminished concurrently.

Sodium and potassium. Duke-Elder (1969) considered the majority of potassium ions, in the normal lens, were bound with protein and other complexes. He concluded that cellular death in cataract formation liberated these ions which then diffused out of the lens. Simultaneously, sodium levels in the lens increased as a result of diffusion from the aqueous humor. Lebensohn (1936) stated that the

potassium-sodium ratio was a reliable index of tissue vitality. He reported a ratio of 1.65 in the normal cortex and 1.34 in the normal nucleus. Cataractous lenses had a ratio of 0.41 in the cortex and 0.32 in the nucleus, due to decreased potassium and elevated sodium levels.

Glutathione. Dische *et al.* (1956) observed reduced glutathion levels in cataractous lenses. Moses (1970) stated that it was not known whether the disappearance of glutathion was the cause of cataract formation or if it was secondary to the cataractous process. He added, however, that the ability of the lens to consume oxygen depended on the presence of glutathion.

Protein. Salit (1939) reported gradual loss of total protein in the process of cataract formation and considered the loss proportional to the degree of damage to the lens. He observed that soluble proteins (alpha- and beta-crystallins) decreased and the insoluble albuminoids were retained.

Pirie and van Heyningen (1956) stated that increased proportions of insoluble proteins were a general rule for all types of cataracts.

The fate of reduced levels of soluble proteins in cataracts is not known; however, Sippel (1967) postulated that low molecular weight proteins escaped through the capsule following breakdown of lens fibers.

Pathology of Cataracts. Jubb and Kennedy (1969) stated that pathologic reactions of the lens were limited, due to an intact capsule impermeable to leukocytes, and the absence of blood vessels. They considered the lens capable of 2 main reactions, namely proliferation of capsular epithelium and necrosis of lens fibers.

Hogan and Zimmerman (1962) classified cataractous alterations according to the way the capsule, epithelium and lens substance were affected. Capsules of cataractous lenses were either thickened, or thinned, and occasionally ruptured. Changes associated with the subcapsular epithelium were either degenerative or proliferative. Degeneration of epithelial cells resulted in cloudy swelling, cytoplasmic vacuolation, pyknosis and cell death. Epithelial proliferation resulted in metaplasia, migration of cells to the posterior capsule, and sometimes a new hyaline capsule was formed which encircled groups of epithelial cells. Alterations associated with cataractous lens substance were degenerative or sclerotic, the latter regarded as a part of the normal aging process.

Cogan (1962) described the histologic alterations of cataractous lens fibers. Cortical cataracts were characterized by the formation of large round cells or aberrant lens fibers. Lens substance was occasionally transformed into eosinophilic vacuoles, called Morgagnian globules, which aggregated in clefts between lens fibers. It was suggested that cortical cataracts were characteristically spotty and did not affect the lens uniformly.

Kuwabora *et al.* (1969) studied the ultrastructural alterations in galactose-induced cataracts in rats. They observed increased reactivity of the epithelium, edema of lens fibers and intercellular cyst formation.

Cataracts in Avian Species

Experimental Cataracts

Viruses. Several viruses have been utilized to study the experimental production of cataracts in chick embryos.

Blattner *et al.* (1951) observed defective development of the lens and auditory vesicles in chicken embryos infected with Newcastle disease. Williamson *et al.* (1956) reported similar defects in chick embryos infected with influenza A virus. Enders strain of mumps virus produced cataracts in chick embryos inoculated prior to separation of the lens vesicle from the surface ectoderm (Robertson *et al.*, 1964).

Dinitrophenol. Dinitrophenol (DNP), a metabolism-stimulating drug, was used in the 1930's for the treatment of human obesity but it produced toxic reactions and cataracts in some patients (Horner, 1942). Attempts to produce experimental cataracts in laboratory mammals by various and repeated doses of DNP were unsuccessful. However, Robbins (1944) experimentally induced cataracts, for the first time, in ducklings and chickens by the oral administration of DNP. Lens lesions produced were reversible and grossly resembled the transitory opacities induced by heat, cold, anoxia and other physiopathologic factors. The mechanism believed to be responsible for the formation of DNP cataracts was the decreased incorporation of inorganic phosphate into high energy phosphate compounds (uncoupling of oxidative phosphorylation).

Vitamin E deficiency. Cataracts were observed in turkey embryos obtained from hens fed a diet low in vitamin E (Ferguson *et al.*, 1956). Ocular changes were characterized by areas of lens liquefaction and focal areas of corneal degeneration in affected embryos. Capsular changes were not observed; however, degeneration and focal areas of proliferation were evident in epithelial cells. Liquefaction necrosis was observed in part or all of the lens fibers. Some of the turkeys permitted to hatch from vitamin E-deficient hens had bilateral cataracts.

Spontaneous Cataracts

Lymphomatosis. Ocular lesions consisting of iritis and iridocyclitis with lymphocytic infiltration were reported by several investigators (Doyle, 1928; Lee and Wilcke, 1941; Nelson and Thorp, 1943) in chickens infected with the virus of lymphomatosis.

Rigdon (1959) also studied ocular lesions of chickens with lymphomatosis; however, he observed lens degeneration with cataract formation. Lens changes were characterized by coagulation necrosis of fibers, cyst formation, and epithelial proliferation and degeneration. Chickens with lens lesions usually had an associated iritis and some birds also had visceral and neural lymphomatosis.

Rigdon *et al.* (1959) reported similar cataracts in turkeys; however, iritis was not observed. Turkeys that developed cataracts were in contact with chickens infected with lymphomatosis, and the investigators suspected interspecies viral transmission as the cause.

Avian encephalomyelitis (AE). Peckham (1957) presented a case report in which he described lens opacities in young fowl which exhibited central nervous signs. Ocular histopathologic changes included cataracts with epithelial proliferation, uveitis with mononuclear infiltration and posterior synechia. The investigator suspected AE, due to the central nervous manifestations and compatible histopathologic brain lesions.

Bridges and Flowers (1958) observed similar lesions in several flocks; however, their attempts to isolate a viral agent were unsuccessful and attempts to reproduce the condition were inconclusive. Barber and Blow (1963) and Halpin (1967) also observed cataracts in chickens following outbreaks of what was thought to be AE.

Several investigators have studied chickens experimentally infected with AE. Feibel *et al.* (1952) produced iridocyclitis in chicks by intraocular inoculation of AE virus; however, cataracts were not reported. Butterfield *et al.* (1969) exposed chickens to AE and observed the incidence and longevity of histopathologic lesions. They reported the highest incidence of lesions in the brain, gizzard and pancreas; however, cataracts were not reported.

Cataracts associated with artificial light. Lauber and McGinnis (1966) reported that extreme buphthalmos developed in galliform birds, maintained under continuous light from the time of hatching through the growing period. Reduced corneal curvature, decreased pupillary size, shallow iridocorneal angle and equatorial asymmetry of the eye were also observed. Cataracts developed in many birds after prolonged exposure to continuous light but were rarely seen in diurnal birds.

Cataracts associated with *Leukocytozoon*. Chew (1968) observed unilateral cataracts in 2 five-month-old hens with leg paralysis. Characteristic cells, suggestive of Marek's disease, were observed in the liver; however, megaloschizonts of *Leukocytozoon* were observed in several ocular and extraocular tissues.

Cataracts of undetermined etiology. Devolt (1944) observed cataracts in 18% of a group of 3-1/2-month-old chickens. Birds were raised in a cellar and electric light bulbs were used for brooders. These birds were fed the same ration utilized by other growers whose flocks were unaffected. A subsequent brood raised under similar conditions, except for shorter cellar confinement, did not develop cataracts. The investigator suggested 2 possible causes, either excess light intensity or a ration altered by wartime conditions.

Brion *et al.* (1960) observed cataracts in chickens and ducks enzootic in France. Opacities appeared at less than 1 month of age and usually affected both eyes equally. Lesions were also observed in the cerebrum, liver or pancreas of some animals. It was suggested that subclinical AE and lymphoid leukosis may have been involved.

Cataracts in Fish

Prince (1956) considered the lenses of fish and avian species similar in that both were spherical and had thickened annular pads of epithelial cells. Reports of cataracts in fish have revealed causes similar to those of cataracts in birds.

Hess (1937) compared the incidence of cataracts in trout fed pig spleen with those fed beef liver and heart. Fish fed pig spleen developed a high incidence of cataracts and those fed beef liver and heart, or a combination of both diets, had normal lenses. The absence of cataracts in the 2 latter groups suggested a nutritional inadequacy in the pig spleen diet.

Allison (1962) observed that direct sunlight affected the lens of young lake trout and prolonged exposure induced cataracts. Steucke *et al.* (1968) conducted a 3-year study on the effects of light and diet on hatchery-reared lake trout. They observed cataracts in 2.6% of fish raised in covered tanks compared with a 76% incidence of cataracts in fish raised in non-covered tanks. The second year a portion of the fish in covered tanks was transferred to non-covered tanks. An 87% incidence of cataracts was observed in the transferred fish. Only 9.1% of those kept in covered tanks developed cataracts by the end of the second year.

Van Duijn (1967) described 2 types of cataracts in fish. Gray cataracts, observed particularly in goldfish, were diffuse unilateral opacities of undetermined cause. Worm cataracts were distinguished by minute multifocal opacities, produced by trematode larvae situated between the capsule and lens cortex. These larvae were less than 0.5 mm in size and were classified as *Diplostomum sp.*

MATERIALS AND METHODS

Experimental Animals

Bobwhite quail (*Colinus virginianus*) were utilized as the experimental animals in this study. These birds were chosen because they (1) were readily available, (2) were easily handled for ophthalmoscopic examination, and (3) were known to have a high incidence of spontaneous cataracts.

Selection and Care. Birds used in this study were a part of the Bobwhite quail colony maintained by the Poultry Science Department, Michigan State University. Quail eggs were hatched and birds were examined periodically for the development of cataracts. These birds (designated young quail) were less than 13 months old and were used primarily for experiments designed to evaluate nutrition, population density and environment. Aged quail ranging in age from 1-1/2 to 4-1/2 years were divided into 3 subgroups based on an average age of 2, 3, or 4 years. Aged quail were used primarily for the chemical analyses in this investigation. Both young and old quail were used in the ophthalmoscopic, histopathologic, microbiologic and ultrastructural studies.

Animals were housed in Petersime Model 2-SD batteries which had heavy wire-mesh floors and thermostatically controlled heating elements. The batteries were kept in a large windowless room and continuous artificial light was provided by incandescent ceiling fixtures. Quail less

than 1 month of age also received light from tubular fluorescent fixtures located in each battery compartment. The room was ventilated by a negative air-pressure system, and insulated walls and ceiling provided protection from severe fluctuations in outside temperature.

Standard population density was maintained at not more than 3 birds per square foot of floor space, for all adult birds except those on comparative population density experiments (Experiments II and III).

Rations and Feeding Practices. Quail were fed and watered daily in trough-type feeders and waterers attached to the sides of each battery compartment. The standard Michigan State University quail breeder ration was fed to all birds except those on comparative diet experiments. The standard MSU quail breeder ration is given in Table 1.

Some of the birds in Experiment II received a fortified diet. The standard quail breeder ration was used as a basal ration and specific ingredients were added as presented in Table 2.

Some of the birds in Experiment III received a commercially prepared diet designed for game birds. The exact composition is not available; however, the feed tag analysis is presented in Table 3.

Ophthalmoscopic Examinations

Clinical ophthalmoscopic examinations were conducted on live birds at the times specified in each experiment. All birds were examined initially with intensified focal illumination in a darkened room. Representative birds, from each group, were selected for more detailed slit lamp biomicroscopy and photography. These re-examinations were conducted to more accurately assess lens changes with the aid of special optical equipment.

Table 1. MSU quail breeder ration (25% protein)

Ingredients	Percent
Ground yellow corn	41.25
Soybean oil meal (dehulled 50% protein)	37.00
Alfalfa meal, 17% protein	5.00
Dried whey	2.50
Meat and bone scraps, 50% protein	2.50
Fish meal, menhaden, 60% protein	2.50
Ground limestone ($CaCO_3$)	5.00
Dicalcium phosphate	1.50
Salt, iodized	0.50
Fat	2.00
Vitamin mix (layer-breeder #5001)*	0.25
	100.00

*Vitamin-mix, Layer-Breeder #5001:

Micronutrients	Per pound of mix
Vitamin A, U.S.P. units	800,000.0000
Vitamin D_3, I.C. units	250,000.0000
Riboflavin, mg.	700.0000
Pantothenic acid, mg.	1,200.0000
Niacin, mg.	2,500.0000
Choline chloride, mg.	39,000.0000
Folic acid, mg.	100.0000
Vitamin B_{12}, mg.	1.2000
Vitamin E, U.U.	500.0000
Menadione sodium bisulfite, mg.	150.0000
Manganese, %	1.2870
Iodine, %	0.0201
Copper, %	0.0810
Cobalt, %	0.0051
Zinc, %	1.0000
Iron, %	0.5025

Table 2. Fortified quail breeder ration

Ingredient	Level in std. quail breeder ration	Recommended level by Scott* NRC**	Level in fortified ration
Vitamin A[1]	8,800 IU/kg.	13,000 IU/kg.**	22,000 IU/kg.
Vitamin E[2]	5.5 IU/kg.	20 IU/kg.*	130 IU/kg.
Choline	1,980 mg./kg.	1,300 mg./kg.*	2,800 mg./kg.
Niacin	48 mg./kg.	50 mg./kg.*	100 mg./kg.
Riboflavin	10.6 mg./kg.	8 mg./kg.*	16.5 mg./kg.
Methionine[3]	0.5%	0.5%**	1.0%
Cystine[4]	0.37%	0.35%**	0.45%
Ascorbic acid[5]	---	---	200 mg./kg.
B_{12}	13.2 mcg./kg.	12.0 mcg./kg.	25.0 mcg./kg.
Selenium[6]	---	---	0.3 mg./kg.
Stabilizer (butylated hydroxy toluene) added			0.5 gm.

[1]Robimix A-325.

[2]DL alpha tocopherol.

[3]Feed grade 98% (Dow Chemical Co.).

[4]l (-) cystine.

[5]Based on guinea pig requirements.

[6]Based on guinea pig requirements.

*Scott, M. L., Proc. Cornell Nutr. Conf., Buffalo, N.Y., 1966.

**National Academy of Science NRC Publication 1345, Nutrient Requirements of Poultry.

Table 3. Purina game bird finisher 5425

Guaranteed analysis	
Crude protein not less than	20.0%
Crude fat not less than	3.0%
Crude fiber not more than	4.0%

Grading. Cataracts were classified according to the extent of lens opacity, and grades were assigned as follows:

Grade I — Lens opacities which appeared as minute focal opacities limited to less than 1/3 the lens diameter.

Grade II — Diffuse lens opacities which appeared to involve 1/3 to 2/3 the lens diameter.

Grade III — Complete opacity of the lens.

Grade IV — Complete lens opacity with adhesions between anterior lens capsule and iris.

Both lenses were graded; however, each bird received a single grade. Due to the occasional occurrence of unilateral or bilateral dissimilar cataracts, each bird was assigned the grade of the lens with the most extensive opacity.

Survey of Aged Quail. An initial survey was conducted on 404 aged quail, and birds were separated on the basis of lens change into one of the following groups: normal, grade I, II, III, IV cataracts. Lenses from birds within these grades were used for subsequent studies.

Histopathology

Histopathologic examinations were conducted on ocular and extraocular tissues, including brain, brachial plexus, sciatic nerve, cardiac and skeletal muscle, skin, lung, liver, spleen, trachea, esophagus, proventriculus, muscular stomach, duodenum, pancreas, ceca, cloaca and bursa, adrenal, thyroid, parathyroid and pituitary glands.

Extraocular tissues were routinely fixed in 10% buffered formalin solution. They were infiltrated and embedded in a paraffin-base medium*

*Paraplast, Sherwood Medical Industries, Inc., St. Louis, Mo.

and sectioned at 6 microns. Sections were stained with hematoxylin and eosin and, in selected cases, special stains were used to aid differentiation.

Ocular tissues were prepared by placing freshly removed, intact eyeballs in a 200 F solution of 37% formaldehyde (4%), 70% alcohol (44%), glacial acetic acid (2%), and saline (50%) for 5 minutes. Eyes were briefly washed in running water and then the lenses were removed from the scleral shells and each was processed separately. Lenses and scleral shells were dehydrated, infiltrated, and embedded in a paraffin-base medium* under vacuum. Six micron serial sections were stained in the same manner as extraocular tissues.

Sampling Methods. Two young quail with clinically normal lenses were euthanatized and necropsied at 2, 4, 7, and 10 months of age and both ocular and extraocular tissues were collected. Similar tissues were collected from young quail with cataracts at 4, 7, 10, and 13 months of age.

Aged quail were grouped for histopathologic studies on the basis of cataract grade. Three birds from each of the 4 grades of cataracts were necropsied and ocular and extraocular tissues were collected for histopathologic examination. Similar tissues were collected from 2 aged birds with normal eyes.

Chemical Analyses

Chemical analyses were conducted on normal lenses and cataracts (Grades I, II and III). Due to extensive degenerative changes, Grade IV cataracts were not chemically analyzed.

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*Paraplast, Sherwood Medical Industries, Inc., St. Louis, Mo.

Cations [Sodium (Na) and Potassium (K)] and Water Content. Lenses obtained from aged quail were individually analyzed for their respective levels of cations and water content. Analyses were conducted on 7 normal lenses, 11 Grade I, 10 Grade II, and 12 Grade III cataracts. The protocol used in these analyses is presented in detail in Appendix A.

Soluble and Insoluble Lens Protein. The analysis for soluble and insoluble protein was performed in accordance with the technique described by Lowry *et al.* (1951). Values were obtained from normal lenses taken from birds 4 and 10 months and 2 and 4 years of age. Values were also obtained from 8 Grade I, 8 Grade II and 6 Grade III cataracts. All lenses were individually analyzed and the results averaged for each age or grade. The protocol used in these analyses is presented in detail in Appendix B.

Glutathione Content. Levels of glutathione were obtained from 6 normal lenses, 10 Grade I, 8 Grade II, and 8 Grade III cataracts. Individual lenses were analyzed by the technique described by Ball (1966). The protocol used in these analyses is presented in detail in Appendix C.

Serum Glucose. Blood for serum glucose studies was collected by intracardial puncture at the time of euthanasia. Samples were allowed to clot and were then centrifuged. Serum was removed and glucose levels were determined by the glucose oxidase method (Sigma #510). Analyses were done on 13 normal birds and 12 birds with Grade III cataracts.

Microbiology. The interior of intact globes, removed from 3 young and 5 aged quail, were cultured for bacteria, myocplasma and fungi. Young birds had either Grade I or II cataracts and aged birds had either Grade III or IV cataracts. Another group of young and aged quail,

with similar grades of cataracts, was cultured for viruses, rickettsial and for chlamydial agents by tissue culture and chick embryo inoculations. The detailed protocol used in the microbiologic studies is presented in Appendix D.

Electron Microscopy. Ultrastructural studies were done on 2 normal birds (2 weeks and 4 months old) and 4 aged birds (2 with Grade I and 2 with Grade IV cataracts). These birds were chosen because they represented 2 stages of normal lens growth, a very early and a very advanced cataractous stage.

Double fixation was accomplished by the use of 3% glutaraldhyde for 2 hours and 1% osmium tetroxide for 1-1/2 hours. Both of these fixatives were buffered with 0.4 M s-collidine adjusted to pH 7.4.

Immediately after birds were euthanatized, the lenses were removed and placed in cold fixative. Under magnification the lenses were cut anterior to posterior so that 3 sections nearly equal in width were obtained. Only the central sections were used because ultrastructural studies were confined to the central anterior capsule, subcapsular epithelium and subjacent cortical lens fibers.

Ultrathin sections were cut and stained with uranyl acetate for 30 minutes and lead citrate for 15 minutes. Tissues were embedded in Epon 812 and polymerized for 12-hour periods at 35, 45, and 60 C.

Experiments

Experiment I. This experiment was designed to study the time and rate of cataract development in a single group of birds over a 1-year period. These birds were fed, handled and maintained in the same manner as aged birds with a high incidence of cataracts.

Forty-five birds were used for this experiment and all were individually examined at 2, 3, 4, 7, 10 and 13 months of age. Lens changes were classified and recorded for each bird at each examination.

Experiment II. This experiment was designed to study the effect of diet and population density on the incidence of cataracts. Four groups of birds were used to compare the standard diet with a fortified diet and a high population density with a low population density. Two days after hatching, 108 birds were randomly distributed into experimental groups as given in Table 4. All birds were individually examined at

Table 4. Experimental design, Experiment II

Group	Diet	Pop. Density	No. of Birds
A*	Standard	4 birds/sq.ft.	40
a*	Standard	1.5 birds/sq.ft.	14
B	Fortified	4 birds/sq.ft.	40
b	Fortified	1.5 birds/sq.ft.	14

*Not completed due to excessive death loss.

1, 2, 3, 4, 6, 9 and 12 months of age and lens changes were classified and recorded for each examination. Due to excessive mortality in birds fed the standard ration, a valid test of groups A and a could not be accomplished and this portion of Experiment II was terminated. Death losses were attributed to management problems which did not affect the remaining birds. The experiment was completed as planned with the 2 groups fed the fortified ration.

Experiment III. This experiment was designed to study in a natural light environment the effect of diet and population density on the incidence of cataracts.

Quail used in this study were isolated in a small room with a large window, and kept under infrared heat lamps for the first month of life. Birds were raised on wire mesh floors, and jar-type waterers and trough-type feeders were placed inside the cages. The light to which these birds was exposed was continuous for the first month and consisted of natural daylight, infrared and fluorescent light. Between the ages of 1 and 6 months light exposure was cyclic and consisted of natural daylight only.

The dietary portion of this experiment was designed so that the standard quail ration could be compared with a commercial ration for game birds (Purina 5425).

Population densities were higher than those used in previous experiments and a low population density group on a standard diet was not included.

When the 75 birds in this experiment were 1 month old they were randomly distributed into experimental groups given in Table 5. All birds were individually examined at 2, 3, 4, 5, and 6 months of age. Due to usual mortality, population density decreased slightly from the original level; however, the original proportions were maintained.

Analysis of Data

Chemical data were examined by one-way analysis of variance. The statistical significance between normal and cataractous lenses was determined by Scheffe's confidence intervals and the F test of Snedecor (1956).

Table 5. Experimental design, Experiment III

Group	Diet	Pop. Density	No. of Birds
S	Standard	6 birds/sq.ft.	37
P	Commercial*	9 birds/sq.ft.	27
p	Commercial*	3 birds/sq.ft.	11

*Purina Game Bird Finisher #5425.

Data from the comparative incidence of cataracts in 6-month-old birds were analyzed by the chi square distribution and the statistical significance between groups was determined.

RESULTS

Clinical Characteristics of Cataracts

The clinical appearance of corresponding grades of cataracts were similar in young and old quail. A single clinical characterization, applicable to all ages, is given for each grade.

Grade I. Grade I cataracts appeared as a minute focal opacity in the anterior central lens cortex (Figure 1). The opacities were limited to 1 mm. or less than 1/3 of the lens diameter and in some birds were accompanied by a concomitant posterior cortical opacity. Quail with Grade I cataracts had normal pupillary light responses and vision.

Grade II. Grade II cataracts were more extensive and appeared as diffuse cloudy masses or reflective rays in the anterior central cortex of the lens (Figure 2). These opacities were asymmetrical and involved 1/3 to 2/3 of the visible lens area. Quail with Grade II cataracts had normal pupillary light responses and some birds appeared to be clinically blind.

Grade III. Lenses from quail with Grade III cataracts were completely opaque (Figure 3). The most distinctive feature of these cataracts was lens liquefaction. Degenerate segments of lens fibers floated freely inside the capsule. Pupils of quail with Grade III cataracts were slightly dilated and pupillary light responses were slower than normal. All of these birds were clinically blind.

Figure 1. Grade I cataract with anterior central opacity. x 15.

Figure 2. Grade II cataract with diffuse central and radial linear opacity. x 15.

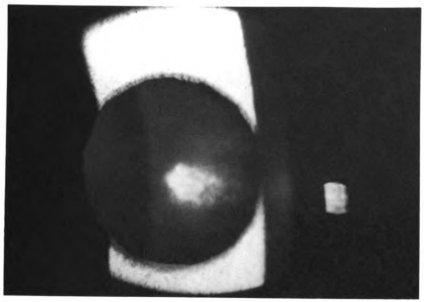

Figure 1

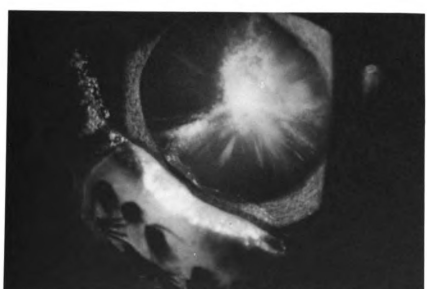

Figure 2

Figure 3. Grade III cataract with complete lens opacity. Reflective spots within the lens are light reflections from fragmented lens fibers. x 15.

Figure 4. Grade IV cataract with crystalline opacity and iris adhesions (arrow). x 15.

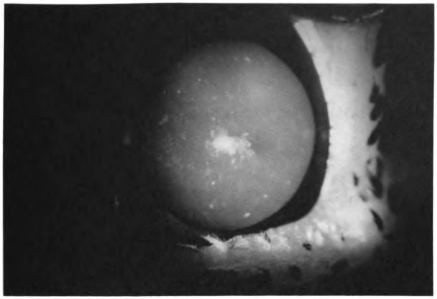

Figure 3

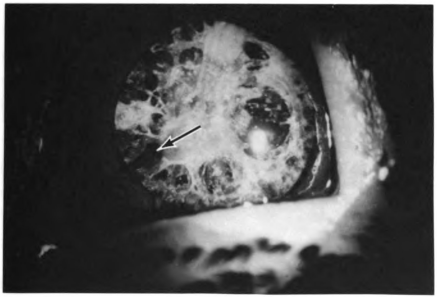

Figure 4

Grade IV. Grade IV cataracts were observed in aged quail only and were considered the last stage of cataractous change. Crystalline opacities were readily apparent on the anterior surface of the lens which was either flat or concave (Figure 4). Posterior synechia was observed due to adhesions between the anterior lens capsule and the iris. These adhesions, and opacity of the lens, appeared to interfere with pupillary light responses.

Incidence of Cataracts in Aged Quail

The percentage incidence of all grades of cataracts in aged quail is presented in Table 6, according to the average age of the birds. The 2-year-old age group had the highest percentage of normal lenses and Grade I cataracts. Three-year-old quail had the highest incidence of Grade II cataracts. The 4-year-old age group had the highest incidence of Grade III and Grade IV cataracts.

Table 6. Incidence of cataracts in aged quail

Age	Total	Normal (%)	Grade I (%)	II (%)	III (%)	IV (%)
2 years	124	23 (*18*)	85 (*69*)	11 (*9*)	2 (*2*)	3 (*2*)
3 years	74	3 (*4*)	38 (*51*)	11 (*15*)	9 (*12*)	13 (*18*)
4 years	206	7 (*3*)	88 (*43*)	22 (*11*)	40 (*19*)	49 (*24*)

Histology

Normal Lenses. Lenses from normal birds were characterized by a single layer of low cuboidal epithelium in the central anterior subcapsular region. This epithelium was transformed into tall columnar cells at the lens equator and formed a wide "annular pad." Nuclei were prominent

in lens fibers of recently hatched birds and extended from the cortex to the nucleus of the lens (Figure 5). Cells in the posterior lens bow changed from epithelium to lens fibers and nuclei were altered from round to flattened structures that disappeared as fibers matured (Figure 6). Cortical lens fibers were closely joined to one another in a regularly arranged lamellar pattern which was striking in young birds (Figure 7). The regular pattern of individual fibers was less evident, and distinct separation of cortical layers was more pronounced in normal lenses of aged birds (Figure 8).

Histopathology

Histopathologic alterations were confined to ocular tissues of young and aged quail with cataracts. Microscopic lens changes in corresponding grades of cataracts were similar at all ages; thus, a single histopathologic characterization is given for each grade. Grade IV cataracts were not seen in young birds and the microscopic description represents the most advanced cataractous stage of aged quail.

Grade I. Microscopic alterations in Grade I cataracts were characterized by metaplasia and hyperplasia of the anterior central subcapsular epithelium (Figures9, 10, 11 and 12). Low cuboidal cells were transformed to columnar in some lenses; however, most of these epithelial changes resulted in irregularly shaped cells. Subjacent cortical lens fibers were interrupted by proliferative groups of cells with prominent nuclei and vacuolated cytoplasm (Figure 9).

Other Grade I cataracts had less extensive epithelial metaplasia and only a few individualized nuclei were noted between cortical lens fibers. Granular material in the interfibrillar spaces of these lenses

Figure 5. Normal lens from a 2-day-old quail illustrating the wide annular pad (P) of epithelial cells and persistent nuclei (N) in young lens fibers. Hematoxylin and eosin. x 50.

Figure 6. Posterior lens bow from normal 4-month-old quail. Note the transition from round to flattened nuclei with maturity of lens fibers. Hematoxylin and eosin. x 250.

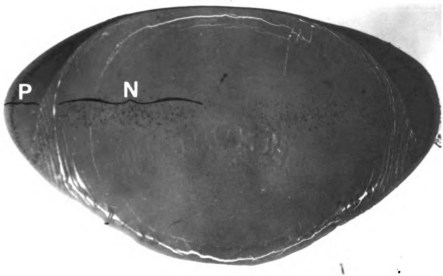

Figure 5

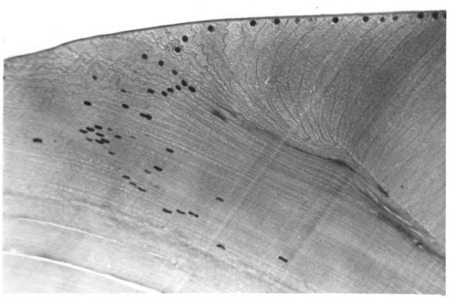

Figure 6

Figure 7. Central anterior capsule, epithelium and cortical lens fibers from a normal 4-month-old quail. Hematoxylin and eosin. x 250.

Figure 8. Central anterior capsule, epithelium and cortical lens fibers from a normal 4-year-old quail. Hematoxylin and eosin. x 250.

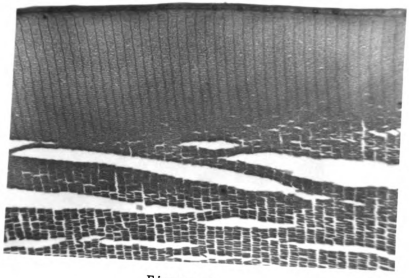

Figure 7

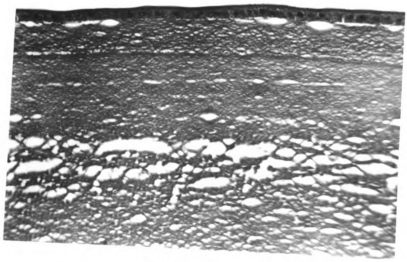

Figure 8

Figure 9. Central anterior epithelium with metaplasia from low cuboidal to high columnar cells. Aberrant cells and vacuoles (arrow) interrupt cortical lens fibers. Tissue (c) above the capsule is an artifactual corneal adhesion. Hematoxylin and eosin. x 250.

Figure 10. Epithelial hyperplasia (E) subjacent to the central posterior capsule (arrow) accompanied by partial liquefaction (L) of the posterior cortex. Loose matrix below the posterior capsule is vitreous humor (V). Hematoxylin and eosin. x 250.

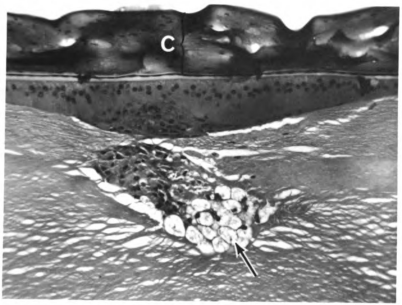

Figure 9

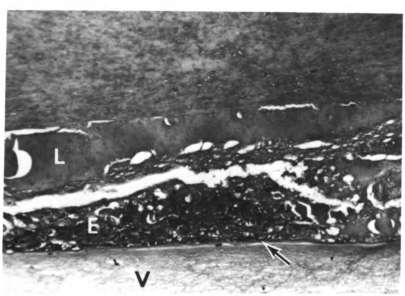

Figure 10

Figure 11. Central anterior interfibrillar spaces containing granular degenerative lens material (arrow). Grade I cataract in aged quail. Hematoxylin and eosin. x 250.

Figure 12. Hydropic degeneration and liquefaction of anterior cortical lens fibers. Note the swollen cells with pyknotic nuclei. Grade I cataract in aged quail. Hematoxylin and eosin. x 250.

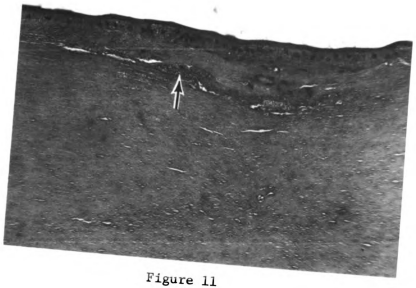

Figure 11

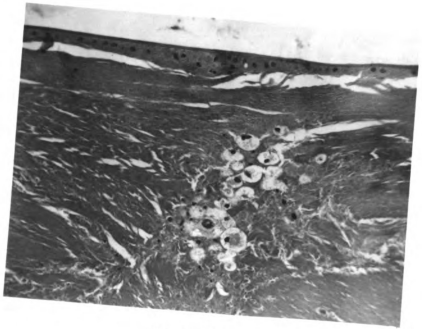

Figure 12

was indicative of an early degenerative change in the anterior cortex (Figure 11). The most extensive Grade I cataracts had partially liquefied fibers in the central anterior cortex suggestive of early hydropic degeneration. Swollen cells with pyknotic nuclei were also observed in these areas (Figure 12).

Some Grade I cataracts also had focal areas of epithelial hyperplasia which extended partway around the posterior capsule. Focal clusters of cells, similar to those under the anterior capsule, were occasionally observed immediately under the center of the posterior capsule (Figure 10).

Grade II. Histologic changes in Grade II cataracts were similar to, but more extensive than, Grade I cataracts. Subcapsular epithelial proliferation was more pronounced and vacuolar spaces occurred in the anterior cortex accompanied by partial liquefaction of lens fibers. Hydropic degeneration of lens fibers was observed in the posterior cortex and lens bow of Grade II cataracts (Figures 13 and 14).

Grade III. Grade III cataracts were characterized by severe degeneration of lens fibers and epithelial hyperplasia. Early changes in this grade resulted in fragmentation and individualization of lens fibers accompanied by epithelial hyperplasia, especially prominent in the lens bow (Figure 15). The lens substance of more advanced cataracts had an eosinophilic granular appearance suggestive of liquefaction necrosis (Figure 16). In some of these lenses the anterior capsule was thickened and the subcapsular epithelium was vacuolated.

Grade IV. Lenses classified as Grade IV cataracts were markedly shrunken and extensive adhesions were observed between the iris and anterior

Figure 13. Vacuolation and granules in the lens bow. Note swollen cells with granular appearance (arrow). Grade II cataract in a 10-month-old quail. Gomori's trichrome stain. x 250.

Figure 14. Cluster of hyperplastic epithelial cells in the lens bow. Grade II cataract in aged quail. Hematoxylin and eosin. x 250.

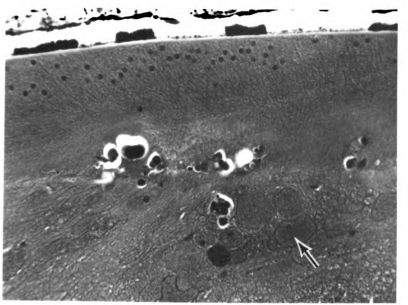

Figure 13

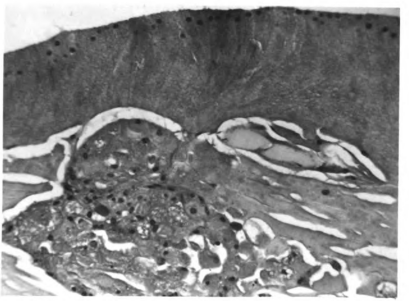

Figure 14

Figure 15. Epithelial hyperplasia in the lens bow accompanied by separation of lens fibers. Grade III cataract in aged quail. Gomori's trichrome stain. x 250.

Figure 16. Thickened and folded anterior lens capsule, epithelial degeneration and lens fiber liquefaction. Grade III cataract in aged quail. Hematoxylin and eosin. x 250.

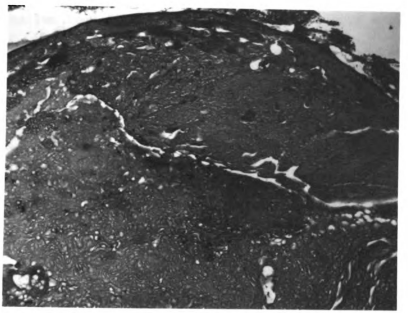

Figure 15

Figure 16

capsule of the lens (Figures 17 and 18). Degenerative changes in subcapsular epithelium lens fibers and anterior capsule were more severe than in Grade III cataracts. Proliferation of subcapsular epithelium usually extended beyond the lens bow across the posterior capsule. Occasional mononuclear cells were identified in the adhesions of the anterior capsule and iris; however, no other inflammatory changes were observed in eyes with Grade IV cataracts.

Electron Microscopy

Normal Ultrastructure

Capsule. The ultrastructural appearance of the central anterior lens capsule in normal 2-week-old and 4-month-old quail was that of a homogeneous lamellar membrane lacking organelles. The outer border was slightly irregular and the deep border conformed to the outer surface of the underlying epithelial cells. The only difference between capsules representative of the 2 ages was the comparative thickness. The capsule of 4-month-old quail was approximately twice as thick as the capsule of 2-week-old quail when compared at similar magnifications (Figures 19 and 20).

Epithelium. The epithelium of normal quail lenses consisted of a single layer of flattened cells located immediately below the anterior lens capsule. These cells were joined laterally with outfoldings of the plasma membrane and dense segments occasionally obliterated the intercellular space.

The cytoplasm of epithelial cells was homogeneous and small numbers of free ribosomes were observed. Mitochondria and rough endoplasmic reticulum (RER) were readily evident in cells of both age groups.

Figure 17. Shrunken lens with degenerate lens substance. Grade IV cataract in aged quail. Gomori's trichrome stain. x 50.

Figure 18. Higher magnification of Figure 17 showing lamellar separation of anterior capsule with iris adhesion above and epithelium below. Grade IV cataract in aged quail. Gomori's trichrome stain. x 500.

Figure 17

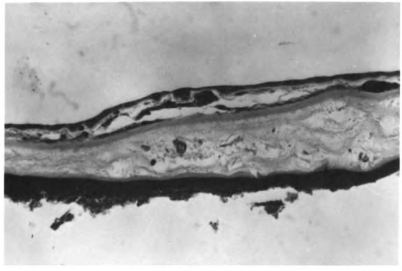

Figure 18

Figure 19. Central portion of the anterior lens capsule (C) of a normal 2-week-old quail. The surface is slightly irregular and the inner border conforms to the contour of the underlying epithelial cell. A portion of the epithelial cell nucleus (N) and plasma membranes (PM) are identified. (x 10,200)

Figure 20. Central portion of the anterior lens capsule (C) of a normal 4-month-old quail. Note the plasma membrane (PM) and the increased thickness of the capsule compared to the capsule in a 2-week-old quail. (x 10,200)

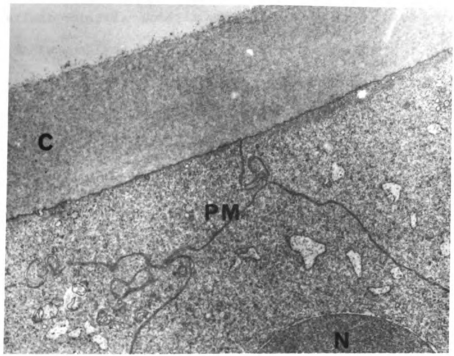

Figure 19

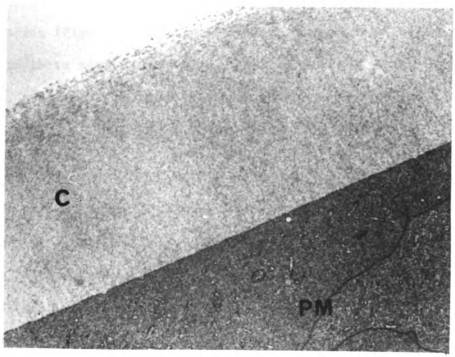

Figure 20

Those in lenses from 2-week-old quail appeared slightly swollen and lacked distinct cristae. Golgi's apparatus and microtubules were more prominent in lenses from 4-month-old birds (Figure 22).

The primary difference in epithelial cells representative of the 2 ages was the shape of the nuclei. Epithelial nuclei at 2 weeks of age were circular and present in nearly every cell (Figure 21). The nuclei at 4 months of age appeared flattened and occurred in approximately half of the cells (Figure 22).

Ultrastructure of Cataracts

Grade I. Ultrastructural studies of the earliest stage of quail cataracts were confined to the central anterior capsule and underlying epithelium. The capsule of these cataracts was normal; however, epithelial cells were characterized by extensive and elaborate outfoldings of the lateral cellular membranes (Figure 23). Golgi's apparatus was especially prominent and numerous microtubules were evident at higher magnification (Figure 24). Organelles near the capsular surface were similar to those seen in epithelial cells of normal lenses. Swollen vesicles, suggestive of early hydropic degeneration, were apparent in areas bordering the interdigitations of the deep epithelium (Figure 25).

Grade IV. Ultrastructural features of the central anterior capsule and epithelium of Grade IV cataracts were variable. The capsule was often irregular, and fibrillar strands of tissue with particles of electron dense pigment adhered to the outer surface (Figure 26).

Epithelial cells did not stain uniformly and interdigitating processes were not as prominent as those seen in Grade I cataracts. Normal epithelial cells were observed in Grade IV cataracts (Figure 26)

Figure 21. Central anterior epithelium and capsule (C) from the lens of a normal 2-week-old quail. Mitochondria (M) lack definitive inner structure and rough endoplasmic reticulum (RER) appears dilated. Clusters of free ribosomes (r) are numerous in the cytoplasm. Note the rounded nuclei (N) and nuclear pores (P). (x 17,000)

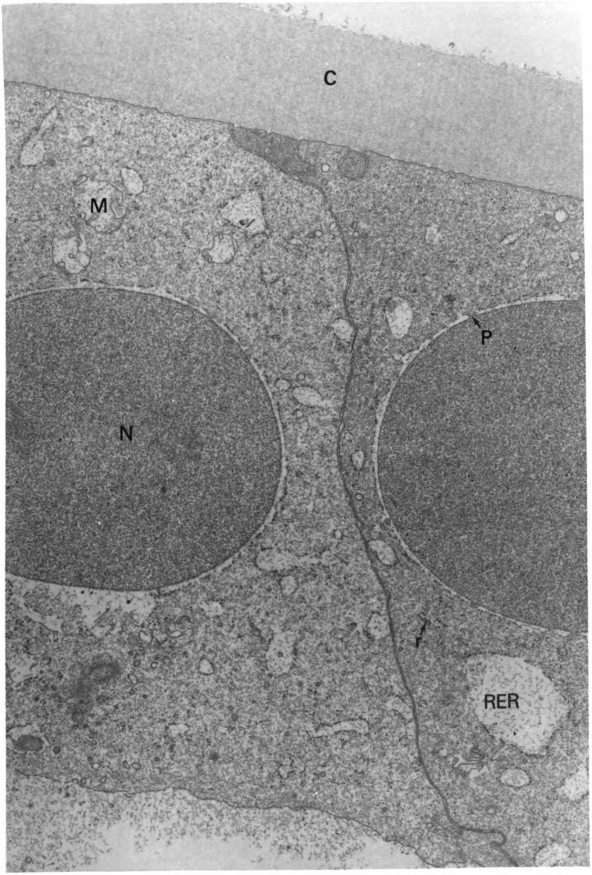

Figure 21

Figure 22. Central anterior epithelium and capsule (C) from the lens of a normal 4-month-old quail. Cytoplasmic organelles include mitochondria (M), rough endoplasmic reticulum (RER), clusters of free ribosomes (r), Golgi's apparatus (G) and microtubules (mt). Note the flattened nucleus with homogeneous chromatin and mass (n) suggestive of a nucleolus. The lens fiber (LF) adjacent to the epithelial cell is nearly devoid of organelles. (x 17,000)

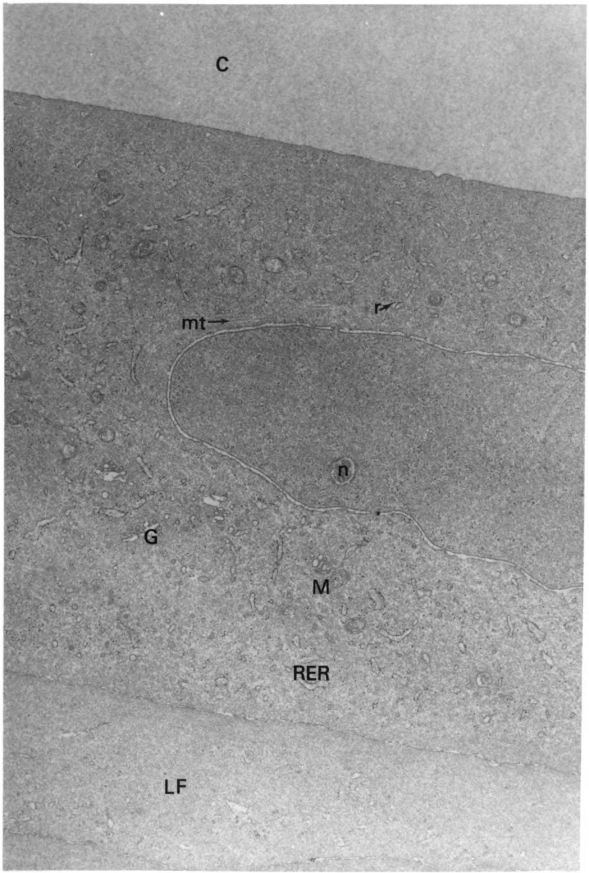

Figure 22

Figure 23. Central anterior epithelium from a lens with a Grade I cataract. Elaborate outfoldings of plasma membranes (PM) are evident and Golgi's apparatus (G) is abundant. Note the swollen vesicles (sv) and decreased cytoplasmic density bordering plasma membranes in the deep epithelium. Rough endoplasmic reticulum (RER) and small mitochondria (M) are prevalent near the capsular border (C). (x 8,100)

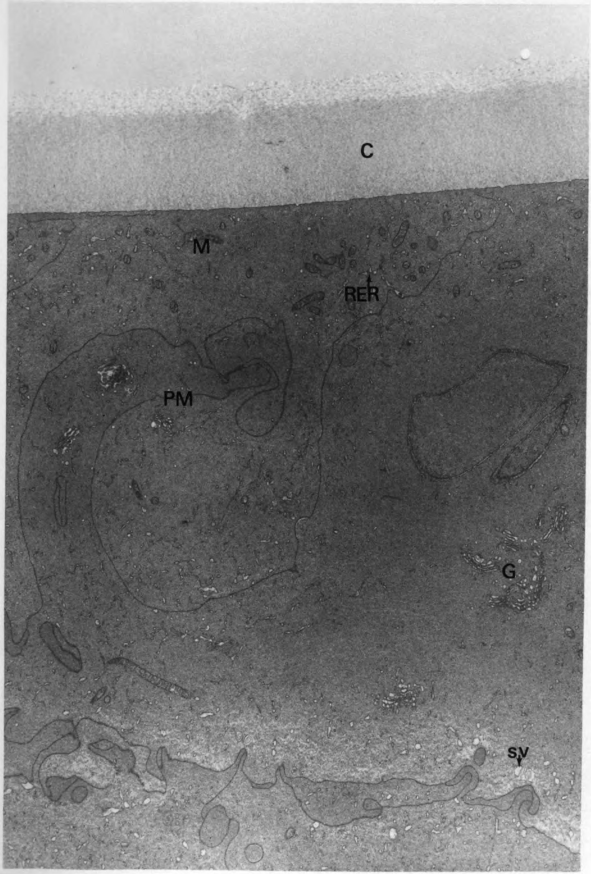

Figure 23

Figure 24. Higher magnification of Golgi's apparatus (G) as shown in Figure 23. Microtubules (mt), mitochondria (M), rough endoplasmic reticulum (RER), and clumps of free ribosomes (r) are irregularly scattered throughout the homogeneous cytoplasm. (x 31,500)

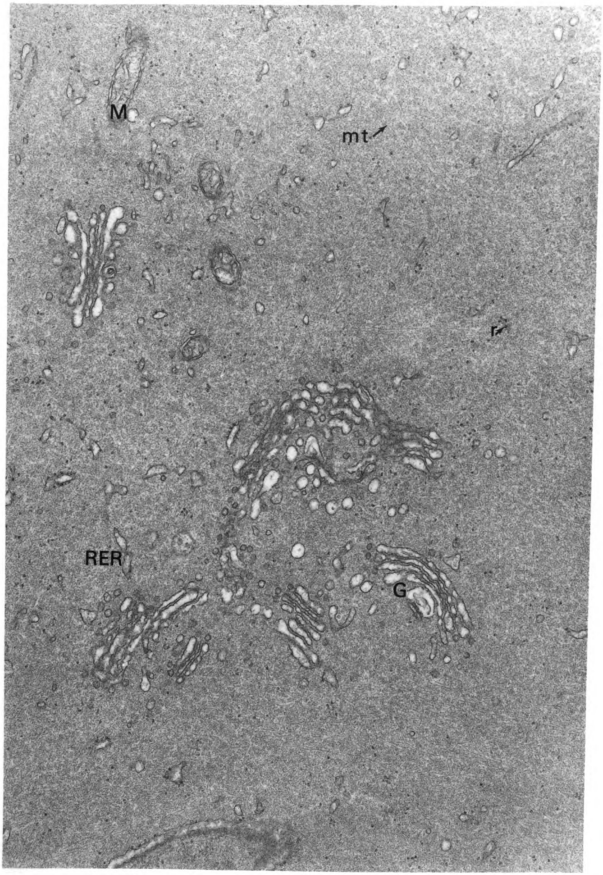

Figure 24

Figure 25. Higher magnification of Figure 23 showing a loss of cytoplasmic matrix in areas bordering the interdigitations of the deep epithelium. Pale areas in the cytoplasm and swollen vesicles (sv) probably represent early degenerative change. Free ribosomes (r) and microtubules (mt) are identified. Short segments of plasma membrane (PM) suggest interdigitations cut in another plane. (x 31,000)

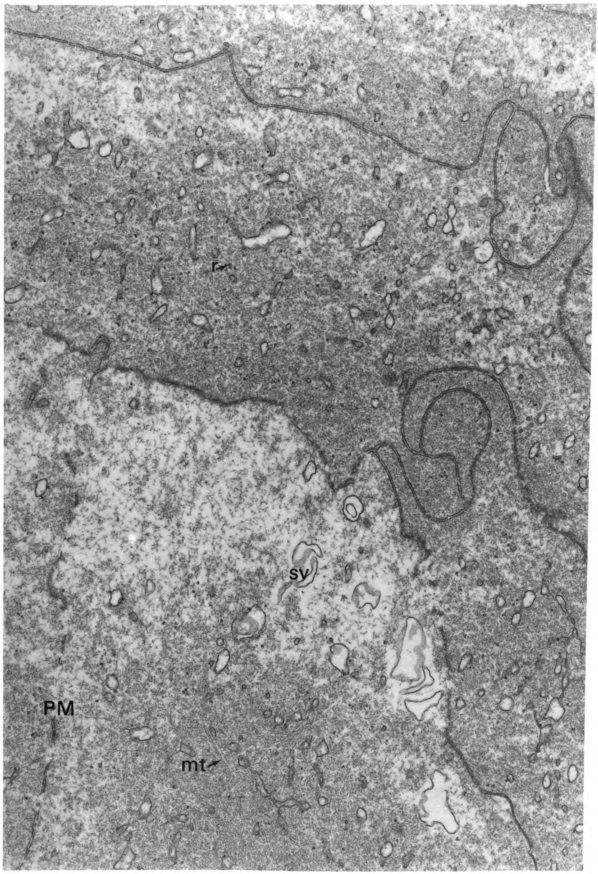

Figure 25

Figure 26. Central anterior capsule (C) and epithelium from a lens with a Grade IV cataract. Fibrillar strands of tissue with particles of pigment are adhered to the outer surface of the capsule. Relatively normal epithelial cells with variable density are shown. Cell nucleus (N), mitochondria (M), rough endoplasmic reticulum (RER), and plasma membrane (PM) are identified. (x 8,100)

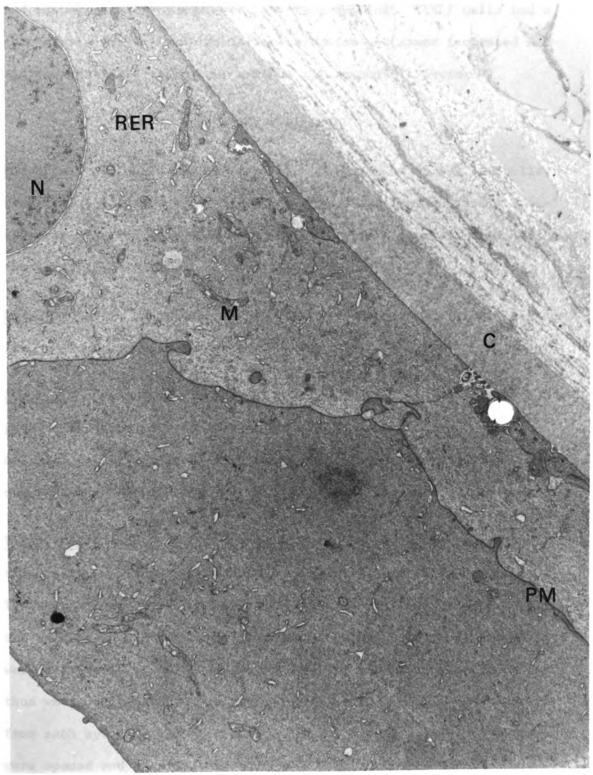

Figure 26

and in the same lens some cells appeared to be undergoing extensive degeneration. The cytoplasm in these cells was nearly devoid of matrix and the organelles present were severely swollen. Other cells had a more nearly normal cytoplasmic density which contained increased RER and comparatively infrequent swollen mitochondria (Figure 27).

Microbiology

Bacteria. No growth was obtained on blood agar or in the semisolid brain heart infusion (BHI) agar from any of the eyes cultured. Subcultures on blood agar made from the semisolid media were also negative for bacterial organisms.

Mycoplasma. No organisms were observed in any of the 3 weekly passages from enriched mycoplasma broth or agar media.

Fungi. No pathogenic fungi were observed. However, 3 different saprophytic organisms were identified in 3 of 8 eyes cultured. Based on mycelial morphology in direct mount preparations, the organisms were classified as *Penicillium sp.*, *Scopulariopsis sp.*, and *Cladosporium sp.*

Virus, Rickettsial and Chlamydial Agents. No embryonic deaths occurred that could be attributed to viral, rickettsial, or chlamydial agents. There were some early deaths (12 to 15 hours postinoculation) in both the yolk-sac and CA-membrane inoculation techniques. (These deaths were attributed to weakened embryos and the inoculation procedure and thus were not considered in the final interpretation.) Some embryos from each eye cultured remained viable for a week and, at that time, were opened and examined for lesions. No microscopic changes were observed in stained preparations of membranes from these eggs.

Figure 27. Central anterior capsule (C) and epithelium from a lens with a Grade IV cataract. Note the loss of cytoplasmic matrix and the swollen vesicles (sv) in cell toward the bottom of the picture. This cell appears to be undergoing an extensive degenerative change. The adjacent cell contains an abundance of rough endoplasmic reticulum (RER) and mitochondria are infrequent. (x 9,100)

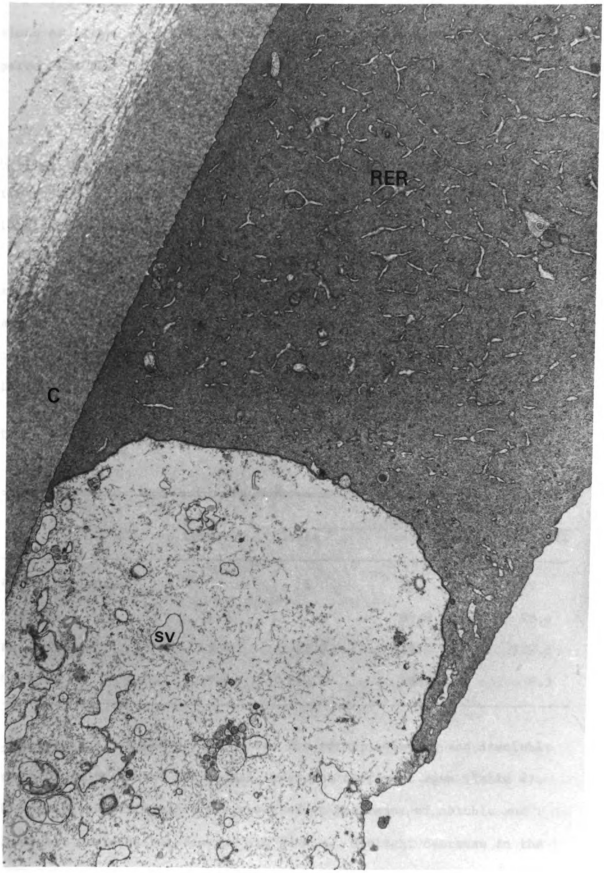

Figure 27

Cytopathogenic changes were not seen in either the daily examinations of tissue cultures or in the monolayers of stained cells prepared on cover slips.

Chemical Analyses

Cations (Na and K) and Water Content of Lenses. A summary of values for sodium, potassium, and water content in normal and cataractous lenses is presented in Table 7.

The mean values for sodium and potassium ions in normal lenses and Grade I cataracts were similar. Levels of these cations were markedly altered in Grade II and III cataracts. Sodium was significantly increased and potassium was significantly decreased ($P<.0005$) in both grades.

Table 7. Summary of lens HOH, Na, and K in normal and cataractous lenses

	Normal Lenses	Cataracts		
		Grade I	Grade II	Grade III
Number of lenses	7	11	10	12
% water	71.3	71.5	80.6	90.4
Na mEq/l.	15.4	17.9	166.7	172.3
K mEq/l.	119.4	119.1	19.7	9.3

Lens Proteins. A summary of values for total, soluble, and insoluble proteins is presented for normal lenses at different ages (Table 8). No significant difference was observed in the means of soluble and insoluble proteins between any age groups. A slight decrease in the mean total protein was observed between young quail (4 and 10 months)

and 2-year-old quail. A significant reduction ($P<0.05$) was noted in the mean total lens protein of 4-year-old quail when compared with young quail.

Table 8. Summary of total, soluble and insoluble proteins in normal lenses at different ages

	Average age of birds			
	4 months	10 months	2 years	4 years
Number of lenses	7	8	8	7
Total protein (µg/mg wet weight)	379.3	376.3	356.7	332.4
% sol. prot.	79.2	82.6	81.1	82.7
% insol. prot.	20.8	17.4	18.9	17.3

A summary of values for total soluble and insoluble proteins is presented for normal and cataractous lenses in aged quail (Table 9). A significant reduction ($P<0.0005$) was observed in all protein values when normal lenses and Grade III cataracts were compared. However, Grade II cataracts had a significantly higher ($P<0.05$) mean value for total protein when compared with normal lenses.

Glutathione. A summary of values for glutathione in normal and cataractous lenses is presented in Table 10. The mean values of glutathione were similar in normal lenses and Grade I cataracts. Values for Grade II cataracts varied widely from 0.5 to 4.1 micromoles per gram; however, there was a slight reduction in the mean value when compared with normal lenses. A significant reduction ($P<0.0005$) in the means of glutathione was observed when normal lenses and Grade III cataracts were compared.

Table 9. Summary of total, soluble and insoluble proteins for normal and cataractous lenses in aged quail

	Normal Lenses	Cataracts		
		Grade I	Grade II	Grade III
Number of lenses	7	8	8	6
Total protein (µg/mg wet weight)	332.4	366.4	399.1	176.1
% sol. prot.	82.7	85.0	80.5	57.6
% insol. prot.	17.3	15.0	19.5	42.4

Table 10. Summary of glutathione in normal and cataractous lenses

	Normal Lenses	Cataracts		
		Grade I	Grade II	Grade III
Number of lenses	6	10	8	8
Glutathione (µmoles/gm)	3.47	3.76	2.25	0.41

Serum Glucose. A summary of serum glucose levels for normal and cataractous quail is presented in Table 11. No significant difference was observed in the mean serum glucose levels of normal and cataractous quail.

Experiment I. The percentage incidence of cataracts in young quail fed a standard ration is presented in Table 12. Cataracts were not observed in quail less than 3 months of age. Grade I cataracts were identified at 3 months of age and the percentage increased with each subsequent examination. Grade II and Grade III cataracts were apparent at 7 and 10 months, respectively. The percentage of Grade II cataracts increased;

Table 11. Serum glucose levels in normal and cataractous quail

	Normal Quail	Quail with Cataracts
Number of samples	13	12
Serum glucose (mg/100 ml)	324	332

Table 12. Incidence of cataracts in young quail on the standard quail ration

Age	Total	Normal (%)	Grade I (%)	II (%)	III (%)	IV (%)
2 mo.	45	45 (*100*)	0	0	0	0
3 mo.	45	42 (*93*)	3 (*7*)	0	0	0
4 mo.	45	31 (*69*)	14 (*31*)	0	0	0
7 mo.	33	21 (*64*)	11 (*33*)	1 (*3*)	0	0
10 mo.	28	13 (*46*)	11 (*40*)	2 (*7*)	2 (*7*)	0
13 mo.	26	10 (*38*)	12 (*46*)	3 (*12*)	1 (*4*)	0

however, a decrease in the percentage of Grade III cataracts resulted from the death of one 10-month-old bird with this grade of opacity.

Lens opacification was progressive in some birds and comparatively stable in others. Some birds with Grade I cataracts at 4 months remained Grade I at 13 months of age. Other birds with Grade I cataracts at 4 months had developed Grade II or Grade III opacities by 13 months of age.

Experiment II. The incidence of cataracts in birds fed a fortified ration is presented in Table 13. The first evidence of lens opacity was seen in both groups at 3 months of age. There was a 30% incidence of cataracts

Table 13. Incidence of cataracts in young quail on a fortified ration at 2 population densities

Age	Total	Normal (%)	Cataracts		
			Grade I (%)	Grade II (%)	Grade III (%)
Group B P.D.=4*					
5 weeks	31	31 (*100*)	0		
2 months	31	31 (*100*)	0		
3 months	30	21 (*70*)	9 (*30*)		
4 months	30	16 (*53*)	14 (*47*)		
6 months	27	6 (*19*)	21 (*81*)		
9 months	19	3 (*16*)	16 (*84*)		
12 months	19	3 (*16*)	14 (*74*)	2 (*10*)	
Group b P.D.=1.5*					
5 weeks	11	11 (*100*)	0		
2 months	11	11 (*100*)	0		
3 months	11	10 (*91*)	1 (*9*)		
4 months	11	6 (*55*)	5 (*45*)		
6 months	10	3 (*30*)	7 (*70*)		
9 months	10	1 (*10*)	8 (*80*)	0	1 (*10*)
12 months	10	1 (*10*)	8 (*80*)	0	1 (*10*)

*Population density in birds/sq.ft. of floor space.

in the high population density group (HPD) at 3 months of age. The low population density (LPD) group had only a 9% incidence at the same age. The percentage incidence of cataracts was nearly the same by the end of the 4th month and remained comparatively similar for the balance of the experiment. Grade II cataracts were observed in the HPD group and Grade III cataracts were observed in the LPD group. The incidences, however, were comparatively low in both groups.

Experiment III. A summary of the incidence of cataracts in young quail in a natural light environment is presented in Table 14. Cataracts were not observed in quail from any of the 3 groups until birds were

Table 14. Incidence of cataracts in young quail in a natural light environment

Age	Commercial Ration Group p P.D.=3.3*	Commercial Ration Group P P.D.=8	Standard Ration Group R P.D.=5
2 months	0/11**	0/27	0/37
3 months	0/10	0/27	0/34
4 months	0/10	0/25	0/32
5 months	0/10	0/25	0/30
6 months	+1/10 (10%)	+2/25 (8%)	+1/29 (3.4%)

*Population density in birds/sq.ft. of floor space.

**Cataracts/normal birds.

+ = Grade I.

6 months of age. The percentage incidence of cataracts in 6-month-old birds was similar in both groups on the commercial ration. The percentage incidence was slightly lower in quail fed the standard ration. Those cataracts observed were all Grade I and higher grades did not occur in this experiment.

Comparative Incidence of Cataracts

A statistical comparison of the incidence of cataracts in Experiments I, II and III is presented in Table 15. This analysis was based on the percentage incidence of cataracts of each group of quail at 6 months of age.

A significant difference ($P<.001$) was observed between all birds in a continuous light environment when compared with birds in a natural light environment. A significant difference ($P<.01$) was also noted

Table 15. Statistical comparison of the incidence of cataracts in 6-month-old quail

		Subgroup	No. of Birds	No. of Cataracts
Continuous Light Group A	1	Std. ration	33	12
	2	Fort. ration	27	21
	3	Fort. ration	10	7
Natural Light Group B	4	Comm. ration	11	1
	5	Comm. ration	25	2
	6	Std. ration	29	1

Group Comparisons

A vs B (all quail, continuous vs natural light)	P<.001
1 vs 6 (continuous vs natural light, quail on std. ration)	P<.01
4&5 vs 6 (natural light, comm. vs std. ration)	P>50
1 vs 2&3 (continuous light, std. vs fort. ration)	P>5

when only those quail fed the standard ration were compared in both continuous and natural light environments.

No significant difference occurred in the incidence of cataracts when groups with different population densities or different rations were compared.

DISCUSSION

This study utilized an avian model, with naturally occurring cataracts, to provide a new approach to comparative cataract research. A great deal of effort has been expended in the study of cataracts in mammalian species and some pathogenic mechanisms have been identified. Data obtained from this investigation revealed that the basic physiologic and histopathologic changes which occurred in quail cataracts were similar to those seen in mammalian species.

Incidence of Cataracts

The absence of cataracts in birds less than 3 months of age suggested that quail had normal lenses when hatched and lens opacities developed sometime later. When cataracts were seen in young birds, they advanced with age and more of the higher grade cataracts occurred in older birds. This observation suggests a progressive and age-related pattern in the development of cataracts in Bobwhite quail.

A difference in the incidence of cataracts was noted at 3 months of age in 2 population densities (Experiment II). Subsequent examinations revealed a similar percentage at the different density levels. Greater numbers of birds per square foot were evaluated in Experiment III, and the occurrence of lens opacities was nearly identical. It was concluded that the influence of population density had little if any effect on cataract formation.

Three different rations were used in this study to evaluate the influence of nutrition in the cataractous process. A commercial ration was used in Experiment III to ascertain the presence of a cataractogenic agent in the standard or fortified rations. The occurrence of cataracts in quail fed each of the 3 diets indicated that lens opacities were not related to nutritional components.

Environment significantly influenced the percentage incidence and the age at which cataracts appeared in Bobwhite quail. The major difference in the 2 environments was the quality and quantity of light. Those birds exposed to continuous artificial light had greater numbers of cataracts at an earlier age than did those birds exposed to cyclic natural light.

Histopathology

The absence of extraocular lesions in cataractous Bobwhite quail was consistent with the general health of the birds studied.

Duke-Elder (1969) reported that regardless of the cause or type, the general pathologic changes of cataracts were similar. These consisted of degeneration of lens fibers, aberrant epithelial proliferation and alteration in capsule thickness. Changes in lens fibers and epithelium were observed in early opacities and all 3 processes occurred in advanced cataracts.

Histologic changes were progressive and appeared initially in the anterior polar cortex and epithelium. There was occasional simultaneous involvement of the posterior polar cortex with epithelial hyperplasia. It is believed that concomitant anterior and posterior polar opacification led to the most rapid formation of complete cataracts. Aged birds with Grade I cataracts seldom had posterior polar

involvement. This suggests that the difference in rate of progression was related to the bipolar involvement in initial opacification.

Cogan (1962) suggested that lens epithelium could be stimulated to proliferation by irritants, such as heat, ultraviolet light and capsular injury. The consistent occurrence of epithelial reactivity in quail cataracts suggests that an unknown irritant may have initiated lens opacification. The decreased incidence of cataracts in quail exposed to natural cyclic light implies that light, in some way, may be a potential cataractogenic irritant. Support for this consideration is provided by Lauber and McGinnis (1966), who reported that prolonged exposure to continuous light does induce ocular changes, including cataracts. Excessive light has also been shown to be cataractogenic in fish, as reported by Allison (1962) and Steucke *et al.* (1968).

Severe histopathologic alterations in Grade III and Grade IV cataracts were consistent with advanced degeneration of the lens as described by Cogan (1962) and Duke-Elder (1969). Adhesions of the iris and anterior capsule were always associated with lenses in the advanced stages of liquefaction necrosis. Capsular changes were frequently observed in Grade IV cataracts and altered permeability allowed the loss of fluid which resulted in shrunken lenses. Lens protein may also have escaped through the altered lens capsule. The presence of lens proteins in the anterior chamber may have initiated a mild uveitis and thus induced posterior synechia.

Electron Microscopy

A study of the central anterior lens capsule and epithelium of Bobwhite quail was conducted to establish the normal ultrastructural features of these tissues.

The central anterior capsule of quail lenses had a uniform granularity and lacked a prominent fibrillar pattern. This finding concurred with the studies of Jakus (1962) on the fine structure of lens capsules. She examined several mammalian species in which the anterior lens capsule had a comparatively homogeneous appearance centrally and a more pronounced fibrous structure near the equator.

The difference in lens capsule and epithelial nuclei at 2 weeks and 4 months of age was thought to be related to tissue maturity. Dukes (1955) reported that the water content of the animal body is at its highest concentration in the young and that a stage of decline occurs until a plateau is reached early in life. The change in capsular thickness with the flattening and loss of epithelial nuclei was probably a normal alteration associated with the maturation process of the lens.

Reddan *et al.* (1970) noted that epithelial injury evoked a sequence of events involving cellular elongation, migration and increased macromolecular synthesis. Lateral outfoldings of epithelium were particularly evident in Grade I cataracts and may have been indicative of early cellular proliferation.

Golgi's apparatus, seen in normal epithelium, was particularly prominent in cells undergoing early degeneration. Kuwabora *et al.* (1969) noted a similar prominence in proliferative epithelial cells of galactose-induced cataracts. The normal function of Golgi's apparatus is secretory and it forms lysosomes and enzymes including some which are autolytic. The increased prominence of this organelle may be an important factor in early cellular change leading to cataract formation.

The most significant ultrastructural alteration in the central anterior epithelium was decreased cytoplasmic density accompanied by swollen vesicles. This change was observed in Grade I cataracts but was most apparent in lenses with Grade IV opacity. There was a noticeable lack of uniformity in the extent to which individual cells were affected, and some relatively normal epithelium remained even in well advanced cataracts. Light microscopy revealed similar changes, in that individual or small groups of cells were altered, while other portions of epithelium appeared unchanged. These observations suggest that epithelial degeneration in quail cataracts is focal and individual cells are not uniformly affected.

Microbiology

Three different fungi were isolated from cataractous eyes; however, these were saprophytes and were considered common laboratory contaminants. Routine cultural procedures for bacteria and mycoplasma were negative for these classes of organisms.

Early postinoculation deaths in chick embryos were attributed to the inoculation procedure. No significant alterations were observed in other embryos inoculated and subcultures of all eggs resulted in no growth of microorganisms. In addition, cytopathogenic effects were not observed in tissue cultures, thus discounting the presence of viral, rickettsial or chlamydial agents.

These observations indicate that microorganisms were not involved in the etiology of cataracts observed in this study.

Chemical Alterations

The evaluation of proteins in normal quail at different ages was done to establish the distribution pattern of these substances in the

lens aging process. Relative proportions of soluble and insoluble protein remained similar and total protein gradually decreased with advancing age. These results differ from the protein distribution in normal aging rat lenses (Lerman, 1964). This difference is not remarkable in view of the variation in protein composition and metabolism between avian and mammalian lenses (van Heyningen, 1969).

Sturkie (1965) reported that domestic fowl had serum glucose values which were variable and approximately twice the level of most mammals. While high glucose values were obtained from normal quail used in this study, they were considered normal. The similarity of serum glucose values in normal and cataractous quail indicated that the cataracts formed were not metabolic in origin.

Chemical changes observed in quail cataracts were similar to those observed in man (Salit, 1938) and experimental animals (Moses, 1970). The marked increase in sodium and decrease in potassium was abrupt and occurred between the transition from cataractous Grades I to II. Water content of the lenses increased gradually in Grades II and III cataracts. Decreases in glutathione and alterations in lens protein were most apparent in Grade III cataracts. Total and soluble lens protein decreased while insoluble protein increased with complete lens opacity. Grade IV cataracts could not be chemically analyzed due to the wide variation in degenerative changes and the loss of lens substance.

The sequence of chemical changes in quail cataracts parallels the morphologic alterations. Minor histologic changes occurred with focal opacities in Grade I cataracts and no chemical changes were noted. Grade II cataracts had greater tissue damage and as cellular degeneration progressed there was a marked change in cation levels. Grade III cataracts were extensively altered morphologically and similarly there

were pronounced chemical changes in levels of glutathione, total, soluble and insoluble proteins. Increased water content with each grade of opacity corresponded to the progression of liquefaction necrosis.

Ultrastructural alterations were also consistent with chemical changes. In the early stages of lenticular opacity the apparent increase in cytoplasmic organelles was thought to be a proliferative response of injured epithelial cells. The lack of chemical change suggests that metabolic alterations were subtle and not detectable by the analytic procedures used. Grade IV cataracts had numerous swollen vesicles and some cells appeared to have lost considerable cytoplasmic matrix. These degenerative alterations are frequently associated with cellular death and lethal metabolic disturbance. Thus the extensive chemical changes seen in advanced cataracts were compatible with the ultrastructural alterations.

The alteration in proportion of soluble and insoluble protein was related to opacification. Normally the lens has a high percentage of soluble protein which is essential to lens transparency (van Heyningen, 1969). Degeneration of lens fibers resulted in conversion of protein from a soluble to an insoluble form. The accumulation of insoluble protein results in opacification and the formation of cataracts.

SUMMARY

This investigation was conducted to characterize the pathologic and physiologic alterations of spontaneous cataracts in Bobwhite quail. Birds 1-1/2 to 4-1/2 years of age were examined clinically and cataracts were graded on the extent of lens opacity. Cataractous lenses from these birds were then chemically analyzed for water content, sodium and potassium ions, glutathione, and soluble and insoluble proteins.

Three experiments were conducted on young quail and the incidence of cataracts was recorded for each study. The influence of diet, population density and environment was evaluated.

Morphologic changes in the cataracts of young and aged quail were examined by slit lamp, light, and electron microscopy. Microbiologic studies were also conducted on quail in both age groups to identify or eliminate microorganisms as etiologic agents in cataract formation.

The first change noted in cataract formation was focal degeneration in the anterior polar cortex and epithelium. There was occasional simultaneous involvement of the posterior polar cortex with epithelial hyperplasia. Histopathologic changes were progressive and resulted in lens liquefaction with epithelial proliferation and thickening of the capsule in advanced cataracts.

Ultrastructural alterations in the central anterior epithelium of cataracts consisted of increased outfoldings of the cell membranes and early degeneration of cytoplasmic organelles.

Chemical changes of cataracts paralleled morphologic alterations and decreases were seen in the potassium ions, glutathione, total and soluble proteins. Sodium ions, water content, and insoluble protein increased with lens opacification.

Lens opacities could not be attributed to congenital causes, metabolic disturbances, or infectious microorganisms. Population density and nutrition did not affect the incidence of cataracts, but environment significantly influenced cataract formation. The primary difference in the 2 environments studied was the type and duration of light exposure. Those birds exposed to continuous artificial light had greater numbers of cataracts at an earlier age than did those birds exposed to cyclic natural light.

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APPENDICES

APPENDIX A

Protocol for Na and K Determination and Water Content

1. Birds were euthanatized by decapitation and globes were removed from orbits.

2. The posterior 1/2 of the globe was removed by incising the sclera circumferentially.

3. The vitreous was removed to expose the posterior portion of the lens.

4. A round point spatula was inserted between the lens and the zonular fibers, which were broken by blunt dissection.

5. The lens was then removed from its normal position in the globe and placed on filter paper.

6. The lens was rolled on the paper to remove excess tags of vitreous and to attain a uniform dryness.

7. The lens was then placed in a preweighed 10-ml. Erlenmeyer flask which had been chemically cleaned. The flask was covered with aluminum foil.

8. The flask and lens were then weighed and wet weight recorded.

9. The flask containing the lens was then placed in a drying oven and maintained at 105 C. for 48 hours to achieve a uniform dryness.

10. After the drying period was completed the flasks with the lenses were removed and placed immediately into a desiccator to dry.

11. Flasks and lenses were reweighed when cool and a dry weight obtained.

12. The dry remains of the lens and capsule were digested with 0.75 ml. of 1:3 dilution of concentrated HNO_3. This digestion process was carried out on a hot plate at approximately 80 to 100 C. for approximately 1 hour.

13. Two standards and 2 controls were prepared with each group of lenses and were treated with HNO_3 in the same manner as the test samples. Standards and controls contained a known amount of Na and K in 50 lambdas of HOH.

14. After digestion 10 ml. of lithium standard were added to each flask and the amount of Na and K was determined by flame photometry utilizing an IL Model 143 digital Na/K flame photometer.

15. The average of 2 readings was used for computations. Values obtained were expressed in milliequivalents and were converted to mEq/liter of lens water by computation with the percent water in each lens.

16. Percent water content was calculated by utilizing wet and dry lens weights.

APPENDIX B

Protocol for the Determination of Lens Protein

1. Birds were euthanatized by decapitation and globes were immediately removed from the orbits.

2. The lens was removed from each globe with the capsule remaining intact.

3. The lens was rolled on filter paper until dry and then weighed.

4. The lens was placed into a tissue grinder and ground for approximately 5 minutes with 2 ml. of double-distilled water.

5. The homogenate was removed and placed into a 12-ml. plastic highspeed centrifuge tube (International Equipment Corporation).

6. The tissue grinder was then rinsed with 2 ml. of double-distilled water.

7. This rinse was then added to the homogenate in the centrifuge tube.

8. An additional 2 ml. of double-distilled water were used to rinse the tissue grinder and this was also added to the homogenate in the centrifuge tube. (A total of 6 ml. double-distilled water were used for each lens.)

9. The homogenate was then centrifuged at 12,000 rpm for 15 minutes at 4 C. (12,000 x g).

10. Three milliliters of the centrifuged supernate were then removed for analysis of soluble protein.

11. The remaining supernate was discarded and the insoluble protein remaining in the bottom of the centrifuge tube was used for analysis of insoluble protein.

12. The analysis of soluble and insoluble protein was then performed (Lowry *et al.*, 1951). Bovine serum albumin was used as a standard and all analyses were run in duplicate.

APPENDIX C

Protocol for the Determination of Lens Glutathione

1. Birds were euthanatized by decapitation and globes were immediately removed from the orbits.

2. The lens was quickly removed from each globe with the capsule remaining intact.

3. The lens was rolled on filter paper until dry and then weighed.

4. The lens was then homogenized in 1 ml. of ice-cold 5% sulfasalicylic acid.

5. The homogenate was then centrifuged at approximately 1000 x g for 10 minutes.

6. Five milliliters of 0.5 molar phosphate buffer* were added to 1 ml. of supernate.

7. The supernate and buffer were then incubated at 60 C. for 5 minutes.

8. Samples were then quickly cooled to room temperature and 1 ml. of Ellman's reagent** was added.

9. Samples were then read with a Beckman Spectrophotometer (DU-2400) at 412 mμ and results were expressed as micromoles of sulfhydryl per gram of wet tissue.

10. One normal rat lens was analyzed with each group of quail lenses. This served as a reference control for each series of determinations.

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*To each liter of phosphate buffer (Gradwohl's) 1 gm. of ascorbic acid and 0.5 gm. of glyoxylic acid were added and a final pH of 6.8 was established. Ascorbic acid was added to the phosphate buffer a few minutes prior to each series of determinations in an attempt to minimize the extent of oxidation and color change in the buffer.

**150 mg. DTNB (5,5' dithiobis(2-nitro)benzoic acid) in 100 ml. phosphate buffer (Ellman, 1959).

APPENDIX D

Protocol for Microbiologic Techniques

I. Bacteria, Mycoplasma and Fungi

1. Intact globes were removed from quail and placed in 0.5% Dakin's hypochlorite solution for 5 minutes (Parker, 1961).

2. The globe to be cultured was placed on top of a small metal tube which was connected to a vacuum line and held in a vertical position. The globe was held rigid by placing the corneal surface on top of the metal tube and turning on the vacuum.

3. The posterior 1/3 of the globe was removed with sterile scissors.

4. Sterile cotton swabs were introduced into the interior of the globe and portions of the vitreous, aqueous and lens material were obtained.

5. A blood agar plate was streaked with the first swab and it was then placed into semisolid brain heart infusion (BHI) agar media (Difco Manual, 1965).

6. An enriched mycoplasma agar plate was streaked with a second swab and it was then placed in enriched mycoplasma broth (Allen *et al.*, 1967).

7. A Sabouraud's (Difco Manual, 1965) plate was streaked with a third swab in an attempt to isolate potentially pathogenic fungi.

II. Viruses, Rickettsial and Chlamydial Agents

A. Tissue culture

1. Eyes from another group of quail were removed and aseptically cultured. One swab was placed in 1 ml. of BHI broth and a second swab was placed in antibiotic-enriched Hanks balanced salt solution (HBSS) with lactalbumin hydrolysate (Kalter, 1963). These samples were subsequently frozen and held for tissue culture and chick embryo inoculation.

2. A cell culture medium was prepared from trypsinized 12-day-old chick embryos as outlined by Kalter (1963).

3. Approximately 1.5 ml. of the prepared cell suspension were placed in tissue culture test tubes containing a 5 x 12-mm. cover slip.

4. After a monolayer of cells was obtained, each of the tubes was inoculated with 0.2 ml. of the enriched HBSS with lactalbumin hydrolysate in which an intraocular swab had been placed.

5. Cell monolayers in the inoculated tubes were examined daily for cytopathogenic effects and on the fifth day cover slips were removed, fixed in Bouin's solution and stained with Giemsa's stain for microscopic examination.

B. Chick embryo inoculation

1. Samples of the frozen BHI inoculum from each eye of the same bird were pooled and 4 eggs were inoculated for each pair of eyes cultured.

2. Eight-day-old embryos were inoculated by the yolk sac technique and 12-day-old embryos were used for the chorio-allantoic (CA) membrane inoculations (Kalter, 1963).

3. Each egg was inoculated with 0.1 to 0.2 ml. of inoculum and control eggs were used with each batch of eggs inoculated.

4. Daily postinoculation candlings were done and embryos that died within 24 hours of inoculation were discarded and not considered in the final evaluation.

5. Yolk sacs and CA membranes were collected from inoculated eggs for a second passage by the same technique.

6. Yolk sac smears were made and stained with Macchiavello's stain (Thompson, 1966) for microscopic examination.

VITA

VITA

The author was born in Pretty Prairie, Kansas, on April 17, 1936. He received his primary and secondary education in the public schools of Pretty Prairie, Kansas. After graduation from high school in 1954 he continued his education at Bethel College, North Newton, Kanasa. In 1956 he transferred to Kansas State University and entered the College of Veterinary Medicine in September 1958.

He received his D.V.M. degree in June of 1962 and entered small animal practice in association with Dr. R. L. Russell in Mesa, Arizona. In August 1963 he moved to El Cajon, California, where he was in general practice, associated with Drs. W. J. Dedrick and A. L. Davis.

In 1964 he entered the U.S. Air Force and served as base veterinarian for 2 years at Andersen Air Force Base, Guam. During his tour of duty on Guam he also provided veterinary service to the Department of Agriculture on the island.

After completing his military service he joined the Department of Small Animal Surgery and Medicine at Michigan State University. While serving in that department he entered the graduate school, majoring in pathology. He received his M.S. degree in pathology in 1968.

He served as an instructor in the Department of Small Animal Surgery and Medicine for 3 years and then taught for a year in the Department of Pathology, Michigan State University.

The author was the recipient of a Special Postdoctoral Fellowship from the National Eye Institute in the National Institutes of Health.

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The author married Linda Jean Bunch in 1963 and they have three children: Douglas Jan, 7, Carrie Ladell, 4, and Keith Bryan, 6 months.


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