Glycohistochemical, Immunohistochemical and Electron Microscopic Examination of the Bovine Eyeball

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A thesis
submitted for the
doctor degree in veterinary medicine
Faculty of Veterinary Medicine
Ludwig-Maximilians-University Munich

From
Khaled Aly
Assiut-Egypt

Munich 2003

Gedruckt mit Genehmigung der Tierärztlichen Fakultät der
Ludwig-Maximilians-Universität München

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Tag der Promotion: 18. Juli 2003

To My Wife Riham

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1 INTRODUCTION

Ophthalmology is an important and recognized discipline of veterinary medicine and ocular examination is important in most clinical examinations.

Understanding of ocular disorders is based on knowledge of normal ocular structure and physiology. In my investigation on normal structure of bovine eyes I used advanced light (glycohistochemistry, immunohistochemistry) and ultrastructural methods to get a more detailed picture of the morphology of this important bovine sense organ. The main aim of the present work is to provide new glycohistochemical and immunohistochemical data that may help to explain some of the cellular functions of bovine eye and can provide a better understanding of cellular changes of ophthalmic disorders.

2 REVIEW OF LITERATURE

2.1 Development of the bovine eyeball

The embryonic material of the eye comes from three sources: (1) the optic nerve and retina are derivatives of the fore-brain; (2) lens arises from the surface ectoderm of the head; and (3) the accessory tunics, which provide support, nutrition and accommodation and differentiate from adjacent mesenchyme (Arey, 1965; Patt and Patt, 1969; Hopper and Hart, 1985; Noden and Lahunta, 1985; Rüsse and Sinowatz, 1998; Cook, 1999; Slatter, 2001a).

The neural part of the eye first appears early in the third week as a pair of optic grooves (shallow pits) on either side of the midline at the expanded cranial end of the still-open neural folds. As the neural folds close, the eyes begin their development in ontogenesis from bilateral outpocketings of the forebrain named optic vesicle (Arey, 1965; Patt and Patt, 1969; Hopper and Hart, 1985; Noden and Lahunta, 1985).

Intracellular filaments and microtubules within the cytoskeleton of the optic vesicle alter cell shape and allow cell movement. In addition to the mechanical influences of cytoskeleton and extracellular matrix, localized proliferation and cell growth contribute to expansion of the optic vesicle (Cook, 1999).

The optic vesicle in the developing bovine eye is distinct at the 6-mm CRL stage (Bistner et al., 1973). The invagination that converts the optic vesicle into the optic cup does not occur in the exact center but is also extended to the midventral line (Hopper and Hart, 1985). The optic vesicles become indented along their lateral and ventral surface vesicle, brought about by rapid, marginal growth. The result is a doubled-layered cup connecting to the diencephalon by a tubular optic stalk (Arey, 1965; Patt and Patt, 1969; Noden and Lahunta, 1985; Rüsse and Sinowatz, 1998; Cook, 1999; Slatter, 2001a)

The opening of the eye-cup is very large at first; fusion of the edges proceeds out over the optic cup until only a uniformly round opening is left.

It is then constricted and reduced to its final relative dimensions. The rim of the eye-cup later becomes the edge of the pupil (Patt and Patt, 1969; Balinsky, 1970; Noden and

Lahunta, 1985; Hopper and Hart, 1985; Rüsse and Sinowatz, 1998; Cook, 1999; Slatter, 2001a).

The optic cup is imperfect from the beginning, because of a notch in its double wall. This defect is brought about by the original invagination involving also the underside of the cup and then continuing as a groove that extends along the optic stalk. The complete defect comprises the optic fissure (choroid fissure). Blood vessels that develop in the nearby mesenchyme form the hyaloid artery, which enters the optic fissure to supply the inner surface of the optic cup and the lens. The hyaloid vein drains this area. These vessels provide an intraocular vascular system for the developing eye. However, this system atrophies completely later, and new intraocular supply develops. The fissure normally closes during embryonic life (Arey, 1965; Balinsky, 1970; Hopper and Hart, 1985; Noden and Lahunta, 1985; Cook, 1999; Slatter, 2001a).

The edges of the grooved optic stalk soon come together and fused, making a tube that later will serve as sheath for the optic nerve, and fibers of the optic nerve grow back from the retina to the brain (Arey, 1965; Patt and Patt, 1969; Noden and Lahunta, 1985; Rüsse and Sinowatz, 1998; Cook, 1999; Slatter, 2001a). The accessory coats (vascular and fibrous) of the eyeball organize from the surrounding mesenchyme. During the seventh week the mesenchyme surrounding the optic cup begins to specialize into two accessory coats. The outer one is the more compact and becomes a definitely fibrous tunic, the sclera and cornea. The inner, looser covering organizes into the vascular choroid; it also contributes the ciliary body and iris (Arey, 1965; Patt and Patt, 1969; Hopper and Hart, 1985; Slatter, 2001a).

2.1.1 Development of the cornea

The remaining ectoderm of the skin after separation of the lens vesicle is transparent and differentiates into the corneal epithelium (Patt and Patt, 1969; Rüsse and Sinowatz, 1998; Slatter, 2001a).

The surface ectoderm overlying the optic cup (i.e. the presumptive corneal epithelium) secrets a thick matrix, the primary stroma (Hay, 1980). Loosely arranged mesenchyme fills the future anterior chamber. It gives rise to corneal endothelium and stroma. Type I

collagen fibrils and fibronectin are secreted by the developing keratocytes from secondary corneal stroma. Subsequent dehydration results in a considerable loss of the fibronectin and in 50% reduction in stromal thickness (Allen et al., 1955; LeDouarin and Teillet, 1974).

2.1.2 Development of the sclera

The more superficial layer of the mesenchyme surrounding the optic cup becomes densely fibrous and differentiates into the sclera of the eye, a tough, thin, opaque layer of very high tensile strength. (Arey, 1965; Patt and Patt, 1969; Hopper and Hart, 1985; Rüsse and Sinowatz, 1998; Slatter, 2001a). Fibrous, scleral tunic extended around the bovine eye by the 58-mm CRL (Bistner et al., 1973).

2.1.3 Development of the choroid

The choroid is the inner of two primary, mesenchymal tunics of the eyeball. It is located between the sclera and pigment layer of the retina. The choroid primordium acquires a high vascularity in embryos as young as six weeks; moreover, its cell becomes satellite and pigmented, so that the tissue is loose and spongy (Arey, 1965; Patt and Patt, 1969; Hopper and Hart, 1985; Rüsse and Sinowatz, 1998; Slatter, 2001a). Pigment in the choroids of bovine eye is seen in the 90-mm CRL fetus (Bistner et al., 1973).

2.1.4 Development of the iris and ciliary body

The part of uvea that lies at the rim of the optic cup differentiates into ciliary muscle, ciliary processes, and the outer layer of the iris (Arey, 1965; Patt and Patt, 1969; Hopper and Hart, 1985).

The two layers of optic cup consists of an inner, nonpigmented layer and an outer, pigmented layer. Both the pigmented and non-pigmented epithelium of the iris and ciliary

body develop from the anterior aspect of the optic cup; the retina develops from the posterior optic cup.

The iris stroma originates from the anterior segment of mesenchymal tissue (that is neural-crest in its origin). The pigmented and non-pigmented epithelium of the iris originates from the neural ectoderm of the optic cup. The smooth muscle of the pupillary sphincter and dilatator muscles ultimately differentiate from these epithelial layers, and they represent the only mammalian muscles of neural ectodermal origin. Differential growth of the optic cup epithelial layers results in folding of the inner layer, representing early, anterior ciliary processes. The ciliary body epithelium develops from the neuroectodem of the anterior optic cup. The underlying mesenchyme differentiates into the ciliary muscle. Extracellular matrix secreted by the ciliary epithelium develops into lens zonules (Arey, 1965; Balinsky, 1970; Hopper and Hart, 1985; Rüsse and Sinowatz, 1998; Cook, 1999; Slatter, 2001a).

The margin of the bovine optic cup begins to grow forward at the 33-mm CRL and becomes the double-layered posterior iridal surface. From this neuroectodermal tissue the sphincter and dilatator muscles originate; mesenchymal tissue gives rise to the iris stroma.

The bovine ciliary body can be divided posteriorly into the orbiculus ciliaris and pars ciliaris retinae. The ciliary processes become distinct at the 125-mm CRL and they touch the equator of the lens by 230-mm CRL (Bistner et al., 1973).

The pars iridica retinae plus the mesoderm associated with it, differentiate into the iris. A thin layer of mesoderm adherent to the outer layer of the pars iridica retinae extends beyond the inner margin of the iris over the surface of the lens forming the pupillary membrane. This membrane is resorbed before birth. Smooth muscle fibers develop outside the pars iridica retinae in the mesenchyme covering it. Eventually, they differentiate into dilatator and constrictor muscles of the iris. They have long been considered to be formed from the outer layer of the pars iridica retinae rather than from overlying mesoderm. The sphincter muscle forms a group of muscle cells close to the margin of the iris, and the dilatator muscles consist of radially oriented muscle cells closer to the ciliary body (Hopper and Hart, 1985).

2.1.5 Development of the retina

The optic cup is destined to become the retina or the essential sensory lining of the eye. The invaginated wall of the optic cup is much thicker than the remaining external wall. The first develops into sensory retina of the eye, the second into pigmented coat of the retina (tapetum nigrum) (Arey, 1965; Patt and Patt, 1969; Balinsky, 1970; Noden and Lahunta, 1985). The invagination of the optic cup in bovine eye is evident by the 6-mm CRL (Bistner et al., 1973). The inner layer of the optic cup transforms into the retina, while the rim of the optic cup represents the border of the future iris. The circular opening into the cup is the primitive pupil.

The combined layer of the early retina soon shows two zonal regions: (1) the thicker pars optica, a truly nervous portion that lines most of the cup; (2) the thinner pars caeca, a zone bordering the rim of the cup. The line of demarcation between these two regions makes a wavy circle and is called ora serrata. The ora serrata in bovine eye is present by 180-mm CRL and the orbiculus ciliaris is distinct at the 200-mm CRL (Bistner et al., 1973).

The outer thinner component of the optic cup becomes an epithelium known as the pigmented layer of the retina, but in the iris it also gives rise to the pupillary muscles (Arey, 1965; Hopper and Hart, 1985; Rüsse and Sinowatz, 1998; Cook, 1999; Slatter, 2001a).

The internal thick layer of the optic cup differentiates mostly into photoreceptive and impulse-transmitting neurons. In the pars optica, or nervous portion of the retina, this differentiation begins near the optic stalk. An outer nucleated layer and inner clear layer can be distinguished in 12 mm CRL embryos. These correspond to the cellular layers (ependymal and mantle) and marginal layer of the neural tube respectively. At two months the retina shows three strata. The neuroblasts (including early ganglion cells) in the meantime have migrated inwards from the outer neuroblastic layer. In a fetus of six months all the layers of the adult retina can be recognized, also the developing, photoreceptive rods and cones (Arey, 1965; Hopper and Hart, 1985; Cook, 1999; Slatter, 2001a).

Bruch's membrane (i.e., the basal lamina of the retinal pigmented epithelium) is first seen during this time, and becomes well developed when the choriocapillaries ~~is~~ developing. After that the retinal pigmented epithelium cells take a hexagonal cross-sectional shape and develop microvilli that interdigitate with projections from photoreceptors of the nonpigmented layer of the optic cup.
Retinal ganglion cells develop first within the inner neuroplastic layer, and axons of the ganglion cells collectively form the optic nerve. Cell bodies of the Müller and amacrine cells differentiate in the inner portion of the outer neuroblastic layer. Horizontal cells are found in the middle of this layer. The bipolar cells and photoreceptors mature last (Arey, 1965; Spira and Hollenberg, 1973; Hopper and Hart, 1985; Rüsse and Sinowatz, 1998; Cook, 1999; Slatter, 2001a).

2.1.6 Development of the optic nerve

Nerve fibers that arise from ganglion cells covering radially to a point where the optic stalk leaves the cup, and growing back in the tissue of its inner tube toward the brain. The cells of the optic stalk convert into a scaffolding of neuroglial supporting tissue, and the canal in the stalk is rapidly obliterated. The optic stalk is thus transformed into the so called optic nerve, containing the central artery and vein which originally coursed along the open groove of its optic fissure. Their branches will vascularize the neural retina (Arey, 1965; Rüsse and Sinowatz, 1998; Slatter, 2001a).
Axons from the developing ganglion cells pass through vacuolated cells from the inner wall of the optic stalk. A glial sheath forms around the hyaloid artery. As the hyaloid artery regresess, the glial sheath becomes enlarged. Bergmeister's papillae represent a remnant of this glial cells are found around the hyaloid artery.
Glial cells migrate into the optic nerve and form the primitive optic disc. The glial cells around the optic nerve and the glial part of the lamina cribrosa come from the inner layer of the optic stalk, which is one of the neuroectodermal origins. Later the mesenchymal portion of the lamina cribrosa develops. Myelinization of the optic nerve begins at the optical chiasm, progresses toward the eye, and reaches the optic disk after birth (Cook, 1999).

In the central part of the bovine retina, a distinct nerve fiber layer is seen in the 20-mm CRL embryo, and a distinct optic nerve in the 24-mm CRL embryo. An inner plexiform layer and retinal vasculature in the posterior pole is evident by 180-mm CRL, and by 410-mm CRL all layers of the retina are seen, although rods and cones were still developing (Bistner et al., 1973).

2.2 Macroscopical anatomy of the bovine eyeball

The eyeball encloses several compartments, containing refractive media, and various adnexa. The latter are accessory structures such as the ocular muscles that move the eyeball, the lid that protect it, and the lacrimal apparatus that keeps its exposed parts moist. Most of these are housed in the orbit where the eyeball is embedded in generous quantities of fat.

The position in the head is related to the animal's environment, habits, and method of feeding. In general, predatory species for example (cat, dog) have eyes set well forward, whereas those that are hunted (herbivores; like horse, ruminants, rabbits) carry their eye more laterally. The former position of the eyes provides a large field of binocular vision that allows concentration on near objects and for perception of depth (Dyce et al., 1987).

The eyeball of the domestic mammals is nearly spherical but with some anterioposterior compression in horse and in cattle located in a bony orbit. The bovine bony orbit is larger than the equine, yet having a much smaller globe giving more room for any surgical invasion of the orbit (Prince et al., 1960).

The eyeball is a slightly asymmetrical sphere, somewhat flattened from top down. The central points of the corneal and sclera curvatures are termed the anterior and posterior poles, and the line joining them is the geometrical axis. This must not be confused with optic or visual axis, which is the line joining the centre of the pupil and the fovea, the latter being the spot of most distinct vision. The posterior pole of the geometrical axis lies between the fovea and optic papilla (Leeson and Leeson, 1970).

The average diameter of the globe in a fully grown bovine is 34 to 37 mm anteroposteriorly, 37 to 42 mm vertically, and 38 to 43 mm transversely (Prince et al.,

1960). The anteriorposterior diameter of the eye is about 24 mm, being slightly greater in the male than in the female. It is about 16 to 17 mm at birth, and increases postnatally rapidly in size. At age of thirteen it is of adult size (Leeson and Leeson, 1970).
The eyeball consists of three layers: (1) outer fibrous tunic, which can be subdivided into (a) sclera, the white tough posterior portion of the eyebulb, and (b) cornea, the transparent portion of the fibrous tunic, which bulges slightly in the centre of the rostral pole of the eye, (2) middle vascular tunic, composed of (a) choroid, (b) ciliary body and (c) iris; and (3) inner nervous tunic of the eye or retina, with (a) an optic portion containing sensory receptors and (b) a blind portion that is epithelial in nature and covers the ciliary body as well as and the iridial posterior surface (Prince et al., 1960; Dyce et al., 1987).
The anterior compartment is filled with aqueous humor and is located between cornea and vitreous body. It is further subdivided into (1) anterior chamber located between cornea and iris and (2) posterior chamber between iris and vitreous body. The posterior compartment of the eye, located between the lens and retina, is filled with the vitreous body (Dellmann and Brown, 1976).

2.2.1 Outer fibrous tunic

The fibrous tunic of the eyeball is made up of very dense collagenous tissue which protects the delicate inner structures of the eye and, together with the intraocular fluid pressure, serves to maintain the shape and turgor of the eyeball.

2.2.1.1 Cornea

The cornea forms the anterior 1/6 of the fibrous tunic of the eye. It is transparent, permitting the rays of light to enter (Bloom and Fawcett, 1970 and Leeson and Leeson, 1970). The functions of the cornea include support of intraocular contents, refraction of the light (because of its curvature), and transmission of the light (because of its transparency).

The cornea has a horizontal measurement of 27 to 32 mm and a vertical dimension of 22 to 24 mm. The average difference between the meridians is about 5 mm. It varies slightly in thickness (from 0.75 to 0.85 mm) (Prince et al., 1960). Corneal thickness varies also from species to species, from breed to breed, and from individual to individual. In most domestic animals, it is less than 1 mm. In the bovine, it is 1, 5-2 mm thick centrally and 1, 5-1, 8 mm in the periphery (Samuelson, 1999).

The transparent cornea acts as a convex-concave lens, thicker at periphery than the center, and with a smaller radius of curvature centrally than peripherally. Because it also has a smaller radius than the sclera, it is more curved than the sclera. The normal cornea is completely devoid of blood vessels. It receives nutrients by diffusion from the blood vessels located in the area of transition, where the sclera overlaps the cornea rostrally. This region is called limbus (Dellmann and Brown, 1976).

The surface of the cornea is very sensitive owing to the presence of free nerve endings near the anterior epithelium. These arise from long ciliary nerves, branches of the ophthalmic nerve. Their axons form the afferent limb of the corneal reflex which closes the lids, when the cornea is touched (Dyce et al., 1987).

The cornea is elliptical in shape, with a horizontal diameter greater than the vertical. In most ungulates, the differences between these diameters is much more pronounced, allowing a remarkable horizontal field of view that is further complemented by the lateral positioning of their orbits within their skulls. The combination of the exaggerated corneal dimensions and orbital positions in these grazing animals appears to be the adaptive result of their feeding behaviour, affording them greater protection from predacious enemies (Samuelson, 1999).

The anatomic factors that contribute to the transparency of the cornea are: (1) lack of blood vessels, (2) nonkeratinized surface epithelium maintained by a preocular moisture film, (3) lack of pigmentation and (4) size and organization of stromal collagen fibrils. In addition, physiologic factors such as state of hydration are important (Dyce et al., 1987 and Samuelson, 1999).

2.2.1.2 Sclera

The sclera is a white, tough layer of dense connective tissue which forms the posterior 5/6 of the fibrous tunic (Leeson and Leeson, 1970) that protects the eye and maintains its form. The tendons of extrinsic muscles are attached to the sclera. Nerve fibers and blood and lymph vessels pierce the sclera at various locations. The site where the optic fascicle penetrates the sclera is referred to as the lamina cribrosa sclerae. It is a sieve-like area composed of a network of reticular, collagen and elastic fibers. Through its meshes the nerve fibers of the optic fascicle pass (Dellmann and Brown, 1976; Tortora and Anagnostakos, 1981; Dyce et al., 1987).

The sclera is quite thin at the equator (1 mm), thicker at the corneoscleral junction (1.2 to 1.5 mm) and at the posterior pole (2 mm). Its colour varies from one animal to another, from almost white to light greenish or grey. Much of the coloration is due to a pigmentation of the episclera and the presence of many chromatophores within the sclera. Although these chromatophores become denser nearer to the choroid, it is certain that the choroidal pigment shows through them and contributes to the blue tint of the sclera, especially where the sclera is thinner.

The bovine sclera has areas of varied strength where the muscles are inserted and the blood vessels penetrate the globe (Prince et al., 1960; Bloom and Fawcett, 1970; Samuelson, 1999).

The region of the intrascleral venous plexus is the thickest area in animals with a well developed plexus like dog and cat, whereas in ungulates, the region of the optic nerve entrance or posterior pole is the thickest. At the point where the nerve passes through the sclera, it becomes sieve-like in the area known as the lamina cribrosa. Abnormal tension in this region due to of glaucoma disrupts the axoplasmic flow in individual nerve fibers of the optic nerve (Samuelson, 1999).

Around the exit of the optic nerve, the sclera is pierced by the ciliary nerves and the short posterior ciliary arteries, arranged as a ring around the optic nerve. Further forward, two long posterior ciliary arteries pierce the sclera, one on each side of the horizontal meridian. Four veins (the vortex veins) draining the choroid emerge a little behind the equator, one for each quadrant, and anterior ciliary arteries and veins pierce the eye just

posterior to the corneoscleral junction (Leeson and Leeson, 1970; Dyce et al., 1987; Samuelson, 1999).

2.2.2 Middle vascular tunic

Between the outer fibrous layer and the retina is a vascular, nutrient layer analogous to pia and arachnoid of the brain. The vascular tunic is composed of three portions: the posterior choroids, the anterior ciliary body and the iris. Collectively, these three structures are also called uvea (Leeson and Leeson, 1970; Tortora and Anagnostakos, 1981; Dyce et al., 1987). The choroid and ciliary body are both attached to the internal surface of the sclera. The iris originates from the anterior portion of the ciliary body, and it extends centrally to form a diaphragm in front of the lens. The iris and ciliary body are termed the anterior uvea and the choroid the posterior uvea (Samuelson, 1999).
The uvea is concerned with the nutrition of the ocular tissues and also provides mechanisms for visual accommodation and reduction or exclusion of light (Bloom and Fawcett, 1970).

2.2.2.1 Iris

The iris is a thin continuation of the ciliary body projecting over the anterior surface of the lens with its free edge outlining the pupil. The horizontal axis of the pupil is longer than the vertical. The iris divides the anterior ocular compartment into anterior and posterior chambers, which communicate through the pupil. In bovine, the diameter of the iris is approximately 1.2 mm. Its opening, the pupil, can be reduced or expanded through the contraction or relaxation of the constrictor and dilatator muscles of the iris. In this way, the iris functions as an adjustable optic diaphragm regulating the amount of the light entering the eye (Prince et al., 1960; Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Dellmann and Brown, 1976; Tortora and Anagnostakos, 1981 Dyce et al., 1987; Samuelson, 1999).
The anterior iris is composed of a central pupillary zone and a peripheral ciliary zone. The demarcation between these two zones is the collarets, which is best demonstrated

with moderate pupillary dilation. The portion of the ciliary zone adjacent to the pupil is sometimes more pigmented than the rest of the iris (Samuelson, 1999).

The shape of the moderately dilated pupil varies among species. The pupil is round in primates, dogs and pigs. It is vertical when constricted in cat, and is oval in horizontal plane in herbivores (horses, bovine, sheep, and goats) (Samuelson, 1999).

The iris has usually a very dark colour, the inner surface being deeply pigmented to a thickness of from 20 to 80 µm and smooth except for some short shallow striations near the free edge. The other tissues of the iris are fairly universally infiltrated by pigment cells. Black corpora nigra are attached to both upper and lower pupillary margins. The upper ones are quite large, especially in the center, while those of the lower edge are very small.

The sphincter muscle of the iris appears to be usually weak, but there is very extensive vascularization and a powerful peripheral arterial arcade (Prince et al., 1960; Samuelson, 1999).

The iris is suspended between the cornea and the lens and is attached at its outer margin to the ciliary body. When the eye is stimulated by bright light, the circular muscle of the iris contracts and decreases the size of the pupil. When the eye must adjust to dim light, the radial muscles of the iris contract and increase the pupil's size (Tortora and Anagnostakos, 1981; Samuelson, 1999).

Iridal colour varies considerably among individuals and among breeds or species of animals. Colour depends on the amount of pigmentation of the iridal stroma. The variation of colour primarily results from the amount and type of pigmentation present, and the degree of vascularization. In many instances, coloration of the irides of domestic animals tend to be dark, but colour can vary from dark brown to gold brown, gold, blue, and blue-green (Samuelson, 1999).

2.2.2.2 Ciliary body

The ciliary body, the largest component of the anterior uvea, is triangular in sagittal section, with its apex continuing into the choroid, the inner side facing the lens and vitreous body, and the outer side facing the sclera (Prince et al., 1960; Tortora and

Anagnostakos, 1981; Dyce et al., 1987; Samuelson, 1999). It begins caudally at the ora serrata, a sharply outlined dentate border, which marks the transition between the optic part (pars optica retinae) and the blind or ciliary part (pars ciliaris reinae) of the retina. Rostrally, it is continuous with the iris and participates in the formation of the trabecular meshwork of the iris angle (Bloom and Fawcett, 1970; Dellmann and Brown, 1976; Tortora and Anagnostakos, 1981). The ciliary body is widest temporally (10.7 mm) and superiorly (10.4 mm). It narrows considerably medially (6.6 mm) and little inferiorly (9.1 mm). The distance from the iris root to the ora ciliary's retinae is 6.5 mm. The greater arterial circle is very well developed in the bovine (Prince et al., 1960).

The caudal portion of the ciliary's body contains the ciliary muscle which is a smooth muscle that alters the shape of the lens for near and far vision (Bloom and Fawcett, 1970; Dellmann and Brown, 1976; Tortora and Anagnostakos, 1981; Dyce et al., 1987). Topographically, the ciliary body is divided into an anterior pars plicata (corona ciliaris) and a posterior pars plana (orbiculus ciliaris). The pars plicata consists of a ring of 70 to 100 ciliary processes, depending on the species. In bovine 90 to 110 large ciliary processes occur, each from 3 to 5 mm long and of varying width, with intervening valleys, which protrude into the posterior chamber and to which the suspensory ligaments of the lens are attached (Prince et al., 1960; Dellmann and Brown, 1976; Samuelson, 1999). The innermost portion within the ciliary processes is a highly vascularized connective tissue (Dellmann and Brown, 1976).

The processes increase greatly the production area of aqueous humor, and are generally more prominent and numerous in animals with larger anterior chambers (like the bovine with 100 processes) than in animals with smaller anterior chambers. Among lower vertebrates, they are often absent. In addition to aqueous production, ciliary processes play variable roles in lenticular accommodation, because these structures are intimately associated with the crystalline lens (Samuelson, 1999).

The ciliary body provides nourishment and removes wastes for the ocular structures that focus or refract light (cornea and lens). Nutrients for the refractive structures are primarily supplied by the aqueous humor of the of the eye, which is an optically clear fluid originating from vascular sinuses within the folds and processes of the ciliary body and draining into the iridocorneal or anterior chamber angle, which forms the anterior

boundary of the ciliary body. In the continuous process of aqueous humor formation and drainage, intraocular pressure (IOP) is created, which is responsible for providing the eye with most of its rigidity (Samuelson, 1999).
The fibrous and vascular tunics are attached firmly at the corneoscleral junction anteriorly and at the exit of the optic nerve posteriorly. Between these two regions, they are separated by the perichoroidal or subchoroidal space (Leeson and Leeson, 1970).

2.2.2.3 Choroid

The choroid follows the standard mammalian pattern in being highly vascular and densely pigmented. Several of the short posterior ciliary arteries enter the choroid well away from the optic nerve entrance (Prince et al., 1960; Bloom and Fawcett, 1970). Peripherally, the choroid is connected with the sclera; centrally it is adjacent and intimately attached to the pigmented epithelium of the retina (Dellmann and Brown, 1976).
The choroid appears as thin dark brown membrane that lines the most of the internal surface of the sclera. It contains numerous blood vessels and a large amount of pigment. The choroid absorbs light rays so they are not reflected back out of the eyeball. Through its blood supply, it nourishes the retina. The optic nerve also pierces the choroid at the back of the eyeball (Tortora and Anagnostakos, 1981; Dyce et al., 1987).
The choroid is supplied by the posterior ciliary arteries and is drained by the vorticose veins. A flat sheet of capillaries on the internal surface is responsible for nutrition of the external layers of the nervous tunic (retina). The blood of these capillaries produces redness of the fundus seen when the eye is examined with an ophthalmoscope (Dyce et al., 1987).
In the dorsal part of the fundus, the choroid forms a variously coloured, light reflecting area known as the tapetum. This is an avascular layer (cellular in carnivores, fibrous in ruminants and horses) between the capillaries and the network of larger vessels. The tapetal cells contain crystalline rods arranged in such a way that light striking them is split into its components, resulting in the characteristic iridescence. The tapetum makes the eyes of the animals shine when they look toward a light, such as headlights of an

oncoming car. Our eyes, and those of the pig, do not have a tapetum and therefore do not give this reaction. It is believed that the tapetum is a nocturnal adaptation since by reflecting incident light it increases the stimulation of the light-sensitive receptor cells in the overlying retina and thus aids vision in dark places (Dyce et al., 1987).

2.2.3 Inner nervous layer

2.2.3.1 Retina

The third inner coat of the eye lies only in the posterior portion , and covers the choroid. The retina is composed of a sensory portion, also referred to as the pars optica retinae, and a non sensory portion, which begins at the ora serrata and covers the ciliary body, as the pars ciliaris retinae, and the iris, as the pars iridis retinae. The primary function of the varying portions is image formation (Prince et al., 1960; Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Dellmann and Brown, 1976; Tortora and Anagnostakos, 1981; Dyce et al., 1987; Samuelson, 1999).

The thickness of the retina is about 0.22 mm (Prince et al., 1960). The optic disc in cattle is somewhat smaller than that of the horse and it is horizontal oval in shape. The disc margin is fairly indistinct and short. Radial striations extend from the disc on the retina. The spot where the nerve enters the eyeball, the papilla of the optic nerve, is a pink disk approximately 1.4 mm in diameter. It is situated 3 mm medial to the posterior pole of the eye (Bloom and Fawcett, 1970).

The macula lutea or yellow spot is in the exact center of the retina. The fovea is the area of sharpest vision because of high number of cones. Rodes are absent from the fovea and macula, but they increase in density toward the periphery of the retina (Tortora and Anagnostakos, 1981).

2.2.4 Optic nerve

The optic nerve in the most domestic animals lies inferior and lateral to the posterior pole. Surrounding the optic nerve are many ciliary nerves and short posterior ciliary arteries. The posterior ciliary nerves pursue a long intrascleral course up to 12 mm before entering the suprachoroidal space to reach the iris, ciliary body, and limbus (Leeson and Leeson, 1970; Samuelson, 1999).

About 15% of the optic nerve fibers from each eye remain on the ipsilateral side of the head, and in this respect the ratio of fibers not crossing at the chiasma is about the same as in the horse. In every respect the bovine optic nerve seems to conform to the general mammalian pattern. The lamina cribrosa is not as dense and powerful as might be expected, but is evidently strong enough to withstand the degree of intraocular pressure to which the eye is subjected. In a transverse section the septa which extend into the optic nerve from the dura can be seen to be well supplied with capillaries (Prince et al., 1960).

2.3 Microscopical anatomy of the bovine eyeball

The eyeball consists of three coats: Outer fibrous coat, middle vascular coat, inner nervous coat.

2.3.1 The outer fibrous layer

2.3.1.1 Cornea

The cornea is divided into three layers, each of which plays a role in keeping it transparent: outer multicellular epithelial layer, middle dense connective tissue-stromal layer and inner endothelial single cell layer. The stroma must be maintained at a specific level of dehydration to remain transparent.

The cornea in domestic animals is composed of five layers: anterior epithelium, subepithelial basement membrane, substantia propria or stroma, posterior limiting

membrane (Descemet's membrane), and posterior epithelium (corneal endothelium) (Diesem, 1975; Dellmann and Collier, 1987; Banks, 1993; Slatter, 2001b).

Corneal thickness in most domestic animals is 0.56 - 1 mm. The centers of bovine, canine, feline and porcine corneas are thicker than the peripheries; the reverse is true in horses (Gelatt, 1991; Banks, 1993, Slatter, 2001b).

The thickness of cattle cornea, 0.75-0.85 mm (Prince et al., 1960), does not vary so much as in other species of domestic animals; the periphery measures 1.5- 1.8 mm, the middle of the cornea usually measures 1.5 - 2 mm (Diesem, 1975).

The available literature shows that many factors influence corneal thickness: hydration, intraocular pressure, age, sex, closed or opened eye, dead or living cornea, method of measurements, diseased conditions of the cornea and many other factors. For biomechanical studies of various tissues, it is known that changes in hydration or redistribution of water within tissue affect the mechanical properties of tested specimen (Fung, 1981).

Thickness of cornea is largely determined by degree of hydration. Normal cornea during life maintains a fairly constant thickness, and it keeps its water content at a steady level of about 75% to 80% of its weight. However, excised pieces of corneal tissue have a marked affinity for water when immersed in isotonic solutions. Under such conditions the cornea becomes swollen and loses transparency (Moses, 1975).

The intraocular pressure is also a factor influencing corneal thickness. Corneal oedema is a well known clinical sign in glaucoma. The intraocular pressure within normal ranges has little influence on corneal thickness in the normal eye (Yttenbarg and Dohlman, 1965).

The outermost layer is the corneal epithelium which is stratified squamous epithelium consisting of 5 to 20 layers of cells (Patt and Patt, 1969; Diesem, 1975; Dellmann and Collier, 1987; Banks, 1993; Slatter, 2001b).

Thickness of the epithelium in bovine cornea is 14-17 rows of epithelial cells usually measuring about 90 μm in thickness (Prince et al., 1960).

Superficial cells are highly irregular in surface view, with many processes that interdigitate with one another. A profuse network of bare nerve endings ramifies between the cells, particularly at basal and intermediate levels (Patt and Patt, 1969).

As the cornea is a nonvascular structure, blood vessels loop around its borders. Only in the foetus they advance near its centre. The cornea receives nerve supply from the ciliary nerves (Raghavan and Kacharoo, 1964).

The epithelium is completely avascular and is nourished by lacrimal secretion, as well as by aqueous humor of the anterior chamber. The epithelium is characterized by a remarkable capacity for rapid repair in case of injury (Patt and Patt, 1969). The epithelial cells of the cornea may have lymph spaces between them in their more posterior rows (Diesem, 1975).

The sensitivity of the cornea is due to the fact that great numbers of free nerve endings are found in this layer. The regenerative capability of the corneal epithelium is pronounced and, together with cell movements, assures a rapid return to normal of an injured epithelium. An intact corneal epithelium is necessary for maintenance of its transparency (Diesem, 1975; Dellmann and Collier, 1987; Banks, 1993).

The next layer encountered is the external limiting membrane or membrane of Bowman. The membrane may not be distinct in domestic species but is prominent in primates. It is more intimately attached to the substantia propria and is considered to be a part of that portion of the cornea (Raghavan and Kacharoo, 1964; Patt and Patt, 1969; Diesem, 1975; Dellmann and Collier, 1987; Banks, 1993).

The subepithelial basement membrane consists of a basal lamina and an underlying layer of reticular fibers. Frequently, this layer can be distinguished by light microscope. It should not be confused with the anterior limiting lamina (Bowman’s membrane) (Dellmann and Collier, 1987).

Underlying the epithelium is a thick substantia propria that accounts for more than 90% of the thickness of the cornea. In corneal stroma, the relationship between thickness and hydration is linear (Ehlers, 1966). The substantia propria itself is mainly collagenous, and fibers are arranged in regular layers. All fibers within a given layer lie flat and run parallel to each other, but the orientation of fibers of adjacent layers is oblique to each other, as in plywood. There is some intermeshing of fibers between adjacent layers, which serves to tie the layer together. Between neighbouring layers is a glycoprotein cementing substance and flattened irregular fibroblasts. Like the corneal epithelium, the substantia propria is avascular containing distributed nerves (Raghavan and Kacharoo,

1964; Patt and Patt, 1969; Diesem, 1975; Dellmann and Collier, 1987; Banks, 1993; Slatter, 2001b).

The corneal stroma in the cow has sometimes a light dusting of pigment but is quite highly pigmented around the peripheral areas (Prince et al., 1960).

Occasional elastic fibers are observed at the periphery of the cornea. The predominating cell type of the corneal substantia propria is the fibroblast, located mainly between collagen layers. These cells are elongated and branched, with little cytoplasm. Toward the limbus, other cells such as histiocytes are present (Dellmann and Collier, 1987).

Cells and fibers are embedded in the amorphous ground substance that stains metachromatically due to the presence of sulfated glycosaminoglycans (chondroitin sulfate, keratan sulfate). The ground substance plays an essential role in transparency of the cornea by maintaining an optimal degree of hydration; excessive water content causes opacification of the cornea (Dellmann and Collier, 1987).

The internal limiting membrane or Descemet's membrane is a fairly thick, glassy, homogeneous membrane. It consists of unusual protein fibers resembling collagen. The membrane may have protuberances near its periphery (Patt and Patt, 1969; Diesem, 1975). Thickness of the Descemet's membrane in the cow varies from 10 to 25 µm (Prince et al., 1960).

In H&E-stained preparations, the posterior limiting lamina appears as a highly refractile, thick amorphous layer. It gives a positive PAS reaction and stains with dyes specific for elastic fibers (Dellmann and Collier, 1987; Banks, 1993).

The Descemet's membrane is the basement membrane of the corneal endothelium and is laid down throughout life, increasing in thickness with age. It does not stain with fluorescein and appears as a relatively dark, transparent structure (Slatter, 2001b).

The bovine Descemet's membrane is a homogenous elastic layer. It glistens and breaks off at the margin into three sets of fibers: anterior, middle and posterior one. The anterior fibers join the sclera and the middle set to the ciliary muscle; the posterior set penetrates the iris and forms the ligamentum pectinatum iridis (Raghavan and Kacharoo, 1964).

The inner most or posterior layer of the cornea is an epithelium of mesenchymal origin that is sometimes referred to as corneal endothelium. It consists of a single layer of low cuboidal cells or flattened cells with the nuclei lying parallel to the internal limiting

membrane; it is in direct contact with the aqueous humor of the anterior chamber, separated from the substantia propria by the narrow refractile membrane (Descemet's membrane) (Raghavan and Kacharoo, 1964; Patt and Patt, 1969; Diesem, 1975; Dellmann and Collier, 1987; Banks, 1993; Slatter, 2001b).
The thickness of the posterior layer of the endothelium can be up to 6 µm (Prince et al., 1960). The endothelium functions through maintenance of the transparency of the cornea; indeed, defects in the endothelium cause oedema and opacification of the cornea, which disappear rapidly after regeneration of the endothelium. Endothelial regeneration occurs through increased mitosis in the vicinity of the wound. The regenerative ability appears to vary within species and age of animals (Dellmann and Collier, 1987).

2.3.1.2 Sclera

The sclera is thin in most vertebrates (about 0.5 mm in human eye), being strong and inelastic (Patt and Patt, 1969). The sclera consists of flat ribbons of collagenous bundles which run in various directions parallel to the surface. Between these bundles are fine elastic nets as well as fibroblasts and occasionally melanocytes (Maximow and Bloom, 1955; Patt and Patt, 1969; Leeson and Leeson, 1970; Diesem, 1975; Dellmann and Brown, 1976; Dyce et al., 1987; Gelatt, 1991; Banks, 1993; Slatter, 2001b).
The fibers in the sclera may intertwine with each other; even though they run in identical directions they may form a thicker sclera in some areas than in others (Diesem, 1975; Dyce et al., 1987).
Rostrally, fibers of the sclera are oriented in a circular direction around the optical axis providing firm attachment points for insertion of extraocular muscles (Banks, 1993).
The sclera can be subdivided into three layers. The outermost layer, the episcleral tissue consists of loose fibroelastic tissue that is continuous externally with dense connective tissue of Tenon's capsule. Its deeper surface blends with the middle layer, the sclera proper, where bundles of collagenous fibers are oriented mainly parallel to the surface with some branching and interweaving. The innermost layer, termed the lamina fusca or dark layer, is composed of much smaller bundles of collagenous fibers (Leeson and Leeson, 1970).

Branching chromatophores containing melanin are situated between the fibers. These can be found in the deeper layers, especially in the entrance region of the optic nerve which gives the inner aspect of the sclera a brown colour. There are very few blood vessels in the sclera, no lymphatic vessels, and a few nerve fibers originating from the ciliary nerves (Maximow and Bloom, 1955; Leeson and Leeson, 1970 and Dellmann and Brown, 1976; Dyce et al., 1987; Gelatt, 1991; Banks, 1993).

2.3.2 The middle vascular layer

2.3.2.1 Iris

The iris is attached by its root or basis to the ciliary body. It is narrowed toward the ciliary body and the pupillary opening (pupil) in its center (Dellmann, 1971).
The iris consists of three layers: an anterior epithelial layer continues across the iridocorneal angle into the posterior epithelium of the cornea; a middle layer of connective tissue stroma contains two smooth muscles (musculus sphincter and musculus dilatator pupillae), and the posterior layer of the pigmented epithelium (Dyce et al., 1987).
The first and most anterior layer, called the endothelial layer consists of two cell types: fibroblasts and melanocytes (Samuelson, 1999).
The anterior border layer faces the anterior chamber. It is an incomplete pavement of thin, delicate cells very difficult to discern in vertical sections and continuous with that of the corneal endothelium (Maximow and Bloom, 1955; Patt and Patt, 1969; Leeson and Leeson, 1970; Dellmann, 1971; Bloom and Fawcett, 1970; Dellmann and Brown, 1976).
The endothelium of the iris is non-continuous being pierced by numerous intercellular spaces and pores, which communicate with channel-like intervals in the rostral limiting layer. Present toward the papillary margin are occasional invaginations, so called crypts, of varying depth whose functional significance is still unknown (Dellmann and Brown, 1976).
Underlying this layer, the rostral limiting layer or rostral stromal sheath, is found. Situated under the epithelium, this layer is rich in mucopolysaccharides, reticular fibers,

collagenic fibers and pigmented cells which generate the colour of the iris. The rostral limiting layer is avascular (Maximow and Bloom, 1955; Patt and Patt, 1969; Leeson and Leeson, 1970; Bloom and Fawcett, 1970; Dellmann, 1971; Dellmann and Brown, 1976). The third iridial layer is the stroma, which consists of a rather loose network of collagenous and elastic fibers, chromatophores, fibroblasts, and a large number of blood vessels with unusually thick walls.

Near the pupil, the stroma includes smooth muscle fibers of the pupillary sphincter, which derive from cells of pigmented retina in this area (Maximow and Bloom, 1955; Patt and Patt, 1969; Leeson and Leeson, 1970; Bloom and Fawcett, 1970; Dellmann, 1971; Dellmann and Brown, 1976; Samuelson, 1999; Hees and Sinowatz, 2000).

The stroma is loosely arranged except around blood vessels and nerves, where it can form dense sheaths (Shively and Epling, 1969).

The blood vessels of the iris are completely surrounded by spirally wound collagen bundles which belong to several different arcuate bundles. This way, the blood vessels change their position during contraction or dilatation of the iris in accordance with the collagen bundles that protect them against compression and bending. Fibroblasts, melanocytes, mast cells, histocytes and a few chromatophores represent the majority of the cells in the iris stroma.

The melanocytes are prominent around the adventitia of the blood vessels (Dellmann and Brown, 1976; Samuelson, 1999). The iridal color varies among individuals and among various breeds or species of animals. Variation of color primarily results from the amount of pigmentation present, the type of pigmentation, and the degree of vascularization (Samuelson, 1999).

Underlying this layer is connective tissue. It contains smooth muscle fibers, arranged as sphincter and dilatator pupillae. The sphincter muscles lies around the pupillary margin, closely associated with pigment epithelium on the posterior surface of the iris. The shape of the sphincter muscle varies among species according to pupillary shape (Prince, 1956). It is supplied by parasympathetic fibers of the third brain nerve which have synapsed in the ciliary ganglion. The dilatator fibers appear to be more primitive in structure and contain some pigment, but both groups of fibers are ectodermal in origin, being derived from pigment epithelium. The dilatator muscle is situated posteriorly and blends with the

sphincter fibers near the pupillary margin, radiating peripherally from it like the spokes of a wheel. The muscle is thicker peripherally at the ciliary margin of the iris. The size of the dilatator muscle varies with species, being well developed in dog and involving full circumference of the iris. In horse, it is less developed, and in species with elongated pupils, it is poorly developed adjacent to the long axis of the pupil (Prince et al., 1960). The dilatator is supplied by the sympathetic nervous system through the superior cervical ganglion (Maximow and Bloom, 1955; Patt and Patt, 1969; Leeson and Leeson, 1970; Bloom and Fawcett, 1970; Dellmann, 1971; Dellmann and Brown, 1976; Hees and Sinowatz, 2000).

The posterior-most and fifth layer of the iris is pigment epithelium which varies in thickness from 20 to 80µm (Prince et al., 1960). This layer represents the anterior continuity of the nervous retina between the ciliary body and the pupil. It consists of two layers, the inner layer is a heavily pigmented epithelium, and the outer layer is transformed into the myoepithelial cells of dilatator muscle. In this layer, only the part of cells containing the nucleus is pigmented. The contractile portion does not contain pigmented granules. If there is no pigment in any of the iridial layers, as in albinos, the iris appears pink because of the reflection of the light from the choroid layer behind the retina (Maximow and Bloom, 1955; Patt and Patt, 1969; Leeson and Leeson, 1970; Bloom and Fawcett, 1970; Dellmann, 1971; Dellmann and Brown, 1976; Dyce et al., 1987; Samuelson, 1999; Hees and Sinowatz, 2000).

The limiting membrane of the iris is a continuation of the ciliary inner limiting membrane (Dellmann, 1971).

Granula iridis (iris granules), represent proliferation at the papillary edge of the stroma of the iris and the retinal epithelium. In sheep and goat there, is a large cyst filled with fluid, lined by pigmented epithelium and dense capillary network (Dellmann, 1971).

2.3.2.2 Ciliary Body

The ciliary body is the direct rostral continuation of the choroids. It begins caudally at the ora serrata, a sharply outlined dentate border that marks the transition between the optic part (pars optica retinae) and the blind or ciliary part (pars ciliaris retinae) of the retina.

Rostrally, it is continuous with the iris and participates in the formation of the trabecular meshwork of the iris angle (Maximow and Bloom, 1955; Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Dellmann and Brown, 1976; Samuelson, 1999; Hees and Sinowatz, 2000).

The histological structure of the ciliary body:

The outermost layer of the ciliary body is merely a continuation of the suprachoroid layer of the choroid (Dellmann and Brown, 1976).

Adjacent to it are muscle bundles of the ciliary muscle. It comprises three layers of smooth muscle cells with a common origin from the ring like ciliary tendon attached to the scleral spur and the pectinate ligament. Usually three predominant fibers directions are distinguishable, meridional, radial and equatorial. The outermost fibers are the meridional fibers (muscle of Brücke) which originate from substantia propria of the cornea, the adjacent connective tissue of the trabecular meshwork of the iris angle and the sclera. They are attached by elastic tendons to the elastic membrane of the choroid.

The main portion of ciliary muscle consists of meridional fibers in the posterior portion of the ciliary body that are rostrally and peripherally continuous with circular fibers located partially within the sclera. This muscle stretches the choroids and is also called tensor muscle of the choroids.

Radiate fibers have the same origin as the meridional fibers; they are located inside these fibers and radiate into the circular fibers. The bundles of muscle cells radiate fan like from the region of the scleral roll toward the cavity of the eyeball. This is the radial or reticular portion of the ciliary muscle.

Circular fibers (Müller's muscle) are less numerous than meridional fibers. However, they are predominant in the nasal portion of the ciliary body, where they are the only existing fibers in the pig (Maximow and Bloom, 1955; Bloom and Fawcett, 1970; Patt and Patt, 1969; Leeson and Leeson, 1970; Dellmann and Brown, 1976; Samuelson, 1999). The bovine ciliary muscle is relatively lengthy. Circular fibers appear to be very rudimentary, and the radial fibers are also few in numbers (Prince et al., 1960). Between the bundles of smooth muscles there is a meshwork rich in elastic fibers and containing melanocytes (Leeson and Leeson, 1970).

Vessels and basal plate layer are the continuation of the same layer of the choroids. Veins are predominant and are interspersed with capillaries. Some arteries are located in the periphery. This layer extends as dense network of capillaries into the ciliary processes. The connective tissue of the basal plate is a moderately dense, irregular connective tissue (Patt and Patt, 1969; Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Dellmann and Brown, 1976; Samuelson, 1999).

The internal surface of the vascular layer is lined by a condensation of elastic fibers are directly continuous with the outer elastic lamina or Bruch's membrane. The elastic membrane of the choroids is also continuous into the ciliary body. However, it gradually disappears toward the rostral third of the ciliary body. Some elastin can be found in ciliary processes. Ciliary epithelium is separated from connective tissue by a distinct basement membrane (Maximow and Bloom, 1955; Leeson and Leeson, 1970; Dellmann and Brown, 1976; Samuelson, 1999).

Between the elastic and the cuticular lamina, a narrow zone of collagenous fibers with fibroblasts is developed (Leeson and Leeson, 1970). It is continuous with the Bruch's membrane of the choroid and extends anteriorly to the root of the iris. Here it has a corrugated surface and is the basal lamina of the pigment epithelium which covers it (Leeson and Leeson, 1970). It is the continuation of the pigmented epithelium of the retina and consists of a simple cuboidal or low cuboidal, deeply pigmented epithelium. The base of the cell is characterized by deep invaginations of plasma membrane. Pigmented and non-pigmented epithelial layers are connected through finger-like processes and desmosomes (Maximow and Bloom, 1955; Patt and Patt, 1969; Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Dellmann and Brown, 1976; Samuelson, 1999; Hees and Sinowatz, 2000).

The outstanding morphologic characteristics of the inner epithelial layer, which consists of cuboidal or columnar cells, are numerous, deep apical invaginations of the surface membrane. In addition, there are deep interdigitations between the lateral walls of adjacent epithelial cells. The cells are continue forward on the posterior surface of the iris and become hear heavily pigmented (Maximow and Bloom, 1955; Patt and Patt, 1969; Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Dellmann and Brown, 1976;

Samuelson, 1999). The pigmented and nonpigmented epithelial layers, play an active secretory role in the production of aqueous humor (Dellmann and Brown, 1976).
This fibrillar sheet overlying the epithelium follows closely the irregularities of the surfaces of the ciliary body. Anteriorly, it blends with the condensation of fibrillar material forming the zonule of the lens. It is continuous with the internal limiting membrane of the optical portion of the retina (Maximow and Bloom, 1955; Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Dellmann and Brown, 1976 and Samuelson, 1999).
Ciliary processes are the site of aqueous humor formation. The covering pigmented epithelium is not present at the tip of a ciliary process (Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Samuelson, 1999).

2.3.2.3 Choroid

The choroid is a thick, highly vascularized layer. The outer side of the choroid is connected with the sclera; the inner side is adjacent and intimately attached to the pigmented epithelium of the retina (Maximow and Bloom, 1955; Patt and Patt, 1969; Dellman and Brown, 1976).
The most peripheral layer of the choroid is the suprachoroid layer. It is loosely structured, only 10 to 35 µm thick, and consists of bundles of collagen and some elastic fibers. Toward the sclera, these bundles assume an oblique course, are separated by numerous spaces, the perichoroidal spaces, and are continuous with the connective tissue of the sclera. The cell population of this layer consists of fibroblasts, numerous flat melanocytes, and occasional macrophages (Maximow and Bloom, 1955; Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Dellman and Brown, 1976; Samuelson, 1999; Hees and Sinowatz, 2000).
The melanoblasts are numerous, with scattered macrophages, and some smooth muscle cells. Blood vessels traverse this layer to reach the deeper layers (Bloom and Fawcett, 1970; Leeson and Leeson, 1970).
The function of the suprachoroid is also a posterior component for uveoscleral outflow. Aqueous humor moves along this narrow junction of the sclera, diffuses into the choroid

and sclera and, subsequently, into the systemic circulation. The layer of melanocytes, fibroblasts and interspersing fibres (collagen and elastic fibers) may produce resistance to uveoscleral drainage, even though a cellular barrier has not been found to exist (Koseki, 1992).

The vessel layer consists of intercrossing large and medium size arteries and veins, separated by loose connective tissue stroma rich in chromatophores (Maximow and Bloom, 1955; Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Dellman and Brown, 1976; Samuelson, 1999; Hees and Sinowatz, 2000). The outer part of vessel layer is modified in many vertebrates into a light reflecting (carpet) or tapetum lucidum (Patt and Patt, 1969). The vessel layer contains strands of smooth muscle independent of the arteries (Maximow and Bloom, 1955; Bloom and Fawcett, 1970).

The tapetum lucidum is a light reflecting layer, supposedly increasing light reception under conditions of poor illumination. The tapetum is not present throughout the choroid but is located mainly in the dorsal half of the fundus of the eye. In herbivores the tapetum is fibrous, consisting of intermingling collagen fibers and a few fibroblasts. The thickness of the tapetum varies, being multilayered at its centre and thinning out to a single cell at its periphery. The tapetal cells are packed with bundles of parallel small rods, all of which are oriented with their long axis parallel to the retinal surface. Diffraction of light as a result of spatial orientation of rods (or of collagen fibrils in herbivores) is probably responsible for producing light reflection of the tapetum (Dellman and Brown, 1976; Dyce et al., 1987; Samuelson, 1999).

As in all ruminants the bovine has a fibrous tapetum. Histologically it consists of dense regular connective tissue fibers, the whole membrane being rather homogenous and revealing very clearly the passage of the capillaries. The lamina of the tapetum is up to 8 µm thick, and the entire membrane varies in thickness from 10 µm at the periphery to 50 µm at the centre (Prince et al., 1960; Samuelson, 1999).

The choriocapillary layer is a dense network of capillaries about 2 µm in thickness (Hees and Sinowatz, 2000). It is immediately adjacent to the pigmented epithelial layer of the retina. The wide capillaries often deeply indent these cells and are thus intimately related to them. The intercapillary stroma consists mainly of delicate collagenous and elastic networks, fibroblasts and occasional melanocytes (Maximow and Bloom, 1955; Patt and

Patt, 1969; Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Samuelson, 1999; Hees and Sinowatz, 2000). Furthermore the endothelium is fenestrated. Endothelium nuclei and pericytes are located only toward the choroidal side of the capillaries, and the capillary and the pigmented epithelial basement membranes are fused. These features indicate transport from the capillaries to pigmented epithelium (Dellman and Brown, 1976).

The basal complex is also referred to as Bruch's membrane (Maximow and Bloom, 1955; Bloom and Fawcett, 1970; Leeson and Leeson, 1970). It separates the choroid from the retina. When the basal complex is fully developed, it consists of five layers: (1) basement membrane of the retinal pigmented epithelium, (2) inner collagenous zone, (3) elastic layer, (4) outer collagenous zone, and (5) basement membrane of the choriocapillary layer. In the area over the cellular tapetum, the basement membrane of the retinal pigmented epithelium and choriocapillary often fuse, obliterating the outer three layers (Patt and Patt, 1969; Dellman and Brown, 1976).

2.3.3 The inner nervous layer

2.3.3.1 Retina

The retina is composed of a sensory portion, also referred to as the pars optica retinae, and a non-sensory portion, which begins at the ora serrata, and covers the ciliary body as pars ciliaris retinae, and the iris as the pars iridis retinae (Leeson and Leeson, 1970; Dellmann, 1971; Dyce et al., 1987; Dellman and Collier, 1987; Samuelson, 1999; Hees and Sinowatz, 2000).

The retina consists of the following layers: pigment epithelium, layers of rods and cones, external limiting membrane, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer, optic nerve fiber layer, and internal limiting membrane (Maximow and Bloom, 1955; Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Dellmann, 1971; Dyce et al., 1987; Dellman and Collier, 1987; Samuelson, 1999).

2.3.3.1.1 Retinal pigment epithelium (RPE)

The RPE is a simple squamous or cuboidal epithelium resting on a basal lamina. The basal part of the cells closely adherents to the choroid coat. The base of the cell is characterized by deep infoldings of the plasma membrane (Maximow and Bloom, 1955; Patt and Patt, 1969; Leeson and Leeson, 1970; Dellmann, 1971; Dellman and Collier, 1987; Samuelson, 1999; Hees and Sinowatz, 2000).
Melanin granules which usually occur in great number in the epithelial cells are lacking in the RPE overlying the tapetum lucidum.
Tongue like apical processes extends from the cells to surround the outer segments of the rods and cones. They do not contain any pigment granules according to several investigators (Patt and Patt, 1969; Dellmann, 1971; Dellman and Collier, 1987; Samuelson, 1999) while others recorded that these processes are filled with pigment granules (Maximow and Bloom, 1955).
There is comparative little pigment in the pigment epithelium of bovine retina in the area of the tapetum, but it increases to great density at the periphery (Prince et al., 1960).
The function of the retinal pigmented epithelium is to absorb light after passing though the transparent photosensitive retina, so that the light will not be scattered and blur the retinal image (Patt and Patt, 1969).
There are four groups of cellular elements in the retina: visual receptors (rods and cones), direct conducting neurons (bipolar, ganglion cells), association and other neurons (horizontal, amacrine) and supporting elements (Müller’s cells and neuroglia) (Leeson and Leeson, 1970).

2.3.3.1.2 Layer of rods and cones

Rods and cones alike consist of an outer segment, which is the photosensitive part, and an inner segment, which includes the nucleus and cytoplasmic organelles (Patt and Patt, 1969; Leeson and Leeson, 1970; Samuelson, 1999; Hees and Sinowatz, 2000). The outer segments of the photoreceptive rods and cones (first neuron) are readily distinguished with the light microscope as a layer adjacent to the pigment epithelium. Each outer

segment is connected to its inner segment by a modified cilium (Patt and Patt, 1969; Bloom and Fawcett, 1970; Dellmann, 1971; Dellman and Collier, 1987; Samuelson, 1999).

The rod cells are responsible for vision in dim light, while the cone cells function in bright light and are responsible for color vision. Thus, animals that are mainly active at night show fewer cone cells than day-active animals (Dyce et al., 1987; Dellman and Collier, 1987; Krebs and Krebs, 1991).

The rod cells are elongated cells comprising an outer and an inner segment. The rod cell is long, slender, and highly specialized. The scleral part of the rod cells, the rod proper is situated between the outer limiting membrane and the pigmented epithelium. The vitreal ends of the rod proper extend through the outer limiting membrane into the outer nuclear layer. Each rod proper consists of an outer and inner segment.

Cone cells are the same parts as the rod cells, but they differ in certain details. There is no visual purple in cones. Instead of a slender cylinder, the cone outer segment has a long conical structure, considerably wider than a rod at its base and tapering down to a blunt rounded tip. The cone consists of an outer and inner segment. Proximal to the outer limiting membrane, the inner cone segment merges with its body, containing a nucleus, which is larger and paler than of the rod nucleus. The bodies and nuclei of the cones, in contrast to those of the rods are arranged in a single row immediately beneath the outer limiting membrane. In the outer fovea, the nuclei of the cones are accumulated in several rows (Maximow and Bloom, 1955; Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Dellmann, 1971; Samuelson, 1999; Hees and Sinowatz, 2000). In bovine retina, there is an increasing number of rods toward the center (Prince et al., 1960).

2.3.3.1.3 External limiting membrane

It separates the layer of the rod and cone inner segments from the outer nuclear layer. It is not a true membrane, joining adjacent photoreceptor and Müller’s cells, which surround and support all of the neural elements between the inner limiting membrane of the retina and the outer limiting membrane (Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Dellman and Collier, 1987; Samuelson, 1999; Hees and Sinowatz, 2000).

2.3.3.1.4 Outer nuclear layer

In the outer nuclear layer, the perikarya of the cones are located in the intermediate proximity of the outer limiting membrane and form only a single row, whereas the perikarya of the rods form several layers in the inner portion of this layer (Dellmann, 1971; Dellman and Collier, 1987).
Additional structures in this layer are outer rod and cone connecting fibers, rod and cone axons, and Müller cell processes (Samuelson, 1999).

2.3.3.1.5 Outer plexiform layer

It is composed of rod spherules and cone pedicles. The rode spherules have synaptic contact with the rod bipolar and horizontal cells. The cone pedicles synapse with midget bipolar, flat bipolar and horizontal cell processes (Dellmann, 1971; Dellman and Collier, 1987; Samuelson, 1999).

2.3.3.1.6 Inner nuclear layer

Four cell types, horizontal, bipolar, amacrine and supporting glia (Müller's) cells are found in this layer (Bloom and Fawcett, 1970; Dellman and Collier, 1987).
The horizontal cells are found either in the outer plexiform layer or in the outer zone of the inner nuclear layer (Maximow and Bloom, 1955; Leeson and Leeson, 1970; Dellmann, 1971; Samuelson, 1999).
Horizontal cells possess a round or oval nucleus and with more perinuclear cytoplasm than in bipolar cells. The cell processes are generally thick and one or more may be quite long and run horizontally through the outer plexiform layer (Patt and Patt, 1969). Two types of horizontal cells have been identified, an axonless horizontal cell known as type A synapsing with all kinds of cones (Gallego, 1986; Boycott et al., 1987) and a second type, type B, which has an axon and synapses with rod spherules (Kolb and Famiglirtti, 1974).They are located in the outer zone of the inner nuclear layer. Some may be found adjacent to the outer plexiform layer. Horizontal cell processes are in contact with rod

spherules and cone pedicles (Maximow and Bloom, 1955; Patt and Patt, 1969; Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Dellmann, 1971; Dellman and Collier, 1987; Samuelson, 1999; Hees and Sinowatz, 2000).

Bipolar cells are characterized by large, round nuclei and long processes that connect the cells with both inner and outer plexiform layers. They are located in the center of the inner nuclear layer. These neurons connect the rods and cones with the ganglion cell of the retina (Leeson and Leeson, 1970; Bloom and Fawcett, 1970; Dellmann, 1971; Samuelson, 1999).

Three types of bipolars have been described in primate retina: rod bipolars, flat bipolars and midget bipolars (Patt and Patt, 1969; Leeson and Leeson, 1970; Samuelson, 1999; Hees and Sinowatz, 2000).

Two groups of bipolars can be distinguished: centripetal bipolars, which transmit impulses from rods and cones to ganglion cells, and centrifugal bipolars, which transmit impulses in opposite direction (Maximow and Bloom, 1955). They are located in the center of the inner nuclear layer. These neurons connect the rods and cones with the ganglion cell of the retina (Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Dellman, 1971; Samuelson, 1999).

Dendrites of the rod bipolar ganglion cells contact several rods. The axons have axosomatic and axodendritic synapses with various ganglion cells. The dendrites of the cone bipolar ganglionic cell contact several cones and have axosomatic and axodendritic synapses with other ganglion cells (Leeson and Leeson, 1970; Dellman and Collier, 1987).

Amacrine cells predominate in the inner portion of the inner nuclear layer. Their nuclei are generally characterized by deep invaginations. Their cell processes extend into the inner plexiform layer and establish contact with the dendrites and also perikarya of all ganglion cells, with axon of bipolar cells. Amacrine cells also appear to be interconnected (Dellman and Collier, 1987). The supporting glial cells (Müller cells and radial cells) are elongated, atypical, fibrous astrocytes extending between the inner and outer limiting membrane. The cell bodies are characterized by a homogeneous oval nucleus and a dark cytoplasm; they are located in the outer portion of the inner nuclear layer. Their processes spread radially through the retina. The outer processes terminate in foot-like

enlargements similar to those of the inner processes unit to form continuous layers inside the nerve fiber layer (Maximow and Bloom, 1955; Patt and Patt, 1969; Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Dellman and Collier, 1987; Samuelson, 1999; Hees and Sinowatz, 2000).

2.3.3.1.7 Inner plexiform layer

Axons of bipolar cells, dendrites of ganglion cells and numerous processes of amacrine cells make up this layer (Dellmann, 1971; Dellman and Collier, 1987; Samuelson, 1999).

2.3.3.1.8 Ganglion cell layer

This layer includes nuclei and cell bodies of the retinal ganglion cells of varying sizes, arranged in one or several layers.
The ganglion cell bodies are also large (from 15 to 20 µm in diameter) and have round, eccentric nuclei and abundant cytoplasm with many organelles. The dendritic synapse of the ganglia cells lies in the inner plexiform layer, although somatic synapses of the rod bipolars and amacrine cells occur in the cell bodies of the ganglion cells (Leeson and Leeson, 1970; Maximow and Bloom, 1955; Patt and Patt, 1969; Dellmann, 1971; Dellman and Collier, 1987; Samuelson, 1999). There are two main types, the diffuse type with dendrites contacting several bipolar cells, and the small or monosynaptic type with dendrites synapsing with cones (Leeson and Leeson, 1970).
In bovine retina there are many ganglion cells, one row being most usual, but occasionally there are two. Some of these ganglion cells are very large, however, having a diameter up to 40 µm which is larger than any of the ganglion cells identified in other animals (Prince et al., 1960).

2.3.3.1.9 Nerve fiber layer

It is composed of non-myelinated axons of the ganglion cells arranged parallel to the surface of the retina. The inner most layer of this nerve fiber layer is composed of the

supporting glial (Müller's) cell end feet (Dellmann, 1971; Dellman and Collier, 1987; Samuelson, 1999).

2.3.3.1.10 Inner limiting membrane

It is the basal lamina associated with reticular fiber and lies between the vitreous body and the end feet of the supporting glial cells of the retina (Leeson and Leeson, 1970; Dellman and Collier, 1987; Samuelson, 1999; Hees and Sinowatz, 2000).

2.3.4 Optic nerve

The optic nerve fibers run parallel to each other and are separated by septa which extend from the dura mater. They are well supplied with capillaries. The glial cells of the nerve are uniformly scattered throughout the fiber bundles (Prince et al., 1960).

2.4 Lectin histochemistry of the bovine eyeball

Cell surface saccharides are believed to be involved in a variety of cell functions, including development, growth regulation and cellular locomotion (Hakomori, 1981, Gabius et al., 1988).

Lectins are carbohydrate-binding proteins of non-immune origin derived from both plants and animals that bind to specific carbohydrates residues and provide a valuable tool for the localisation of sugar sequences in oligosaccharides, including glycoproteins and glycolipids (Liner et al., 1986; Sharon and Lis, 1975, 1989, 1990). They can be used as histochemical probes for specific carbohydrate residues found in cell membranes and the cytoplasm (Damjanov, 1987; Bopp et al., 1990; Spicer and Sculte, 1992).

On the basis of their carbohydrate-binding specificity lectins can be classified into: (1)glucose/mannose group (Concanavalin A(Con A)); (2)N-acetylglucose-amine group (*Wheat germ* agglutinin (WGA)); (3)galactose/N-acetylgalactose-amine group (*Dolichos biflorus* agglutinin (DBA), *Helix pomatia* agglutinin (HPA), *Helix aspersa* agglutinin

(HAA), *Psophocarpus tetragonolobus* agglutinin (PTA), *Griffonia simplicifolia* agglutinin-I-B4 (GSA-I-B4), *Peanut agglutinin* (PNA) and *Ricinus communis* agglutinin (RCA-I)); (4)L-fucose group (*Ulex europaeus* agglutinin (UEA-I)); (5)Sialic acid group (*Wheat germ* agglutinin (WGA) (Tuori et al., 1994).

The pattern of lectin-binding sites shows differences not only between the same organs of different species, but also within the organs of the same individual. For some of these differences, a correspondence to various stages of cellular differentiation, neoplastic alteration, and organ development could be shown (Liener et al., 1986).

Lectins have been applied in ocular tissue for various purposes: to study the differences between normal and migrating corneal epithelium (Gipson et al., 1983), to determine the distribution of carbohydrate residues on photoreceptor cell surfaces (Bridges, 1981), and to demonstrate the importance of glycoconjugates in the morphogenesis of the corneoscleral angle where they provide some of the required signals for the differentiation of the trabecular meshwork (Beauchamp et al., 1985).

2.4.1 Cornea

Previous studies have used lectins at light microscopal level to investigate normal cornea in a variety of species (Holmes et al., 1985; Panjwani et al., 1986a; Panjwani and Baum, 1988; Tuori et al., 1994).

In addition, using this technique, changes in patterns of glycosylation have been observed in wounded (Gipson et al., 1983; Gordon and Marchand, 1990) and dystrophic corneas (Panjwani et al., 1986b, 1987; Panjwani and Baum, 1989; Bishop et al., 1991). The cornea consists of a highly ordered stromal collagenous matrix, which is bounded on its anterior surface by a stratified squamous epithelium and on its posterior surface by an endothelial monolayer, which sits on a thick basement membrane, the Descemet's membrane (Maurice, 1984).

Formalin fixed, paraffin embedded sections of normal cornea from several species including human, cat and rabbit, were stained with a panel of nine lectins using an avidin-biotin-complex procedure. WGA stained the plasma membranes of all epithelial cell layers of the cornea of cat and human and the superficial and wing cells of rabbit.

The plasma membrane of the superficial and wing cells of the cat corneal epithelium was also stained with PNA and RCA I. Human and cat keratocytes were stained with WGA and RCA I. Stromal matrices of the all three species showed a distinct binding of ConA and LTA. Corneal sections from three species failed to stain with BSA I, BSA II, UEA I and SBA (Panjwani et al., 1986a).

Differences in lectin staining patterns of frozen and paraffin sections have been obtained. They may be related to differences in the solubility properties of various macromolecules (Panjwani et al., 1986a).

There are differences in the ability of lectins to bind to the components of frozen and paraffin sections from human cornea except for stromal matrix which fails to stain under both conditions. Con A, SJA and WGA intensively bound to the epithelium while the DBA and SGA didn't bind to the epithelium of the paraffin embedded corneal sections. Con A, RCA -120, BSL-I, PHA-L and WGA were intensely bound to the keratocytes in paraffin section, while the other lectins (LCA, PSA, PNA, SJA, DBA and SBA) only moderately bound to keratocytes.

WGA bound strongly to the endothelium of the cornea, while PSA and SBA only showed a weak staining of the endothelium. From the lectins used, WGA bound strongly to the epithelial basal lamina, while LCA, PSA, SBA, PNA, RCA –120 and UEA –I failed to stain it.

SBA, SJA, PNA, RCA –120 and UEA-I also failed to react with the Bowman's layer in paraffin sections of the cornea. The stromal matrix and Descemet's membrane did not react with any of the lectins (Brandon et al., 1988).

The anterior segment of the human eye was screened for differences in the lectin-binding pattern of ConA, PNA, GSA-I, WGA, SBA, DBA and UEA-I to enable cell typing for cell culture purposes (Rittig et al., 1990). All lectins bound to corneal epithelial cells, with some differences in binding intensity and location within the cellular layers. Whereas ConA and WGA are bound through all epithelial layers, binding of SBA, DBA, and UEA-I took place only at the superficial cells. PNA bound only to a few scattered cells within the basal and intermediate epithelial layers. ConA and WGA were bound by the endothelial cells of the cornea (Rittig et al., 1990).

Normal corneas showed staining of their epithelial cells, keratocytes and endothelial cells with the lectins ConA, PSA , LCA , e-PHA and WGA. The epithelium had strong staining of its basal and inner wing cell layers with all of these lectins, but showed progressively less staining of the more superficial layers.

The staining was localized to the plasma membrane and to the cytoplasmic granules, which were predominantly apical in the basal cells, with all these lectins, except ConA, which also stained the nuclear envelope strongly and produced weak diffuse staining of the cytoplasm.

The anterior surface of the cornea stained strongly with all lectins besides 1-PHA that gave only a weak reaction. Resolution at light microscopic level did not allow to determine the subcellular distribution of lectin binding within the keratocytes and endothelial cells. The normal corneal epithelial cell basement membrane and Bowman's membrane variably stained with ConA, PSA, LCA, e-PHA, and WGA. This staining, when present, was weak and often inconstant along the length of the membranes. The corneal stroma demonstrated diffuse moderate staining with ConA, PSA and LCA. No staining of Descmet's membrane was observed (Bishop et al., 1991).

Lectins which recognize N-acetylgalactosamine residues, namely SBA, WGA and VVA were identified as a component of the corneal and limbal, but not of the conjunctival basement membrane. In normal cornea, SBA distinctly labels the basement membrane zone separating epithelial cells from stroma. Although a similar localization pattern was also observed in limbal basement membrane, SBA labelling was not detected in the conjunctival basement membrane (Smithson and Kurpakus, 1995).

In the anterior segment of bovine eye some lectins reacted very intensely with apical cell surfaces of cojunctival and corneal epithelium suggesting a different glycosylation of the glycocalyx of the epithelia. PNA and RCA-I didn't bind at all, and GSA-I-B4 bound very weakly to the epithelium of the cornea. In addition, HPA, HAA, PNA and WGA didn't bind to the corneal basal membrane, but to conjunctiva and vascular basal membranes instead. This suggests that corneal basal membrane is somewhat different from other basement membranes. Lectins with the same carbohydrate specificity (DBA, HPA, HAA and PTA) reacted with sections of the anterior segment almost identically, but some differences were noticed: DBA did not bind to the basal membrane of the conjunctiva and

sclera but rather to the basement membrane of the cornea, whereas other lectins with the same carbohydrate specificities reacted vice versa. GSA-I-B4 reacted with endothelium of blood vessels and did not bind to stroma. This lectin reacted also strongly with corneal endothelium of blood vessels and did not bind to stroma. It made blood vessels especially prominent and it could be used as an endothelial marker. This lectin also reacts avidly with corneal endothelium. Therefore GSA-I-B4 appears to be specific marker for bovine endothelial cells for both blood vessels and corneal endothelium cells (Tuori et al., 1994).
Using frozen sections showed that mouse cornea stains fluorescein-conjugated Bandeiraea simplicifolia I agglutinin (BSA I) (Peters and Goldstein, 1979).
In frozen sections of the human cornea staining is also seen with both LTA and DBA (Bonvicini et al., 1983).
WGA was identified on basal and wing cells of rat corneal epithelium where they may contribute to cell migration (Gipson et al., 1983).
WGA binding sites were also observed throughout the endothelium, Descemet's membrane, stroma and the epithelium. The epithelial glycocalyx and plasmalemma were strongly labelled, while the epithelial basal lamina and intracellular binding sites were moderately stained. The stroma was also moderately and quite homogenously labelled.
GSA-1 binding sites were generally sparse throughout the corneal sections.
The number of ConA binding sites was generally moderate throughout the endothelium, stroma and epithelium (including cytoplasm and glycocalyx), but the density of labelling with ConA was stronger than the one noted with WGA especially within Descemet's membrane, which was heavily labelled (Lawrenson et al., 1998).
Con A conjugate bound to Bowman's membrane and stroma of the cornea, revealing a lamellated structure. The endothelial side of the Descemet's membrane and endothelium reacted with Con A conjugate. Corneal epithelium and BM, stroma, keratocytes, Descement's membrane and endothelium bound WGA conjugate. The apical cells in the corneal epithelium bound TRITC-WGA more strongly than the basal cells and the reaction was confined mainly to cell membranes.
LTA conjugates reacted with the epithelium, stroma, keratocytes, and endothelial side of the Descemet's membrane and endothelium of the cornea (Tuori et al., 1998).

A typical pattern of PNA binding was found in porcine cornea. The corneal epithelium (peripheral and central cornea) displayed strong membrane fluorescence in all cell layers. The basal membrane was positive whereas Bowman's membrane did not stain. The stromal collagen was generally negative. In the anterior stroma, the keratocytes were positive, whereas staining was only faint in the posterior stroma. In all stromal layers, long fibrillar structures, positive for PNA binding, were detected. Their appearance was more pronounced in the posterior stroma, where they seemed to join Descemet's membrane. Thus, they allowed differentiation of strongly positive anterior from a completely negative posterior part of Descemet's membrane. The endothelium showed a moderately positive staining of the cytoplasm, possibly also of the apical cell membrane (Vogelberg et al., 1988).

2.4.2 Sclera

The stroma of the bovine sclera reacted strongly with all conjugates except the TRITC-DBA and TRITC-UEA-I, which revealed only pale fibrils at higher magnification (Touri et al., 1994). Faint lectin staining could be seen with ConA and WGA in parts of the human sclera (Söderström, 1988).

2.4.3 Iris

The iridal muscle of the human iris reacted strongly with ConA and WGA, on the other hand GSA I, PNA and DBA bound weakly while UEA I did not give any reaction (Rittig et al., 1990).

2.4.4 Ciliary Body

The non-pigmented epithelium of formalin fixed and paraffin-embedded human ciliary body reacted intensely with LCA, Con A and RCA-I agglutinin. Positive reaction was also seen with WGA, PNA and SBA. Pre-treatment with neuraminidase to remove sialic

acid resulted in increased binding of PNA and SBA, and decreased binding of WGA. The results indicate that α-mannosyl, β-galactosyl, N-acetyl-D-glucosaminyl and N-acetylneuaraminic acid residues are present in glycocongugates of the nonpigmented epithelium of the ciliary body (Hietanen and Tarkkanen, 1989).
The lectin histochemistry revealed that the inner epithelial layer of the double-layered ciliary body epithelium of the rat eye was rich in sugar residues as shown by its positive reaction to S-WGA, PWA, DSA, GSA-I-B4, PNA, DBA, SBA, WFA, UEA-I, LTA and PHA-E. The reaction with GSA-I-B4, PNA, DBA and SBA were restricted to apical cell parts in the inner layer of ciliary processes. On the other hand, the outer epithelial layer was stained evenly by DSA and Jacalin, and partly by MAA, showing that this epithelial layer is rich in disaccharides. These lectin binding patterns of the ciliary body epithelium suggest a topographical and functional difference in this double cell-layered epithelium (Chan et al., 1999).

2.4.5 Retina

ConA and WGA bound to all canine retinal layers including the tapetum cellulosum, retinal pigmented epithelium, inner and outer segments of the rods and cones, plexiform layer and ganglion cells. Binding became more instense with increased age. s-WGA bound to apical portions of the retinal pigment epithelium and to outer segments of the rods and cones. They also bound ConA, WGA and RCA. DBA labelled all inner segments, while PNA bound to subpopulation of inner segments. RCA bound intensively to retinal vessels, as did PNA after neuraminidase treatment. UEA did not consistently bind to any layers. There was transient binding by some lectins at different developmental stages. This study indicates that canine retina demonstrates differential expression of glycoproteins, as indicated by lectin binding during development and that this expression is temporally and topographically regulated (Whiteley and Scott, 1990).
Proliferating retinal pigmented epithelium cells revealed lectin binding sites for Con A, WGA, PNA, and RCA I. SBA, UEA I, DPA and LPA were not found to react with RPE. These results indicate that lectin histochemistry allows cytochemical identification of

normal and reactive RPE, which shows positive staining with Con A, WGA, PNA and RCA I not found in other cell types of retinal origin (Bopp et al., 1990).

There are notable interspecies differences in lectin binding sites in retina of mammals. In porcine retina, a distinct labelling of all neurons of the stratum ganglion was obtained by using s-WGA. In baboon stratum ganglion didn't label, though the entire inner plexiform layer can be stained with s-WGA. In cat, s-WGA binds exclusively to the outer segment of rod and cones (Lange et al., 1990).

The normal retinal pigmented epithelium has receptors for the lectins Con A, WGA, PNA and RCA I, mostly localized in the apical portion of the cells. Binding sites of these lectins are also found on photoreceptor cells, but with different binding characteristics (Bopp et al., 1989).

Con A stained specifically several human retinal structures. In the ganglion cell layer cytoplasm of some ganglion cells was intensively stained. Both inner and outer plexiform layers were faintly marked. Cell borders in both inner and outer nuclear layers were slightly stained. The outer segment of rods and cones was stained intensely, as well as the inner segment of rods.

WGA faintly stained the inner and outer plexiform layers. Both inner and outer segments of rods and outer segment of the cones bound Con A. The inner segment of rods was less intensely stained than the outer segment. The inner segment of the cones was unstained. PNA selectively stained only inner segments of cones (Söderström, 1988).

In human retinal pigmented epithelium cells, which are in contact with the normal outer retina, binding was observed for Con A, WGA, PNA and RCA I. The positive reaction product was localized in the apical portion of the RPE cells including the microvilli. The basal part of retinal RPE-cells remained unstained, except for Con A which showed a faint basal staining. No binding in retinal pigmented epithelium was detectable with SBA, UEA I, DBA and LPA (Bopp et al., 1992).

The binding of eight fluorescence-labelled lectins to photoreceptors of monkey retina was investigated using a post-embedding staining method (Uehara et al., 1983a). Con A bound to the outer and inner segment of both rods and cones, while the degree of staining was more intense in rods. Outer segments of rods showed patchy fluorescence and proximal portions of inner segments were diffusely stained. WGA bound to the surface of

the outer and inner segments of both rods and cones. RCA-1 stained rod outer segments, particularly strongly in the region dividing outer and inner segments. The cones were also stained, although faintly. The distal halves of the rod and cones showed diffuse weak staining and their proximal halves stained spotty. PNA bound to the cones only scarcely. The external surfaces of both outer and inner segments of cones were uniformly stained. The interior of the cone outer segments was also stained, while the interior of the inner segment was not. Two lectins specific for fucosyl residues namely, UEA-1 and LTA, bound diffusely to the distal halves of the inner segments of both rods and cones. Lectins reacting with N-acetyl-galactosamine residues, DBA and SBA, bound weakly to the distal portions of rods and cones (Uehara et al., 1983a).

In monkey, pig, cat and rabbit, cones were intensely labelled with the lectin PNA but the rods were unlabelled. The cones synaptic pedicles, inner synaptic layer, internal limiting membrane and retinal vessels were also labelled. It has been suggested that galactosyl and/or galactosaminyl residues are present in the mammalian cones, but not in rods (Kawano et al., 1984).

2.5 Electron microscopic examination of the bovine eyeball

2.5.1 Cornea

The mammalian cornea consists of four and sometimes five layers. These are the corneal (anterior) epithelium, Bowman’s layer (present only in primates), corneal stroma, Descemet’s membrane, and endothelium.

The epithelium covering the anterior part of the cornea is of the nonkeratinizing stratified squamous type.

Electron microscopic investigation shows that the basal layer of low columnar cells are firmly attached to the basal lamina by hemidesmosomes. In man and primates, the basal lamina thick and is referred to as Bowman’s membrane (Samuelson, 1999).

Toward the surface of the epithelium the cell gradually change from columnar to polyhedral or wing cells and to flat surface cells. The nuclei of the basal cells are

extremely dense but gradually become less electron dense in cells located close to the surface (Rhodin, 1963).

The basal cells are tall, columnar cells with a flattened base and domed apex. They are crowded together, and as a result, the nuclei, which are located in the apical region, are often forced into two or alternating layers. Mitosis is confined to the basal cells or those cells immediately superficial to the basal cells (stratum germinativum).

Wing cells are s group of polygonal cells on top of the basal cells. They form a various number of layers, depending on the species and the location of the cornea. These layers are a transient zone between the columnar basal cells and the more superficial squamous cells. The flattened superficial cells compose several layers.

The cytoplasm of the superficial cells contains numerous tonofilaments and vesicles, but it generally lacks cell organelles like mitochondria, rough endoplasmic reticulum, and ribosomes. Numerous desmosomal attachments are visible, and the surface cells have zonulae occludentes on their lateral membranes. Microprojections are visible on the surface cells (Rhodin, 1963; Shively and Epling, 1970; Hogan et al., 1971). The origin of the microvillae appears to be from detached desmosomal junctions, but other authors have found no evidence for this (Hogan et al., 1971; Hoffman and schweichel, 1972). Nerves that enter the epithelium lose their ensheathment and terminate in naked nerve endings among the wing cells (Hogan et al., 1971).

The corneal stroma comprises 90% of the thickness of the cornea. It consists of transparent, almost structureless lamellae of fibrous tissue, and these lamellae lie in sheets and split easily into planes. Between the lamellae are fixed and infrequent wandering cells. Occasionally Schwann cells occur in conjunction with thin unmyelinated nerves (Rhodin, 1963). The fixed cells are fibrocytes, which are called also keratocytes. Their cytoplasm shows a predominance of ribosomes, and profiles of rough-surfaced endoplasmic reticulum (Rhodin, 1963). These cells can transform into fibroblasts when deep cell injury occurs, and they may form scar tissue that is not transparent. Wandering cells are usually leucocytes that have migrated from the limbus.

The lamellae are parallel bundles of collagen fibrils, with each lamella running the entire diameter of the cornea. All the collagen fibrils within lamellae are parallel, but between lamellae, they vary greatly in direction (Rhodin, 1963; Samuelson, 1999).

A distinct Bowman's layer is described in large, herbivorous mammals (Murphy et al., 1991). In bovine corneal stroma, collagen fibrils are tightly packed into fascicles in a lamellar fashion. In each lamella, collagen fibrils run in almost the same direction. The diameter of the collagen fibrils is within the range of 25-30 nm and the interfibrillar space appeared to be almost uniform. Keratocytes are located in the interfascicular spaces of collagen fibers, and amorphous materials are found in these interfascicular and pericellular spaces (Takahashi and Tohyama, 1991).

Descemet's membrane is distinctly layered in most animals, usually having a relatively thin anterior unbanded zone next to the stroma. It is characterized by an amorphous substance, however cross striations of coarse bands and filaments occur interconnected by thin filaments (Smolek and Klyce 1993).

The Descemet's membrane of the mouse cornea has been shown to contain filamentous and granular structures. Their size and amount differ significantly (Yamada, 1955; Jakus, 1956, 1964).

The Descemet's membrane of the bovine cornea lies just under the corneal endothelial cells. When cut transversely to the surface, the Descemet's membrane showed a regular organization: extremely fine filamentous structures coursed parallel to the surface of the membrane and dense lines ran vertical to the surface (Sawada, 1982).

The corneal endothelium cells are of a squamous type with elongated nuclei. Desmosomes are not present, but special area of the plasma membrane occur where the intercellular space is decreased locally (Rhodin, 1963). The extensive lateral convoluted interdigitations between adjacent cells in the dog. Along the lateral cell margins, the cell junctions, zonulae occludentes, maculae adherents and nexies occur (Samuelson, 1999).

2.5.2 Sclera

The bulk of the sclera is called the sclera proper. It contains elastic fibers that are interlaced among the collagen fibers, as well as melanocytes (anteriorly) and fibrocytes. The collagen fibers, fibrocytes, and occasional melanocytes are arranged meredionally, obliquely and radially in an irregular fashion (Samuelson, 1999).

2.5.3 Iris

The iris is divided into the anterior border layer, the stroma and sphincter muscle, and the posterior epithelial layer.

The anterior border layer consists of two cell types; fibroblasts, and melanocytes, but more fibroblastic in nature (Tousimis and Fine, 1959; Rohen, 1961; Donovan et al., 1974). The anterior cells, which lack a basement membrane, form an almost continuous layer with their cellular processes, but frequent small openings with large intercellular spaces and extension of underlying melanocytes processes break the continuity. One or more layers of melanocytes are beneath the single layer of fibroblasts. For the most part, the melanocytes are oriented parallel to the iris surface and their processes intermingle with other melanocytes and anterior fibroblasts with no intercellular junctions. The pigment granules in the cat and dog are lanceolate to ovoid in shape, whereas they are round to ovoid in horse (Tousmis, 1963).

The stroma is composed of fine collagenous fibers, many chromatophores, and fibroblasts. The stroma is loosely arranged except around the blood vessels and nerves, at which it can form dense sheaths. The collagen fibrils are organized to some extent in overlapping, wide arcades running from the pupil to the ciliary body (Rhodin, 1963; Shively and Epling, 1969). Fibroblasts and melanocytes are the predominant cell types that are evenly distributed throughout the stroma. In addition to scattered melanocytes in the anterior stroma, dense band of melanocytes can be present in the ciliary zone anterior to the dilatator muscle, extend centrally to the sphincter muscle. The melanocytes are also prominent around the adventitia of blood vessels. The shape of the melanin granules varies with species and with the maturity of granules. The granules are generally smaller and more rod like than the pigment granules of the posterior epithelium. In the cat they are rod like, in dog they are both oval and rod like (Tousmis, 1963).

Particularly in the horse and the dog, large cells containing pigment are associated with capillaries and venules near the sphincter muscle (Tousmis and Fine, 1959; Woberman and Fine, 1972). These are believed to be macrophages of haematogenous origin. In humans, these cells known as the clump cells of Koganei (Woberman and Fine, 1972). The cytoplasmic pigmented inclusions are so dense that they obscure the nucleus.

The smooth muscle cells of the sphincter have a small diameter is located in the stroma near the pupil. In dog and cat, it lies in the posterior stroma, separated from the pigmented epithelium and adjacent to the dilatator muscle by thin layer of connective tissue. In horse it occupies the main portion of the central stroma. In the bovine dilatator muscle is a single layer of unstriated muscle fibers in the posterior iridal stroma. These muscle fibers apically contain pigment around their nuclei. The basal region of each cell contains myofilaments (Samuelson, 1999).

The basal aspect of the posterior epithelium of the iris faces the posterior chamber and has numerous surface projections. The posterior surface of the iris contains radial folds that extend to the base of the ciliary processes. These folds are radially oriented. The posterior pigmented epithelium of the iris contains two layers of cells, inner and outer layer. A basement membrane separates the cells from the posterior chamber but does not follow all the invaginations of the cell surface. The lateral cell surface of the posterior epithelium has numerous slender cell processes with scattered desmosomes (Rhodin, 1963; Donovan et al., 1974). The cytoplasmic density of the superficial layer is greater than that of the deep layer, mostly because of a denser accumulation of ribosomes. The mitochondria are spherical and dense in both cell types, but the pigmented granules are larger in the cells of inner layer (Rhodin, 1963). In general, large spaces occur between the lateral cell membranes, which allow free access to the posterior chamber. The nucleus of the iridal posterior epithelium in the dog is oval and moderately indented, whereas the nuclei in horse are often bizarre-shaped, being indented by adjacent pigment granules. The epithelial (i.e., apical) portion of the dilatator muscle (i.e., anterior epithelium) is located adjacent to the apical portion of the posterior epithelium and contains the cell nuclei. Melanin granules are mostly present in this portion of each cell. In the horse the basement membrane material fills much of the intercellular space between projections of the myoepithelium (Samuelson, 1999).

2.5.4 Ciliary body

Each ciliary process consists of a central core of stroma and blood vessels covered by a double layer of epithelium; an inner nonpigmented, cuboidal epithelium, and outer pigmented cuboidal epithelium.

The nuclei of the inner nonpigmented epithelial cells are large. The cytoplasm displays a fair number of ribosomes. The mitochondria are few and have a spherical shape (Rhodin, 1963).

The lateral cell surface of the nonpigmented epithelium has numerous villous processes along the bottom one-half to two- thirds (Fine and Yanoff, 1979). The lateral intercellular junctions of the nonpigmented epithelium consist of desmosomes, except at the apical end. The apical ends possess gap junctions, zonulae adherentes, and zonulae occludentes, which probably represent the anatomic blood-aqueous barrier (Rhodin, 1963; Smith, 1971; Smith and Rudt, 1973; Shabo et al., 1976; Smith and Raviola, 1983; Streeten, 1988).

Cellular junctions between the nonpigmented and pigmented epithelium of the ciliary process are very important, because this cell layers are tightly locked together in a physiologic way. The types of junctions found here reflected this importance. They consist of many gap junctions interspersed with desmosomes, and unusual junctions termed puncta adherentes, which resemble desmosomes but lack the larger tonofilaments and associated intercellular central band (Streeten, 1988). There are also dilated portions of the apical intercellular space with villous cytoplasmic processes protruding into them. These dilatations have been termed ciliary channels, and they are usually near the apical termination of two adjacent cells.

The pigmented epithelium is generally cuboidal and heavily loaden with round to oval melanin granules. The lateral cell surfaces of the pigmented epithelium are joined by desmosomes, and the basal cell surfaces have no specialized junctions. The nuclei of pigmented epithelium are located apically. The cytoplasm contains rough and smooth endoplasmic reticulum, free ribosomes (i.e. polysomes) and spherical or slightly elongated mitochondria (Rhodin, 1963; Samuelson, 1999).

The smooth musculature of the primate ciliary bodies is generally believed to be the most highly developed among mammals. The muscle has three components (i.e., radial, meridional, and circular), and forms a large, anterior pyramidal structure that provides a strong base plate for iridal attachment.
Transmission electron microscopy of different ungulates has revealed that many of the muscle fibers, especially those located anteriorly, course more circumferentially than meridionally (Samuelson and Lewis, 1995).

2.5.5 Choroid

Histologically the choroid can be divided from externally to internally into the suprachoroid, the stroma with large blood vessels, the stroma with medium-sized vessels, the tapetum and the choriocapillaries.
The suprachoroid consists of elastic, heavily pigmented connective tissue. In coloured rabbits, the choroidal melanocytes are situated predominantly in this region, being intimately associated with elastic fibers (Funata et al., 1990).
The layers of melanocytes and fibroblasts and the interspersing collagen and elastic fibers may produce resistance to uveoscleral drainage, even though a cellular barrier has not been found to exist (Koseki, 1992).
The stroma contains a vascular plexus embedded in loose connective tissue with melanocytes and fibrocytes (Samuelson, 1999).
The medium sized vessels, especially the arteries, branch dichotomously. They are radiating slightly inward in a fanlike manner from the large vessels. The cells surrounding these vessels consist of melanocytes and fibroblasts. In heavily pigmented individuals, the melanocytes are the predominant cell type. In most domestic animals the melanocytes possesses characteristically oval-to-round melanin granules ranging from 0.1 to 4.0 µm in diameter. The extracellular space consists of loosely arranged bundles of collagen mainly running parallel to the choroidal surface, and it is interspaced with numerous elastic fibers (Samuelson, 1999).
In ungulates, closely and regularly arranged collagen fibers comprise the tapetum, which is referred to as a fibrous tapetum. The fibrous tapetum is basically acellular, except for

occasional fibrocytes. The collagen fibrils are organized into well-ordered lamellae that branch and interconnect with adjacent lamellae at the same level, being parallel with the retinal surface. The collagen fibrils within each layer vary little in diameter, being approximately 80 nm, and have regular spatial arrangement similar to that seen in corneal stroma.

Choriocapillaries is the inner most layer of the choroid, forming a thin layer with numerous capillaries. The lumens of the capillaries is fairly wide, allowing red blood cells to pass through. The endothelial lining possesses numerous circular fenestrations, which are often arranged in rows. External to the endothelium is a basement membrane forming the external layer of Bruch's membrane (Samuelson, 1999).

2.5.6 Retina

Histologically the retina consists of ten layers which are usually considered from outside inwards in the following order: retinal pigmented epithelium, visual cell layer (rod and cone layer), outer limiting membrane, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer, nerve fiber layer, and inner limiting membrane.

Retinal pigmented epithelium (RPE) is a layer of flat, polygonal or cuboidal cells with round nuclei cells that form the outermost layer of the retina. There are numerous infoldings of the cellular membrane at the base of each cell. These are indicative of the extensive, ongoing transport between the RPE and the adjacent choriocapillaries (Rhodin, 1963; Samuelson, 1999). Each cell sends cytoplasmic processes inwards to surround the visual receptors, to insulate them from bright light, and to increase their individual sensitivity. They also phagocytose the outer segments of the photoreceptors, as they are continuously shed.

The cells are usually densely pigmented, and mononucleate. The cells near the ora ciliaris retinae are larger and often binucleate.

The nuclei are centrally positioned, round oval, with its long axis parallel to the retinal surface. The cytoplasm is predominately filled with smooth endoplasmic reticulum. Mitochondria are numerous, being concentrated in the basal two-thirds of the cell. In cells

that do not overlie the tapetum lucidum, melanin granules are present. They are either elongated or lanceolate and located in the apical to mid portion of the cytoplasm (Samuelson, 1999). Additionally other bodies, including residual bodies of lipofuscin, lamellated bodies, and phagosomes consisting of phagocytosed outer segment discs of photoreceptor occur (Shively et al., 1970; Hebel, 1971; Nilsson et al., 1973a).

Lysosomes also appear to increase in number in this region of the retinal pigmented epithelium (Braekevelt, 1990). This may indicate enhanced shedding of the outer segment material over the tapetum however, results of recent studies on melanogenesis suggest that the lysosomal system is closely associated with that of the melanosomes, and that the additional lysosomes may be a part of an underdeveloped or partially suppressed melanosomal system (Schraermeyer, 1993; Jimbow, 1995; Orlow, 1995).

The morphology and location of the RPE melanosomes varies to some degree among different animals. The shape of melanosomes is typically oval, but they can be more elliptical in diurnal species (Kuwabara, 1979).

The inner apical surface of the RPE has numerous long villous processes that lie between and surround the outermost and oldest portion of the outer segments of the photoreceptor. Microvilli are especially large and long in the RPE of many non-mammalian animals (Kuwabara, 1979). It is not unusual that the tips of these processes extend to the level of the inner segments, and even make direct contact with the microvilli of the Müller cells.

The lateral RPE cell surfaces near the apical ends have well defined junctional complexes, including zonulae occludentes and a zonulae adherentes.

The basal aspect of the RPE rests on a basal lamina over the choriocapillaries. Marked basal infoldings of the cell membrane contrast sharply with the basal lamina, which runs relatively smooth. The size and organization of the basal infoldings vary to some degree among mammals (Braekevelt, 1986a, 1990).

The basal lamina of the RPE and adjacent choriocapillaries collectively form a complex structure called Bruch’s membrane. When fully developed, Bruch’s membrane consists of five layers: the basal lamina of the pigment epithelium of the retina, an inner collagenous zone, an elastic layer, an outer collagenous zone, and the basal lamina of the choriocapillaries.

In most species the outer collagenous zone is thinner than the inner collagenous zone (Samuelson, 1999). The RPE cells of the bovine are predominantly mononucleate. The intranuclear basophilic bodies are prominent, and the melanin granules are especially numerous.

The retinal photoreceptors comprise two types, rods and cones cells. Each consists of an inner and an outer segment. Their nuclei are forming the outer nuclear layer.

The outer segments of the rods and cones are composed of stacks of membranous discs that consist of double layers of lipid molecules, sandwiched between very thin layers of proteins or glycoproteins surrounded by the cell membrane (Rhodin, 1963). The rods form stacks of uniform width through their length and are longer than the cone outer segments. The cone outer segments also consist of stacks of discs, but these segments are wider at their inner end, producing a cone shape. In the area centralis, these cones are longer and more slender. Nevertheless, they are easily distinguished from the rods. The discs are actually flattened spheres. They consist of two membranes that are continuous at their ends and, in rods, are separated from the cell membrane as well as the adjacent discs (Hogan et al., 1971; Rodieck, 1973; Seifert and Spitznas, 1996).

In some mammalian cones, the proximal discs have continuity with the cell membrane, but it is uncertain if this is a general feature of all mammals (Rodieck, 1973). Nevertheless, these invaginations of the cell membrane, which forms the disc lamellae, constitute the major morphologic difference between rods and cones (Cohen, 1969). Within each mammalian cone outer segment, the discs contain one of the photopigments, which are sensitive to one of three different wavelength ranges. The rod and cones outer segments are connected to the inner segments by a modified cilium, whose basal body lies in the distal inner segment. The cilium extends for a variable distance into the outer segment. The outer portion of the inner segment is filled with long, tubular mitochondria and small cytoplasmic vesicles. It is termed ellipsoid and (Rhodin, 1963). Distally within the ellipsoid and to one side is the basal body, or one of a pair of centrioles, that gives rise to the connecting cilium. The ellipsoid of the cones is broader and more conical, and it contains more mitochondria than the rod ellipsoid (Rodieck, 1973; Fine and Yanoff, 1979; Gelatt and Samuelson, 1982). The vitreal or inner portion of the inner segment is called myoid in lower vertebrates because of the presence of myofilaments and

contractile properties (Rodieck, 1973; Fine and Yanoff, 1979; Gelatt and Samuelson, 1982). Although there is an absence of contractile structures in higher animals, the term myoid is often used for this portion of the inner segment. The vitreal inner segment contains few mitochondria in rods and is relatively void of mitochondria in cones. The cytoplasm mostly consists of smooth and rough endoplasmic reticulum, free ribosomes, Golgi apparatus, small vesicles, and microtubules, and is the principal site of protein synthesis. In the pig, the vitreal portion of the cones also may contain a fairly prominent vesicle.

The inner segments of the rods and cones are separated from each other by long, villous extensions of Müller cells, which are called fiber baskets. These fiber baskets are virtually morphologically identical among the vertebrate species, but they are less numerous in retinae with intraretinal vasculatures (Uga and Smelser, 1973). It has been hypothesized that these cell processes may serve to keep the extracellular portion of the visual cell layer dehydrated and, thus, help to maintain the main proper lignment of the outer segments (Siglman and Ozanics, 1988). These processes are also most likely involved in the exchange of metabolites with the RPE and help to provide a homeostatic environment for the outer segments (Magalhaes, 1976).

The outer segments of the bovine retina are easily distinguishable as rods and cones, with the rod photoreceptors clearly predominating. Approximately 15 rods come for every cone. The cone outer segments are 3-4 μm in length and 0.8 μm in average diameter. The cones are bounded by a plasma membrane distinct from the discs membranes. The saccules in cone outer segments possess intradisc and interdisc spacings which are both larger than the corresponding spacings in the rod saccules.

The rod outer segments are 7-10 μm in length and 1-2μm in diameter. The outer and inner segments are connected by a modified cilium similar to that observed in other vertebrates (Cohen, 1960, 1961; Brown et al., 1963; Cohen, 1965; Holmberg, 1970). The mitochondria of the receptor cells are concentrated at the apex of the inner segment layer. Other supporting material surrounds the mitochondria, namely small osmiophilic vesicles and granules, numerous strands of a granular endoplasmic reticulum, and some free ribosomes and polyribosome. In the vitreal end of the inner segment, mitochondria are sparse, but an extensive Golgi complex is evident, and long cisternae of granular

endoplasmic reticulum predominate in most cases oriented parallel to the long axis of the inner segment.

The central and peripheral region of the bovine retina differs in the frequency of cones. The central region possesses a higher density, but the cone density is relatively low compared to other vertebrates (Mason et al., 1973).

The outer limiting membrane is composed of the densities of the cell junctions, the zonulae adherentes that firmly attach the inner segments of rods and cones to Müller cells and the Müller cells to each other (Samuelson, 1999).

The outer nuclear layer contains the cell bodies and nuclei of the photoreceptors. The nuclei of the cones are generally situated next to the external limiting membrane. In mammals, they are usually larger, oval and more rich in euchromatin (i.e., staining lighter in the TEM) than the rod nuclei. Additional structures in this layer are outer rod and cone connecting fibers, rod and cone axons and Müller cell processes. The rod and cone connecting fibers are continuations of the inner segments to their origin from their respective cells. The axons of rod and cone with cells extend in the outer plexiform layer to synapse with the horizontal and bipolar cells (Rhodin, 1963 and Samuelson, 1999).

The outer plexiform layer consists of the terminal arborisation of the axon of the rod and cone cells that synapse with the dendrites of the horizontal and bipolar cells. The axons of the rods typically end in pear shaped spherule structures whereas those of the cones end in larger, broad pedicles. The rod spherules have one or more invaginations at which ribbon synapses occur whereas the cone pedicles have numerous, more shallow invaginations of ribbon synapses. The cone pedicles usually extend further vitreal into the outer plexiform layer (Shively et al., 1970; Hogan et al., 1971; Rodieck, 1973).

The terminal branchlets of the bipolar dendrites end in an invagination of the plasma membrane of the dense synaptic end of the photoreceptor cells. In the center two large vacuoles are located, and the dendritic branchlets of the bipolar cells establish connection with both vacuoles and the receptor cell membrane. The density of synaptic ends is caused by the presence of numerous small synaptic vesicles, peculiar, ribbon-shaped structures and numerous mitochondria (Rhodin, 1963).

The inner nuclear layer is composed of the soma and nuclei of horizontal cells, bipolar cells, amacrine cells, and Müller cells. The neurons in this layer maintain connections

between the visual cell layer and the ganglion cell layer. The horizontal cell nuclei are positioned along the outer most margin of the inner nuclear layer, whereas the amacrine cells are situated along the inner most margins. Bipolar nuclei and Müller cell nuclei compose the intermediate zone of the inner nuclear layer (Rhodin, 1963; Shively et al., 1970; Gelatt and Samuelson, 1982; Sigelmann and Ozanice, 1988).

Two types of horizontal cells have been identified in most vertebrates, small field horizontal cells and large field horizontal cells with axons (Hu and Mah, 1979). More recently, an axonless horizontal cell, known as type A, has been found in several mammalian species. They are synapsing with all kinds of cones (Gallego, 1986; Boycott at al., 1987). In the second type, type B, the dendritic endings synapse with the same cone pedicles as type A, but have although axons, which synapse with rod spherules (Kolb and Famiglietti, 1974). A third type, which also possesses an axon, is known to exist in the human retina (Kolb et al., 1992). The nuclei of horizontal cells are large, with a single prominent nucleolus. The cells are characterized by their wide, horizontally oriented cell processes.

The bipolar cell is the second most numerous neurons in the retina of the domestic animals, and it constitutes the radial connection between the photoreceptors and the ganglion cells. In cone rich retinas, the numbers of the bipolar cells increase remarkably as do those of amacrine cells. Being radially oriented, their dendritic processes in the outer plexiform layer synapse with photoreceptors and horizontal cells, and their axonal processes terminate in the inner plexiform layer. The bulk synapses with amacrine and ganglion cells. Among mammalian retinas, the inner nuclear layer houses the somata of a single type of rod bipolar cells and a variety of cone bipolar cells.

Rod bipolar cells usually connect only with rod spherules. In the cat, they synapse with two types of amacrine cells in the inner plexiform layer. These bipolar cells, which are in contact with many rod spherules, do not synapse directly with ganglion cells (Samuelson et al., 1992).

Cone bipolar cells by comparison, can be divided into many types, consisting of eleven in the cat and nine in both rabbit and monkey (Mills and Massey, 1992; Boycott and Wassle, 1991; Strettoi and Masland, 1995).

The cytoplasm of bipolar cells can be identified by their microtubules and their nuclei, that are slightly smaller and more osmiophilic than those of horizontal cells.

The amacrine cell has been described as a neuron without an identifiable axon whose processes terminate in the region of the internal plexiform layer (Rodieck, 1973). Ultrastructural studies demonstrated that these neurons are of the pseudounipolar type, having axon with the characteristic synaptic vesicles but also having features in common with dendrites (Sigelman and Ozanics, 1988). The amacrine cells are located vitreally in the inner nuclear layer. They are recognized by indented euchromatic nuclei. Their cytoplasm is more copious than that of bipolar cells, being filled with polysomes, rough endoplasmic reticulum (i.e. Nissl bodies), mitochondria, neurofilaments, and microtubules (Samuelson, 1999).

Müller cells are the principal non-neuronal cell of the vertebrate retina and serve as supportive cells for most neurons in the retina. They tend to have more cytoplasm and to lie in the inner portion of the inner nuclear layer. Their nuclei are angular and have denser chromatin than other nuclei in the inner nuclear layer (Samuelson, 1999). In animals with an intraretinal vascular system, the outer processes are scanty in cellular cytoplasmic organelles and light-staining, whereas the inner fibers contain more organelles. Numerous filaments are present, especially near the internal limiting membrane, and a very well-developed smooth-surfaced endoplasmic reticulum is present (Uga and Smelser, 1973; Nilsson et al., 1973b). The vitreal ends of the Müller cells possess end feet, which have the ability to phagocytose foreign substance and, consequently may play an important role in normal retinal function (Nishizono et al., 1993).

The inner plexiform layer comprises the cell processes of the inner nuclear layer and ganglion cell layers, at which synapses between bipolar, amacrine, and ganglion cells occur.

The bipolar axons contain numerous synaptic vesicles and mitochondria, and they are the only structures to possess synaptic ribbons. The ganglion cell dendrites are the only processes in the plexiform layer without synaptic vesicles. They are pale, with smooth and rough endoplasmic reticulum, small mitochondria, and microtubules. The amacrine processes, which are also pale and possess large mitochondria and synaptic vesicles, are the most numerous in the retina and show an extensive arborisation of their axons

(Rhodin, 1963; Samuelson, 1999). Aside from the neurochemical synapses, gap junctions occur between bipolar axons and ganglion cell somata as well as between rod AII amacrine cells and midget cone bipolar cells (Kolb, 1979).
The ganglion cell layer usually contains ganglion cells of three different types (Boycott and Wassle, 1974) described as α, β and γ ganglion cells in the retina on the basis of dendritic fields. These morphologic types correspond to the three physiologic types of ganglion cells (W, X, and Y) (Boycott and Wassle, 1974; Levick, 1975).
Ultrastructurally, the different types of ganglion cells have not been differentiated (Seifert and Spitznas, 1996). In general they have a fairly large nucleus and light cytoplasm (Rhodin, 1963). The cytoplasm is characterized by aggregates of rough endoplasmic reticulum and ribosomes, scattered through the cytoplasm, a smooth-surfaced endoplasmic reticulum, dense membrane-bound bodies, and mitochondria (Shively et al., 1970; Hogan et al., 1971; Beauchemin, 1974).
In the nerve fiber layer Müller cells, are found. These astrocytes have long, branching processes and are found in the supportive role structurally and, perhaps nutritionally around blood vessels and nerve axons (Hogan et al., 1971; Smith and Rudt, 1973; Rodieck, 1973).
The inner limiting membrane is formed by the fused terminations of Müller cells.

2.5.7 Optic nerve

The optic nerve is formed by the axons of ganglion cells, by glial cells, and septae. The axons within the optic nerve are easily distinguished by their tubular processes, which contain evenly dispersed neurofilaments, neurotubules, occasional vesicles and mitochondria. These organelles and other cytoplasmic materials are being continuously moved within the axon toward the synaptic ending. Movement of these materials, which is known as axonal flow, is responsible for the constant supply of newly synthesized proteins to the synapses (Samuelson, 1999).

3 MATERIAL AND METHODS

3.1 Examined materials

Eyeballs were obtained from 13 adult clinically healthy animals of both sexes. These materials were collected from the Munich slaughterhouse.

3.2 Light microscope

The eyeballs were isolated from the head and then dissected by a sharp scalpel into two halves. Specimens were taken from the cornea, sclera, iris, ciliary body, choroid, retina and optic nerve, with a side length of 0.3 - 0.5 cm. The samples were fixed in Bouin's solution (Romeis, 1989) and in 7% buffered formalin solution. Fixation took 24 hours. They were then preserved in 70% ethanol for 24 hours until embedding.

3.3 Embedding

After fixation the specimens were dehydrated in a graded series of alcohol then embedded automatically by using a Shandon-Duplex-Processor. Finally the prepared tissue specimens were embedded in paraplast blocks. Sections were cut using a Leitz rotatory microtome (type 1516) at 4-5µm.

3.4 Histological Staining

Sections were deparafinized in xylene, then rehyderated in ethanol of descending concentration down to distilled water. After staining they were dehydrated in ascending concentrations of ethanol, clarified in xylene and covering with Eukit® (Romeis, 1989). Different staining procedures were performed, Haematoxylin and Eosin staining (H&E) (Harris, 1898) method according Romeis (1989) for detection the general histological structure of the examined bovine eye. Periodic Acid Schiff reaction (PAS), Drury and

Wallington, (1980) after McManus, (1948) and Periodic Acid Schiff reaction after amylase digestion, Alcian blue 8 G X (pH 2.5) and (pH 1.0) Steedman, (1950) for detection the mucopolysaccharides. Trichrom staining according to Goldner, (1938), Resorcinfuchsin-Nuclear red (Elastic stain) after Weigert, (1898) for detection of the elastic fibers, and van Gieson's method, (1889), after Gabe, (1976).

3.5 Light microscopic examination

The stained sections were examined using Leitz Dialux 20 Microscope. Photos were taken by using Agfa Pan 25 ASA, Kodak, Tri-X pan, 400ASA, Kodak, or Ectachrome plastic, 64 ASA films.

3.6 Lectin histochemistry (Glycohistochemistry)

Detection of the glycoconjugates was done by using Fluoresceinisothiocyanate (FITC)-labelled lectins (Sigma;-Munich) the used lectin is listed in (Table 1). Deparaffinization was performed in xylene for 20 minutes, rehydration in alcohols isopropanol; 96% ethanol; 90% ethanol; 80% ethanol; 70% ethanol; distilled water for 5 minutes each. After washing with 0.05 M Tris buffered saline (TBS) at pH 6.8 for 30 minutes at room temperature. The sections were incubated for 12 hours at 4°c with following FITC-conjugated lectins (Con A, LTA, ECA, SBA, VVA, WGA, PNA, GSA I, UEA I) (Table 1). The lectin concentration was 33µg/ml buffer. After incubation the sections were washed with Tris buffered saline (TBS) at pH 6.8 three times and covered with polyvinylalcohol and ethylenglycol in Tris buffer at pH 8.5 (Serva, Heidelberg). The control group was incubated with PBS only. Another group of sections was incubated with different FITC-conjugated lectins and their inhibitory specific sugars (i.e. Con A + glucose/mannose group; WGA or VVA + N-Acetylglucosamine group; PNA or SBA or LTA or ECA or GSA I + N-Acetylgalactosamin/Galactose group and UEA I + fucose group) to confirm the specificity of the results. The sections were studied using a Leitz Dialux 20 microscope. The photos were taken by using Kodak T max film 400.

Table 1: FITC-labelled lectins

Abbreviations	Lectins	Carbohyderate specifity
I Glucose/mannose group		
Con A	*Concanavalin A*	Mannose (α/β-Man>α-GLc>α-GLcNAc)
II N-Acetylglucosamine group		
WGA	*Wheat germ* agglutinin	N-Acetylglucosamine (GlcNAc-[(β1,4)-GlcNAc]$_{1-2}$> [β-GlcNAc]$_n$>Neu5Ac)
III N-Acetylgalactosemin/ Galactose Group		
PNA	*Peanut* agglutinin	Galactose (Gal(β1,3)-GalNAc)
ECA	*Erythrina cristagalli* agglutinin	Galactose (Gal(β1,4)-GalNAc)
SBA	*Glycine maximus* agglutinin	N-Acetylgalactosemin (α-GalNAc>β-GalNAc>α-/β-Gal)
GSA I	*Griffonia simplicifolia* agglutinin I	N-Acetylgalactosemin (α-GalNAc> α-Gal)
VVA	*Vicia villosa* agglutinin	N-Acetylglucosamine (GalNAc-(α1,3)-Gal = GalNAc-(α1,6)-Gal = GalNAc-Serin)
VI L-Fucose group		
UEA-I	*Ulex Europaeus* agglutinin	L-Fucose (α-L-Fuc)
LTA	*Lotus tetragonolobus* agglutinin	L-Fucose (α-L-Fuc)

3.7 Immunhistochemistry

The immunohistochemical studies were performed using the Avidin-Biotin-Complex Method (ABC- Method) according to Hsu et al. (1981).

Sections were deparaffinized in xylene for 20 minutes, rehydrated in a graded series of alcohol (isopropanol, 96% ethanol, 90% ethanol, 80% ethanol, 70% ethanol) and distilled water. After washing three times in phosphate buffer saline (PBS), pH 7.4 for 5 minutes each. Endogenous peroxidase was inhibited with 1% H_2O_2 for 10 minutes. After intense washing in normal tap water for 10 minutes, and washing in PBS (three times, 5 minutes each).

The sections were covered with DAKO protein block serum-free (DAKO, Hamburg, Germany) for 10 minutes at room temperature. They were then incubated with the specific primary antibody (Table 2) for 12 hours at 4ºC.

After washing with PBS for 5 minutes, incubation with a secondary antibody (Table 2) for 30 minutes at room temperature was performed.

After washing in PBS (three times 5 minutes for each) the sections were incubated with streptavidin-biotin horseradish peroxidase complex (DAKO, Hamburg, Germany) for 30 minutes at room temperature. The reaction was developed using diaminobenzidin (DAB) solution for 10 minutes in room temperature.

Counter staining of the nuclei was done with Haematoxylin for 30 seconds. Sections were dehydrated by using graded series of alcohols (70% ethanol, 80% ethanol, 90% ethanol, 96% ethanol, isopropanol), cleared with xylene, and covered with (DPX).

Table 2: Antibodies used in immunhistochemistry.

	Primary antibody against	**Dilution**	**Source**	**Origin of primary Ab**	**Secondary Antibody**	**Dilution**
1.	**Laminin**	1:500	Institute of Veterinary Anatomy II, LMU-Munich	Chicken	Antichicken IgG Biotin from Rabbit (Rockland, USA)	1:400
2.	**Galactosyltransferase**	1:1000	Institute of Veterinary Anatomy II, LMU-Munich	Chicken	Antichicken IgG Biotin from Rabbit (Rockland, USA)	1:400
3.	**Vascular Endothelial Growth Factor**	1:800	DAKO, Hamburg	Rabbit	Antirabbit IgG Biotin from pigs (DAKO, Hamburg)	1:300
4.	**Angiotensin Converting Enzyme**	1:500	Institute of Veterinary Anatomy II, LMU-Munich	Chicken	Antichicken IgG Biotin from Rabbit (Rockland, USA)	1:400
5.	**Smooth Muscle Actin**	1:40	DAKO, Hamburg	Mouse	Antimouse IgG Biotin from Rabbit (DAKO, Hamburg)	1:300

3.8 Transmission electrone microscopic examination

Small tissue blocks about 1-2 mm in diameter were taken from the cornea, sclera, iris, ciliary body, choroid, retina and optic nerve and fixed in a solution after Karnovsky (1965) (2.5% formaldehyde–2% glutaraldehyde in 0.1 cacodylate buffer, pH 7.2 mixture)

for 4 hours at 4°C. After that the specimens were washed in 0.1 M sodium cocodylate buffer at pH 7.4 for 24 hours.

The specimen were dehydrated in a graded series of alcohol and embedded in Epon (Polysciences, Eppelheim, Germany). Semithin sections (1µm) were cut using a ultramicrotome (Ultratome Nova, LKB, Bromma, Switzerland) and stained with methylene blue-azur II (Richardson et al., 1960). Ultra thin sections (60 nm) were cut on a Reichert ultramicrotome, mounted on a cupper grids and contrasted with unenyl acetate and lead citrate. They were examined and photographed using a Zeiss EM 902 electron microscope.

3.9 Chemicals

- PBS Buffer (phosphate buffered saline):

  42.5 g Sodiumchlorid
  6.35 g Disodiumhydrogenphosphate-dihydrate
  1.95 g Sodiumdihydrogenphosphate-monohydrate
  in 5 liters distilled water at pH 7.4 to 7.6.

- DAB preparation

  Solution A: 50 ml TBS buffer + 0.15 ml 30% H_2O_2
  Solution B: 60 ml PBS + 3 DAB tablets 0.4 ml of solution A
  are mixed with solution B. Filtration of the mixture.

- Karnovsky solution (Karnovsky, 1965)

Paraformaldehyde	10 g
Distilled water	100 ml
NaOH, 1M	6 drops

- Contrasting solution for electron microscopy

OsO_4, 4%	2 ml
(Polysciences Inc., Warrington/USA)	
Distilled water	6 ml
Potassiumferrocyanide	0.12 g
(Sigma, Deisenhofen)	

- Cacodylate Buffer

  *Solution A

  $Na(CH_3)2AsO_2 3H_2O$ 8.56 g

  (Polysciences Inc., Warrington/USA)

  Distilled water 200 ml

  *Solution B

  HCl, 0, 2 (Merck, Darmstadt)

  for Cacodylate buffer, 0, 2 M, pH 7.2:

  Solution A 50 ml

  Solution B 4.2 ml

  Distilled water 100 ml

  for Cacodylate buffer, 0,1 M, pH 7.2:

  0.2 M solution 50 ml

  Distilled water 100 ml

4 RESULTS

4.1 Microscopical anatomy of the bovine eyeball

4.1.1 Cornea

As in other species the bovine cornea is composed of five layers, the corneal epithelium, subepithelial basement membrane (Bowman's membrane), substantia propria or stroma, posterior limiting membrane (Descemet's membrane), and posterior epithelium (corneal endothelium) (Fig. 1).

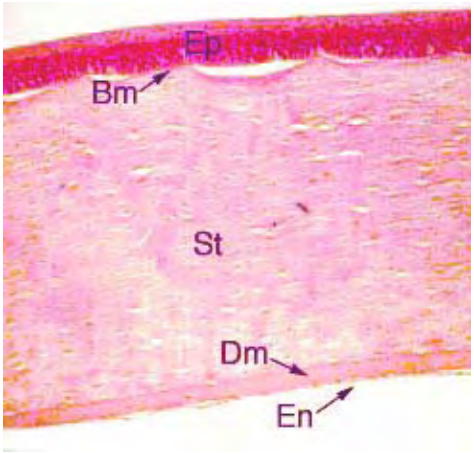

Fig. 1: Staining of the bovine cornea
with H&E (×750).
Ep = Epithelium.
Bm = Bowman's membrane.
St = Stroma.
Dm = Descemet's membrane.
En = Endothelium

The outermost layer, the corneal epithelium (98± 1.5 µm), is a stratified squamous epithelium. It consists of 14-17 layers of epithelial cells.

The basal cell layer of corneal epithelium is represented by columnar cells resting on a wavy, thin and distinct basement membrane that stain positively with PAS (Fig. 2) and

Alcian blue. The height of the basal cell layer is variable depending upon the wavy character of the basement membrane, being relatively high when the basement membrane curves posteriorly and vice versa. The cells have vesicular oval or rounded basely located nuclei, which contain a distinct nucleolus. In H&E stain their cytoplasm is lightly eosinophelic.

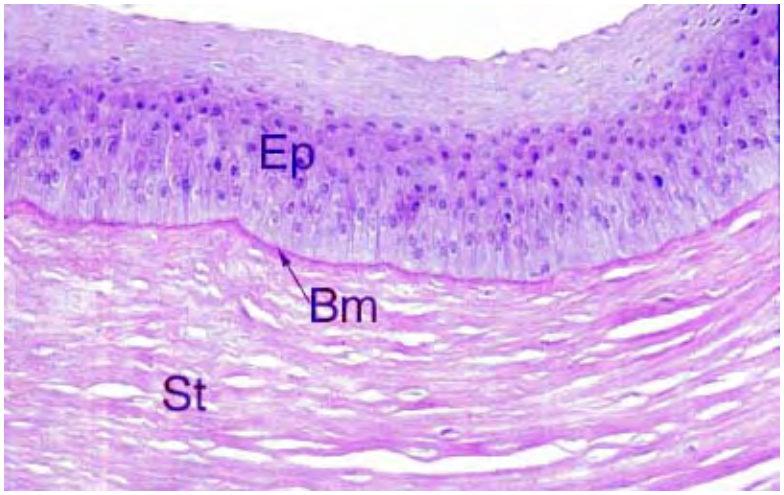

Fig. 2: Staining of the bovine cornea with PAS (×750).
Ep = Epithelium.
Bm = Bowman's membrane.
St = Stroma.

The polyhedral cells are arranged into 7-8 layers having oval or rounded darkly stained nuclei and deeply stained acidophilic cytoplasm. The squamous cells are represented by 5-6 layers of flattened cells with darkly stained nuclei. With H&E the cytoplasm is stained lightly eosinophilic in the deep cells but it is more deeply stained in the superficial ones.

Peripherally, the epithelium contains pigmented granules. The basal cells are highly pigmented but the degree of pigmentation decreases gradually anteriorly (Fig. 3).

The next membrane encountered is the external limiting membrane or membrane of Bowman about (9 ± 1 µm) which is prominent in bovine cornea. It is more intimately attached to the substantia propria and is considered to be a part of that portion of the cornea. This membrane, stains positively with PAS (Fig. 2) and Alcian blue because it is rich in mucopolysaccharides.

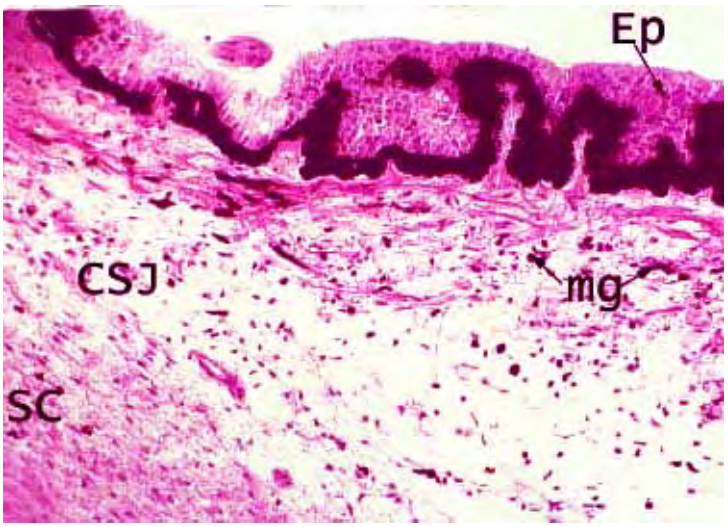

Fig. 3: Staining of the bovine corneoscleral (limbus) with H&E (×470).
CSJ = Corneoscleral junction (Limbus).
Mg = Melanin granules.
Ep = Epithelium of the cornea.

Underlying the epithelium is a thick substantia propria (580 ± 4 µm) that accounts for more than 90% of the thickness of the cornea. The substantia propria itself is composed of collagenous fibers which are stained light green with the Trichrom staining; the fibers are arranged in regular layers parallel to each other. Like the corneal epithelium, the substantia propria is avascular. The bovine corneal stroma has sometimes a light dusting of pigment in most cases, but appears highly pigmented in the peripheral areas near the corneoscleral junction (Fig. 3).

The predominating cell type of the corneal substantia propria is the fibroblast, located mainly between the collagen layers. These cells are elongated and branched, with comparatively little cytoplasm.

The internal limiting membrane or Descemet's membrane is a fairly thick (30 ± 1 µm), glossy, homogeneous membrane, reacts positively with PAS and Alcian blue indicating that this membrane is rich in mucopolysaccharides. Descemet's membrane does not gives any reaction with Resorcin Fuchsin. This indicates that the Descemet's membrane is free from elastic tissue. It consists mainly of collagen fibers which appear light green with

Trichrom staining. In H&E-stained sections, the posterior limiting lamina appears as a highly refractile, thick amorphous layer (Fig. 1).

The inner most or posterior layer of the cornea is corneal endothelium (8 ± 0.3 µm). It consists of single layer of low cuboidal cells or single layer of flattened cells with the nuclei lying parallel to the internal limiting membrane, which is in direct contact with the aqueous humor of the anterior chamber. It is separated from the substantia propria by the narrow, refractile Descemet's membrane.

4.1.2 Sclera

The sclera consists of flat ribbons of collagenous bundles which appear light green with Trichrom staining and run in various directions. Between these bundles are fine elastic nets which appear as blue fine filaments with Resorcine-fuchsin stain fibroblasts and occasional melanocytes.

As in the other species H&E stained preparation of the bovine sclera can be subdivided into three layers (Fig. 4): the outermost layer, the episcleral tissue (52 ± 1.5 µm), consists of loose fibroelastic tissue. It is continuous externally with the dense connective tissue of Tenon's capsule.

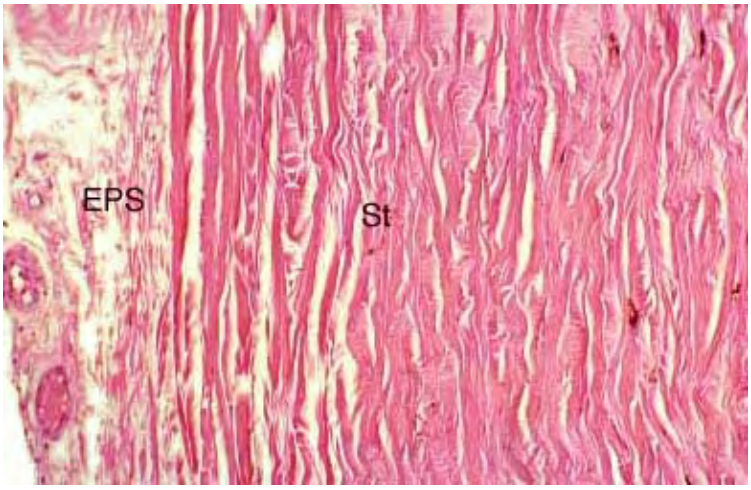

Fig. 4: Staining of the bovine sclera with H&E (×190).
St = Stroma
EPS= Episclera

In the middle layer, the sclera proper (substantia propria, 1036 ± 20.7 µm), bundles of collagenous fibers are oriented mainly parallel to the surface but with some interweaving. Fine bundles of smooth muscle fibers can be demonstrated as yellow bundles mainly by Van Gison's staining. The innermost layer, termed the lamina fusca or dark layer (44 ± 2.2 µm), is composed of much smaller bundles of collagenous fibers. Between the fibers are branching chromatophores containing melanin. They can be found in the deeper layers, especially in the region of the entrance of the optic nerve which give the inner aspect of the sclera a brown colour. There are very blood vessels in the sclera.
By using PAS technique and Alcian blue, the sclera reacts faintly indicating a low mucopolysaccharides content of the interstitial cellular matrix (Fig. 5).

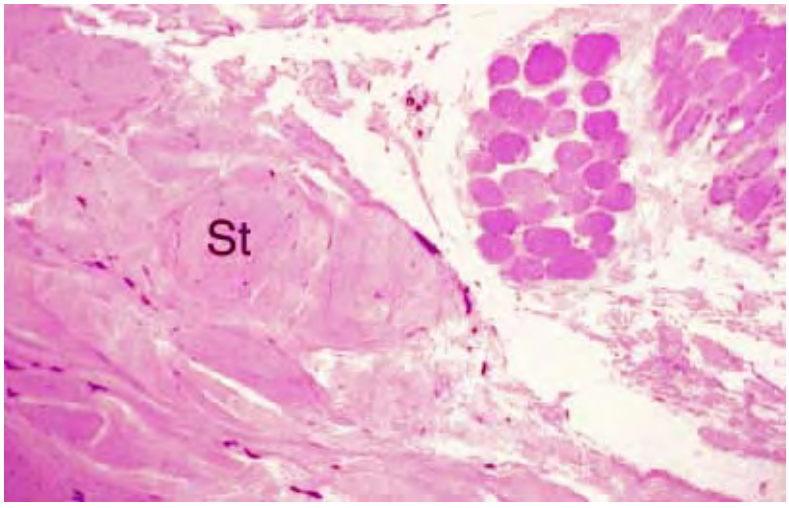

Fig. 5: Staining of the bovine scleral stroma (St) with PAS after digestion of glycogen with amylase (×470).

4.1.3 Iris

Histologically the bovine iris consists of three layers: an anterior epithelial layer (endothelial layer) continues across the iridocorneal angle into the posterior epithelium of the cornea, a middle layer of connective tissue stroma, which contains two smooth muscles (dilatator and sphincter pupillae muscles), and the posterior layer of the pigmented epithelium (Fig. 6). The forward extension of the pigmented layer of the

retina, known as iridic part of the retina, is closely associated with the dilatator pupillae muscle.

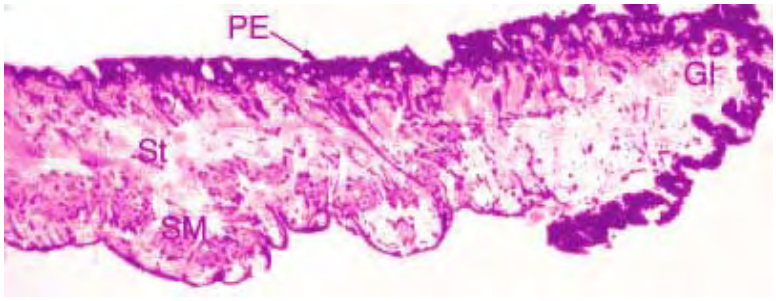

Fig. 6: Staining of the bovine iris with H&E (×470).
PE = Pigmented epithelium.
St = Stroma.
SM = Sphincter muscle.
GI = Granula iridica

The endothelial layer (8 ± 0.3 µm) consists of two cell types: fibroblasts and melanocytes. It is facing the anterior chamber, and its thin, delicate cells are continuous with that of the corneal endothelium. Underlying this layer is the rostral limiting layer or rostral stromal sheath. This layer is rich in mucopolysaccharides which is demonstrated by PAS stain and Alcian blue stain, collagenic fibers appear light green by Trichrom stain and the pigmented cells which contribute to the color of the iris. This layer is an avascular layer.

The stroma (460 ± 12.4 µm) of the bovine iris consists of a rather loose network of collagen that appears light green by Trichrom staining, elastic fibers can be demonstrated as fine dark blue fibers with Resorcine -Fuchsin. Additionally chromatophores, fibroblasts, and a large number of blood vessels with unusually thick walls, that are generally radially arranged occur within the stroma. The stroma is loosely arranged except around blood vessels and nerves where it forms dense sheaths.

The blood vessels of the iris are completely surrounded by spirally wound bundles of collagen fibers which belong to several different arcuate bundles. The melanocytes are essentially prominent around the adventitia of the blood vessels (Fig. 7).

Near the pupil the stroma includes smooth muscle fibers, which appear as brownish yellow bundles by van Gison’s staining of the pupillary sphincter (58 ± 1.3 µm).

The dilatator muscle fibers (24 ± 0.9 µm) appear to be more primitive in structure and contain some pigment. The dilatator muscle is situated posterior and blends with the sphincter fibers near the pupillary margin. The muscle is thicker peripherally (62 ± 2.2 µm). Its fibers are distributed throughout the stroma of the iris at the ciliary margin.
The pigment epithelium (54 ± 1.1 µm) represents the anterior continuity of the pars caeca retina. It consists of heavily pigmented cells.

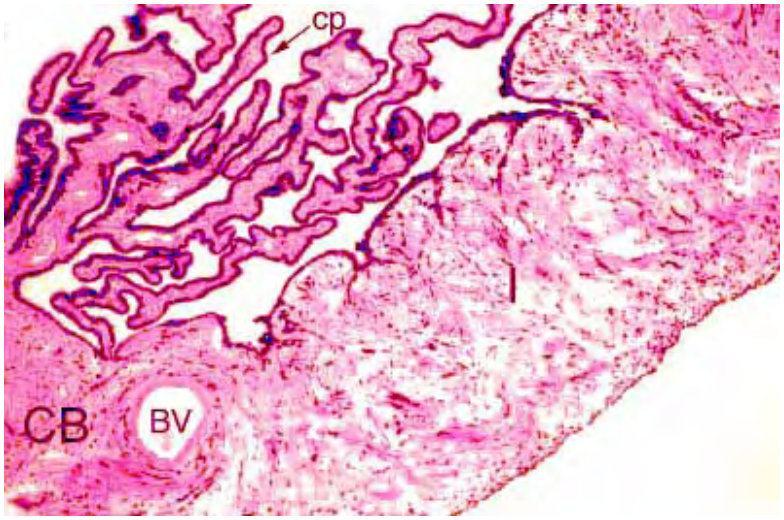

Fig. 7: Staining of the bovine iris and ciliary body with H&E (×190).
I = Iris.
CB = ciliary body.
BV = Blood vessels
cp = ciliary processes.

Granula iridis (iris granules) represent proliferations at the pupillary edge of the stroma of the iris. They are large cysts filled with fluid, lined by pigmented epithelium and show a dense capillary network (Fig. 6).

4.1.4 Ciliary Body

The ciliary body is the rostral continuation of the choroidea. It begins caudally at the ora serrata.

The bovine ciliary body consists of following layers; the supraciliaris (10 ± 1 µm) layer is the most peripheral layer of the ciliary body. It is loosely structured, consisting of bundles of collagen and some elastic fibers. The cell population of this layer consists of fibroblasts, numerous flat melanocytes, some smooth muscle cells and occasional macrophages.

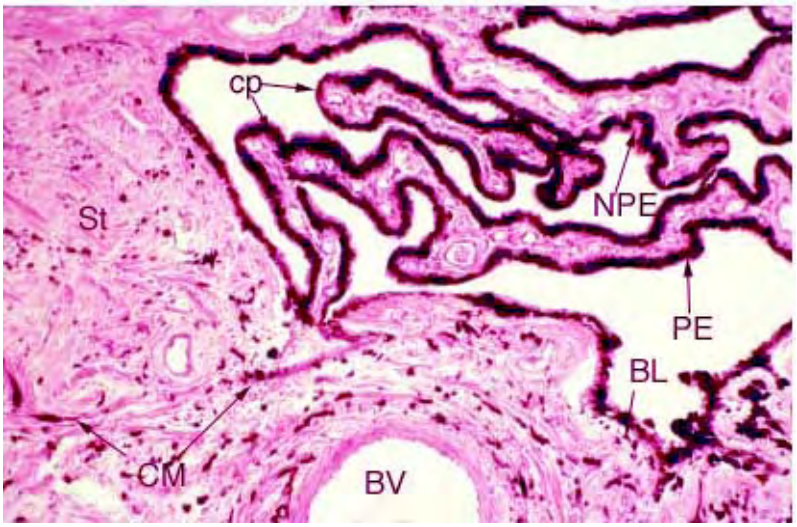

Fig. 8: Staining of the bovine ciliary body with H&E (×470).
PE = Pigmented epithelium.
NPE = Non pigmented epithelium.
St = Stroma.
CM = Ciliary muscle.
BL = Basal Lamina (Bruch's membrane).
BV = Blood Vessels.

The ciliary muscle comprises three layers of smooth muscle fibers (meridional, circular and radial fibers) with a common origin. The outermost fibers are the meridional fibers (muscle of Brücke). The main portion of the ciliary muscle (58 ± 1.9 µm) consists of meridional fibers in the posterior portion of the ciliary body that is rostrally and peripherally continuous with circular fibers (Müller's muscle). Radiate fibers are located inside the meridional fibers and meet with the circular fibers. Between the bundles of smooth muscles there is a meshwork rich in elastic fibers appearing as fine dark blue fibers by Elastic staining (Fig. 8).

The stroma of the ciliary body (656 ± 7.1 µm) contains a large number of blood vessels, arteries and veins (Fig. 8). Veins are predominant and are interspersed with capillaries.

Some arteries are located in the periphery. This layer extends as dense network of capillaries into the ciliary processes.
The Bruch's membrane of the ciliary body (5 ± 0.1 µm) is continuous with the Bruch's membrane of the choroidea and extends anteriorly to the root of the iris. It has here a corrugated surface under the basal lamina of the pigment epithelium which covers it (Fig. 8).
The pigmented epithelium layer (22 ± 0.8 µm) is the continuation of the pigmented epithelium of the retina. Anteriorly it continues as the anterior pigmented epithelial layer of the iris. It consists of simple cuboidal or low columnar cells with rounded nuclei. It is heavily loaded with round or oval melanin granules. The base of the cell is characterized by deep invaginations of the plasma membrane.
The nonpigmented epithelial layer (15± 0.5 µm) is the internal cellular lining of the ciliary body (Fig. 8). Its cuboidal or low columnar cells contain oval nuclei. The surface membrane of this layer shows deep apical invaginations. The cells proceed forward on the posterior surface of the iris. Here they become heavily pigmented.
Each ciliary process consists of a central core of connective tissue stroma and blood vessels covered by a double layer of epithelium: an inner, pigmented, cuboidal epithelium and an outer nonpigmented cuboidal epithelium (Fig. 7, 8).

4.1.5 Choroid

The choroid is a thick, highly vascularized layer that is continuous with the ciliary body stroma. The ora serrata (junction between retina and ciliary epithelium) overlies the junction between the choroid and ciliary body. The outer side of the choroid is connected with the sclera; the inner side is adjacent and intimately attached to the pigmented epithelium of the retina.
The choroidea (Fig. 9) can be subdivided into four layers as follows: the suprachoroid layer (46 ± 1.5 µm), the most peripheral layer of the choroid, is loosely structured, consists of bundles of collagen. Toward the sclera these bundles assume an oblique course. They are separated by numerous spaces, the perichoroidal spaces, and are continuous with the connective tissue of the sclera. The cell population of this layer

consists of fibroblasts, numerous flat melanocytes, and occasional macrophages. The melanocytes are numerous and some smooth muscle cells occur.

The vessel layer consists of intercrossing large (72 ± 2.5 µm) and medium sized (40 ± 1.4 µm) arteries and veins, separated by loose connective tissue stroma rich in chromatophores, similar to that of the suprachoroid. The lamellar arrangement here is much less. It contains strands of smooth muscle cells

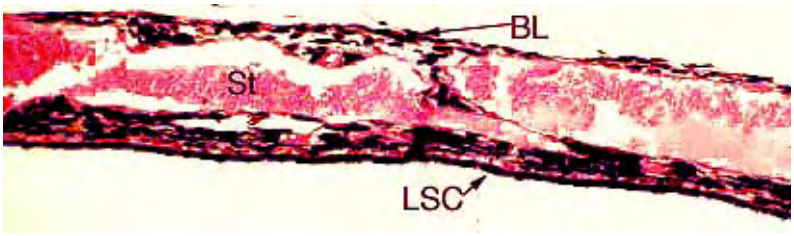

Fig. 9: Staining of the bovine choroid with H&E (×470).
BL = Basal lamina (Bruch membrane).
St = Stroma.
LSC = Lamina suprachorioida

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In bovine the tapetum is fibrous, consisting of dense regular connective tissue fibers.

The choriocapillary layer (50 ± 1.5 µm) contains a dense network of capillaries. It is immediately adjacent to the pigmented epithelial layer of the retina. The intercapillary stroma consists mainly of delicate collagenous and elastic networks, fibroblasts and occasional melanocytes.

The basal complex is also referred to as Bruch's membrane (0.6 ± .005 µm) which separates choroid from retina (Fig. 9).

4.1.6 Retina

The retina is composed of a sensory portion, also referred to as the pars optica retinae, and a nonsensory portion, which begins at the ora serrata and covers the ciliary body as pars ciliaris retinae, and the iris as pars iridis retinae.

The retina consists of following layers: pigment epithelium, layers of rods and cones, external limiting membrane, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer, optic nerve fiber layer, and internal limiting membrane (Fig. 10, 11, 12).

The retinal pigment epithelium (9 ± 0.9 µm) is a simple squamous or cuboidal epithelium resting on a basal lamina. The basal part of the cells includes a spherical nucleus lying peripherally to the basal lamina and a number of round granules of melanin. The base of the cells is characterized by deep infoldings (tongue like apical processes) of the plasma membrane extending from the cells to surround the outer segments of the rods and cones. They do not contain pigment granules.

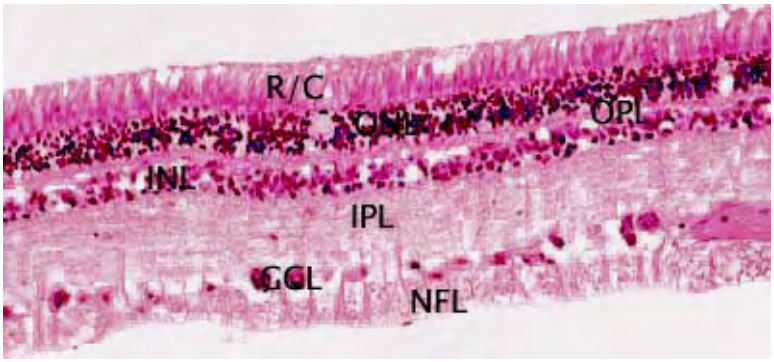

Fig. 10: Staining of the bovine retina with H&E (×750).
R/C= Rods and cones layer.
ONL = Outer nuclear layer.
OPL = Outer plexiform layer.
INL. = Inner nuclear layer.
IPL = Inner plexiform layer.
GCL = Ganglion cell layer.
NFL = Nerve fiber layer

Rod and cone cells (20 ± 1.2 µm) consist of an outer segment, which is a photosensitive part, and an inner segment, which includes the nucleus and cytoplasm. The outer segments of the photoreceptive rods and cones can be distinguished with the light microscope as a layer adjacent to the pigment epithelium (Fig. 11).

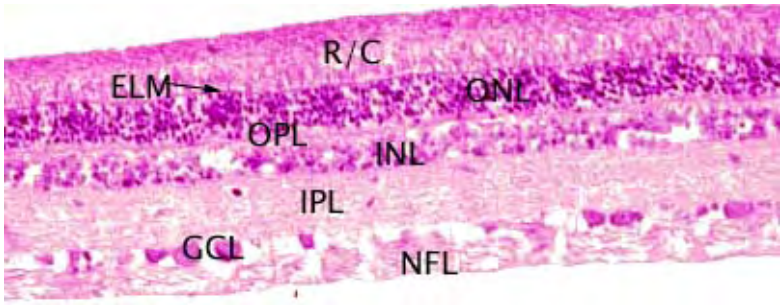

Fig. 11: Staining of the bovine retina with PAS after digestion of glycogen with amylase (×750).
R/C= Rods and cones layer.
ELM = External limiting membrane
ONL = Outer nuclear layer.
OPL = Outer plexiform layer.
INL = Inner nuclear layer.
IPL = Inner plexiform layer.
GCL = Ganglion cell layer.
NFL = Nerve fiber layer.

The rod cells are long, slender, highly specialized cells and comprise an outer and inner segment. The peripheral part of the rod cells is situated between the outer limiting membrane and the pigmented epithelium while the inner end of the rod cells extends through the outer limiting membrane into the outer nuclear layer. The rod nuclei represent the majority of the nuclei of the outer nuclear layer.

The cone cells also consist of an outer and inner segment. The cone outer segment is a long conical structure, considerably wider than a rod at its base and tapering down to a blunt rounded tip. Proximal to the outer limiting membrane, the inner cone segment is found containing the nucleus that is larger and paler than the rod nucleus. The nuclei of the cones, in contrast to those of the rods are arranged in a single row immediately beneath the outer limiting membrane.

The external limiting membrane (5 ± 0.7 µm) separates the layer of rod and cone outer segments from the outer nuclear layer. This membrane contains elastic material.

The outer nuclear layer (30 ± 1.7 µm) (Fig. 12) is composed mainly of rod's and cone's nuclei arranged in 6 rows. The nuclei of the cones are located in the proximity of this layer and form only a single row, whereas the nucleus of the rods forms several layers in the inner portion of this layer. Additional structures in this layer are rod and cone axons, and Müller cell processes.

The outer plexiform layer (11 ± 0.8 µm) is a thin layer that separates the outer nuclear layer from the inner nuclear layer. It is composed mainly of the horizontal cell processes. The inner nuclear layer (9 ± 1.1 µm) that reacts positively with Resorsin-Fuchsin staining (Fig. 12), is thinner than the outer nuclear layer. Its nuclei are arranged in 3 rows. It is composed mainly of four cell types, horizontal, bipolar, amacrine and supporting glia (Müller's) cells.

The axons of the bipolar cells and the dendrites of the ganglion cells form the inner plexiform layer which is a thick layer (34 ± 2.1 µm) (Fig. 11).

The ganglion cell layer (12 ± 0.6 µm) includes the nuclei and cell bodies of the retinal ganglion cells of varying sizes, arranged in one or several layers. The ganglion cell bodies are very large and have round, eccentric nuclei and abundant cytoplasm. The dendritic synapses with the bipolar cells lies in the inner plexiform layer.

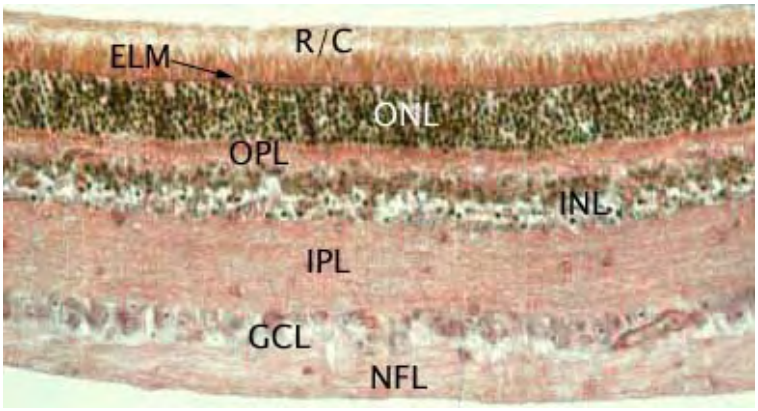

Fig. 12: Staining of the bovine retina with Resorcin- van Gieson stain (×750).
R/C= Rods and cones layer.
ELM = External limiting membrane.
ONL= Outer nuclear layer.
OPL= Outer plexiform layer.
INL= Inner nuclear layer.
IPL= Inner plexiform layer.
GCL= Ganglion cell layer.
NFL= Nerve fiber layer.

The nonmylinated axons of the ganglion cells are arranged parallel to the surface of the retina, forming the optic nerve fiber layer. It is a thick layer (25 ± 1.4 µm). The inner

most layer of this nerve fiber layer is composed of the end feet of the supporting glial (Müller's) cells.

The inner limiting membrane (6 ± 0.3 µm) lies between the vitreous body and the end feet of the supporting glial cells of the retina and contain elastic material.

4.1.7 Optic nerve

The optic nerve is formed by ganglion cell axons, glial cells and septa of connective tissue which arise from the pia mater.

The area cribrosa is formed by lamellae of collagenous fibers which, run in different directions forming a mesh like arrangement and are penetrated by the axons of the optic nerve (Fig. 13).

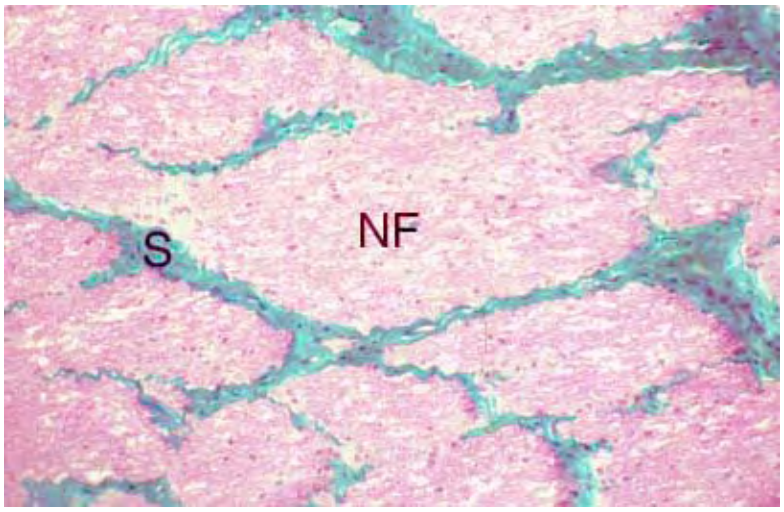

Fig. 13: Staining of the bovine optic nerve with Trichrom stain (×470).
S = Connective tissue septa.
NF = Nerve fiber

In sagittal sections stained with H&E the bundles of the fibers of the optic nerve (110 ± 3.2 µm) run parallel to each other separating by collagenous bundles septa (17 ± 1.2 µm) of the area cribrosa (Fig. 14). These bundles fade out in posterior directions.

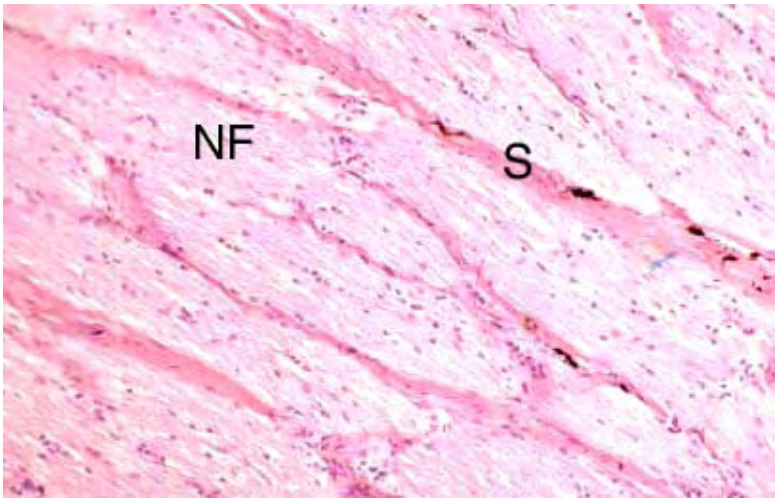

Fig. 14: Staining of the bovine optic nerve with H&E (×470).
S = Connective tissue septa.
NF = Nerve fiber

In the area cribrosa of bovine optic nerve there is a cup shaped structure lined by a plaque of glial cells known as the central supporting tissue meniscus of Kuhnt (Fig. 15).

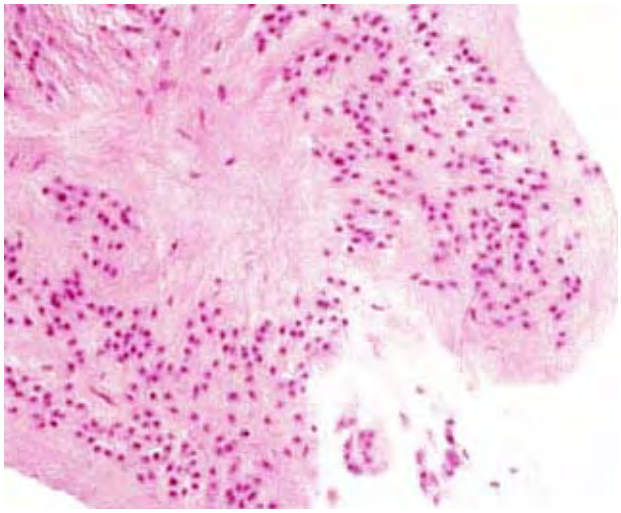

Fig. 15: Staining of the central miniscus of Kuhnt
in bovine optic nerve with H&E (×470).

4.2 Lectin immunohistochemistry

4.2.1 Cornea

All lectins bound to the cornea with some differences in binding intensity. Whereas Con A, WGA and ECA bound to all layer of the cornea, binding of LTA, SBA, VVA, PNA, GS I and UEAI was observed only in epithelial layer of the cornea. Details of the lectin binding and the influence of fixation are found in Table 3 and 4.

Con A, WGA and UEA-I reacted strongly with the corneal epithelium (Fig. 16, 17), while the reaction of LTA, ECA, GSA I, SBA, VVA, and PNA was very weak.

ECA, VVA, Con A, PNA and GSA I were bound mainly to the apical epithelial cells (Fig. 16) whereas WGA bound throughout all epithelial layers of the cornea (Fig. 17).

The binding of the Con A, WGA and PNA to the Bowman's membrane fixed by Bouin was strong (Fig. 16, 17) while the other lectins did not bind significantly.

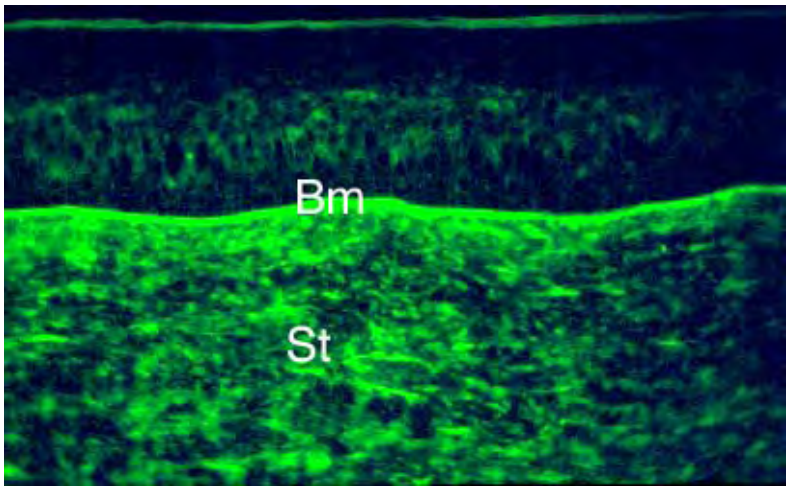

Fig. 16: Distinct labelling of the Bowman's membrane (Bm) and stroma (St) of the bovine cornea with Con A; fixation with Bouin's fluid (×600).

Section fixed with Bouin's fluid show the corneal stroma reacted mainly with Con A and WGA. Other lectins did not show any reaction or gave only a weak reaction. On the other

hand in formalin fixed materials the corneal stroma reacted strongly with Con A and WGA (Fig. 18) which intensely bound to the keratocytes.

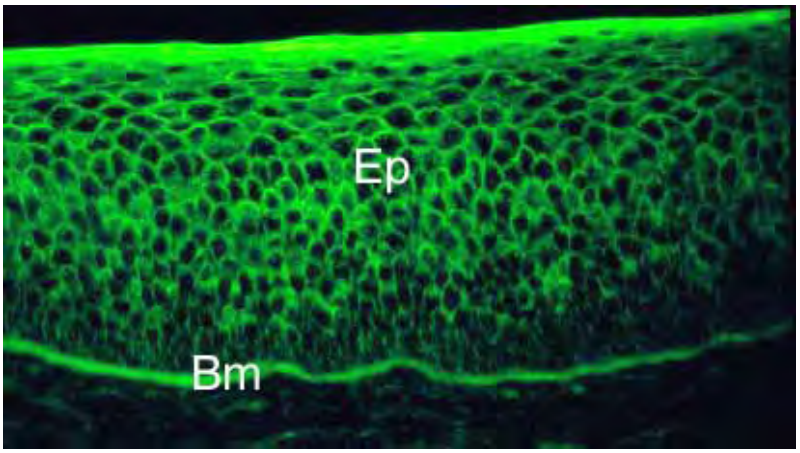

Fig. 17: labelling of the epithelium (Ep) and Bowman's membrane (Bm) of the bovine cornea with WGA; fixation with Bouin's fluid (×600).

The lectin binding pattern of the Descemet's membrane varied with different lectins. Con A and WGA were the most reactive whereas LTA, VVA and PNA only weakly stained the Descemet's membrane.

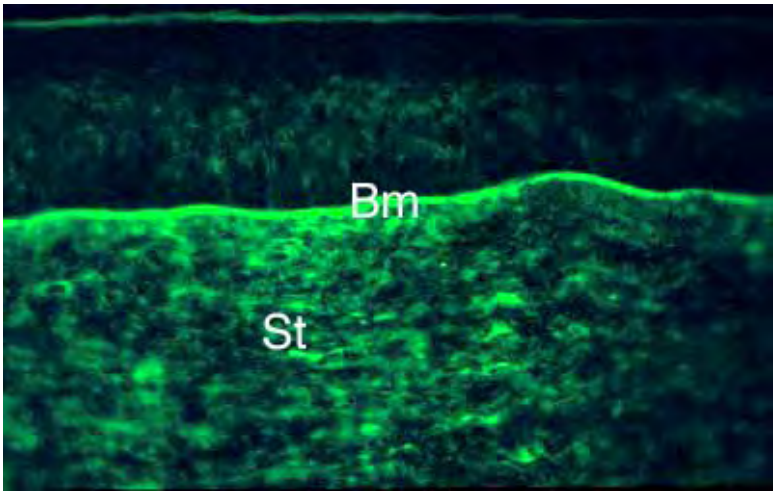

Fig. 18: labelling of the Bowman's membrane (Bm) and stroma (St) of the bovine cornea with Con A; fixation with 7% formalin (×600).

Con A and WGA were distinctly bound only to the endothelial cells of the cornea while ECA, SBA, VVA and PNA gave only a very weak reaction (Table 3, 4) with this cells.

4.2.2 Sclera

The lectins ECA and WGA bound only to the connective tissue of the sclera if the material was fixed by Bouin's solution. In formalin fixed material only Con A and WGA showed a positive reaction (Table 4).

4.2.3 Iris

The lectins Con A and WGA bound strongly to the endothelium of blood vessels and to smooth muscle cells of the iris (Fig. 19, 20). ECA and GS I reacted only with the capillaries of the iris either fixed by Bouin's solution (Fig. 21) or formalin 7%. Other lectins were not reacting or gave only a weak reaction.

A detail examination of the iris gave the followings results:

Con A, WGA, VVA and ECA reacted strongly with the stroma of the iris (Fig. 19, 20).

The binding was mostly to the connective tissue fiber. GSA I lectin did not react with the iridal stroma.

The binding of the lectin to the different layers of blood vessels varied depending on the lectin used. Con A and WGA lectin stained the two inner most layers of the tunica intima and the tunica media (Fig. 19, 20).

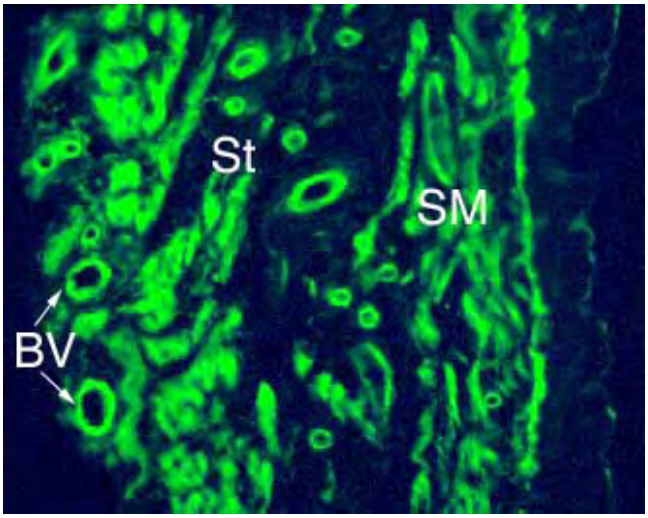

Fig. 19: labelling the endothelium of the blood vessels (BV), iridal sphincter musscle (SM) of the stroma (St) of the bovine iris with Con A; fixation with Bouin's fluid (×600).

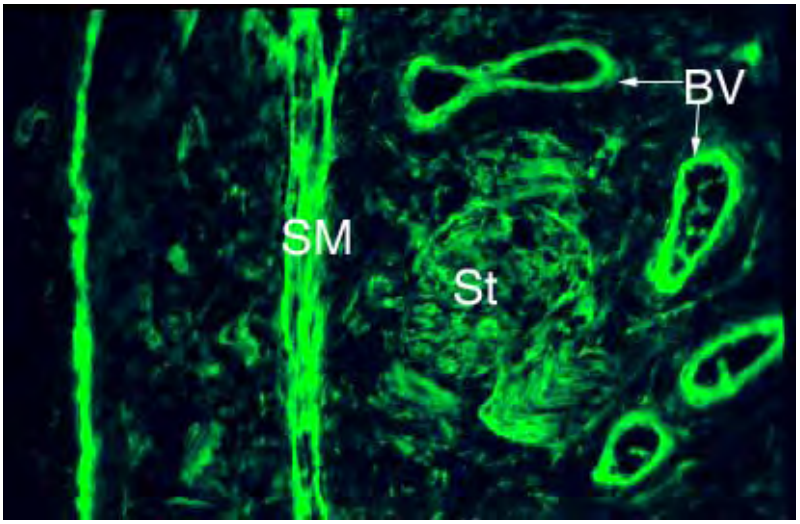

Fig. 20: labelling of the endothelium of blood vessels (BV) and iridial sphincter muscle (SM) of the stroma (St) of the bovine iris with WGA; fixation with Bouin's fluid (×600).

The VVA and ECA bound to all layers of the blood vessels, but the staining was not particularly prominent. GSA I agglutinin bound strongly to the endothelium of all blood vessels. The reaction appeared very distinct because the adjacent stroma was negative (Fig. 21).

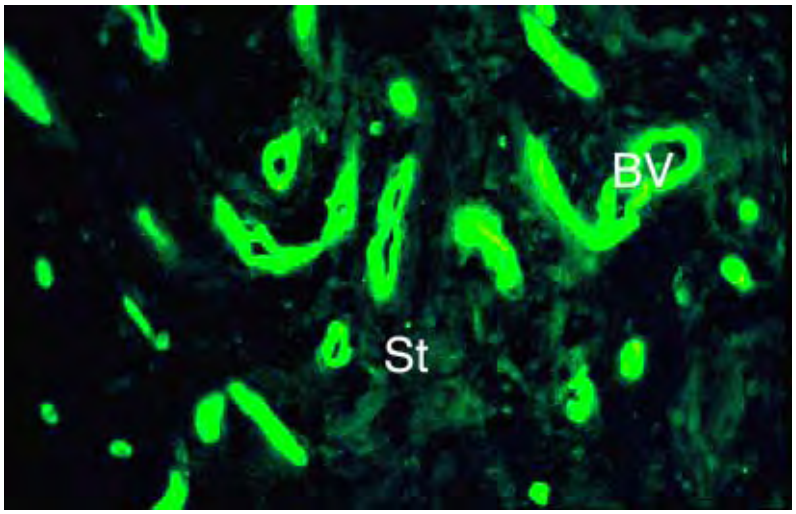

Fig. 21: labelling of the endothelium of blood vessels (BV) of the stroma (St) of the bovine iris with GSA I; fixation with Bouin's fluid (×600).

Con A, WGA and VVA bound strongly to the sphincter iridal muscles (Fig. 19, 20). Also ECA bound to it but to lesser degree. ECA bound strongly to the dilatator muscle of the iris. Results with Con A were similar. Other lectins did not show any reaction with iridal muscles.

Con A and WGA reacted with the posterior pigmented epithelium of the iris. The reaction was not very strong. Others lectins did not stain the pigmented epithelium at all (Table 3, 4).

4.2.4 Ciliary Body

Ciliary body of material either fixed with Bouin's solution or formalin reacted strongly with Con A and WGA while ECA and PNA bound only to samples fixed with Bouin's solution. The other lectins did not give any positive reaction with samples either fixed by Bouin's solution or formalin (Table 3, 4). Con A, WGA and PNA also labelled the smooth muscle cell of the ciliary muscles. The nonpigmented layer of the ciliary epithelium bound all lectins except UEA I. Con A, WGA, and PNA showed a stronger staining than the other lectins.

4.2.5 Choroid

Basal lamina and blood capillaries of the choroid, either fixed by Bouin's solution or formalin reacted strongly with Con A, ECA, VVA and WGA. PNA bound to capillaries which were fixed with Bouin's solution (Fig. 22).

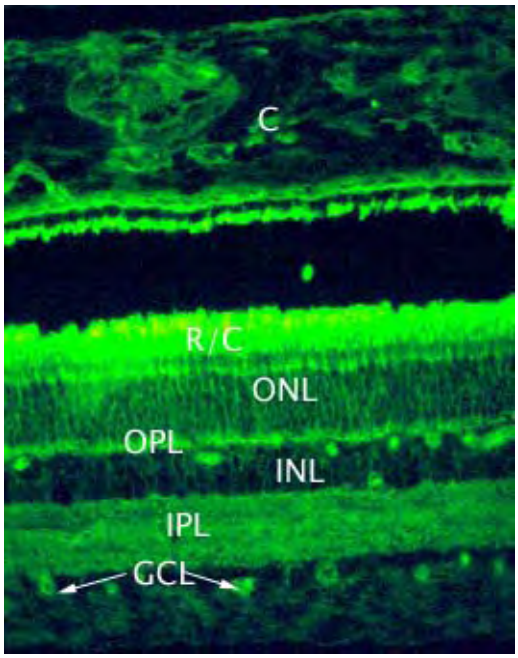

Fig. 22: labelling of the bovine choroid (C), layer of rods and cones (R/C), outer nuclear layer (ONL), outer plexiform layer (OPL) and inner plexiform layer (IPL) with WGA; fixation with Bouin's fluid (×600).
INL = Inner nuclear layer.
GCL = Ganglion cell layer.

GSA I bound strongly to all capillaries, fixed with either formalin solution or Bouin solution. Other lectins were not reacting with the choroid, independent of the fixation (Table 3, 4).

Table (3): lectin binding sites in the bovine eyeball fixed with Bouin's solution.

Sites	*Con A*	*LTA*	*ECA*	*SBA*	*VVA*	*WGA*	*PNA*	*GSA I*	*UEA I*
Cornea									
Ep	+++	+	+	+/-	+/-	+++	+/-	+	++
Bm	++	-	-	-	-	++	++	-	-
St	++	-	+/-	-	-	++	+/-	-	-
Dm	++	+/-	+	-	+/-	++	+/-	-	-
En	++	+	+/-	+/-	+/-	++	+/-	-	-
Sclera	-	-	+	-	+/-	+/-	-	-	-
Iris									
PE	+++	-	+/-	-	-	+/++	-	-	-
St	++	-	++	-	++	++	+	-	+
BV	+/++	+/-	+/++	+/-	++	+/++	++	+++	-
SM	++	-	+	-	+/++	++	-	-	-
Ciliary body									
CM	+	-	-	-	-	+	+	-	-
NPE	++	+	+	+	+	++	++	+	-
Choroid									
BL	++	-	++	-	++	++	-	-	-
BV	++	-	++	-	++	++	++	++	-
Retina									
RPE	++/+++	-	-	-	++/+++	++/+++	++/+++	+/-	-
R&C	++/+++	-	-	-	++/+++	++/+++	++/+++	++	-
ELM	++	-	-	-	-	++	++	-	-
ONL	+	-	-	-	-	+	-	-	-
OPL	+	-	-	-	-	+	+	-	-
INL	+	-	-	-	-	+	-	-	-
IPL	+	-	-	-	-	+	+	-	-
GCL	+	-	-	-	-	-	-	-	-
NFL	+	-	-	-	+	+	+	-	-
ILM	++	-	-	-	-	++	++	-	-
BV	+	-	-	-	+	+	+	+++	-
Optic nerve									
NF	-	+	+	-	+	-	+	-	+
S	++	+	++	-	-	++	+	-	-
BV	++	-	++	++	+	++	-	++	-

- = negative reaction + = week reaction ++ = moderate reaction +++ = strong reaction
Ep = Epithelium, Bm = Bawman's membrane, St = Stroma, Dm = Descemet's membrane, En = Endothelium, PE = Pigmented epithelium, NPE = Non pigmented epithelium, BV = Blood vessels, SM = Sphincter muscle, CM = Ciliary muscle, BL = Basal lamina, RPE = Retinal pigmented epithelium, R&C = Rods and cones, ELM = External limiting membrane, ONL = Outer nuclear layer, OPL = Outer plexiform layer, INL = Inner nuclear layer, IPL = Inner plexiform layer, GCL= Ganglion cell layer, NFL = Nerve fiber layer, ILM = Internal limiting membrane, S = Septa, NF = Nerve fiber.

4.2.6 Retina

WGA and Con A bound strongly to all layers of the retina, mainly to the rods and cones (Fig. 22, 23). LTA, SBA and UEA I did not give any reaction with retina either fixed by Bouin's fluid or formalin solution. In detail, the retina showed following staining pattern:
RPE revealed strong lectin binding sites for Con A, VVA WGA, and PNA (Fig. 23), while GSA I agglutinen showed a weak reaction with RPE. Other lectins did not give any positive reaction with RPE.

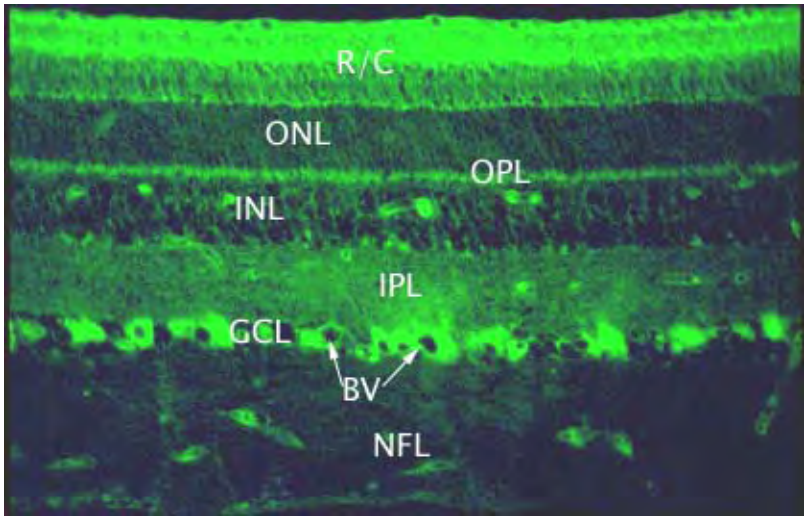

Fig. 23: labelling of the rods and cones (R/C), outer plexiform layer (OPL), inner plexiform layer (IPL) and the endothelioum of blood vessels (BV) of the bovine retina with Con A; fixation with Bouin's fluids (×600).
ONL = Outer nuclear layer.
INL = Inner nuclear layer.
GCL = Ganglion cell layer.
NFL = Nerve fiber layer.

WGA, Con A, VVA and PNA (Fig. 22, 23 24) bound strongly to the rod and cone photoreceptor cells. GSA I agglutinin reacted only strongly with the outer segment of the rods and cones (Fig. 25).
Con A, WGA, and PNA bound strongly to the external and internal limiting membrane of the retina (Table 3, 4), but the reaction was more prominent in external limiting

membrane. Other lectins did not give any staining, neither of the external or the internal limiting membrane.

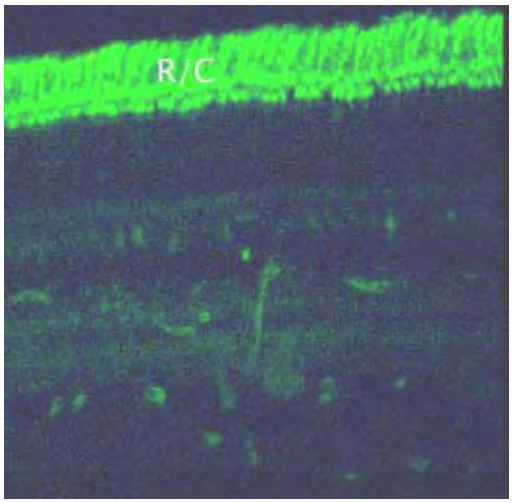

Fig. 24: labelling of the rods and cones (R/C) of the bovine retina with VVA; fixation with Bouin's fluid (×600).

WGA, Con A and PNA labelled the outer and inner plexiform layers (Fig. 22, 23). WGA reacted strongly with both outer and inner plexiform layers fixed by formalin (Table 4), whereas WGA and Con A reacted weakly with both outer and inner nuclear layers of the retina (Fig. 22, 23).

Con A was the only lectin which showed moderate binding to the ganglionic cell layer in retina fixed by Bouin's solution (Fig. 23). All others did not give any reaction. WGA lectin labels the ganglionic cell layer in retina fixed by formalin solution.

WGA, Con A, VVA and PNA reacted weakly with the optical nerve fiber layer of the retina when fixed in Bouin's solution (Fig. 22, 23). On the other hand retina fixed by formalin solution gave a prominent positive reaction with WGA (Table 4).

GSA I agglutinin reacted strongly with the endothelium of all blood vessels in the retina (Fig. 25). Con A, VVA, WGA, and PNA showed the endothelium of the retinal blood vessels weakly. All other lectins were negative (Table 3, 4).

Table (4) lectin binding sites in bovine eyeball fixed by 7% Formalin.

Sites	*Con A*	*LTA*	*ECA*	*SBA*	*VVA*	*WGA*	*PNA*	*GSA I*	*UEA I*
Cornea									
Ep	+/-	-	-	-	-	-	-	-	-
Bm	++	-	-	-	-	-	-	-	-
St	++	-	-	-	-	+	-	-	-
Dm	+	+/-	+	-	-	+	-	-	-
En	+/-	+	-	+/-	-	+/-	-	-	-
Sclera	-	-	-	-	-	-	-	-	-
Iris									
PE	+/-	-	+/-	-	-	+/-	-	-	-
St	-	-	+/-	-	-	+/-	-	-	-
BV	+	+/-	+	+/-	-	++	-	++	-
CM	+	-	-	-	-	+/-	-	-	-
Ciiaryl.body									
CM	-	-	-	-	-	-	-	-	-
NPE	+/-	-	-	-	-	+	-	-	-
Choroid									
BL	+	-	-	-	-	-	-	+	-
BV	++	-	-	-	-	+	-	+	-
Retina									
RPE	++	-	-	-	+	++	-	+	-
R&C	++	-	-	-	+	++	-	+	-
ELM	-	-	-	-	-	-	-	-	-
ONL	-	-	-	-	-	+	-	-	-
OPL	-	-	-	-	-	++	-	-	-
INL	-	-	-	-	-	+	-	-	-
IPL	-	-	-	-	-	++	-	-	-
GCL	-	-	-	-	-	-	-	-	-
NFL	-	-	-	-	+	-	-	-	-
ILM	-	-	-	-	-	-	-	-	-
BV	+/++	-	-	-	+	+	-	+	-
Optic nerve									
NF	-	-	-	-	+	+	-	-	-
S	++	+	-	-	-	++	-	-	-
BV	+	-	-	+	+	-	-	-	-

- = negative reaction + = week reaction ++ = moderate reaction +++ = strong reaction
Ep = Epithelium, Bm = Bawman's membrane, St = Stroma, Dm = Descemet's membrane, En = Endothelium, PE = Pigmented epithelium, NPE = Non pigmented epithelium, BV = Blood vessels, SM = Sphincter muscle, CM = Ciliary muscle, BL = Basal lamina, RPE = Retinal pigmented epithelium, R&C = Rods and cones, ELM = External limiting membrane, ONL = Outer nuclear layer, OPL = Outer plexiform layer, INL = Inner nuclear layer, IPL = Inner plexiform layer, GCL= Ganglion cell layer, NFL = Nerve fiber layer, ILM = Internal limiting membrane, S = Septa, NF = Nerve fiber.

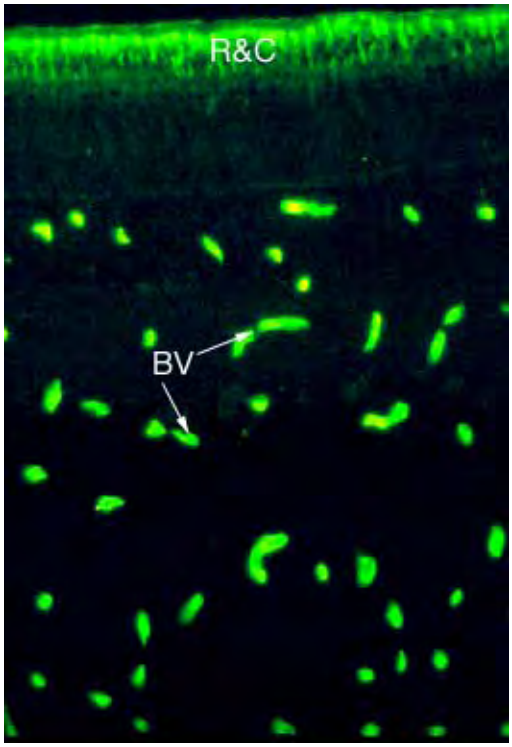

Fig. 25: Labelling of the rods and cones (R/C) and the endothelium of blood vessels (BV) of the bovine retina with GSA I; fixation with Bouin's fluid(×600).

4.2.7 Optic Nerve

Nerve fibers of the optic nerve fixed with Bouin's solution bound strongly ECA, VVA, PNA and UEA I. There was no positive reaction with Con A, WGA, LTA, SBA and GSA I (Fig. 26). VVA and WGA lectins label the optic nerve fibers when the material was fixed with formalin (Table 4).

Bundles of the collagen fibers of the connective tissue septa in the area cribrosa, when fixed with Bouin's solution, reacted strongly with Con A, WGA and ECA (Fig. 26). They showed a weak reaction with LTA and PNA and did not stain with VVA, SBA, GSA I and UEA I. Collagenous fibers in the area cribrosa, when fixed with formalin, reacted strongly with Con A and weakly with WGA.

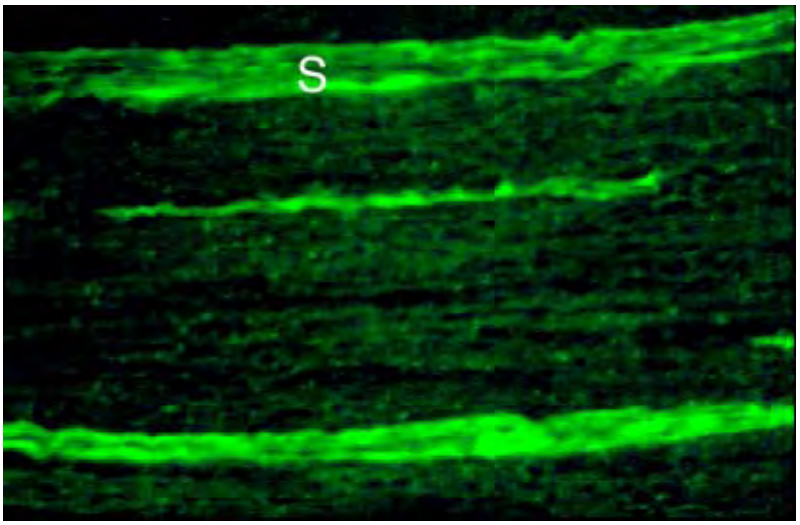

Fig. 26: Labelling of the connective tissue septa (S) of the bovine optic nerve with WGA; fixation with Bouin's fluid (×600).

The staining with ECA of the collagenous fibers from the area cribrosa was not particularly prominent. Blood capillaries of the optic nerve, when fixed with Bouin's solution, reacted strongly with Con A, ECA, SBA, WGA and GSI (Fig. 27) while bound weakly VVA, and no reaction was seen with LTA, PNA and UEA I. On the other hand samples fixed with formalin bound only Con A and VVA and did not give any positive reaction with other lectins (Table 3, 4).

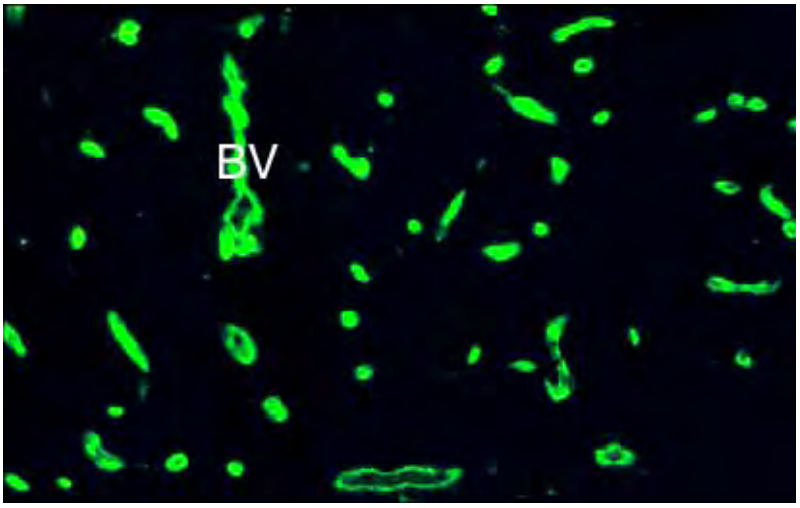

Fig. 27: Labelling of the endothelium of blood vessels (BV) in bovine optic nerve with GSA I; fixation with Bouin's fluids (×600).

4.3 Immunohistochemical studies on the bovine eyeball

4.3.1 Distribution of laminin in the bovine eyeball

The immunostaining techniques used in this study allowed us to determine the distribution of the laminin within bovine ocular tissues.

4.3.1.1 Cornea

The corneal epithelium showed moderate staining for laminin in the basement membrane of the epithelium. The Bowman's membrane and corneal stroma revealed no immuno-staining for laminin. The outer most layer of the Descemet's membrane, facing the endothelium, showed a striking linear immuno-staining.The remaining part of the Descemet's membrane was unstained.The corneal endothelium showed a diffuse immunostaining for laminin (Table 5).

4.3.1.2 Sclera

The immunostaining of the scleral stroma for laminin was negative.

4.3.1.3 Iris

The stroma of the iris as well as M. sphincter and M. dilatator pupillae revealed no staining for laminin. The pigmented epithelium showed circumferential immunostaining for laminin. The basal lamina of the blood vessels of the iris showed a very strong reaction for laminin (Table 5).

4.3.1.4 Ciliary body

The stroma of the ciliary processes contains many blood vessels. The basal lamina of the endothelium exhibited a strong staining for laminin. The stroma of the ciliary body and the ciliary muscle showed diffuse, very faint immuno-staining (Fig. 28). The nonpigmented ciliary epithelium displayed faint surface staining, whereas its basement membrane facing the vitreous body a strong reactive.

4.3.1.5 Choroid

Bruch's membrane and a thin layer of choriocapillaris reacted positively for laminin. The wall of the choroidal blood vessels in the substantia propria (lamina vasculosa) stained heavily for immunoreactive laminin.

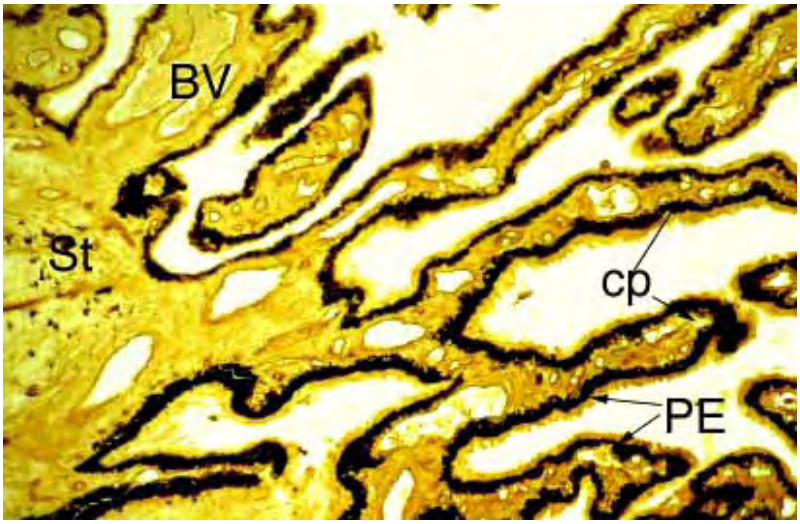

Fig. 28: Immunolocalization of laminin in the ciliary processes (cp) of the bovine ciliary body (×470).
PE = pigmented epithelial cells in ciliary process
St = Stroma of the bovine ciliary body.
BV = Blood Vessels.

4.3.1.6 Retina

The basal membrane of the retinal blood vessels showed a distinct immunostaining for laminin (Fig. 29). Immunoreactive laminin was also present on the inner limiting membrane of the retina, although the intensity of staining was weaker than that of the vessels walls.

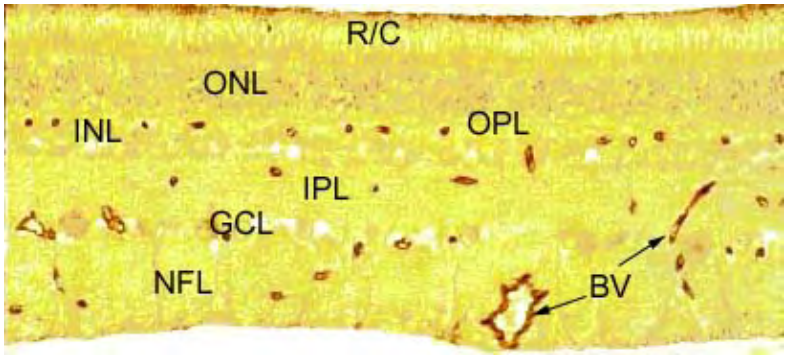

Fig. 29: Immunolocalization of laminin in the endothelium of the blood vessels (BV) of the bovine retina (×750).
R/C = Rods and cones layer.
ONL = Outer nuclear layer.
OPL = Outer plexiform layer.
INL = Inner nuclear layer.
IPL = Inner plexiform layer.
GCL = Ganglion cell layer.
NFL = Nerve fiber layer

4.3.1.7 Optic nerve

The inner surface of the optic disk stained in a linear, border-like pattern for laminin. Blood vessels walls and the septum of the optic nerve also stained in a border-like pattern for laminin (Fig. 30). The lamina cribrosa gives a band-like staining for laminin.

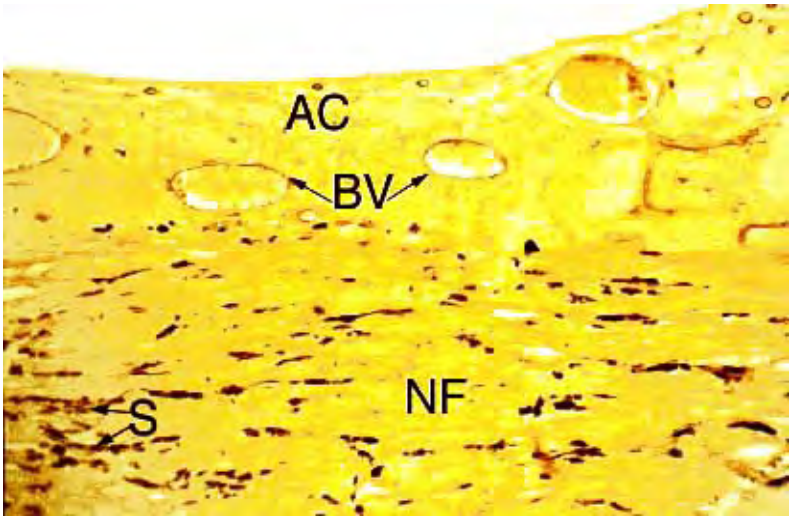

Fig. 30: Immunolocalization of laminin in the bovine optic nerve (×470).
AC = Area cribrosa.
NF = Nerve fiber of the bovine optic nerve.
S = Connective tissue septa
BV = Blood Vessels.

4.3.2 Distribution of smooth muscle actin in the bovine eyeball

4.3.2.1 Cornea

All layers of the cornea were negative for the smooth muscle actin (SMA) except the endothelial layer of the cornea that gave a moderate reaction.

4.3.2.2 Sclera

No immunostaining for smooth muscle actin was detectable in the stroma of the sclera except in the smooth muscle cells of the scattered blood vessels (Fig. 31).

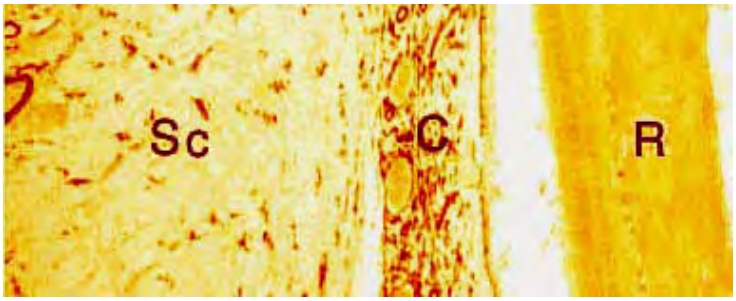

Fig. 31: Immunolocalization of SMA in the bovine sclera (Sc) choroid (C) and retina (R) (×750).

4.3.2.3 Iris

The pigment epithelium and stroma of the iris did not show any immune reaction for SMA. On the other hand, the sphincter and diltator muscles of the iris show high intensity of immunostainig for SMA (Fig. 32, 33).

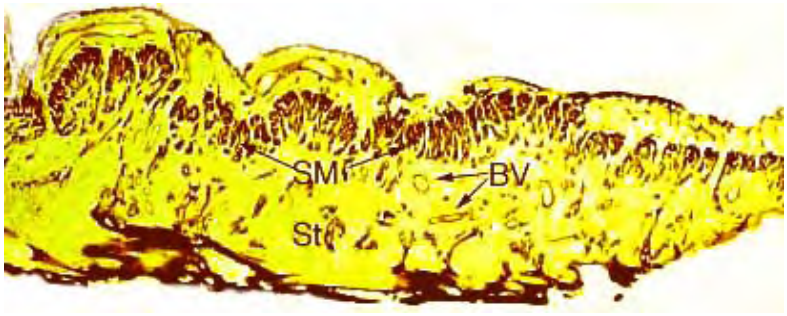

Fig. 32: Immunolocalization of SMA in bovine iris (×190).
St = Stroma of the bovine iris.
SM = sphincter iridal muscle.
BV = Blood Vessels.

Also the blood vessels gave a strong positive reaction for immunoreactive SMA (Fig. 33).

4.3.2.4 Ciliary body

The non pigmented epithelium, and its basement membrane, as well as the stroma of the ciliary process did not show immunostaining for SMA. The stromal blood vessels and the ciliary muscles displayed strong staining for immunoreactive SMA (Table 5).

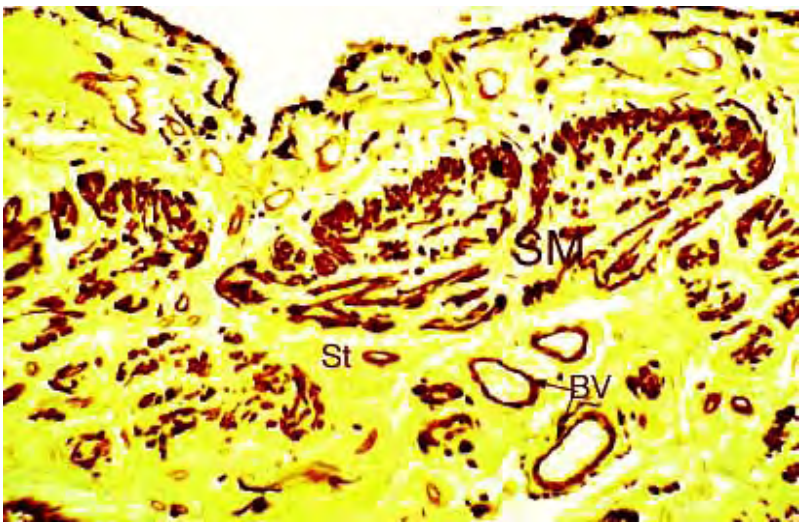

Fig. 33: Immunolocalization of SMA in stroma of the bovine iris (×470).
St = Stroma of the bovine iris.
SM= sphincter iridal muscle.
BV = Blood Vessels.

4.3.2.5 Choroid

The Bruch's membrane layer and the lamina choriocapillaris did not give any staining for SMA. A strong reaction for SMA was detected only in smooth muscle cells of the blood vessels that are scattered throughout the lamina vasculosa (Fig. 34).

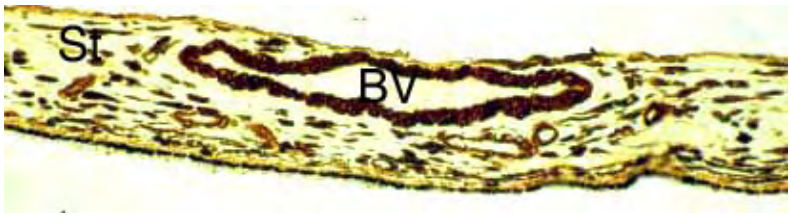

Fig. 34: Immunolocalization of SMA in the endothelium of blood vessels of the bovine choroid (×470).
St = Stroma of the bovine choroid.
BV = Blood Vessels.

4.3.2.6 Retina

SMA showed as dense band of immunoreactivity only in the internal limiting membrane while the other layers of the retina did not give any reaction. The smooth muscle cells of retinal blood vessels were distinctly stained for SMA (Fig. 35).

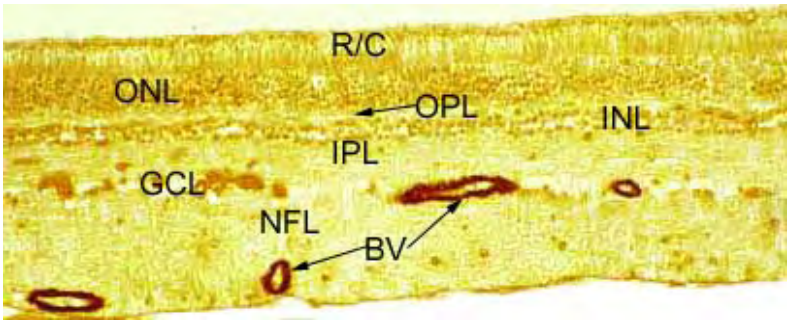

Fig. 35: Immunolocalization of SMA in the endothelium of blood vessels (BV) of the bovine retina (×470).
R/C= Rods and cones layer.
ONL = Outer nuclear layer.
OPL = Outer plexiform layer.
INL = Inner nuclear layer.
IPL = Inner plexiform layer.
GCL = Ganglion cell layer.
NFL = Nerve fiber layer.

4.3.2.7 Optic nerve

The smooth muscle cells of the blood vessels of the wall and septum stained strongly for SMA, while the septum itself and area cribrosa did not give any reaction to SMA antibodies.

4.3.3 Distribution of galactosyltransferase in the bovine eyeball

4.3.3.1 Cornea

The corneal epithelium and endothelium showed moderate staining for galactosyltransferase while the other layers of the cornea were negative (Table 5).

4.3.3.2 Sclera

Scleral stroma did not show any staining for galactosyltransferase.

4.3.3.3 Iris

All layers of the iris were consistently negative, even the blood vessels (Fig. 36).

4.3.3.4 Ciliary body

The ciliary body did not stain with antibodies to galactosyltransferase.

4.3.3.5 Choroid

The Bruch’s membrane layer, choriocapillaries and the stromal choroidal blood vessels did not show any immunostaining for galactosyltransferase.

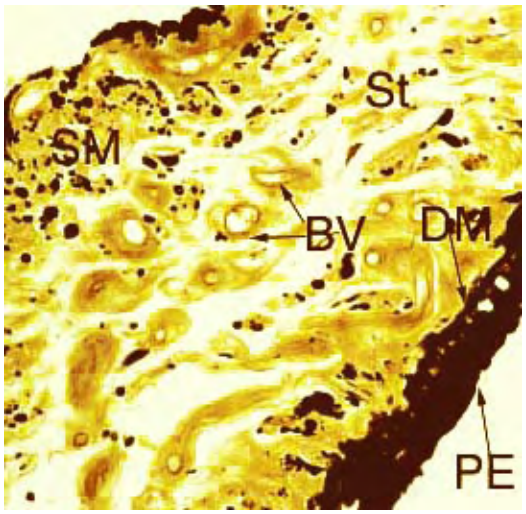

Fig. 36: Immunolocalization of galactosyltransferase in the bovine iris (×750).
PE = Pigmented epithelium of the iris.
St = Stroma of the bovine iris.
SM = sphincter iridal muscle.
DM = Dilatator iridal muscle.
BV = Blood Vessels.

4.3.3.6 Retina

The inner segment of the rods and cones and the ganglion cell layer give very faint positive staining for the galactosyltransferase (Fig 37).

4.3.3.7 Optic nerve

The optic nerve septa reacted weakly for galactosyltransferase.

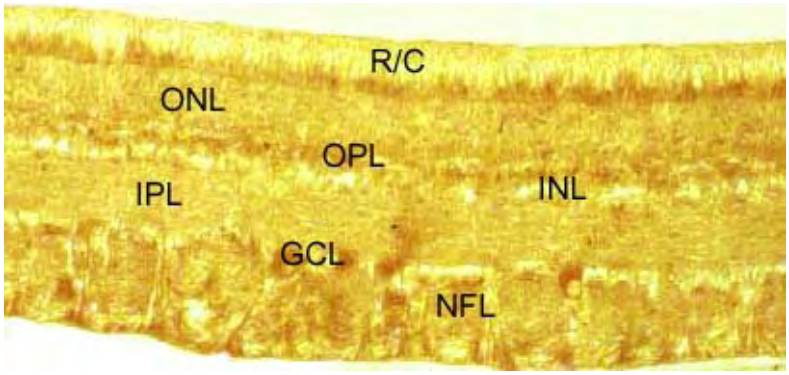

Fig. 37: Immunolocalization of galactosyltransferase in the bovine retina (×750).
R/C= Rods and cones layer.
ONL = Outer nuclear layer.
OPL = Outer plexiform layer.
INL = Inner nuclear layer.
IPL = Inner plexiform layer.
GCL = Ganglion cell layer.
NFL = Nerve fiber layer.

4.3.4 Distribution of angiotensin converting enzyme in the bovine eyeball

4.3.4.1 Cornea

The cytoplasm of corneal epithelium cells stained positively for ACE. The corneal stroma gave very faint positive reaction for ACE, while the endothelium appeared strongly positive. The Descemet's membrane did not give any reaction with the ACE antibodies (Table 5).

4.3.4.2 Sclera

The scleral stroma reacted weakly with ACE-antibodies.

4.3.4.3 Iris

The iridal blood vessels showed strong staining for immunoreactive ACE. The other layers of the iris did not react for ACE.

4.3.4.4 Ciliary body

Immunostaining for ACE was observed only in the ciliary blood vessels, whereas the other layers of the ciliary body did not react for ACE (Fig. 38).

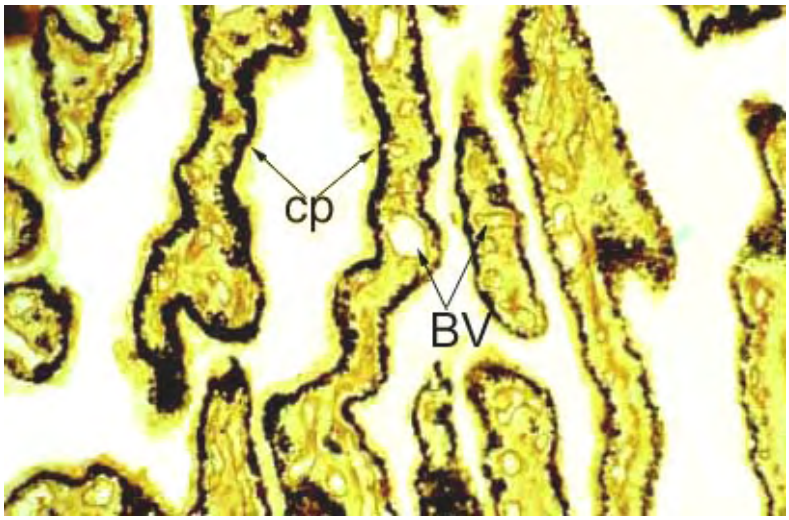

Fig. 38: Immunolocalization of ACE in the endothelium of the blood vessels (BV) of ciliary processes (cp) of the bovine ciliary body (×750).

4.3.4.5 Choroid

The choroidal blood vessels reacted strongly with ACE. The other layers of the choroid did not give any reaction with ACE-antibodies.

4.3.4.6 Retina

The inner segment of the rods and cones, the cytoplasm of the nerve cells of the outer and inner nuclear layer, cells of the ganglion cell layer and the nerve fiber layer stained positively with ACE. The retinal blood vessels reacted strongly with ACE-antibodies (Fig. 39).

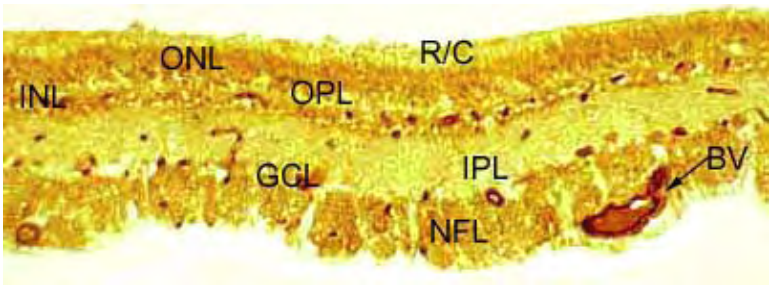

Fig. 39: Immunolocalization of ACE in the bovine retina (×470).
R/C = Rods and cones layer.
ONL = Outer nuclear layer.
OPL = Outer plexiform layer.
INL = Inner nuclear layer.
IPL = Inner plexiform layer.
GCL = Ganglion cell layer.
NFL = Nerve fiber layer.
BV = blood vessels

4.3.4.7 Optic nerve

The inner surface of the optic disk stained in a linear, border-like pattern for immunoreactive ACE. Blood vessels walls and the septum of the optic nerve also stained for ACE (Fig. 40).

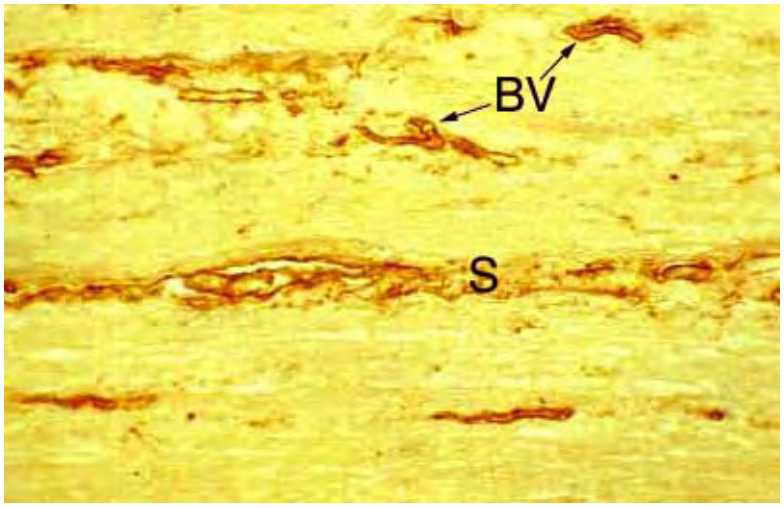

Fig. 40: Immunolocalization of ACE in the endothelium of the blood vessels (BV) in connective tissue septa (S) of the bovine optic nerve (×470).

4.3.5 Distribution of vascular endothelial growth factor in the bovine eyeball

4.3.5.1 Cornea

The corneal epithelium was specifically stained for immunoreactive VEGF. Staining was most intense in the cell layers closer to the surface. The corneal endothelial cells showed a weak positive immunostaining for VEGF.

4.3.5.2 Sclera

Scleral stroma and blood vessels did not stain for VEGF.

4.3.5.3 Iris

All layers of the iris were negative for VEGF.

Table (5) immunohistochemical reaction in the bovine eyeball

Sites	Laminin	Smooth Muscle Actin	Galactosyltrans-ferase	Angiotensin converting enzyme	Vascular endothelium growth factor
Cornea					
Ep	+	-	+	+	++
Bm	-	-	-	-	-
St	-	-	-	+/-	-
Dm	+/-	-	-	-	-
En	+/++	+	+	++	+
Sclera	-	-	-	+/-	-
Iris					
PE	+	-	-	-	-
St	-	-	-	-	-
BV	++	++	-	++	-
SM	-	++	-	-	-
Ciliary Body					
CM	-	++	-	-	-
NPE	+	-	-	-	-
Choroid					
BL	+	-	-	-	-
BV	++	++	-	++	-
Retina					
RPE	-	-	-	-	-
R&C	-	-	+ (In seg)	+ (In.seg)	-
E LM	-	-	-	-	-
O N L	-	-	-	+	+
O PL	-	-	-	-	-
I N L	-	-	-	+	-
I P L	-	-	-	-	-
G C L	+	-	+	+	-
N F L	-	-	-	+	-
I L M	+	+	-	-	-
BV	++	++	-	++	+
Optic nerve					
N F	-	-	-	-	-
S	+	-	+/-	+	-
BV	++	++	-	++	-

- = negative reaction + = week reaction ++ = moderate reaction +++ = strong reaction
Ep = Epithelium, Bm = Bawman's membrane, St = Stroma, Dm = Descemet's membrane, En = Endothelium, PE = Pigmented epithelium, NPE = Non pigmented epithelium, BV = Blood vessels, SM = Sphincter muscle, CM = Ciliary muscle, BL = Basal lamina, RPE = Retinal pigmented epithelium, R&C = Rods and cones, ELM = External limiting membrane, ONL = Outer nuclear layer, OPL = Outer plexiform layer, INL = Inner nuclear layer, IPL = Inner plexiform layer, GCL= Ganglion cell layer, NFL = Nerve fiber layer, ILM = Internal limiting membrane, S = Septa, NF = Nerve fiber.

4.3.5.4 Ciliary body

The ciliary processes and ciliary muscles did not show any immunostaining for VEGF.

4.3.5.5 Choroid

Bruch's membrane, lamina choriocapillaris and lamina propria did not give any reaction for VEGF.

4.3.5.6 Retina

The outer nuclear layer of the retina and some retinal blood vessels gave a moderate positive reaction for VEGF. The other layers of the retina were negative (Table 5).

4.3.5.7 Optic nerve

The optic nerve septa and the optic nerve fibers were not stained with VEGF-antibodies.

4.4 Electron microscopic examination of the bovine eyeball

4.4.1 Cornea

The cornea of the cow consists of five distinct layers. These are the corneal epithelium, Bowman's layer, corneal stroma, Descemet's membrane, and endothelium (i.e., mesothelium). The epithelium covering the anterior part of the cornea is of non keratinizing stratified squamous type. Its basal layer consists of columnar cells that are firmly attached to the basal lamina by hemidesmosomes. Towards the surface of the epithelium the cells gradually change from columnar to polyhedral or wing cells and to flat surface cells.

The basal cells possess a flattened base and a domed apex. They are crowded together, and their nuclei are located in the apical region. Mitosis is confined to the basal cells or

those cells immediately superficial to the basal cells (stratum germinativum). Adjacent cell surfaces have small infoldings with numerous desmosomal attachments (Fig. 41). Occasional lymphocytes are found in the basal layer and in the more superficial layers of the corneal epithelium.

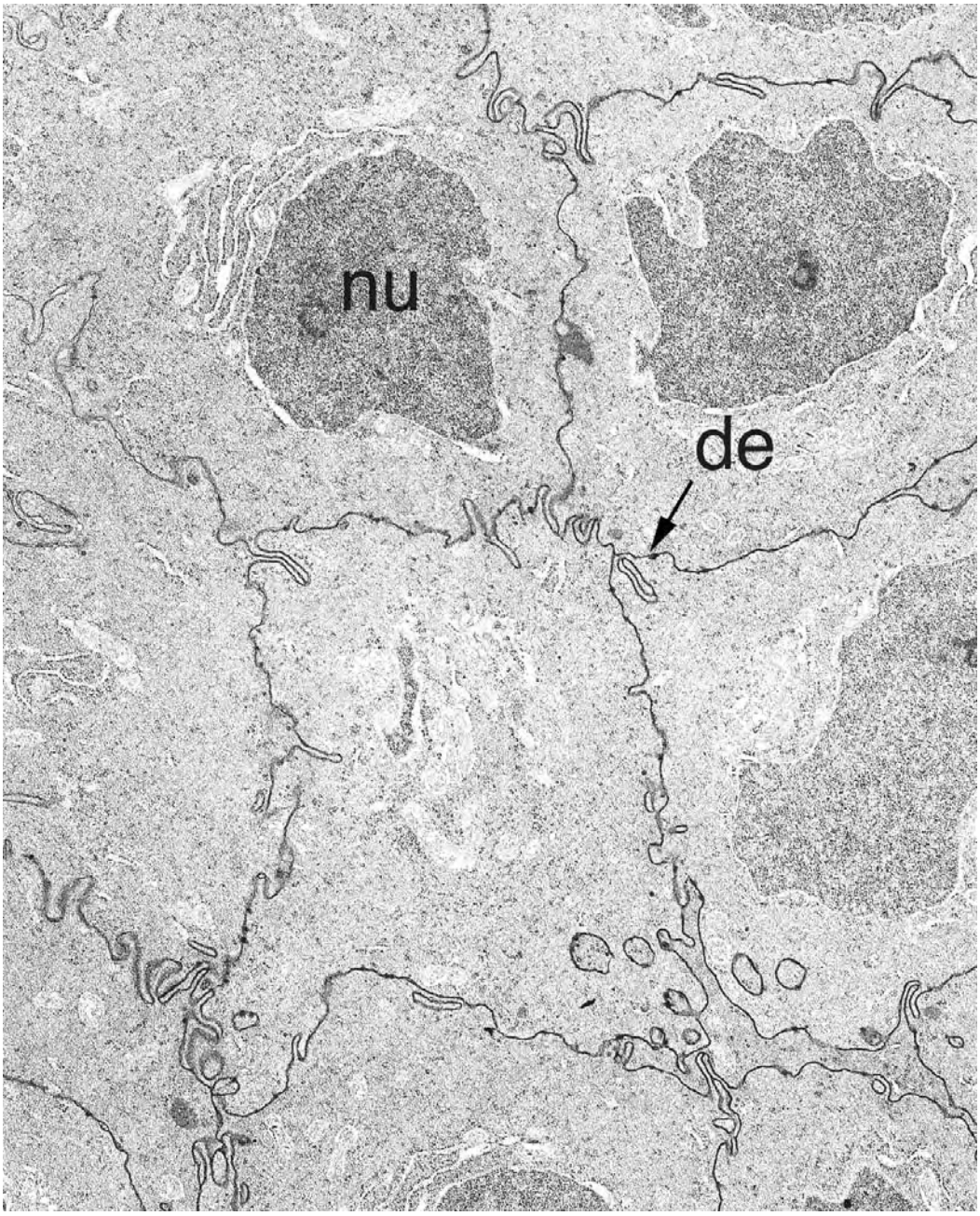

Fig. 41: Corneal epithelial cells of the bovine cornea with distinct nucleus (nu). Note the multiple infoldings of cytoplasmic membranes with numerous desmosomes (de) (×7500).

Wing cells are a group of polygonal cells on top of the basal cells. These layers form a transient zone between the columnar basal cells and more superficial squamous cells. The flattened superficial cells comprise several layers.

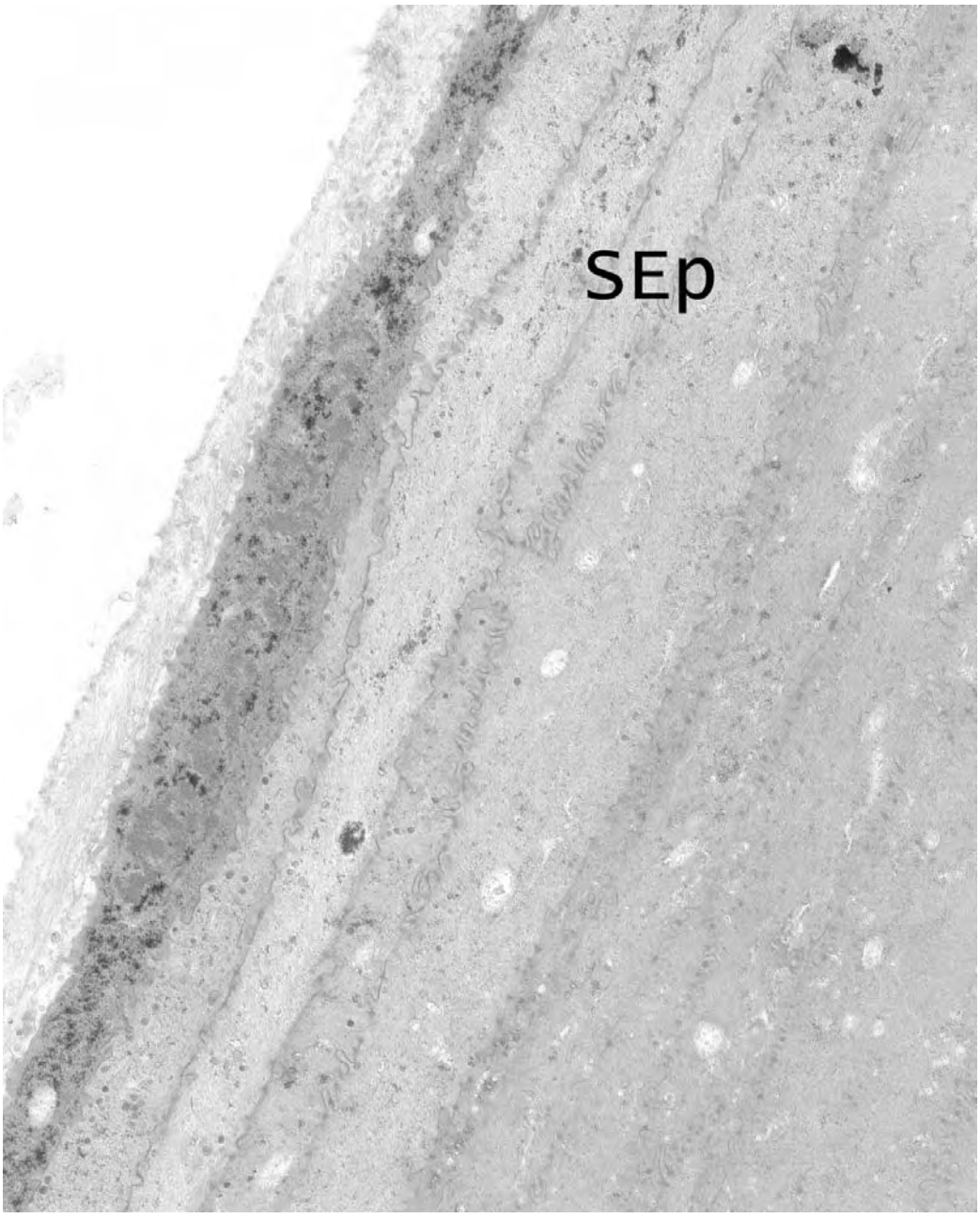

Fig. 42: Superficial corneal epithelium (SEp) of the bovine cornea (×7500).

The cytoplasm of the superficial cells (Fig.42) contains numerous tonofilaments and vesicles, but it generally lacks the mitochondria, rough endoplasmic reticulum, and ribosomes that occur in the basal and wing cells.

Numerous desmosomes are visible and the surface cells have zonulae occludentes on their lateral membranes. Microprojections are visible on the surface cells. At the limbus, pigment granules are scattered in all layers except the superficial squamous cells. Nerves that enter the epithelium lose their ensheathment and terminate in naked nerve endings among the wing cells.

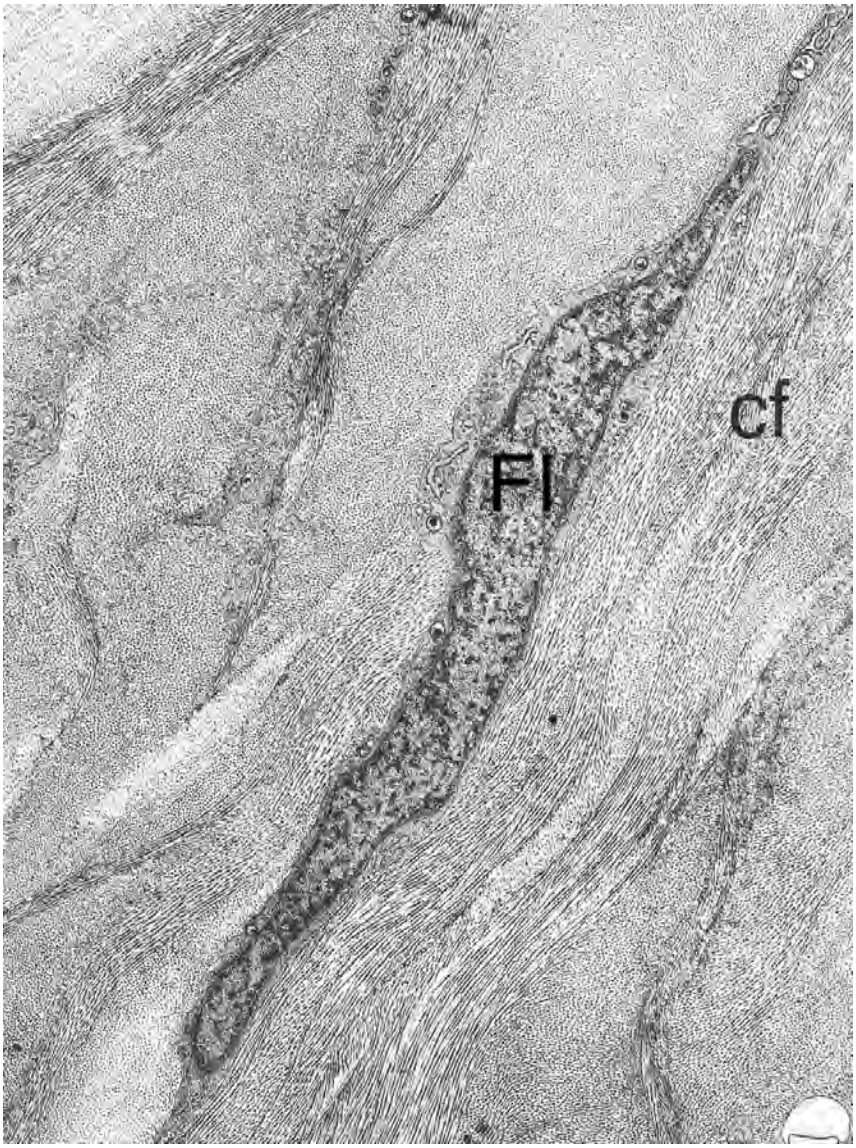

Fig. 43: Stroma of the bovine cornea consists of arranged lamellae of collagen fibers (cf) which are interspersed with fibroblasts (keratocytes) (FI) (× 7500).

The corneal stroma comprises 90% of the thickness of the cornea. It consists of transparent, almost structureless lamellae of fibrous tissue. These lamellae lie in sheets

and split easily into planes. Fixed and infrequently wandering cells are located between the lamellae.The fixed cells are fibrocytes, which are called keratocytes in the cornea (Fig. 43).

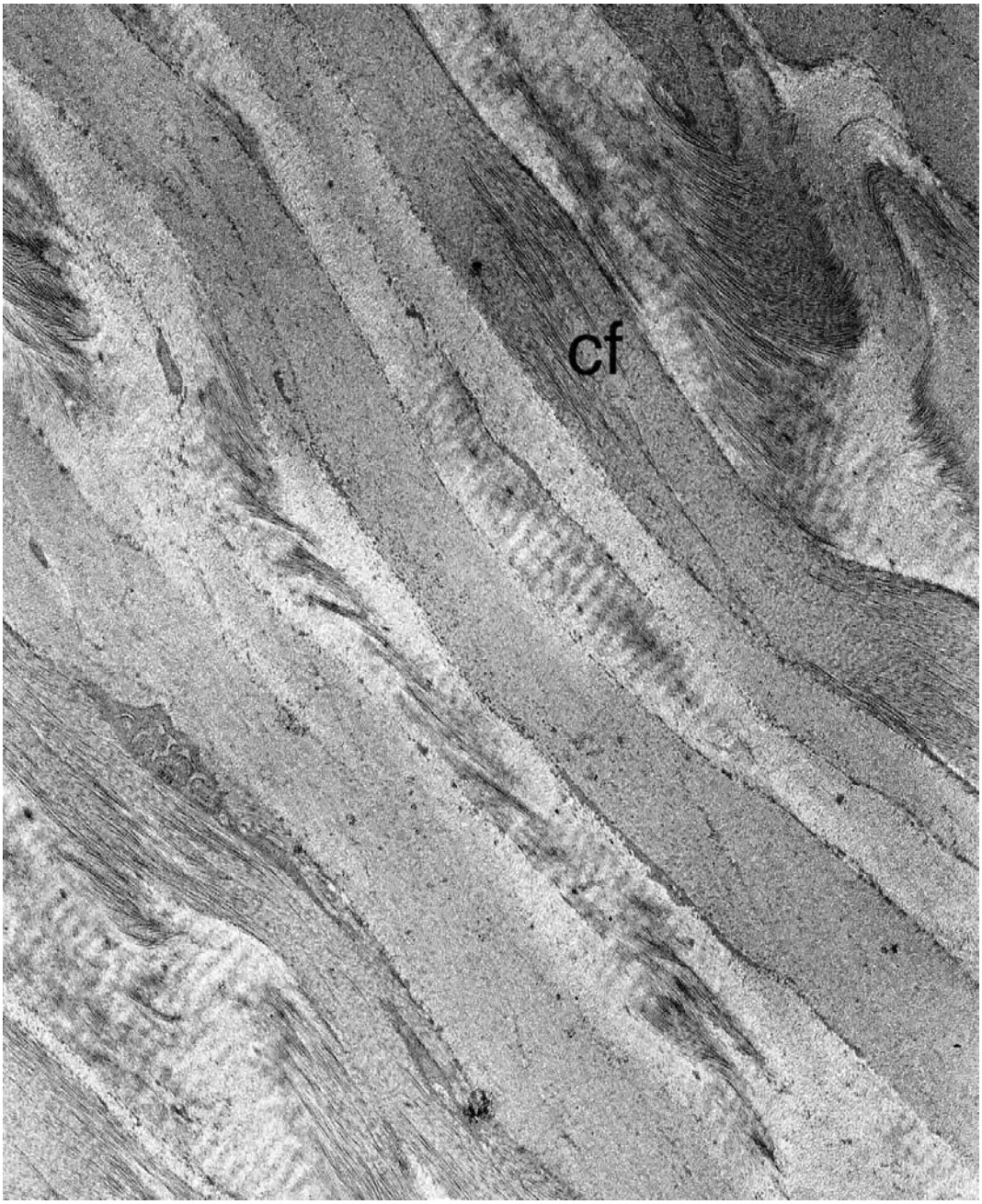

Fig. 44: Stroma of the bovine cornea consists mainly of lamellae of collagen fibers (cf) (× 7500).

These cells contain a thin nucleus occupying a major part of the cell. The cytoplasmic

inclusions are sparser than in fibroblasts from other locations. There is a predominance of ribosomes and profiles of the rough-surfaced endoplasmic reticulum. Wandering cells are usually leucocytes that have immigrated from the limbus.

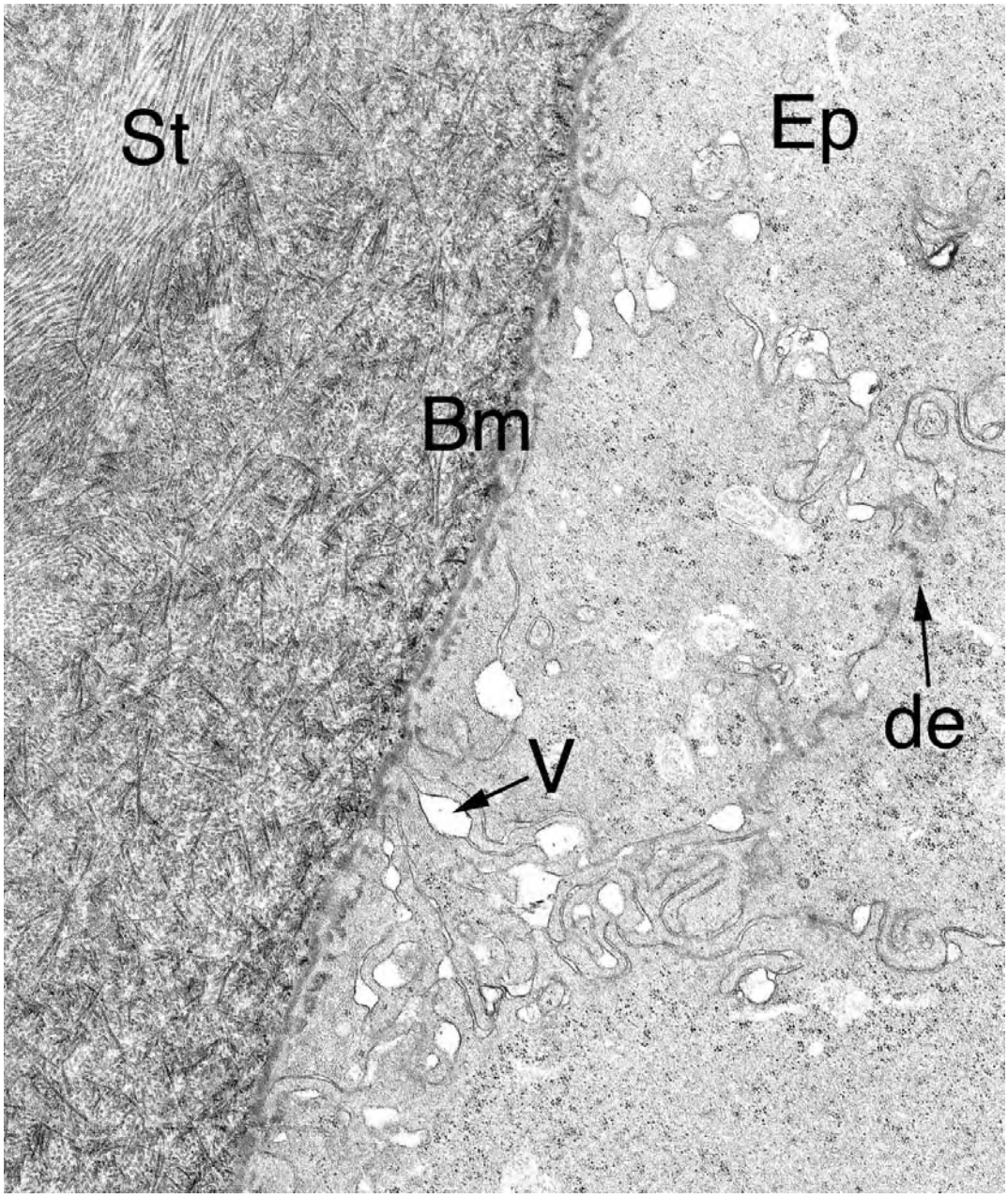

Fig. 45: Basal corneal epithelium and the anterior stroma (St) of the bovine cornea. Bowman's membrane (Bm) is well developed with randomly oriented thin collagen fibers. The basal cell of the corneal epithelium (Ep) contain many vacuoles (V) and the cell membranes have desmosomal attachment (de) (x 17500).

The lamellae are parallel bundles of collagen fibrils that are tightly packed into fascicles in a lamellar fashion. Each lamella is running through the entire diameter of the cornea (Fig. 44). All the collagen fibrils within lamellae are parallel, but different lamellae vary greatly in direction. The lamellae of the posterior stroma are more regular in arrangement than those of the anterior third of the stroma. The anterior lamellae are more oblique to the surface, and they show more branching and interweaving.

The most anterior stroma has a thin, cell free zone corresponding in location to the anterior limiting membrane, also known as Bowman's membrane. The collagen fibrils in this layer are randomly dispersed and are smaller in diameter than in the other layers (Fig. 45).

The Descemet's membrane consists of three zones. The thin anterior unbanded zone next to the stroma is characterized by an amorphous material. It is followed by a broad banded zone that has cross striation and another broad posterior unbanded zone.

The cells of the corneal mesenchymal epithelium are of a squamous type with elongated nuclei.The cytoplasm contains abundant mitochondria, many ribosomes, smooth and rough endoplasmic reticulum, and a variety of vesicles, including pinocytotic vesicles.

4.4.2 Sclera

The bulk of the sclera is called the sclera proper. It contains elastic fibers that are interlaced among the collagen fibers with melanocytes and fibrocytes. The collagen fibers, fibrocytes, and occasional melanocytes are arranged meridional, obliquely and radially in an irregular fashion. The spaces between scleral collagen fibrils or bundles appear very limited. The amount of amorphous materials filling the spaces between them is very small.

4.4.3 Iris

The bovine iris is divided into the anterior border layer, the stroma and sphincter muscle, and the posterior epithelial layers.

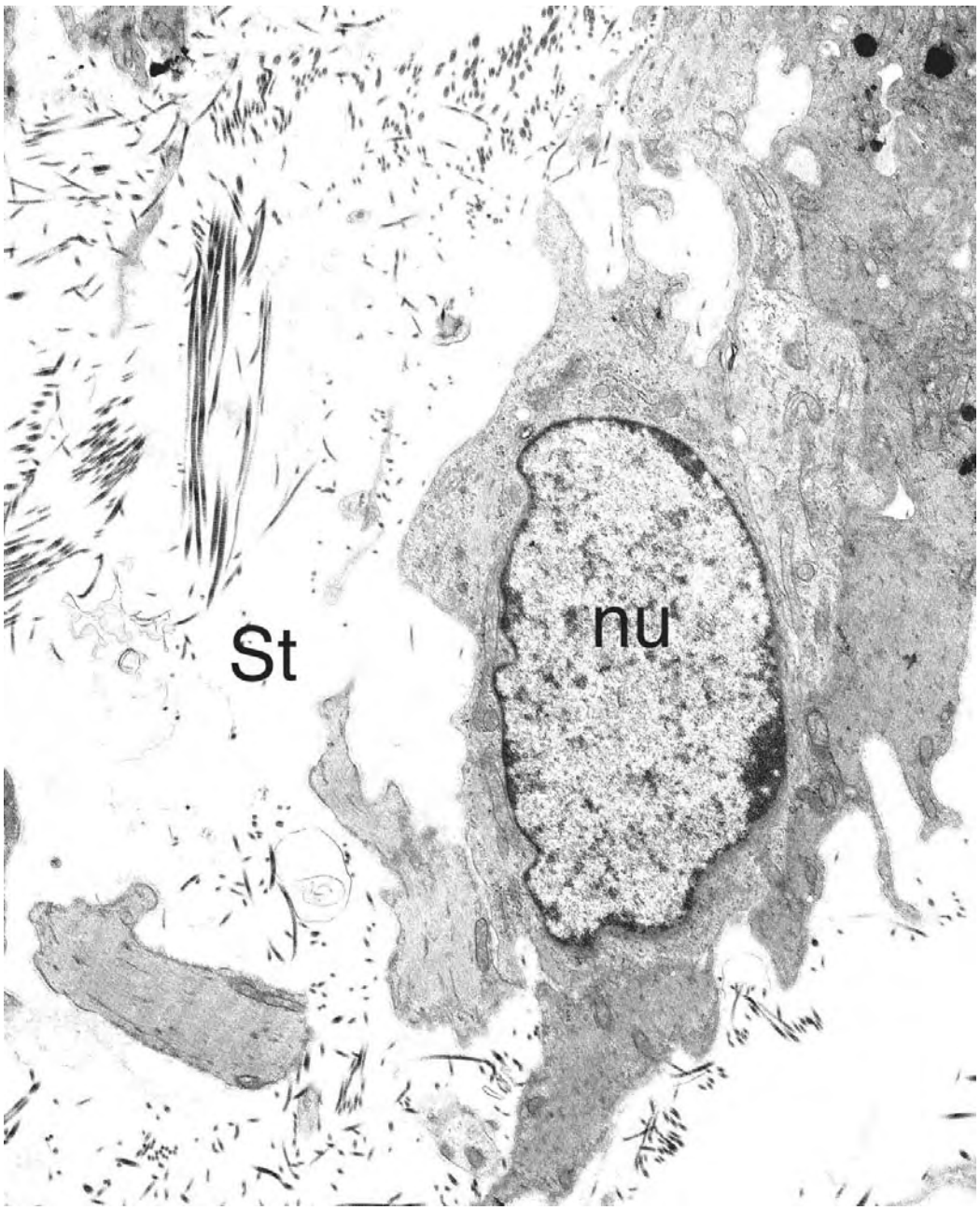

Fig. 46: Stroma of the bovine iris (St). Note the nucleus (nu) of a cell of the iridal epithelium (× 7500).

The anterior border layer consists of two cell types fibroblasts and melanocytes. One or more layers of melanocytes with round to oval pigmented granules are beneath the single layer of fibroblasts. For the most part, the melanocytes are oriented parallel to the iris surface and their processes intermingle with other melanocytes and anterior fibroblasts. No intercellular junctions are seen (Fig. 46).

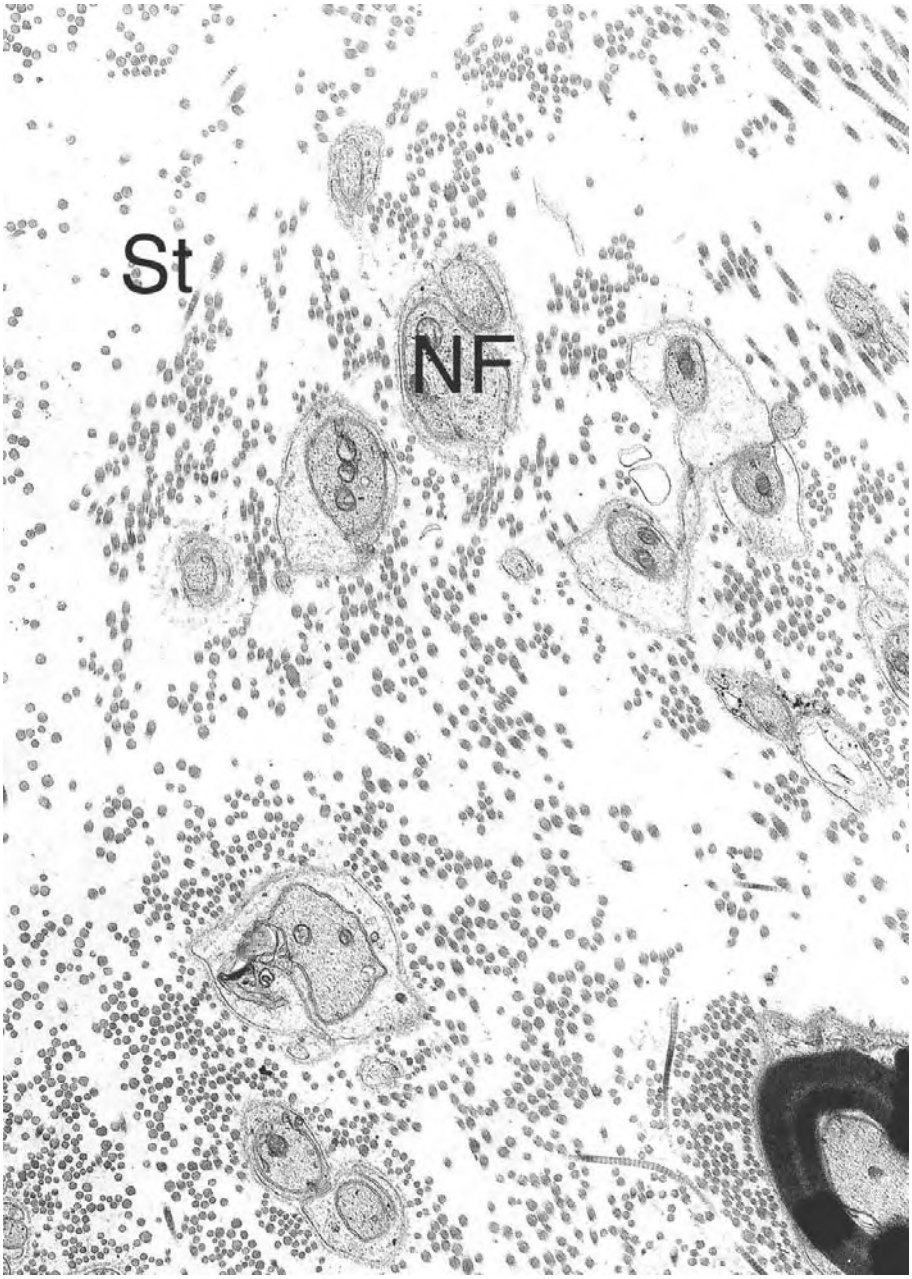

Fig. 47: Stroma of the bovine iris (St) with nonmyelinated nerve fiber (NF) (×11000).

As in other animals the iridal stroma is composed of fine collagenous fibers, many chromatophores and fibroblasts.

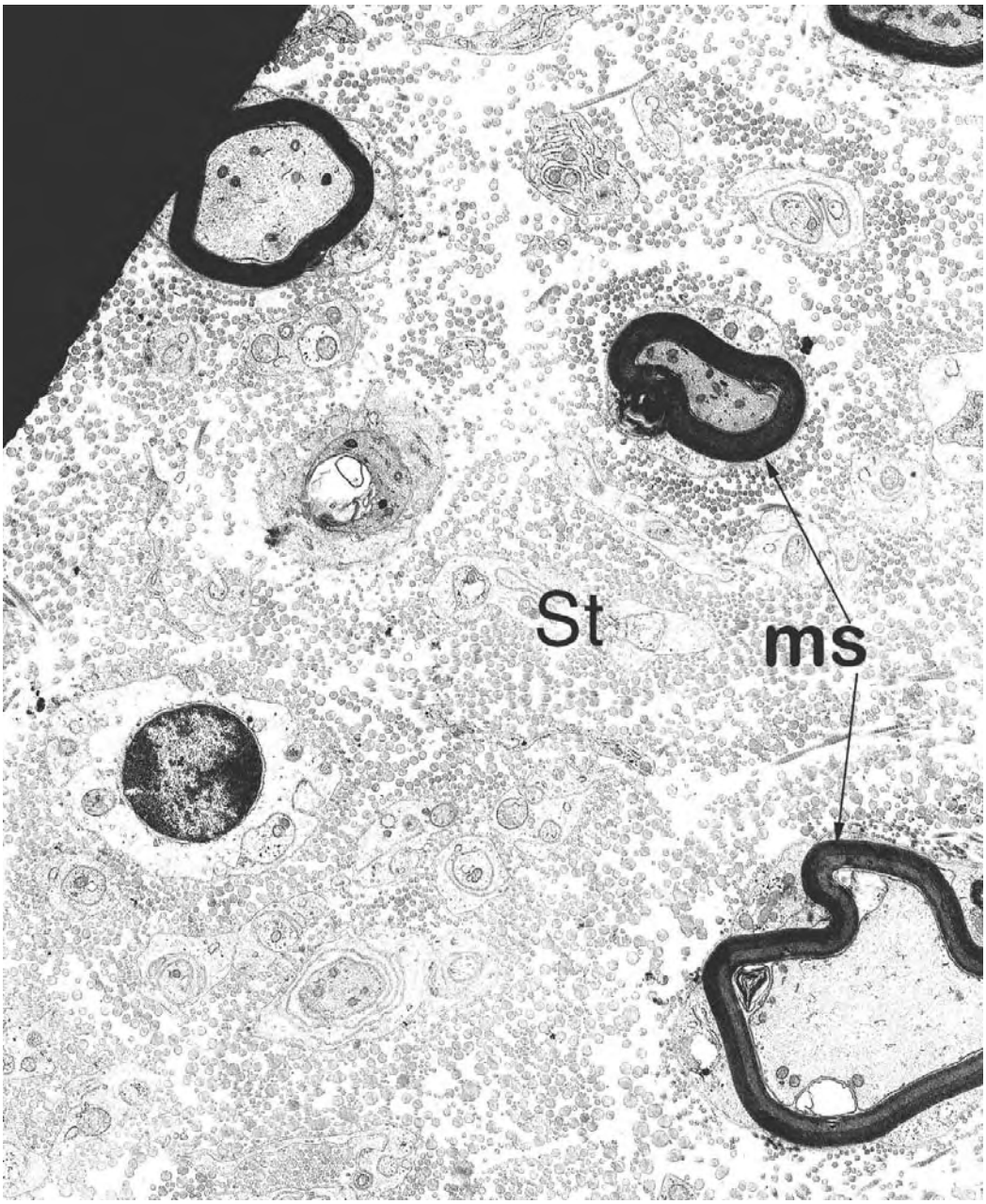

Fig. 48: Stroma of the bovine iris (St) with nerve fiber covered by myelin sheath (ms) (× 7500).

The iridial stroma is rich in myelinated and non-myelinated nerve fibers (Fig. 47, 48). The stroma is loosely arranged (Fig. 46) except around the blood vessels and nerves, where it forms dense sheaths (Fig. 47, 48, 49). Fibroblasts and melanocytes are the

predominant cell types that can be evenly distributed throughout the stroma. They are more concentrated in the anterior and posterior parts.

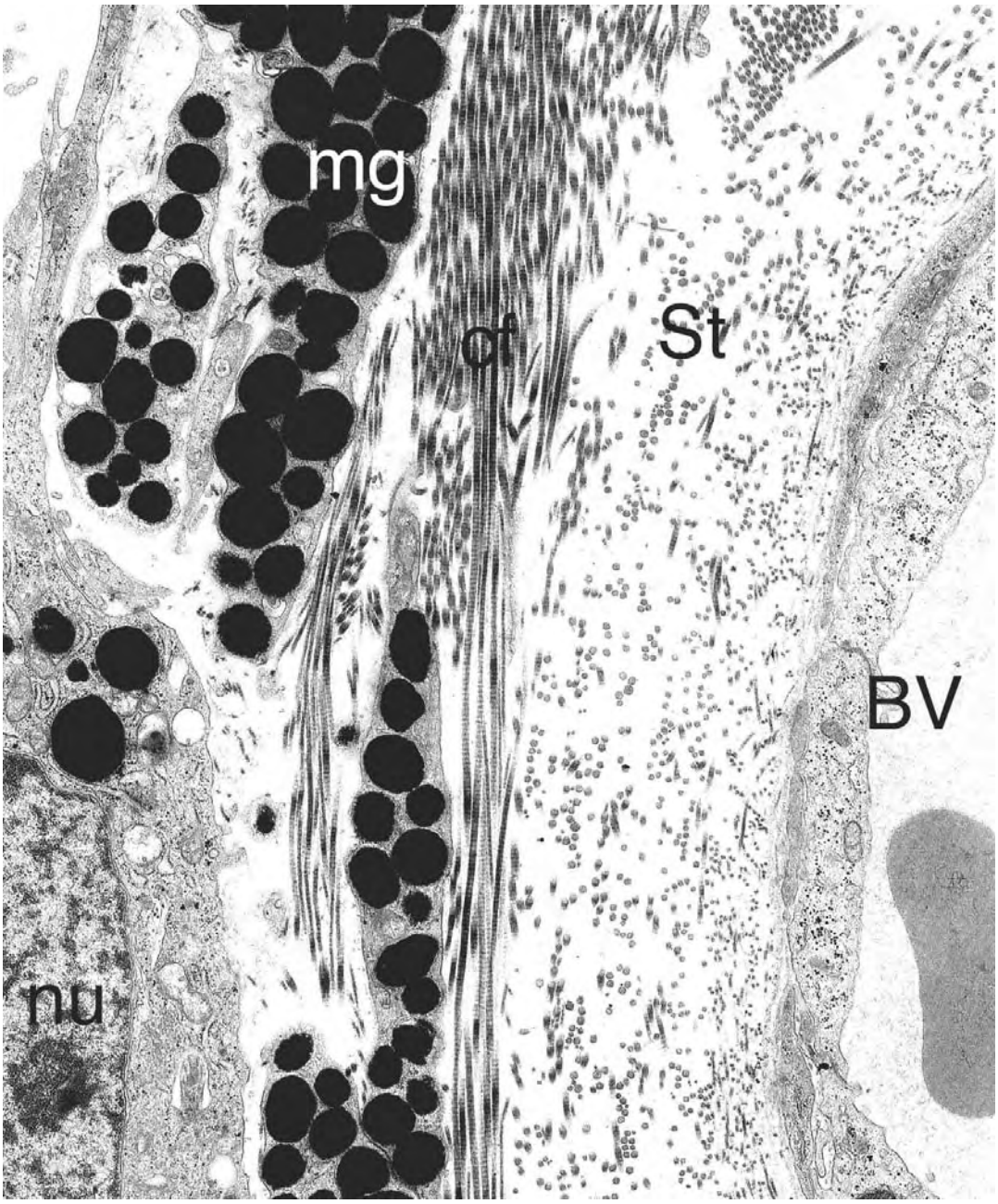

Fig. 49: Pigmented epithelium in the bovine iris with distributed melanin granules (mg). Note the stroma (St) rich with blood vessels (BV) (× 11000).

The melanocytes are also prominent around the adventitia of blood vessels. The pigmented granules are generally small and round or oval. The cytoplasm is occupied

mainly by filaments intermingled with some mitochondria. The iridal sphincter muscle, consisting of flat thin, circular arranged bundles of smooth muscle fibers, is located in the stroma near the pupil.

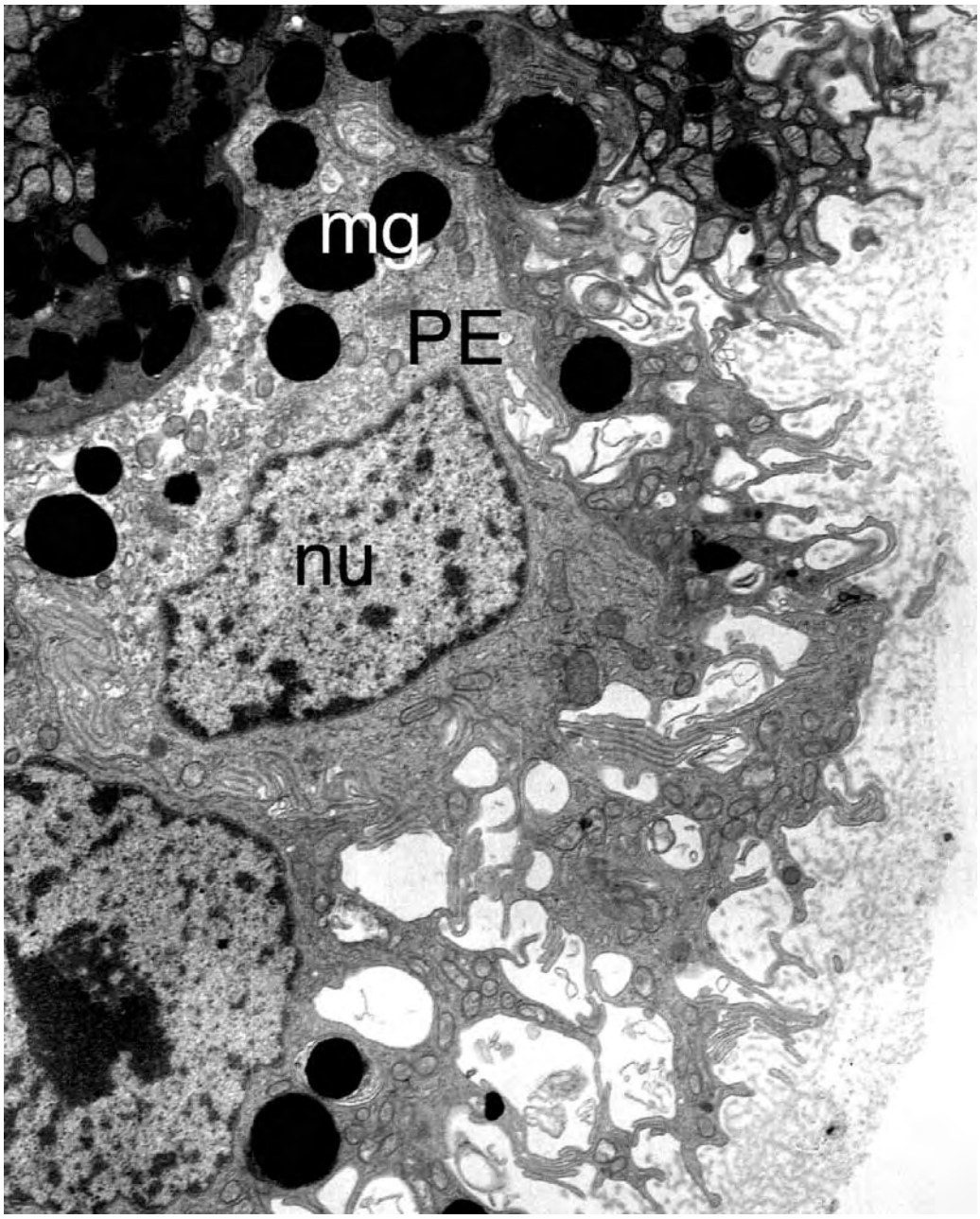

Fig. 50: Pigmented epithelial cell (PE) in bovine iris, nucleus (nu); melanin granules (mg) (x 11000).

The dilatator muscle is a single layer of smooth muscle fibers. These muscle fibers contain pigment around their nuclei. The basal region of each cell contains myofilaments. The basal aspect of the posterior epithelium of the iris faces the posterior chamber and has numerous surface projections. A basement membrane separates the cells from the posterior chamber. The lateral cell surfaces of the posterior epithelium have numerous slender cell processes with scattered desmosomes. The cytoplasmic density of this layer is greater than that of the deep layer, mostly because of a more compact accumulation of ribosomes. The mitochondria are spherical in both cell types.The pigmented granules are larger in the cells of inner layer. In general, large spaces occur between adjacent lateral cell membranes (Fig. 50).

4.4.4 Ciliary body

The bovine ciliary process consists of a central core of stroma and blood vessels covered by a double layer of epithelium an inner pigmented, cuboidal epithelium, and an outer non-pigmented cuboidal epithelium (Fig. 51). Nuclei of outer non-pigmented epithelial cells are large (Fig. 52).

Cytoplasm displays a fair number of ribosomes. Mitochondria are few and have a spherical shape. Lateral cell surfaces of the pigmented epithelium have numerous villous processes. The lateral intercellular junction of the pigmented epithelium consists of desmosomes, except at the apical end, where gap junctions, zonulae adherentes, and zonulae occludentes can be observed.

Cellular junctions between the non pigmented and pigmented epithelium of the ciliary process are very important, because these cell layers are tightly locked together in a physiologic way. The types of junctions found here reflect this important feature. They consist of many gap junctions interspersed with desmosomes and unusual junctions termed puncta adherentes, which resemble desmosomes but lack the larger tonofilaments and the intercellular central band of dense material.

The pigmented epithelium is generally cuboidal and heavily loaded with round to oval melanin granules (Fig. 52). The lateral cell surfaces of the pigmented epithelium are

joined by desmosomes. The basal cell surfaces show no specialized junctions. The nuclei of pigmented epithelia are located apically (Fig. 53).

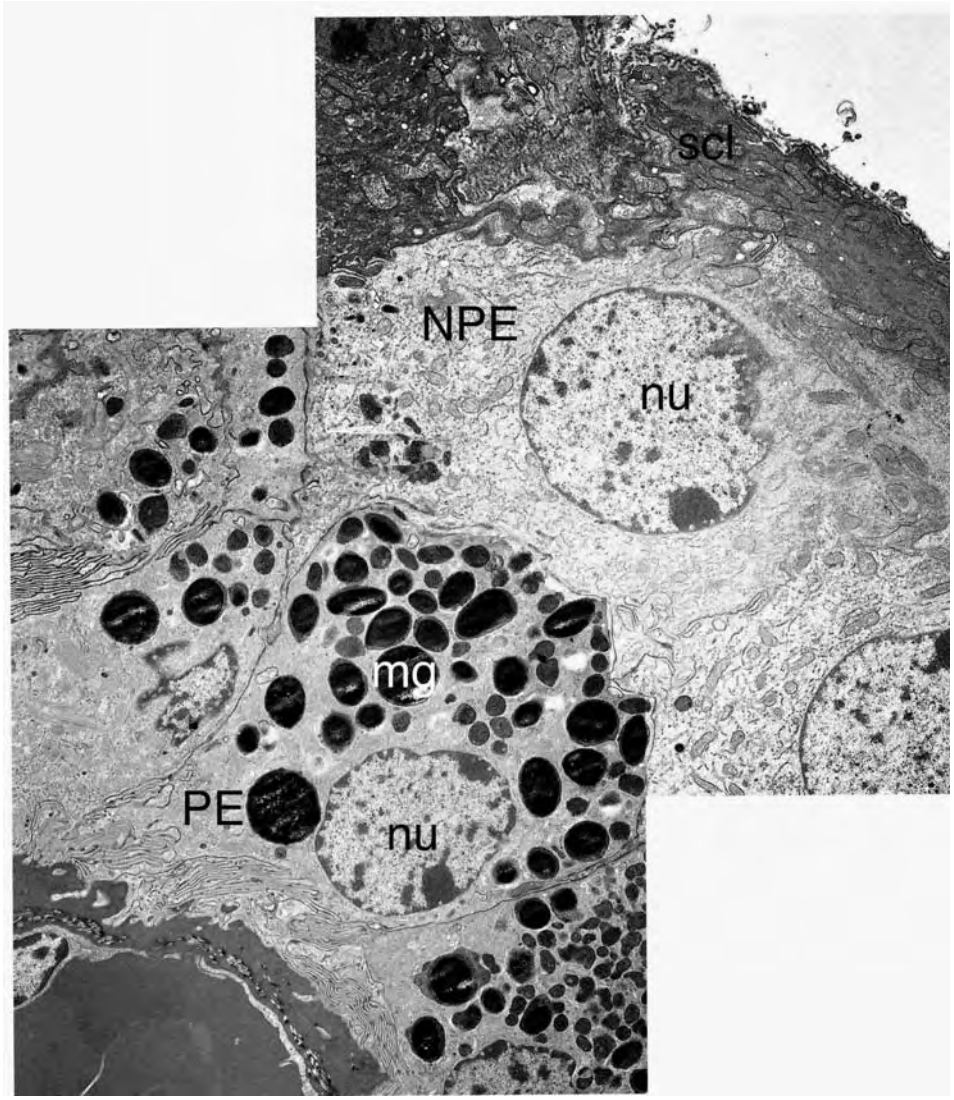

Fig. 51: Layers of the ciliary process in the bovine ciliary body showing supraciliary layer (scl), non pigmented epithelial cell (NPE) with a prominent nucleus (nu) and a pigmented epithelial cell (PE) with characteristic melanin granules (mg) and distinct nucleus (nu) (×4500).

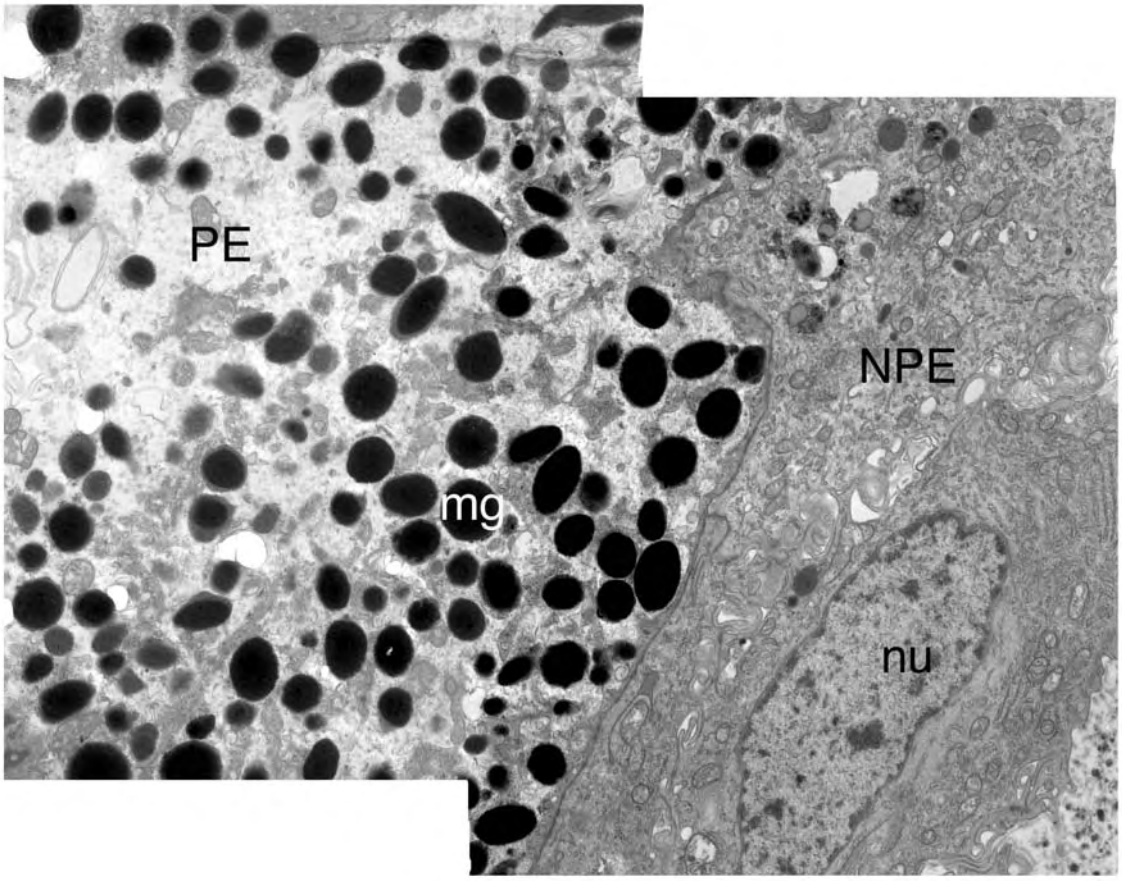

Fig. 52: Non pigmented epithelial cell (NPE) with an oval nucleus (nu) rich in euchromatin and pigmented epithelial cells (PE) with characteristic melanin granules (mg) in the ciliary process of the bovine ciliary body (× 6600).

The smooth muscule of the ciliary bodies is highly developed (Fig. 55). The muscle has three components (i.e., radial, meridional, and circular), and forms a large, anteriorly pyramidal shaped structure that provides a strong base plate for iridal attachment. The muscle fibers, especially those located anteriorly, course more circumferentially than meridional.

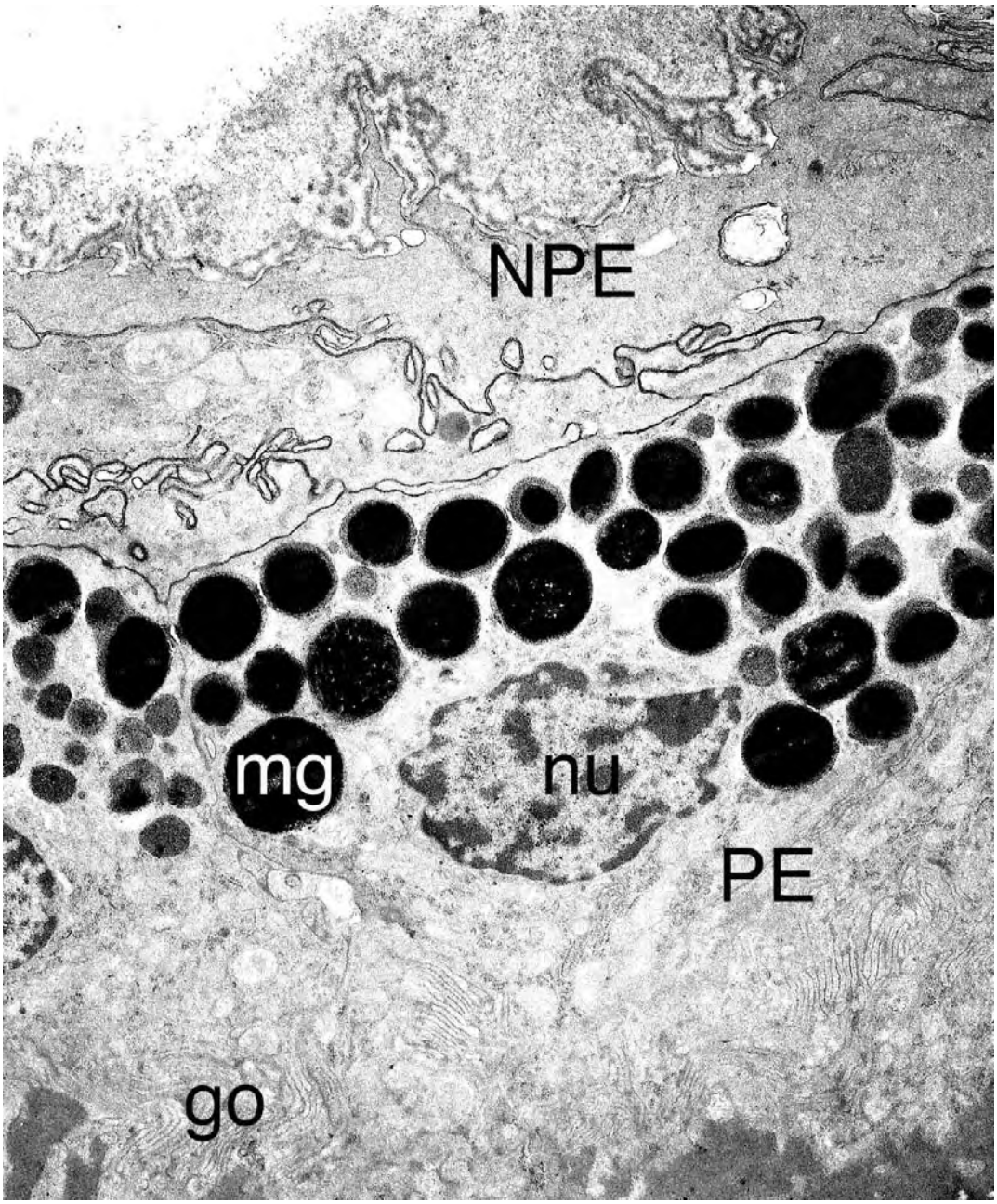

Fig. 53: A non-pigmented epithelial cell (NPE) and a pigmented epithelial cell (PE) in a ciliary process of the bovine ciliary body. Note the melanin granules (mg), nuclei (nu) and Golgi complex (go)in the pigmented ciliary epithelium (×11000).

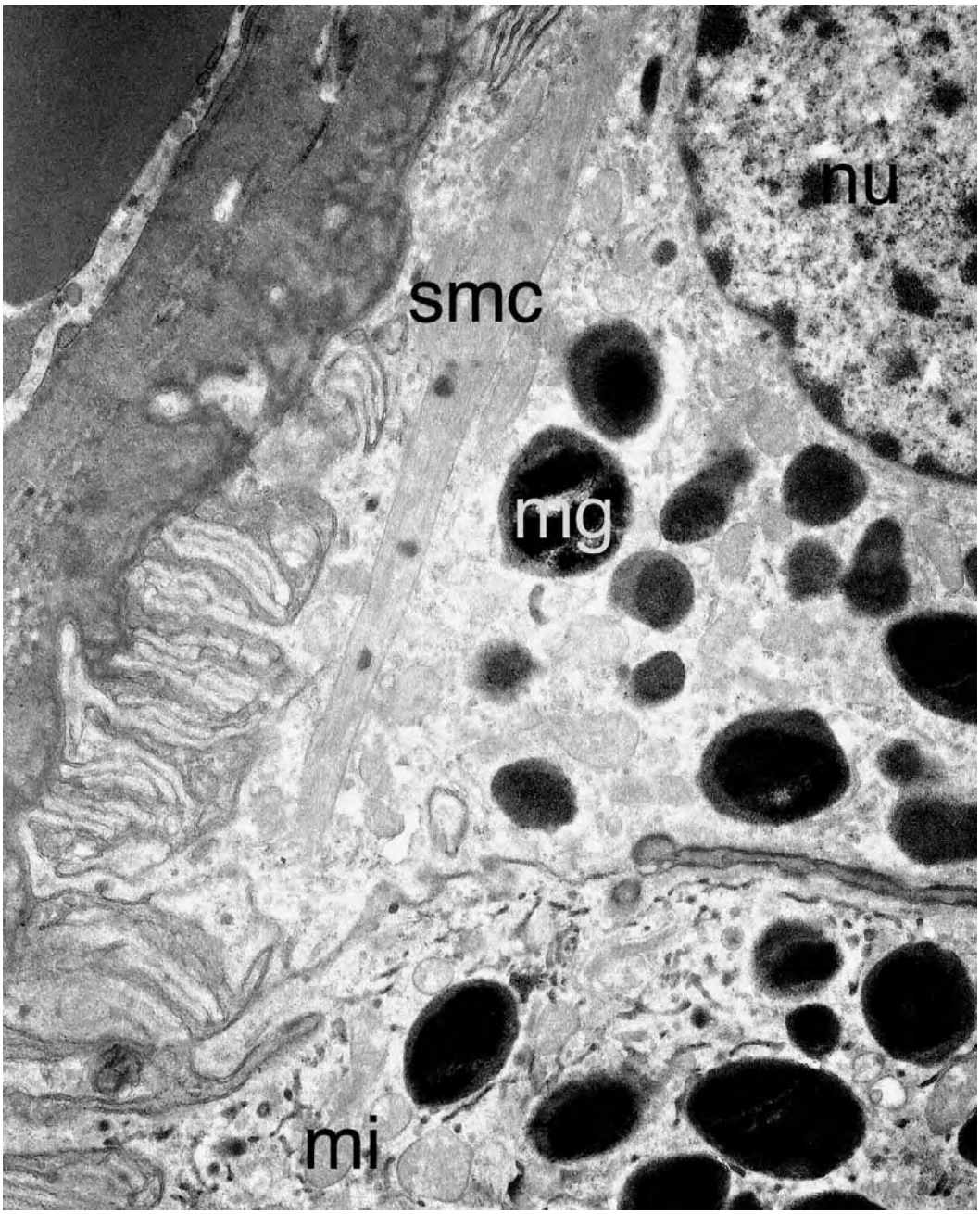

Fig. 54: Pigmented ciliary epithelium in a ciliary process of the bovine ciliary body containing melanin granules (mg), nucleus (nu) and distributed mitochondria. Note the presence of ciliary smooth muscle cells (smc) (× 17500).

The cytoplasm contains numerous melanin granules, rough and smooth endoplasmic reticulum, free ribosomes (i.e. polysomes) and spherical or slightly elongated mitochondria (Fig. 54).

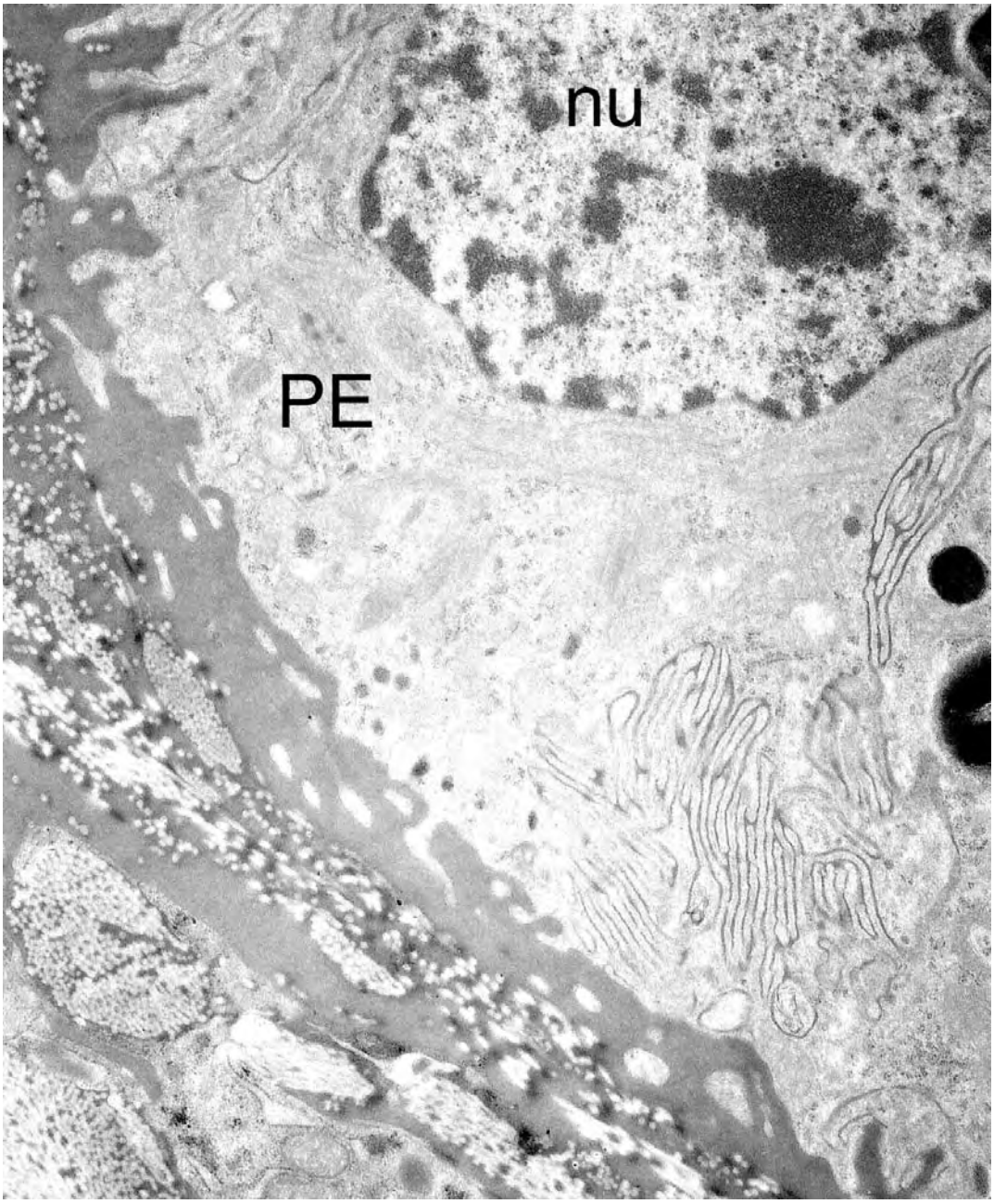

Fig. 55: The pigmented ciliary epithelial cell (PE) in a ciliary process of the bovine ciliary body with prominent nucleus (nu) (× 17500).

4.4.5 Choroid

Histologically the choroid composed of suprachoroidea, stroma with large and medium-sized blood vessels, tapetum and choriocapillaries.

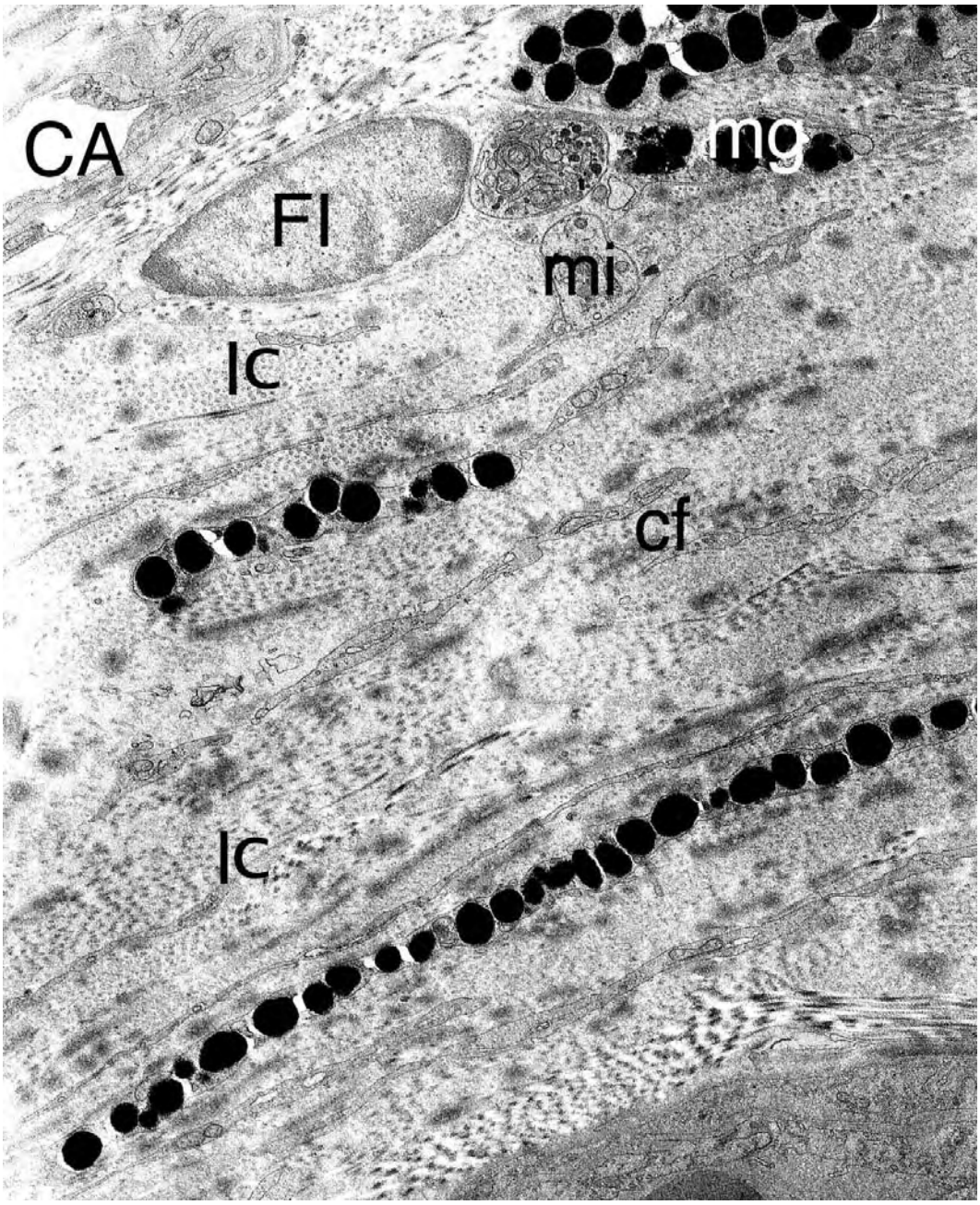

Fig. 56: Choriocapillaries in the bovine choroid contains capillaries (CA) and distributed melanin granules (mg). The stroma of the choroid consists mainly of collagen fibes (cf) and iridocytes (Ic) or tapetal cells bordered by fibroblast (FI) mitochondria (mi) (× 7500).

The bovine suprachoroidea consists of elastic, heavily pigmented connective tissue. The choroidal melanocytes are round to oval. They are predominantly situated in this region, being intimately associated with elastic fibers (Fig. 56).

The large sized blood vessels in the choroidal stroma form a vascular plexus embedded in loose connective tissue containing melanocytes and fibrocytes.
The medium sized vessels, especially the arteries, are branching and, radiating slightly inward in a fanlike manner from the large vessels. The cells surrounding these vessels comprise melanocytes and fibroblasts. The extracellular space contains loosely arranged bundles of collagen fibers, mainly running parallel to the choroidal surface. Interspersed are numerous elastic fibers.
Closely and regularly arranged collagen fibers form the tapetum, which is referred to as fibrous tapetum. The fibrous tapetum is basically acellular, except for occasional fibrocytes. The collagen fibrils are organized into well-ordered lamellae that branch and interconnect with adjacent lamellae at the same level, running parallel to the retinal surface (Fig. 56).
The choriocapillaries is the inner most layer of the choroid, forming a thin layer of capillaries (Fig. 56). The lumen of the capillaries is fairly wide. The capillaries possesses numerous circular fenestrations, which are often arranged in rows. External to the endothelium is a basement membrane forming the external layer of Bruch’s membrane.

4.4.6 Retina

Histologically the retina consists of ten layers which are usually considered from outside inward in the following order: retinal pigmented epithelium, visual cell layer (rod and cone layer), outer limiting membrane, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer, nerve fiber layer, and inner limiting membrane.
The retinal pigmented epithelium is a layer of cuboidal cells that forms the outermost layer of the retina. There are numerous infoldings of the cellular membrane at the apex (vitreal end) of each cell. All retinal epithelial cells possess numerous finger-like processes which enclose the outer segment of the visual receptors. The lateral borders of the retinal pigmented epithelium cells near the apical end have well defined junctional complexes, including zonulae occludentes and a zonulae adherentes.

The nuclei are oval to round and basally located with its long axis parallel to the retinal surface.

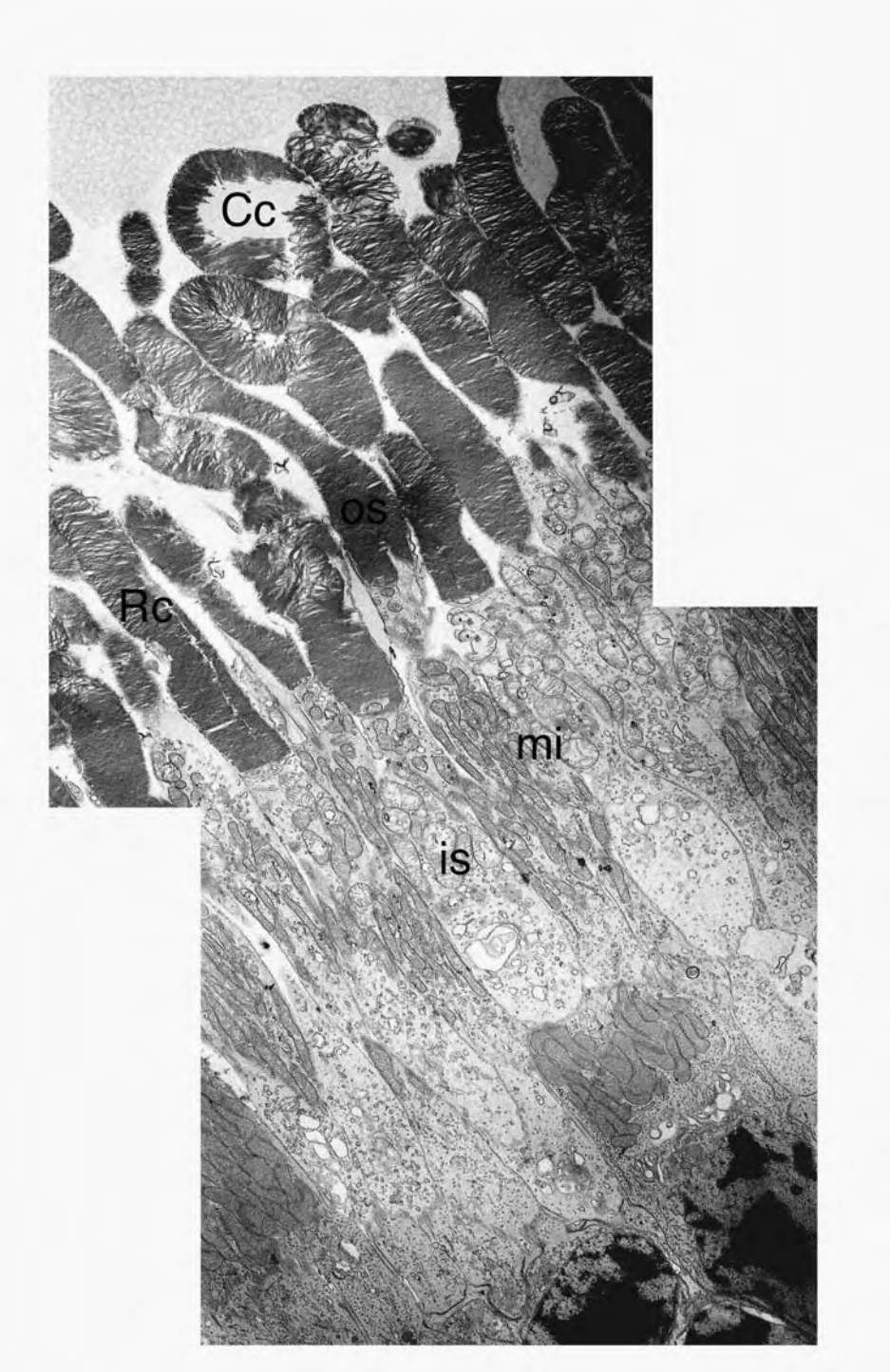

Fig. 57: The visual cell layer of the bovine retina contains cone cells (Cc) and rod cells (Rc). Each cell consists of outer segment (os) and inner segment (is) which contains mainly mitochondria (mi) (× 4500).

The cytoplasm is predominantly filled with smooth endoplasmic reticulum, however discrete groupings of rough endoplasmic reticulum are also seen, as well as scattered polysomes. Mitochondria are small, oval and evenly distributed throughout the entire epithelial cells.

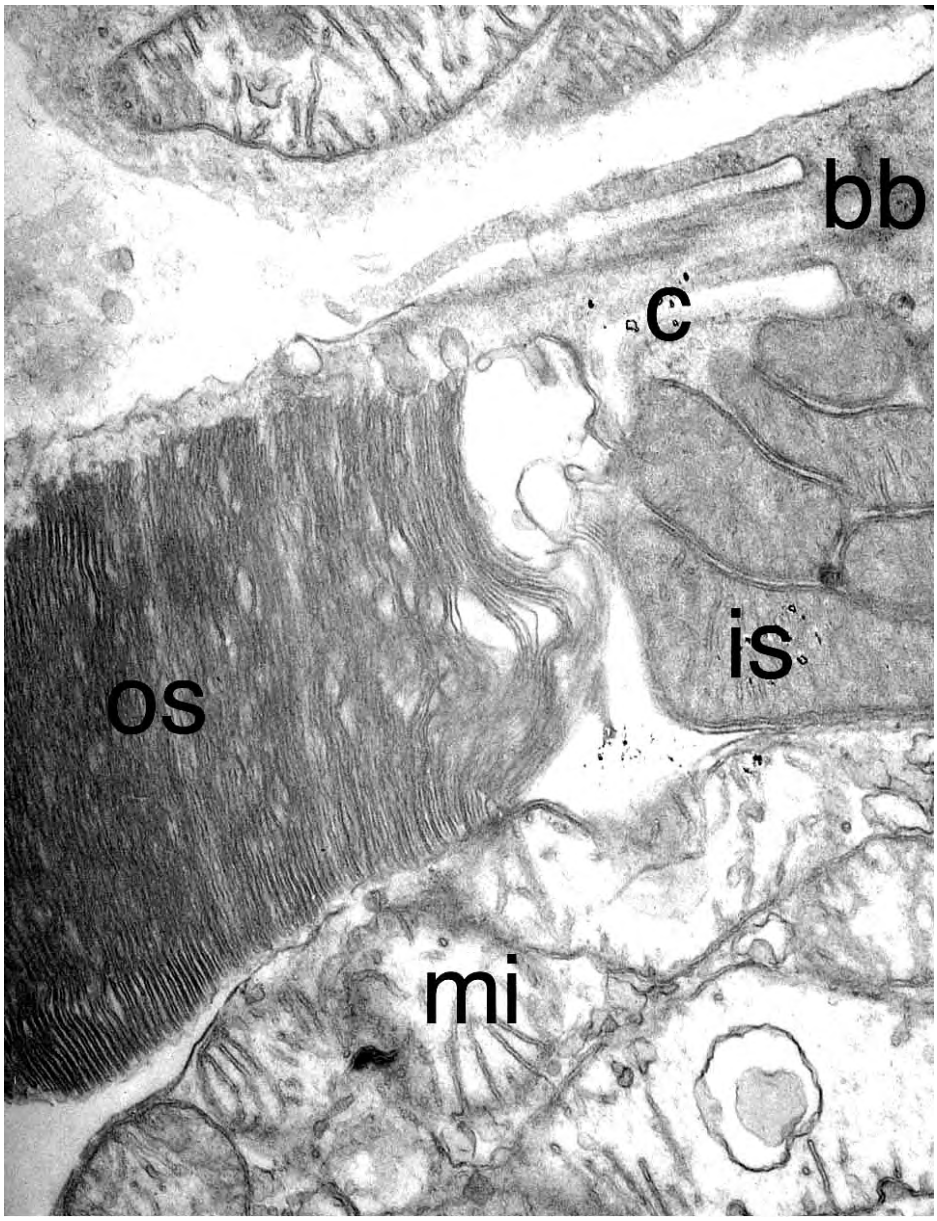

Fig. 58: Outer segment (os) and inner segment (in) of the photoreceptor cell in the bovine retina is connected by a connecting cilium (c). It contains a basal body (bb). Note the presence of numerous mitochondria (mi) (× 21000).

Also other bodies, including residual bodies of lipofuscin and lamellated bodies, or phagosomes consisting of phagocytosed photoreceptor outer segment discs are frequently found.

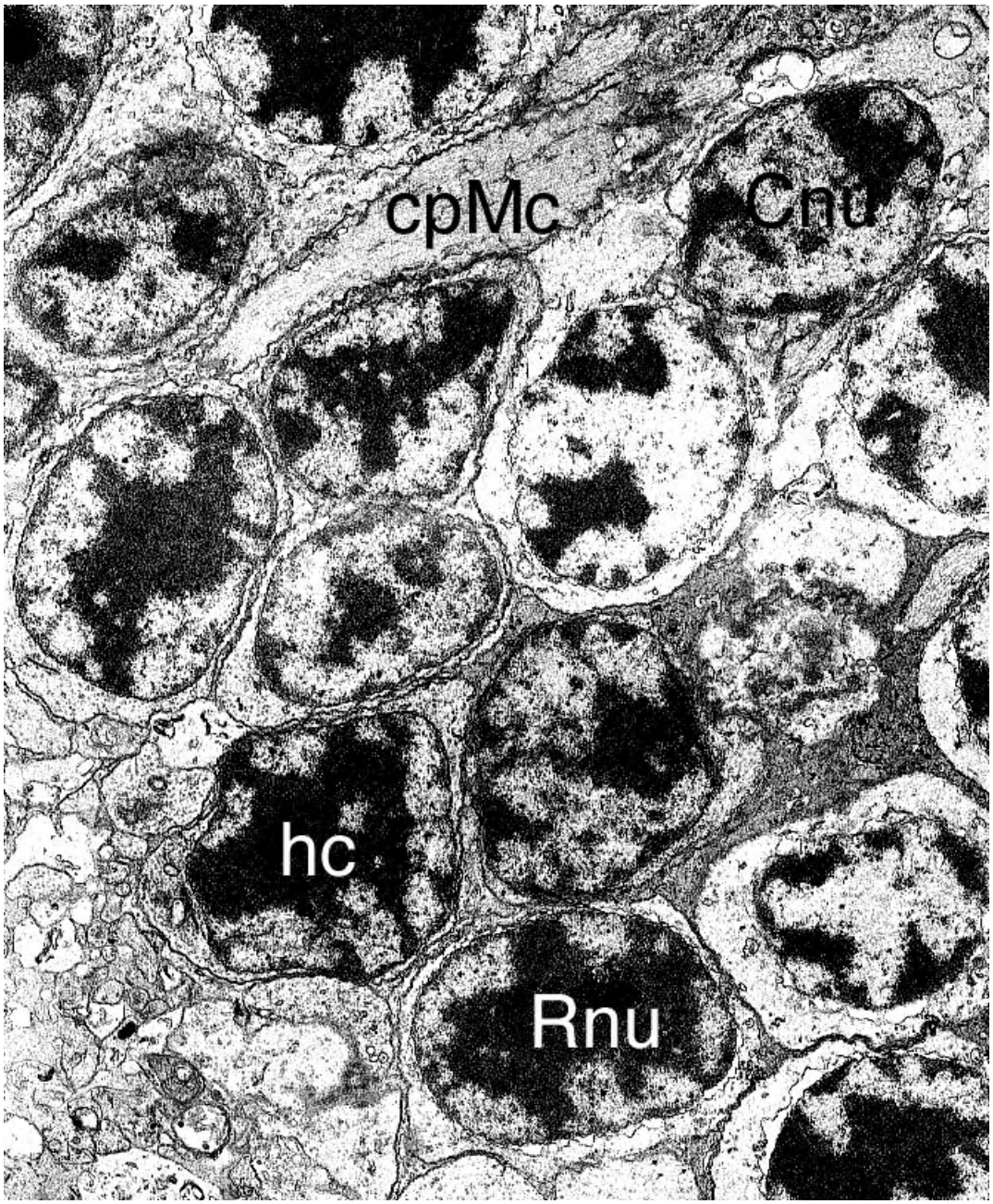

Fig. 59: Within the outer nuclear layer of bovine retina rod nuclei (Rnu) are more heterochromatic (hc) than cone nuclei (Cnu). Note the presences of cytoplasmic processes of the Müller cell (cpMc) (× 7500).

Lysosomes are abundant in the apical region of the retinal pigmented epithelium. The melanosomes in the retinal pigmented epithelium are numerous and their shape is typically oval.

The basal (scleral) aspect of the retinal pigmented epithelium rests on a complex basement membrane called Bruch's membrane, When fully developed, Bruch's membrane consists of a pentalaminate structure: the basal membrane of the pigment epithelium of the retina, an inner and outer collagenous zones, an elastic layer that lie between then, and the basal membrane of the choriocapillaries.

Retinal photoreceptors comprise two types, rods and cones cells. Each consist of inner and outer segments, with their nuclei being in the outer nuclear layer (Fig. 57). Outer segments of rods and cones are composed of stacks of bi-membranous discs. The rods form stacks of uniform width through their length and are longer than the cone outer segments. Cone outer segments also consist of stacks of discs, but these segments are wider at their inner end, producing a cone-like shape.

The discs consist of two membranes that are continuous at their ends and, in rods, are separated from the cell membrane as well as the adjacent discs.

Proximal discs of the cones have continuity with the cell membrane. Nevertheless, this invagination of the cell membrane, which forms the disc lamellae, constitutes the major morphologic difference between rods and cones.

Rod and cones outer segments are connected to the inner segments by a modified cilium (Fig. 58). The outer portion of the inner segment is filled with long, tubular mitochondria and is termed the ellipsoid. The cone ellipsoid is more broad and conical, and it contains more mitochondria than the rod ellipsoid. The vitreal inner segment contains few mitochondria in rods and is relatively void of mitochondria in cones.

The cytoplasm contains smooth and rough endoplasmic reticulum, free ribosomes, a well developed Golgi apparatus, small vesicles, and microtubules. The inner segments of rods and cones are separated from each other by long, villous extensions of Müller cells.

The outer limiting membrane is actually composed of densities of the cell junction, the zonulae adherentes that firmly attach the inner segments of rods and cones to Müller cells and also the Müller cells to each other.

The outer nuclear layer contains the cell bodies and nuclei of the photoreceptors. The nuclei of the cones are usually larger, oval and more euchromatic (i.e., staining lighter) than the rod nuclei.

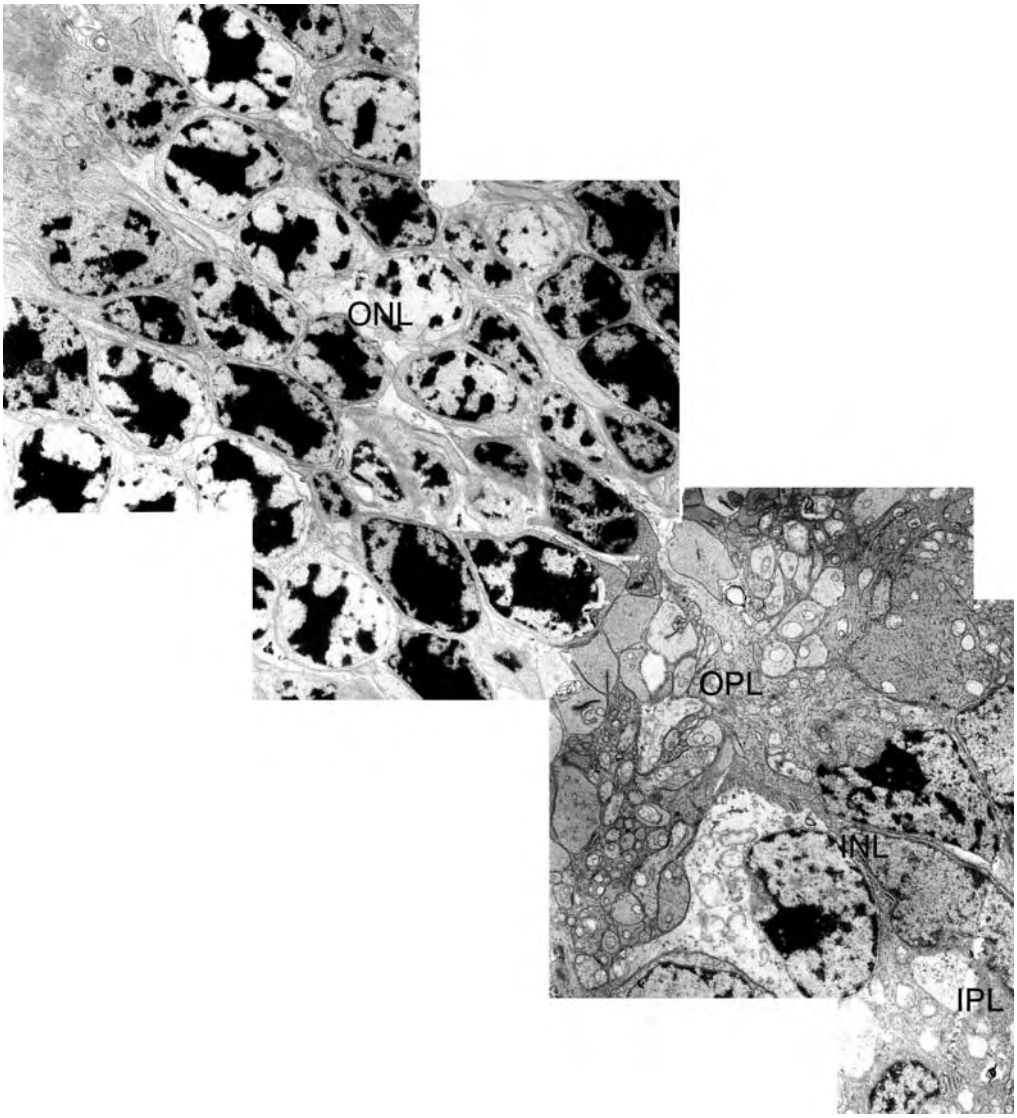

Fig. 60: Outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL) and inner plexiform layer (IPL) in the bovine retina (× 3000).

The rod nuclei are smaller, round to oval and darker, being more heterochromatic (Fig. 59). Additional structures in this layer are outer rod and cone connecting fibers.
The outer plexiform layer (Fig. 60) consists of axons of the rod and cone cell that synapse with the dendrites of the horizontal and bipolar cells. Double layered retinal blood capillaries lie mostly within the outer plexiform layer and extend to ganglion cell and

nerve fiber layer. With the TEM can be seen that the first layer is formed by one to four endothelial cells, and the second layer is a basement membrane that covers the endothelium as well as the surrounding pericytes (Fig. 61).

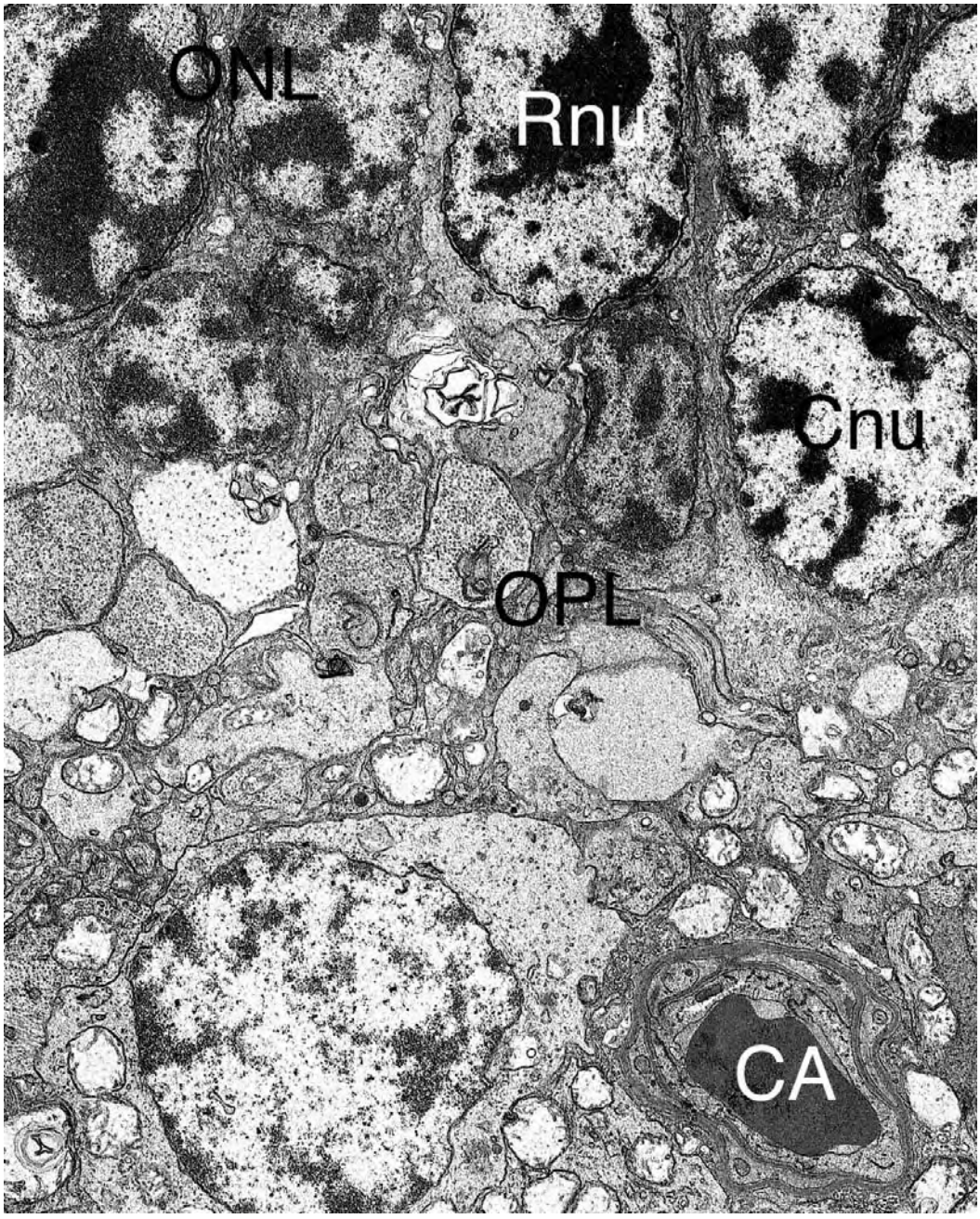

Fig. 61: Outer nuclear layer (ONL) of the bovine retina with rod nuclei (Rnu) and cone nuclei (Cnu). The outer plexiform layer (OPL) contains double layered blood capillaries (CA) which are characteristic for bovine retina (× 7500).

The rod axons typically end in a pear shaped spherule structure whereas those of the cones end in larger, broad pedicles. The terminal branchlets of the bipolar dendrites end in an invagination of the plasma membrane of the dense synaptic end of the photoreceptor cells.

The inner nuclear layer is composed of the soma of the horizontal cells, bipolar cells, amacrine cells, and Müller cells. The neurons in this layer maintain connection between the visual cell layer and the ganglion cell layer.

The horizontal cell nuclei are situated along the outer most margin of the inner nuclear layer, whereas the amacrine cells are positioned along the inner most margins. Bipolar nuclei and Müller cell nuclei compose the intermediate zone of the inner nuclear layer.

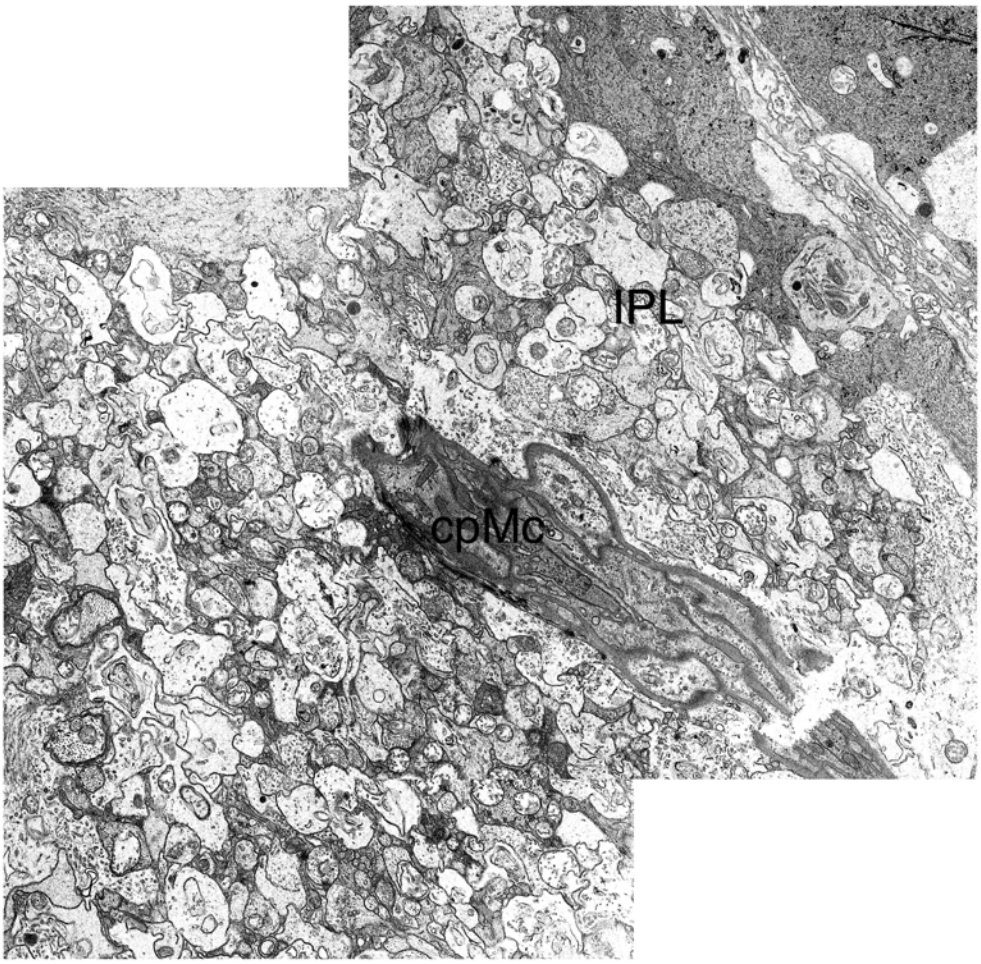

Fig. 62: Inner plexiform layer (IPL) in bovine retina with the cytoplasmic processes of the Müller cells (cpMc) (×6600).

The nuclei of horizontal cells are large, with a single prominent nucleolus. The cells are characterized by their wide, horizontally oriented cell processes.

The bipolar cells are the second most numerous neurons in the retina. They constitute the radial connection between photoreceptors and ganglion cells. These cells are radially oriented. Their dendritic processes in the outer plexiform layer synapse with photoreceptors and horizontal cells, and their axonal processes terminate in the inner plexiform layer synapsing with amacrine and ganglion cells. The cytoplasm of bipolar cells can be identified by their relatively large number of microtubules. Their nuclei are slightly smaller and more osmiophilic than those of horizontal cells.

The amacrine cell has been described as a neuron without an identifiable axon whose processes terminate in the region of the internal plexiform layer. The amacrine cells are located in the inner nuclear layer and they are recognized by occasional, indented euchromatic nuclei. Their cytoplasm is more filled with polysomes and rough endoplasmic reticulum, mitochondria, neurofilaments, and tubules.

Müller cells are supportive cells for most neurons in the retina. They tend to have more cytoplasm and to lie in the inner portion of the inner nuclear layer. Their nuclei are angular and have denser chromatin than other nuclei in the inner nuclear layer. The vitreal ends of the Müller cells possess end feet (Fig. 62).

The inner plexiform layer comprises the cell processes of the inner nuclear layer and ganglion cell layers, where synapses between bipolar, amacrine, and ganglion cells occur (Fig. 62).

The bipolar axons contain numerous synaptic vesicles and mitochondria, and they are the only structures to possess synaptic ribbons. The ganglion cell dendrites are the only processes in the plexiform layer without synaptic vesicles. They are pale, with smooth and rough endoplasmic reticulum, small mitochondria and microtubules. The amacrine processes are pale and possess large mitochondria and synaptic vesicles.

The cells of the ganglion cell layer (Fig. 63) have fairly large nuclei that are spherical and are located eccentrically in the cytoplasm. The cytoplasm is characterized by aggregates of rough endoplasmic reticulum, ribosomes smooth-surfaced endoplasmic reticulum, dense membrane-bound bodies, and irregular shaped mitochondria.

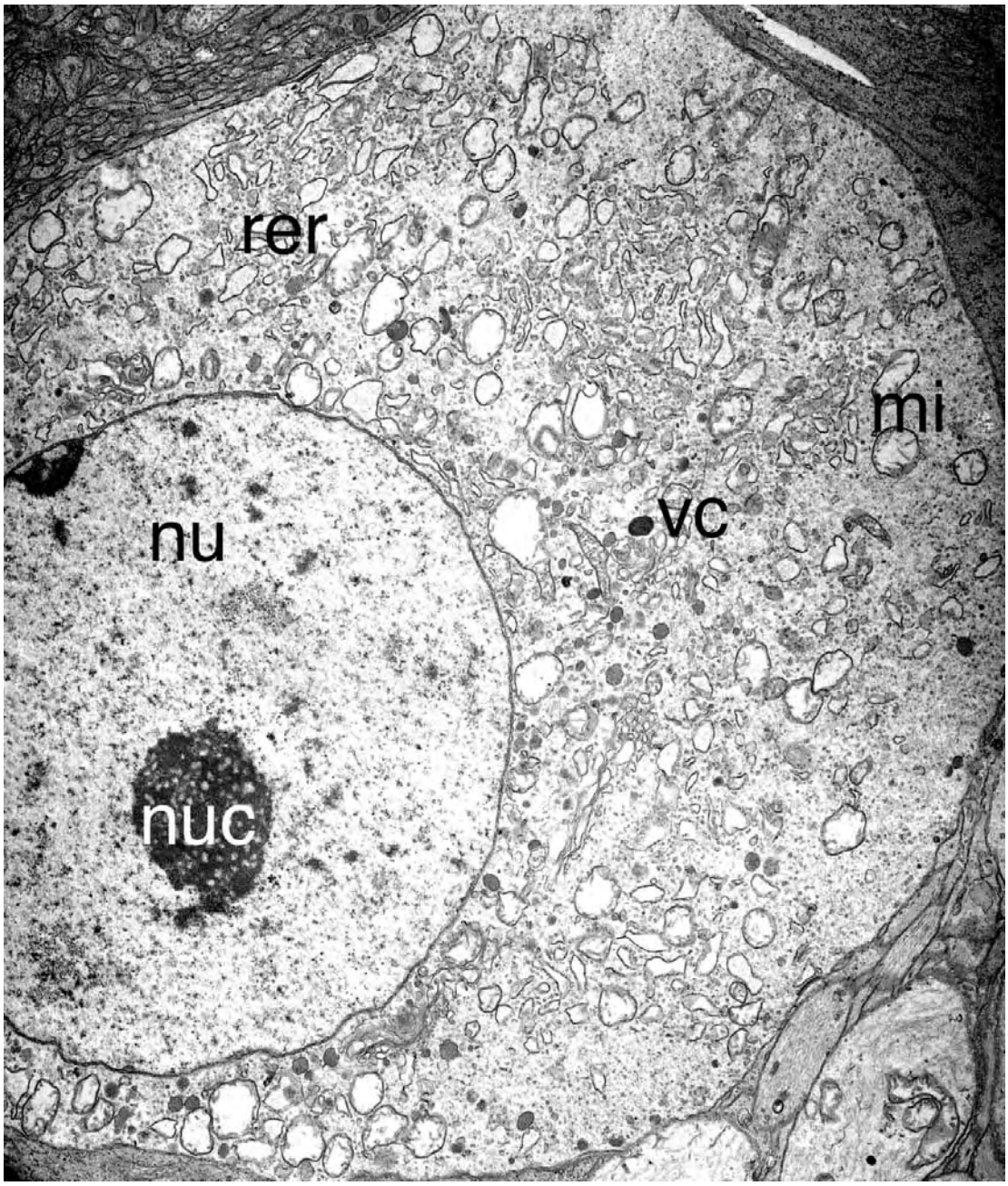

Fig. 63: Ganglion nerve cell within the ganglionic cell layer in the bovine retina. Nucleus (nu) with distinct nucleolus (nuc); the cytoplasm contains scattered mitochondria (mi), vesicles (vc), and rough endoplasmic reticulum (rer) (× 7500).

The nerve fiber layer as well as the inner plexiform layer and ganglion cell layers, contain Müller cells and glial cells (Fig. 64)

The inner limiting membrane is formed by the fused terminations of Müller cells.

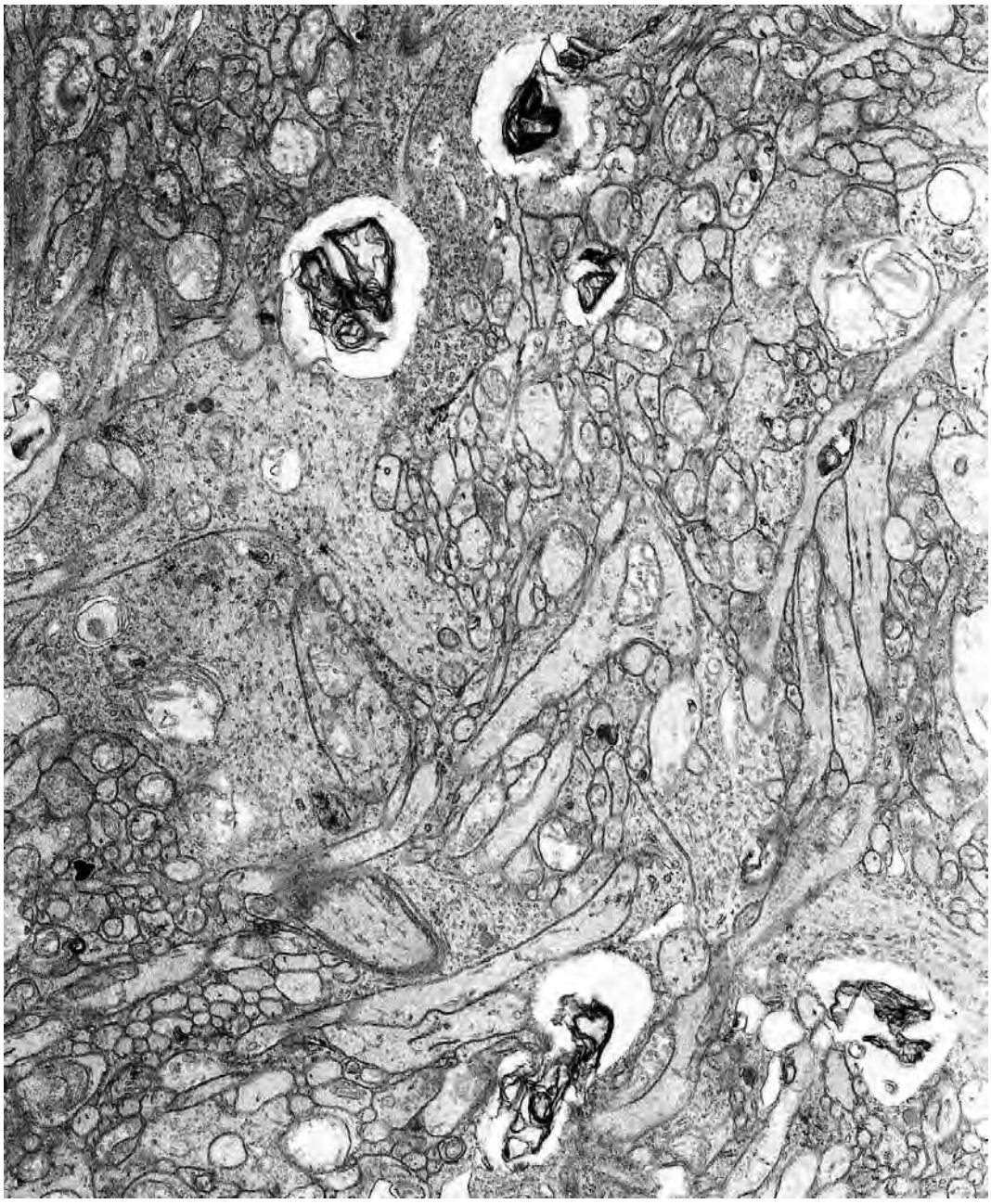

Fig. 64: The nerve fiber layer in bovine retina comtaining the axons of the ganglion cells (× 11000).

4.4.7 Optic Nerve

The optic nerve is formed by ganglion cell axons, glial cells, and septae. The axons within the optic nerve are easily distinguished by their tubular processes, which contain evenly dispersed neurofilaments and neurotubules and occasional vesicles and mitochondria.

5 DISCUSSION

5.1 Microscopical anatomy of the bovine eyeball

The present work shows that the bovine corneal epithelium is formed of 14-17 layers of epithelial cells. These results agree with the finding of Prince et al., (1960) and Diesem, (1975) who mentioned that the bovine corneal epithelium is made up of 14-18 layers of cells.

The pigmentation of the corneal epithelium is observed only in the peripheral part of the cornea. Dyce et al., (1987) reported that the bovine cornea is transparent although some pigments are found, especially towards the corneoscleral junction that tend to increase with age. However, the previous authors didn't indicate whether the pigment is located in the epithelium or in the stroma.

The Bowman's membrane is intimately attached to the substantia propria. This finding is similar to the results of Raghavan and Kachroo (1964); Patt and Patt (1969); Diesem (1975); Dellmann and Collier (1987); Banks (1993) and Samulson (1999). My results are different to those of Murphy et al., (1991), who reported that the large herbivorous mammals possess a thickened basement membrane under the anterior corneal epithelium. Our results show that the bovine Bowman's membrane rarely exceeds several micrometers in thickness and clearly does not possess the morphologic features of the Bowman's layer of bird and primates.

The collagen bundles of the bovine cornea have a wavy appearance superficially but in the deeper parts of the cornea, they run parallel to the basement membrane. Similar results were described by Gelatt, (1991). Peripherally, the substantia propria contains melanocytes. These results are in agreement with those of Prince et al., (1960) and Diesem, (1975) who mentioned that the pigmentation of the bovine corneal stroma increases towards the periphery. In addition, these authors did not observe any blood vessels in the substantia propria of the bovine cornea, whereas thin walled blood vessels were demonstrated peripherally in present work. Occasionally some elastic fibers are

observed at the periphery of the cornea. This has also been reported by Dellmann and Collier, (1987).

The Descemet's membrane is relatively thin in the investigated animals. This membrane is composed of homogenous elastic membrane according the results of Ragavan and Kacharoo, (1964) who named it lamina elastica posterior. This finding does not agree with our present work, in which the Descemet's membrane did not react with Resorcin-Fuchsin stain. In my study this membrane gave positive staining with PAS. This was also demonstrated by Dellmann and Collier, (1987), Banks, (1993) and Smuelson, (1999).

The endothelium consists of a single layer of low cuboidal cells. It is in direct contact with the aqueous humor of the anterior chamber of the eye. These observation was similar to the findings of Raghavan and Kachroo, (1964); Patt and Patt, (1969); Diesem, (1975); Dellmann and Collier, (1987), Banks, (1993) and Samuelson, (1999). The endothelium is important for the maintenance of the transparency of the cornea. Defects in the endothelium cause oedema and opacification of the cornea, which disappear rapidly after regeneration of the endothelium. Endothelial regeneration occurs through increased mitosis in the vicinity of the wound. The regenerative ability appears to vary with species and the age of the animals (Dellmann and Collier, 1987).

Generally, the bovine sclera is composed of collagenous fibers and fibroblasts. The collagen fibers differ in size and shape and run in different directions. Similar results were reported by Diesem (1975), Dyce et al., (1987), Samuelson, (1999) and Slatter, (2001b) in other domestic animals. On the other hand, the fibers of the sclera are not arranged as regular as those of the substantia propria of the cornea (Slatter, 2001b). Moreover the mucopolysaccharide content of the sclera in the present work was lower than that of the cornea. The melanocytes of the bovine sclera increase in number towards the choroid. This was also found by Prince et al., (1960) and Diesem, (1975) in the bovine.

Bundles of smooth muscle fibers were observed in the deep part of the sclera. These muscle fibers have not been described previously in the literature.

The tough fibrous structure of the sclera protects the intraocular content from trauma and mechanical displacement. Snell and Lemp, (1989) concluded that firmness and strength of the sclera, together with intraocular pressure, maintains the shape of the eyeball and

the exact position of different parts of the optic system. Firmness of the sclera provides also a rigid insertion point for extraocular muscles.

The present work shows that blood vessels and nerves are observed between collagenous bundles of the sclera. Blood vessels were especially numerous in the episclera. Similar results were obtained by Snell and Lemp, (1989), in human. While blood supply to the stroma of sclera is poor, the episclera has a rich arterial supply (Snell and Lemp, 1989). This is particularly important from a clinical point of view, as an episcleral plexus-formed by branches of anterior ciliary arteries exists beneath the conjunctiva. Normally, this plexus is inconspicuous, but during inflammation involving cornea, iris, and ciliary body, marked vasodilatation may occur, especially in limbal area surrounding the cornea. This pronounced vasodilatation is known as ciliary flush. On the other hand rich blood supply of episclera allows a rapid healing of surgical incisions. The sclera receives profuse sensory innervations. Inflammations of sclera therefore cause a dull, aching pain. Since the extraocular muscles insert the sclera, pain becomes worse by ocular movement.

In the present study the endothelial layer of the iris consisted of two cell types, fibroblasts and melanocytes facing the anterior chamber of eye. These results are in accordance with Leeson and Leeson, (1970).

The iridial stroma in bovine eyes appear loosely arranged except around blood vessels where it is spirally arranged. By this arrangement the blood vessels can change the position during contraction or dilatation of the iris.

Stroma of the iris contains the sphincter muscle, which is a flat, thin, circular bundle of unstriated muscle fibers in mammals and of striated muscle fibers in non-mammals. It is located in the stroma of the iris, near the pupil in bovine. In dog and cat, it is situated in the posterior stroma, separated from the pigmented epithelium and the dilatator muscle by a thin layer of connective tissue. In horse, the musculus sphincter pupillae occupies the main portion of the central stroma and is capped by granula iridica. The shape of the sphincter varies among the species according to the shape of the pupillae (Prince, 1956).

The posterior surface of the iris covered by a two layered, of epithelium that continues the epithelium of the ciliary body. The anterior layer, which forms the muscle dilatator, is directly continuous with the pigmented epithelium of the ciliary body. The posterior layer is densely pigmented.

It is directly continuous with the non-pigmented epithelium of the ciliary body (Samuelson, 1999).

Size of the dilatator muscle varies between the species, being especially well-developed in dog and involving full circumference of the iris. In horse, it is less developed, and in species with elongated pupils, it is poorly developed adjacent to the long axis of the pupil (Prince et al., 1960). The present study demonstrates that the dilatator muscle of bovine iris is well developed and fills the circumference of the iris. In herbivores, posterior and anterior layers extend anteriorly along iridal margin to form the granula iridica. These were also seen in the present work. In young animals, the granula iridica are smaller and more vacuolated (Samuelson, 1999). In ungulates, iris granules (granula iridica) are found at ventral and dorsal iridal margins. They represent highly vascularized proliferations of stroma and pigmented epithelium of the iris. They are cystic formations (small cysts in the horse; large cysts in goat and sheep) lined by pigmented epithelial cells and associated with a complicated glomus-like capillary network. These granules may function in the production of aqueous humor (Dellmann, 1971; Dellmann and Collier, 1987).

Each ciliary process consists of a central core of connective tissue and blood vessels and is covered by a double layer of epithelium an inner non-pigmented cuboidal, and an outer, pigmented, cuboidal epithelium. This finding does not agree with the results of Samuelson, (1999) who stated that in ungulates the double layer epithelium is more columnar than cuboidal.

The ciliary muscle comprises three layers of smooth muscle fibers; meridional (muscle of Brücke), circular fibers (Müller's muscle) and radiate fibers. Generally, the bovine ciliary muscle is relatively long. The circular and radial fibers are rudimentary and few in numbers. This was also found by Prince et al., (1960) in bovine eye. Several authors (Maximow and Bloom, 1955; Patt and Patt, 1969; Bloom and Fawcett, 1970; Leeson and Leeson, 1970; Dellmann and Brown, 1976; Dellmann and Collier, 1987 and Samuelson, 1999) noted that circular fibers are less numerous than meridional fibers in different species. Dellmann and Collier, (1987) added that circular fibers are predominant in the nasal portion of the ciliary body, where in pig they are the only existing fibers.

Between the bundles of smooth muscle cells a meshwork rich in elastic fibers and melanocytes occurs (Leeson and Leeson, 1970). These finding agrees with our present work.

The Bruch's membrane in bovine choroid is a limiting layer, separating choroid from retina. There is species variation among domestic animals with respect to the degree of development and the thickness of basal complex. When fully developed, the basal complex consists of five layers: (1) basement membrane of retinal pigmented epithelium, (2) inner collagenous zone, (3) elastic layer, (4) outer collagenous zone, and (5) basement membrane of the choriocapillary layer. In the area over the cellular tapetum, the basement membrane of retinal pigmented epithelium and choriocapillaries often fuse, obliterating the other three layers (Dellmann and Collier, 1987; Samuelson, 1999). There are other descriptions of the Bruch's membrane by Maximow and Bloom, (1955); Bloom and Fawcett, (1970); Leeson and Leeson, (1970), who mentioned that it comprises two laminae. The outer one is a dense network of fine elastic fibers which is continuous with those of choriocapillaries. The inner cuticular lamina is homogenous to the basal lamina of the pigmented epithelium of the retina.

The tapetum fibrosum, already described by Prince et al., (1960); Dellmann, (1971); and Samuelson, (1999) in ox, consists of dense regular connective tissue fibers.

Retinal pigmented epithelium of bovine retina is a simple or cuboidal epithelium with single spherical nuclei laying peripherally to the basal lamina. It also contains round granules of melanin. These findings are in accordance with Mo and Friedman (1967), who report, the retinal pigmented epithelium in most species, except in rat and rabbit, is generally mononucleate. Pigmented epithelium of retina furnishes important metabolites to photoreceptor segments as they are shed during normal outer segment renewal (Herron and Riegel, 1974).

The photosensitive part of bovine retina consists of rods and cones. The outer segment is the photosensitive part and the inner segment contains the nucleus (Patt and Patt, 1969, Lesson and Lesson, 1970; and Samuelson, 1999). Outer segments of the photoreceptive rod and cone could be readily distinguished with the light microscope in our study, as a layer adjacent to the pigmented epithelium. Photoreceptor cells of the retina contain photopigments that change on exposure to light and produce chemical energy. This

energy is then converted to electrical energy, which is ultimately transmitted to the visual cortex of the brain (Herron and Riegel, 1974).

The inner segments of the examined bovine visual cell layer are separated from the nuclei of the photoreceptors by an extremely thin elastic membrane, named the external limiting membrane. These observations are similar to the finding of Patt and Patt, (1969), Leeson and Leeson, (1970) and Samuelson, (1999) in different animal species and in human. The function of the external limiting membrane is still speculative. In addition to holding the outer retina together, it forms a barrier between extracellular spaces of the visual cell layer and the rest of the sensory retina (Samuelson, 1999).

The present work shows that the outer nuclear layer of bovine retina contains the cell bodies of the photoreceptors. It consists of six nuclear layers. Several authors (Fix and Arp, 1991; Samuelson et al., 1984) recorded that the number of rows of nuclei varies greatly according to species and location in the retina. The central retina in dog and cat possess the greatest numbers of rows, whereas others have fewer (5 in horse and pig, and 10 in cow).

The outer plexiform layer of the examined bovine retina consist of terminal arborizations of the axon of rod and cone cells, while the cell bodies of horizontal cells, bipolar cells, amacrine cells and Müller cells formed the inner nuclear layer which maintains connection between visual cell layer and ganglion cell layer. These cells are involved in modification and integration of stimuli. Cell processes of inner nuclear layer and ganglion cell layers form the inner plexiform layer (Samuelson, 1999).

In examined bovine retinae, the inner plexiform layer is thicker than the outer plexiform layer. This is similar to the finding of Samuelson, (1999) in retina of several domestic animals species.

The ganglion cell layer consist of large numerous cells with retinal blood vessels arranged in one or two layers between them. These results show an excellent correlation with Samuelson, (1999) who mention that the ganglion cell layer is the inner most cell layer of retina, consisting of a single layer of cells, except in the area centralis and visual streak, where it can be two or three cell layers thick.

The axons of the ganglion cell layers gather in the fiber layer, and then turn at right angles and course to the posterior pole of the eye. Large retinal blood vessels occur in the nerve fiber layer as well as ganglion cells (Samuelson, 1999).
The axon of retinal ganglion cell leaves the nerve fiber layer and form the optic nerve head or the optic papillae. The optic nerve is formed by ganglion cell axons, glia cells, and septae, which arise from the pia mater. This finding agrees with studies of Rodieck, (1973) on the vertebrate retinae.
Within the optic papillae is a central depression called physiologic cup. This cup is lined by a plaque of glial cells known as central supporting tissue meniscus of Kuhnt (Samuelson, 1999).

5.2 Lectin histochemistry

Glycoproteins occur mainly intracellular and may also be found in cell membranes with a variety of important biological functions. Lectins are sugar binding proteins that can be useful for the localization of glycoproteins in cells. This could contribute to a better interpretation of the physiological and pathological processes in the corneal tissue (Bonvicini et al., 1983).
Post-translational glycosylation of proteins and lipids play an important role for the cellular functions. Lectin histochemistry enables the morphological evaluation of the distribution of the saccharide residues within the tissue sections (Spicer and Schulte, 1992). Thus the present data, demonstrating the lectin binding sites in the bovine eyeball, propose a basis for further analyses of the role of saccharide residues in the eye under different experimental and pathological conditions.
The pattern of lectin binding in the corneal epithelium suggests the presence of glycoconjugates containing terminal α-mannose, N-acetylglucosamine and sialic acid residues and sparse terminal α-galactose and β-N-acetylgalactosamine residues (Panjwani et al., 1986a; Rittig et al., 1990; Bishope et al., 1991 and Lawrenson et al., 1998). This generally agrees with my results.
Lectin binding to the cornea in different species, e.g. calf has been studied previously by Panjwani and Baum, 1989. The results of this study are generally in accordance with their

findings but there are also some differences: Panjwani and Baum, 1989 mentioned that Con A and PNA binds to the corneal epithelium, whereas our results show that Con A binds throughout all epithelial layers of the cornea. Also PNA is bound mainly to the apical epithelial cells (Panjwani and Baum, 1989) rather than to basal cells as demonstrated in my study. Our results are different to the findings of Tuori et al., 1994 who reported no binding also of the Con A and PNA to the corneal epithelium. Tuori et al., 1994 demonstrate that WGA, UEA-I and GSA-I binds to the corneal epithelium and that apical cells of the epithelium of the cornea display more α-GalNAc, GlcNAc, sialic acid and α-L-Fuc residues than the basal cells. This agrees with our results. The different staining pattern between apical and basal cells of the corneal epithelium is described previously by several authors in different species (Gipson et al., 1983; Bonvicini et al., 1983; Panjwani et al., 1986a; Rittig et al., 1990), and it is related to the differentiation of epithelial cell, as they move to the apical layers of the epithelium (Nemanic et al., 1983).

Furthermore, Con A and WGA binds to the corneal stroma in my experiment, but no binding of this lectins is seen in the study of Panjwani and Baum, 1989 who report that PNA is the only lectin that binds to the corneal stroma. This result agrees with findings of Tuori et al., 1994 and our results.

Our findings on corneal tissue do not support those of (Spiro and Bhoyroo, 1984), but show excellent correlation with results of Panjwani and Baum, 1988. The observed species differences in the expression of the corneal stromal GSA-I binding sites probably does not reflect differences in blood group antigens among different species, because, at least in humans, such antigens are not found in stromal matrix or on stromal cells (Herold, 1972).

Con A and WGA bind to the Descemet's membrane of the bovine cornea. This result is generally in accordance with the studies of Panjwani and Baum, 1989 and Tuori et al., 1994, but with a little difference. Con A reacts with the anterior part of the Descemet's membrane, while WGA reacts mainly with the posterior border of the Descemet's membrane. Heterogeneity in the distribution of the glycoproteins within the Descemet's membrane has been demonstrated previously (Gordon, 1990; Ljubimov et al., 1995 and Lawrenson et al., 1998). The thin basement membranes show a marked presence of N-acetylgalactosamine residues, whereas a low concentration of this sugar residues is found in thick basement membranes (Salamat et al., 1993).

Our observations show binding of Con A and WGA to the corneal endothelium. This finding is not in accordance with the results of Panjwani and Baum, 1989 and Tuori et al., 1994 who reported that the corneal endothelium tend to bind only GSA-I-B4.

Some of the results from previous lectin binding studies in the human cornea are somewhat different in various investigations (Bonvicini et al., 1983; Panjwani et al., 1986a; Brandon et al., 1988 and Bishop et al., 1991).

Brandon et al., 1988, compare lectin-binding patterns in frozen and paraffin sections and find marked differences between native and paraffin embedded materials. This explains some of the differences observed. Furthermore, Brandon et al., 1988 suggested that some of the variation in the histochemical lectin binding studies are due to different staining procedures or post-mortem changes of the tissues. This could also explain the difference observed between our investigation and the studies of Panjwani and Baum, 1989 and Tuori et al., 1994.

PNA is a lectin that preferentially detects α-galactose. It is also used as a biological marker to detect the T antigen. Due to the neoexpression of T antigen in malignant cells, PNA and other lectins of the same specificity are used as tools in the diagnosis of cancer. In our work PNA reacts weakly with the bovine cornea.

Contrary Con A, WGA and PNA show a distinct reactivity with Bowman's membrane, demonstrating α-mannose, N-acetylglucosamine and α-galactose residues in this structure. The lectin binding to the sclera does not differ significantly to that of the stroma of the cornea.

The binding of lectins to the blood vessels of the anterior uvea demonstrates the presence of glycoconjugates containing terminal N-acetylglucosamine and α-galactose in the vascular endothelium. The weak staining with these lectins reveal also the presence of some α-mannosyl, N-acetylgalactosamine and sialic acid residues in the endothelium. The vascular endothelium has been previously studied using some of these lectins in bovine tissues (Alroy et al., 1987, Tuori et al., 1994). Our demonstration of presence of α- and β- galactose and sialic acid and the absence of fucose are in agreement with the results of Alroy et al., 1987, and Tuori et al., 1994. We also find mannosyl residues in the endothelium similar to finding of Tuori et al., 1994 and in contrast to the results of Alroy et al., 1987. Previous studies have suggested that GSA-I-B4 is an endothelial marker in mouse tissues (Laitinen, 1987) in the

same way as UEA-I is for human tissues (Holthöfer et al., 1982). The present results show that GSA-I is and endothelium marker in bovine tissues and these findings are in accordance with observations of Tuori et al., 1994.

The stroma of the iris is abundant in collagen fibers. They are stained by Con A, ECA, VVA, WGA and PNA. The presence of sialic acid and β-galactose has been noticed previously by Pena et al., 1981. Similar results were also obtained by Tuori et al., 1994.

Posterior pigmented epithelial cell membrane of the iris and nonpigmented epithelium cell of the ciliary body have α-mannose and N-acetylglucosamine residues. This is in accordance with the studies of Tuori et al., 1994.

α-mannosyl and N-acetylglucosamine residues are abundant in the bovine iridal and ciliary muscle, whereas the Gal-(β1,3)-N-GalNAc residues are also present in the ciliary muscle. Identical results were obtained by Tuori et al., 1994. Lectin histochemistry has been applied to human and rat skeletal muscle previously (Pena et al., 1981) and it has been shown that Con A react with muscle cells whereas UEA-I is negative. However, the other lectins used in the present study (PNA and WGA) stained iridial and ciliary smooth muscle cells differently.

The results of our study show clearly that specific structures in the bovine retina can be stained with different lectins (Table 3). The binding of the retinal structures is dependent on sugar-binding specificities of different lectins, demonstrating presence of different glycoconjugates in specialized parts of the retina.

The binding of Con A and WGA to bovine retinal structures is in agreement with previous studies in frog (Bridges, 1981), in monkey (Uehara et al., 1983a) and in human (Söderström, 1988). However, there are some differences in staining pattern of PNA and UEA-I that may result from species differences or from variation in tissue preparation, which are known to affect lectin histochemistry (Brasitus et al., 1982 and Söderström et al., 1984). For example, PNA staining especially has been shown to be negatively affected by formalin fixation (Malmi and Söderström, 1987).

In our study, normal bovine retinal pigmented epithelium shows lectin binding sites for Con A, WGA, VVA and PNA. These results are in accordance with the findings of (Bopp et al., 1992) in human retinal pigmented epithelium.

The biochemical role of lectin binding sites in RPE still remains unclear, especially the significance of presence or absence of certain cellular sugar residues for structure and function of RPE-cells (Bopp et al., 1992). Con A also binds with high affinity to rhodopsin, the photoreceptor molecules of rods (Fukuda et al., 1979; Liang et al., 1979 and Bridges and Fong, 1980). Rhodopsin contains a special oligosaccharide-chain GlcNAcβ1-2Manα1-3(Manα1-6) Manβ1-4GlcNacβ1-4GlcNac-Asn (Fukada et al., 1979 and Liang et al., 1979). Its high content of both terminal N-acetylglucose and α-mannose residues explain the binding of Con A to rods in bovine retina. Thus, the distribution of Con A-binding sites in bovine rods might reflect distribution of rhodopsin within these cells. However, there are also other glycoconjugates in rods, such as outer segment protein, with a molecular weight of 291,000 (Dreyer et al., 1972; Bownds et al., 1974) and glycoprotein of the interphotoreceptor matrix (Adler and Klucznik, 1982) that binds Con A and WGA.

Rod and cone discs are formed by infolding of plasma membrane. In rods these infoldings are sealed off to form stacks of flattened bimembranous discs, so that the oligosaccharide layer that normally resides on the extracellular surface (Hirano et al., 1972) is sequestrated into the disc interior.

Con A stained material in the bovine rods seem to be intracellularly located and its concentration was higher in outer than in inner segments.

There are membranous disks inside the rod outer segment that contain rhodopsin (Jan and Revel, 1974 and Basinger et al., 1976). It has been suggested that rod outer segments bind fucosyl and galactosyl residues prior to disk shedding and phagocytosis by cells of the pigment layer (McLaughlin and Wood, 1980; O'Brien, 1976). In this way the cells of the pigment layer can recognize the differences between shed and intact disks. Previous studies have given conflicting results concerning this theory. In monkey retina, RCA I, which identifies terminal galactosyl residues, binds to outer segments of rods, indicating the presence of galactosyl residues. However, in monkey retina, UEA I and LTA which identify fucosyl residues bind only to inner segments of rods and cones (Bunt and Klock, 1980 and Uehara et al., 1983a). In our study we could not find any binding of ECA, UEA I or LTA in rods. These findings are in accordance with those of Bridges, 1981 and could be due to species differences, indicating a different processing of rhodopsin in different species. However, in the rods of the frog, RCA I staining is seen on the surface of the outer segments

(Bridges and Fong, 1979). Those studies were done with cell suspensions in which cell surface is easier to study. In the present investigation tissue sections were used. The stained material is primarily intracellular. If the cell surface is only slightly stained, it may be difficult to observe it in tissue sections. Sheets and occasional chunks of this matter were sometimes found on detached retina and probably represent the interphotoreceptor matrix, which is known to contain fucose residues (Feeney, 1973).

Since Con A and WGA also bind to the outer segment of the cones, their visual pigments may contain sugar sequences that resemble those found in rhodopsin, but show different lectin affinities. For instance, rhodopsin and iodopsin both bind to Con A columns but they are eluted at different concentrations of α-methyl-mannopyranoside (Fager and Fager, 1978).

WGA is bound to terminal GlcNac or sialic acid residues (Goldstein and Hayes, 1978). WGA does not bind to rhodopsin (Yamamoto et al., 1983), so the binding of WGA to the retina must be explained by the presence of other glycoconjugates. These are present in the cytoplasm of rods and cones but are not seen in the internal segment of cones. Quite interesting is the band-like staining by WGA within the outer plexiform layer, for which no clear morphological equivalents can be seen with conventional staining methods.

Lectin PNA is known to have high affinity for α-D-galactose and N-acetylgalactosamine (Lotan et al., 1975). It is, therefore, possible to assume that these carbohydrate residues are present at terminal oligosaccharides of membrane glycoconjugates in rods and cones. Concerning the binding sites of PNA, it is noticeable that the labelling was uniform throughout outer and inner segments of rods and cones.

In monkey retina, PNA also selectively stained the cones, but the binding was concentrated to the outer segment (Uehara et al., 1983b).This was also found in human, (Söderström, 1988) in pig and cat and in rabbit retina (Kawano et al., 1984). These observations are different to our results. We show that PNA binds strongly to the rods and cones layer. Our finding agrees with results reported by Kawano et al., (1984) that mammals including rat and bovine and non-mammals including birds and goldfish strongly bind PNA to rods and cones.

In addition to the layer of rods and cones, Con A stains many other structures of the retina. External and internal limiting membrane is only slightly stained in contrast to the outer

nuclear layer, where the nuclei of rods and cones are located. The cell surfaces of neurons in the inner nuclear layer are marked. Both inner and outer plexiform layers are diffusely stained with Con A. This finding agrees with results in the human retina (Söderström, 1988). Internal and external limiting membranes and the wall of retinal vessels are labelled with PNA. The vessel wall contains collagen fibrils (Hogan et al., 1971) which are abundant in α-galactose (Muir and Lee, 1969).

In summary, our study shows that normal bovine eye contains a distinct distribution pattern for several lectins. These results on normal bovine eye may form the basis for future studies concerning changes in lectin staining occurring in different diseases of the eye.

5.3 Immunohistochemistry

The basement membrane consists mainly of type IV collagen and laminin. These two components play important roles in the attachment of the epithelial cells to the basement membrane and in cellular migration, proliferation and differentiation of the epithelium (Martinez-Hernandez and Amenta, 1983; Martin and Timpl, 1987; Kleinman et al., 1993).

Laminin has been shown to be involved in attachment of epithelial cells preferentially to type IV (basement membrane) collagen (Terranova et al., 1980). In the present study laminin is located mainly to the basal membranes. This is in concordance with previous observations (Kohno et al., 1983).

Laminin and other extracellular matrix proteins are classified into many isoforms. A change in the distribution of the isoforms has been observed in different lesions or diseases. Recently, several investigator using antibodies to laminin isoforms reported an abnormal composition of the basal lamina in corneal disease (Tuori et al., 1997; Ljubomov et al., 1996).

Using immunohistochemical techniques, we examined the distribution of laminin in the bovine eye. In general, the distribution pattern is similar to the findings of Kohno et al., (1983, 1987) in bovine and human eyes, but there are also some differences. In my study, I found immunoreactive laminin in all layers of the cornea except the stroma. This is in accordance with Kohno et al., (1983) and Scott et al., 1983 in the bovine cornea, but

disagrees with the finding of Kohno et al., (1987) in human cornea where a positive immunoreaction for laminin was only found in the Descemet's membrane. The Descemet's membrane is a specialized matrix that separates the corneal endothelium and the stromal matrix. This membrane is called the basement membrane of the corneal endothelium because of its location and ultrastructural appearance. Unlike other basement membranes, which are predominantly composed of laminin and type IV collagen, Descemet's membrane contains mostly type VIII collagen and additional type IV collagen (Sawada et al., 1990; Ljubimov et al., 1995).

In vitro, Gospodarowicz et al., (1981), discovered that both fibronectin and laminin are synthesised by bovine corneal endothelium cells. My present work demonstrates laminin in the Descemet's membrane of bovine corneal endothelium, so this protein may also play an additional role in the attachment of endothelium to its underlying matrix.

The present work indicates that in bovine ciliary body laminin is present in basal laminae associated with smooth muscle bundles, blood vessels as well as pigmented and non-pigmented epithelium. These observations are similar to the result of Wang et al., (1994) in the human ciliary body.

Laminin was is also detectable in the inner limiting membrane of bovine retina. Russell et al., (1991) detected fibronectin and laminin in the retinal inner limiting membrane of humans at both young and older adulthood. This result also confirms the finding of Nagy et al., 1986, and Kohno et al., 1987, that laminin is a constituent of retinal inner limiting membrane.

The presence of laminin in internal limiting membrane suggests that laminin is intimately involved in maintaining integrity of this layer and may contribut to the structural stability of the outer portion of vitreous cortex. The latter serves as immediate connection between vitreous body and internal limiting membrane, and inner limiting membrane and Müller cell processes (Kohno et al., 1983).

In the present study galactosyltransferase give a distinct reaction with bovine retinal photoreceptors cells. This agrees with the results of Young, (1976) who suggested that the outer segment of the retinal rod photoreceptor consists of a stack of membranous discs surrounded by plasma membrane. The glycoproteins of the outer segments are synthesised in the inner segment of cells and transported to the proximal end of the outer

segment. In vitro studies have demonstrated the transfer of galactose to several rod outer segment glycoproteins by galactosyltransferase, (O'Brien, 1976, 1978; Keegan et al., 1984).

My results show that the vascular endothelial growth factor (VEGF) is present in bovine eyeball, mainly in corneal epithelium and endothelium. This finding is in accordance with results of Phillip, (1997), Schlingemann and van Hinsbergh, (1997), van Setten, (1997) and Cursiefen et al., (2000) in human cornea. Positive reaction for VEGF may be due to a normal basal production or may be in some cases associated with an underlying corneal disease causing new vascularization (Cordell et al., 1984; Monacci et al., 1993; van Setten, 1997).

VEGF was shown to be present in human corneal epithelium (van Stetten, 1997). The author suggested that corneal epithelial VEGF might be involved in corneal wound healing and can be considered as an important factor in the cascade leading to the onset of corneal neovascularization (van Setten, 1997).

Corneal epithelium hence does not only have the potential to produce VEGF but it seemingly contains also detectable amounts of VEGF under physiological condition, (Bednarz et al., 1995). The involvement in corneal neovascularization could be due to the unique features of VEGF to increase the micro-vascular permeability and endothelial fenestration (Roberts and Palade, 1995).

VEGF acts directly on endothelial cells, initiating and mediating formation of capillaries, (Phillips et al., 1994). It is a potent and highly selective vascular endothelial mitogen and angiogenic factor as well as a modulator of vascular permeability (vascular permeability factor). It seems to be the key angiogenic factor mediating retinal neovascularization in diseases with retinal hypoxia/ischemia (e.g. retinal detachment, central retinal vein occlusion) (Casey and Li, 1997; Boulton et al., 1998).

VEGF production was demonstrated to occur in normal ciliary body and retinal pigmented epithelium, (Phillip, 1997; Schlingemann and van Hinsbergh, 1997; van Setten, 1997). This disagrees with our results.

Production of VEGF is increased by hypoxia in retinal pigmented epithelial cells, retinal endothelial cells, retinal pericytes (Adamis et al., 1993; Simorre-Pinatel et al., 1994; Aiello et al., 1995), Müller cells (Pierce et al., 1995). Retinal endothelial cells possess

numerous high affinity VEGF receptors (Simorre-Pinatel et al., 1994). Recent clinical studies have demonstrated a close correlation between active ocular neovascularization and elevated intraocular VEGF concentration in case of retinopathy, central retinal vein occlusion and rubeosis iridis (Aiello et al., 1994).

Angiotensin converting enzyme ACE has been localized in high concentration in various endothelial and epithelial cells (Erdös, 1977; Caldwell et al., 1976). Gospodarowicz et al., 1977, report the presence of ACE activity in ocular tissues. This finding is similar to our results where the corneal epithelium and endothelium stained positively for ACE. Neels et al., 1983, reported significant ACE activity in rabbit corneal endothelial cells and they suggested that angiotensin II may play a role in normal ocular physiology.

The endothelial cells which line the blood vessels in examined bovine eye tissues stain also positively for ACE. Its presence may provide a useful endothelial cell marker. Recent immunohistochemical evidence has indicated that pulmonary ACE may be concentrated on the luminal surface of endothelial cells (Ryan et al., 1975).

I found in the present study that the cornea, retina, and the optic nerve react strongly with antibodies to ACE, while the iris, ciliary body, and choroid are negative. Igic and Kojovic, (1980) reported that retina, choroid and ciliary body of man and several animal species contain ACE. Dancer et al., (1994) found highest concentration of angiotensin I and II in the retinal pigmented epithelium and choroid layer. We found ACE immunoreactivity of the microvessels in examined bovine tissues (i.e iris, ciliary body, choroid, retina and optic nerve). Ward et al., (1979); Igic and Kojovic, (1980) reported that the highest activity of ACE was found in blood vessels isolated from pig retina. Ferrari-Dileo et al., (1988) showed that highly vascular choroid and blood vessels of the retina have high concentration of ACE in feline and human ocular tissues, which may affect the blood flow in these tissues by formation of angiotensin II and activation of bradykinin. As noted previously by Rockwood et al., (1987), angiotensin I administration into the vitreous body is able to induce contraction in retinal blood vessels of the anesthetized cat.

On the basis of this findings and our present study we can conclude that various eye structures are equipped for local production of angiotensin II, a potent vasoconstrictor agent that may also affect ion transport.

We may speculate that in various conditions such as hypercapnia and hypoxia, inhibition of the enzyme that is present in ocular vasculature, provides a mechanism for an increase in blood flow to hypoxic tissues. Inhibition of this key enzyme for metabolism of vasoactive peptides leads to accumulation of kinins and a decrease of angiotensin II formation.

The strongest immunoreaction for smooth muscle α-actin (SMA) is observed in the smooth muscle layers of small arteries, arterioles, and venules (Nehls and Drenckhahn, 1991). We also found that smooth muscle cells of all blood vessels of bovine eyeball, i.e. in sclera, iris, ciliary body, choroid, retina and blood vessels show strong staining for immunoreactive SMA.

Smooth muscle cells of the iris and ciliary body of the examined bovine eyeball display strong staining for immunoreactive SMA. This is similar to the findings of Gabbiani et al., (1984); Skalli et al., (1986, 1987) who revealed that the antibodies to SMA are specific for smooth muscle and smooth muscle related cells.

5.4 Electron microscopy of the bovine eyeball

My present work on the bovine eyeball demonstrates that in the thin, cell-free zone beneath the corneal epithelium, known as the Bowman's membrane, collagen fibrils are randomly dispersed. This agrees with the findings of Murphy et al., (1991) who described a Bowman's membrane in large, herbivores mammals. This membrane is not distinct in most animals, with exception of avian and human corneas. It has been considered to be part of corneal stroma. It is relatively acellular, and mainly composed of collagen fibrils. Although different types of collagen are found in stroma of the cornea, the collagen fibrils of Bowman's membrane are smaller in diameter and less uniform than those of the corneal stroma (Gordon et al., 1994).

It is generally believed that the transparency of the cornea is mainly due to the regular arrangement of the collagen fibrils in the corneal stroma (Maurice, 1957; Farrel et al, 1973). Furthermore, Maurice (1957) has shown that, in addition to their parallel arrangement, collagen fibrils must have the same diameter to keep the cornea transparent.

Balazs (1965) has proposed that the parallel arrangement of the collagen fibrils is maintained by macromolecules in the interfibrillar spaces, mainly by glycosaminoglycans.

My electron microscopic study showed that bovine corneal stroma consists mainly of regularly arranged lamellae of collagen fibers. These lamellae are arranged in sheets and split easily into planes. These observations agree with the results of Meek and Fullwood, (2001). They found that collagen fibrils in cornea display in transmission electron microscope a regular diameter and are arranged with a high degree of lateral order. Takahashi and Tohyama, (1991) could demonstrate that in bovine corneal stroma, the fibrils are tightly packed into fascicles, which are arranged in a lamellar fashion. In each lamella, collagen fibrils run in almost the same direction. The diameter of the collagen fibrils is within the range of 25-30 nm and the interfibrillar spaces appear to be almost uniform. The collagen fibrils are much thinner in human corneal stroma than in the other connective tissues. The diameter of the collagen seems to be unaffected by aging (Kanai and Kaufman, 1973). In addition to the fibril diameter, the spatial organization of the collagen fibrils in the cornea is thought to be of major importance for its transparency (Maurice, 1957).

Present work demonstrates that between the lamellae, fixed cells, fibrocytes (keratocytes), occur. Takahashi and Tohyama, (1991) found that in bovine corneal stroma the keratocytes are located in the interfascicular spaces of collagen fibers. Amorphous material was located in these interfascicular and pericellular spaces.

Descemet's membrane is an extremely thick basal lamina. It is thought to be produced by endothelial cells of cornea Hay and Revel, (1969); Kefalides et al., (1976). The bovine Descemet's membrane in present work appears as a layer having three zones: A thin anterior unbanded zone lies next to the stroma, followed by a broad banded zone and then by another broad, posterior unbanded zone, located next to the endothelium. These observations agree with the results of Smolek and Klyce (1993).

The bovine corneal endothelium cells has many pinocytotic vesicles, indicating that these cells are metabolically very active.

The bovine sclera is mainly composed of collagen fibrils. They are very thick and run in various directions. The spaces between them are very limited so that the amount of

amorphous material filling the spaces is small. My results are in accordance with Takahashi and Tohyama, (1991), but disagree with the description by Raspanti et al., (1992) who stated that the collagen fibrils of the sclera are large and non-uniform in diameter, similar to those commonly found in tendons. In the inner most layers of the sclera they appeared finer, with small elastic fibers interspersed. In the most superficial layers the collagen fibers are very large and densely packed and contain elastic fibers. The anterior border layer of the bovine iris appears highly pigmented. This was also found by Tousimis and Fine (1959); Rohen, (1961); Donovan et al., (1974). The pigmented granules are round to ovoid as in horse (Tousmis, 1963). Melanocytes as the most prominent cell type are evenly distributed throughout the bovine iridal stroma. They are concentrated either anteriorly or posteriorly, and are also prominent in the adventitia of the blood vessels. The pigmented granules are generally rod like to ovoid. The shape of the melanin granules in the stroma varies with species and with maturity of the granules. In the cat, they are rod like, in dog they can be either oval or rod like (Tousmis, 1963). Particularly in horse and dog, large cells containing pigment are associated with capillaries and venules near the sphincter muscle (Tousimis and Fine, 1959; Woberman and Fine, 1972). These cells are probably macrophages. In the human these cells have been described as clump cells of Koganei, (Woberman and Fine, 1972).

As in most mammalian species, Bruch's membrane of bovine choroid is a pentalaminate structure. This finding has previously reported by several authors (Nakaizumi, 1964a; Kuwabara, 1979; Wouters and De Moor, 1979; Braekevelt, 1983a, 1986a, b). The five layers are basal lamina of choriocapillaries, basal lamina of retinal pigmented epithelium, an inner collagen layer, an outer collagen layer with a central discontinuous elastic layer. In species with a distinct choroidal tapetum lucidum it displays a trilaminate structure, with the central elastic layer being lost, (Nakaizumi, 1964b; Braekevelt, 1982). Bruch's membrane, when associated with profiles of the choriocapillaries, can become even further reduced to a single basal lamina produced by the fusion of the two basal laminae of the retinal pigmented epithelium and the choriocapillaries (Nakaizumi, 1964b; Lesiuk and Braekevelt, 1983; Braekevelt, 1986b).

The choriocapillaries in vertebrates consists of a single layer of large-calibre capillaries, Guyer et al., (1989). The choriocapillaries of the bovine choroid is heavily fenestrated on

the side facing the retinal epithelium as well as toward the tapetum. This feature has also been reported in other species (Lesiuk and Braekevelt, 1983; Braekevelt, 1990; Altunay, 2000). It indicates that the choriocapillaries may have role in the metabolic support of the relatively poorly vascularized tapetal region (Braekevelt, 1983b; Korte et al., 1989; Greiner and Weidman, 1991). In species with tapetum cellulosum, the choriocapillaries often protrudes into the retinal epithelium layer (Nakaizumi, 1964b; Braekevelt, 1982, 1986b, 1990). In the examined bovine choriocapillaries, as in other species with tapetum fibrosum, such as sheep, goat and pig (Braekevelt, 1983b, 1984b, 1986a; Altunay, 1997, 2000), the choriocapillaries does not protrude into the retinal pigmented epithelium. This may indicate that the tapetum fibrosum is less rigid than a tapetum cellulosum and thus does not force the choriocapillaries out of the reflective layer.

The pigmented epithelium cell of the bovine retina resembles that found in other vertebrate retinae (Moody and Robertson, 1960; Cohen, 1960, 1961).

My transmission electron microscopic study show that the retinal pigmented epithelium of the bovine retina consists of single layer of cells. Numerous basal (scleral) infoldings in retinal pigmented epithelial cells is a constant feature of the pigment epithelium in many species (Steinberg and Miller, 1973; Spitznas, 1974 and Nilsson, 1978). They increase the overall membrane surface area, (Boulton, 1991). The infoldings are presumed to indicate an active role in material transport from choriocapillaries to the photoreceptor cells (Dowling and Gibbons, 1962; Steinberg and Miller, 1973). The fact that the infoldings are deeper in the central part of the bovine retinal epithelium may indicate that this area is more heavily involved in transport functions than the peripheral region.

Apical (vitreal) processes of the retinal epithelium which interdigitate with outer segments of the photoreceptor are incorporated in phagocytosis of shed outer segment discs (Young, 1976, 1978), the architectural stabilization of the outer segments (Bernstein, 1961; Enoch, 1979), and in internal adhesion of the neurosensory retina (Zinn and Benjamin-Henkind, 1979). This has been reported in all investigated species. In some species two types of apical process have been described (Braekevelt, 1982). One type consists of finger like processes associated mainly with rod outer segments. The second type is a larger, leaf-shaped process which wraps around normally shorter cone cells

(Steinberg and Wood, 1974; Braekvelt, 1982, 1988). Only finger-like processes associated with rod outer segments have been noted in the bovine retina and the retinae of other ungulates like sheep (Nilsson et al., 1977; Braekevelt, 1983a), pig, (Braekevelt, 1984b) and horse (Altunay, 2000).

The lateral borders of the retinal epithelial cells of most species are relatively smooth and are connected by a series of tight junctions (collectively forming Verhoff's membrane), Cohen, 1965, 1968, Kuwabara, 1979. Several studies have shown that this series of cell junctions play a role as a selective barrier for metabolites and ion transport, and forms part of the blood-ocular barrier (Wouters and De Moor, 1979; Hewitt and Adler, 1989; Boulton, 1991). As in other mammalian species, in the bovine retinae these junctions are located apically between the retinal pigmented epithelial cells (Wouters and De Moor, 1979; Braekevelt, 1986a, 1990; Altunay, 1997 and 2000).

The large vesicular nucleus found in the retinal epithelial cells in all locations, coupled with an abundance of mitochondria in the cytoplasm presumably indicates highly active cells. Altunay (2000), demonstrated that mitochondria are distributed in the mid region of the retinal cells in horses, whereas in bovine retinae they are distributed throughout the epithelial layer. Mitochondria in bovine retinal pigmented epithelial cells are ovoid in shape, whereas in the equine the retinal pigmented epithelial cells have ring shaped mitochondria (Altunay, 2000). This is similar to various avian species (Lauber, 1983; Braekevelt, 1984a).

As is noted in most species, in the bovine much smooth endoplasmic reticulum is also seen in retinal epithelial cells, in both tapetal and non-tapetal areas(Braekevelt, 1982; 1983a). Presence of only small amounts of rough endoplasmic reticulum and polysomes indicates that little protein is produced by these cells (Kuwabara, 1979; Braekevelt, 1986a; Boulton, 1991). Alberts et al., (1989) mentioned that the presence of numerous polysomes reflects that these cells use the produced proteins for internal requirements. Smooth endoplasmic reticulum is involved in lipid biosynthesis (Enders, 1962), and storage and processing of vitamin A (Dowling, 1960; Boulton, 1991; Bok, 1993).

Young and Bok, (1969), reported that the retinal pigmented epithelium phagocytoses the terminal discs of outer segments of the photoreceptors. It seems reasonable to assume that

some retinal diseases of unknown etiology may be due to defects of the phagocytose mechanism in retinal pigmented epithelium (Feeney, 1973).

The melanosomes of the retinal pigmented epithelial cells of the bovine retina are round to spindle shaped and highly electron dense. They are distributed throughout the cells in the non-tapetal area, but are absent in the tapetal area. This has been also noted in other species. Melanosomes are important in the absorption of the light which has passed through the photoreceptor layer. This phenomenon inhibits the back-reflection and increases the visual activity (Braekevelt, 1991).

The high number of lysosomes in the epithelial layer of the examined bovine retina indicate that perhaps the most important function of this layer is the phagocytosis (Young and Bok, 1969; Young, 1978).

Feeney-Burns and Mixon (1979) have shown that in the bovine eye the retinal epithelium during development is initially pigmented but that the portion of the epithelium overlying the tapetum is secondarily depigmented by lysosomal digestion of melanosomes. The presence of lysosomal-melanosomal complexes in the adult retinal epithelial cells near the border of the tapetum may also indicate a continuous breakdown of melanosomes to prevent the pigmented portion of this layer from encroaching on the reflective area of the tapetum.

The presence of more lysosomes in the retinal epithelial cells overlying the tapetum, coupled with the presences of more phagosomes in this region, indicates an elevated shedding of outer segment material by these photoreceptors.

The traditional separation of the retinal photoreceptors into either rods or cones was originally proposed by Schultze, (1866). In this classical division, rods have cylindrical inner and outer segments of much the same diameter, while typical cones have a shorter conical outer segments and inner segment of greater diameter (Walls, 1942).

The outer segment of both rods and cones consists of a stack of bimembranous discs. In rods normally only a few of the more basally located (newest formed) discs are not enclosed by the cell membrane while in cones a number of the inner disc spaces along the length of the outer segment may be open to the exterior (Cohen, 1964, 1970; Braekevelt, 1990).

It is a fairly consistent finding that the bovine cone photoreceptors are shorter than rod cells (Cohen, 1964, 1972; Braekevelt, 1975, 1983a, b, 1990). Both rods and cones are now known to periodically shed apical portions of their outer segments which are then picked up and degraded by the retinal pigmented epithelium cells (Young and Bok, 1969, 1970; Box and Young, 1979). While the tips of the rod outer segments are normally in intimate contact with the cell bodies of the retinal pigmented epithelium cells, cones are not. In bovine, elongated processes of the retinal pigmented epithelium cells extend to the tips of the cone outer segment. This finding was also obtained in many other species (Fine and Yanoff, 1972; Steinberg and Wood, 1974; Braekevelt, 1983b, 1990). This is believed to be a method of maintaining a close physical relationship between the retinal pigmented epithelium cells and the cones. Such elongated, cone-associated processes are not, however, noted in all mammalian species (Braekevelt, 1983a, 1987) and it is not known how outer segment material shed from the cones of these species is picked up by the retinal pigmented epithelium cells.

In examined bovine retinae, a connecting cilium is located between the inner and outer segment of the photoreceptors. This is a constant feature of all vertebrate photoreceptors (Cohen, 1960, 1972; Braekevelt, 1973, 1983a, b, 1987, 1990).

The inner segment of photoreceptors is well established as the metabolic center of cells, and it is here that the material for new outer segment discs and other metabolic requirements are produced.

The cell organelles consists of numerous ellipsoid mitochondria, polysomes, profiles of rough endoplasmic reticulum and prominent Golgi fields (Young and Bok, 1970; Young, 1976).

Mason et al., (1973), described the distribution of mitochondria in the inner segment of bovine retinal photoreceptors and reported that rod inner segments only possesses 10% of the number of mitochondria contained in cone inner segments. Our present study agrees with this observation and also explains that the difference may be reflecting greater need of cones for energy. The abundance of mitochondria in inner segments is also of interest from a different point of view. Cone outer segments contain less than 20% of the disc material found in rod outer segments; nevertheless cones require a greater metabolic supply than the rods. Further, the ratio of mitochondrial volume to the outer segment

volume is approximately 100 times greater in cones than in rods. From this we conclude that cone receptor segments possess a more active metabolic system than rod receptor segments (Mason et al., 1973). The presence of a large concentration of mitochondria in the rod inner segment has been noted by Hagins, (1973), to be associated with an active sodium pump. Mitochondria in the cone inner segment probably have a similar function. It is possible that some of the energy production is associated with rapid pigment turnover at high light intensities.

The presence of vacuoles within the inner segment of examined bovine retina, also noted in cat and other species, appears to be a regular finding and probably reflects normal turnover within these metabolically very active cells (Reme and Sulser, 1977; Reme and Knop, 1980).

The paraboloid is an accumulation of glycogen found in the cone inner segment of some birds, fish, amphibian and reptiles (Cohen, 1972). Also in the present work, numerous glycogen granules were found in the inner segment. This observation agree with findings of Mason et al., (1973), who reported that the presence of granules of high electron densities in both cytoplasm and inner segment of bovine retina are suspected to be either glycogen or mucopolysaccharides.

The presence of mucopolysaccharides in the intercellular space between outer segments has been documented by Sidman, (1958) and Young and Bok, (1969). Holmberg (1970) has detected glycogen in hagfish retina and has suggested that the glycogen granules may support metabolic activity of mitochondria in the inner segment.

Hyperboloid is an accumulation of glycogen in the rod. This are noted in crow (Braekevelt, 1994) and in some other avian species (Meyer and Cooper, 1966; Meyer, 1977). These observations are different to our findings in bovine rods. While early workers felt that these glycogen bodies (paraboloid in cones, hyperboloid in rods) were refractile structures it is now considered that these glycogen accumulations may act as energy source for visual cell metabolism (Meyer, 1977). The presence of a large number of mitochondria (in the ellipsoid), a large amount of glycogen (in the paraboloid or hyperbolboid), numerous polysomes and profiles of rough endoplasmic reticulum, several Golgi zones and autophagic vacuoles within the inner segments of this photoreceptors are

all indicative of the high metabolic activity of these cells (Cohen, 1972; Reme and Sulser, 1977).

As in most other species, the external limiting membrane in bovine retina is formed by series of zonulae adherentes (Uga and Smelser, 1973; Braekevelt, 1990). It has been also noted in several other species that the larger and more vesicular nuclei of the cone cells invariably occur immediately below (vitreal to) the external elastic membrane, while the more numerous rod nuclei are scattered throughout the outer nuclear layer (Walls, 1942; Cohen, 1972; Braekevelt, 1983a, b, 1987, 1990).

Also in the bovine retinae, as is seen in many other species, the Müller cells form a series of villous processes which project though the external limiting membrane and surround the base of the inner segments of the photoreceptors (Braekevelt, 1990, 1994). In this region, the photoreceptors may display a number of vertically oriented lateral fins which interdigitate with Müller cell processes and presumably are important in increasing the surface area for exchange (Crescitelli, 1972; Braekevelt, 1990).

Within the outer plexiform layer the synaptic pedicles of the cones are normally larger, more electron lucent and display more synaptic sites than the spherules of rods (Cohen, 1972; Crescitelli, 1972). Synaptic sites on the vertebrate retinal photoreceptors are both invaginated and associated with a synaptic ribbon (Missotten, 1965) or are of the more conventional flat type, which involves only superficial membrane densifications (Dowling, 1968; Cohen, 1972). While bipolar and horizontal cells are both involved in invaginated synaptic sites (Kolb, 1970), flat synapses occur between photoreceptors and bipolar cells and between photoreceptors (Cohen, 1964; Missotten, 1965; Kolb, 1970). In a few species only superficial contacts are reported (Dowling and Werblin, 1969). Domestic cats display both typical invaginated (ribbon) and superficial (conventional) synaptic sites on both rods and cones.

The bovine retina at the level of the outer and inner nuclear layer appears to possess a large amount of granular endoplasmic reticulum in the vitreal end of the inner segment. This may be the storage site of newly synthesized material which is then transported to the outer nuclear layer. It has been shown that membrane protein for the disc membranes originates in the inner segment (Young, 1967).

The cytoplasm of cells of the ganglion cell layer of bovine retina is characterized by aggregations of smooth and rough endoplasmic reticulum, ribosomes and irregular shaped mitochondria. This indicates that ganglion cells are highly metabolic active (Shively et al., 1970; Hogan et al., 1971; Beauchemin, 1974).

Retinal blood capillaries in the examined bovine retina are distributed thoughout the outer and inner plexiform layer, ganglion cell layer and nerve fiber layer. On a cross-section usually one to four endothelial cells lines the capillary lumen and a basement memebrane cover the endothelium. This observation agrees with finding of Shively et al., (1970) in canine retinae.

6 SUMMARY

In the present work, eyeballs from 13 sexually mature, healthy cows (Deutsches Fleckvieh) were investigated using conventional light- and electron microscopical methods as well as glycohistochemical and immunohistochemical methods. Cellular carbohyderate structures were examined using Fluoreszeinisothiozyanat-(FITC)-labelled lectins (Con A, LTA, ECA, SBA, VVA, WGA, PNA, GSA I, UEA I).

Con A, WGA and ECA bound to all layer of the cornea while the binding of other lectins was observed only in the epithelial layer of the cornea. The connective tissue of bovine sclera bound only ECA and WGA. A specific binding of Con A, WGA, ECA, VVA and GSA I was mainly seen in the endothelium of iridial blood vessels, iridial muscle and stroma of the iris. Con A and WGA bound strongly to the ciliary body fixed by Bouin's solution or formalin solution, while ECA and PNA bound only with the samples fixed with Bouin's solution. Basal lamina and blood capillaries of the choroid reacted strongly with Con A, ECA, VVA and WGA. Con A and WGA bound strongly to all layers of the retina mainly rods and cones while LTA, SBA and UEA I did not give any reaction with retina either fixed by Bouin's solution or formalin solution. Nerve fiber of the bovine optic nerve fixed with Bouin's solution bound strongly with ECA, VVA, PNA and UEA I while other lectins did not give any reaction, VVA and WGA labelled the optic nerve fiber when the material was fixed with formalin, the collagen fibers of the connective tissue septa fixed with Bouin's solution reacting strongly with Con A, WGA and ECA. GSA I lectins bound strongly to endothelium lining the blood vessels of the bovine eyeball and can be considered as an endothelial marker in this species.

The immunohistochemical studies used the Avidin-Biotin-Complex method for the immunolocalization of laminin, galactosyltransferase, smooth muscle actin, angiotensin converting enzyme and vascular endothelial growth factor.

The basement membrane of the corneal epithelium, pigmented epithelium of the iris and ciliary body and the non pigmented epithelium of the ciliary body showed moderate immuno-staining for laminin. The Bruch's membrane and a thin layer of the choriocapillaries reacted positively for laminin. Connective tissue septa of the optic nerve stained in a border like pattern for laminin. The basal membrane of blood vessels in iris,

ciliary body, choroids, retina and optic nerve showed distinct immunostaining for laminin. The sphincter and dilatator iridial muscle, ciliary muscle, and smooth muscle cells of the scattered blood vessels showed high intensity of immunostaining for smooth muscle actin. Corneal epithelium and endothelium, inner segment of the rods and cones and ganglion cell layer and the connective tissue septa of the optic nerve gave very faint positive staining for galactosyltransferase. An immunostainig for ACE was observed only in blood vessels of the iris, ciliary body, choroid, retina and optic nerve. The corneal endothelial cells, outer nuclear layer of the retina and some retinal blood vessels gave a moderate positive reaction for VEGF.

In conclusion, our study showed that the light and ultrastructure of the all components of the bovine eyeball. The lectin binding pattern in the bovine eyeball indicated the presence of glucose, mannose, fucose, N-acetylglucosamin and N-acetylgalactosamin residues. This would be useful as histochemical markers for diseased condition, also as biochemical tools for further isolation of glycoprotein and for cell typing.

The immunostaining for laminin, glactosyltransferase, smooth muscle actine, vascular endothelial growth factor and angiotensin converting enzyme could be detected in the bovine eyeball.

7 ZUSAMMENFASSUNG

Glykohistochemische, immunhistochemische und elektronenmikroskopische Untersuchung am Auge des Rindes *(Bos taurus)*.

In der vorliegenden Arbeit wurden mit licht- und elektronenmikroskopischen Methoden der Bulbus oculi von 13 geschlechtsreifen, klinisch gesunden Rindern (Deutsches Fleckvieh) untersucht.
Mit 9 verschiedenen Fluoreszeinisothiozyanat (FITC)-markierten Lektinen wurde die Topographie von Kohlenhydratstrukturen im Augeapfel studiert.
Die Lektine Con A, WGA und ECA zeigten ein spezifisches Bindungsmuster in allen Schichten der Cornea. Alle anderen Lektine reagierten nur mit den Epithelzellen der Cornea.
Das Bindegewebe der bovinen Sklera band nur ECA und WGA. Eine spezifische Bindung von Con A, WGA, ECA, VVA und GSA I wurde in den Endothelzellen der Irisgefäße, im Musculus dilatator pupillae und Musculus sphincter pupillae sowie im Irisstroma beobachtet.
Die Basallamina und die Blutgefäße der Choroidea reagierten deutlich mit Con A, ECA, VVA und WGA. Con A und WGA banden stark an allen Schichten der Retina, bevorzugt an die Stäbchen- und Zapfenzellen.
GSA I reagierte deutlich mit den Endothelzellen aller Gefäße des bovinen Augapfels. Es kann somit als Marker für die Endothelzellen dieser Spezies betrachtet werden.
Immunhistochemische Untersuchungen wurden mit der Avidin- Biotin- Komplex-Methode durchgeführt. Damit wurde die Histotopik von Laminin, Galactosyltransferase, α-Smooth Muscle Actin (α-SMA), Angiotensin converting enzyme (ACE) und Vascular endothelial growth factor (VEGF) studiert.
Der Antikörper gegen Laminin markierte die Basallamina des Cornealepithels, des Pigmentepithels der Iris, des Ziliarkörpers, des nicht pigmentierten Epithels des Ziliarkörpers, die Bruch Membran der Choriocapillaris, die Bindegewebsepta des Nervus opticus und die Basallamina aller intraokulären Gefäße. Der Musculus sphincter und

Musculus dilatator pupillae, der Musculus ciliaris und die glatten Muskelzellen der intraokular Gefäße zeigten eine intensive Immunreaktion auf α-Smooth Muscle Actin. Der Antikörper gegen Galactosyltransferase markierte vor allem das Epithel der Cornea, sowie die Schicht der Stäbchen- und Zapfenzellen.
ACE wurde zudem von den Endothelzellen aller intraokularen Gefäße exprimiert. Endothelzellen der Cornea, die äußere Kernschicht der Retina und einige retinale Blutgefäße zeigten eine schwach positive Reaktion für VEGF.
Die ultrastrukturelle Untersuchung der Rinderaugen zeigte zu anderen Tierarten viele Übereinstimmungen aber auch einige Besonderheiten. So zeigte sich, dass in der Cornea des Rindes besonders zahlreiche Hemidesmosomen zwischen der Basallamina und dem Corneaepithel vorkommen. Das Stroma der Iris ist locker und enthält myelinisierte und nicht myelinisierte Nervenfasern. Im Zytoplasma der Melanozyten finden sich Melaningranula, glattes und raues endoplasmatisches Reticulum, freie Ribosomen, und elongierte Mitochondrien. Im Pigmentepithel der Retina finden sich charakteristisch ovoide Mitochondrien. Die äußere plexiforme Schicht der Retina enthält charakteristische ´´double layer``-Blutkapillaren.

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9 ABBREVIATIONS

ACE = Angiotensin converting enzyme

bb = Basal body

BL = Basal lamina

Bm = Bowman’s membrane

BV = Blood vessels

C = Choroid

C = Cilium

CA = Capillaries

CB = Ciliary body

Cc = Cone cells

cf = Collagen fiber

CM = Ciliary muscle

Cnu = Cone nuclei

Con A = *Concanavalin A*

cp = Ciliary process

cpMc = Cytoplasmic process of Müller cells

CRL = Crown rump length

CSJ = Corneoscleral junction

de = Desmosomes

Dm = Descemet’s membrane

DM = Dilatator muscle

ECA = *Erythrina cristagalli* agglutinin

ELM = External limiting membrane

En = Endothelium

Ep = Epithelium

EPS = Episclera

FI = Fibroblast

GCL = Ganglion cell layer

GI = Granula iridica

go = Golgi complex

GSA I = *Griffonia simplicifolia* agglutinin I

H&E = Haematoxylin and Eosin

hc = Heterochromatic

I = Iris

Ic = Iridocyte

ILM = Internal limiting membrane

INL = Inner nuclear layer

IOP = Intraocular pressure

IPL = Inner plexiform layer

is = Inner segment

LSC = Lamina suprachroidea

LTA = *Lotus tetragonolobus* agglutinin

mg = Melanin granules

mi = Mitochondria

mm = Millimeter

Ms = Myelin sheath

NF = Nerve fiber

NFL = Nerve fiber layer

nm = Nanometer

NPE = Non pigmented epithelium

nu = Nucleus

nuc = Nucleolus

ºC = Degree centigrade

ONL = Outer nuclear layer

OPL = Outer plexiform layer

os = Outer segment

PAS = Periodic acid schiff

PE = Pigmented epithelium

PNA = *Peanut* agglutinin

R/C = Rods and cones

Rc = Rod cells

rer = Rough endoplasmic reticulum

Rnu = Rod nuclei

RPE = Retinal pigmented epithelium

S = Connective tissue septa

SBA = *Glycine maximus* agglutinin

Sc = Sclera

Scl = Supraciliary layer

SEp = Superficial epithelium of the cornea

SM = Sphincter muscle of the iris

SMA = Smooth muscle actin

smc = Smooth muscle cells of the ciliary body

St = Stroma

UEA I = *Ulex Europaeus* agglutinin

V = Vacuoles

VC = Vesicle

VEGF = Vascular endothelial growth factor

VVA = *Vicia villosa* agglutinin

WGA = *Wheat germ* agglutinin

µm = Micrometer

10 CURRICULUM VITAE

I. Personal Data:

Name:	Khaled Hamdy Zaky Aly
Date and place of birth:	20-8-1971
Place of birth:	Assiut, Egypt
Nationality:	Egyptian
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Profession:	Assistant lecturer in department of Anatomy and Histology, Faculty of Veterinary Medicine, Assiut University, Egypt.

II. Education:

1977-1982: Primary school, result: (Excellent).

1983-1985: Preparatory School, result: (Excellent).

1986-1988: Secondary school, result: (Very Good).

1989-1993: Student in Faculty of Veterinary Medicine - Assiut University - Egypt, Grade: (Very Good with Honour).

III. Degrees:

1993: Bachelor of Veterinary Medicine Sciences from Assiut University, Faculty of Veterinary Medicine – Assiut, Egypt.
General Grade: Very Good with Honour.

1997: Master Degree in Veterinary Anatomy from Assiut University.
Title of Master Thesis: Surgical Anatomical studies on the fibrous coat of the eyeball in some domestic animals.

IV. Positions Held:

Current:

1.9.1999 - 1.9.2003: Scholarship from Egyptian government for a Ph.D. study at the in Institute, of Anatomy, LMU Munich, Germany.

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1994-1997: Demonstrator in Department of Anatomy and Histology-Faculty of Veterinary Medicine - Assiut University, Assiut, Egypt.

1997 (May): Assistant lecturer in Department of Anatomy and Histology, Faculty of Veterinary Medicine Assiut, University, Egypt.

V. Scientific Activities & Researches:

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Teaching the veterinary medical students of Assiut and South Valley Universities the anatomical sciences.

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11 ACKNOWLEDGEMENT

First and foremost all thanks to [Allah] who is the only beneficial and merciful.
It is a please to indicate my deep appreciation and sincerity to ***Professor Dr.Dr. Dr. habil Fred Sinowatz*** for his help in suggesting the subjects of this thesis, and his valuable assistance, continuous encouragement and guidance and untiring and patient efforts in direction and supervision of this work.
I wish to express my deep thanks to ***Miss C. Neumüller*** for her greatly useful help in electron microscopic works.
I would like to express my immense gratitude to ***Mr. M. Kosarian*** for his great help in lectin histochemistry techniques.
Miss G. Boie and G. Rußmeier, thanks for great help in immunohistochemical techniques.
I would like to express my deep thanks to ***Miss M. Vermehren*** for her great help.
I wish to express my deep thanks to ***Mr. S. Baindl*** for his greet help in photo and computer works.
It is pleasure to acknowledge the efforts of all staff members at the Institute of Veterinary Anatomy II who have contributed in this work.
My closed friends ***Alkafafy Mohamed*** and ***Ahmed Shawky***, thanks for your help.
My father ***Hamdy,*** my mother ***Wedad*** and my brother ***Amen*** thanks very much for your support of this work.
Lastly, I wish to thank my wife ***Riham*** for efforts, support and help along the last four years.

Khaled Hamdy Zaky Aly