STRUCTURAL ALTERATIONS OF THE RAT IRIS ASSOCIATED WITH FUNCTION AND GROWTH

by

WAN CHENG LIM

B.A., Wellesley College, 1968

M.Sc., University of British Columbia, 1970

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Department of ANATOMY

The University of British Columbia
Vancouver 8, Canada

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ABSTRACT

1. The structural alterations of the adult rat iris associated with its function of dilating and constricting the pupil were examined. The rat eyes were regularly dilated or constricted with a few drops of a mixture of phenylephrine hydrochloride and cyclopentolate, or echothiophate iodide, respectively. The eyes were prepared for examination with the light, transmission and scanning electron microscopes by well-known methods.

In pupillary dilation, scanning electron microscopic studies reveal that the posterior epithelial cells are arranged in circumferential ridges which bifurcate, taper down and join with adjacent ridges. On the anterior surface of the iris, blood vessels, generally circumferentially oriented, bulge prominently outwards.

In transmission electron micrographs the epithelial cells are high and are discretely separated from each other. The nuclei show indentations of the nuclear envelope. Bundles of intracellular filaments form a 'hammock' around the nucleus. The dilator muscle layer is thick. Dilator hillocks and dilator processes are found all along the boundary zone with the stroma. The nuclei of the dilator muscle cells also show nuclear indentations.

With the light microscope, it is clearly shown that the stromal components and the collagen network appear to be arranged in columns perpendicular to the posterior surface of the iris.

A low magnification scanning electron micrograph of the rat iris in pupillary constriction shows that most of the posterior surface of the iris is smooth. In extreme pupillary constriction, where the pupil is a mere pinhole, the posterior epithelial cells around the pupil are arranged in radial ridges which peter out peripherally. Bulbous structures are

often seen in amongst the radial epithelial ridges. On the anterior surface of the iris the blood vessels do not bulge out as prominently, as they zig-zag from the periphery to the pupillary margin. The morphology of the iridic crypts and pores are well illustrated.

In transmission electron micrographs, the posterior epithelial cells in pupillary constriction are low and they form a continuous layer. The nuclei, with smooth nuclear outlines, and the intracellular filaments are disposed parallel to the length of the cells. The dilator muscle cell layer is low. Dilator hillocks and dilator processes are absent. The nuclei of the dilator muscle cells have a smooth outline and lie along the length of the cells.

The stromal elements and the connective tissue framework are oriented parallel to the posterior surface of the iris, as seen light microscopically.

2. The overall developmental changes in the structure of the various components of the fetal and post-natal rat iris were observed on toluidine blue stained plastic sections. In the immature iris, the brick-shaped posterior epithelial cells form a continuous layer. By two weeks after birth, the epithelial cells have acquired characteristics of the adult iris. The anterior epithelium develops to give rise to the sphincter and dilator muscles. The stromal elements stream into the iris parallel to the posterior surface of the iris in very close association with the stromal surface of the developing dilator muscle cells. With development, the stromal elements move away from the dilator.

A scanning electron microscopic study of the posterior surface of the developing rat iris shows the changes in the surface configurations of the posterior epithelial cells. Initially the posterior surface of the

iris is smooth. By a gradual process, the epithelial cells begin to bulge out posteriorly. By two weeks after birth, the epithelial cells are beginning to be arranged in rows.

The topography of the pupillary membrane and its relationship with the hyaloid system is shown. Most of the blood vessels of the pupillary membrane appear to come from the iris stroma with perhaps some contribution from the hyaloid system. The thin-walled blood vessels are suspended within a scaffolding of connective tissue fibers. The rat pupillary membrane is still present during the first few days after birth.

Changes in the permeability of the iris blood vessels to an intravascularly injected solution of HRP were investigated. The iris capillaries of the fetal and early post-natal rats, up to two weeks after birth, are readily permeable to HRP. They then become impermeable to HRP, as in the adult iris.

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for

my parents

Explanatory Note:

The terms "anterior" and "posterior" are considered with respect to the eye alone and not with respect to the whole rat, as the rat eye does not face directly anteriorly.

"Centrally" is used synonymously with "pupillary" to indicate direction towards the center of the pupil.

"Peripherally" indicates direction towards the root of the iris.

I. INTRODUCTION

A. Historical

Stephen Polyak (1957) has given us an interesting account of the history of the study of the eye. Even as early as the 4th century B.C. (460-370 B.C.) Democritos of Abdera and Hippocrates described the eye as consisting of a number of concentric tunics; an outer, white, fibrous tunic (the sclera) with an anterior transparent cornea; a delicate, spongy, middle tunic with an anterior colored portion (the iris), a crown (the ciliary body and iris root) and a central opening (the pupil); and an inner tunic (the retina). However, it was not until the time of Herophilos (344-280 B.C.) that the first scientific description of the eye appeared. His observations were based on actual animal dissections. Early diagrams of the structure of the eye are found in not only Greek but also Arab anatomy texts. The Arabic diagrams were probably derived from Greek sources as the Arabs, being Muslims, were not allowed to dissect animals. Over the years, changes were made to these early eye diagrams as anatomists gained more knowledge of the relative proportions and relationships of the various parts of the eye.

Rufos and Galen, two Greek anatomists from Classical times, in studying the eye, described the iris as a grape-like tunic. The word "iris" meaning a "rainbow" in Greek was first used by Rufos (Duke-Elder and Wybar, 1961). The iris is appropriately so named because of the amazing variations in color that do occur in this structure. In a large number of fishes, amphibians, reptiles, birds, and man, the iris exhibits diverse variegated color schemes.

The pupil, the aperture formed by the iris, means "maiden" or "little girl" in Greek. The reason for this nomenclature is speculative.

It has been suggested that "the pupil was probably so named from the diminutive image seen reflected from the cornea standing out on the black background of the pupil" (Duke-Elder and Wybar, 1961, p. 167).

B. Functions of the Iris

The iris is a delicate and movable diaphragm similar to those found in other optical systems. It separates the anterior and posterior chambers of the eye. The size and shape of the pupil, the aperture formed by the iris, is controlled by movements of the iris resulting in variations in its area. The main function of the iris is to regulate the amount of light entering the eye and impinging on the retina until the retina has satisfactorily adjusted itself to the light. This is needed as there are variations in the sensitivity of different retinas to external illumination. In general, the image on the retina will be sharper with a decreased aperture or pupil size. A small pupil prevents light coming from the periphery of the cornea from passing through the lens. It channels the light through the central, optically good part of the lens, thus resulting in a sharp image on the retina. However, with a smaller aperture the amount of light entering the eye may not be sufficiently intense to stimulate the retina. In such an instance, the pupil must be capable of being widely dilated. The sharpness and clarity of the retinal image is reduced but at least an image is perceived.

The iris serves a secondary function in some amphibious vertebrates where by the very nature of their habits and habitats a wide range of accommodation is required (Walls, 1942; Rochon-Duvigneaud, 1943; Prince, 1956). Having a dual habitat, aquatic and terrestial, a means must be found to ensure that as perfect a focus is attainable so as to give the

animals reasonable vision both when the head is in the air and when it is immersed in water. The range of accommodation has to be greatly increased. In the turtles (Walls, 1942, pp. 436-437; Rochon-Duvigneaud, 1943, pp. 126, 387-388; Prince, 1956, pp. 237, 374), we see a superb example of how this is accomplished. The lens is extremely soft and pliable. It projects through the pupil so that the iris sphincter surrounds the anterior peripheral part of the lens. In accommodation, the powerful iris sphincter contracts and grips the lens. The front of the lens is squeezed and distorted to give a very short radius of curvature to the lens. The iris sphincter also aids in accommodation by deformation of the lens in some aquatic snakes (Prince, 1956, pp. 235, 375), for example the Natrix tessellatus (Walls, 1942, p. 438); in some diving birds, for example, the shags and cormorants (Walls, 1942, p. 440; Rochon-Duvigneaud, 1943, pp. 472-473; Prince, 1956, p. 238); and even in an aquatic mammal, the sea otter (Walls, 1942, p. 444; Rochon-Duvigneaud, 1943, pp. 669-670; Prince, 1956, p. 240). The cormorant is an aquatic bird which dives deeply into the water and remains submerged for a considerable length of time. It has the most muscular iris of all the vertebrates so far studied. The iris muscles are particularly well-developed. The circular bundles of the sphincter muscle fibers are found extending from the pupillary margin to the root of the iris (Rochon-Duvigneaud, 1943, pp. 472-473). At the iris root, the sphincter muscle fibers are found intermingled with some radial fibers of the dilator muscle. Wherever and whenever warranted, the iris not only acts to regulate the amount of light entering the eye but it also assists in the accommodation process. This latter function, though present in only a few vertebrates, is nevertheless important. It may oftentimes supercede the other means of accommodation present in the eye.

C. Iris Colors

Although iris coloration may appear meaningless in terms of its physiological importance to man or animal, it nevertheless has a significant role to play in the expression and ornamentation of the eye. The varying color of the vertebrate iris is the result of pigmentation within the iris tissue and optical phenomena. Unlike other vertebrates, where the iris may exhibit a range of brilliant hues, the color of the iris in man is either light blue or grey to dark brown (Coque, 1927; Walls, 1942; Rochon-Duvigneaud, 1943; Duke-Elder and Wybar, 1961; Hogan, Alvarado and Weddell, 1971). The color of the iris is genetically transmitted where blue eyes are recessive to brown or dark eyes. The color of the human iris depends on the amount and location of the iridic pigment, melanin, as well as on the density of the stromal tissue. There are two kinds of pigment in the human iris, that found in the posterior pigment epithelial cells and that contained in the branched melanocytes of the iris stroma. The pigment of the posterior epithelial cells is very opaque and shows a certain resistance to chromic acid decoloration (Rochon-Duvigneaud, 1943, p. 86). The pigment granules will turn yellow but not white. Pigment is almost always present in the posterior pigment epithelial cells except in extremely rare cases of true albinism. In blue eyes, pigment is present mainly in the epithelial cells. A few of the stromal cells may also contain varying amounts of pigment. The deep blue color of the iris of Caucasions at birth is the result of the absorption of the long wavelengths of light by the still fairly translucent iris stroma. Thus only the shorter blue wavelengths are transmitted to the observer's eyes after being reflected from the posterior pigment epithelial layer. If the iris stroma is thick but still devoid of melanocytes, the iris appears grey. In dark irises, pigment is present in varying amounts in the stromal melanocytes. The

coloration of the iris may not be uniform due to differences in the degree of pigmentation in various parts of the iris. Freckles or dark brown spots on the iris denote areas of hyperpigmentation, that is, where there are clusters of melanocytes. A large number of such spots will give the iris a mottled appearance. Heterochromia is also a result of the uneven distribution of stromal melanocytes. In such cases, one part of the iris is of a different color from that of the other parts. There may even be striking differences between the two irises of the same individual. It appears that there are documented cases of freaks of nature where the arrangement of the pigmented spots and markings on the iris resemble letters or numbers. As is clearly apparent, the possible variations in the coloration of the human iris are enormous. Attempts have been made to classify the colors of the individual irises and to use such a classification as a means of identifying criminals (Duke-Elder and Wybar, 1961).

When compared to the relatively sober colors of the human iris, those of the other vertebrates appear almost ostentatious. Rochon-Duvigneaud (1943) in his vertebrate ocular anatomy text "Les yeux et la vision des vertebres" discusses the whole gamut of iris colors - shades of orange, yellow, green, blue, red, white, gold, etc. - encountered in the different groups of vertebrates. Some have a metallic silvery or gold sheen to the iris while others show only a silver or gold lining on the pupillary edge. What is present in these irises to impart to them such brilliant and variegated color schemes? In man and the Cyclostomes (Rochon-Duvigneaud, 1943, p. 185), only melanin pigment is present in the stromal melanocytes. However, in the other vertebrate groups, a varying quantity of crystals and pigments, other than melanin, are found within some of the other stromal cells. The type and quantity of these other stromal elements is what makes the difference. In the Elasmobranchs (Rochon-Duvigneaud, 1943, p. 214) the

color and metallic lustre of the iris is not as distinctive as in the higher Teleosts (Rochon-Duvigneaud, 1943, pp. 245-275). There is no definitive guanophore layer although some guanine crystals are present. The golden or bronzed color of the anterior surface of the amphibian eye (Rochon-Duvigneaud, 1943, p. 340) owes its coloration to both carotinoid pigments and guanine crystals. The carotinoid pigments are seen as red or yellow (in the oxidised state) granules in certain stromal cells. The guanine crystals belong to other cell types. These crystals diffract and reflect the light entering the eye. It was once thought that there were xantholeucophores in the iris but the research of Schmidt and Millot have refuted this. The melanocytes beneath the brilliantly colored layer also help to modify the color of the iris. Crocodiles (Rochon-Duvigneaud, 1943, p. 368) have buff or yellow irises. They only have a thin and discontinuous layer of cells containing guanine on the anterior iris surface. In many reptiles (Rochon-Duvigneaud, 1943, pp. 393-394) guanine-containing cells cover the anterior surface of the iris and impart to it a brilliant and metallic hue. Differences occur according to the species. Birds (Rochon-Duvigneaud, 1943, p. 472) generally have deep brown or black irises because of the melanocytes in the stroma. However, some birds do have brightly colored eyes. Then, the iris shows a layer of vesiculated cells which contain red or yellow pigments. Birds are interesting, in that of all the vertebrates, with a few exceptions, sexual differences are expressed in the colors of the irises. The female pheasant and condor have brown eyes while their respective males have yellow and red eyes. In the booby (Walls, 1942) the male has a yellow iris but the female has, in addition, a ring of black spots along the pupillary margin. Other than the birds, the irises of the male adder and box turtle are red in color while those of the females are light brown and yellow brown, respectively. Among the birds, too, the intensity

of the iris colors changes with heightened sexual activity. Various other pheonmena also seem to affect the color of the iris in some vertebrates (Walls, 1942, p. 550; Rochon-Duvigneaud, 1943, p. 245), for example, emotion, age, the seasons and noxious stimuli.

D. Pupillary Patterns

The anatomy of the eye of man or animal is adapted so as to best meet the needs of the individual in performing its life-sustaining activities. This is also subject to the demands of the environment, whether it be water, air or the ground. The information in the following discussion has been gleaned from numerous sources (Walls, 1942; Rochon-Duvigneaud, 1943; Prince, 1956; Prince, Diesen, Eglitis and Ruskell, 1960).

The whole animal world can very broadly be divided into four main activity groups; those which are diurinal, those which are nocturnal, those which are arrhythmic and those which are crepuscular. Naturally there is much overlapping of the categories. The conditions wherein the animals have to interact are varied. Anatomically differences are seen in the eye. Modifications occur so as to allow the animal to function efficiently. The shape, size and mobility of the pupil partly does signify to which activity group an animal belongs. However, this is only partly so. The pupillary form must always be considered in relationship to the structure of the retina. Both iris and the retina work together in harmony to ensure that as clear a retinal image as possible is obtained.

Certain requirements must be met in order for an animal to operate effectively within a particular activity group. Animals which are diurnal, for example, man and a large number of birds, move about chiefly during the daytime. There is an increase in visual acuity owing to the greater number of cones relative to the number of rods in the retina. Animals which feed

on insects or seeds, for example, lizards, primates and birds, must of necessity be diurnal. They must be able to discriminate between that which is suitable and that which is unsuitable as food. Predators and prey animals also need the acute vision afforded by a cone rich retina. Diurnal animals usually have a circular pupil. In dim light, the pupil dilates widely to allow as much light as possible to enter the eye to compensate for the paucity of the rods in the retina.

Nocturnal animals, like owls, bats, crocodiles and nocturnal foxes, are more active at night. During the day they either hide from the light or they may bask in the sun with eyes closed. They are totally passive during the day and have no need of the use of their eyes. Nocturnal animals normally have a much more sensitive retina where the number of rods predominate over the number of cones. Being so sensitive to light, there must be an efficient means of pupillary control to regulate the amount of light striking the retina especially during the hours of daylight. Glare must be eliminated, or at least diminished as much as possible. The slit pupil is associated with nocturnal habits. The slit pupil, as compared with the circular pupil, is capable of a greater degree of closure owing to the arrangement of the sphincter muscle fibers within the substance of the iris. At times a slit pupil can be closed completely, or a pin-hole or two may remain. Complete closure of the pupil is not possible with circular pupils. As the pupil constricts, the tissue around the pupillary edge, namely, the sphincter and the overlying stroma, becomes bunched together. This bunching together of the tissue is the limiting factor in preventing a maximum degree of closure. Slit pupils do not encounter this mechanical problem. Slit pupils which can close completely in bright daylight, can also on the other hand, dilate widely in dim light conditions. Thus it is the most efficient type of pupil and is best suited to nocturnal animals.

Although the generalisation is made that circular pupils are associated with diurnality, and slit pupils with nocturnality, this is not always so. In nature, there can be no hard and fast rules. Exceptions always occur. Among the mammals, the Monotremes are nocturnal and yet they possess round pupils.

Arrhythmic animals, for example, the larger terrestial animals like the ungulates and the large carnivores like the wolves, lions and cougars, have a twenty-four hour habit. They can be active both during the day or during the night, although there is usually a preference as to the maximum period of activity. Here the retina is not highly specialised for acuity as in strictly diurnal animals, or for sensitivity as in nocturnal animals. However, the retina is sensitive enough for its purposes. A highly mobile pupil which can be precisely controlled is very important. It may be circular or it may be some form of a slit pupil.

Crepuscular animals, for example, badgers and foxes, show little or no adaptation of the eye to cope with extremes of illumination of night and day. They carry out most of their activities only during the hours of dawn and dusk.

In discussing the habits of the animal world, we have only dealt with how the visual apparatus has adapted to serve the individual effectively in its quest for food and also in performing other of its life's activities. But all animals do not depend entirely, or at least to a great extent, on vision alone, even though some would be incapacitated if vision were removed. The other senses, notably hearing and smell, work in harmony with vision.

The form of the pupil, circular or slit, with its countless variations, is the result of the arrangement of the sphincter and dilator muscles within the iris tissue (Figure 1 - composite of Walls, 1942, p. 218; and

Prince, 1956, p. 186). In an iris with a round pupil, the sphincter is arranged concentrically around the pupillary margin. When the sphincter contracts, the pupil size is reduced without a change in pupil shape. As afore mentioned, because of the bunching together of the tissue around the pupillary edge, an iris with a round pupil is incapable of complete closure. The dilator muscles are arranged symetrically in a radial fashion. When these fibers contract, the pupil dilates uniformly. The degree of dilation attainable will, of course, depend on the amount of dilator muscle fibers present. The round pupil is seen in man as well as in the rat.

The simple slit pupil is characteristic of many nocturnal animals. The slit may be disposed vertically, horizontally or even at an angle. Some of the sphincter muscle fibers do surround the pupillary aperture. The majority form two bundles which criss-cross above and below and are anchored at the periphery of the iris. When these muscle fibers contract, they exert a "scissor-action" on the pupil. The pupil is constricted into a slit. There is no bunching of the tissue as in circular pupils, and thus there is no mechanical hindrance for complete pupillary closure. The dilator fibers are symetrically radially arranged so that in dilation the pupil assumes a circular form. The slit pupil is capable of extreme dilation in nocturnal conditions. In strictly nocturnal animals, like the lemur, tarsier and chinchilla, this ability of the pupil to dilate maximally is coupled with a large cornea. In dim light, the iris is pushed out of sight of the observer and a large round pupil comprises the whole visible eye.

Other pupil forms are present. In ungulates, the pupil constricts to a horizontal rectangle but dilates to a circle because of the radial positioning of the dilator muscle fibers. The sphincter fibers are also radial but are anchored laterally in iris connective tissue which is free of dilator muscle. In contracting, they draw the pupil into a rectangular

form. In some fish, neither the dilator nor the sphincter encircles the pupil completely so that unusual pupil shapes occur in dilation or constriction.

There is a multiplicity of pupillary patterns. It is not feasible nor entirely useful to deal with them here in too much detail, except to mention that pupils, being round or a slit, may be diamond-, dumbell-, or tear-drop-shaped. Also, the pupillary shape may change with the extent of dilation or constriction. The king penguin is one instance where this happens. In the constricted state the pupil is a perfect square whereas in the dilated state the pupil is a large circle. In dilating from its square form, the iris assumes a series of polygonal shapes until the circle is attained. Prince (1956) deals extensively with pupillary patterns and their functions. In Walls (1942), Rochon-Duvigneaud (1943) as well as in Prince, Diesem, Eglitis and Ruskell (1960), there are relevent sections dealing with pupillary shapes. Taxonomically, the form of the pupil is not used as an element in classification simply because it is not a very constant characteristic in a particular group of animals. Pupil patterns may be very similar in different families and yet differ in very closely related families. The over-riding rule is that the pupil will take on a form which will be most advantageous to the individual in its environment, disregarding its taxonomic or evolutionary position.

The pupil changes in size in response to changes in the intensity of the external illumination. In man, the pupil also constricts during accommodation for near vision. Pupils also respond to emotional states - fear, anger, or excitement - as well as to respiratory conditions. The horse's pupils dilate when it is being exercised and the oxygen content of the blood is reduced. The pupils of a cat dilate in fear but constrict in anger or excitement. All these pupillary excursions occur automatically.

However, it appears that certain amphibians, reptiles and birds can voluntarily control the size of their pupils (Walls, 1942). The intracacies of the mechanisms of how all this takes place are not entirely known.

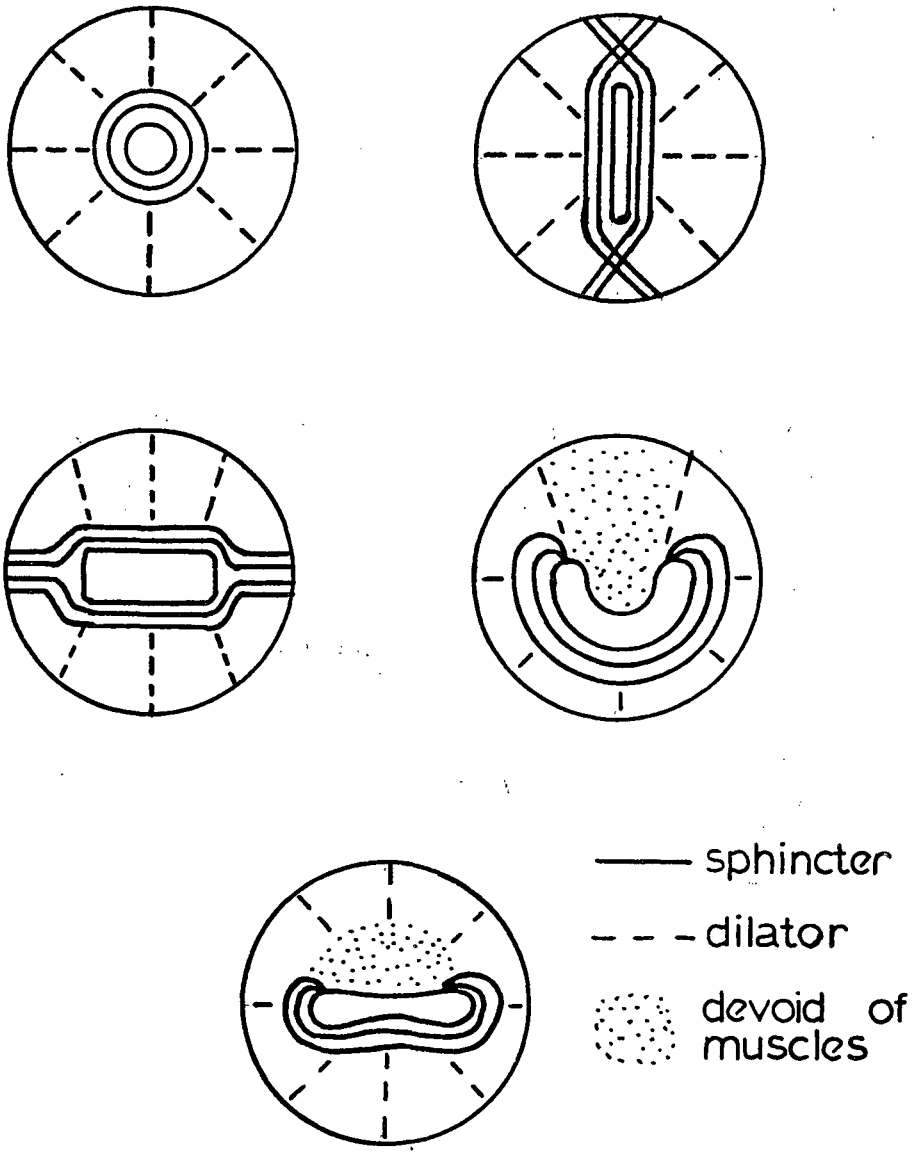


Figure 1 Pupillary Patterns

(Walls, 1942, p. 218 and Prince, 1956, p. 186)

E. Comparative Anatomy of the Vertebrate Iris

It is not only for intellectual but also for practical reasons that the human eye has been extensively and intensively studied since the earliest days of scientific endeavors. An adequate understanding of the normal morphology and physiology of the human eye is very useful when encountering the eye in diseased states. Besides, the human eye can serve as an example of a typical vertebrate eye. The main area of interest here is the histology of the iris (Coque, 1927; Walls, 1942; Rochon-Duvigneaud, 1943; Duke-Elder and Wybar, 1961; Hogan, Alvarado and Weddell, 1971). The microscopic anatomy of the human iris will serve as the basis on which we will compare the other vertebrate irises since it is devoid of any outstandingly unusual features.

The iris is the forward and centrally directed non-sensory portion of the retina and of the uvea. The iris is attached by its root to the ciliary body, which intervenes between the sensory retina and the mobile iris. In the iris, the uvea and the neuroectoderm are very intimately associated. The posterior surface of the iris is covered by a double layer of epithelium, the posterior pigment epithelium which is the forward extension of the sensory retina, and the anterior epithelium, which is the extension of the retinal pigment epithelium. The posterior pigment epithelium is the innermost posterior layer nearest to the lens. It is so heavily pigmented that cellular details are not visible except in depigmented specimens. In very light-colored eyes, the pigmentation of the posterior epithelium makes up almost the totality of the pigmentation present in the iris. At the pupillary edge the posterior pigment epithelium may bulge and be apparent anteriorly. This results in a thin, deeply pigmented rim or ruff at the pupillary margin which becomes more prominent when the pupil is constricted. It may almost be non-visible when the pupil is widely dilated.

At the periphery of the iris, the posterior pigment epithelium loses its pigments as it becomes continuous with the non-pigmented ciliary epithelium. A thin basement membrane, or posterior limiting membrane as it is also called, covers all of the posterior surface of the pigment epithelium.

Closely adherent to the anterior surface of the posterior pigment epithelium is the anterior epithelium. It is the direct continuation of the retinal and ciliary pigment epithelium. However, unlike these, the anterior epithelium contains few, if any, pigment granules. It is a modified epithelium. It has retained its epithelial character in its apical portion. Cell organelles and pigment granules, whenever present, are found within this portion of the anterior epithelium. The basal portion has differentiated into smooth muscle tissue, the dilator, and is filled with myofilaments. Some myofilaments may also be found in the apical epithelial portion of the epithelium. The anterior epithelium is thus, by definition, a myoepithelial layer. The dilator processes are oriented radially in a number of layers, depending on the degree of dilation or constriction of the pupil. By its contractile action, the dilator muscle causes the pupil to open widely, as in the response to dim light conditions, pain or excitement. A thin fibrillar layer, the basement membrane of the anterior epithelium, separates the dilator processes from the iris stroma. This basement membrane layer is also variously called the membrane of Bruch or the membrane of Henle (Duke-Elder and Wybar, 1961). However, in some anatomy texts (Coque, 1927), the term the membrane of Henle embraces the dilator processes as well.

The antagonist of the dilator muscle is the sphincter muscle, a band of smooth muscle cells in the stroma oriented concentrically around the pupillary margin. As has been mentioned previously, the disposition of the sphincter muscle fibers and of the dilator muscle fibers within the iris

tissue determines the shape of the pupil in dilation or constriction. Unlike the dilator muscle fibers, which are the contractile portions of myoepithelial cells, the sphincter muscle cells are fully developed smooth muscle fibers. Medially the sphincter does not extend to the very edge of the pupil. Laterally it merges with the fibers of the dilator. In amongst the sphincter muscle mass are collagen bundles, nerves and blood vessels. A basement membrane is also found in the sphincter.

Embryologically, both the sphincter and dilator in man are derived from neuroectoderm, as is usual in most vertebrates. The sphincter muscle cells have become detached from the lining epithelium of the iris and have developed into typical smooth muscle cells located in the stroma. However, the dilator has only gone half-way in the process of differentiation. For the longest time, there was much controversy as to whether the sphincter was not actually a mesodermal rather than a neuroectodermal derivative, when it is considered in terms of its innervation and its position in the stroma in the adult iris (Duke-Elder and Wybar, 1961). However, recent transmission electron microscopic studies of the developing human eye (Ruprecht and Wulle, 1973) have shown that myofilaments are very early detected in the basal parts of the anterior layer of neuroepithelial cells at the rim of the optic cup. These cells later formed a bundle, the sphincter, within the iris stroma.

The mesodermal portion of the iris, the stroma, consists of a loose connective tissue, variably pigmented, highly vascular and well innervated by both sympathetic and parasympathetic nerves. The supporting connective tissue framework consists of a delicate, loosely arranged network of collagen fibers. It is debatable whether elastic fibers are present (Duke-Elder and Wybar, 1961) or are absent (Hogan, Alvarado and Weddell, 1971). Fibroblasts, melanocytes, clump cells and mast cells comprise the cellular

components of the stroma. The varying shades and patterns of coloration seen in the human iris is attributed to the number and distribution of the melanocytes, filled with melanin pigments, within the stroma. Blood vessels of different calibers are present. Fine plexuses of mainly unmyelinated nerves are found associated with the blood vessels and the musculature of the iris.

The above brief discussion of the histology of the human iris will serve adequately as a means of comparison in the following survey of the iris in the other vertebrate groups. We will be able to note the similarities and differences between these other irises and that of the "typical vertebrate iris". Walls (1942) and Prince (1956) deal with certain comparative aspects of the vertebrate iris. Prince, Diesem, Eglitis and Ruskell (1960) are primarily concerned with domestic animals. However, Rochon-Duvigneaud's "Les yeux et la vision des vertebres" (1943), represents a gold mine of comparative vertebrate ocular histology, including the histology of the iris. The following discussion is based on information obtained from the above sources.

At the other end of the evolutionary scale from man are the Cyclostomes, namely the hag fishes and the lampreys. These have relatively immobile pupils. This is understandably so since there is no iris musculature. The scheme of pigmentation harks back to that of the retina. The anterior epithelium is, like the retinal pigment epithelium, deeply pigmented and cellular details are not discernible without prior depigmentation. The posterior epithelium, in reverse to that in man, is not pigmented except in the peripupillary zone. A marginal sinus may be more or less apparent. The thin stroma which contains a blood vessel layer and little or no pigment, is not as adherent to the posterior epithelia as in the other vertebrates. There are also some radial collagenous fibers in the

stroma. An annular ligament, consisting of a mass of large, irregularly polyhedral cells, is seen at the irido-corneal angle. Although it is not really an integral part of the iris, it is nevertheless very closely associated with it morphologically and may at times affect the movements of the iris. The iris has a slight metallic lustre owing to the presence of guanine crystals in some of the stromal cells. Guanine, a purine, is a pale, yellow powder. However, when it is deposited in cells either as simple guanine or as its calcium salt, it imparts to the tissue a silvery or golden metallic lustre.

The iris of the Chondrosteans (sturgeons), Holosteans (fresh water dogfish) and Dipnoans (Protopterus) is very much like that of the Cyclostomes. There are no iris muscles. The anterior epithelium is squamous and pigmented whereas the posterior epithelium shows no pigmentation. The stroma on the whole is thin. There is an argentea, a guanine-laden layer of cells, towards the anterior surface of the iris. In sturgeons, the argentea varies in the different sectors of the eye. The annular ligament, containing glycogen deposits, is present in the Holosteans.

Elasmobranchs in general, have irises very similar to those of the Cyclostomes. There are a few sphincter and dilator muscle fibers but these may be non-functional as the pupils are observed to be quite immobile. Their epithelial origin was ascribed to as early as 1897 by Vialleton. The anterior epithelium is wholly pigmented although the posterior epithelium is only pigmented at the pupillary region. The stroma consists of a connective tissue network, small non-pigmented cells, large melanocytes, nerves and blood vessels. Cells laden with guanine crystals may be seen scattered in the iris stroma, or as an argentea. Annular ligaments are absent. Some Elasmobranchs, for example, the skates and rays, have pupillary opercula to protect the eyes from the effects of direct sunlight. They are amuscular

prolongations of the superior pupillary border shaped to look like the palm of a hand with the digits spread apart.

The Teleosts, or bony fishes, show no or restrained movements of their irregularly circular or oval pupils. This may be due to two features of the iris histology. Firstly, although there are dilator fibers (so named because of their radial direction), as well as a sphincter, which may indeed be quite large, these appear to be non-contractile. Secondly, there is almost always a well-developed annular ligament at the iris angle. It consists of an accumulation of swollen, vesiculated cells, which may contain glycogen granules, pigment cells, blood vessels and an arcade of fibrils. It is a relatively firm structure holding the iris at a fixed angle to the cornea. It would mechanically hinder any great excursion of the iris. There is a thick argentea just below the mesothelium lining the anterior surface of the iris. The argentea is what gives the glint seen in all fish eyes. The stroma is pigmented and highly vascularised. The anterior epithelium and the peripupillary zone of the posterior epithelium are heavily pigmented.

In dilation, the amphibian pupils are circular but in constriction, they assume numerous forms, vertical and horizontal slits, triangles, etc. The color schemes of the iris follow quite closely the pigmentation patterns of the skin, namely, black and gold. The iris is most often large and thin. Both the anterior and posterior epithelium contain pigment granules, unlike the situation seen in the fishes. The dilator, derived from the anterior epithelium, appears as a thin line with delicate fibrils which are neatly, radially oriented. The sphincter is a small compact mass which rests in close relationship to the anterior epithelium. These cells, besides being filled with myofilaments, are also filled with pigment granules. The stroma is very thin so that blood vessels often bulge from the anterior surface of

the iris. In places the stroma may be absent so that the iris is reduced to two epithelial layers. There is no argentea in the stroma but melanocytes and other cells containing yellow pigments or guanine crystals are present. One distinguishing characteristic of the amphibian iris is the pupillary nodules, which are variously placed along the pupillary margin. These nodules are, in essence, a mass of epithelial cells resulting from the proliferation of the posterior epithelium of the pupillary border. It will later be seen that ruminants have a similar structure known as the corpora nigra. It is speculated that the function of the pupillary nodules is to lift the iris off the lens so as not to impede the flow of aqueous humor. A pigmented and vascular pectinate ligament is present in the iris angle.

In the reptilian iris both the anterior and posterior epithelial layers are pigmented. There is some controversy over the question of the musculature of the iris. It is generally agreed that a sphincter muscle, of neuroectodermal origin, is present in all reptilian irises. However, the presence of a dilator is still in debate. The sphincter consists of striated muscle fibers, unlike that in many vertebrates which consists of smooth muscle fibers. This perhaps suggest that there might be some voluntary control of the sphincter in reptiles. The sphincter muscle fibers, oriented mostly circularly but a few are oriented obliquely, are seen throughout the whole extent of the iris but are concentrated at the pupillary margin, except in the Sphenodon where it is concentrated at the periphery of the iris. In the tortoise eye, the iris sphincter muscle is especially powerful and aids in accommodation by squeezing on the lens. The presence of a dilator in tortoises, crocodiles and lizards is questionable. In the Sphenodon, it has been suggested that there are some muscle fibers derived from the sphincter which possess the function of a dilator. It could conceivably be that the dilator is present in a rudimentary form,

that is, it is comprised of cells containing some myofilaments which are not readily visible with the light microscope. Electron microscopic studies would help to elucidate this question. The stroma is vascular and pigmented. Blood vessels, nerves, lipophores (in crocodiles), melanocytes and cells containing red or yellow pigments or guanine crystals, are also present in the stroma. There is a loose connective tissue network, the pectinate ligament, at the irido-corneal angle.

Snakes show certain characteristics which are different from those in the other reptilian irises, especially in relation to the musculature. The dilator consists of a thin layer of striated muscle fibers pressed flat against the anterior surface of the anterior epithelium. There is also a network of fine striated muscle fibers seen throughout the whole iris. They increase in number in two regions of the iris to form two compact bundles. One bundle is found at the pupillary border and is morphologically correctly placed to act as a sphincter. The other muscle bundle is found at the root of the iris. These muscle fibers run obliquely as well as circularly. This is used in accommodation to press on the lens to push it towards the cornea. Unlike the condition in the other vertebrates, both the sphincter and dilator are surmised to be of a mesodermal origin.

There is a multiplicity of colors in the bird iris due to the pigments and crystals in the iris stroma. The dark or black irises are generally thinner than the colored irises. In most birds, the iris is thin at the pupillary margin and at the root, where it is attached to the cornea by a pectinate ligament. The middle of the iris is thick. Both the anterior and posterior epithelia are pigmented. The anterior epithelium is myoepithelial in nature, consisting of an apical pigmented portion and a basal muscular portion. The striated muscle fibers are radially arranged and thus act as a dilator. In most cases, the sphincter muscle is well-

developed, as is exemplified by the cormorant. The circularly and obliquely disposed striated muscle fibers act as a strong sphincter for light reflexes, as well as for accommodation in certain instances. Blood vessels, nerves, collagen fibers and melanocyte processes are found in amongst the muscle fibers. In some birds, there are no muscle fibers and the iris is then reduced to two layers of epithelia and a thin stroma. The stroma is oftentimes so thin that blood vessels protrude from the anterior surface of the iris.

Of the mammals, the Monotremes and Marsupials have simple irises consisting of the anterior and posterior epithelia (either one or both of which may be pigmented), a large sphincter of smooth muscle fibers, no dilator, and a simple pigmented and vascularised stroma. The iris of the Placentals is very similar to that in man with the exception of a few differences which are noted here. The most distinctive feature is the modification that occurs along the pupillary edge. The camel and giraffe possess an umbraculum or flocculi. These are folds or pleats of the superior and inferior borders of the iris which fit into each other when the pupils are constricted. The dolphin and porpoises have an operculum. It is generally held that the umbraculum is amuscular whereas the opercula of the dolphins and porpoises are essentially enlargements of the sphincter. Pigmented epithelial cysts, formed from the proliferations of the posterior epithelium are known as corpora nigra. They belong almost exclusively to ruminants with horizontally oval pupils. They are seen in the irises of the horse, cattle, sheep and goats. All these modifications of the pupillary margins may serve as eye shades to shield the retina from glare when the pupils are open. When the pupils are constricted, they may ensure a more complete closure of the pupillary aperture in situations where the sphincter is not powerful enough or, when, because of the nature of the

pupillary shape, complete pupillary closure is not inherently possible.

F. Areas of Research on the Iris

Various aspects of the structure of the iris have been of interest to researchers over the years and continue to be of interest at the present time. The basic histology of the iris as being made up of two layers of epithelium, some contractile elements, namely the dilator and the sphincter muscles, and a mesodermally-derived stroma consisting of blood vessels, nerves and stromal cells, has been known for a very long time. But interest was and is focussed on various particular aspects of the iris structure, for example, the epithelium, the pigments, the muscles, and the blood vessels. This is based on the premise that a greater and more detailed knowledge of the iris structure itself would help elucidate the role or roles the iris plays in the overall functioning of the eye. Such studies have been carried out using the light microscope. In addition, recent advances in the field of electron microscopy have resulted in an instrument with a greatly increased magnification factor and resolving power compared with the light microscope. Studies using the electron microscope have given us an insight into the fine ultrastructural details of the cytology of the iris, not only in man but also in the other vertebrates. The study of the innervation of the iris is a viable field of research. Histo-chemical methods at the light and electron microscopical levels have attempted to work out the intricate details of iris innervation. These observations are interpreted with regard to pharmacological studies of the iris. Other investigators are interested in the dynamics of iris movements and have brought a mathematical approach towards the understanding of a biological problem. This, in brief, is what is happening in the field of iris research, at the present time. Each aspect of the above mentioned will be treated in greater

detail in the following discussion.

1. General Considerations of the Iris

The human iris is an easily observable pigmented structure. In certain diseased conditions of the eye, for example, uveitis, gross changes occur in the iris. Norn (1971a, 1971b) in studying the human iris observed that certain pigment defects are associated with uveitis (Norn, 1971b) as well as in normal eyes (Norn, 1971a). In normal eyes, defects of the pigment layer are only found in people who are over 45 years of age. These defects, seen as small depigmented holes, are found near the pupillary ruff infero-nasally, or they may actually be at the ruff itself. If such defects are seen in the irises of young people they are considered to be pathological and indicative of a diseased state. In older people these defects would have to be large to be pathological. It is postulated that normal physiological movements of the iris over the years expose the pigment epithelium to much wear and tear and result in the destruction of the epithelial cells. Regeneration does not or cannot keep up so that depigmented areas are grossly seen. Uveitis accelerates the depigmentation process in young people and aggravates that in old people.

Using a gonioscope, an observer is able to look at different parts of the iris, especially towards the root and at the chamber angle. Processes of iris tissue (Lichter, 1969) are seen attaching to the angle wall. The number of such iris processes may vary from one individual to the next, being more prominent in dark irises than in pale irises, and also within an individual eye. There may be areas with an abundant number of iris processes, and yet other areas in the same eye are devoid of iris processes. The iris processes can insert at any level on the angle wall although in any one eye they all tend to insert at about the same level. Presumably, the iris

processes are mesodermal remnants from cleavage of the chamber angle during development. These processes, when present in large numbers, may be of diagnostic importance when considering glaucomatous conditions of the eye.

As with all human organs, and the iris is no exception, individual variations are common. However, in our discussions of the human iris, the average most generally occurring structure of the iris is taken as the norm. Hervouet (1962) divides the human iris as belonging to one of three categories depending on the amount of stromal tissue that is present. The normal eye would have an average amount of stroma, melanocytes and numerous easily visualised crypts. Then there are the other two extremes. On the one hand, there are irises where the stroma is very dense, heavily pigmented and where there are very few crypts. On the other hand, the iris may possess very little stroma, as to be almost non-existent, and crypts which are both large and numerous.

Before considering the fine ultrastructural details of the various components of the iris, it would be of interest to first consider the iris as a whole in its milieu. The iris is completely bathed by aqueous humor. In the adult eye the aqueous humor is produced by the ciliary processes and released into the posterior chamber of the eye. The aqueous humor than circulates forward centrally through the pupil into the anterior chamber. It leaves the eye via the trabecular meshwork and the canal of Schlemm. The aqueous humor is continuously produced at a constant rate. Its outflow, too, is well regulated so as not to unduly increase the intra-ocular pressure. Of course, in disease, this balance is disturbed. It is reasonable to conjecture that the iris itself is in some way intimately involved with this constant flow of aqueous humor, especially since there are communications between the iris stroma and the surrounding aqueous humor by means of the iridic crypts and iridic pores (Vrabec, 1952; Gregersen, 1958a, 1958b,

1959a, 1959b, 1961; Coulombre, 1961; Klika and Kloucek, 1962; Purtscher, 1962; Newsome and Loewenfeld, 1971). Such crypts and pores are not only found in human beings but are also commonly seen in the irises of many vertebrates (Rohen and Voth, 1960; Rohen, 1961). At one time it was thought that there was a continuous endothelial layer covering the anterior surface of the iris so that the iris tissue was, in essence, structurally cut off from the surrounding aqueous humor. However, Vrabec, (1952) showed that in fetal human eyes and in human eyes at birth, the anterior surface of the iris is indeed covered by a continuous endothelium. With development, discontinuities occur and crypts and pores are formed. Fibroblasts replace the endothelial cell layer. Through these crypts and pores, aqueous humor can easily circulate into the stroma, and conversely, materials from the iris may be added to the aqueous humor. In other words, the iris may be involved in aqueous humor dynamics (Gregersen, 1958a).

Most of the studies on the relationship between the iris stroma and the aqueous humor were done by Gregersen (1958a, 1958b, 1961). Slit lamp observations of the iris shows that the iris very often looks like a sponge saturated with fluid. This aspect of the appearance of the iris may be lost in histological preparations. Gregersen perfused the anterior chambers of human and rabbit eyes with the polysaccharide Dextran (Gregersen, 1958a), killed cocci (Gregersen, 1958b), or a suspension of red blood cells (Gregersen, 1958b). Dextran molecules are about the same order of magnitude as the proteins found in the aqueous humor. The Dextran is very rapidly imbibed by the iris tissue of both human and rabbit irises. The Dextran passes through the anterior border layer via the iridic crypts and pores and is widely distributed in the stroma, including the spaces around the blood vessels. The pigment epithelium does not participate in this imbibition of the iridic tissue by Dextran, but instead acts as a barrier to the

further spread of the Dextran. Cocci are larger than Dextran molecules, being ½ - 1μ in size. In human eyes, the cocci are found in the region of the crypts, and often, throughout the stroma as well. Where the stroma is dense, no cocci are present. Rabbit irises, unlike human irises, lack crypts. Cocci are only found on the anterior iridial surface but not within the stroma. This demonstrates clearly that iridic crypts are necessary for the penetration of large molecules or particles into the iris stroma, whereas, iridic pores are sufficiently large enough to let protein molecules through. Red blood cells, 7-8μ in size, rarely enter the iris tissue. Even then, the red blood cells are located toward the anterior border layer of the iris. Thus, the iris crypts and iris pores must act as openings into a system of inter-communicating, probably widely branched tissue channels in the iris stroma. These tissue channels are filled with aqueous humor. During pupillary movements, there is, conceivably, a free passage of fluid between the tissue spaces of the iris stroma and the anterior chamber. Whether the aqueous humor is modified in some way while in its passage through the iris tissue is not known. Such fluid movements in and out of the iris stroma would necessarily considerably reduce the tension changes that would otherwise occur in the iris stroma during pupillary dilation and constriction.

It seems that this system of tissue channels is organised in some sort of fashion. Francois, Neetens and Collette (1960) perfused human eyes for 10 minutes with Thorotrast and then examined the iris by microradiography. They found a seemingly canalicular network which is completely different from the vascular network of the iris. This canalicular network is localised in the stromal layer. It consists of two relatively distinct parts; in the peripupillary region, the canaliculi are seen as parallel radial marks, whereas in the peripheral region they are seen as circular

marks. In the middle of the iris, the canaliculi are radial with some interconnecting circular canaliculi. This canalicular network revealed by microradiography most probably represents the system of tissue channels in the iris stroma capable of imbibing aqueous humor from the anterior chamber.

2. Posterior Pigment Epithelium

The fine structure of the pigment epithelium covering the posterior surface of the iris has been studied in various vertebrates, for example, man (Tousimis and Fine, 1959a; Mizuno, 1961; Tomita, Matsuo and Kato, 1961; Tousimis and Fine, 1961; Tousimis, 1963; Kaczurowski, 1965; Hogan, Alvarado and Weddell, 1971), monkey (Tousimis and Fine, 1959a, 1961; Tousimis, 1963), rabbit (Tousimis, 1963; Richardson, 1964; Hvidberg-Hansen, 1971a), ox and gecko (Tucker, 1971), newt (Dumont and Yamada, 1972), Elasmobranch (Kuchnow and Martin, 1970), and skates (Kuchnow and Martin, 1972). However, the electron microscopic picture of the pigment epithelial cells from these diverse vertebrates, is amazingly similar. Embryologically, the posterior pigment epithelium of the iris is the forward and central extension of the retina and ciliary epithelium. Unlike the anterior epithelium which is transformed into myoepithelial and muscle elements, the posterior epithelium has maintained its epithelial nature even in adult irises. In pigmented eyes, this layer is heavily pigmented, which is not so in albinos. The overall shape of the posterior epithelial cells is variously described as cuboidal (Tousimis and Fine, 1961; Dumont and Yamada, 1972), columnar (Kuchnow and Martin, 1972), cylindrical (Tousimis and Fine, 1961), rectangular, truncated and pyramidal (Hogan, Alvarado and Weddell, 197;). Naturally the shape of the posterior epithelial cells would vary somewhat according to the location on the iris itself. In the human iris there are structural folds and furrows and the shape of the epithelial cells varies to fit their

positions (Hogan, Alvarado and Weddell, 1971). Also during the excursion of the iris in miosis and mydriasis changes in the shape of the epithelial cells would occur to accommodate the changes in the total surface area of the iris (Alphen, 1963; Kaczurowski, 1965). Thus, the described shape of the epithelial cell would very much depend on the degree of pupillary dilation or constriction of the iris when the tissue was removed and placed in the fixative. Kaczurowski (1965) has tried to reconstruct a three dimensional picture of the iris pigment epithelial cell based on numerous observations. He visualises an epithelial cell with a varyingly polygonal base whose walls are neither parallel nor flat as a result of the numerous and continuous movements of the iris.

Posteriorly, the iris epithelium is bounded by a typical basement membrane which is continuous with that covering the ciliary body. The basement membrane may vary in thickness from 120-140Å (Hvidberg-Hansen, 1971a) to 250Å (Kuchnow and Martin, 1970). Rarely, a series of fine, electron dense particles may be seen at the interface between the basement membrane and the aqueous humor, the significance of which is not known (Richardson, 1964).

The basal plasma membrane of the epithelial cells shows many infoldings which may extend quite deeply into the cell cytoplasm. These infoldings are irregular in shape, size and orientation. The basement membrane, however, does not follow the course of the infoldings but only covers the external surface of the epithelium. Most often there is a clear-looking space between the filamentous basement membrane and the epithelial plasma membrane. The basement membrane does not appear to be tightly or closely adherent to the plasma membrane. The lateral walls of the epithelial cells show deep, complicated and irregular outlines of the interdigitations between adjacent cells. The intercellular spaces may be 200Å (Hogan,

Alvarado and Weddell, 1971), or as much as 1μ in width (Tousimis and Fine, 1961). This discrepancy may be due to the mode of fixation as well as, perhaps, the degree of miosis or mydriasis of the tissue under observation. There may be some maculae adherentes or occludentes here and there along the lateral wall. The apical cell wall, that is, the surface in apposition with the anterior epithelium, may be relatively smooth and in close contact with the anterior epithelium (Richardson, 1964), or undulating (Hogan, Alvarado and Weddell, 1971). At intervals between the anterior and posterior epithelial layers, there would be relatively large spaces with microvilli projecting into them from both the epithelial layers (Tomita, Matsuo and Kato, 1961; Richardson, 1964; Hogan, Alvarado and Weddell, 1971). In skates (Kuchnow and Martin, 1972) there is a heterogenous intercellular matrix between the anterior and posterior pigment epithelial layers.

The most conspicuous components of the pigment epithelium in pigmented eyes are the large number of pigment granules. Tousimis (1963) compared the pigment granules in the pigment epithelium and stromal melanocytes in man, rabbit, cat, dog and monkey. He found that the pigment granules in the epithelium of all these five species are very similar, being round or oval in shape and larger than those in the stromal melanocytes. Shearer (1969), with the scanning electron microscope, has given us a three dimensional view of isolated pigment particles in man, rabbit, sheep, ox, pig, rat, pigeon, crab, fish and lizard. There is some diversity in the shape and size of the pigment particles but there is also an underlying basic similarity between them all.

The nuclei are round and smooth and may at times be indented, or they may be elongated (Dumont and Yamada, 1972), There is generally a paucity of intracellular cytoplasmic organelles. The mitochondria are few in number. Smooth and rough endoplasmic reticulum is present in limited amounts. An

occasional Golgi apparatus may be discerned. Glycogen is also present in normal human iris pigment epithelium (Berkow and Fine, 1970). Most often, it is extracted during the usual preparative procedures for electron microscopy. The glycogen particles are widely distributed, although they might have a tendency to accumulate near to dense melanin granules.

In man it is unfortunate that the iris pigment epithelium is unable to contribute towards regeneration of the lens, but in the newt this is observed following lentectomy (Dumont and Yamada, 1972). Iris epithelial cells from the mid-dorsal margin of the iris are able to first dedifferentiate and then redifferentiate to form lens cells. Changes in cell morphology and physiology occur during this process. (Yamada and Roesel, 1969).

3. Anterior Epithelium and Dilator Muscle

The anterior epithelial layer of the iris is myoepithelial in nature in various mammals that are commonly studied in the laboratory, for example, man (Tousimis and Fine, 1961; Hogan, Alvarado and Weddell, 1971), monkey (Tousimis and Fine, 1961), cat (Geltzer, 1969), rat (Nilsson, 1964; Roth and Richardson, 1969; Kelly and Arnold, 1972) and rabbit (Richardson, 1964). However, in Elasmobranchs the question is under dispute (Kuchnow and Martin, 1970). In the adult Elasmobranch iris the anterior epithelium and the dilator form two distinctly separate layers. Other investigators do consider this layer as being myoepithelial.

The anterior epithelial layer consists of an apical epithelial and a basal contractile portion. It is, in common usage, usual to refer to the epithelial portion as the anterior epithelium and the contractile portion as the dilator muscle, although they are both really part of one cell layer. This will be the terminology that will be used in this discussion.

The anterior epithelial cells are generally not as high as the cells

in the posterior epithelium. The nucleus is flattened or elongated in a radial direction and situated towards the apical or posterior poles of the cell. Sometimes the nucleus may be irregularly indented (Nishida and Sears, 1970). Numerous pigment granules similar to those in the posterior epithelium are present. The usual cell organelles are seen. Rough endoplasmic reticulum in stacks and smooth endoplasmic reticulum in the form of tubules or vesicles are present. In man (Hogan, Alvarado and Weddell, 1971), the Golgi apparatus is not well developed, whereas in the hen (Nishida and Sears, 1970), the Golgi apparatus can be quite sizeable. Free ribosome clusters are observed over most of the cytoplasm, with a greater concentration in the perinuclear region. Mitochondria are large (Tousimis and Fine, 1961), rod-shaped (Nishida and Sears, 1970) but are generally not plentiful. There is a moderate amount of interdigitation of the lateral cell membranes. At the apices of the anterior epithelial cells there are desmosomes and tight junctions which hold the anterior epithelium to the posterior epithelium. As mentioned before, there are large intercellular gaps between the two epithelial layers into which microvilli and occasional cilia from both epithelial layers project. Myofilaments may also be found in the anterior epithelium.

The dilator muscle consists of a series of muscular processes from the anterior portions of the anterior epithelium projecting into the stroma. These dilator muscle processes are radially arranged in numerous stacks. The dilator muscle has a highly complicated outline so that it is extremely difficult to trace out all the dilator muscle processes that stem from any one individual cell. The dilator muscle processes are separated from each other by an intercellular gap of about 200Å (Richardson, 1964; Hogan, Alvarado and Weddell, 1971). However, the dilator muscle processes also adhere to each other by junctional specialisations. It appears that only

zonulae occludentes and zonulae adherentes are present. Desmosomes are rarely found (Richardson, 1964; Nishida and Sears, 1970). The dilator muscle processes are filled with contractile myofilaments oriented radially in the iris. The myofilaments are of two types - the thin myofilaments are about 30Å in diameter (Hogan, Alvarado and Weddell, 1971) while the thick filaments are about 180Å in diameter (Kelly and Arnold, 1972). Dense bodies are found amongst the myofilaments as well as along the plasma membranes. At times, the myofilaments appear to insert into these dense bodies. The sarcoplasm is quite electron-dense. In the human dilator muscle processes there are a few pinocytotic vesicles (Hogan, Alvarado and Weddell, 1971) but Geltzer (1969) in his observations of the cat dilator found many pinocytotic vesicles at the borders of the cells. Long mitochondria are oriented radially within a matrix of myofilaments (Tousimis and Fine, 1961). In the Elasmobranch, the presence of pinocytotic vesicles is used as a means of identifying the dilator muscle processes which are widely dispersed in the iris stroma (Kuchnow and Martin, 1970). A typical basement membrane covers all of the surfaces of the dilator processes but stops short at the epithelial portion of the anterior epithelium. As with the basement membrane covering the posterior surface of the iris, there is a clear space of about 500Å (Nishida and Sears, 1970) which separates the basement membrane from the plasma membrane of the dilator muscle processes.

Embryologically, the dilator muscles are derived from neuroectodermal cells which have remained in their original position but have acquired a myoepithelial structure (Duke-Elder and Wybar, 1961; Lowenstein and Loewenfeld, 1969; Mann, 1964; Hogan, Alvarado and Weddell, 1971; Imaizumi and Kuwabara, 1971; Lai, 1972b).

4. Sphincter Muscle

The sphincter muscle, as its name suggests, acts as the constrictor of an orifice, namely, the pupil. It consists of an annular band of typical smooth muscle fibers, concentric with the pupillary margin, in man (Tousimis and Fine, 1961; Lowenstein and Loewenfeld, 1969; Hogan, Alvarado and Weddell, 1971), monkey (Tousimis and Fine, 1961), rat (Hokfelt and Nilsson, 1965; Kelly and Arnold, 1972), guinea pig (Nishida and Sears, 1969b), newt (Tonosaki and Kelly, 1971), Elasmobranch (Kuchnow and Martin, 1970), and skate (Kuchnow and Martin, 1972). However, in birds and reptiles, for example, the alligator (Reger, 1966), the sphincter muscle consists of an admixture of both smooth and striated muscle cells. It will be considered separately from the more usually occurring smooth muscle sphincter of most vertebrates.

The sphincter is located more or less in the iris stroma at the pupillary margin. These muscle cells show a remarkable degree of contractility as compared to the other smooth muscle cells. The sphincter muscle cells are elongated and spindle-shaped and are oriented concentrically around the pupil. The muscle cells are intimately and firmly attached to each other and to the surrounding stroma, so that if a segment of the iris is removed surgically due to disease, the rest of the sphincter can function unimpaired (Lowenstein and Loewenfeld, 1969; Hogan, Alvarado and Weddell, 1971). A basement membrane separates the individual muscle cells from the surrounding connective tissue stroma. This basement membrane follows the contours of the cells except in regions where two cells are in very close apposition, as at a nexus or tight junction. Hogan, Alvarado and Weddell (1971), in their observations of the human iridial sphincter with the electron microscope, note that sphincter muscle cells associate together, by means of tight junctions, in groups of five to eight cells. Each muscle

cell may have more than one region of close apposition with another cell. It is postulated that possibly such morphological groupings represent functional units, where the cells can contract in synchrony in response to a stimulus. The elongated nucleus is located centrally within the spindle-shaped cell. These muscle cells also contain a Golgi apparatus, an endoplasmic reticulum, which may be quite extensive in some cases (Kuchnow and Martin, 1972), some free polyribosomes and elongated mitochondria which are oriented along the long axes of the cells. There are also some melanin pigment granules, or, as in the West Coast newt, a cluster of special R granules. Large irregular vesicles are distributed throughout the cytoplasm. Besides, there are many pinocytotic vesicles along the cell membrane. Some vesicles open into the extracellular space. Sometimes, there are densities along the cell membrane. Being a contractile tissue, the most abundant components within the cell cytoplasm are the myofilaments. It was once thought that only one type of myofilaments is present (Reger, 1966; Hogan, Alvarado and Weddell, 1971). In the Elasmobranch sphincter, Kuchnow and Martin (1970) found myofilaments with transverse diameters of 40-100Å, depending on the species. These would comprise the thin filaments. However, Kelly and Arnold (1972) found that if the rat iris tissue is well fixed by perfusion, both thick and thin filaments are consistently observed. The thin filaments are 70-80Å while the thick filaments are 220-230Å in diameter.

The sphincter of the Alligator mississippiensis (Reger, 1966) contains not only smooth but also striated muscle cells. In addition, there are some myoblast-like cells which probably represent a stage in the differentiation of striated muscle. Smooth and striated muscle cells are often in contact, as are myoblast-like cells with striated cells. The smooth muscle cell components of the sphincter possess the characteristics of typical smooth muscle cells, as described above. The striated muscle cells show

features of a slow-acting muscle, that is, a poorly organised sarcoplasmic reticulum and T system. Thick and thin filaments and Z lines are present. Myoblast-like cells are in many respects similar to the striated muscle cells.

Any discussion of the sphincter must necessarily include some comments on its embryological derivation. In the adult iris, the sphincter is structurally a smooth muscle but embryologically it develops from neuroectoderm (Mann, 1964) rather than from mesoderm. This was the generally accepted view although some were not convinced, their arguments being based on the position of the sphincter in the adult (in the stroma), and the innervation of the sphincter muscle cells (parasympathetic, which is similar to the innervation of the mesodermally-derived ciliary muscles). Recent investigations on the development of the sphincter muscle in man (Ruprecht and Wulle, 1973), rabbit (Tamura and Smelser, 1973), rat (Lai, 1972a; Imaizumi and Kuwabara, 1971) and newt (Tonosaki and Kelly, 1969, 1970, 1971) with the electron microscope have shown definitively that the mature smooth muscle cells of the sphincter do arise from the neuroepithelial cells of the anterior layer of the optic cup near to its rim. Except for minor differences in terms of the sequence and timing of the events that take place during the differentiation of the epithelial layer into muscle tissue, this process essentially involves the formation of myofilaments within the cells. The shape and orientation of the cells also alter to best fit its function in adult life. The sphincter muscle as a whole may also shift to occupy a stromal position. The degree of movement depends on the species.

5. Stroma

Apart from the posterior and anterior epithelial layers and the two muscles, the rest of the bulk of the iris comprises the stroma. The width

of the stroma from the anterior iridial surface to the dilator varies from individual to individual (Hervouet, 1962) as well as with the degree of pupillary dilation or constriction (Alphen, 1963). There are also species differences in terms of the density of the stroma (Rohen, 1961). The number and distribution of the cellular and intercellular components of the iris stroma is characteristic for each species, as is amply shown by Rohen (1961).

The cells of the iris stroma are the non-pigmented fibroblasts and mast cells (Hogan, Alvarado and Weddell, 1971) and the pigmented melanocytes and clump cells (Iwamoto, 1961, 1962; Tousimis, 1963; Kaczurowski, 1965; Hogan, Alvarado and Weddell, 1971). In addition, there are some ciliated stromal cells (Vrabec, 1971). Blood vessels and nerves are also seen coursing through the stroma. The blood vascular supply and the innervation of the iris will be considered separately. All the cells are surrounded by a mucopolysaccharide ground substance and fluid milieu. The intercellular spaces are also filled with a connective tissue framework of filaments and collagen fibrils (Ringvold, 1970b). The structure of the connective tissue and the arrangement of the cells within this connective tissue framework varies with the species (Rohen, 1961).

At the anterior surface of many primate irises, the stroma is modified to form the anterior border layer. This is a dense accumulation of cells and connective tissue (Rohen, 1961; Hogan, Alvarado and Weddell, 1971; Purtscher, 1972). The thickness of this layer varies for different segments of the same eye (Hogan, Alvarado and Weddell, 1971). This variation is also observed between eyes from different species of mammals (Rohen, 1961). The anterior border layer, according to current thinking, does not perform any important functions but does influence eye color to a certain extent. The principal types of cells found in the anterior border layer are fibroblasts and melanocytes. These are oriented parallel to the anterior surface of the

iris and may form a relatively continuous layer. An endothelium covers the anterior iridial surface incompletely in the adult (Vrabec, 1952). Localised defects of the anterior border layer and the anterior endothelium give rise to the iridial crypts (Vrabec, 1952; Gregersen, 1959a; Rohen and Voth, 1960). Gregersen (1959a) has made a detailed study on the structural variations seen in the crypts of the human iris. Spanning the openings of some, but not all, of the crypts are the so-called "bridge trabeculae". These are non-cellular fibrillar (presumably collagenous) tissue components. They may be remnants of the fetal pupillary membrane.

Fibroblasts are seen throughout the stroma but they very often congregate around blood vessels and nerves. They possess the usual cell organelles. The melanocytes (Iwamoto, 1962; Tousimis, 1963; Kaczurowski, 1965) are also distributed throughout the stroma and especially towards the anterior surface in man. The melanocytes are small and elongated and possess a few branching processes. It appears that the melanocytes situated towards the anterior iridial surface and at the crypts may possess heavily pigmented "globes" at the ends of the cytoplasmic processes (Kaczurowski, 1965). This feature is lacking in melanocytes found in the other more posterior parts of the stroma. The melanocytes are nucleated and possess mitochondria, ribosomes and both smooth and rough endoplasmic reticulum. Melanin granules in different stages of differentiation are observed within the melanocytes. Unlike the pigment granules of the epithelial cell layers, there are distinct differences between the pigment granules of the stromal cells of different mammalian species (Tousimis, 1963). Generally, the stromal pigment granules are smaller than those in the epithelium and are more rod-like in shape. They may be specifically or randomly oriented within the cell cytoplasm.

Clump cells (Iwamoto, 1962; Tousimis, 1963; Kaczurowski, 1965; Hogan, Alvarado and Weddell, 1971) are large, pigmented stromal cells varying in

size from 3 to 12μ. They are mostly found in the pupillary zone in the vicinity of the sphincter muscle. They are spherical with numerous microvilli on the cell surface but no cytoplasmic processes, as is characteristic of the normal melanocytes. The cell cytoplasm is engorged with two types of pigment granules (Tousimis, 1963). Some granules are small and are similar to those of the other stromal melanocytes, while others are larger and are similar to those in the pigment epithelium. They may be displaced neuroectodermal cells or macrophages (Hogan, Alvarado and Weddell, 1971).

In the anterior border of the human iris, there are sometimes nevus-like structures (Hogan, Alvarado and Weddell, 1971). Dieterich and Franz (1972) observed that the nevus cell morphologically resembles the melanocyte. The cytoplasm is filled with pigment granules. Mitochondria, ribosomes, filaments and a fairly well-developed Golgi complex are present. There may be pinocytotic vesicles at the cell membrane.

Mast cells containing specific granules are sometimes found in the iris stroma.

In electron microscopic studies, mention is sometimes made of ciliated cells in the iris stroma (Hogan, Alvarado and Weddell, 1971). The cilia may be seen on fibroblasts. But it is impossible to study the extent of the occurrence of ciliated cells in electron microscopic studies because of the inherent limitations of the method, that is, the small size of the tissue sample under observation and the thinness of the tissue section. Vrabec (1971), using flat, frozen sections of rabbit and human irises, observed that almost all of the superficial cells from the anterior surface of the iris possess typical long cilia which might end in a spherule at the tip. Cilia are rarely found on cells deeper in the stroma, but when present, they are short. The cilia are capable of beating synchronously. These cilia are probably embryonic remnants. It is interesting to speculate that since

the cilia are quite commonly found, they might be involved in the movement of aqueous humor across the surface of the iris.

The ground substance of the stroma is a hyaluronidase-sensitive acid mucopolysaccharide. Within this ground substance are different types of fibrils which comprise the connective tissue skeleton of the stroma (Tousimis and Fine, 1959; Ringvold, 1970b; Hogan, Alvarado and Weddell, 1971). Collagen fibrils predominate in the stroma. The collagen fibrils form a network which, in some mammals, is regularly arranged in interweaving arcs (Rohen, 1961). In electron micrographs, they appear to be distributed at random except around the blood vessels. The collagen fibrils are between 200-700Å in width and show an axial periodicity of about 600-640Å. In addition, Ringvold (1970b) found two other types of fibrils in the iris stroma. Some appear in bundles which taper and split. These are unevenly wide along the length and also show cross-banding with a periodicity of 650Å. The third type of fibril is about 130Å in diameter, do not branch, associate in groups and also show cross-banding. These latter two types of fibrils may represent native collagen fibrils (Ringvold 1970b).

6. Iris Blood Vessels

The blood supply to the iris has been studied using various methods by different investigators. The iris of albino rats or mice, two animals commonly used in the laboratory, is thin and membranous, non-pigmented and highly vascularised. The albino rat and mouse iris is then very suitable for vital microscopic examination of the iris vascular pattern and its blood flow behavior (Bensley, 1960; Castenholz, 1965, 1966, 1971). Bensely (1960) developed a method of transilluminating the iris using a quartz rod. Castenholz (1965, 1966, 1971) developed another method for studying the living iris using the principle of the ophthalmoscope. By means of a

series of prisms and mirrors, vertically directed light is reflected off the fundus of the eye so that the outlines of the blood vessels are quite distinctly set off against an illuminated background. With this method the eye is observed without being handled so that, possibly, comparative physiological studies can be made.

Dyes and contrast media (Castenholz, 1965; Saari, 1971b, 1972), fluorescein (Craandijk and Aan d Kerk, 1970; Harris, Toyofuku and Shimmyo, 1972), and Neoprene latex (Wong and Macri, 1964; Saari, 1971b) are sometimes injected into the orbital blood vessels which supply the iris. The passage of the dyes or fluorescein through the iris is visualised by reflected light (Castenholz, 1965) or by fluorescein angiography (Craandijk and Aan de Kerk, 1970; Harris, Toyofuku and Shimmyo, 1972). The velocity of the blood flow in the different vessels of the iris can then be studied under different conditions (Castenholz, 1965; Craandijk and Aan de Kerk, 1970; Castenholz, 1971; Harris, Toyofuku and Shimmyo, 1972). In the case of Neoprene injections of the eye (Wong and Macri, 1964), casts are made and studies are made on the casts.

Saari (1970, 1971a, 1971b, 1972) has developed over the years a flat preparation method for studying the blood vessels of the pig iris. The non-vascular components of the iris are first digested away with trypsin so as to thin out the section of the iris. The pigment granules are bleached in solutions of potassium permanganate and oxalic acid. The preparations are floated onto a slide and stained with PAS-hematoxylin. Blood vessels stain pale purple in contrast to the dark purple myelinated nerves.

From the above studies, there has emerged a pattern of the blood supply to the iris in man (Craandijk and Aan de Kerk, 1970), pig (Saari, 1970, 1971a, 1971b, 1972), cat (Wong and Macri, 1964), rabbit (Harris, Toyofuku and Shimmyo, 1972), and the albino rat (Castenholz, 1965, 1966,

1971). The long posterior ciliary artery and its branches enter the iris to form the major arterial circle situated in the stroma near the iris root. Branches from the major circle course radially through the iris stroma towards the pupillary margin. Capillaries branch off from these radial iris vessels and anastomose to form a fine network in the iris stroma. In man (Hogan, Alvarado and Weddell, 1971), some of the larger branches of the radial iris vessels anastomose at the region of the collarette to form the minor circle. From thence, capillaries course to the sphincter and pupillary margin, where capillary arcades are seen. The paths of the venous channels for the return of blood to the general circulation follow closely those of the arterial vessels. There are some slight species differences from the basic vascular pattern as seen in man. In the albino mouse (Bensley, 1960), there is a large arterial anastomosis midway in the iris. Branches arising from here radiate centrally towards the pupil and peripherally towards the limbus. In the albino rat (Castenholz, 1965, 1966, 1971) and in the rabbit (Harris, Toyofuku and Shimmyo, 1972), there is no minor arterial circle. Among the larger blood vessels, arterio-venous shunts are common. The minor circle is not purely arterial but is formed by arterio-venous anastomoses (Calmette, Lazorthes, Deodati, Bec and Bechac, 1959). In disease, local vascular changes may occur. Highly tortuous vessels are associated with pigmented iris tumors (Craandijk and Aan de Kerk, 1970).

The rate of flow of blood through the iris vessels can be measured by observing the passage of a dye or fluorescein through the vessels. Castenholz (1965) found that 2.3 to 2.8 seconds after the injection of lissamine green into the femoral vein of the rat, dye is seen in the iris root vessels. It takes 5 to 11 seconds for the dye to be drained out of the iris. Harris, Toyofuku and Shimmyo (1972) injected 5% sodium fluorescein into an ear vein of the albino rabbit and observed fluorescence in the

major arterial circle 4 to 5 seconds later.

In the rabbit, iris blood flow is responsive to stimulation of the cervical sympathetic nerve fibers (Cole and Rumble, 1970). Vasoconstriction occurs. The alterations in the blood flow is measured in terms of the changes in the amount of heat that is dissipated into the surrounding aqueous humor. This is done by implanting thermocouples in the anterior chamber just in front of the iris. Thus the iris blood vessels are demonstrated to be adrenergically innervated.

Early light microscopic studies showed that arterioles, venules and capillaries make up most of the blood vessels of the human iris stroma, with capillaries being predominant. In all of these vessels, it is observed that there are tubular tissue spaces surrounding the endothelial channels (Gregersen, 1959b; Lassman, 1964). External to this clear space are circularly oriented collagen fibrils. Thus, the iris vessel is visualised as two tubes separated by a space. Such a unique configuration of the iris blood vessels may be useful for maintaining a constant and unimpaired circulation of blood in the iris despite the extreme movements of the iris tissue in miosis and mydriasis. Imbibition studies show that molecules like Dextran, when introduced into the anterior chamber, find their way into this clear space around the endothelium (Gregersen, 1959b). These tissue spaces are not present in baby eyes but they slowly increase in width during development into adulthood. In adults, variations do occur between individuals with respect to the size and number of the tubular spaces around the iris blood vessels.

Electron microscopic studies (Ikui, Mimatsu, Maeda and Tomita, 1960; Tomita, 1960; Purtscher, 1966; Ringvold, 1969; Tamura, 1969; Ringvold, 1970a; Vegge and Ringvold, 1969) have elucidated the nature of this clear perivascular space seen in light micrographs. It is really a light collag-

enous zone (Hogan, Alvarado and Weddell, 1971). Sparse collagen fibrils, about 300Å in diameter, embedded in a fine granular ground substance, are oriented longitudinally, parallel to the length of the vessels. External to this is a thick layer of collagen fibrils measuring 1000Å in diameter. These fibrils may be circularly (Hogan, Alvarado and Weddell, 1971) or obliquely and longitudinally (Ringvold, 1969) oriented around the vessel lumen. This collagenous layer is visible with the light microscope. In eyes with exfoliation syndrome, changes occur in the light collagenous zone immediately adjacent to the endothelium (Ringvold, 1969; 1970a). In normal eyes, this zone appears clear in toluidine blue stained plastic sections (Ringvold, 1907a). However, in eyes with exfoliation syndrome, a homogenous light blue material is seen. Electron microscopic examination of this blue zone shows exfoliation material in the vessel wall (Ringvold, 1969). It consists of an abnormal amount and type of extracellular material. Membrane-bound round to oval granules make up the non-fibrillar elements. Besides the normal fibrils, fibrils of different thicknesses with occasional cross-banding are found in groups.

A single layer of endothelial cells surrounds the lumen of the iris blood vessels (Ringvold, 1969; Hogan, Alvarado and Weddell, 1971; Smith, 1971; Vegge, 1971, 1972). The endothelium is non-fenestrated in man (Ringvold, 1969; Hogan, Alvarado and Weddell, 1971), monkey (Vegge, 1971b, 1972), rabbit (Harris, Toyofuku and Shimmyo, 1972) and mouse (Smith, 1971). There are conflicting reports on the iris capillaries of the rat. It is non-fenestrated according to some investigators (Saari, 1972) but Castenholz (1971) reports that there are fenestrations in the endothelium. In the cytoplasm are the usual organelles, such as, the nucleus, mitochondria, rough and smooth endoplasmic reticulum, ribosomes and microtubules. In addition, characteristic tubular bodies are found in the endothelial cyto-

plasm (Matsuda and Sugira, 1970). They are rod-shaped, possess an internal structure and are delimited by a unit membrane. Each rod consists of 10 to 30 tubules. They are postulated to be produced by the Golgi complex. The functional significance of these bodies is obscure. Tight junctions are present between adjacent endothelial cells. A relatively thick basement membrane is always present.

Pericytes or typical smooth muscle cells may be present outside the endothelial layer.

In fetal eyes, there is another vascular system associated with that of the iris, that is, the vessels of the pupillary membrane (Matsuo and Smelser, 1971). However, these become non-functional and finally atrophy. When functioning, the blood vessels consist of a layer of endothelial cells enclosing a lumen, a basement membrane external to it and pericytes.

7. Innervation of the Iris

Both myelinated and unmyelinated nerves are seen in the iris stroma. The nerves enter the iris at its root. These nerves arise from the trigeminal, the superior cervical sympathetic and ciliary ganglia (Schaeppi, 1966). There is some indication that there might be ganglion-like cells within the iris tissue itself. Thus not all the ganglia which innervate the eye are found extra-ocularly (Macri, 1971). The fine unmyelinated branches of the trigeminal are sensory and are diffusely distributed throughout the iris stroma. They may also be vasomotor to the iris blood vessels. The sympathetic and parasympathetic nerves are motor nerves and they not only innervate the sphincter and dilator muscles but also the blood vessels (Cole and Rumble, 1970).

The classical concept of the innervation of the iridial musculature is that the sphincter muscle is supplied by parasympathetic cholinergic

nerve fibers, while the dilator muscle is supplied by sympathetic adrenergic nerve fibers. However, this concept of a simple and separate innervation of the iris musculature now has to be modified. There is much evidence to show that cholinergic parasympathetic fibers also supply the dilator muscle cells and that adrenergic sympathetic fibers innervate the sphincter muscle as well. These conclusions on the dual innervation of the iris sphincter and dilator are based on studies employing a variety of techniques, anatomical, physiological and pharmacological. For light microscopic observations, the Falck-Hillarp fluorescence technique is used to demonstrate the cellular localisation of noradrenalin. Acetylcholinesterase is localised by Koelle's or Karnovsky's methods. Methylene blue is not a highly specific stain but is nevertheless used for demonstrating cholinergic nerve fibers. For electron microscopic studies, it is found that with potassium permanganate fixation, adrenergic sympathetic nerve endings can be differentiated from cholinergic parasympathetic nerve endings at the ultrastructural level. All these cytological studies are often coupled with selective denervations either of the sympathetic or parasympathetic. From these anatomical studies, a fairly good idea of the intricacies of the innervation of the iris is obtained. Concomitant physiological and pharmacological studies help to complete the picture.

The adrenergic innervation of the iris has been studied extensively in the rat (Ehinger, 1964; Csillik and Koelle, 1965; Ehinger and Falck, 1965; Eranko and Raisanen, 1965; Malmfors, 1965a, 1965b; Malmfors and Sachs, 1965a, 1965b; Ehinger, 1966b; Ehinger and Falck, 1966; Ehinger, Sporrong and Stenevi, 1967, 1968; Staflova, 1969b; Farnebo and Lidbrink, 1971). The rat iris is thin and has a dense network of adrenergic fibers associated with the dilator. Because of the thinness of the albino rat iris, it can be stretched out flat and mounted whole on a microscope slide and the non-

terminal and terminal axons to be studied can be examined in their entirety. The adrenergic innervation of the iris of numerous other animals, including man, has also been studied (Ehinger, 1964; Malmfors, 1965b; Ehinger, 1966a, 1966b; Laties and Jacobowitz, 1966; Ehinger, 1967; Staflova, 1969a, 1969b; Ehinger and Sjoberg, 1971). The histochemical fluorescence method of Falck and Hillarp is used to visualise the adrenergic transmitter, noradrenalin, at a cellular level. The method is highly specific and sensitive and the chemical bases of the reactions are known. The iris tissue is dried and exposed to an atmosphere of formaldehyde vapor. Noradrenalin, like other catecholamines, forms the highly fluorescent compound, 4, 6, 7-trihydroxy-3, 4,- dihydroisoquinoline, with formaldehyde. This fluorescence, indicating the presence of endogenous noradrenalin in the adrenergic nerves, is then examined with a fluorescence microscope. It appears green to yellow-green and varies in intensity. The methodology is discussed in detail by Malmfors (1965a).

In the rat iris, the adrenergic supply to the dilator is a dense, intermeshing plexus of main, preterminal and terminal axons. The fibers are so dense that it is not possible to follow all of the ramifications of the system of terminals belonging to any one individual adrenergic neuron. However, Malmfors and Sachs (1965a) found that following destruction or extirpation of the superior cervical sympathetic ganglion, the adrenergic ground plexus of the iris is much reduced. Only a few postganglionic fibers remain and are still fluorescent. The other postganglionic fibers lose their noradrenalin contents and cannot be seen with the fluorescence microscope. The morphology and pattern of distribution of the branches of a single postganglionic fiber can then be followed from when it enters the iris to its terminals (Malmfors, 1965a; Malmfors and Sachs, 1965a). The terminals are easily visualised as they are the storage sites of noradrenalin. The

intensity of the fluorescence in the other non-terminal axons can be increased by treating the experimental animals with noradrenalin prior to sacrifice. The main axon is smooth and is only weakly fluorescent in untreated animals. At varying distances from its entry at the iris root, many branches are given off the main axon. These are the preterminal axons which run to all parts of the iris. The preterminal axons are similar in appearance and in the degree of fluorescence as the main axons, except at its distal extremities where a few enlargements may be seen. The preterminal axons branch profusely to give the terminal axons. The terminal axons possess characteristic, strongly fluorescent enlargements or varicosities. A terminal axon may or may not end in a varicosity. Noradrenalin is stored in the terminal varicosities. If an isolated rat iris is stimulated by an electric field, noradrenalin is released and there is a slight decrease in the fluorescence of the slightly less prominent varicosities (Farnebo and Lidbrink, 1971). If noradrenalin synthesis is inhibited prior to sacrifice, the effect is much enhanced. Terminals from more than one neuron run together and converge to innervate the same group of dilator cells.

Adrenergic terminals are not only found in the dilator but also in the sphincter region and in the walls of the small iris blood vessels. In the sphincter, the adrenergic terminals run alongside and in between the muscle cells. Sometimes a single preterminal axon gives rise to one system of terminal axons which end at the dilator and another set of terminal axons which end at the sphincter or blood vessel. The adrenergic innervation to the dilator is excitatory while, in all probability, that to the sphincter is inhibitory. Recently it has been shown that there appears to be functionally different types of sympathetic neurons in one and the same ganglion (Edvinsson, Owman, Rosengren and West, 1972). They may thus selectively innervate the different components of the iris.

Comparative studies on the adrenergic innervation of the iris have been carried out using the Falck-Hillarp fluorescence technique (Ehinger, 1964; Malmfors, 1965a, 1965b; Ehinger, 1966a, 1966b; Laties and Jacobowitz, 1966; Staflova, 1969; Ehinger and Sjoberg, 1971). Such comparative anatomical studies of the adrenergic innervation are useful and necessary when attempts are being made to correlate physiological and pharmacological studies done on one species to another species. From denervation studies it is seen that almost all of the adrenergic innervation to the iris is from the ipsilateral superior cervical ganglion (Malmfors, 1965b; Ehinger, 1966b). Most of the adrenergic terminals go to supply the dilator muscle. In the rat and mouse, the adrenergic terminals are primarily on the anterior surface of the dilator (Malmfors, 1965a; Ehinger, 1966b). Since the iris is so thin in the rat and mouse, it is possible to get an overview of the total adrenergic innervation to the iris. The nerves form arcades (Ehinger, 1966b). In the cat, rabbit, dog and pig, the nerves are not only very dense to the anterior dilator surface (as in the mouse and rat), but branches are seen in between the muscle cells themselves (Malmfors, 1965b; Ehinger, 1966b). The latter configuration is similarly seen in man and the Cynomologus monkey (Ehinger, 1966a). In the rat and guinea pig, several varicose nerve terminals run together in the same strand, whereas in the mouse the terminals are found singly (Malmfors, 1965b; Ehinger, 1966b).

The sphincter muscle always receives an adrenergic supply but to differing degrees according to the species. There are few adrenergic fibers in the rat sphincter. The pig and dog possess more than do the rats. Cats, however, have a very rich adrenergic innervation to the sphincter. This shows up as a very intense fluorescence with the Falck-Hillarp technique (Ehinger, 1964). In the monkey, cat and rabbit, the fibers to the sphincter are always parallel to the long axis of the sphincter cells (Laties and

Jacobowitz, 1966). In pigmented animals, there are more adrenergic fibers to the sphincter than in albinos (Malmfors, 1965b).

In phylogeny, it is seen that primates have overall fewer adrenergic fibers than the lower animals (Staflova, 1969). Man has the least number of all (Ehinger, 1966a). Differences also exist in the rate of degeneration of the adrenergic network within the iris following ganglionectomy. In the rabbit it only takes four to five days, whereas in the monkey it might be as long as twenty days (Staflova, 1969).

In developmental studies on man and on the guinea pig (Ehinger and Sjoberg, 1971) it is observed that in young eyes the nerves have a smooth appearance. The varicosities on the nerve terminals develop in time. The nerves do not have any inductive effect on the development of the dilator and sphincter muscles as these develop before the nerves appear on the scene. The adrenergic nerves do not enter the eye together with the blood vessels. Innervation to the blood vessels develops later.

Noradrenalin is the adrenergic transmitter at the nerve terminals. Its presence is detected by means of the Falck-Hillarp fluorescence technique. Acetylcholine is the cholinergic transmitter but there are no histochemical methods to directly stain for acetylcholine. Rather, the enzyme which breaks down the transmitter, acetylcholinesterase, is stained by the thiocholine method of Koelle (1955). A combination of the Falck-Hillarp fluorescence technique for the localisation of noradrenalin and the Koelle technique for the histochemical localisation of acetylcholinesterase has been used to study the adrenergic and cholinergic network of nerves in the cat (Ehinger, 1967) and rat (Csillik and Koelle, 1965; Eranko and Raisanen, 1965; Ehinger and Falck, 1965, 1966) iris. Pairs of micrographs from acetylcholinesterase staining and from fluorescence microscopy are superimposed on each other so as to be better able to compare the networks of

nerves demonstrated by the two methods (Eranko and Raisanen, 1965). It is found that the pattern of acetylcholinesterase staining is generally similar to the pattern of fluorescence, although there is a much greater density of acetylcholinesterase towards and in the sphincter region (Csillik and Koelle, 1965; Ehinger and Falck, 1966; Ehinger, 1967). Both noradrenalin and acetylcholinesterase are contained within the terminal varicosities (Csillik and Koelle, 1965). From these studies it is observed that there are three types of nerves (Eranko and Raisanen, 1965; Ehinger and Falck, 1965, 1966). Many fibers show both fluorescence and histochemical staining for acetylcholinesterase. Some fibers are fluorescent but show no acetylcholinesterase activity, while others show acetylcholinesterase activity but are not fluorescent. The question then arises as to whether some nerve fibers are both cholinergic and adrenergic at one and the same time, or whether the fibers that show both acetylcholinesterase activity and fluorescence at the light microscope level are in reality closely associated but distinctly separate fibers. The former view is suggested by the work of Eranko and Harkonen (1964) using fluorescence microscopy and acetylcholinesterase staining on the superior cervical ganglion cells. It is shown that there are some ganglion cells which show both fluorescence and also much acetylcholinesterase activity. These neurons may then issue forth nerve fibers with dual characteristics. This would support the theory of Burn and Rand (1962, 1965) that there is a cholinergic component in the adrenergic transmission of a nerve impulse. It is postulated that the release of noradrenalin is initiated or triggered by the release of acetylcholine. The latter view that adrenergic and cholinergic fibers only accompany each other very closely is favored (Ehinger, 1967). This is based on electron microscopical studies of the nerve endings themselves, as will be discussed later, and on bioassays for the acetylcholine content in the iris following selective

denervation (Ehinger, Falck, Persson, Rosengren and Sporrong, 1970).

Essentially the same pattern of innervation is obtained using a combination of the Falck-Hillarp fluorescence technique and staining with methylene blue (Ehinger, Sporrong and Stenevi, 1967, 1968; Ehinger, 1971; Takkunen, 1971). Methylene blue is an axon stain but its reliability and selectivity is sometimes questionable (Richardson, 1969; Takkunen, 1971). Although both adrenergic and cholinergic nerves take up the stain, there is enough difference to distinguish between the two at the light microscopic level.

From these light microscopic observations, it is seen that both the sphincter and dilator muscles are dually innervated. The sphincter muscle receives predominantly a cholinergic nerve supply although there is a substantial degree of adrenergic innervation as well. The reverse is true of the dilator.

The nerve terminals have also been examined with the electron microscope, which has a much greater magnification range and resolving power than the light microscope, to see if there are any ultrastructural differences between the adrenergic and cholinergic nerve terminals (Richardson, 1964; Nilsson, 1964; Hokfelt, 1966; Richardson, 1966; Hokfelt, 1967; Richardson, 1968, 1969; Ochi, Konishi, Yoshikawa and Sano, 1968; Geltzer, 1969; Matsuda, 1969; Nishida and Sears, 1969a, 1969b; Roth and Richardson, 1969; Ehinger, Falck and Sporrong, 1970; Nishida and Sears, 1970). The axon terminals may come into very close contact with the effector cells, namely, the dilator and sphincter, leaving only a gap of 170-220Å (Nishida and Sears, 1969b), or the plasma membranes of the muscle and the axon terminal may be fused (Nilsson, 1964). Nerve terminals appear as enlargements or varicosities containing mitochondria and different types of vesicles. The cholinergic and adrenergic nerve terminals can be differentiated from each other by

their populations of vesicles. Early studies on the rat iris revealed that there were granular and agranular vesicles in the nerve terminals. But they were not correlatable as to the nature of the terminals, either sympathetic or parasympathetic (Nilsson, 1964). With the technique used, there are no ultrastructural differences between adrenergic and cholinergic nerve terminals. But in 1966, Richardson found that fixation of the iris in potassium permanganate, instead of in glutaraldehyde or in osmium, produces consistent results in terms of the vesicular populations of the adrenergic and cholinergic nerve terminals. There are two types of vesicles. Adrenergic terminals contain predominantly 400-500Å vesicles with dense cores (granular vesicles). Cholinergic terminals contain small agranular vesicles. Noradrenalin is stored in the form of dense granules in the membrane-bound vesicles.

With the light microscope, it is seen that methylene blue is an axon stain relatively specific for cholinergic nerves (Ehinger, Sporrong and Stenevi, 1967, 1968; Ehinger, 1971; Takkunen, 1971). With the electron microscope, methylene blue is seen to be not highly specific for cholinergic axons except under well-controlled conditions which are pH dependent (Richardson, 1969). The methylene blue is toxic to cholinergic terminals and ultrastructural changes occur (Richardson, 1968, 1969). The axon may or may not swell and be deformed. Most often the varicosities are swollen and vacuolated. Scattered membrane densities are present. These swollen varicosities contain a population of agranular vesicles, thus distinguishing them as cholinergic terminals.

The adrenergic and cholinergic nerve terminals run together in bundles. They are in close association but they are separate entities. At times, there are cellular axo-axonal contacts between two types of terminals (Ochi, Konishi, Yoshikawa and Sano, 1968; Ehinger, Falck and Sporrong, 1970).

Thus, it is possible for the cholinergic and adrenergic nerves to mutually influence each other.

From both light and electron microscopic studies, it is evident that the sphincter (Richardson, 1964; Hokfelt and Nilsson, 1965; Ochi, Konishi, Yoshikawa and Sano, 1968; Geltzer, 1969; Hirano, 1969; Nishida and Sears, 1969b) and dilator (Nilsson, 1964; Richardson, 1964; Geltzer, 1969; Hirano, 1969; Nishida and Sears, 1969a, 1970) are dually innervated. The extent of dual innervation varies with the species, with either the adrenergic or cholinergic predominating. One system predominates while the other modulates (Ehinger, 1971). Early physiological studies had shown that this dual innervation is present. Joseph (1921) produced a relaxation of the sphincter of cats and dogs by stimulating the cervical sympathetic. Thus the sphincter has an excitatory parasympathetic nerve supply from the occulomotor via the ciliary ganglion, as well as an inhibitory sympathetic nerve supply from the cervical ganglion. Neuropharmacological studies show that there are two types of adrenergic receptors, α and β . Activation of the α receptors results in muscle contraction while activation of the β receptors results in muscle relaxation. Whether it is the α or the β receptors which are activated depends on the type of stimulation applied (Schaeppi and Koella, 1964a, 1964b). Both the sphincter and dilator are dually innervated, by cholinergic and adrenergic (α and β) nerve fibers. In pupillary constriction or dilation both systems work in harmony rather than as two discrete entities (Apter, 1956). During pupillary constriction, cholinergic parasympathetic nerve fibers stimulate the sphincter muscle cells to contract while at the same time inhibiting the action of the dilator muscle cells, thus resulting in the relaxation of the dilator (Ehinger, Falck and Persson, 1968). During pupillary dilation, the adrenergic sympathetic nerve fibers cause dilator muscle contraction while

the terminals cause sphincter muscle relaxation.

The dilator and the sphincter muscles are not the only components of the iris that are innervated. As mentioned previously, the iris blood vessels are also innervated almost exclusively by the sympathetic system (Cole and Rumble, 1970). Melanophores of rats (Ehinger and Falck, 1970), skates (Kuchnow and Martin, 1972), monkey and man (Ehinger, 1966a) also possess a nerve supply. In the monkey and man some adrenergic fibers come very close to the melanophores but not to the clump cells. In rats, melanophores appear to have both a cholinergic and an adrenergic nerve supply (Ehinger and Falck, 1970). The functional significance of a nerve supply to the pigmented cells of higher animals is not readily perceptible. In lower vertebrates which can change their coloration, such a nerve supply would be useful.

8. Miscellaneous Studies on the Iris

Some enzyme histochemical studies have been carried out on the iris, spurred on by the idea that perhaps the iris does not merely act as a shutter but may also be involved in other metabolic activities, for example, a contribution towards the production of aqueous humor (Berkow and Patz, 1964; Lessell and Kuwabara, 1964; Hvidberg-Hansen, 1971a). Numerous oxidative enzymes (Berkow and Patz, 1964; Hvidberg-Hansen, 1971a), alkaline and acid phosphatase (Lessell and Kuwabara, 1964; Hvidberg-Hansen, 1971a) and specific phosphatases (Lessell and Kuwabara, 1964) are localised to the iris and are indicative of the metabolic potential of the iris. The histochemical pattern of the distribution of many of the dehydrogenases are essentially similar (Berkow and Patz, 1964). The reaction precipitate is denser in the posterior epithelium than in the stroma both in the adult and in the developing eyes. This enzymatic activity is detectable at 17 days fetal

and reaches the adult pattern by 10 days post-natal (Berkow and Patz, 1964). In the iridic epithelium as a whole, there are some slight differences in the distribution of the dehydrogenases in terms of the intensity if the reaction (Hvidberg-Hansen, 1971a). In numerous vertebrate species, both alkaline and acid phosphatases are present in the iris epithelium (Lessell and Kuwabara, 1964). Hvidberg-Hansen (1971a) demonstrated that in the rabbit iris epithelium there are regional differences between the localisation of alkaline and acid phosphatase. This is not shown by Lessell and Kuwabara (1964). On the basis of alkaline and acid phosphatase staining, the rabbit iris epithelium can be divided into two zones, a central zone concentric to the pupillary margin and of about the same extent as the sphincter, and a peripheral zone. The central zone is rich in both alkaline and acid phosphatases. Ultrastructurally, these enzymes are localised to the membranes of the numerous pinocytotic vesicles associated with the lateral interdigitations of the iridic epithelium. The peripheral zone has a lesser amount of both phosphatases. Acid phosphatase is found in the lysosomes. The iris epithelium also shows intense adenosine triphosphatase activity (Lessell and Kuwabara, 1964). Thus the iris epithelium appears to be a highly metabolic epithelial layer.

The iris is also very active in lipid synthesis (Culp, Cunningham, Tucker, Jeter and Deiterman, 1970). If C^{14} sodium acetate is administered into the anterior chamber of the rabbit eye, it is taken up by the iris tissue and incorporated into fatty acids. These lipogenic pathways may be important as a source of metabolic energy (for the sphincter and dilator muscles, perhaps), or for replenishing membrane constituents that are lost as a result of the wear and tear that might occur during the incessant movements of the iris.

In lower vertebrates, Wolffian lens regneration occurs, that is, if

the lens is extirpated certain epithelial cells from the iris are able to be transformed in a series of steps into lens cells. This is seen in the adult newt (Dumont and Yamada, 1972). This regenerative capacity of the iris epithelium is lost as one ascends the evolutionary scale. However, the iris and the lens may still be similar in some ways, for example, in its immunological properties. By using various immunochemical methods, Brahma, Bours and van Doorenmaalen (1971) show that there are certain proteins (antigens) in the chick iris which are immunologically similar to the antigens extracted from the lens. The chick iris epithelium antigens, present in small but detectable amounts, have antigenic properties of lens α-crystallin.

Another aspect of iris research concerns the dynamics of pupillary movements (Mapstone, 1970; Loewenfeld and Newsome, 1971; Tucker, 1971; Davanger, 1972). As is well known, changes in pupillary size occur in response to changing light conditions, certain emotional and psychological phenomena and to direct or indirect stimulation of the associated ganglia and nerves (Mapstone, 1970; Pierau, Alexandridis, Spaan, Oksche and Klussmann, 1970; Borthne and Davanger, 1971; Davanger, 1971; Loewenfeld and Newsome, 1971). The resting size of the pupil is dependent on the age of the individual and on the color of the iris. Senile atrophic changes occur in the iris stroma. The sphincter and dilator muscles lose their tonus with age. Thus with increasing age, there is a concomitant decrease in the resting pupil size (Borthne and Davanger, 1971). The average resting pupil size of dark irises is also smaller than that of light, blue irises. This may be related to the density of the stroma.

Mydriatics and miotics, that is, drugs used for dilating or constricting the pupils respectively are often used in experimental conditions to study the mechanics of iris movements. When cyclopentolate or phenyle-

phrine, two mydriatics, are applied to the eyes, it is seen that the pupils dilate at a speed which is proportional to the difference between the resting and maximum pupillary size (Davanger, 1971). However, the effects of the mydriatics are not identical. Pupillary dilation occurs quicker with cyclopentolate than with phenylephrine (Borthne and Davanger, 1971). Also cyclopentolate results in greater dilation than does phenylephrine in young people, but the reverse is the case with old people. Such age differences in drug effects is probably related to senile changes that occur in the iris tissue.

The pupil has a linear range of movement in response to bright light or dim light conditions (Loewenfeld and Newsome, 1971). When a bright light is shone into the eye, the pupil constricts very promptly at a certain velocity. However, at a particular pupil size, which is not the limit of constriction, the rate of pupillary constriction slows down. The diameter of the pupil at which this happens is fixed for each individual but varies from individual to individual. In pupillary dilation, the same phenomenon is observed, that is, the pupil would dilate linearly until a certain pupillary diameter is attained. Then the dilation process slows down in velocity and in extent. There seems to be a mechanical barrier at a particular pupil diameter which slows down pupillary dilation or constriction.

Mathematical attempts are made to analyse the forces which determine pupil size (Mapstone, 1970; Davanger, 1972). According to Mapstone (1970), the forces due to the pull of the sphincter and dilator muscles determine the pupil size. The sum of these forces is greatest when the pupil is constricted but diminishes when the pupil dilates. The pull of the sphincter at its maximum is twice that of the dilator. Davanger (1972) however, starts with a different set of assumptions. The size of the pupil is determined by the tension of the sphincter and dilator muscles. The result-

ant constricting force of the sphincter is centripetally directed and is equal to S/r, where S is the total force of the sphincter and r is the radius of the pupil. On the other hand, the resultant dilating force of the dilator is centrifugally directed and is equal to $1/2\,\pi \cdot D/r$, where D is the total force of the dilator and r is the radius of the pupil. At equilibrium, that is, when the pupil is at rest, the constricting and dilating forces must be equal, so that $S/r = D/2\,r$. The pupil can be at equilibrium at any pupil size, but the force of the dilator will always inherently be $2\pi(6.28)$ times the force of the sphincter. This is also exemplified if there is a small change in the radius of the pupil, Δr. The change in the dilator force is $D \times \Delta r$, while the change in the constrictor force is $S \times 2\pi\Delta r$. These changes balance each other at equilibrium so that again $D = 2\pi S$. So if the force of either the dilator or sphincter is changed, the pupil size is adjusted until equilibrium is reached, that is, when the force due to the dilator is 2π that of the sphincter.

According to current thinking on pupillary movements, pupillary constriction is a result of the contraction of the sphincter muscle and relaxation of the dilator muscle, while the opposite is true during pupillary dilation. The active contraction of one muscle is accompanied by a concomitant relaxation of the other muscle. There appears to be much evidence to support this point of view. However, this view is not shared by all (Tucker, 1971). It is felt that the muscles do not act reciprocally. Rather, when all the muscles contract, the iris shortens and results in pupillary dilation. When the muscles relax, fluids from the aqueous humor rush to occupy the tissue spaces in the iris stroma, thus, expanding the iris and constricting the pupil. Here the sphincter and dilator muscles only act as stabilisers, respectively, of the pupillary margin and the iris as a whole. Such a view on pupillary dilation and constriction, however,

does not take into account the results obtained from many selective denervation, physiological and pharmacological studies.

G. Thesis Proposal

1. The histology of the iris in man as well as in other vertebrates has been studied over the years. However, in all the investigations, except for one (Alphen, 1963), the histology of the iris is described as if it were a static rather than a dynamic structure. No attempts have been made to compare the structure of the iris during different degrees of pupillary dilation and constriction. The iris is capable of extensive excursions both in response to changing light conditions and in response to miotics and mydriatics. The changes in the overall size of the iris control the changes in the size of the pupil. These changes in pupillary diameter are highly precise and occur very rapidly. During extreme pupillary constriction, the pupil appears only as a mere pin-hole. On the other hand, during extreme pupillary dilation, the pupil is enlarged many times over. The iris appears as a thin, barely visible rim of tissue at the edge of the cornea. Obviously, this must entail structural changes within the iris tissue to accommodate for the great changes in total surface area from pupillary constriction to pupillary dilation. Also, the iris architecture must be structurally adapted to facilitate these movements so that they occur as smoothly and as efficiently as possible. Thus, the first major part of the present investigation deals with the alterations in iris structure during the extremes of pupillary size which occur in dilation and constriction. The changes observed are pharmacologically induced.

The rat, a common laboratory experimental animal, is used in this study. It has a typical mammalian iris with a round pupil. The basic histological components of the iris is well known. The rat iris consists

of a posterior epithelial layer and a modified anterior epithelial layer. In the adult rat, the anterior epithelium has differentiated into two contractile masses, the sphincter muscle and the dilator muscle, which are readily detectable at the light and electron microscopic levels. Anterior to both the posterior epithelium and the dilator is the stroma consisting of blood vessels of varying sizes, myelinated and unmyelinated nerves of the sensory, sympathetic and parasympathetic systems, and numerous stromal cells which are all enmeshed within a connective tissue framework and a mucopolysaccharide ground substance. With the aid of drugs, the rat iris is maintained in pupillary dilation and constriction. The rat iris tissue, in the dilated or constricted state, is examined with (a) the light microscope, (b) the transmission electron microscope, and (c) the scanning electron microscope. Interest is focussed on:

(a) what are the structural changes that are observed in the iris when the pupil is constricted and when the pupil is dilated,

(b) what structural features of the various components of the iris would facilitate, or at least not hinder, the excursions of the iris, and

(c) what new information can be gleaned from scanning electron microscopic studies on the posterior and anterior surfaces of the rat iris.

(a) Changes in the shape and orientation of the posterior epithelium, the dilator and the stromal elements are examined with the light microscope on toluidine blue stained plastic sections which have been prepared by the conventional methods for transmission electron microscopy. The relationships of the stromal elements to the epithelium and dilator are examined.

It is known that there is a collagenous connective tissue framework in the iris stroma, although it is by no means abundant (Tousimis and Fine, 1959b). The overall organisation of both the cellular and intercellular

(collagen) components in the iris stroma during pupillary dilation and constriction are observed. Particular emphasis is placed on the relationships of the collagen network to the stromal cells during these rapid changes in pupillary size. With ultrathin sections, the overall organisation of the collagen network is not readily apparent since collagen is not abundantly present. Thus, parafin sections (7-10μ) are specifically stained for collagen using a modified Mallory's Trichome Stain to reveal the general orientation of the collagen during miosis and mydriasis.

(b) The investigation is carried one step further. The rat iris, either dilated or constricted, is examined with the transmission electron microscope to see what ultrastructural changes, if any, occur, especially in the posterior epithelium and the dilator. The intimate relationships of the collagen to the surrounding cells is noted. Kelly and Arnold (1972) have studied the sphincter muscle of the rat iris in the constricted and dilated state with the transmission electron microscope. However, their main interest is the demonstration of both thin and thick filaments within the sphincter muscle, which, according to them, is dependent on the means and mode of fixation used.

(c) In recent years, a new tool has been introduced for the study of biological materials, the scanning electron microscope. A recent review article deals with the potential usefulness of the scanning electron microscope for biological research (Hollenberg and Erikson, 1973). The main advantage of the scanning electron microscope lies in its great depth of focus so that clear three dimensional pictures of the surface features of cells and tissues can be obtained. The basic principle for the construction and operation of the scanning electron microscope has been discussed in numerous investigations (Nixon, 1971; Oatley, Nixon and Pease, 1965). The scanning electron microscope also uses an electron gun, as does the trans-

mission electron microscope, which sends out a beam of electrons towards the specimen. However, the electrons do not pass through an ultrathin section but strike the surface of the specimen which has been previously coated with a metal. Secondary electrons are emitted from the specimen surface and are amplified and displayed on a cathode ray screen.

During pupillary dilation and constriction, changes must inevitably occur on the anterior and posterior surfaces of the iris. To reconstruct the surface configurations of the iris in these two pupillary conditions would entail an inordinate number of serial sections. Using the scanning electron microscope drastically reduces the labor involved, gives us a view of the iris surface in its totality and in much greater detail. New information is obtained on the anterior and posterior surface structures of the iris. The ultrastructure of the rat iris surface has been examined with the scanning electron microscope (Hansson, 1970). The tissues were frozen in isopentane-propane and freeze-dried. However, no attempts were made to compare the ultrastructural images obtained in pupillary dilation and constriction. These studies are presented here. Both the camphene method (Watters and Buck, 1971) and the critical point drying method (Boyde and Wood, 1969; Smith and Finke, 1972) are used for preparing the iris tissues for examination with the scanning electron microscope. The specimens are coated with a thin layer of gold.

2. Structural alterations of the rat iris are not only associated with its function but also with its growth and development. This is the concern of the second major part of this investigation.

Embryological studies show that the development of any tissue or organ is a composite of processes, cell division, cell differentiation and often cell rearrangements. The relationships of cells to each other alter

in the process of growth and development. Developmentally, the iris is unique in one aspect. In the iris, as in many tissues, both mesodermal and ectodermal components come together and are closely inter-related. In the iris, the mesoderm gives rise to the stromal elements. However, the posterior epithelium, the dilator muscle and the sphincter muscle are neuroectodermal derivatives. The posterior epithelium retains the epithelial character of the neuroectoderm. The anterior neuroectodermal layer differentiates in an unusual direction and gives rise to a myoepithelium, the dilator, and to smooth muscle, the sphincter. Over the years, there has been much controversy over the embryological origin of the sphincter, as to whether it is derived from mesoderm, as is the usual case with smooth muscle, or whether it is indeed derived from neuroectoderm. Reports on the development of the rat iris observed with the transmission electron microscope have recently appeared (Imaizumi and Kuwabara, 1971; Lai, 1972a, 1972b; Tamura and Smelser, 1973). In these papers special emphasis is placed on the development of the sphincter and dilator muscles. Their neuroectodermal rather than mesodermal origin is affirmed. Information, however, is scarce on the overall development of the iris, that is, the changes that take place with respect to the histology of the various components of the iris and their relationships to each other. Such developmental studies would be useful when attempting to correlate the development of both the structure and function of the iris. The second part of this study deals with the following aspects of development of the iris in terms of its histology and function:

(a) To observe, with the light microscope on toluidine blue stained plastic sections, the structural alterations taking place in the iris as it develops from the rim of the optic cup in fetal stages to the adult form.

(b) To observe the changes in the configuration and arrangement of

the posterior epithelial cells of the developing rat iris, and the architecture of the peri-natal vascular system and its regression with development, using the scanning electron microscope.

(c) To observe, with the light microscope, the changes in the permeability of the iris capillaries to an intravenously injected tracer substance, Horse-radish Peroxidase (HRP), with development. These tracer studies would also give some indication as to the role of the iris vascular system in the production of aqueous humor. In adult eyes, aqueous humor is produced in the ciliary body. The path of the aqueous humor can be followed by an intravenous injection of a tracer substance. It is found that in the Vervet monkey (Vegge, 1971a) and in the mouse (Smith, 1971), the capillaries of the ciliary processes are readily permeable to the tracer HRP. The ciliary capillaries are fenestrated (Pappas and Smelser, 1961; Smith, 1971; Vegge, 1971a). However, the iris capillaries are non-fenestrated and present a barrier to the HRP (Smith, 1971; Vegge, 1971b, 1972). The HRP is only seen within the lumens of the iris vessels but not external to the endothelium. In the rat iris, there are no known HRP tracer studies. There are also conflicting reports as to the nature of the rat iris capillaries, whether they be fenestrated or non-fenestrated (Saari, 1972). In the present study, HRP is injected intravenously into fetal, young post-natal and adult rats and the localisation of the reaction precipitate is observed on toluidine blue stained plastic sections.

II. MATERIALS AND METHODS

A. A Light Microscopic Study of the Rat Iris in Pupillary Dilation and Constriction

Adult Wistar rats were used. Their pupils were dilated with a few drops of a mixture of 5% phenylephrine hydrochloride and 0.5% cyclopentolate, or constricted with a few drops of 0.125% echothiophate iodide. The rats were anesthetised with ether and the eyes were removed immediately. A small meridional slit was made in the eyes extending from the cornea anteriorly to the ora serrata posteriorly. This was done to facilitate the inflow of fixative into the interior of the eye to ensure rapid fixation. The eyes were fixed in 4% glutaraldehyde buffered with 0.1M sodium cacodylate at pH 7.2, or in formalin, held at room temperature. After 30 minutes in the respective fixatives, the eyes were cut in half, the lenses were removed and fixation was continued for a total of 4 hours in glutaraldehyde or 5 hours in formalin. The tissues were rinsed with and stored in 0.1M sodium cacodylate buffer at $4^{o}C$. The tissues were then processed as follows: (1) for demonstrating the overall histological characteristics of the iris in pupillary dilation and constriction, and (2) for demonstrating the collagen network in the stroma of the rat iris in pupillary dilation and constriction.

1. For Demonstrating the Overall Histology of the Iris

The anterior halves of the eyes were trimmed into smaller pieces while in the cacodylate buffer wash. The tissues were post-fixed in 1% osmium tetroxide in 0.1M cacodylate buffer for 1 hour, stained *en bloc* in uranyl acetate for 1 hour, dehydrated through a graded series of alcohols, infiltrated and embedded in epon-araldite. Thick (about 1μ) epon-araldite

sections were cut on a Porter-Blum MT-2 microtome and stained with toluidine blue. The sections were examined with and light micrographs were taken on a Zeiss Photomicroscope II.

2. For Demonstrating the Collagen Network in the Iris Stroma

The glutaraldehyde- or formalin-fixed tissues were dehydrated through a graded series of alcohols, cleared in chloroform and embedded in parafin. 7-10μ sections were stained with a modified Mallory's Trichrome Stain (Culling, 1963). The collagen network within the stroma was examined.

B. A Transmission Electron Microscopic Study of the Rat Iris in Pupillary Dilation and Constriction

Adult albino rats of the Wistar strain were used. Their pupils were dilated or constricted with a few drops of a mixture of 5% phenylephrine hydrochloride and 0.5% cyclopentolate, 0.125% echothiophate iodide, respectively. Various methods were tried to obtain good preservation of the tissues for transmission electron microscopy.

1. The rats were anesthetised with ether and the eyes were removed immediately. A small meridional slit was made extending from the cornea anteriorly to the ora serrata posteriorly and into the vitreous humor. The eyes were immersed in the fixatives within 30 seconds from the moment that they were enucleated. The following fixatives were used:

(a) 4% glutaraldehyde in 0.1M cacodylate buffer (Sabatini, Bensch and Barnnett, 1963).

(b) Full strength Karnovsky's fixative (Karnovsky, 1965).

(c) Karnovsky's fixative diluted to 50% with 0.1M cacodylate buffer (Graham and Karnovsky, 1966).

(d) Millonig's fixative (Millonig, 1961).

After about 30 minutes in the respective fixatives, the eyes were cut in half, the lenses were removed and fixation was continued for a total of 4 hours at room temperature.

2. The rats were anesthetised with ether. A slit was made in the cornea and half strength Karnovsky's fixative was dripped on to the eye *in situ* for 5 minutes. Then the eye was rapidly removed, immersed in the fixative and treated as in (1).

3. The rats were anesthetised by an intraperitoneal injection of sodium pentabarbitol and a subcutaneous injection of sodium phenobarbitone. The left common carotid artery was exposed and cannulated. The cannula was connected to a 20 ml syringe containing one of the following fixatives:

(a) 4% glutaraldehyde in 0.1M cacodylate buffer.

(b) 4% glutaraldehyde in 0.2M cacodylate buffer.

(c) Full strength Karnovsky's fixative.

(d) 1:1 Karnovsky's fixative - diluted with 0.1M cacodylate buffer.

The inferior vena cava was also exposed. The fixative was perfused through the carotid artery at a pressure of 100mm Hg. As soon as perfusion started, the inferior vena cava was cut just below the renal vessels for drainage to take place. The perfused eyes were then immersed in fixative and treated as in (1).

The iris tissues were stored in the respective buffers at $4^{o}C$. The anterior portions of the eyes, with the attached iris, were trimmed into smaller pieces while in the buffer wash. The tissues were post-fixed for 1 hour in 1% osmium tetroxide in 0.1M cacodylate buffer, stained *en bloc* in uranyl acetate for 1 hour, dehydrated through a graded series of alcohols, infiltrated and embedded in epon araldite. Thin sections of well-preserved tissues, as determined light microscopically on thick sections, were cut on a Porter-Blum MT-2 microtome. The sections were stained with lead citrate

for 2 to 2½ minutes and examined on a Philips EM 200 operating at 60kV.

C. A Scanning Electron Microscopic Study of the Rat Iris in Pupillary Dilation and Constriction

METHOD:

Adult rats of the Wistar strain were used in these studies. Their pupils were dilated with a few drops of a mixture of 5% phenylephrine hydrochloride and 0.5% cyclopentolate, or constricted with a few drops of 0.125% echothiophate iodide. These drugs were allowed to act for 15 to 20 minutes at which time the pupils were widely dilated or much constricted, as the case may be. The rats were etherised and the eyes were removed immediately and immersed into the fixative of 4% glutaraldehyde in 0.1M cacodylate buffer at pH 7.2 at room temperature. An antero-posterior slit was made through the corneal-scleral junction extending from the anterior and posterior chambers into the vitreous humor to facilitate the penetration of the fixative into the iris tissue. This step was very critical when dealing with eyes in pupillary constriction. Almost as soon as the eye is detached from the rat, "death dilation" occurs. This seems to be somewhat arrested if fixation is almost instantaneous. After about 30 minutes in the fixative, the posterior halves of the eyes and the lenses were removed and discarded. The eyes were fixed for a total of 4 hours, washed with and stored in 0.1M cacodylate buffer at pH 7.2 at 4^{o}C.

The tissues were prepared for examination on the scanning electron microscope in the following way:

1. Camphene Method (Watters and Beck, 1971)

From the buffer the tissues were transferred through a graded alcohol series to be dehydrated. While in absolute alcohol, the tissues were cut into pieces suitable for examination on the scanning electron

microscope. In order to view the anterior surface of the iris, the cornea was cut away as close to the corneal-scleral junction as possible. It was found that it was better to remove the cornea while the tissue was in absolute alcohol rather than when it was in buffer. The iris, being very delicate, was slightly stiffer at this point and therefore held its shape much better even though the cornea was absent. From absolute alcohol, the tissues were transferred into propylene oxide. They were then infiltrated with a mixture of equal volumes of propylene oxide and camphene for ½ hour, followed by another ½ hour in pure camphene. Both the propylene oxide-camphene mixture and camphene were kept in a water bath at 45°C to prevent recrystallisation from occurring. The tissues were removed from the camphene, mounted and oriented on tissue stubs and left in the vacuum evaporator (Edwards) overnight to evaporate the camphene from the tissues. These were then coated with a thin layer of gold.

2. Critical Point Drying Method (Boyde and Wood, 1969; Smith and Finke, 1972)

The eyes, stored in 0.1M cacodylate buffer, were either post-fixed in 1% osmium tetroxide in 0.1M cacodylate buffer at pH 7.2 for 1 hour and then dehydrated, or they were dehydrated immediately without previous post-fixation in osmium. Dehydration was done in a graded alcohol series. The tissues not treated with osmium were appropriately trimmed down while in absolute alcohol, whereas those tissues to be post-fixed in osmium were trimmed down while in the buffer wash. The latter would be too brittle after osmium tetroxide fixation. Thus any manipulation might be liable to cause mechanical damage to the tissues. The absolute alcohol was then substituted by iso-amyl acetate (Fisher) in a stepwise manner. The iso-amyl acetate is a polar solvent miscible with carbon dioxide and it is

also a protective agent against ice crystal formation during the critical point drying process. Carbon dioxide was used as the transitional fluid in our Critical Point Drying Apparatus (Parr). When the tissues were dried, they were mounted on tissue stubs, and coated with gold in a vacuum evaporator.

All the specimens were examined on a Cambridge Stereoscan (Model S4) microscope. Scanning electron micrographs were taken on Agfa Isopan ISS film.

D. A Light Microscopic Study of the Development of the Rat Iris Using Toluidine Blue Stained Epon Sections and Horse-radish Peroxidase (HRP) Studies of the Iris in Fetal, Post-natal and Adult Rats

METHOD:

Fetal and post-natal rats of different ages, as well as adult rats, of the Wistar strain, were used in these studies.

1. Fetal Rats

The ages of the fetal rats were determined by the method of Christie (1964). Pregnant female rats were anesthetised by an intraperitoneal injection of sodium pentobarbitol (0.4-0.7 ml of a solution of 200 mg sodium pentabarbitol dissolved in 6 ml saline) and a subcutaneous injection of sodium phenobarbitone (0.3-0.6 ml of a solution of 240 mg sodium phenobarbitone dissolved in 12 ml saline). Small doses of sodium phenobarbitone, given subcutaneously, were administered during the course of the experiment whenever warranted, that is, when the female rat became a little sensitive

to manipulations within the abdominal cavity. The rat was held down firmly onto a dissecting board. As small as possible a slit was made to expose the abdominal cavity. Each fetus was exposed individually beginning with the fetus at the very tip of the uterine horns. Only that part of the uterus pertaining to the fetus being used was cut open to reveal the fetus still held within its own amniotic sac and attached to its placenta. The rest of the uterus containing the other fetuses was kept within the abdominal cavity at all times. Whenever possible the amniotic sac was left intact during the experiment. In several instances the umbilical vessels were too small and it was technically very difficult to infuse the Horseradish Peroxidate (HRP) into the umbilical veins without breaking the amniotic sac. Small amounts of HRP dissolved in saline (Table 1) were injected into the fetuses via the umbilical vein which was in general recognisably larger and lighter in color than the umbilical artery. After the HRP injection, the fetus was put back into the abdominal cavity to keep warm. The incision in the abdominal cavity was closed with a hemostat. This method was found to be very effective. The fetuses felt warm to the touch and looked pink and healthy. The fetuses were sacrificed by decapitation at various times from 1 minute to 45 minutes after injection of the HRP. The eyes were removed and fixed whole in 4% glutaraldehyde in 0.1M cacodylate buffer at pH 7.2 held at room temperature. After ½ to 1 hour in the fixative, the eyes were cut in half, the lenses were removed and fixation was continued for a total of 4 hours at room temperature. The eyes were washed with and stored in 0.1M cacodylate buffer at pH 7.2 at 4^{o}C.

2. Post-natal and Adult Rats

Rats, aged 1 to 10 days after birth, were anesthetised by being

placed in the freezer for 10 minutes. This was sufficient to lower their metabolism to such a low level that the rats were easily manageable. The rats were placed on a bed of ice cubes during injection of the HRP. Various doses of HRP (Table 1) were injected into the most readily accessible vein, the retro-orbital, jugular and saphenous veins. These post-natal rats were easily aroused from anesthesia by warming them up in-between the palms of the hands. At various times from 1 to 45 minutes after injection of HRP the rats were sacrificed by decapitation and the eyes removed.

Rats older than 10 days after birth and adult rats were lightly anesthetised with ether. HRP was injected into the saphenous veins and allowed to circulate in the body from 5 to 45 minutes. The rats were re-anesthetised and the eyes were removed.

In all adult and post-natal rat eyes, an antero-posterior slit was made through the ora serrata. The eyes were fixed by immersion in a solution of 4% glutaraldehyde in 0.1M cacodylate buffer at pH 7.2 for ½ to 1 hour. Then the eyes were cut into smaller pieces and fixation continued for a total of 4 hours at room temperature. The eyes were washed overnight in a 0.1M cacodylate buffer at pH 7.2 at 4°C.

3. Processing of the Tissues

All fetal, post-natal and adult rat eyes were incubated in open petri dishes for 40 to 60 minutes at room temperature in a solution made up of 5 mg 3,3' diaminobenzidine tetrahydrochloride (DAB - Reagent grade, Nutritional Biochemicals Corp.) dissolved in 0.05M Tham buffer, pH 7.6, containing 0.01% H_2O_2 (Karnovsky, 1967). The tissues were washed in distilled water and post-fixed for 1 hour in 1% osmium tetroxide in 0.1M cacodylate buffer at pH 7.2. The tissues were then dehydrated in a graded

TABLE 1

HRP TRACER STUDIES

Age	Anesthesia	Route of Intervenous Injection	Amount of HRP (ml) (40 mg/ml saline)
19 df	None	Umbilical vein	0.08
20 df	None	Umbilical vein	0.10
21 df	None	Umbilical vein	0.10
21 df	None	Umbilical vein	0.10
1 dpn	Ice	Retro-orbital vein	0.10
3 dpn	Ice	Jugular vein	0.10
4 dpn	Ice	Jugular vein	0.11
5 dpn	Ice	Jugular vein	0.11
7 dpn	Ice	Saphenous vein	0.15
10 dpn	Ice	Saphenous vein	0.20
12 dpn	Ether	Saphenous vein	0.20
15 dpn	Ether	Saphenous vein	0.14
22 dpn	Ether	Saphenous vein	0.25
Adult	Ether	Saphenous vein	0.50

N.B. The fetal rats were not directly anesthetised. However, they must, in effect, have been anesthetised by the sodium pentobarbitol and sodium phenobarbitone administered to the mother rat crossing the placenta. They were relatively quiescent. If a pregnant female rat is etherised and the abdominal cavity opened, it will be found that the fetuses are extremely active as compared to the fetal rats we were working with.

HRP = Horse-radish Peroxidase, Type II - salt free powder (Sigma)
df = days fetal
dpn = days post-natal

alcohol series, infiltrated and embedded in an epon-araldite mixture. The tissue blocks were polymerised in a 65°C oven overnight. Thick sections were cut on a Porter-Blum MT-2 ultramicrotome and stained with toluidine blue.

4. Examination of the Tissues

The sections were examined on a Zeiss Photomicroscope II. It was found that the toluidine blue stain on the sections often obscured the sites of deposition of the brownish-black HRP reaction product. To overcome this, a sliding monochromator was used. By altering the wavelengths of the light falling onto the sections on the slides, it was then possible to study both the histology of the iris tissue as well as the sites of deposition of the HRP reaction product. Light micrographs were made on Kodak Plus-X Pan film.

E. A Scanning Electron Microscopic Study of the Posterior Surface of the Developing Rat Iris

METHOD:

Fetal and post-natal rats of the Wistar strain were used. The ages of the fetal rats were determined by the method of Christie (1964). The fetal rats were removed individually from the uterus while the mother rat was kept under anesthesia either with ether, or with injections of sodium pentobarbital(administered intraperitoneally) and sodium phenobarbitone (administered subcutaneously). The fetuses were sacrificed by decapitation. The eyes were removed immediately and fixed whole in the fixative of 4% glutaraldehyde in 0.1M sodium cacodylate buffer at pH 7.2 at room temperature for a total of 4 hours.

Rats, aged up to six days after birth, were anesthetised by being placed in the freezer till they were quiescent and then they were placed on ice cubes. Older rats were anesthetised by ether. The eyes were removed and placed in the fixative of 4% glutaraldehyde in 0.1M sodium cacodylate buffer at pH 7.2. After ¼ to ½ hour in the fixative, the posterior halves of the eyes and the lenses were removed and fixation was continued for a total of 4 hours.

The fetal and post-natal eyes were stored in a 0.1M cacodylate buffer at 4°C. The eyes were dehydrated through a graded series of alcohols. While in 70% alcohol, the eyes were trimmed as much as possible so as to best display the posterior surface of the developing iris. The absolute alcohol in the tissues was then substituted by iso-amyl acetate (Fisher) in a stepwise fashion. The tissues were dried using the Critical Point Drying method, as has been previously described. The tissues were then mounted on tissue stubs, coated with a thin layer of gold and examined on a Cambridge Stereoscan (Model S4).

III. RESULTS

A. A Light Microscopic Study of the Rat Iris in Pupillary Dilation and Constriction

1. The Iris in Pupillary Dilation (Figures 2-7)

When the pupil is well dilated, the iris is short in terms of its dimensions from the root to the tip of the iris, as seen in meridional sections. It may only be about 1mm or less across. The iris tends to be disposed in a slight convex arch towards the surface of the cornea. It seems to buckle in the middle (Figure 2). This is in part due to the fact that the stroma in the middle one third of the iris is always thicker than that at the tip or at the root of the iris (Figure 2).

In meridional sections, the posterior epithelial cells form a layer covering the posterior surface of the iris from the pupillary edge to the root of the iris where it is continuous with the epithelium of the ciliary processes. Each individual cell may be discretely separated from its neighboring cell thus giving the posterior surface a deeply convoluted appearance (Figures 2-3, 5-7). The peg-like epithelial cells then appear like the myriad legs of a millipede (Figures 2-3, 5-7). On the other hand, the posterior epithelial cells may be so closely abutted on each other that there are no gaps in between the cells. This is most prominently seen in the middle one third of the iris (Figure 4), whereas the epithelial cells at the root of the iris may be separated one from another.

The posterior epithelial cells are most often large, columnar in shape and they have a highly irregular cell outline (Figures 3, 5-7). The height of the epithelial cells may vary from being high cuboidal to low columnar to high columnar depending on the degree of pupillary dilation.

The anterior border of these cells is in apposition with the dilator muscle cells but the posterior and lateral surfaces are generally free and not touching other cells except in the instances mentioned above. The posterior surface of the cells show large or major cell processes. Oftentimes such a major cell process imparts to the cell a bifurcate appearance. The major cell processes may in turn show minor processes (Figures 5 and 6).

In the center of each cell is the nucleus. The nuclear structure and staining characteristics with toluidine blue seem to be in some way related to the configuration of the posterior epithelial cells. If the epithelial cells are discretely separated from each other, the nuclei stain uniformly intensely with toluidine blue. They stain much darker than the nuclei of the dilator muscle layer. Not much nuclear detail is visible at all. These elongated nuclei have a very irregular outline. They are oriented such that the long axis of the nucleus lies along the long axis of the cell. On the other hand, if the posterior epithelial cells are in close apposition, the nuclei are round to oval in shape and only sometimes elongated (Figure 4). The nuclei stain lightly and consequently they show a little more nuclear detail. The nuclear envelope stains intensely. Specks of darkly staining chromatin material are found scattered within the nuclear substance. These nuclei generally stain lighter than those in the adjacent dilator. The nuclear outlines are relatively smooth with a few clefts and indentations. If the nuclei are larger and slightly elongated, then they are situated parallel to the long axis of the cells (Figures 3, 5-7).

The cytoplasm of the posterior epithelial cells appears vesicular, especially so at the anterior poles of the cells where they come in contact with the dilator muscle cells. Whether these vesicles are a consequence of fixation or whether they serve as a membrane pool to accommodate to changes in cell shape and size during pupillary constriction and dilation is

speculative.

At the junction of the dilator and sphincter, there is an abrupt change from a columnar to a squamous epithelium lining the whole posterior aspect of the sphincter region up to the pupillary border (Figures 2, 7). The nuclei stain similarly to those of the epithelial cells. However, they are oriented radially with respect to the whole iris. The cell outlines are irregular and there are many cell processes. The cytoplasm is thin and appears vesicular too.

The dilator muscle layer is relatively thick and quite readily discernible with the light microscope (Figures 2-7). The width of the dilator may range from one half to two thirds the height of the columnar cells of the posterior epithelium. Sometimes the dilator may be as wide as the epithelial cells are high. The posterior surface of the dilator is apposed onto the anterior surface of the posterior epithelium. There does not seem to be a tight line of fusion between the two layers of cells. The junction region looks vesicular (Figures 3, 5, 6). It could be that here cell processes of the two layers interdigitate loosely leaving relatively large intercellular spaces. This would be deemed desirable in view of the fact that rapid changes in relationships between the two cell layers might have to take place during pupillary constriction and dilation. Unlike the posterior epithelial cells, the boundaries between individual dilator muscle cells are not readily apparent (Figures 3, 5, 6).

The nuclei, placed more or less in the center of the cells, vary both in shape and size. Also, the nuclei are not as regularly placed within the entirety of the muscle layer as in the posterior epithelium (Figure 6). These observations are a result probably of the plane of sectioning of the material. The nuclei are irregular in shape, sometimes somewhat rounded, with grooves and indentations so that they may even appear lobulated. These

usually exhibit a slightly more intense staining along the nuclear envelope with some fine dense particulate chromatin within the rest of the nucleoplasm.

On the whole, the cytoplasm of the dilator muscle cells appear less vesicular than that of the posterior epithelial cells. It is especially noticeable in the anterior two thirds of the cells where the cytoplasm has a smooth and more solid appearance. This is where all the myofilaments are located. At the boundary between the dilator and the stroma, the cytoplasm stains much more intensely probably owing to an accumulation of myofilaments in this region (Figures 3, 4, 6). At intervals all along this boundary zone, the dilator muscle cells send out a series of arborescent processes (Figures 3, 6). These cell processes, simple or complex, also stain darkly. Large or major processes with their many minor processes which radiate and spread out in a fan-like manner, protrude into the stroma (Figure 6). At times it seems that not only dilator cell processes but whole groups of dilator muscle cells encroach on the stroma (Figures 5, 7). One receives the impression that during extreme pupillary dilation there is just not enough room within the dilator muscle layer to accommodate all of the cells so that some of the cells are squeezed out of position, as it were, by partial buckling of specific areas of the dilator muscle layer (Figures 5, 7). Such muscle spurs, when present, are primarily seen in the medial half of the iris (Figure 2).

At the junction between the dilator and sphincter, some of the cells of the dilator seem to intrude into sphincter muscle territory. Sometimes they appear to spread out and envelope the laterial extents of the sphincter muscle fibers (Figure 7). Thus there does not seem to be a very sharp clear-cut demarcation between the dilator and sphincter, as seen by light microscopy. Most probably the myofilaments are disposed in their respective

directions within the muscle cells despite their proximity.

The sphincter muscle is a distinct, but not a compact, bundle found at the pupillary tip of the iris. It is bounded anteriorly by a small amount of stroma and posteriorly by a double-layered squamous epithelium (Figures 2, 7). The posterior epithelium lining the sphincter region seems to be a continuation of the dilator and the columnar posterior epithelial cells which have abruptly changed their form. In the stroma overlying the sphincter, there are few stromal cells. This part of the stroma is primarily occupied by numerous capillaries, presumably for efficient exchange of materials between the sphincter cells and blood. Small capillaries are also found in among the sphincter muscle cells themselves. Being contractile elements, the energy requirements must be high and thus they need to be well vascularised. The individual muscle cells may be closely associated with each other or they may be far apart. In meridional sections, the uniformly darkly staining muscle fibers are cut in cross-section and appear round to polygonal. The nuclei are small, round or elliptical in shape and are irregularly distributed. There are very many more muscle cells seen than there are nuclei owing to the plane of sectioning.

The stroma extends from the root to the tip of the iris. In pupillary dilation, its antero-posterior dimensions vary depending on the particular region of the iris. There is usually just a small amount of stromal tissue over the anterior face of the sphincter. In the middle third of the iris the stroma is thick and highly cellular. The stroma slowly tapers off towards the root of the iris. This uneven distribution of stroma over the extent of the iris during pupillary dilation imparts to the iris a convexly curved appearance.

No attempt is made to stringently identify the various cell types within the stroma. This has been adequately done in numerous other works.

The main object of concern within this study is to examine the overall arrangement of the cellular elements within the stroma rather than the interrelationships of individual types of cells. Suffice it to mention that in the stroma there are blood vessels of various sizes, both myelinated and unmyelinated nerves and stromal cells.

The stroma over the sphincter is loose and not very cellular (Figures 2, 7). Large intercellular spaces are present. This area is primarily occupied by numerous blood vessels. Most of these are capillaries; some consist of a single endothelial cell lining the lumen and thus large enough to accommodate only one erythrocyte (Figure 7), while others may be a little larger and may have in addition a single layer of pericytes encircling the endothelial lining. The stromal cells, when present, appear haphazardly arranged.

In the middle of the iris, the stromal cells are very closely packed together. It is difficult or nearly impossible to trace the continuity of each individual cell. However, the numerous cytoplasmic cell processes seem to be disposed perpendicularly with respect to the posterior layers of the iris, that is the posterior epithelium and dilator. The spaces in between the cell nuclei and their processes give the impression of vertical linearity (Figures 3,6). The stromal cells then appear to be arranged in vertical columns streaming down from the anterior to the posterior surface of the iris. Also, the nuclei of the stromal cells seem to be kept away at a distance from the dilator muscle cell processes. Only the stromal cell cytoplasmic processes come into close proximity to the dilator muscle layer. The nuclei, having more bulk and perhaps being less deformable, seem to be held aloof from the dilator almost so as not to impede the folding up of the dilator during pupillary dilation (Figures 3, 5, 6). Occasionally a nucleus might come fairly close to the dilator especially where muscle spurs are

present.

Nerves are found singly or in bundles of various sizes within the stroma. They may be myelinated or unmyelinated. They are often associated with blood vessels (Figure 5). However, it is not possible to tell if there are nerve terminals in close association specifically with the dilator or sphincter since toluidine blue is not a preferential stain for nervous tissue.

Most of the blood vessels are large. Their lumina are open and filled with erythrocytes (Figures 2, 7). The lumina may be round or oval in shape. When the lumina are oval, the length of the oval is oriented similarly to the cells, that is, perpendicular to the posterior surface of the iris (Figures 3, 5, 6). These blood vessels thus appear to be squeezed in from side to side, but not sufficiently to occlude the lumina. This is important for the maintenance of vital cell functions even in extreme pupillary dilation. The walls of the iridial blood vessels are not very thick in relationship to their size. This is probably for greater flexibility during movements of the iris in pupillary dilation and constriction.

The stroma at the root of the iris is in most respects similar to the stroma in the middle of the iris except for the fact that the width of the stroma is decreased. Oftentimes the division of the stroma into vertical columns of cells is much more evident (Figure 3). A fair sized number of blood vessels make up the bulk of the stroma here.

The anterior surface of the iris usually shows a scalloped appearance. Sometimes it is more pronounced (Figures 2-4, 7) than at other times (Figure 5). This could be due to the plane of sectioning. The anterior surface of the stroma directly over the sphincter is in general smooth. The scalloped appearance over the rest of the stroma is the result of columns of cells protruding outwards or dipping inwards. The protrusions are most often due

to the bulging out of large blood vessels at the anterior surface. The dips occur in between the bulging blood vessels. These dips may be quite deep. The blood vessels do not ever abut directly onto the anterior stromal surface. The cytoplasm of other stromal cells are always interposed between the blood vessels and the anterior chamber. Oftentimes small cytoplasmic blebs may be seen off the anterior surface. These may be a fixation artifact.

Figure 2 The Iris in Pupillary Dilation (LM)

This low magnification light micrograph of the iris in pupillary dilation shows that the iris is short. Both the posterior epithelium (pe) and dilator (d) are very distinctly visible. The stroma (s) is thick, especially in the middle one third of the iris, so that the iris appears to buckle anteriorly. Dilator muscle spurs (arrows) are seen in the pupillary half of the iris. The sphincter (sph) is a mass of small cells at the pupillary tip of the iris. x 90

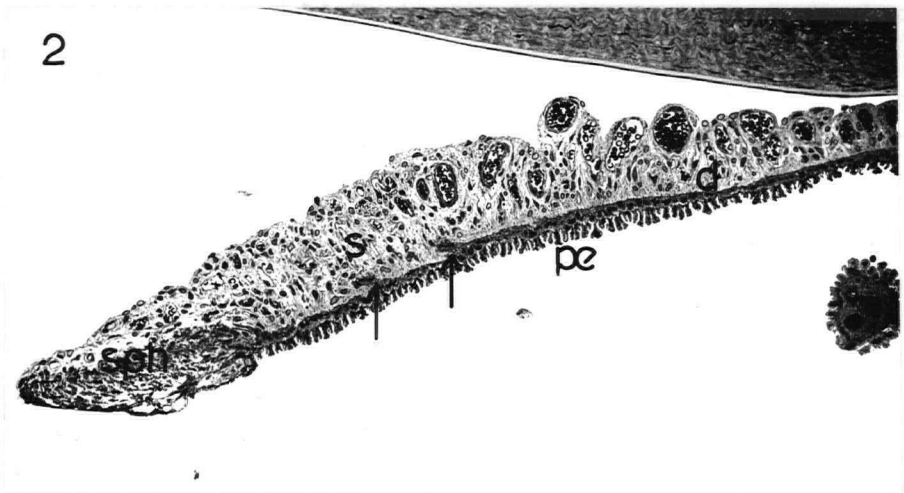
Figure 3 The Iris in Pupillary Dilation (LM)

Each posterior epithelial cell (pe) is discretely separated from the next. The epithelial cells are peg-like giving the posterior surface of the iris a deeply convoluted appearance. The nuclei are large and are situated in the middle of the cells surrounded by a thin vesiculated rim of cytoplasm.

The dilator (d) is a relatively thick layer in apposition with the posterior epithelium (pe) and with the stroma (s). At the stromal surface of the dilator, there is a dense line where all the myofilaments are concentrated. Fine, dark-staining processes from the dilator are seen all along the dilator-stroma boundary (arrows).

The stroma (s) is filled with blood vessels containing masses of red blood cells. The lumens of the blood vessels may be round to oval. When oval, they are perpendicular to the dilator and the posterior epithelium. Some of the blood vessels appear to have only an endothelial lining (bv1) while others possess in addition a layer of pericytes (bv2). The stromal cells are arranged in columns extending from the anterior surface of the iris to the dilator-stroma boundary. The spaces in between the stromal cells emphasise the vertical linearity. The nuclei of the stromal cells are kept at a distance from the dilator. The anterior surface of the iris facing the anterior chamber (AC) is highly scalloped in outline. This is mainly caused by the bulging out of the iris blood vessels.

x 240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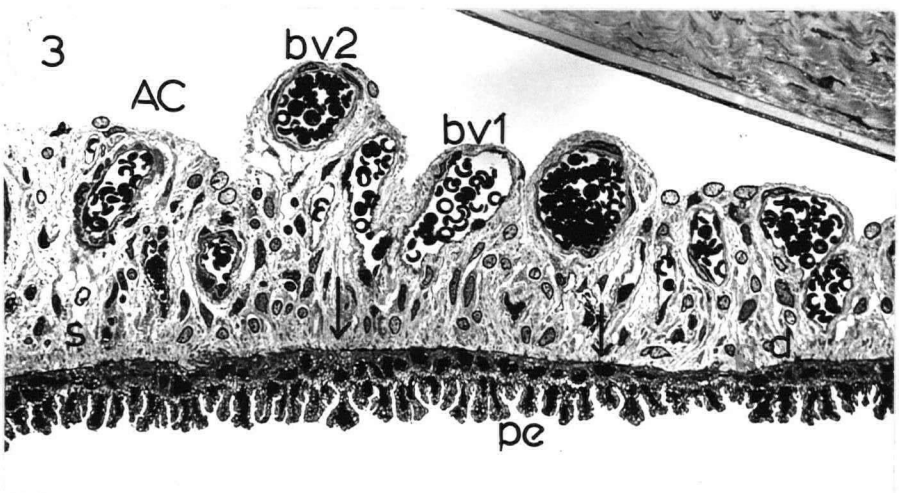
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Figure 4 The Iris in Pupillary Dilation (LM)

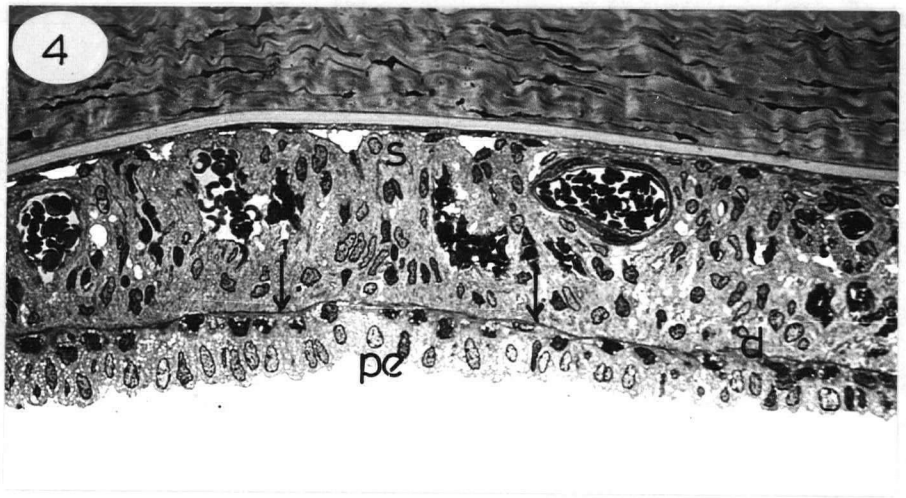
Unlike in Figure 2, the posterior epithelial cells (pe) are quite close together. The posterior surface of the iris is not deeply convoluted but slightly wavy in appearance. The contractile portion of the dilator (d) layer is seen as a very distinct, smooth, dense line (arrows) while the epithelial portion is lighter staining. The cells in the stroma (s) are packed together. The anterior surface of the iris shows a scalloped outline. x 280

Figure 5 The Iris in Pupillary Dilation (LM)

The posterior epithelial cells (pe) have a highly irregular outline. The cells may bifurcate and show numerous cell processes which are devoid of nuclei (arrows). The cytoplasm of the posterior epithelial cells appears vesiculated. The most distinctive feature of this light micrograph are the groups of dilator cells or muscle spurs (ms1, ms2) which encroach on the stroma. They may also show fine dilator processes (ms2).

Blood vessels of different sizes (bv), nerves (n), and stromal cells make up the stroma (s). The nerves are often found in bundles near to blood vessels.

The anterior surface of the iris shows a relatively undulating outline. x 380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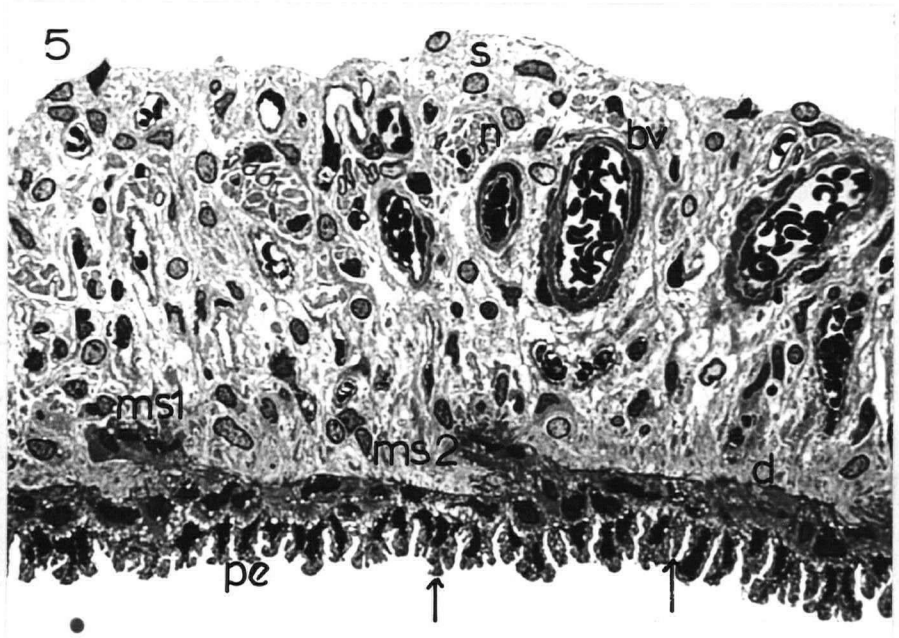
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Figure 6 The Iris in Pupillary Dilation (LM)

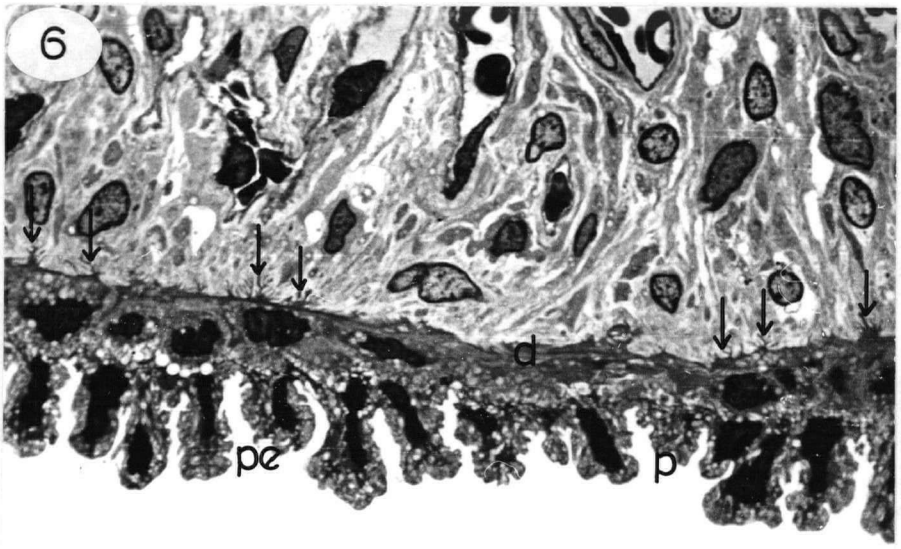
The posterior epithelial cells (pe) are large, high columnar with an irregular cell outline. The nuclei, with irregular nuclear envelopes, are elongated along the long axes of the cells. The cytoplasm has a lace-like appearance. The cells show processes of different sizes (p). The dilator (d) layer is quite thick. The nuclei are irregularly shaped. Small densely staining hillocks of dilator material (arrows) are distributed all along the anterior stromal surface of the dilator. From these hillocks arise numerous, highly branched dilator processes which spread out in a fan-like manner.

The stromal cells, the spaces in between the cells and the lumens of the blood vessels are all perpendicular to the posterior surface of the iris. x 1400

Figure 7 The Iris in Pupillary Dilation (LM)

The sphincter (sph) is a distinct bundle of small, darkly staining cells. It is bounded anteriorly by a thin strip of stroma containing few cells and numerous capillaries (bv). Posteriorly, it is bounded by a barely perceptible squamous epithelium.

At the peripheral extent of the sphincter (arrow), some of the dilator cell processes seem to spread out and envelope the sphincter. Here, too (arrow), there is an abrupt transition between the high columnar epithelium lining the rest of the posterior surface of the iris and the squamous epithelium lining the posterior surface of the sphincter. The stroma (s) is cellular and vascular. As in Figures 2-4, and 6, the stromal elements are arranged perpendicular to the posterior surface of the iris.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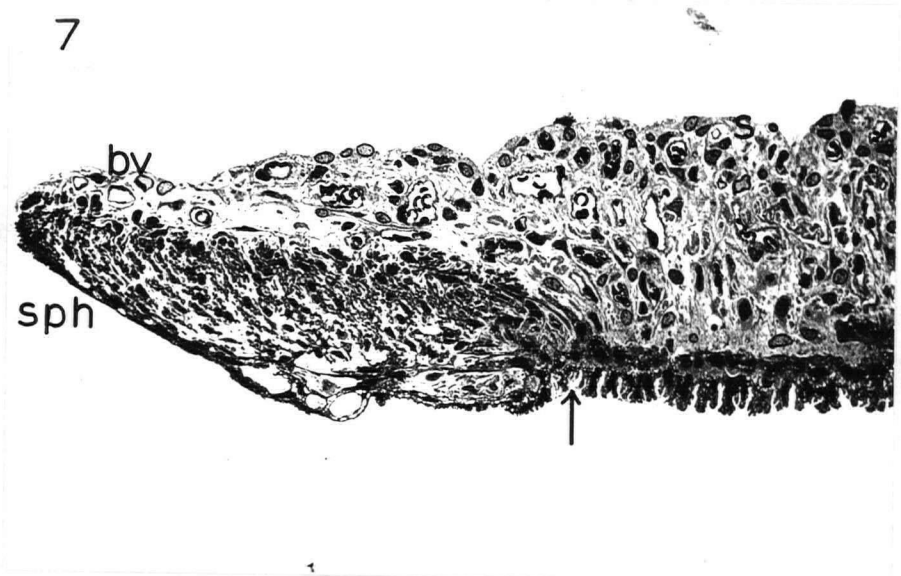
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2. The Iris in Pupillary Constriction (Figures 8-11)

When the pupil is constricted the iris is a thin, delicate and tenuous structure (Figure 8) covering the anterior surface of the lens. In meridional sections, the antero-posterior width of the iris varies in direct relationship to the degree of constriction of the pupil. The iris may be evenly thin from the pupillary border to the root of the iris (Figure 8). At times, the root of the iris is slightly more attenuated than the rest of the iris. Or, at other times, there may be some unevenness along the whole length of the iris and in which the root of the iris is extremely stretched out. The configuration of the posterior epithelium, dilator, sphincter and stroma all contribute to the overall width of the iris. Thus differing changes in cell shape, size and configuration, and changes in inter-cell relationships occur depending on the extent of pupillary constriction.

In extreme pupillary constriction the posterior epithelium is in general a relatively thin layer lining all of the posterior surface of the iris. It has the appearance of a thick squamous epithelium (Figure 9). The nuclei are flattened and there is little discernible cytoplasm which may be vacuolar. Sometimes in amongst the thick squamous epithelium there may be low cuboidal cells. These may be present in groups or they may be present singly (Figure 10). The nuclei are thicker, with an irregular outline and more prominently seen. The cuboidal cells may be pushed close to each other or they may be set apart with the cytoplasm of one cell extending out towards the cytoplasm of an adjacent cell (Figure 10). The cell outlines are irregular. In less extreme states of pupillary constriction, the posterior epithelial cells are squamous only at the root of the iris. Along the rest of the iris the posterior epithelial cells are thicker with an associated relative increase in nuclear size. The cytoplasm may

bulge out over the nucleus giving the posterior surface a very slight scalloped appearance.

The epithelium lining the posterior surface of the sphincter is not easily seen. Since it is such a thin squamous epithelium there is not a sharp transition between this epithelium and that lining the rest of the iris, as is observed when the pupil is dilated.

The dilator is an extremely thin squamous layer which is barely visible (Figures 9-11). It is only about half the thickness of the posterior epithelium. Essentially the dilator muscle layer is seen as a row of flattened nuclei. The cell cytoplasm is attenuated and discernible only in certain areas. The dilator may appear to be closely applied onto the posterior epithelium or there may be spaces in between the two layers. Presumably, the cytoplasmic processes of both the posterior epithelial cells and the dilator muscle cells interdigitate loosely in this region. The anterior surface of the dilator most often appears as a smooth dense line. There are no signs of dilator cell processes (Figures 9, 10) seen when the pupil is dilated. Neither are there clumps of dilator cells being pushed into the stroma. There is no necessity for this to occur. With an increase in total area of the iris in pupillary constriction all of the dilator cells can be accommodated within one layer (Figures 9-11) unlike the condition that is present when the pupil is dilated.

The sphincter is a thick bundle of cells found at the pupillary edge of the iris (Figures 8, 11). It is just possible to make out the squamous epithelium lining its posterior surface. Anteriorly it is covered by a little bit of stroma. The sphincter muscle cells are closely packed together. These muscle cells stain uniformly darkly in comparison to the stromal cells. The numerous small nuclei of the muscle cells do not stain much darker than their cytoplasm.

The stroma is thin (Figures 9-11) as compared to its width when the pupil is in the dilated state. The width of the stroma is in essence a qualitative indicator of the extent of constriction or dilation of the pupil. In pupillary constriction, the stromal cells and the associated intercellular spaces are arranged parallel to the posterior surface (Figures 9, 10). This arrangement is more evident in certain parts of the iris than in others. Likewise, the larger blood vessels, open and filled with red blood cells, are stretched out lengthwise parallel to the posterior surface (Figures 9, 10). Large bundles of myelinated nerves are often found next to the blood vessels. Only a few stromal cells and capillaries are present in the stroma overlying the sphincter.

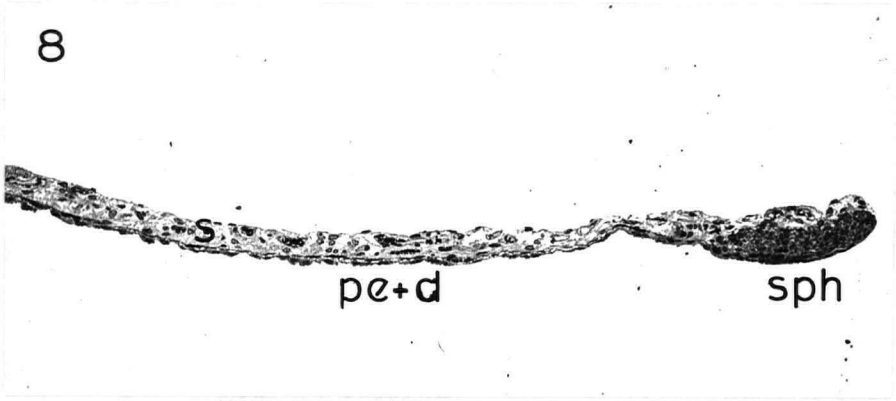
In pupillary constriction, the anterior surface of the iris is quite smooth (Figure 8). There may be a hint of some scalloping in the region of the sphincter. Slight undulations along the rest of the anterior surface of the iris may at times be observed but these are by no means a prominent or constant feature. In the dilated pupil, it is seen that the scalloped appearance of the anterior surface of the iris is in large measure created by the bulging anteriorly of the blood vessels in the stroma. However, in the constricted state, the blood vessels are stretched out parallel to the posterior surface thus rendering the anterior iridial surface relatively smooth (Figure 10).

Figure 8 The Iris in Pupillary Constriction (LM)

In pupillary constriction, the iris is long and thin. The posterior epithelium(ep) and dilator (d) are barely visible but as two rows of flattened nulei. The stroma (s) is thin. The sphincter (sph) is a darkly staining compact bundle at the pupillary tip of the iris. x 90

Figure 9 The Iris in Pupillary Constriction (LM)

The posterior epithelium (pe) is a squamous layer of cells with the nuclei flattened parallel to the iris length. The dilator (d) is an even thinner layer. In most instances, it is seen as a dense line with a few scattered elongated nuclei. The stroma (s) appears relatively loose. There are many large spaces. The blood vessels and stromal cells are generally oriented parallel to the iris length. The anterior surface of the iris is smooth with openings (arrows) leading into the anterior chamber (AC). x 260


8
s
pe+d
sph

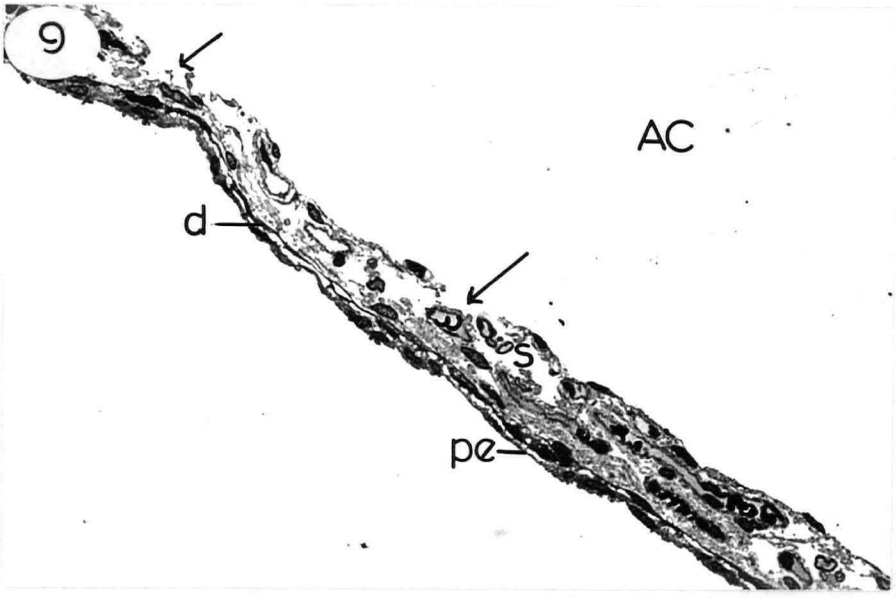

9
AC
d
s
pe

Figure 10 The Iris in Pupillary Constriction (LM)

Some of the posterior epithelial cells (ep), singly or in groups, are cuboidal or thick squamous in shape and they bulge slightly into the posterior chamber (PC). This gives the posterior surface of the iris a slightly scalloped appearance.

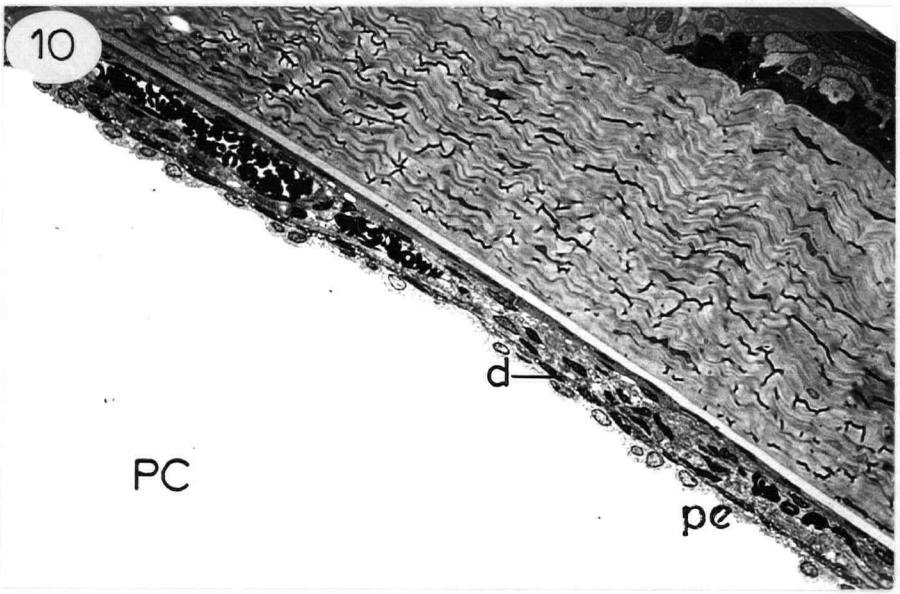
The dilator (d) is a thin layer of dense cytoplasm and extremely attenuated nuclei. The stromal surface of the dilator is smooth.

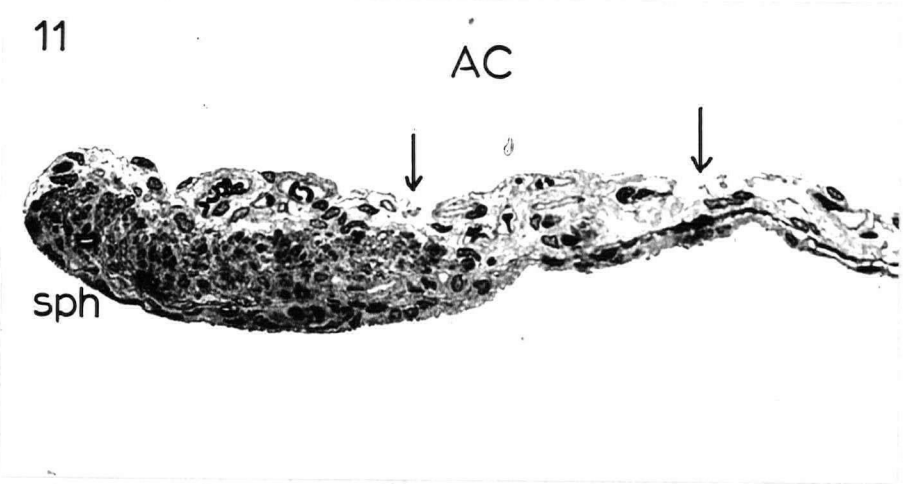
The blood vessels and stromal cells are packed close together parallel to the posterior surface of the iris.
x 260

Figure 11 The Iris in Pupillary Constriction (LM)

The sphincter (sph) is a compact mass of darkly staining cells. Spaces are found in between the cells, giving the sphincter as a whole a cracked glass effect.

The anterior surface of the iris is relatively smooth except over the sphincter where it is convoluted. Crypts (arrows) open into the anterior chamber (AC). x 260


10
d
PC
pe


11
AC
sph

B. A Light Microscopic Study of the Collagen Network in the Stroma of the Rat Iris in Pupillary Dilation and Constriction

Using a modified Mallory's Trichrome Stain, the collagen network in the stroma of the iris is stained blue. The disposition of the collagen in the stroma follows closely the changes in arrangement of the stromal cells during pupillary dilation and constriction. When the pupil is dilated, the stromal cells are arranged perpendicular to the posterior epithelium and dilator muscle. Likewise, the collagen appear as bundles streaming down from the anterior surface of the iris stroma towards the boundary zone between the dilator and stroma (Figure 12). There seems to be a condensation of collagen, as indicated by the relative increase in the intensity of staining, at this boundary zone (Figure 12).

When the pupil is constricted, the iris is long and thin and all of the stromal cells are oriented parallel to the posterior epithelium and dilator muscle. Because of the paucity of the collagen elements in the rat iris stroma and the extreme thinness of the iris as a whole, it is not often easy to visualise the collagen network. However, where visible, it is found that the collagen is indeed arranged parallel to the posterior epithelium and dilator muscle, as are the stromal cells (Figure 13).

Figure 12 The Collagen Network in Pupillary Dilation (LM)

In pupillary dilation, the iris is short. The stromal (s) cells are arranged perpendicular to the posterior epithelium (pe) and the dilator (d). The collagen network, stained blue, are seen as bundles streaming from the anterior surface of the iris to the boundary zone with the dilator muscle. There is a condensation of collagen all along this boundary zone. x 250

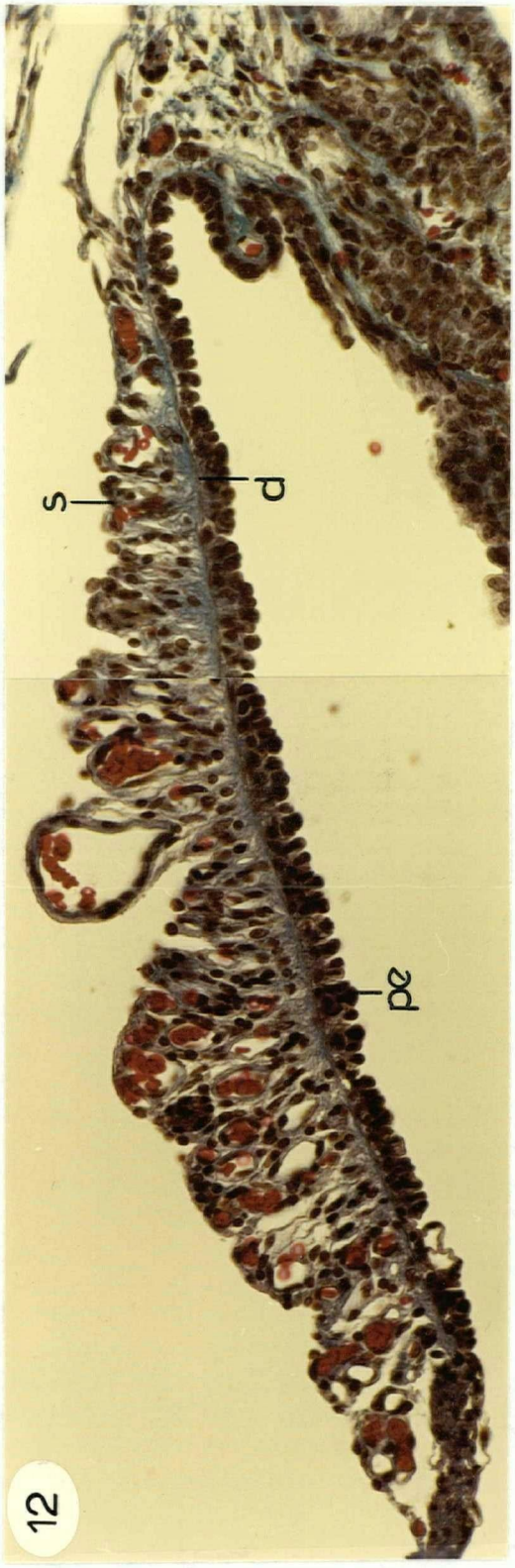
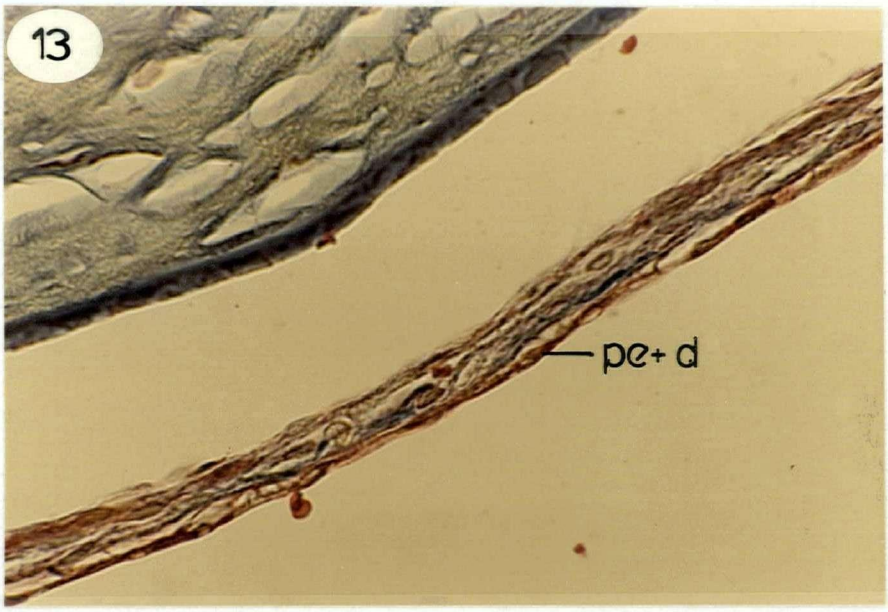

s
d
pe
12

Figure 13 The Collagen Network in Pupillary Constriction (LM)

In pupillary constriction, the iris is long and thin. The posterior epithelium (pe) and dilator (d) cannot be easily distinguished from each other. The stromal cells and the collagen bundles are oriented parallel to the length of the iris. x 400


13
pe+ d

C. A Transmission Electron Microscopic Study of the Rat Iris in Pupillary Dilation and Constriction

1. General (Figure 14)

Various chemical fixatives and methods of fixation are used, as outlined in the method. The eye, in pupillary dilation or constriction, as the result of the topical application of mydriatics or miotics, is removed immediately from the rat. A slit is made through the cornea to facilitate the inflow of fixatives into the anterior chamber and thus to the iris. The eye is then immersed in the respective fixatives. The iris is maintained in the constricted or dilated state. The so called "death dilation" which is normally observed in the pupil when death ensues, does not occur if the iris is fixed right away after being removed from the animal. Light microscopically, the iris usually appears adequately fixed when the immersion method is used. The lumens of the blood vessels are patent although not distended. However, with the transmission electron microscope, it is found that there is some tissue destruction, especially to the mitochondria of the posterior epithelial cells. The mitochondria are broken up. The cristae disintegrate within the mitochondrial membranes leaving a large vesicle with cellular debris. The extent of mitochondrial disruption varies from specimen to specimen and cannot be correlated with either the speed of transfer of the tissue from the animal to the fixative, or with the type of fixatives used.

As an alternate method, the iris is first fixed *in situ* prior to being immersed in the fixative. A slit is made in the cornea and fixative is dripped onto the eye for 5 minutes. *In situ* fixation prior to immersion fixation does not seem to improve the quality of fixation as compared to that obtained with immersion fixation alone.

The best fixation of the iris tissue is obtained by perfusion fixation. The animal stiffens as the fixative circulates through all parts of the body. This is one indication that the whole animal has been thoroughly perfused. Also the pupils remain in the same degree of miosis or mydriasis as perfusion occurs, and when the eyes are enucleated. This is another gross evidence that the initial fixation has been almost instantaneous. Light microscopically, the tissues usually appear well-fixed. The blood vessels are relatively distended and have a smooth outline to their lumens. Observations made with the transmission electron microscope on such specimens reveal that the iris is usually well-fixed. The mitochondria are preserved whole and there are no vesicles with cellular debris, although other types of vesicles are present. The posterior epithelium of the iris is most capricious and difficult to fix well. Even though all other parts of the iris may be adequately fixed, the posterior epithelium may appear slightly broken up. The ultrastructural features of the posterior epithelial cells differ slightly from one part of the iris to the other, suggesting that the posterior epithelial cells are not similarly susceptible to fixation conditions. It is extremely difficult to obtain good fixation throughout all of the posterior epithelium at any one instance. When the fixation is good, both thick and thin myofilaments are observed in the sphincter and dilator muscles (Figure 14). The thick filaments are not present in tissues fixed by immersion. Kelly and Arnold (1972) found that thick filaments can only be demonstrated if good fixation is achieved. This is so in our studies. However, unlike Kelly and Arnold's investigations, the rat does not have to be perfused with a salt solution prior to fixation to maintain all of the ultrastructural features of the iris.

In our study, attention is focussed only on the posterior epithelial and dilator layers, as it is here that the ultrastructural changes are

observed between pupillary dilation and constriction.

2. The Iris in Pupillary Dilation (Figures 15-23)

In a low magnification transmission electron micrograph, the posterior surface of the iris is seen to be highly convoluted or scalloped (Figures 15, 16). Towards the root or periphery of the iris, the grooves in between the individual posterior epithelial cells and their processes are deep and quite wide (Figure 15). Usually, towards the mid-portion of the iris, the posterior epithelial cells are separated by deep but narrow grooves (Figure 16). The cells may appear to be in apposition in light micrographs, but in electron micrographs, a narrow space and the basement membrane of the posterior epithelial cells always separates out the individual cells.

Following the contours of the posterior surface of the iris at the bases of the posterior epithelial cells there is a basement membrane (Figures 15-19). The basal cell membranes of the posterior epithelial cells show numerous complicated infoldings which extend relatively deeply into the cytoplasm (Figures 15-19). In fact, there is only a thin strip of cytoplasm around the nucleus. The cell infoldings are found along the posterior and lateral walls of the cells. They interdigitate with each other in a three dimensional fashion so that these small, fine, basal cell membrane infoldings are always sectioned in different planes. Besides these basal cell infoldings, the posterior epithelial cells in pupillary dilation also show large or major cytoplasmic processes (Figures 15, 19). These processes do not contain a nucleus and consist almost exclusively of the basal cell infoldings and some cell organelles (Figure 15). The cytoplasmic cell processes are of different sizes (Figure 15). The thin basement membrane follows the outlines of the cytoplasmic processes but it does

not follow the outlines of the basal cell infoldings. The basement membrane is only loosely associated with the surface of the posterior epithelial cells. There is always a space of varying widths between the electron dense basement membrane and the cell membrane. This is very clearly shown in some of the electron micrographs.

The cytoplasm contains the usual organelles (Figures 15-19). There are quite a number of mitochondria, rough and smooth endoplasmic reticulum, golgi apparatus and free ribosomes. There are a number of vesicles in the cell cytoplasm (Figure 16). The number of vesicles vary for different epithelial cells. Occasionally, there are some dense bodies which are membrane bound (Figures 16, 17, 19). Another component of the cell cytoplasm which has not been observed previously is the masses of fine filaments (Figures 15, 17-19). The filaments are sometimes very clearly seen in bundles (Figures 15, 17-19). The filaments are usually found around the nucleus (Figure 18). They seem to cascade down to surround the nucleus of the posterior epithelial cells. These filaments are also found in the more apical or anterior part of the cell cytoplasm (Figure 17). They form bundles which curve around the cell organelles. Very rarely is a bundle of filaments cut in cross-section (Figure 19). Here, it is clearly seen that the filaments do form a distinct bundle devoid of cell organelles situated in the middle of a large cell process.

The nuclei of the posterior epithelial cells assume different shapes but in all instances, they are highly indented (Figures 15-19). The nuclei are situated in the posterior portions of the cells surrounded by a thin layer of cytoplasm, and occasionally, filaments, and external to that, a layer of cell infoldings. Dense heterochromatin is usually associated with the nuclear envelope but there are also patches of heterochromatin throughout the nuclear substance. The nuclear material is enclosed within a

double layer of nuclear membranes. However, there always appears to be a gap between the inner and outer nuclear membranes. The nuclear outline is not smooth but is indented to varying degrees, depending on the plane of sectioning. Tongue-like processes of cytoplasm, devoid of cell organelles, occupy the indentations of the nuclear envelope (Figures 15-18).

It is impossible to delineate the boundaries of one cell from the next, as adjacent posterior epithelial cells also interdigitate with each other. Cell junctions are not seen between the individual posterior epithelial cells. The cells appear to associate loosely with each other. In the dilated condition, the boundaries between the posterior epithelium and dilator are also not distinct. The junctions between the posterior epithelium and the dilator are better seen when the iris is observed in the constricted state and will be described then.

The cytoplasm of the epithelial portion of the dilator layer is relatively dense. There are numerous delicate-looking mitochondria scattered throughout the cytoplasm (Figures 20-23). Vesicles and vacuoles of different sizes, rough and smooth endoplasmic reticulum and free ribosomes are found in the cytoplasm. The nuclear outline is much more convoluted than that of the posterior epithelium. Heterochromatin is associated with the nuclear envelope and is present as patches in the center of the nucleus. Usually, but not always, the nuclear membranes appear to be closely adherent to the nuclear material. Except for a small amount of cytoplasm around the nucleus, most of the cell cytoplasm consists of an immense number of microvillous, finger-like cell processes (Figures 20-23). The cytoplasmic processes from adjacent cells interdigitate loosely with each other in all planes. There are large spaces between the cell processes. Occasionally, neighboring dilator cells are joined together by cell junctions (Figures 22, 23). It appears that the membranes are fused to-

gether but this may be due to the plane of sectioning. Probably these are tight junctions. Tight junctions have been observed by other investigators (Hogan, Alvarado and Weddell, 1971).

The contractile portion is confined to the stromal poles of the dilator cells. The sarcoplasm is very dense so that the myofilamentous nature of this layer is not always distinguishable. It is sometimes apparent (Figure 21). There is one constant and interesting feature of the dilator that is always observed in irises in pupillary dilation. Numerous arborescent protrusions are regularly found all along the length of the dilator-stroma boundary (Figures 20-23). These dilator processes may be relatively simple in configuration where they consist of single projections of the dilator into the stroma (Figure 21). More often, the dilator processes are quite complex and branch profusely (Figures 20-23). Small hillocks of dilator muscle are disposed along the length of the dilator at its boundary with the stroma. From these hillocks arise long, delicate, dilator processes which branch extensively in all planes. Pinocytotic vesicles are present both in the dilator processes and in the underlying layer. All of the dilator processes are covered by a basement membrane. Much like the basement membrane of the posterior epithelium, the basement membrane of the dilator cells does not adhere to the cell membranes. There is alwasy a distinctly visible clear region between the cell membrane and the basement membrane (Figures 20, 21). The collagen fibers in the stroma seem quite closely associated with the basement membrane (Figure 20). The basement membrane and the neighboring collagen fibers give to the dilator processes a halo effect.

Figure 14 The Iris in Pupillary Dilation (TEM)

The sphincter muscle cells contain a nucleus (N), numerous mitochondria (m) and both thick and thin myofilaments. The presence of the thick filaments, cut in cross-section, is an indication of adequate fixation of the tissue. The muscle cells come relatively close to each other. There may be some condensation of the adjacent cytoplasm (arrow). x 28,400

14

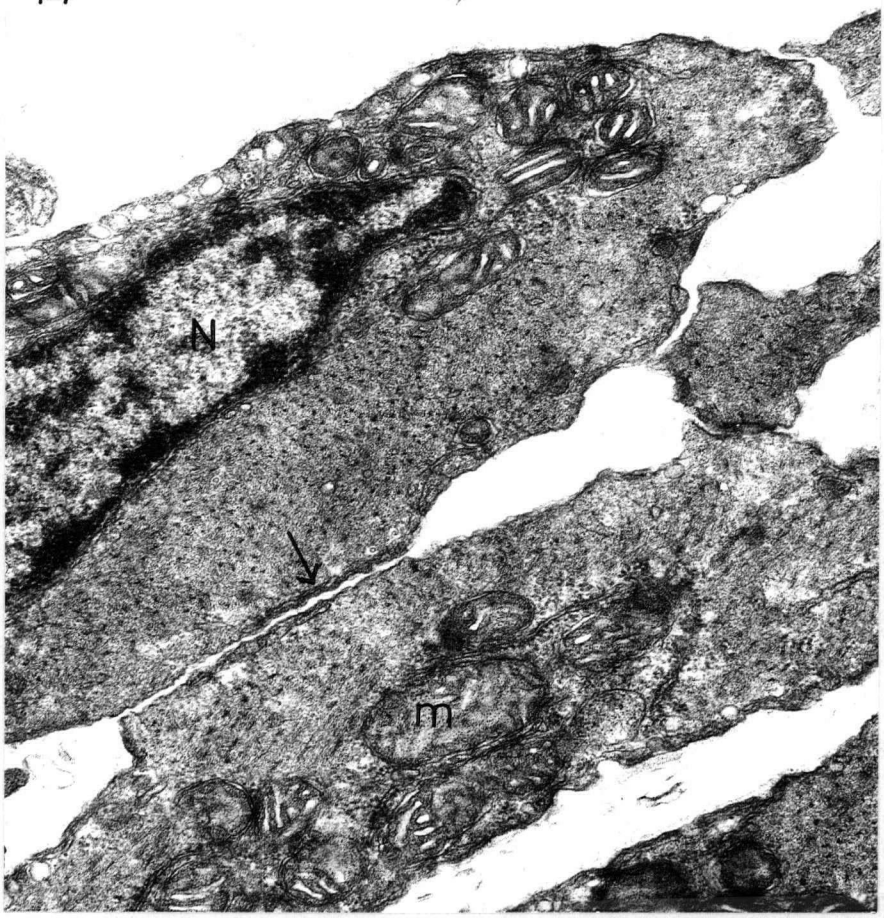


Figure 15 The Iris in Pupillary Dilation (TEM)

In a low magnification electron micrograph, the posterior epithelial cells (pe) are discretely separated from each other by deep grooves (g). The posterior epithelial cells often show large cytoplasmic processes (cp) devoid of a nucleus. The basement membrane (bm) loosely follows the outlines of the posterior epithelial cells and the cytoplasmic processes.

Within the posterior epithelial cells, there is a nucleus (Npe) and numerous mitochondria (m). Bundles of intracellular filaments (if) surround the nucleus. The cell membrane is highly infolded to give numerous cell infoldings (ci) which interdigitate with each other. The cytoplasmic processes consist almost exclusively of cell infoldings. The posterior epithelium is closely related to the dilator layer (d). The nucleus of the dilator (Nd) is highly indented. x 8,900

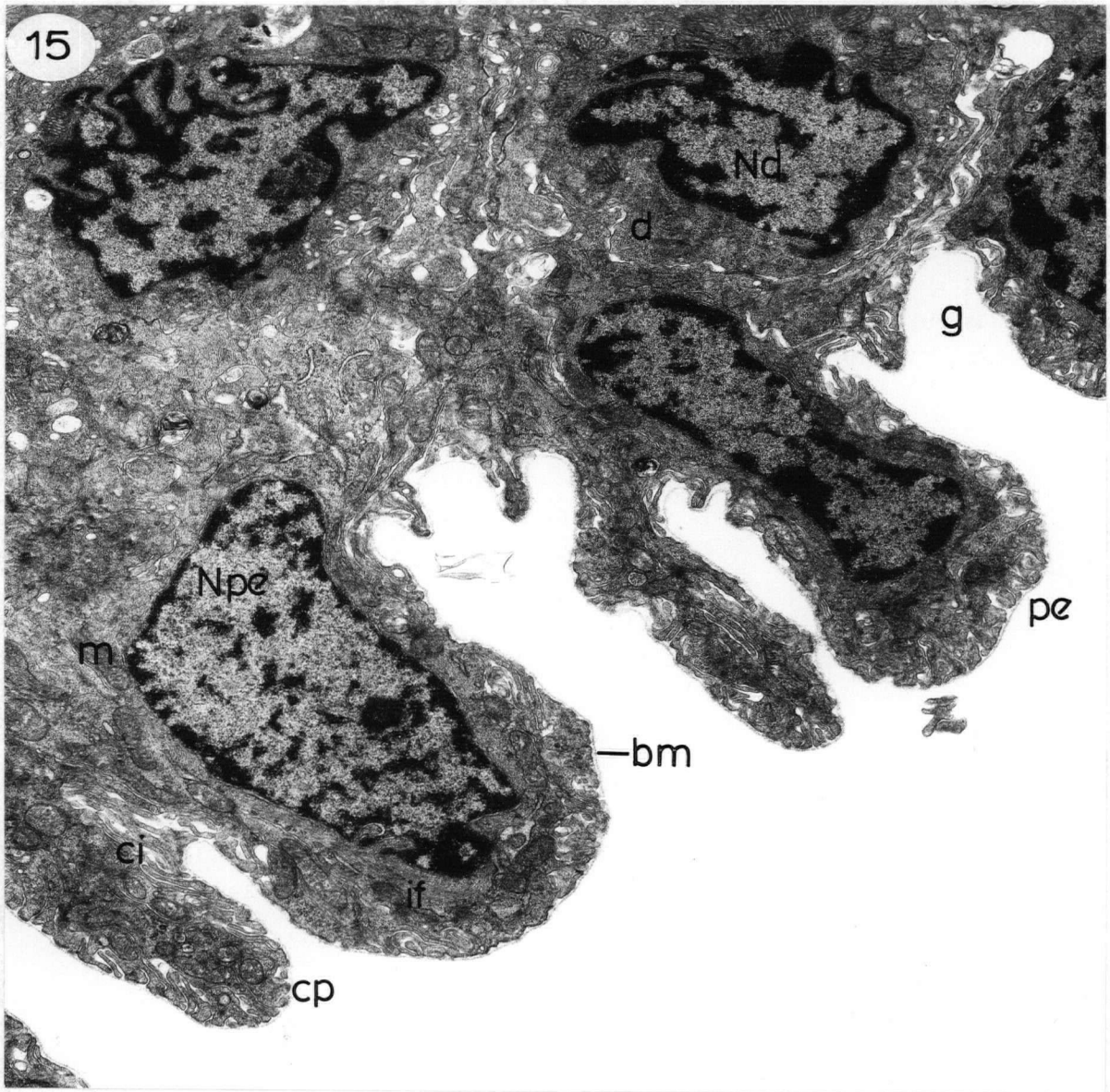

15
Nd
d
g
pe
Npe
m
bm
ci
if
cp

Figure 16 The Iris in Pupillary Dilation (TEM)

The posterior epithelial cells (pe) are very close together. The grooves (g) in between the posterior epithelial cells are very deep and narrow. However, the cells are always separated by the basement membrane (bm). The nuclei of the posterior epithelial cells (Npe) show deep indentations which are occupied by the cytoplasm devoid of cell organelles (*). In the cytoplasm, there are numerous mitochondria (m), vesicles (v) and dense bodies (db). There are also many cell infoldings (ci). There are large spaces in between the posterior epithelium and the dilator (d). x 8,900

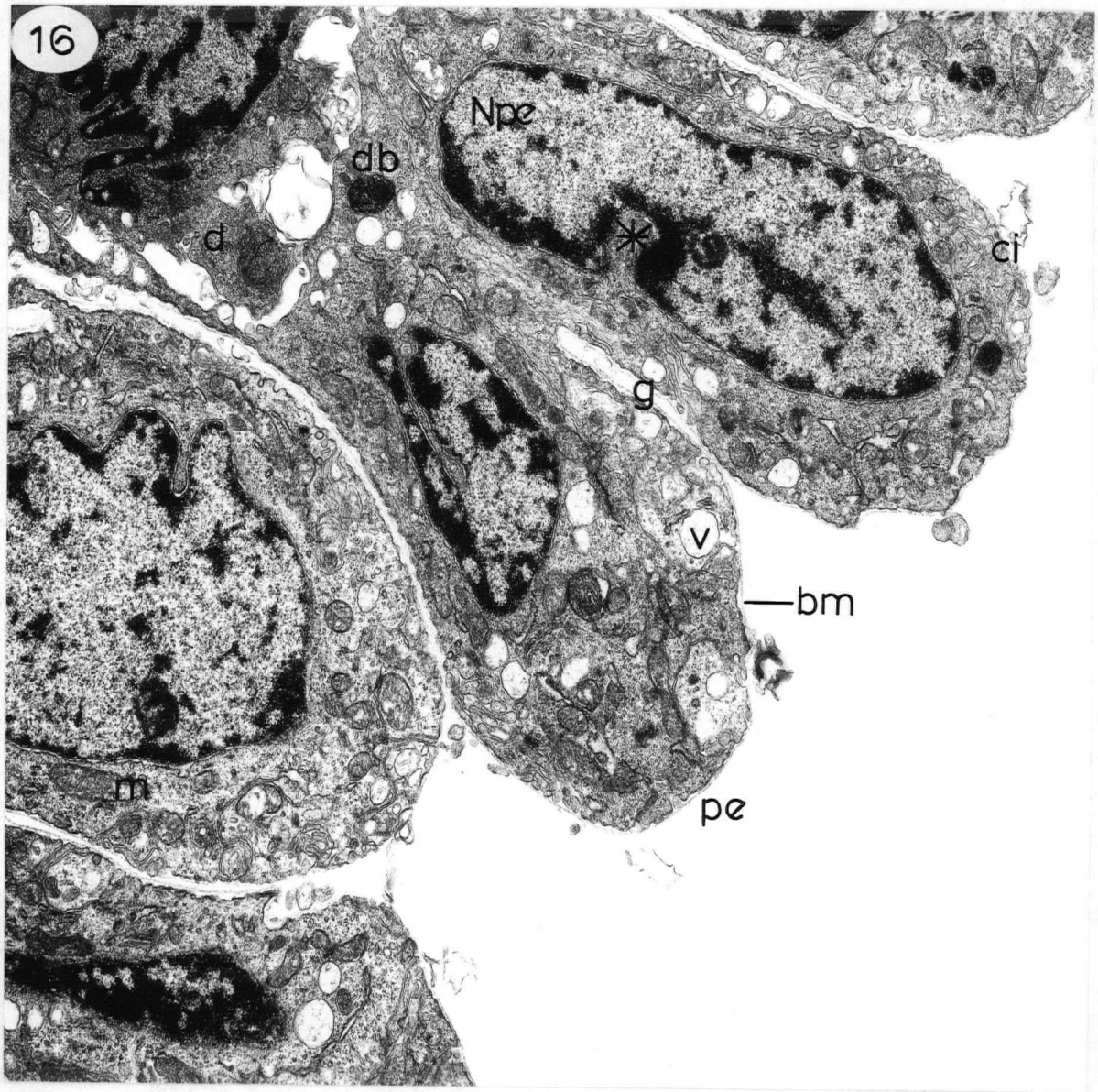

16
Npe
db
d
ci
g
v
bm
m
pe

Figure 17 The Iris in Pupillary Dilation (TEM)

Each posterior epithelial cell is separated from the next by a deep groove (g). There is always a clear space between the basement membrane (bm) of the posterior epithelial cells and the cell membrane. The cell membrane is deeply and complexly infolded (ci). The cell infoldings interdigitate in various planes. Right around the nucleus (Npe) there is a thin rim of cytoplasm containing mainly mitochondria (m) and a few dense bodies (db). In the anterior cytoplasm, there are some bundles of intracellular filaments (if) cascading down. The nucleus of the posterior epithelial cells is located in the middle of the cell. Dense heterochromatin is mainly associated with the nuclear envelope. There is a clear peri-nuclear space present (arrow). The nucleus is indented and the indentation is occupied by cell cytoplasm (*). x 21,300

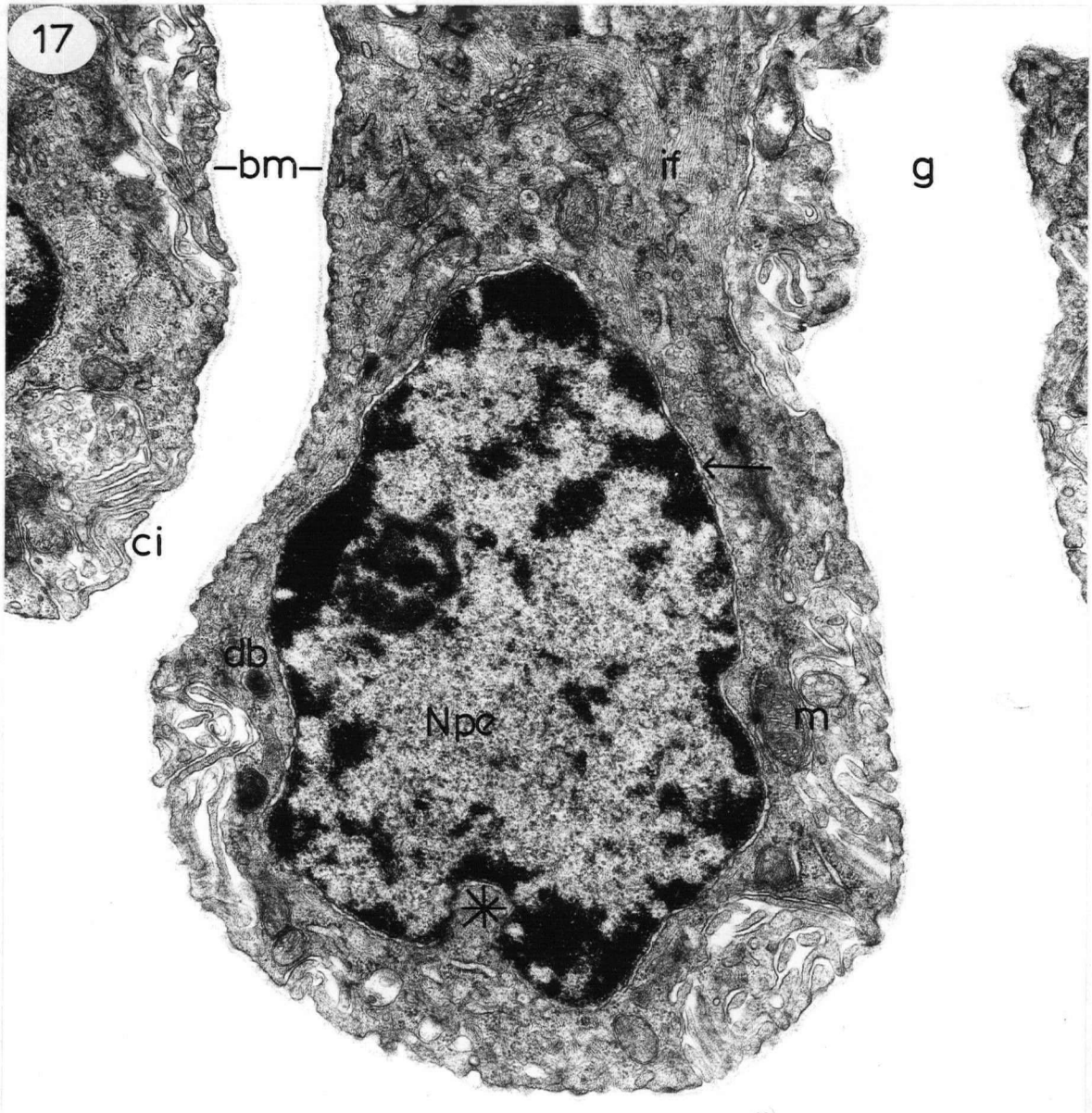

17
–bm–
if
g
ci
db
Npc
m

Figure 18 The Iris in Pupillary Dilation (TEM)

The basement membrane (bm) of the posterior epithelial cell does not follow the contours of the cell infoldings (ci). The cell infoldings are extensive. Where the cell infoldings are absent, there are many mitochondria (m) with delicate cristae. Some free ribosomes (r) are seen. A large bundle of intracellular filaments (if) forms a hammock around the nucleus of the posterior epithelial cell (Npe). The indentations of the nucleus are filled with tongues of cytoplasm (*). x 26,600

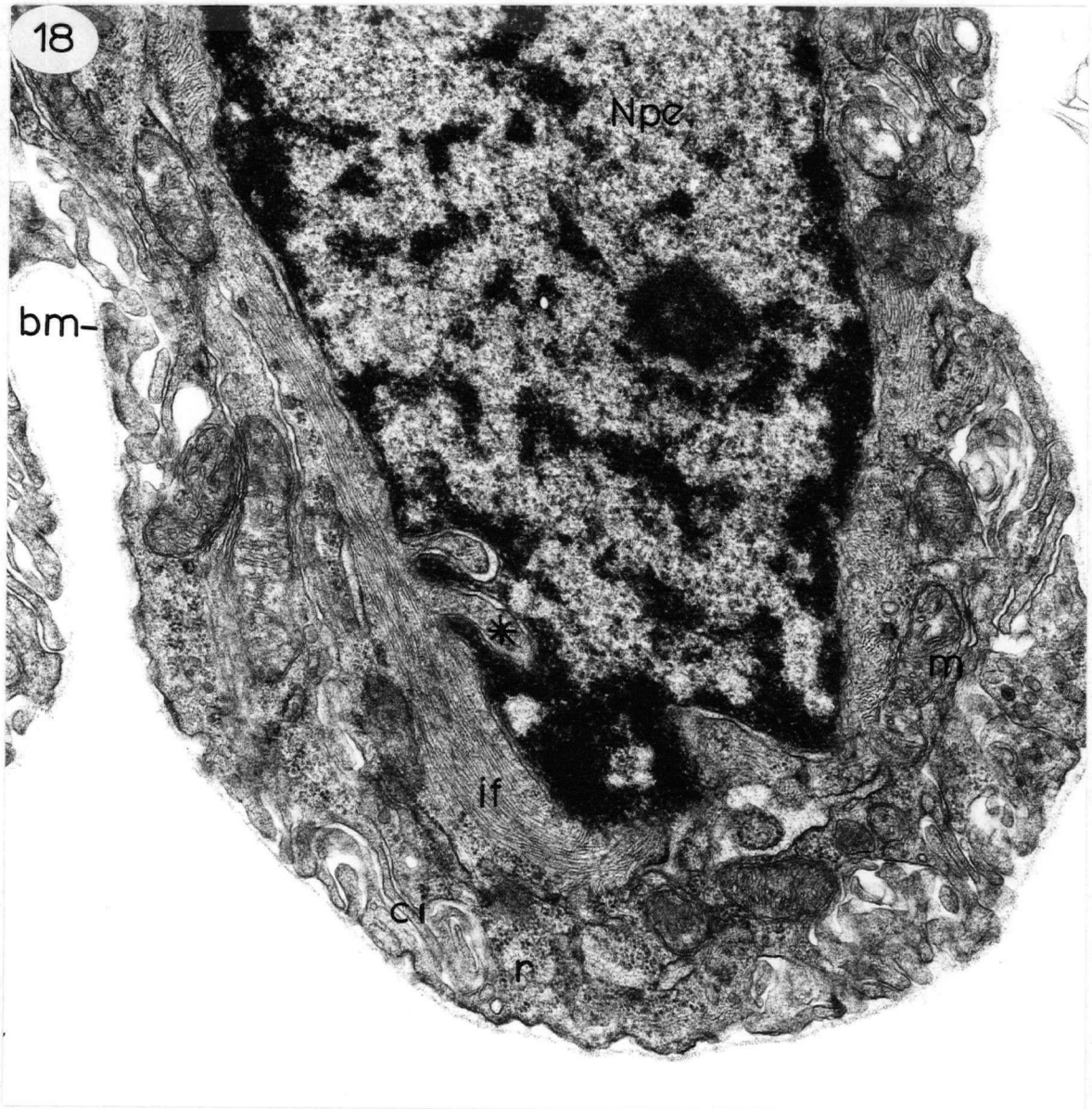

18
Npc
bm
*
m
if
ci
n

Figure 19 The Iris in Pupillary Dilation (TEM)

A large (cp2) and small (cp1) cytoplasmic process of the posterior epithelium are shown. A golgi apparatus (go), and endoplasmic reticulum (er), free ribosomes (r), mitochondria (m), vesicles (v) and dense bodies (db) are seen in the cell cytoplasm. Intracellular filaments sectioned longitudinally (if1) and in cross-section (if2) are present. The intracellular filaments form a large discrete bundle (if2). The basement membrane (bm) is loosely adherent to the posterior surface of the cells. A very clear peri-nuclear space (arrow) is distinctly seen. x 21,300

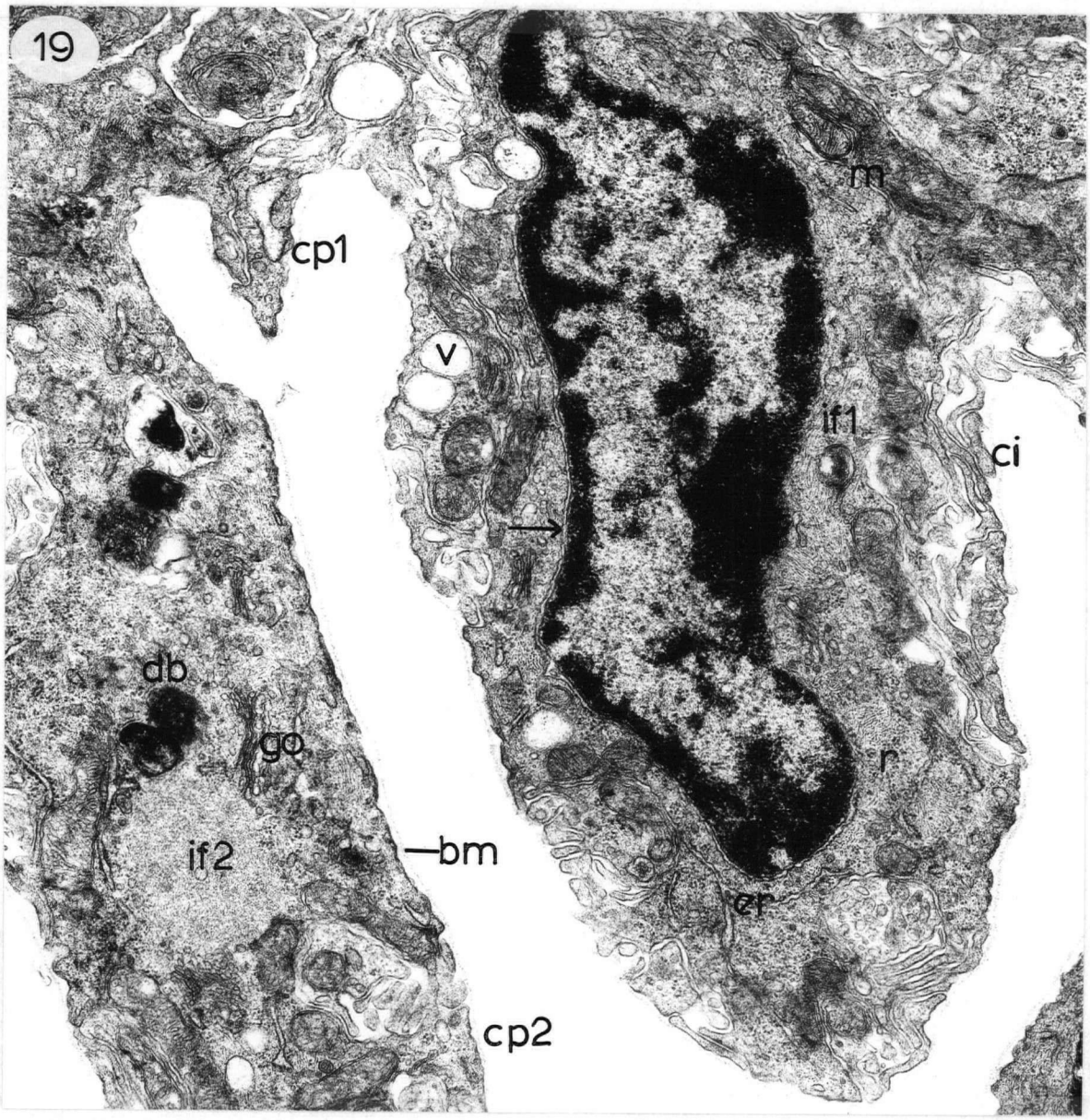

19
cp1
v
m
if1
ci
db
go
r
if2
bm
er
cp2

Figure 20 The Iris in Pupillary Dilation (TEM)

The dilator cells (d) are so complexly interdigitated and interrelated with each other that the cell boundaries are not observable. The nucleus (Nd) has a highly irregular and indented outline. Dense heterochromatin is mainly associated with the nuclear envelope. The cytoplasm is very electron dense, especially in the region of the dilator hillocks (dh) and dilator processes (dp). All along the stromal boundary of the dilator, hillocks of dilator material are seen. From these hillocks arise a number of complex and profusely branching series of dilator processes which protrude into the stroma. The basement membrane (bm) of the dilator follows the contour of the dilator processes loosely. There is a clear space between the basement membrane and the cell membrane of the dilator cells. Small pinocytotic vesicles (pv) are found in the stromal poles of the dilator cells. Larger vesicles are found deeper in the cell cytoplasm.

The iris stroma consists of stromal cells (sc), blood vessels (bv) and a network of collagen fibers (co). The collagen fibers are sectioned in all planes. Occasionally, the collagen fibers appear to be closely associated with the basement membrane (arrow). The stromal blood vessel shows microvillous processes of the endothelium (mi). x 8,900

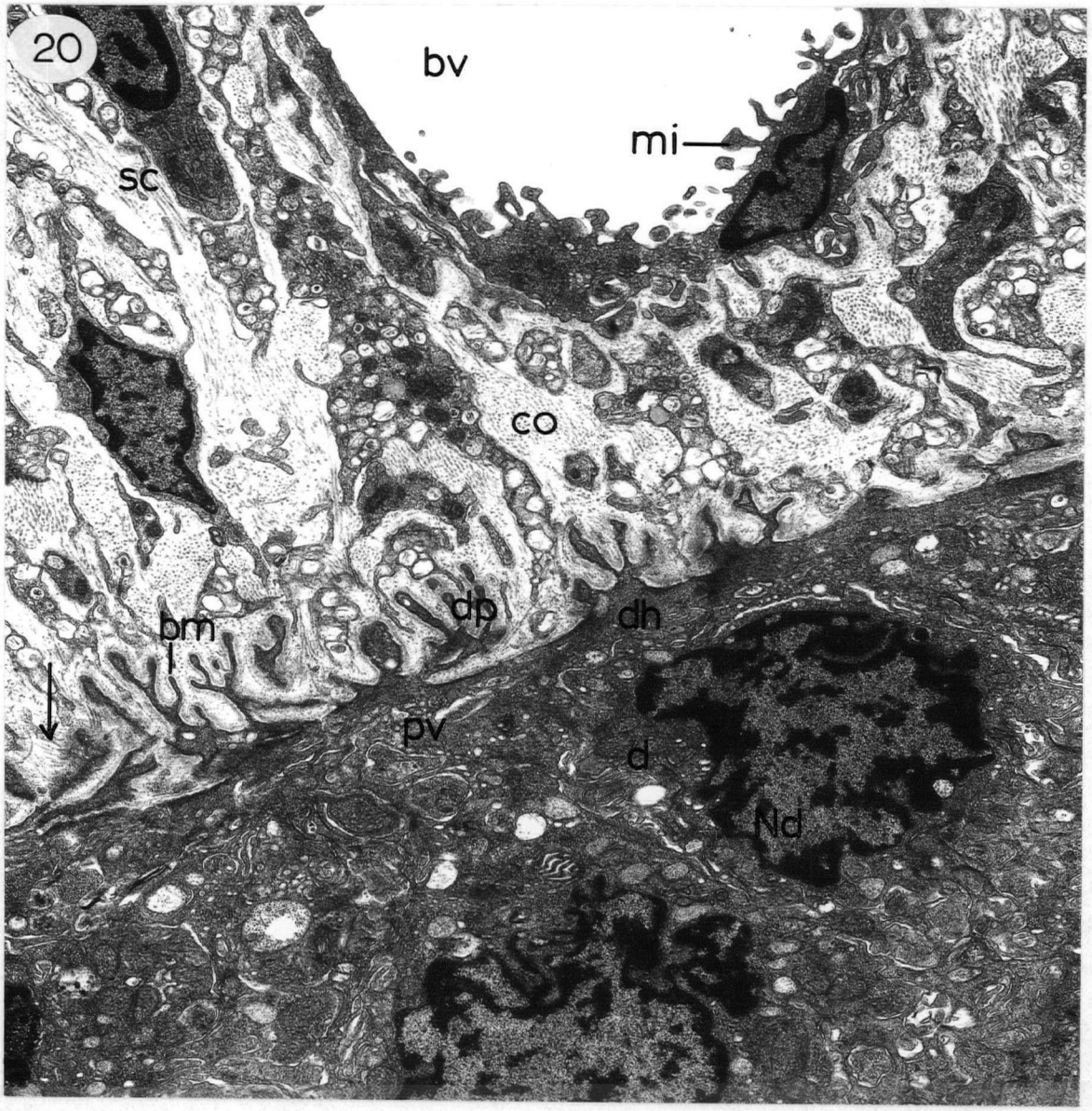

20
bv
mi
sc
co
dp
dh
bm
pv
d
Nd

Figure 21 The Iris in Pupillary Dilation (TEM)

The dilator cytoplasm is very dense. There are few organelles in the stromal parts of the cells except for some mitochondria (m) and vesicles (v). Some myofilaments are seen in one of the dilator hillocks (*). The dilator interdigitations (di) are complex and it is difficult to decipher whether the interdigitations belong to two different cells or to parts of the same cell. The dilator processes may be simple protrusions of the dilator into the stroma (dp1), or they may arise from a dilator hillock (dh) and branch (dp2). The basement membrane (bm) loosely follows the outlines of the dilator processes. The endothelial cells of the blood vessels have numerous microvilli (mi). Stromal cells and collagen fibers (co) make up part of the stroma. x 21,300

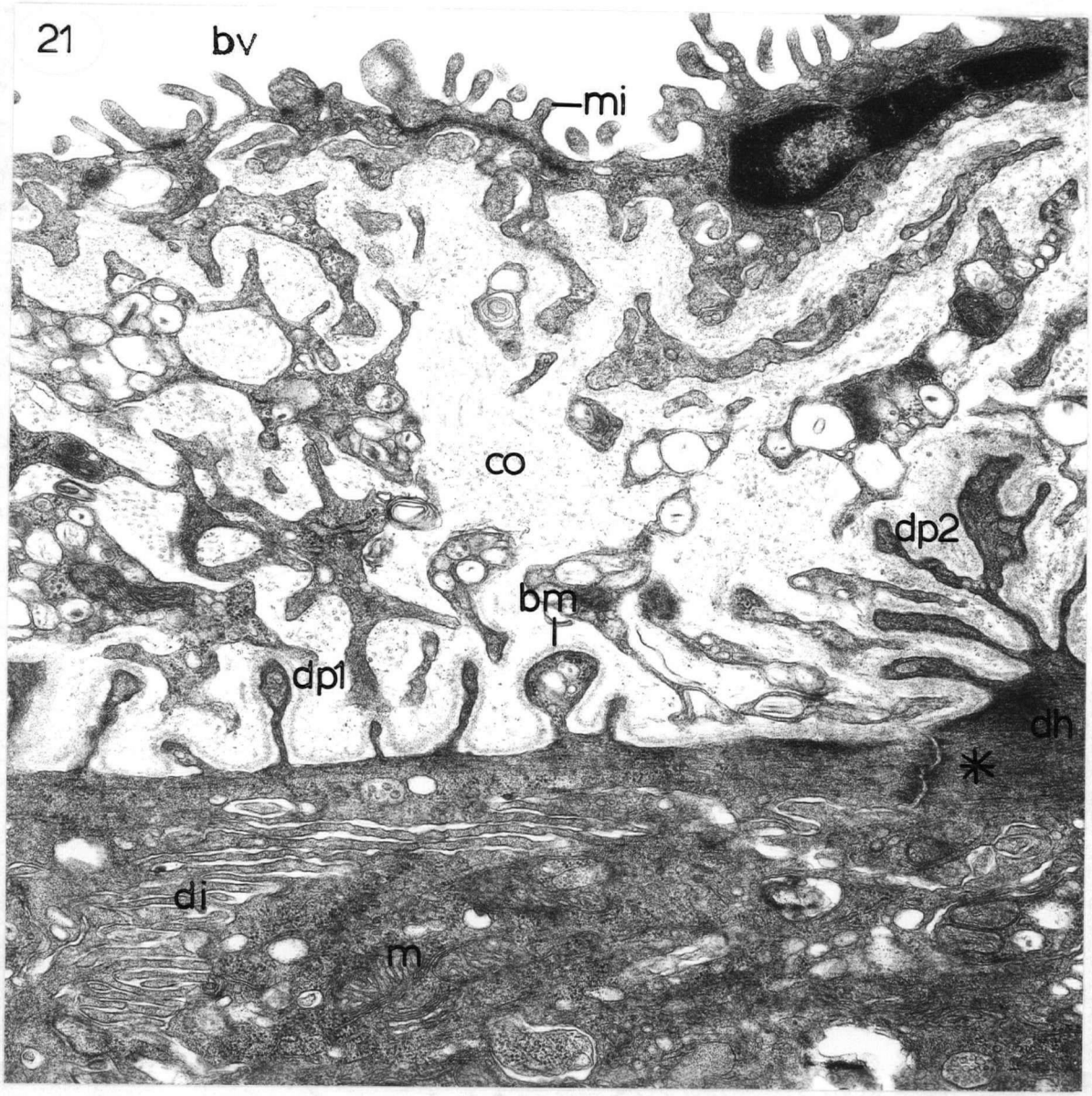

21
bv
mi
co
dp2
bm
dp1
dh
*
di
m

Figure 22 The Iris in Pupillary Dilation (TEM)

There are numerous small pinocytotic vesicles in the dilator processes (pv1) as well as in the dilator hillock (pv2). The characteristic features of the dilator processes (dp) and the dilator hillocks (dh) have been described. The nuclei (Nd) show irregular outlines. Apparent fusions of the cell membranes of two adjacent dilator cells are seen (arrow). The well-fixed mitochondria (m) look delicate. x 21,300

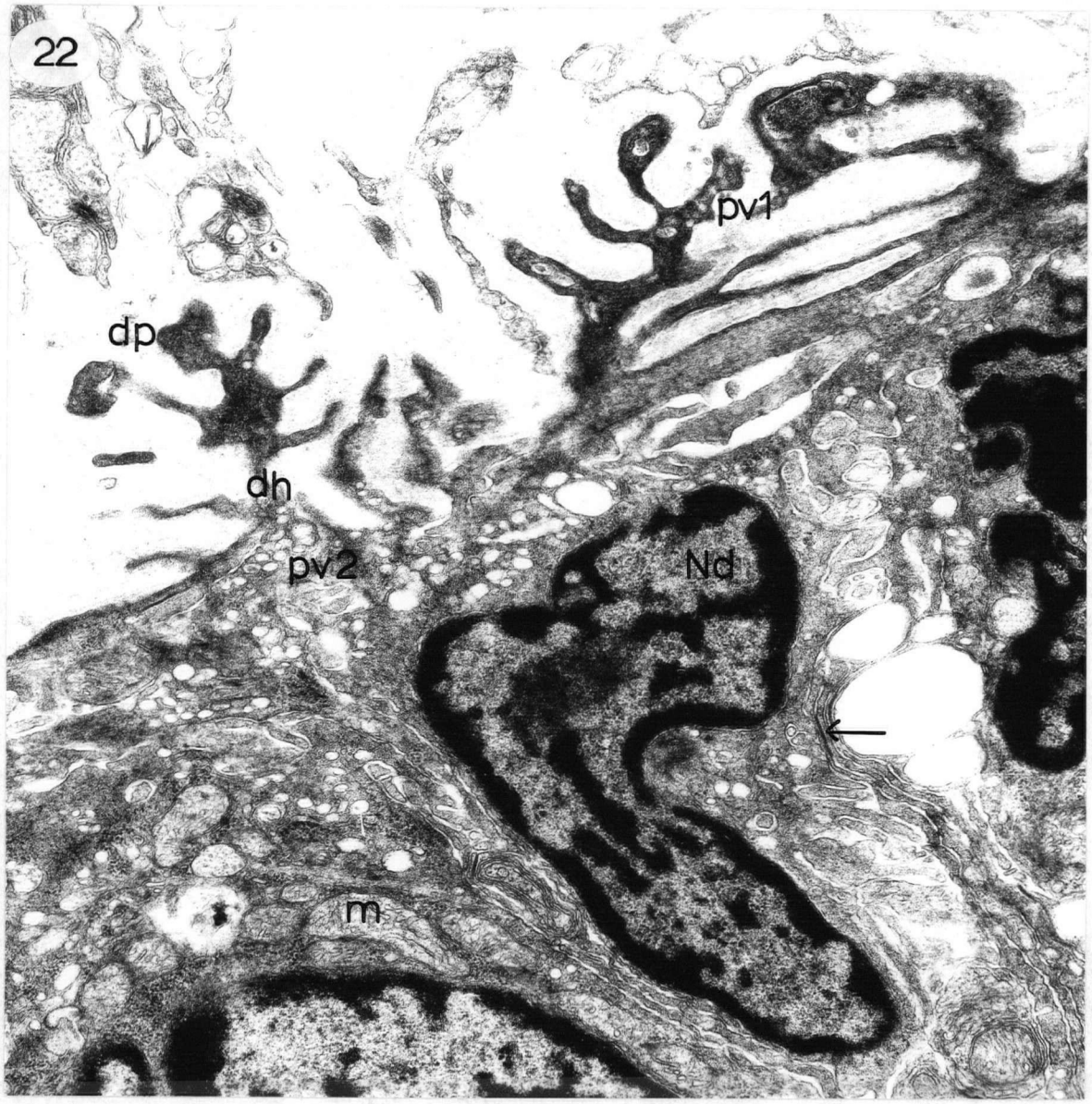
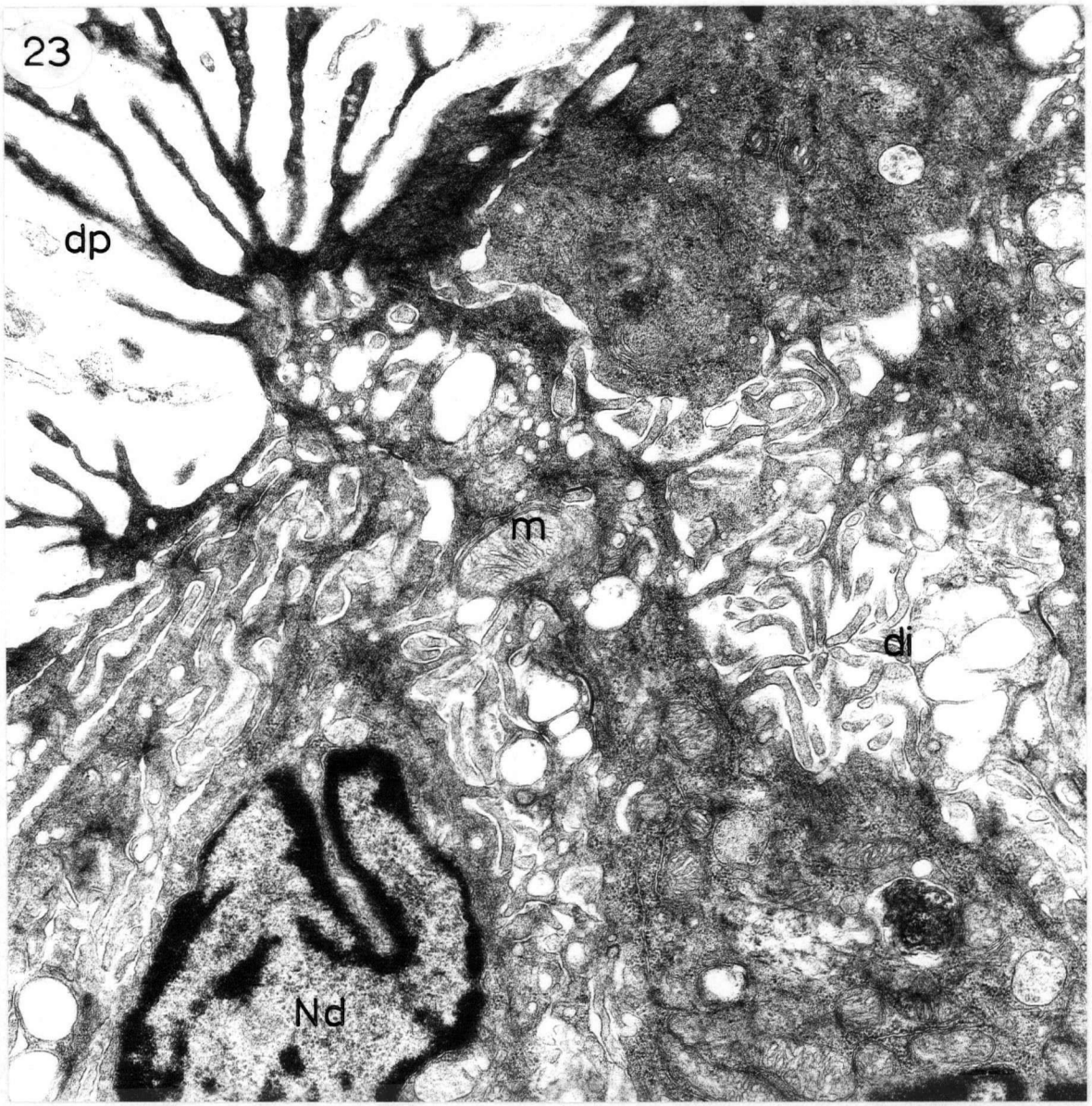

22
pv1
dp
dh
pv2
Nd
m

Figure 23 The Iris in Pupillary Dilation (TEM)

The interdigitations of the dilator cells (di) are clearly shown. Microvillous cytoplasmic cell processes interdigitate loosely in a complicated three dimensional network. The nucleus of the dilator (Nd), mitochondria (m) and pinocytotic vesicles are present in the cell cytoplasm. The characteristic branching of the dilator processes (dp) is clearly shown.
x 21,300


23
dp
m
di
Nd

3. The Iris in Pupillary Constriction (Figures 24-30)

In pupillary constriction, the posterior epithelial and dilator layers are much thinned out. The relationships between the two layers are more clearly seen. Certain changes from that present in pupillary dilation are observed.

The posterior surface of the iris is relatively smooth (Figure 24), except for an occasional posteriorly directed bulge (Figures 25-28). There are no grooves in between the posterior epithelial cells so that one cell cannot be separated from the next. The basal cell membranes appear to show as many infoldings as in pupillary dilation. The basement membrane forms a straight covering for the basal surfaces of the posterior epithelial cells. Within the epithelial cells themselves, the usual organelles are present, including a large number of vesicles which are distributed throughout the cell. The orientation of the organelles within the cells do not appear to be organised in any particular fashion. However, the filaments within the cell cytoplasm seem to be aligned parallel to the length of the cells (Figures 24-25, 27-28). The filaments are longitudinally sectioned. They run in bundles which may branch (Figures 24, 27) and criss-cross (Figure 27). These filaments are quite prominently seen in some cells and less so in others. The filaments are usually located in the posterior portions of the cells (Figures 24, 25, 27, 28). Rarely are filaments seen in the anterior apical portions of the cells near to the boundary with the dilator. Sometimes, densities of the cell membranes are seen (Figure 28). Occasionally, a bundle of filaments appear to attach to the densities of the cell membrane (Figure 28).

Remarkable changes are seen with respect to the shapes of the nuclei of the posterior epithelium and dilator when compared to that observed during pupillary dilation. The nuclei of the posterior epithelial cells

are oval or elongated (Figures 25, 26, 29). The length of the nuclei are oriented parallel to the length of the cells and the posterior surface of the iris. The nuclear outline is relatively smooth. There are no indentations of the nuclear envelope.

At the boundary between the posterior epithelium and the dilator, there are a number of cell junctions. The cell membranes may only come close together (Figure 25), or the membranes may appear to fuse (Figure 24, 26) without any specialisation of the adjacent cytoplasm. Occasionally, the cell membranes are in close apposition and there is an apparent increased density in the surrounding cytoplasm (Figure 24). However, desmosomes are not observed in the rat iris. Oftentimes, in this boundary region, there are a number of vesicles (Figures 24, 26, 29). Also, microvillous cytoplasmic processes from both the posterior epithelial and dilator layers interdigitate with each other (Figures 25, 29). In many instances, it seems as if the interdigitations occur in a plane parallel to the posterior surface of the iris.

The dilator is also thin (Figures 24-26, 29). There is little cytoplasm in comparison to the size of the nucleus. The usual organelles are present. The nucleus of the dilator becomes long and thin and the length of the nucleus is also parallel to the length of the cells. The nuclear outline is smooth and usually shows no indentations (Figures 24, 30). Occasionally, the nuclear envelope may be indented and a spit of cytoplasm is seen occupying the space (Figure 25). But when this occurs, the nuclear indentation is parallel to the posterior surface of the iris (Figure 25).

The muscular portion of the dilator cells is confined to a small region to the basal poles of the cells (Figures 24-26, 29, 30). The sarcoplasm is not as dense as in pupillary dilation. The myofilaments of the dilator are seen running along the length of the stromal poles of the cells

(Figures 24-26, 29, 30). Sometimes, bundles of filaments are seen towards the apical poles of the cells (Figure 29). The dilator may consist of one layer (Figures 25, 26, 29), or it may consist of stacks of a few layers which show interdigitations with each other (Figures 24, 30). The nucleus of the dilator layer is usually surrounded by cell cytoplasm and cell organelles (Figures 24-26). Sometimes, however, the nucleus is embedded in myofilaments (Figure 30). Numerous mitochondria are often present in the myofilamentous region. In addition, there are lots of pinocytotic vesicles within the sarcoplasm and along the cell membranes (Figure 30). Some of the pinocytotic vesicles may be open to the outside.

The anterior stromal surface of the dilator is normally quite smooth (Figures 24-26). Right at the boundary with the stroma, the cell membrane sometimes appears dense (Figures 24-26). Arborescent dilator processes are very rarely seen (Figure 29). They are only present in isolated spots and are very simple in configuration. Small and simple dilator processes jut into the stroma. They do not show the complex branching that is observed in pupillary dilation. If there is a pile up of muscle spurs (Figure 30), the anterior surface of the dilator is no longer smooth.

The basement membrane of the dilator is not as easily visible (Figures 24-26) except where the small dilator processes protrude into the stroma (Figure 29). Collagen fibers are also not as apparent as in pupillary dilation. When present, they tend to be oriented parallel to the length of the iris.

Figure 24 The Iris in Pupillary Constriction (TEM)

Both the posterior epithelial (pe) and dilator (d) layers are thin. The posterior surface of the iris is smooth and is covered by a loosely adherent basement membrane (bm). The cell infoldings (ci) of the posterior epithelium are present. In the cytoplasm of the posterior epithelial cells, there are mitochondria (m), vesicles and intracellular filaments (if). The posterior epithelium and dilator are joined by apparent fusions of the cell membranes (double arrows). Occasionally, the cell membranes are close together and there is some modification of the immediately adjacent cytoplasm (single arrow).

Almost all of the dilator cell is occupied by the cigar-shaped nucleus (Nd). The anterior surface of the dilator is a smooth line. x 21,600

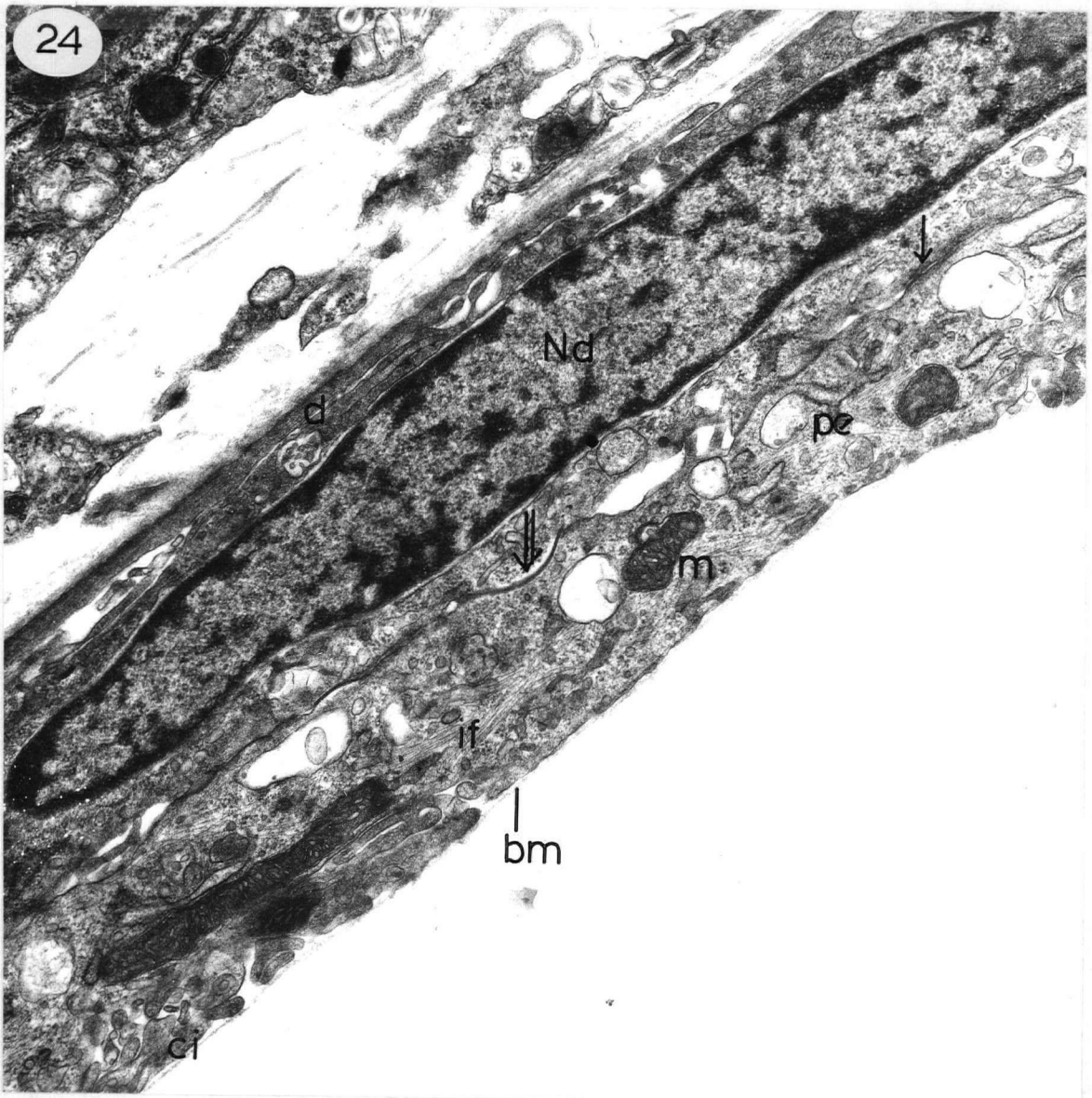

24
Nd
d
pe
m
if
bm
ci

Figure 25 The Iris in Pupillary Constriction (TEM)

There is an occasional bulge of the posterior surface of the iris. Cell infoldings (ci) are numerous. Intracellular filaments (if) are found in the posterior portion of the cells and are closely associated with the nucleus (Npe). The nucleus of the posterior epithelium is oval in shape and has a smooth outline. The nucleus of the dilator (Nd) shows a deep indentation parallel to the length of the iris. Microvillous cytoplasmic processes from both layers interdigitate with each other (*). The contractile myofilamentous portion of the dilator (arrow) is only a thin strip at the stromal pole of the cell. There may be slight condensation of the cell membrane at the stromal boundary. The basement membrane (bm) is perceptible. It is straight, as is the stromal surface of the dilator. x 21,600

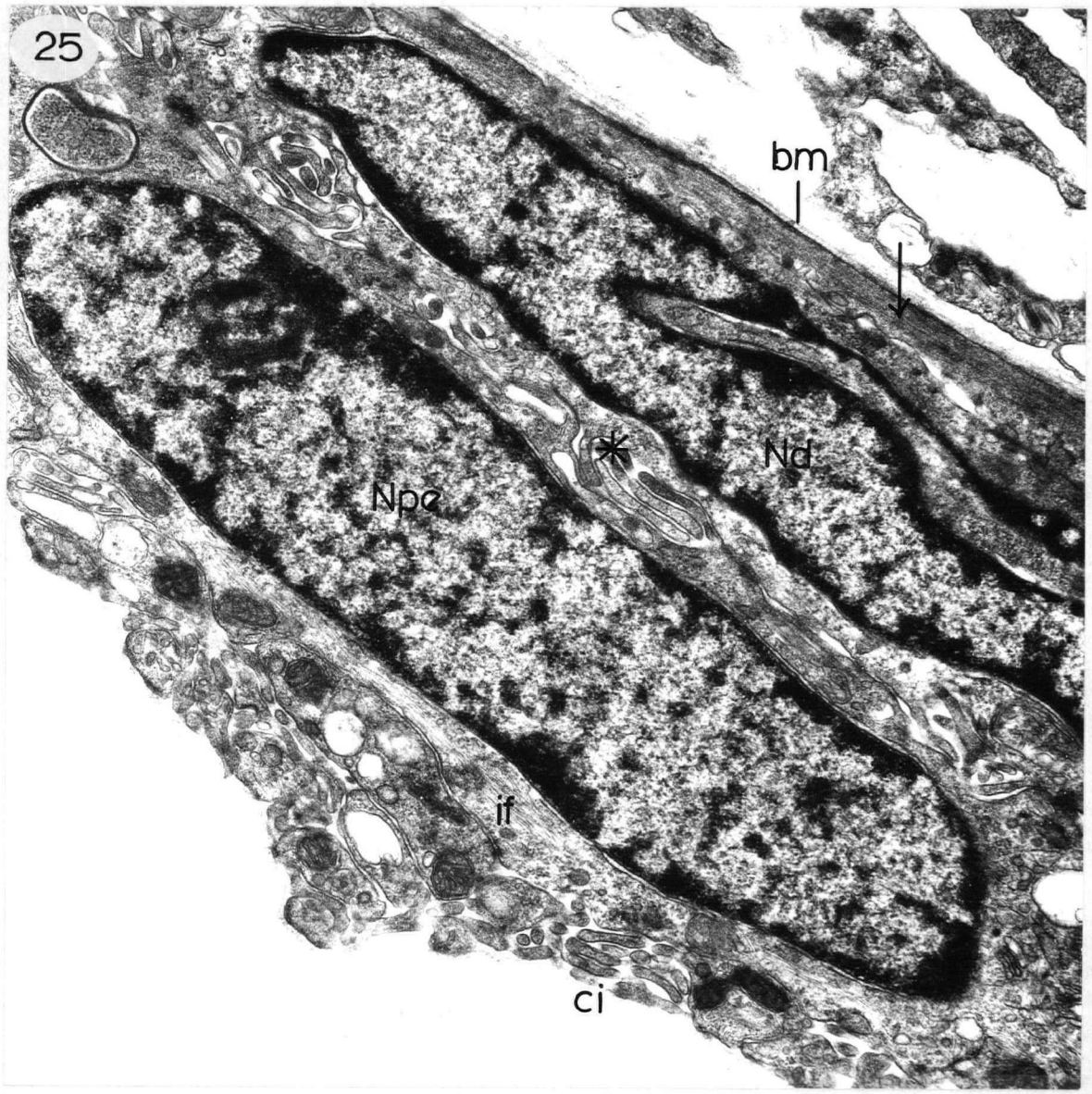
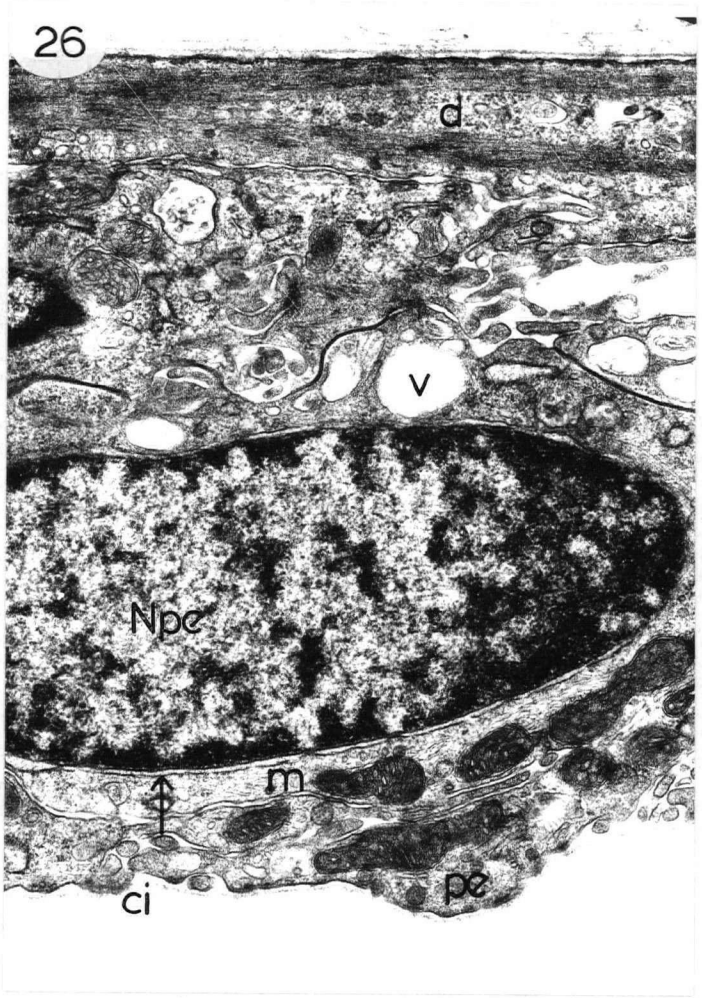

25
bm
Nd
Npc
*
if
ci

Figure 26 The Iris in Pupillary Constriction (TEM)

The nucleus of the posterior epithelium (Npe) is smooth and oval in shape. There is a clear peri-nuclear space (arrow). Mitochondria (m) and cell infoldings (ci) are confined to the posterior portions of the cells. The cell membranes of the posterior epithelium (pe) and of the dilator (d) fuse. Large vesicles (v) are present in this boundary zone. The stromal surface of the dilator is a smooth dense line. Myofilaments in diverging bundles are present in the stromal cytoplasm.
x 21,600

Figure 27 The Iris in Pupillary Constriction (TEM)

Cell infoldings (ci) occupy most of the superficial parts of the posterior aspect of the posterior epithelium (pe). In the posterior cytoplasm, there are large bundles of intracellular filaments (if) which run parallel to the posterior surface of the iris. The filaments may branch or they may come together and intermesh. x 27,000


26
d
v
Npe
m
ci
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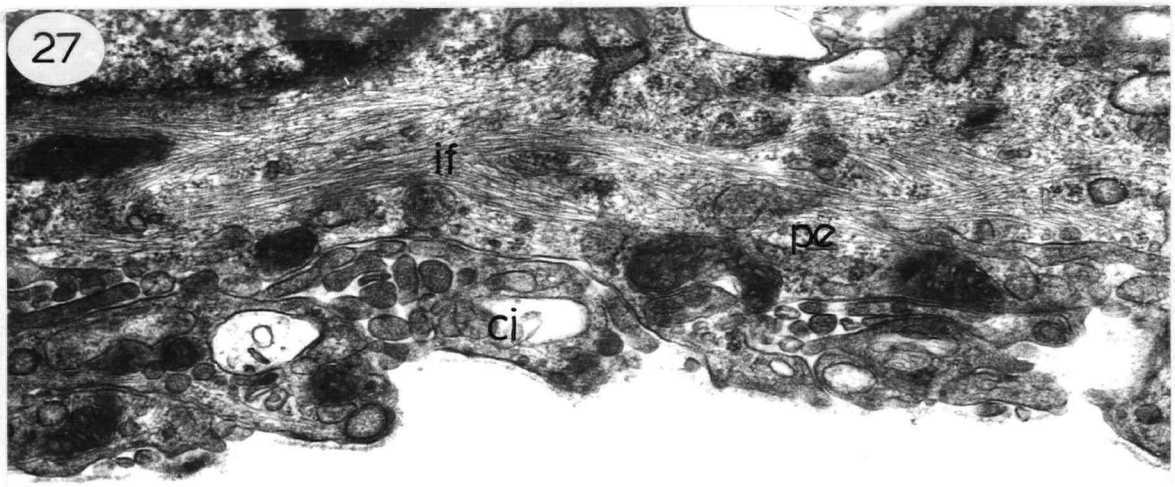
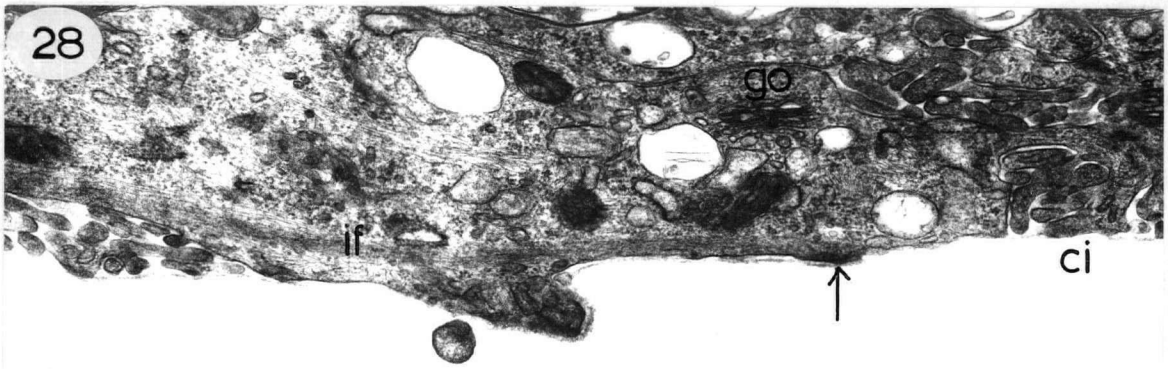

27
if
pe
ci

Figure 28 The Iris in Pupillary Constriction (TEM)

The intracellular filaments (if) run in bundles parallel to the posterior surface of the iris. They sometimes appear to attach to dense areas on the cell membrane of the posterior epithelium (arrow). A golgi apparatus (go) and cell infoldings (ci) are also seen. x 21,600

Figure 29 The Iris in Pupillary Constriction (TEM)

The anterior surface of the dilator (d) is not smooth. There are, instead, a few dilator processes (dp). These are relatively simple protrusions of the dilator into the stroma. The myofilaments of the dilator are seen as longitudinal bundles running along the length of the cells (*). The myofilaments are found in the stromal as well as in the deeper parts of the cell. The basement membrane (bm) is loosely adherent to the dilator layer. There appears to be a wider space between the basement membrane and the dilator processes than between the basement membrane and the smooth part of the dilator. x 21,600


28
go
if
ci

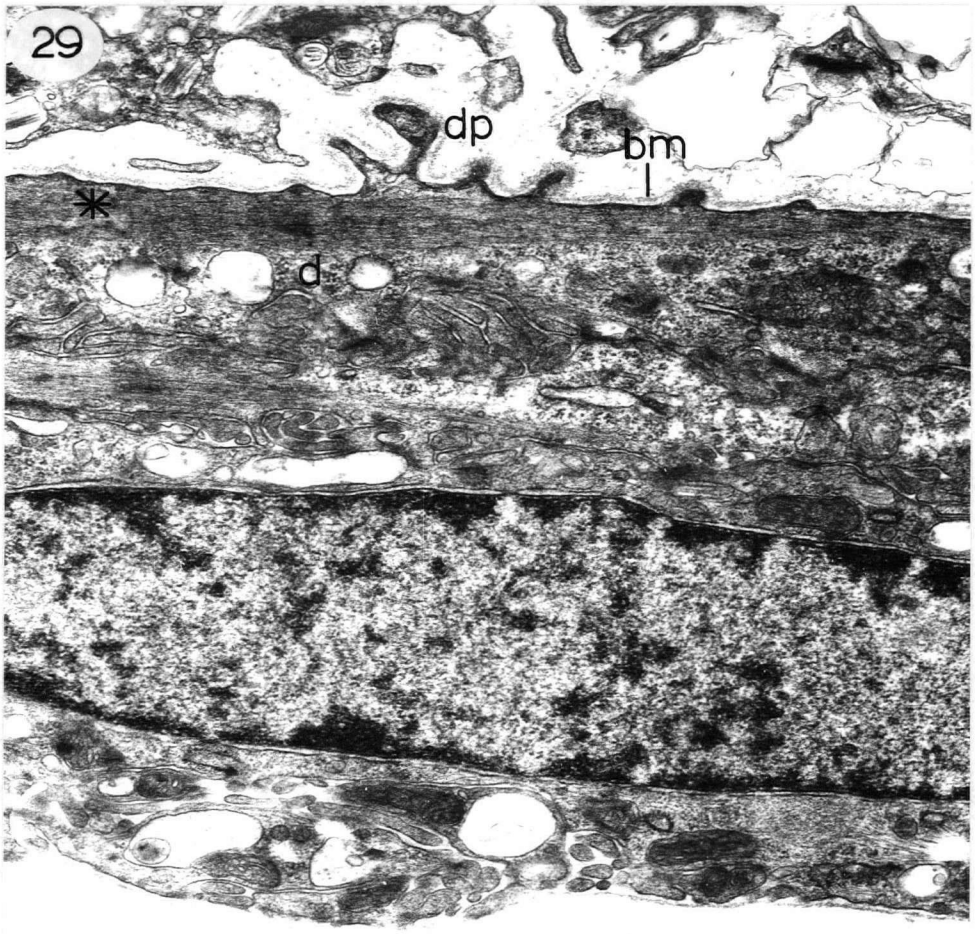

29
dp
bm
*
d

Figure 30 The Iris in Pupillary Constriction (TEM)

Occasionally, there are stacks of dilator muscle spurs filled with myofilaments, mitochondria (m) and pinocytotic vesicles (pv). Some of the pinocytotic vesicles are open to the intercellular space (arrows). The elongated nucleus of the dilator (Nd) is embedded in myofilaments. x 21,600


30
pv
m
Nd

D. A Scanning Electron Microscopic Study of the Rat Iris in Pupillary Dilation and Constriction

1. General

The iris tissues are either only fixed in glutaraldehyde, or in glutaraldehyde followed by post-fixation in osmium tetroxide. Then, both the camphene method (Watters and Buck, 1971) and the critical point drying method (Boyde and Wood, 1969; Smith and Finke, 1972) are used to prepare the specimens for examination with the scanning electron microscope. It is found that these differing methods of preparing the iris tissues do not result in different images obtained with the scanning electron microscope.

2. The Posterior Surface of the Iris in Pupillary Dilation (Figures 31-38)

In a low magnification scanning electron micrograph, the posterior surface of the iris is seen as a circumferentially grooved surface (Figure 31). Peripherally, the ciliary processes overhang the root of the iris. At times, zonular fibers attaching the ciliary processes to the lens capsule are observed on the posterior iris surface (Figure 32). At the pupillary margin, the posterior surface is relatively flat and smooth (Figure 31). Occasionally, there may be some hints of a few shallow ridges and grooves which are not distinctly visible (Figure 31). The rest of the iris, though, is deeply grooved. The ridges represent rows of posterior epithelial cells (Figure 32). The ridges are not uniformly concentrically arranged around the pupil. Rather, a ridge may bifurcate, taper down, or blend with another ridge of cells (Figures 33, 34). These epithelial ridges appear rounded, bulging into the posterior chamber. An amorphous basement membrane layer covers all of the posterior surface of the epithelial cells so that

the cell boundaries are obscured. According to Hansson (1970), the epithelial cells are spindle-shaped or polyhedral. However, with our preparations of the iris in pupillary dilation, the margins between individual cells in an epithelial ridge are not discernible. In man, the cells within any one ridge are arranged in a staggered fashion (Fine and Yanoff, 1972). The basement membrane covering the posterior epithelial cells is not smooth but shows bumps and large and small corrugations. This unevenness of the basement membrane may give a suggestion of the numerous infoldings, interdigitations and grooves of the posterior surface of the individual epithelial cells, as is readily verified in meridional sections of the rat iris in pupillary dilation observed with the light microscope. However, the basement membrane does not extend deeply into the row of cells but covers them superficially. Occasionally a much larger ridge is seen extending from the base of the ciliary processes to midway in the iris. This ridge is radially rather than circumferentially oriented (Figure 34) and it is larger than the circumferential ridges. The circumferential ridges do not extend over this bump but skirt around it, or there might be a break in the continuity of the epithelial ridges (Figure 34).

The grooves in between the epithelial ridges are deep and of varying widths and depths. Like the epithelial ridges, the grooves also bifurcate or blend into one another (Figures 33, 34). At the large ciliary-iris ridge or process (as it will be referred to here), both the epithelial ridges and the intervening grooves are absent.

The epithelial ridges are not always rounded posteriorly. Sometimes they are a little flattened and cordlike (Figures 35, 36). In lesser degrees of dilation, the distinctions between the epithelial ridges and grooves are not as clear-cut although they are still visible (Figures 37, 38). The ridges are not as high and the grooves not as deep. The

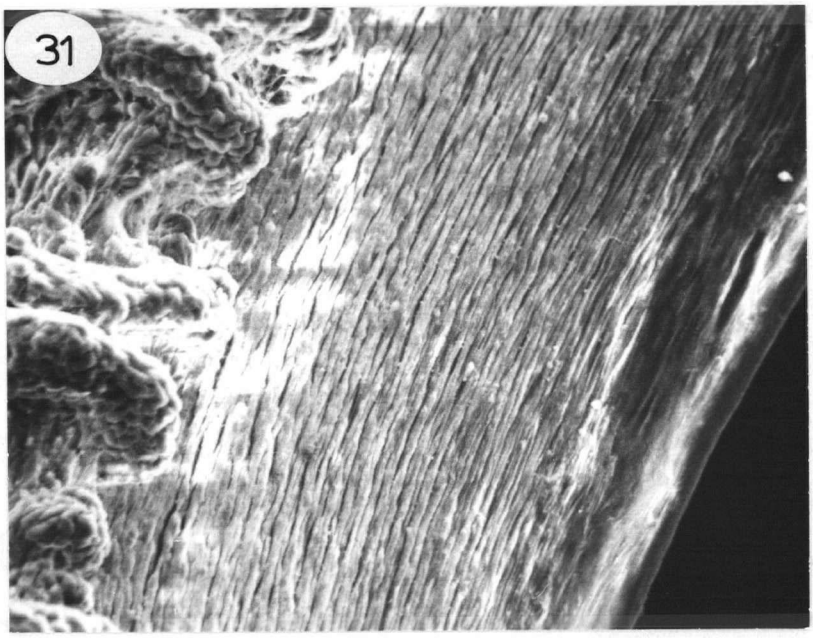
crinklings of the basement membrane always follow the circumferential direction of the ridges and grooves. Occasional spindle shaped bulges are seen along the ridges. These probably represent the nuclei of the posterior epithelial cells (Figure 37). Sometimes wandering cells, presumably white blood cells, are seen attached to the posterior surface of the iris (Figure 38). They are large and may span one or two ridges of epithelial cells.

Figure 31 The Posterior Surface of the Iris in Pupillary Dilation (SEM)

In a low magnification scanning electron micrograph, most of the posterior surface of the iris, except for the pupillary margin, is circumferentially grooved. Around the pupillary margin, the surface is relatively smooth but for a few shallow, circumferentially disposed striations. The ciliary processes overhang the periphery of the iris. x 280

Figure 32 The Posterior Surface of the Iris in Pupillary Dilation (SEM)

Zonular fibers are sometimes encountered. The epithelial ridges represent rows of posterior epithelial cells covered by a basement membrane. These ridges are rounded. The ridges may bifurcate, taper down or join with adjacent ridges of epithelial cells. The ridges are separated by grooves. x 500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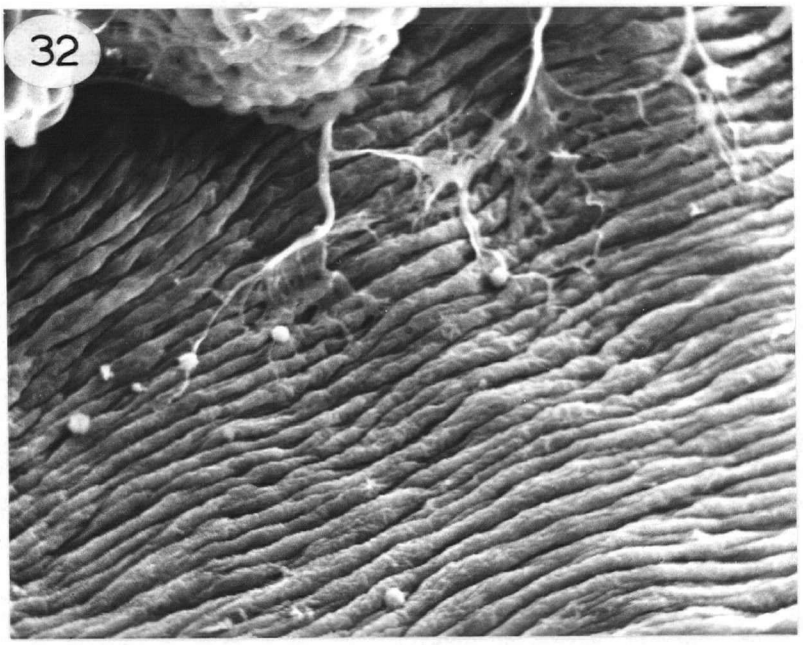
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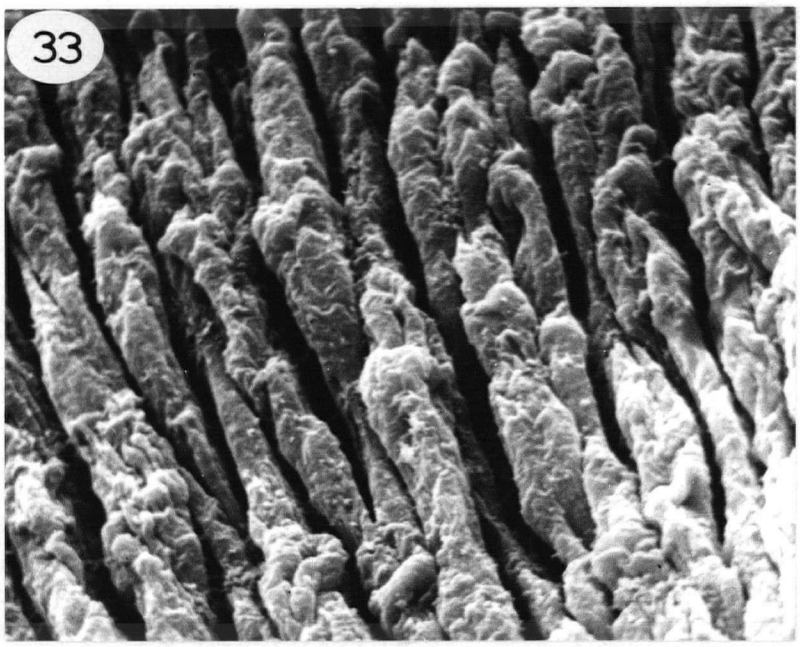
Figure 33 The Posterior Surface of the Iris in Pupillary Dilation (SEM)

At a higher magnification, the epithelial ridges are found to be separated by deep grooves. The basement membrane covering the epithelial ridges dips deep into the grooves. There are bumps and corrugations of the basement membrane giving a suggestion of the infoldings and interdigitations of the posterior epithelial cells. x 2,100

Figure 34 The Posterior Surface of the Iris in Pupillary Dilation (SEM)

A ciliary-iris process is seen here as a radial ridge perpendicular to the circumferential direction of the posterior epithelial ridges. The radial ridge is much larger and higher than the posterior epithelial ridges. The continuity of the rows of posterior epithelial cells is broken or they may skirt around the edge of the radial ridge. x 1,000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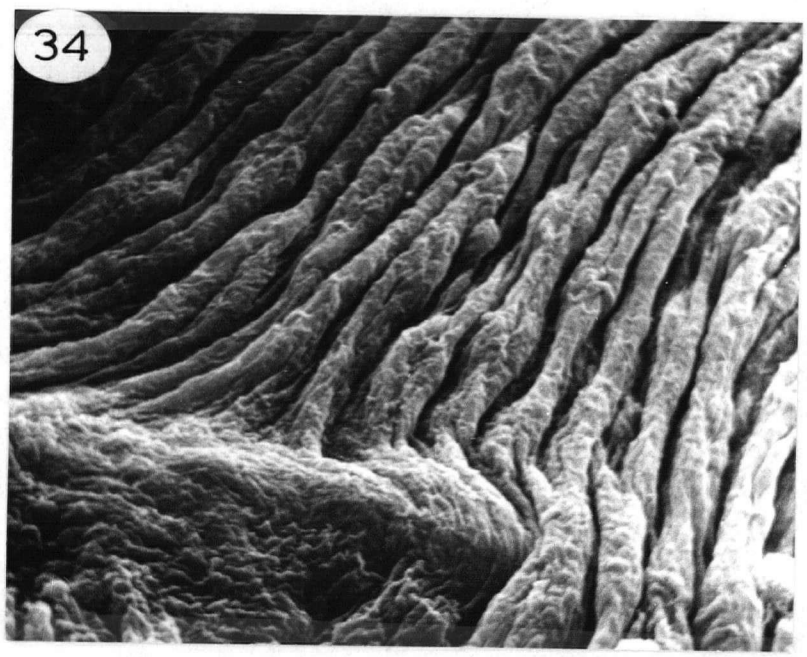
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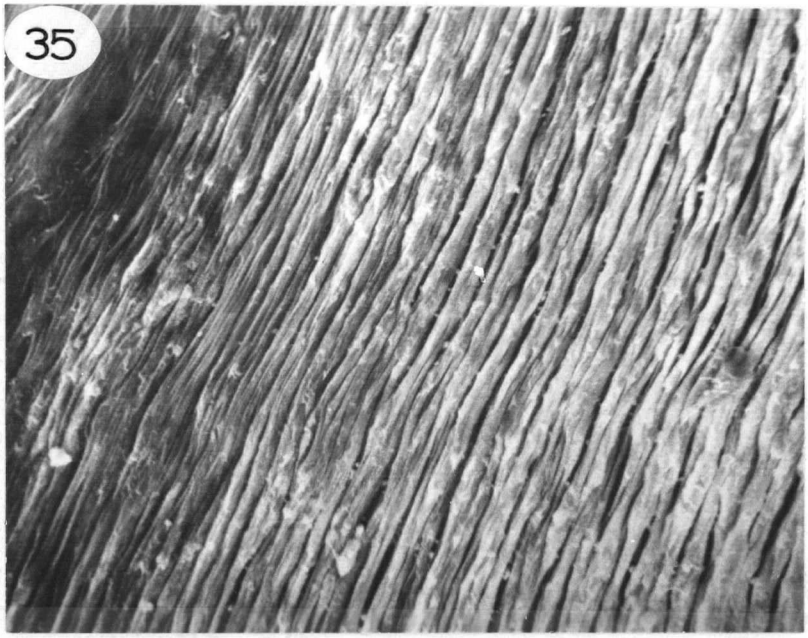
Figure 35 The Posterior Surface of the Iris in Pupillary Dilation (SEM)

The circumferential epithelial ridges are more cord-like rather than rounded (compare with Figures 32-34). x 620

Figure 36 The Posterior Surface of the Iris in Pupillary Dilation (SEM)

The grooves in between the posterior epithelial ridges are deep and narrow. The epithelial ridges have a more cord-like appearance. The basement membrane covering the epithelial ridges is relatively smooth. Occasional bulges along the row of cells may be due to the outward bulging of the nuclei.
x 1,260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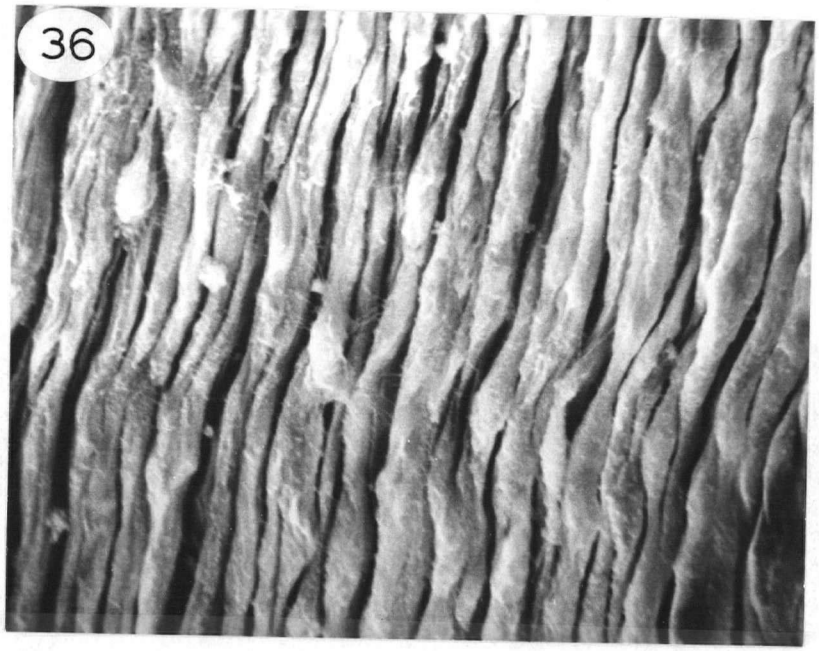
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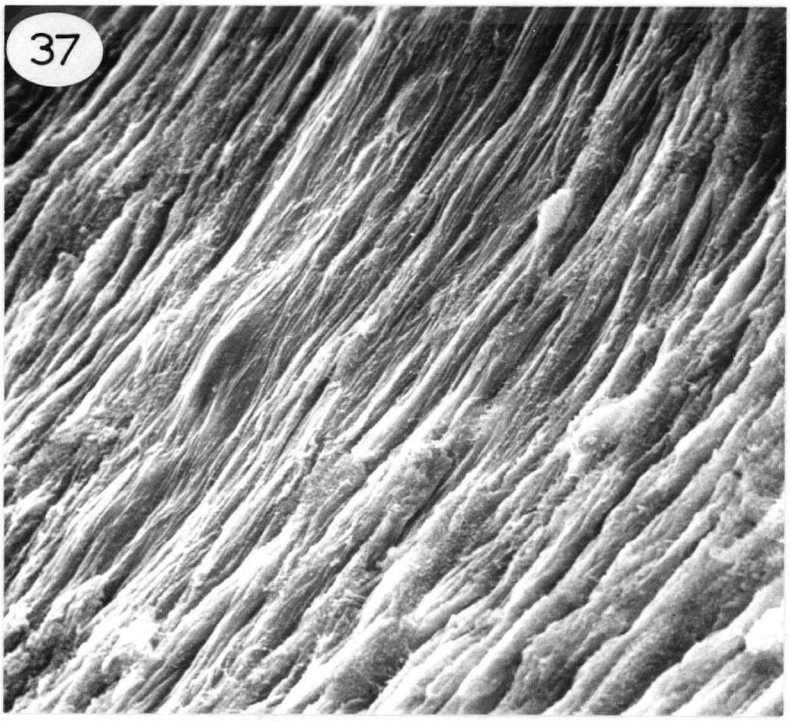
Figure 37 The Posterior Surface of the Iris in Pupillary Dilation (SEM)

The epithelial ridges are not as high nor are the intervening grooves as deep. The continuity of the epithelial ridges is not as obvious. There are occasional spindle bulges of the nuclei of the posterior epithelial cells. The crinklings of the basement membrane covering the epithelial ridges are also circumferentially oriented. x 780

Figure 38 The Posterior Surface of the Iris in Pupillary Dilation (SEM)

Large wandering cells, presumably white blood cells, are seen on the posterior surface of the iris. The epithelial ridges are of varying heights. The ridges divide and rejoin. x 1,560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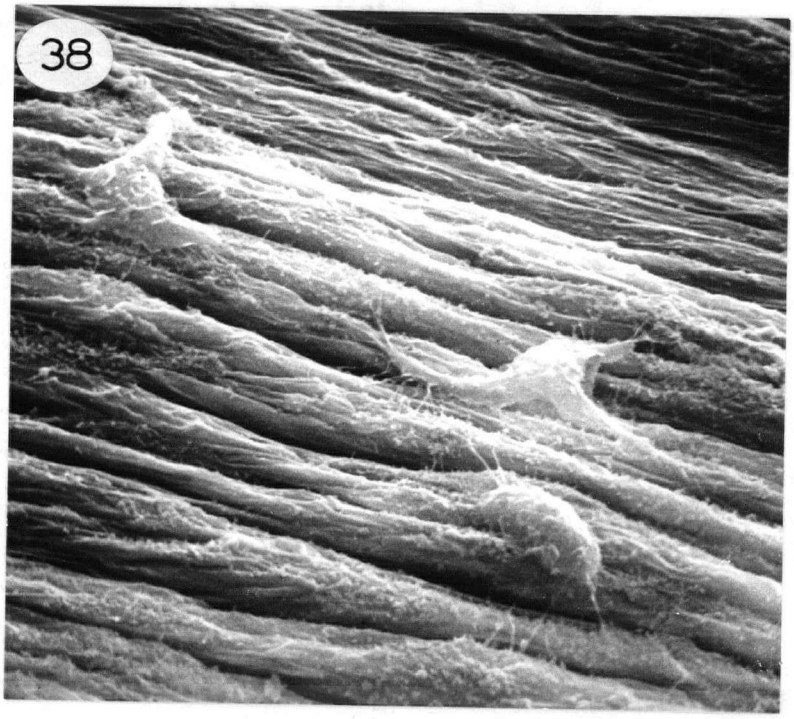
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3. The Posterior Surface of the Iris in Pupillary Constriction (Figures 39-56)

The posterior surface of the iris is observed in two degrees of pupillary constriction. This is only a qualitative distinction based on some of the morphological characteristics that are present. In one condition, referred to here simply as pupillary constriction, the pupil is constricted such that the pupillary diameter is about 2/7 of the total iris diameter (Figures 39, 40). In another condition, referred to as extreme pupillary constriction, the pupil is very small and makes up only about 1/14 of the total iris diameter (Figures 41, 42). These two aspects of the posterior surface of the iris will be treated separately.

In pupillary constriction, the posterior iridial surface is a flat, smooth, circular structure with an aperture, the pupil, situated in the center (Figures 39, 40). In a low magnification scanning electron micrograph, the pupillary margin is smooth (Figure 39), but at a slighter higher magnification, a few blebs may be seen along parts of the pupillary margin (Figure 40). Not much detail of the rest of the iris surface is discernible. There are some tube-like structures which radiate from the pupillary margin to the region of the ciliary processes (Figure 39). At a higher magnification, it is seen that these are actually capillaries running along the posterior surface of the iris. These vessels have not been previously observed or reported. The beginning and termination of these vessels are not well defined (Figure 40). These vessels could presumably be remnants of the peri-natal circulatory system. The number of these vessels that are present varies from iris to iris. At the periphery of the iris, in the region of the ciliary body, there are numerous ciliary-iris connections (Figures 39, 43, 44). They seem to be quite regularly spaced out along the rim of the iris. The lengths of these processes may differ very

slightly. They are tissue processes which, perhaps, anchor the ciliary processes to the iris. Near the ciliary body, it is superficial to the rest of the iris surface but a little centrally, the process goes into the depths of the iris tissue itself. Its presence is only detected as a minor radial ridge as one moves centrally (Figures 43, 44). If an analogy is warranted here, the ciliary-iris process is likened to the buttress root of a tree. It slants up near the trunk but dives into the ground further away. These ciliary-iris processes perhaps act as stabilisers of the root of the iris during the continuous excursions of the pupil.

At a higher magnification, the posterior surface of the iris shows slight corrugations and bulges which are generally oriented circumferentially (Figure 43). There are no distinct grooves or ridges (Figure 45) although the posterior epithelial cells appear to be arranged in circumferential rows in some parts of the iris (Figure 45) but are more haphazardly arranged in other parts of the iris (Figure 46). Each posteriorly directed bulge probably represents a single epithelial cell. They are slightly spindle shaped or polygonal, as has been reported (Hansson, 1970), although the shapes of the cells do vary quite a bit. The posterior basement membrane is probably still present over most of the posterior surface of the epithelial cells. However, it may be thinned out during pupillary constriction or it may be removed during the processing of the material. An enormous number of processes are seen radiating out from the individual cells and intermingling with those of the adjacent cells (Figures 46, 47).

In extreme pupillary constriction, the pupil appears as a mere pinhole (Figures 41, 42). From our present observations, we cannot say whether this degree of pupillary constriction is normally attainable in the usual responses to intense light, or whether it is only attainable with the aid of drugs. Most of the iris appears as a smooth sheet except for a

small area around the pupil (Figure 41). The pupillary edge is not smooth but presents a highly irregular outline (Figure 42) which is clearly seen in higher magnification scanning electron micrographs (Figures 48, 49). It is a purse-string effect, where the string (equivalent to the sphincter) is drawn so tightly that all the tissue around the pupillary edge is gathered together. Numerous ridges and grooves radiate out from the pupillary margin like the rays of the sun. These peter out before the mid-iris is reached. The ridges and grooves are of varying lengths.

Right around the rim of the pupil, are big humps of tissue. This is probably in the sphincter region and the humps represent bundles of much constricted sphincter muscle cells. The surface of these humps is relatively smooth with only a small amount of furrowing (Figures 48, 49). The pupillary margin is not a single layer but consists of a pile-up of these sphincteric humps (Figures 48, 49). A capillary is seen extending from the pupillary edge (Figures 41, 49) to the ciliary region (Figure 41). The capillary lies superficially on the posterior iridial surface. At the pupillary margin, it appears to make a turn anteriorly. It may perhaps continue anteriorly to become one of the blood vessels of the iris stroma.

At a little distance from the pupillary edge, the posterior iris surface shows a series of grooves and ridges which are radially oriented (Figures 48-50). The ridges are higher, rounder and closer together centrally but they become broader and lower peripherally. Eventually, the ridges flatten out so that the rest of the iris posterior surface is smooth. The grooves in between the ridges are deep and narrow centrally but they become shallower peripherally. These ridges are probably rows of epithelial cells covered by a basement membrane (Figure 50). Unlike the epithelial ridges seen in pupillary dilation, these ridges are radially rather than circumferentially oriented. The ridges bifurcate as well as merge.

Within this zone of epithelial ridges and grooves, are some grotesquely large bulbous structures (Figure 42). They are very many times larger than the surrounding epithelial ridges (Figures 51-53). They may be round (Figure 51), or they may be drawn out at both ends (Figure 52). They look bloated at times (Figures 51, 52), but at times they look collapsed (Figures 51-53). When puffed-up, the surface is relatively smooth (Figures 51, 52), but when collapsed the surface shows some irregularities (Figure 53). The basement membrane covering the rest of the posterior iris surface also covers these bulbous structures. The crinkles and folds of the basement membrane over the epithelial ridges are also seen over these bulbous structures. Thus they are indeed part of the posterior iris surface. Mapstone (1970) suggests that past a certain pupillary size, the purse-string effect of the pull of the sphincter no longer produces further miosis but instead produces "eversion of the pigment epithelium". These bulbous structures may be the posterior pigment epithelium of the iris which has been crowded out of its normal position with extreme pupillary constriction.

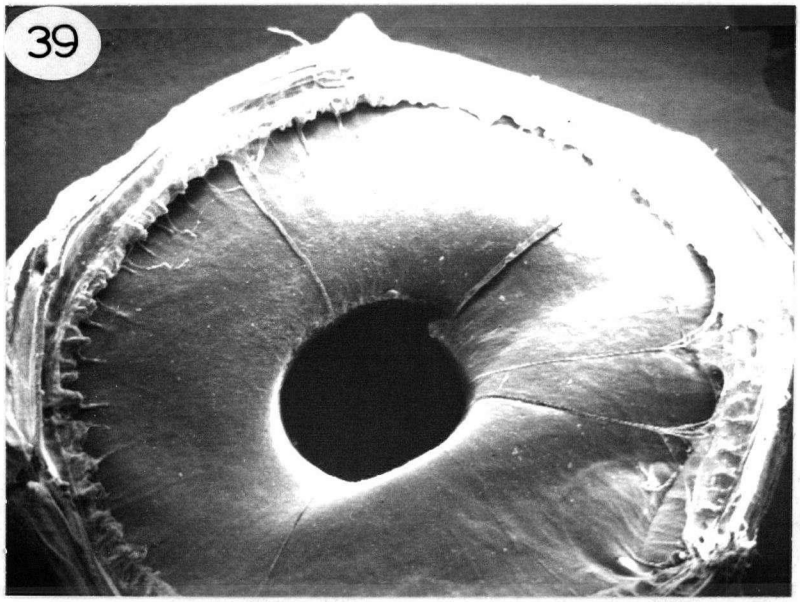
The rest of the posterior iris surface outside of the central ridged area is smooth and quite non-descript (Figures 54-56). The ciliary-iris process may cause a slight, low, wide ridge to be seen at the periphery (Figure 54). Some of the polygonal epithelial cells are distinguishable at times (Figure 56). They are not arranged in any particular order or pattern. Processes of the epithelial cells form a fine meshwork (Figure 55). At other parts of the iris, the surface is very smooth (Figure 56). Cell boundaries cannot be traced but slight variations in the contour of the surface suggest where the outlines of the cells may be (Figure 56). Precipitated proteinaceous material is oftentimes present on the iris surface.

Figure 39 The Posterior Surface of the Iris in Pupillary Constriction (SEM)

At a low magnification, the posterior surface of the iris in pupillary constriction is smooth. The pupil is round. There are a few tube-like structures spanning the posterior surface of the iris from the pupillary edge to the ciliary region. All along the ciliary region at the periphery of the iris are a series of ciliary-iris processes. x 20

Figure 40 The Posterior Surface of the Iris in Pupillary Constriction (SEM)

A high magnification of the pupillary margin shows that the margin is relatively smooth except for a few blebs. The tube-like structures seen in Figure 39 are capillaries which seem to arise from around the pupillary margin. x 50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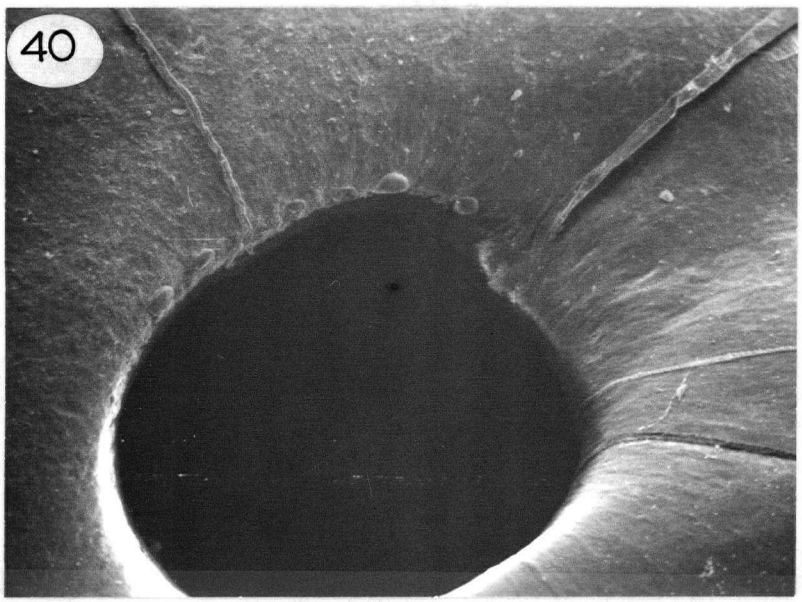
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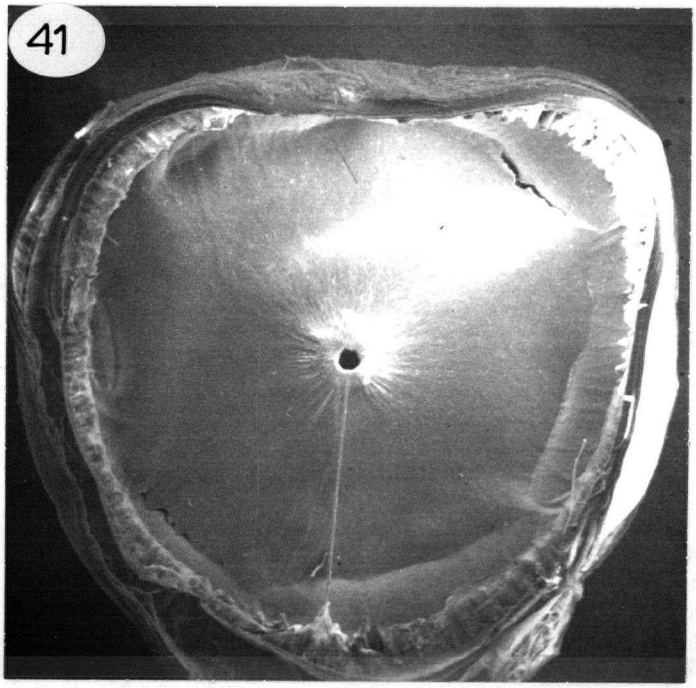
Figure 41 The Posterior Surface of the Iris in Extreme Pupillary Constriction (SEM)

In extreme pupillary constriction, the posterior surface of the iris is smooth except for a small portion around the pin-hole pupil. x 20

Figure 42 The Posterior Surface of the Iris in Extreme Pupillary Constriction (SEM)

A high magnification electron micrograph of the pupillary region shows that the pupillary margin is irregular in outline. There are some humps right around the pupillary rim. Epithelial ridges and grooves and a capillary radiate outwards from the pupil. In amongst the epithelial ridges and grooves are numerous bulbous structures of different sizes. x 170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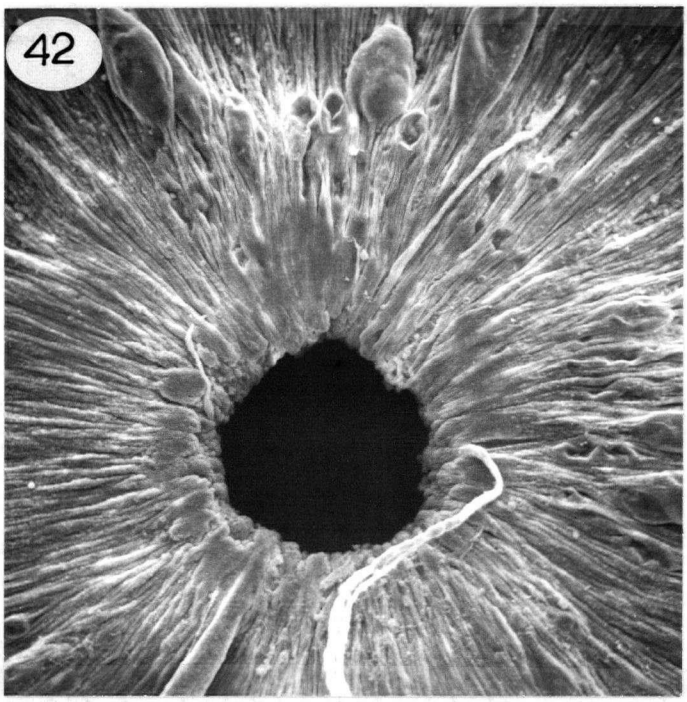
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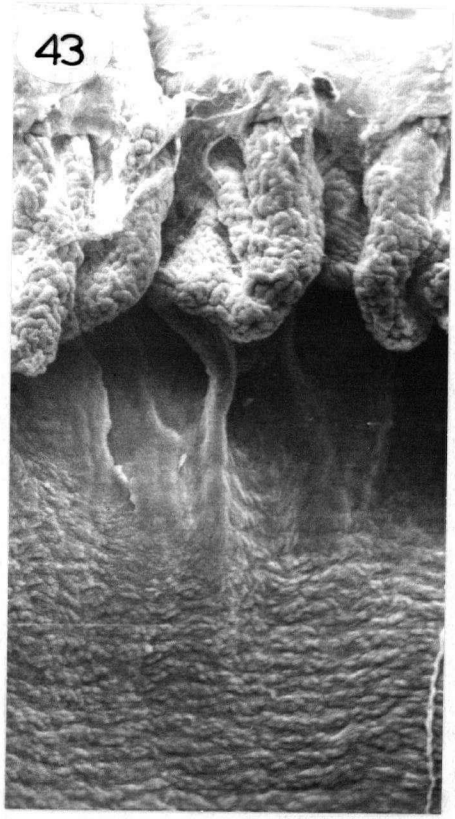
Figure 43 The Posterior Surface of the Iris in Pupillary Constriction (SEM)

In partial pupillary constriction, the posterior surface of the iris shows corrugations and bulges which are generally oriented circumferentially. At the ciliary region, there are ciliary-iris processes of differing lengths. x 200

Figure 44 The Posterior Surface of the Iris in Pupillary Constriction (SEM)

A high magnification electron micrograph of the ciliary-iris process shows that it stands high above the posterior surface of the iris at the ciliary region. It then dives deep into the iris tissue itself more centrally. x 500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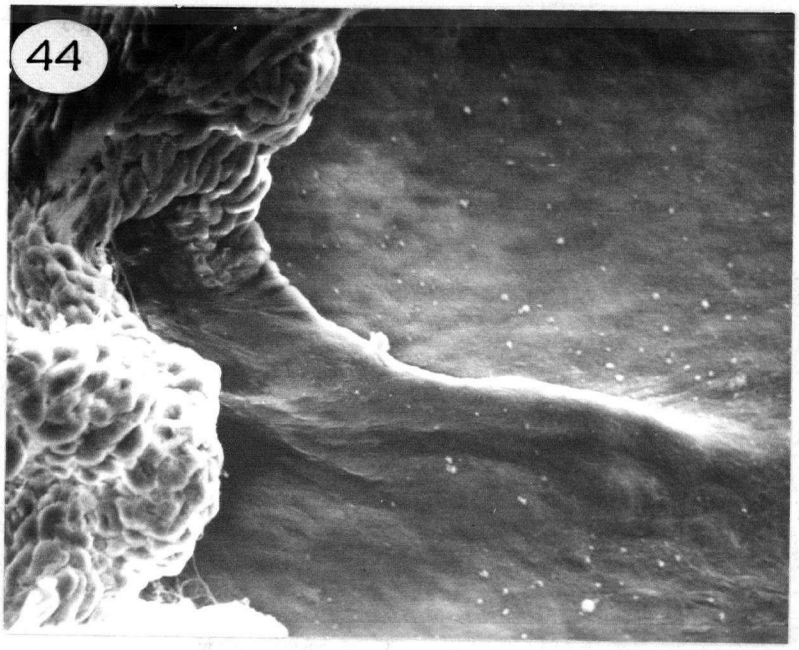
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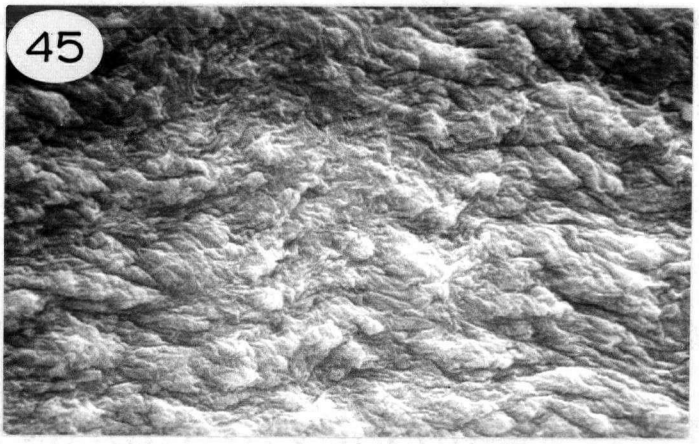
Figure 45 The Posterior Surface of the Iris in Pupillary Constriction (SEM)

The posterior epithelial cells are irregularly shaped bulges which are organised in some semblance of circumferential rows. x 960

Figure 46 The Posterior Surface of the Iris in Pupillary Constriction (SEM)

The posterior epithelial cells are haphazardly arranged. Occasionally some fine interdigitations are seen. x 960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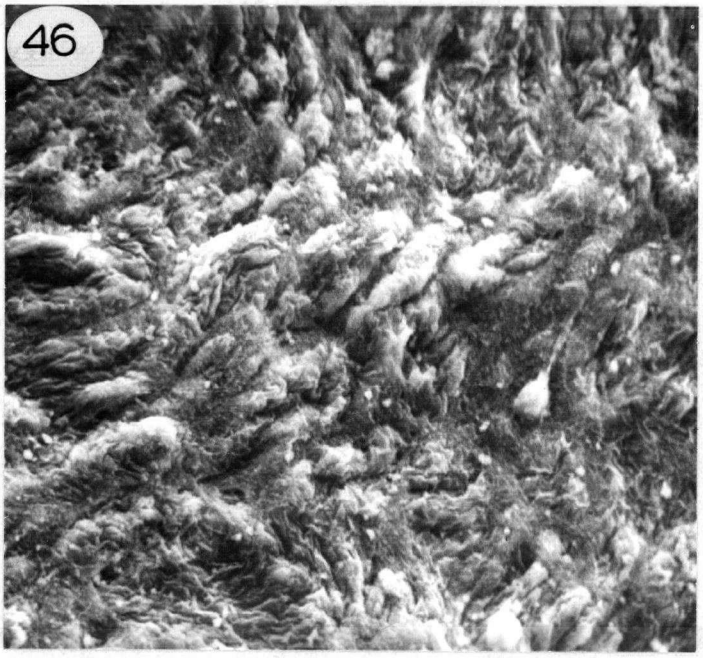
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Figure 47 The Posterior Surface of the Iris in Pupillary Constriction (SEM)

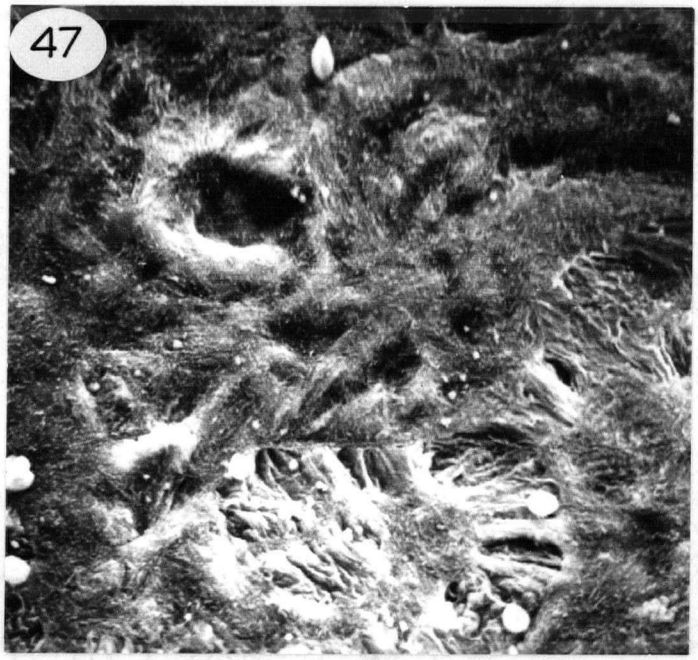
Where the basement membrane has been thinned out or has been removed, a large number of cell processes are observed.

x 960

Figure 48 The Posterior Surface of the Iris in Extreme Pupillary Constriction (SEM)

In extreme pupillary constriction, the pupillary edge of the iris consists of many layers of humps of tissue. These probably represent bundles of sphincter muscle cells. The surface of the humps is smooth. Numerous epithelial ridges radiate from the sphincter region. The ridges are high and rounded near the sphincter but they decrease in height peripherally.

x 430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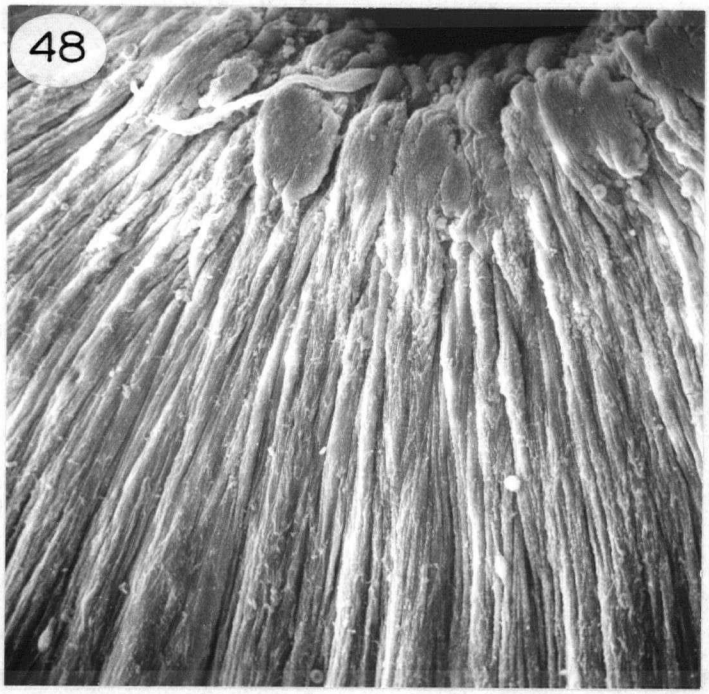
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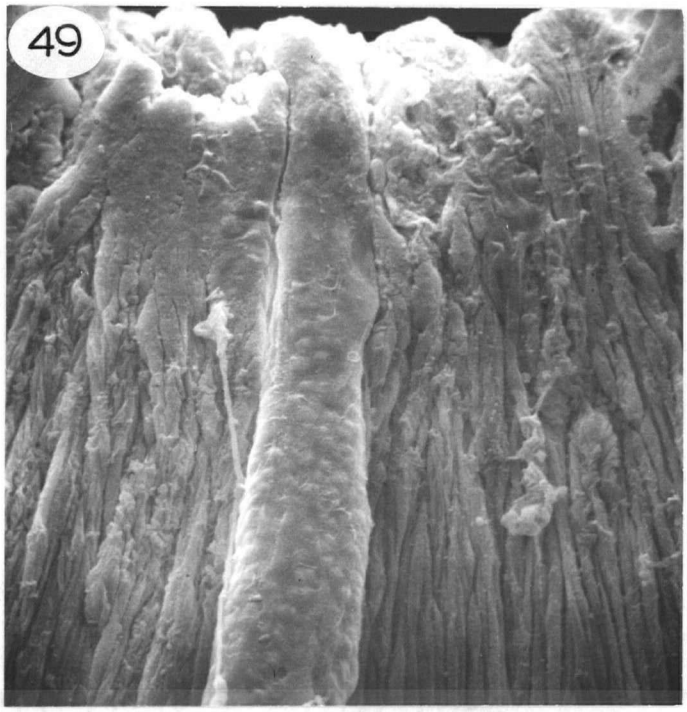
Figure 49 The Posterior Surface of the Iris in Extreme Pupillary Constriction (SEM)

The sphincter humps at the pupillary margin have a smooth posterior surface with only a few fine striations. The capillary lies superficial to the posterior surface of the iris. At the pupillary edge, it appears to turn anteriorly.
x 760

Figure 50 The Posterior Surface of the Iris in Extreme Pupillary Constriction (SEM)

The radial epithelial ridges show numerous branchings. The basement membrane covering the posterior surface of the epithelial cells is highly wrinkled. x 3,900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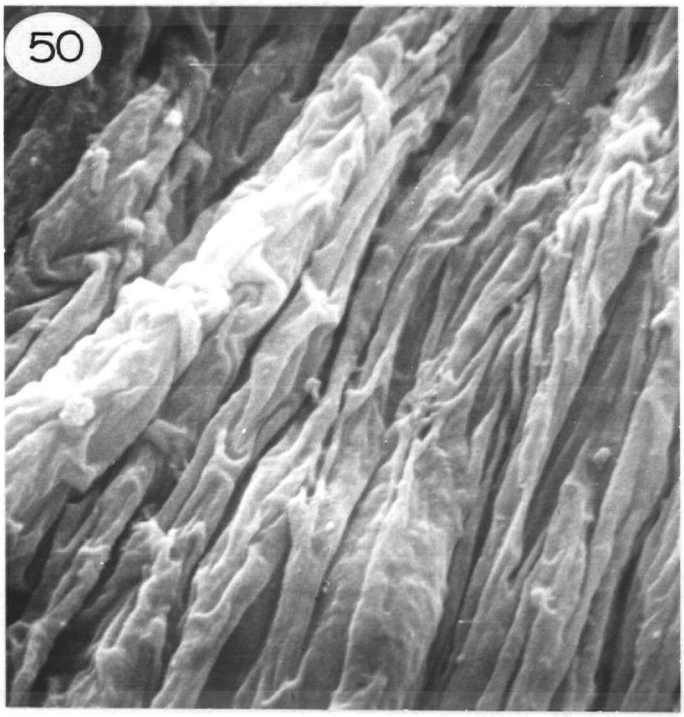
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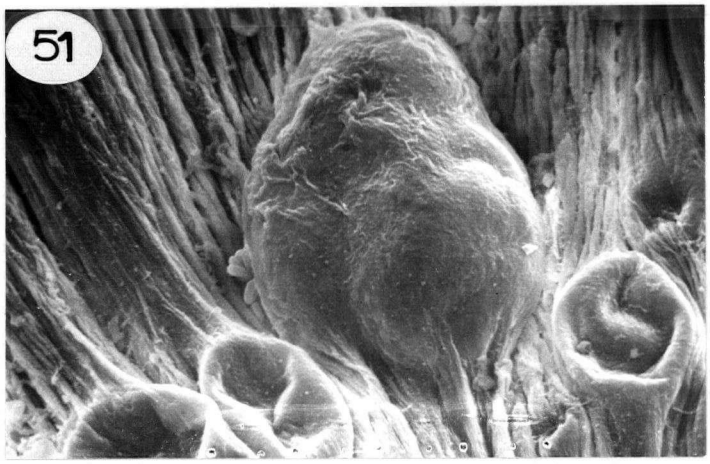
Figure 51 The Posterior Surface of the Iris in Extreme Pupillary Constriction (SEM)

In amongst the radial epithelial ridges are bulbous structures of different shapes and sizes. They are large and bloated or small and collapsed. x 760

Figure 52 The Posterior Surface of the Iris in Extreme Pupillary Constriction (SEM)

The bulbous structures are sometimes drawn out at both ends. It is puffed-up. The posterior surface of the structure is relatively smooth. x 760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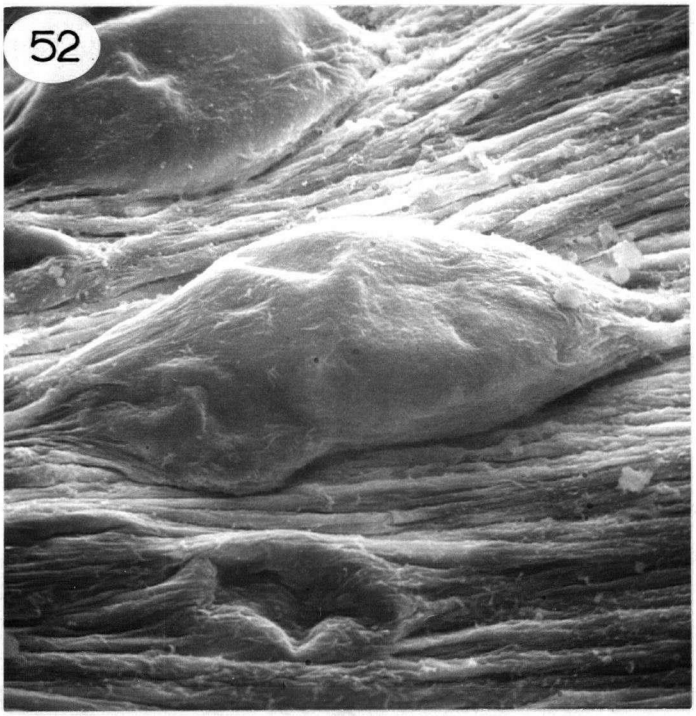
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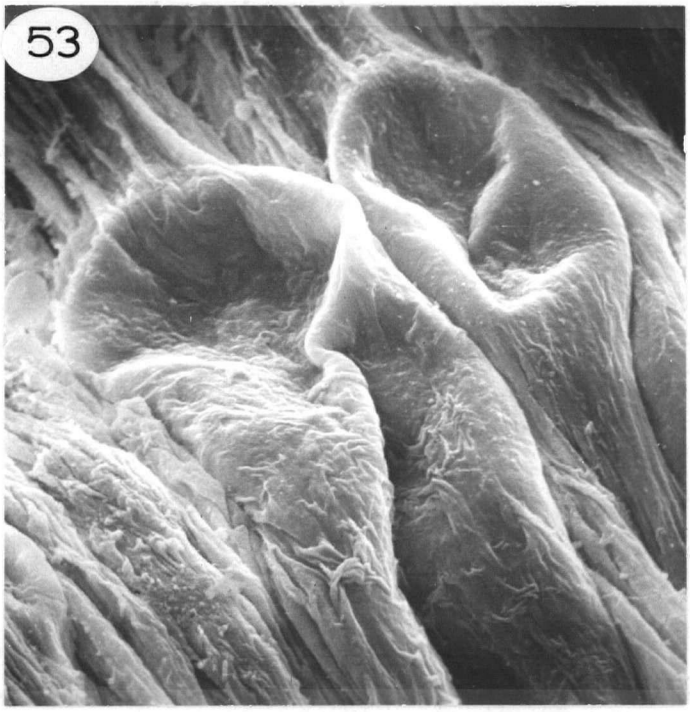
Figure 53 The Posterior Surface of the Iris in Extreme Pupillary Constriction (SEM)

The bulbous structures are sometimes collapsed. The basement membrane over the bulbous structures is continuous with the basement membrane covering the rest of the epithelial cells.
x 1,570

Figure 54 The Posterior Surface of the Iris in Extreme Pupillary Constriction (SEM)

The posterior surface outside of the pupillary region is smooth. A few low ridges are caused by the ciliary-iris processes.
x 90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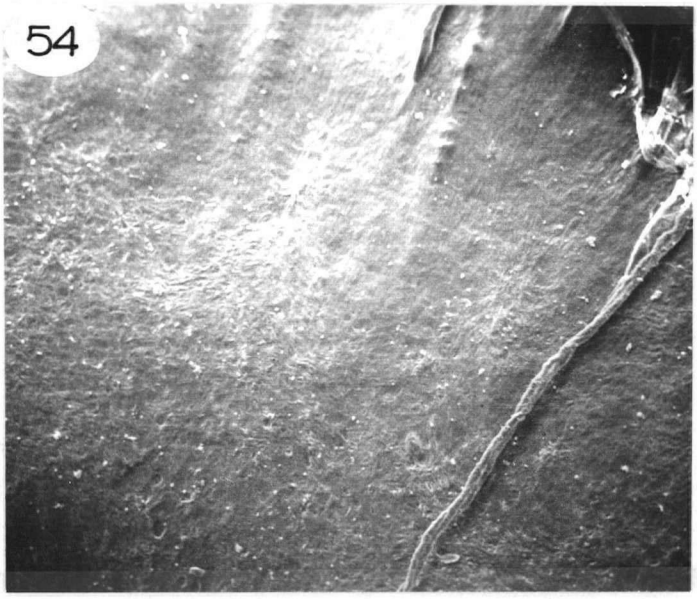
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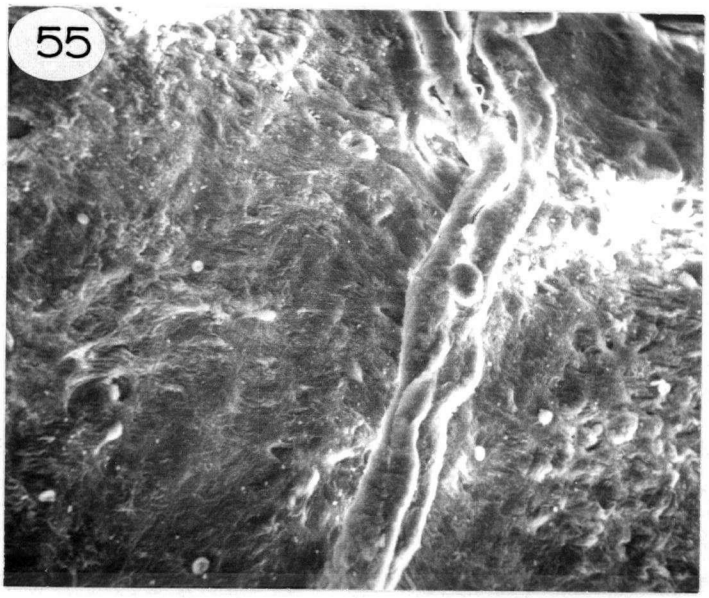
Figure 55 The Posterior Surface of the Iris in Extreme Pupillary Constriction (SEM)

In parts of the posterior surface of the iris, there are numerous, haphazardly arranged polygonal bulges of the posterior epithelial cells. Two delicate blood vessels traverse the posterior surface of the iris. x 430

Figure 56 The Posterior Surface of the Iris in Extreme Pupillary Constriction (SEM)

In extreme pupillary constriction, many parts of the posterior surface of the iris is smooth and non-descript. An occasional bulge suggests the location of the underlying nuclei of the epithelial cells. x 3,900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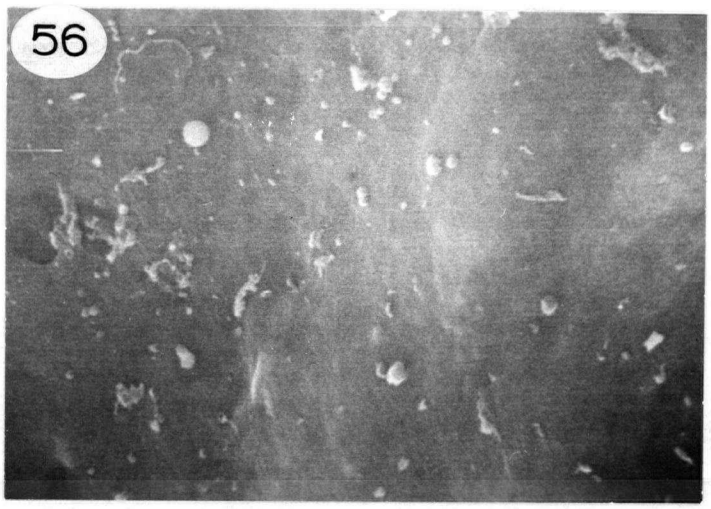
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4. The Anterior Surface of the Iris in Pupillary Dilation

(Figures 57-65)

The most distinctive feature of the anterior surface of the iris in pupillary dilation is the large number of blood vessels which bulge out anteriorly from the iris surface (Figures 57-59). Usually, the anterior surface can be divided into two zones, the central half consists of a series of small-sized blood vessels with intervening crypts, while the peripheral half consists of a mass of large blood vessels of varying calibers (Figures 58, 59). The crypts in the medial half may be quite distinct (Figure 59). However, with a greater degree of pupillary dilation, this zone is telescoped into the peripheral zone (Figure 58) until eventually the anterior iris surface only appears as a mass of large blood vessels (Figure 57). The pupillary margin, if observed edge on, is quite smooth as compared to the rugged contours of the rest of the iris surface (Figure 60).

The iris blood vessels assume different configurations although the overall general orientation is circumferential. Occasionally, a large blood vessel is seen entering the iris at its periphery (Figure 57). It has a radial course. But shortly after entering the iris, it gives rise to a large straight vessel at right angles to it, that is, oriented circumferentially. This would most probably be the vessel of the major arterial circle situated at the iris root (Figure 57). The rest of the iris vasculature forms an interwoven network of large and small blood vessels. The blood vessels may be in the form of an 'X' bifurcating at both ends (Figure 57). Another vessel may be inserted through the arms of a bifurcation and join up with one of the branch blood vessels (Figure 58). At times, a small branch blood vessel curls around its parent vessel (Figure 59). A quite common feature is exemplified in Figure 61. Here a large blood vessel bifurcates. Shortly afterwards, one of its branches bifurcates again while

the other branch dives deep into the iris stroma. The 'tuning fork' configuration is also quite common (Figure 62). The two branch blood vessels are just as large, if not larger, than the originating blood vessel. The junction of these three blood vessels usually bulges out prominently anteriorly. A row of these may be seen from the periphery medially, giving the appearance of a row of sand-dunes. It is very rare that a blood vessel zig-zags its way across the iris. But when it does, it is very distinctive (Figure 63). The path of the zig-zag is not directly perpendicular to the pupillary margin but rather, it is slightly askew.

From light microscopic histological studies, it is known that the iris blood vessels are not freely exposed to the aqueous humor in the anterior chamber. There is an endothelial lining covering the anterior surface. The endothelial cells are not easily visible, but occasionally, an endothelial cell nucleus is seen to bulge anteriorly (Figure 64). Little blebs and microvillus-like processes are seen on the cell surface. The outlines of the cells are not clear but can be faintly made out. The boundary with an adjacent cell is not a tight adhesive zone. It merely consists of an intermingling of cytoplasmic cell processes. These features are observed in much better detail when the anterior surface of the iris is examined in pupillary constriction. At times, a sieve-like structure is observed over one of the bulging blood vessels (Figure 64). It is difficult to say whether this is formed at the junctions of a few endothelial cells, or whether this sieve is within the confines of the ctyoplasm of a single endothelial cell. Whatever it may be, it certainly exposes the underlying blood vessel more directly to the circulating aqueous humor.

The blood vessels in the central half of the iris are small and are presumably capillaries (Figures 58, 59). They do not bulge out as much anteriorly although their course can still be charted. Most of them form

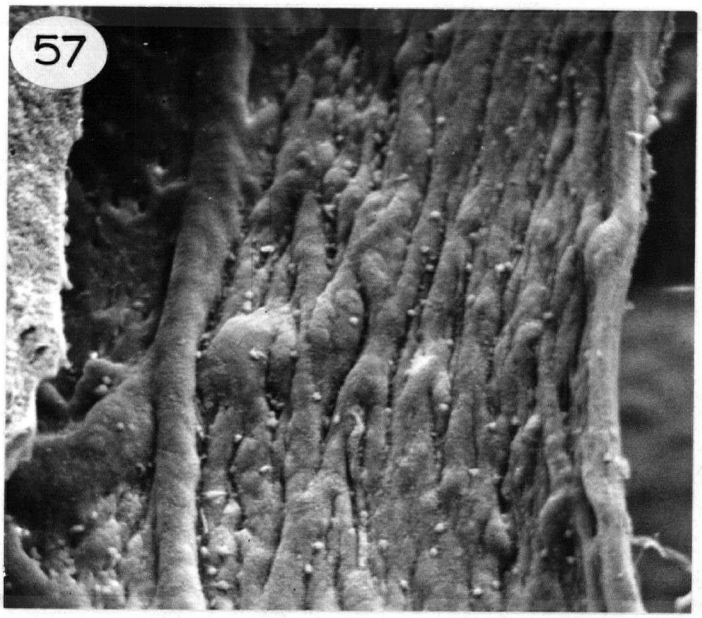
an interconnecting series of X's. Towards the pupillary margin, most of these smaller blood vessels sometimes drain into a larger cord-like blood vessel which follows the pupillary margin (Figures 58, 59). It is not as large as the vessels in the lateral extent of the iris but it is still of a considerable size. In this capillary zone, there are a large number of relatively large crypts (Figures 59, 65). The crypts are usually found in between the arms of a bifurcating set of capillaries (Figure 65). The crypt is not just one large orifice. A series of cell processes divide up the opening into a series of smaller apertures. It appears like a wire mesh over the crypt opening. Deeper down, other cell processes, belonging to the underlying stromal cells, are seen.

Figure 57 The Anterior Surface of the Iris in Pupillary Dilation (SEM)

The anterior surface of the iris consists of a mass of large blood vessels bulging anteriorly. The blood vessels are generally circumferentially oriented. A large blood vessel is seen entering the iris at the periphery and immediately dividing. Other blood vessels may form an X which bifurcates at both ends. x 190

Figure 58 The Anterior Surface of the Iris in Pupillary Dilation (SEM)

The anterior surface of the iris can be divided into two regions, the pupillary region with a series of small blood vessels, and the peripheral region with a series of larger blood vessels interwoven in a complicated network. In general, the blood vessels are circumferentially disposed. Along the pupillary edge, there is a medium-sized, cord-like smooth blood vessel. x 250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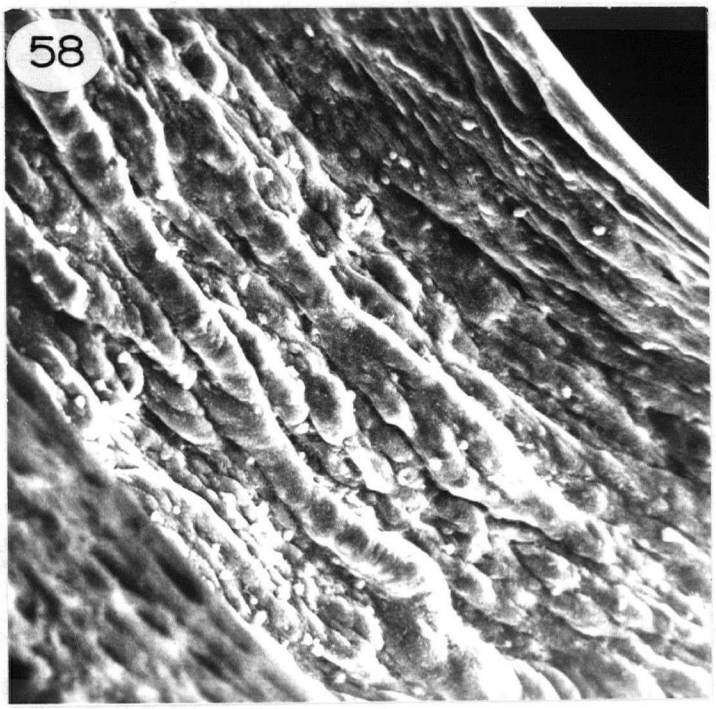
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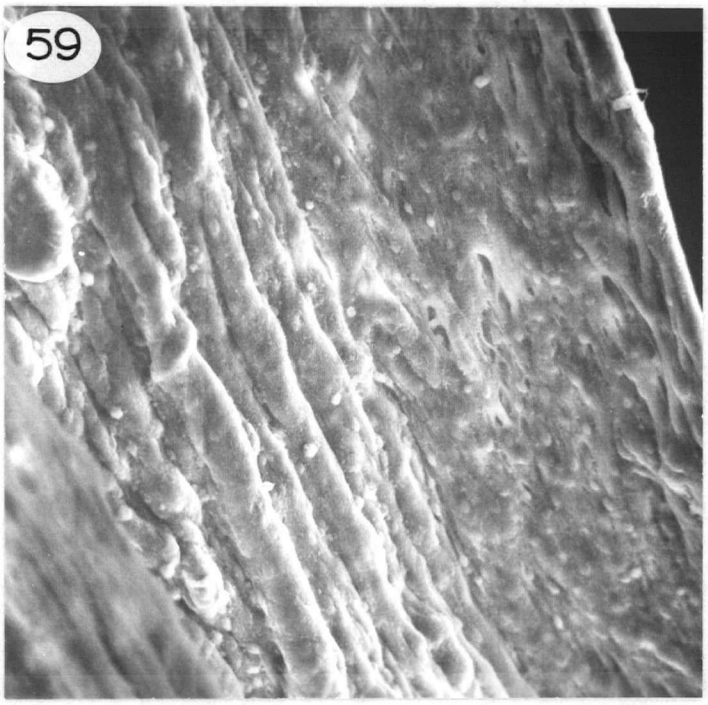
Figure 59 The Anterior Surface of the Iris in Pupillary Dilation (SEM)

The cord-like blood vessel along the pupillary edge is connected to a series of smaller blood vessels which form X's with each other. Sometimes a small branch blood vessel may curl around its parent vessel. In the pupillary region there are many crypts. x 260

Figure 60 The Anterior Surface of the Iris in Pupillary Dilation (SEM)

The pupillary margin viewed edge on is smooth. The blood vessels near the pupillary margin are small and are generally circumferentially arranged. The blood vessels in the other parts of the iris protrude quite prominently anteriorly. They appear highly tortuous. x 280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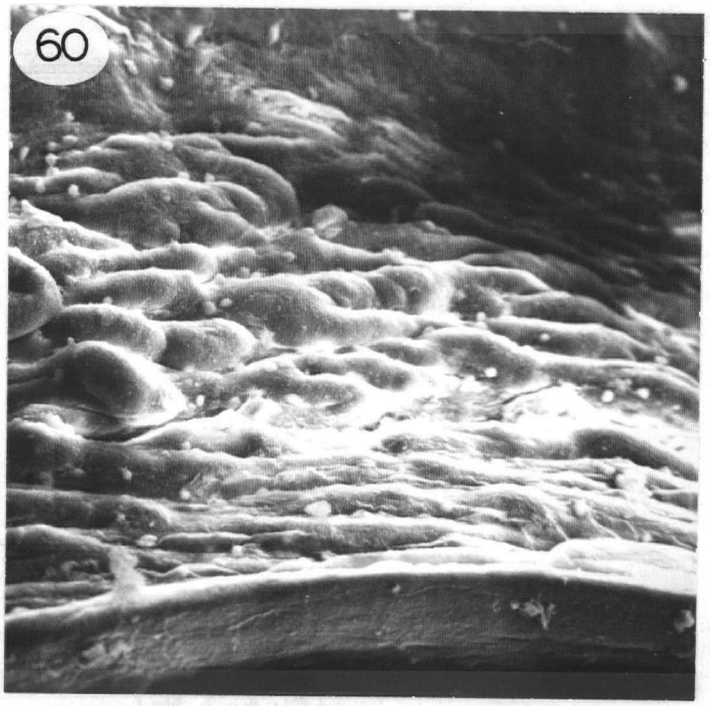
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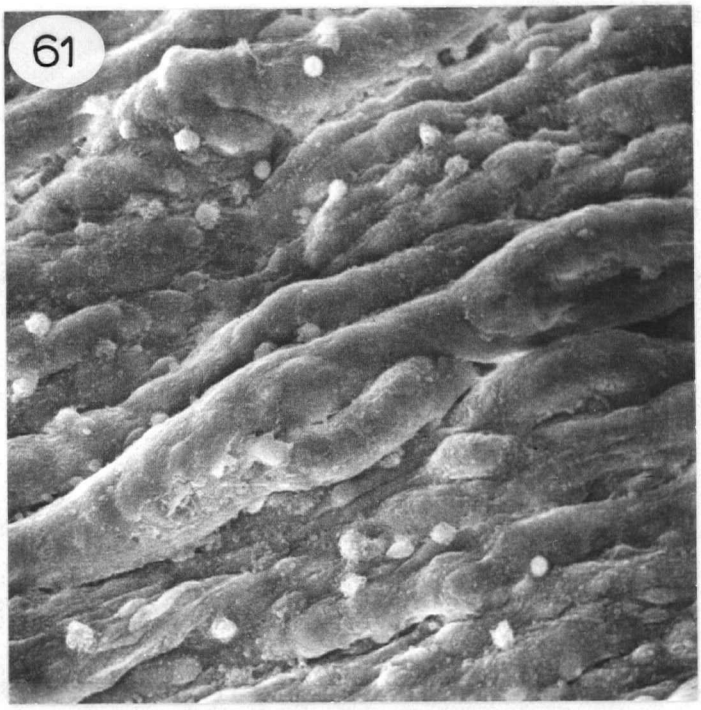
Figure 61 The Anterior Surface of the Iris in Pupillary Dilation (SEM)

A blood vessel bifurcates. One of its branches dives deep into the iris tissue while the other branch continues on to form another bifurcation. x 530

Figure 62 The Anterior Surface of the Iris in Pupillary Dilation (SEM)

The blood vessels are shaped like a tuning fork where each of the branch blood vessels is as large as the parent vessel. x 800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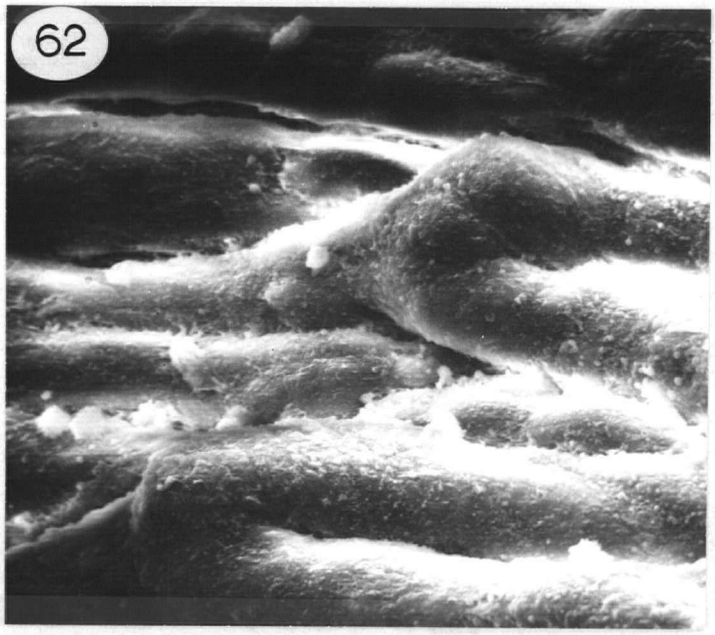
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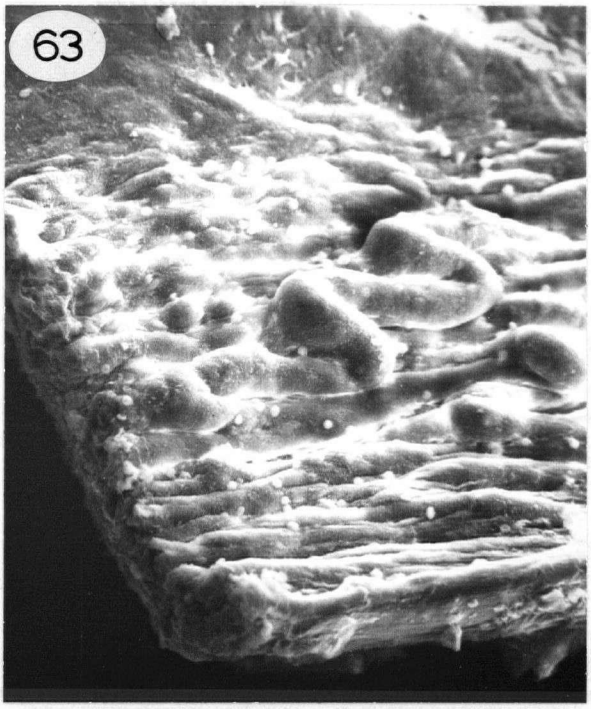
Figure 63 The Anterior Surface of the Iris in Pupillary Dilation (SEM)

One of the blood vessels protruding out anteriorly is zig-zagging obliquely across the anterior surface of the iris. x 260

Figure 64 The Anterior Surface of the Iris in Pupillary Dilation (SEM)

A sieve-like structure covers the surface of a blood vessel which is bulging out anteriorly. x 1,780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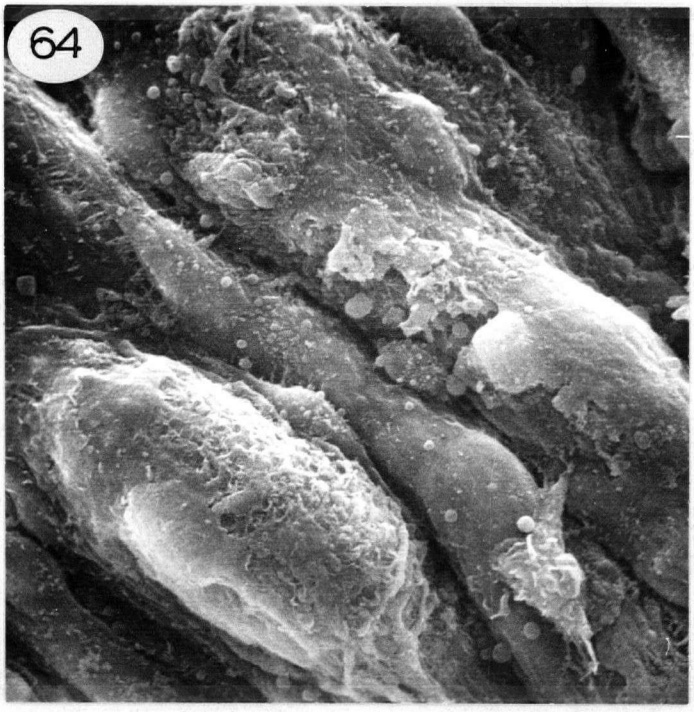
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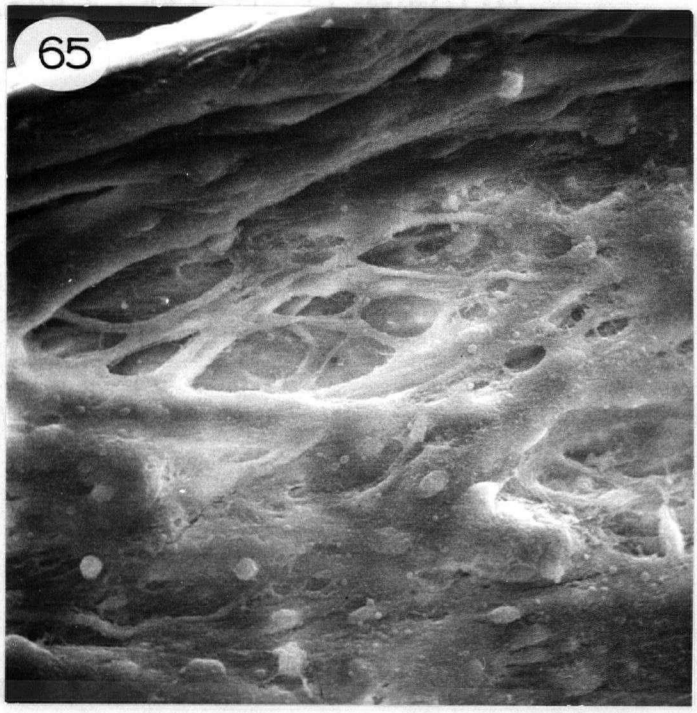
Figure 65 The Anterior Surface of the Iris in Pupillary Dilation (SEM)

The iridic crypts in the pupillary region are often located between the arms of a bifurcating blood vessel. A series of cell processes form a wide mesh over the crypt opening.
x 670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5. The Anterior Surface of the Iris in Pupillary Constriction

(Figures 66-76)

In a low magnification scanning electron micrograph, the anterior surface of the iris is large, relatively smooth and slightly pock-marked, especially towards the pupillary zone (Figure 66). Some of the blood vessels, presumably only the larger ones, are readily seen. They are generally oriented radially. The blood vessels wind their way in a serpentine fashion, from the periphery centrally. At the periphery, the zig-zag course of the blood vessels is very prominent (Figures 66, 67). Often, it appears that branches are given off at each external bend of the parent vessel and these plunge into the deeper parts of the iris stroma (Figure 67). The smaller blood vessels are not easily seen although they too seem to have a twisting course. However, they tend to go deep and disappear. They may perhaps reappear again some distance away (Figures 66, 67).

In the pupillary region, the blood vessels are much more in evidence (Figures 68, 69). They are tortuous and form a complicated pattern. They twist and turn and divide. It is difficult to follow the course of any one vessel. Sometimes, they may appear to form arcades which go over and embrace the pupillary margin itself (Figure 69). In between the blood vessels, there are always holes and spaces. These are the iridic crypts, which are of varying sizes (Figure 68).

The detailed surface structure of the iris can only be studied at higher magnifications. The anterior surface is covered by a thin endothelial layer (Figure 70). The endothelial cells are variously polygonal in shape. The cell size differs between the individual cells, but the nuclear size is relatively constant. The nucleus is oval in shape. It is usually situated in the centre of the cell mass and bulges a little anteriorly into the anterior chamber. Thus, the whole anterior iridial surface

has a slightly undulating aspect to it. The boundaries of the endothelial cells are quite well-defined. This is not so clearly shown in Hansson's studies (1970). This difference could be the result of the different methods used in preparing the tissues for examination with the scanning electron microscope. Each endothelial cell may be associated with its neighbor by various means. But in all cases, there is no tight adhesive boundary between adjacent endothelial cells. In certain instances (Figures 70-72), cytoplasmic processes from adjacent cells bridge the intervening gap (Figures 70-72). The intercellular gap may be large (Figures 70, 71), or it may be quite small (Figure 72). The number of cytoplasmic processes spanning the intercellular gap may be numerous (Figure 71), or it may be few in number (Figure 72). Sometimes tiny holes are present within the endothelial cell cytoplasm (Figure 71), but at other times the cell surface is relatively smooth and non-pitted (Figures 72, 73). Another configuration often seen at the boundary zone between the endothelial cells is the series of large holes or crypts (Figures 72-74). The crypts are of varying sizes. Their relative sizes can be determined by utilising the adhering red blood cells as a comparative means of measure (Figure 74). The crypts are relatively well-defined structures, as compared to the holes seen by Hansson (1970). They are usually irregularly round or oval in shape. The borders of the crypts are quite smooth although occasionally a few small blebs may be present. Sometimes cytoplasmic processes from the endothelial cells making up the crypt border span the openings of the crypts. A network of the cytoplasmic prolongations of the underlying stromal cells are seen at a slightly deeper level. These are the cellular components beneath the crypt entry. A fine fibrillar material is sometimes seen in addition to the stromal cell processes (Figure 73). At times, the fibrillar material may occupy the totality of the crypt opening (Figure 75). The iridic crypts

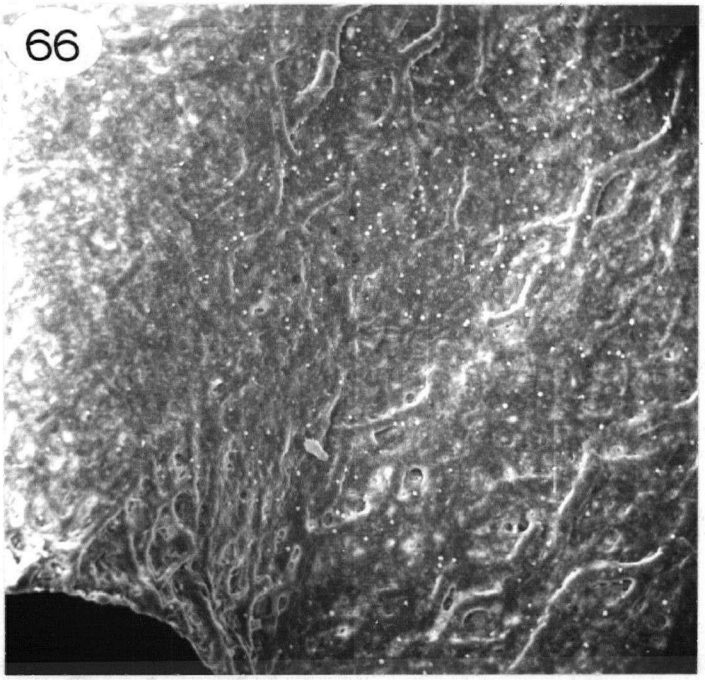
are often found quite closely associated with blood vessels (Figure 76). The proximity of the crypts to the blood vessels, may facilitate any interchange of materials that might ensue between the iris blood vessels and the surrounding aqueous humor.

Figure 66 The Anterior Surface of the Iris in Pupillary Constriction (SEM)

A low magnification scanning electron micrograph shows that the anterior surface of the iris is pock-marked. Numerous blood vessels zig-zag from the periphery to the pupillary edge. x 90

Figure 67 The Anterior Surface of the Iris in Pupillary Constriction (SEM)

At a higher magnification, the zig-zagging blood vessels are seen to be of different sizes and they are found at different depths in the iris tissue. At each external bend of the parent blood vessel, branches appear to be given off, which then immediately dive deep into the iris tissue. x 180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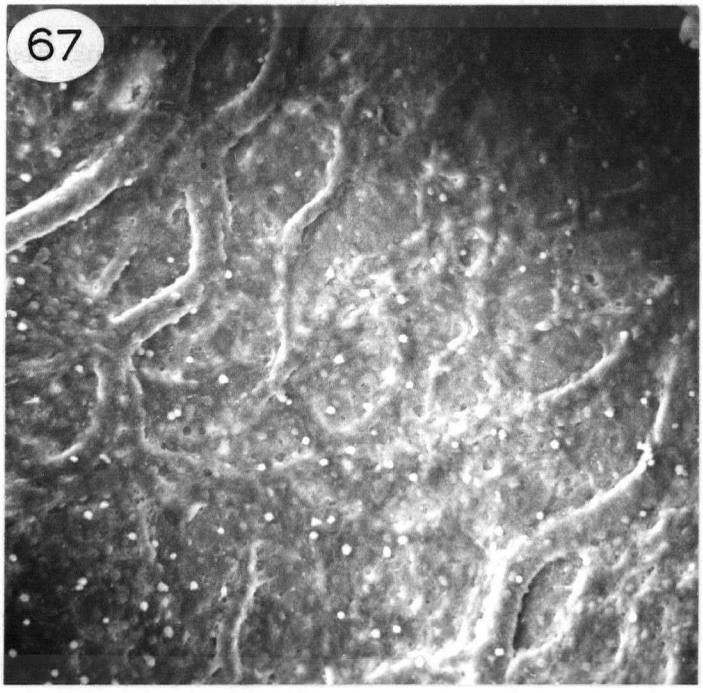
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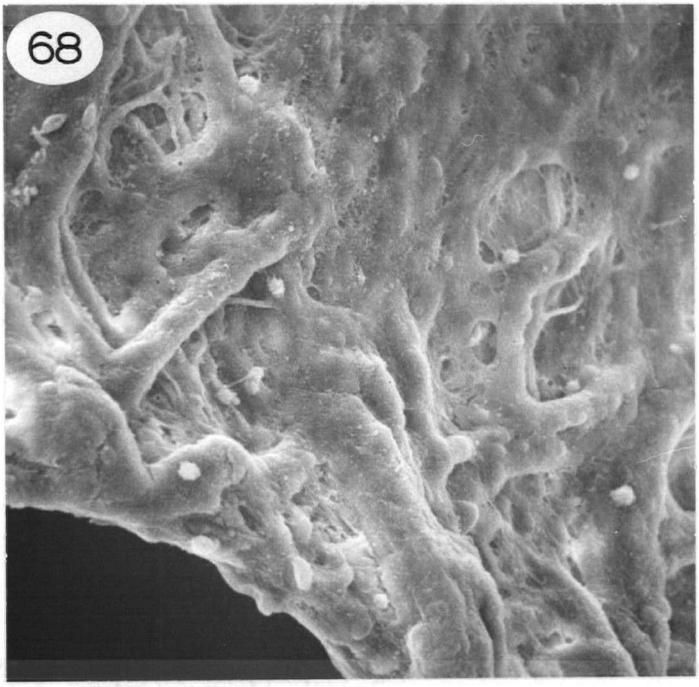
Figure 68 The Anterior Surface of the Iris in Pupillary Constriction (SEM)

The blood vessels at the pupillary region form a complicated pattern. In between the blood vessels, there are holes and crypts of various sizes. x 460

Figure 69 The Anterior Surface of the Iris in Pupillary Constriction (SEM)

Some of the blood vessels at the pupillary region form arcades which embrace the pupillary margin itself. In between the vascular arcades, iridic crypts and pores are found. x 460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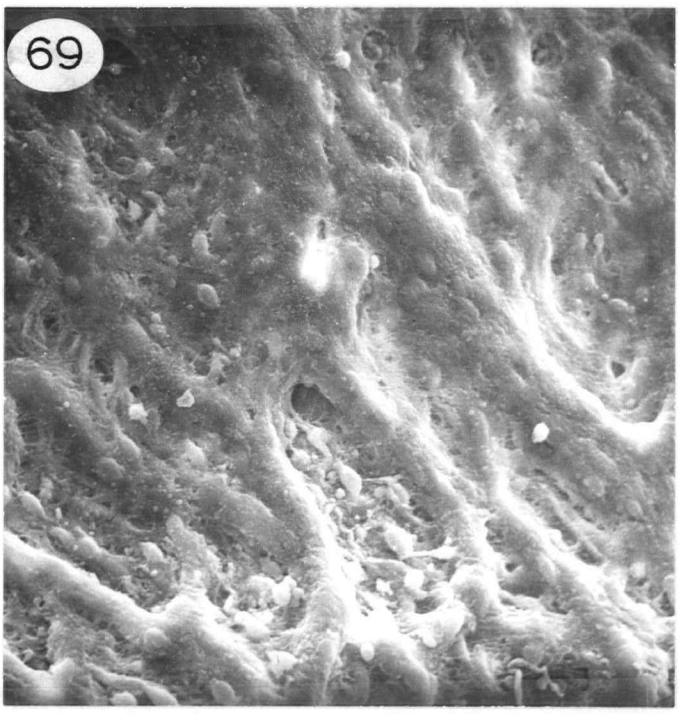
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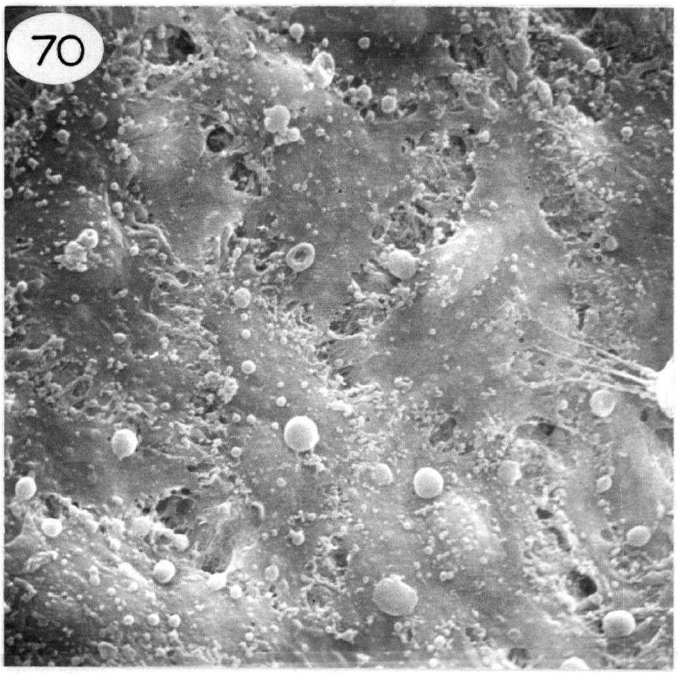
Figure 70 The Anterior Surface of the Iris in Pupillary Constriction (SEM)

The nuclei of the anterior endothelial cells are oval in shape and they bulge slightly anteriorly. The nuclei are located in the middle of the polygonal cells. The anterior endothelial cells do not form a continuous covering for the anterior surface of the iris. Spaces and pores are present in between the individual cells. x 1,800

Figure 71 The Anterior Surface of the Iris in Pupillary Constriction (SEM)

A single anterior endothelial cell is shown in this micrograph. The oval nucleus, in the middle of the cell, bulges slightly. The cytoplasm shows other small bulges, presumably, of the underlying cell organelles. There are some fine holes present in the cell cytoplasm. A gap of varying widths separates the anterior endothelial cell from its neighbors. A large number of cytoplasmic processes from this and adjacent cells span the gap. x 4,600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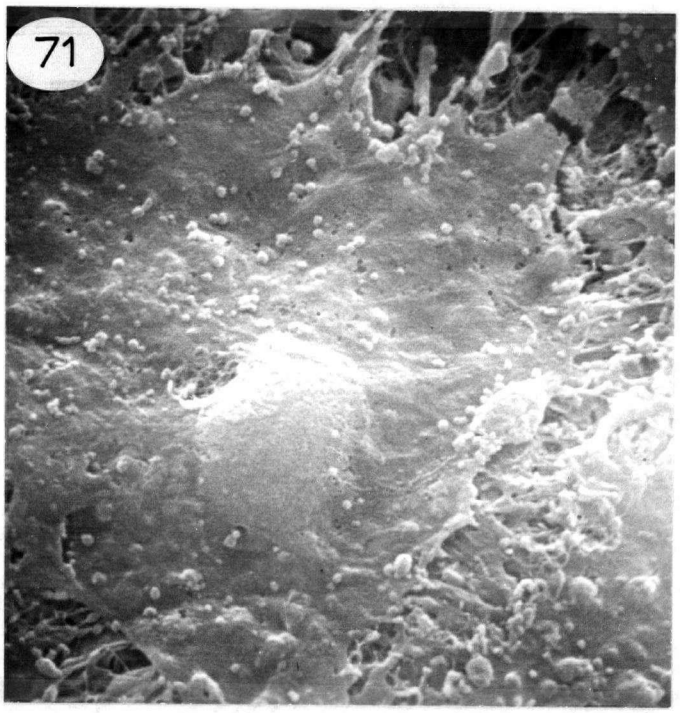
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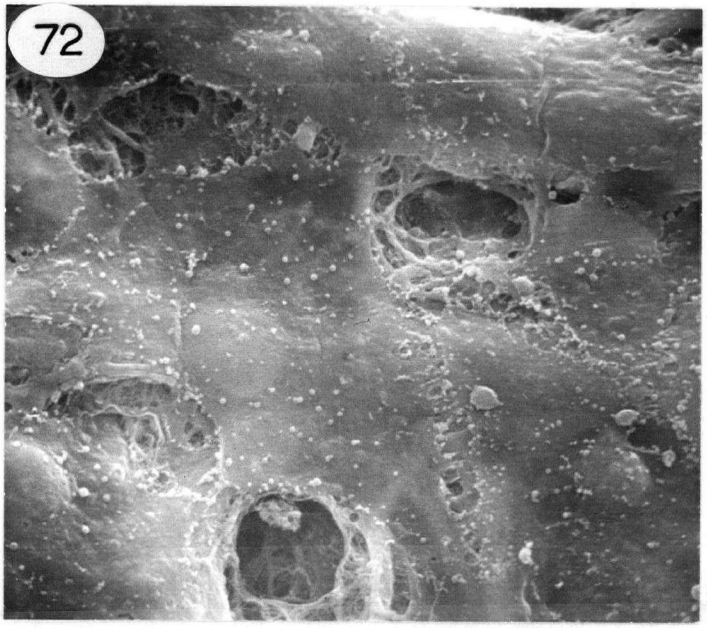
Figure 72 The Anterior Surface of the Iris in Pupillary Constriction (SEM)

The intercellular gaps between the anterior endothelial cells are sometimes small so that the endothelial cells appear to be joined together by loose seams. Crypts, with relatively well-defined margins, are numerous. They are quite often close to a blood vessel. Below the crypt opening, cytoplasmic processes of the stromal cells are present. x 1,800

Figure 73 The Anterior Surface of the Iris in Pupillary Constriction (SEM)

A single endothelial cell, as identified by its nucleus, is almost totally surrounded by a series of crypts. The crypts are round. The margins are smooth and well-defined. Cytoplasmic processes of the underlying stromal cells and a dense network of fibrillar material is seen through the crypt openings. x 4,100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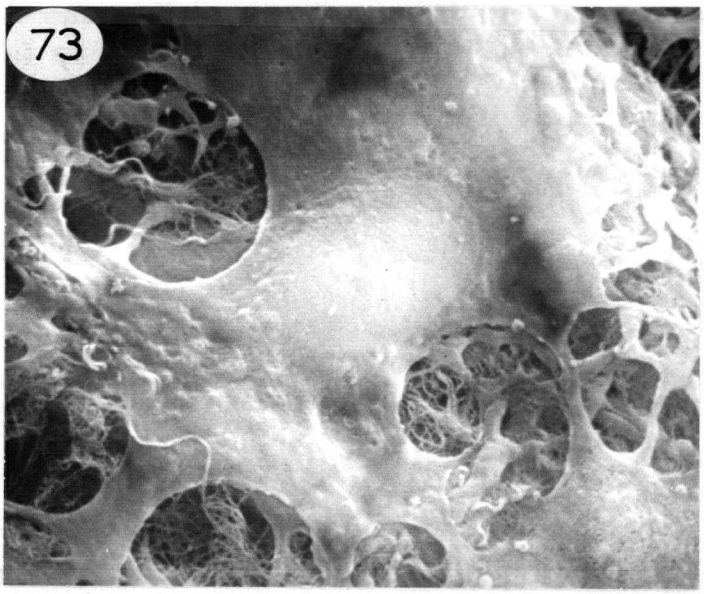
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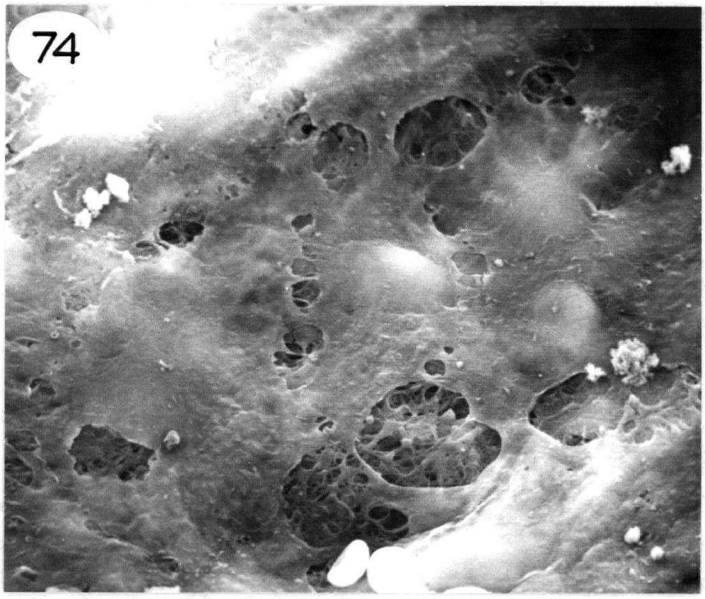
Figure 74 The Anterior Surface of the Iris in Pupillary Constriction (SEM)

The iridic crypts are of different sizes. The erythrocyte can be used as an indication of the relative sizes of the crypts.
x 1,600

Figure 75 The Anterior Surface of the Iris in Pupillary Constriction (SEM)

A very large crypt opening is completely filled with fibrillar material. x 4,100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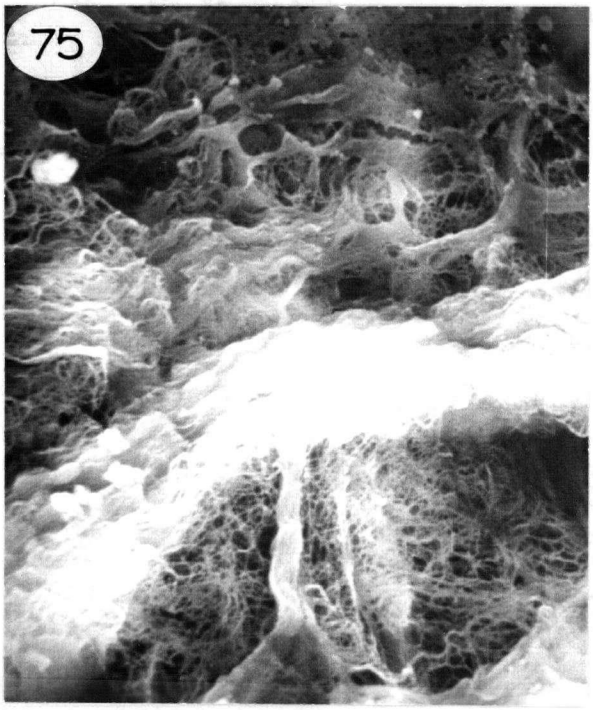
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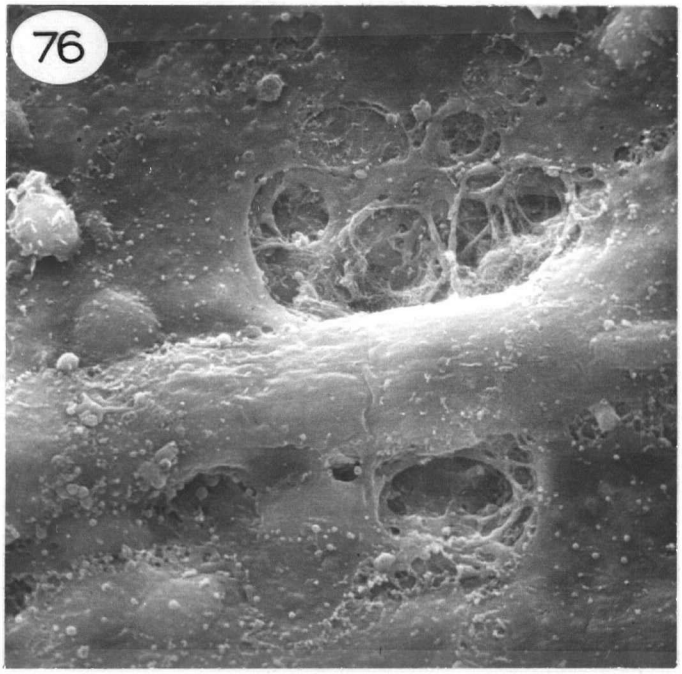
Figure 76 The Anterior Surface of the Iris in Pupillary Constriction (SEM)

Iridic crypts are very often associated with blood vessels.
x 1,800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E. A Light Microscopic Study of the Development of the Rat Iris Using Toluidine Blue Stained Plastic Sections

1. 19 Days Fetal (Figures 77-79)

At 19 days fetal, the ciliary body and iris are not too well developed as yet. In meridional sections the iris and the ciliary body together form a cone-shaped structure with its broad base capping the end of the retina (Figures 77-79). The ciliary body may be seen as a slight bulge interposed between the retina and the developing iris, or, at times there may be the faintest hint of a dip between the retina and ciliary body, and between the ciliary body and the iris, thus demarcating the three regions (Figure 78). However, most often one region just flows onto the next (Figure 77). Therefore, posteriorly there is a smooth line from the retina through the would-be ciliary body and onto the iris.

The ciliary body may not be grossly but it is cytologically delineated from the retina. There is a definite line of demarcation between the cells which are differentiating into the retina and those which are differentiating into the ciliary epithelium of the ciliary body (Figure 78). This is apparent in toluidine blue stained epon sections but not in hematoxylin and eosin stained paraffin sections.

Viewed as a whole in meridional sections, the ciliary epithelium is conical shaped with a wide base at the retinal junction and the apex directed towards the iris (Figures 77-79). At the ciliary-retinal junction there is a thick stratified mass of cells. This tapers down centrally to become a simple columnar epithelium at the ciliary-iris junction. Unlike at the ciliary-retinal junction where the cells of the developing retina and those of the developing ciliary epithelium are cytologically different, there is not a marked difference between the cells of the ciliary epithelium

and those of the posterior epithelium of the iris. Thus the epithelium is arbitrarily assigned as being part of the ciliary body or part of the iris. At the retinal junction, the ciliary epithelial cells are closely packed together in long vertical rows (Figures 77, 78). The nuclei are generally small, elongated and tapered at both ends. They are darkly staining and contain fine chromatin specks and nucleoli. These elongated nuclei occupy practically the whole of the cells. Oftentimes mitotic figures are seen. More centrally towards the iris, the nuclei are larger and are angularly elongated or polygonal in shape (Figure 79). They contain nucleoli as well. The nuclei are irregularly disposed within the cells. The cells of the ciliary epithelium when compared to those of the immediately adjacent retina, are particularly sensitive to the fixation and dehydration procedures used in preparing the tissues. The cytoplasm is highly vacuolated. Smaller vesicles are present in the apical (anterior) and basal (posterior) poles of the cells. The cytoplasm then appears much darker. Anteriorly next to the pigment epithelium, the ciliary epithelial cells seem to send out cytoplasmic processes which invaginate in between each of the pigment epithelial cells, thus giving the anterior surface of the ciliary epithelium a scalloped appearance (Figure 78). Posteriorly, the surface of the ciliary body has a slightly frayed look. Blood vessels, presumably from the hyaloid system, may be found along the posterior ciliary surface (Figure 78). Similar blood vessels are also seen along the posterior surface of the iris.

The pigment epithelium of the ciliary body, a continuation of the retinal pigment epithelium, may be seen as a long straight columnar epithelium (Figure 77), or the epithelium may be bent into a widely open U or V with the shallow trough of the U or V facing the ciliary stroma anteriorly (Figures 78, 79). At the points where the epithelium bends, there is a piling up of cells and thus the simple epithelial nature of this layer is

lost (Figure 79). When the ciliary pigment epithelium is an almost straight columnar epithelium, blood vessels and cells of the ciliary stroma are seen beginning to push into the epithelium (Figure 77). The slight anterior concavity of the epithelium is filled with blood vessels and stromal cells. In practically all of the specimens that were studied, the anterior (basal, stromal) surface of the pigment epithelium is very intimately associated with the endothelium of the blood vessels of the ciliary stroma. The endothelium of the incoming blood vessel closely lines the stromal surface of the pigment epithelium. There is hardly any visible intercellular gap between the two components, as seen light microscopically (Figures 78, 79). Neither are there any stromal cells interposed between the endothelium and the pigment epithelium. In some instances, the endothelium of a large blood vessel not only lines the anterior surface of the ciliary pigment epithelium but also then extends centrally to line the stromal aspect of the anterior epithelium of the iris (Figure 78). In comparison with the ciliary epithelium, the pigment epithelium is lightly staining. Generally, the nuclei are quite large, oval in shape, pale with fine specks of chromatin and contain nucleoli. The nuclei do vary in size and shape but this may be due to the plane of sectioning of the tissue. They are usually placed in the center of the cells but may also be slightly staggered from one cell to the next. In a few cells the nuclei are seen in mitosis. The cytoplasm is uniformly pale with very fine vesicles which are mainly concentrated towards the posterior apical poles of the cells (Figures 78, 79).

The stroma of the ciliary body is not very thick. Half its bulk consists of incoming blood vessels and the other half of stromal cells which appear to stream into the ciliary body from the cornea-scleral junction. The blood vessels, although large, have only a lining endothelium which abuts on the pigment epithelium (Figures 78, 79). The lumen is

widely patent and may be filled with blood cells. Smaller caliber blood vessels are also seen within the stroma. Sometimes the ciliary stromal blood vessels are observed to continue centrally into the stroma of the iris (Figure 78). The stromal cells are small with dark staining nuclei and cytoplasm. They are closely packed parallel to each other. There are a few intercellular spaces between the stromal cells. These cells are fibroblast-like with cigar-shaped nuclei. Some of the stromal cells are dividing.

The surface of the stroma facing the anterior chamber is smooth. It seems to be lined by a layer of squamous cells. Occasionally a cell bubbles outwards into the anterior chamber (Figure 78). This might be just an artifact of fixation.

The iris is not always clearly marked off from the ciliary body. It is short. The developing sphincter, which is a relatively well-defined egg-shaped bulge capping the extreme pupillary tip of the iris, makes up half to one third of the iris length (Figures 77-79).

The posterior epithelium of the iris (Figures 77-79) consists of high columnar cells peripherally near to the ciliary junction. There is a gradual transition to low columnar or high cuboidal cells at the peripheral extent of the developing sphincter. The nuclei are darkly stained and large, occupying most of the cell height and width. Near the ciliary body where the cells are columnar, the nuclei are long and thin with irregular nuclear outlines and they are stretched out between the poles of the cells. Towards the peripheral edge of the sphincter where the cells are lower, the nuclei are more or less angulated or diamond-shaped. The cytoplasm is broken up and lacy throughout the cells except for thin strips of dark staining cytoplasm along the anterior and posterior poles of the epithelium. It also appears that the cytoplasm is more vacuolated in the cells nearer

to the ciliary epithelium. The posterior epithelial cells form a relatively smooth lining for the posterior iridial surface.

Unlike the posterior epithelium, the anterior epithelium is an evenly wide, uniformly pale, simple high columnar epithelium, except in parts where there may appear to be two layers of cells (Figures 78, 79). The nuclei are large, pale, oval to round in shape with prominent nucleoli. They are situated in the center or a little towards the posterior half of the cells. The nuclear envelope is smooth or there may be some indentations. The cytoplasm is pale with some fine vesicles which may be randomly distributed but are generally slightly concentrated towards the posterior poles of the cells at the boundary zone with the posterior epithelium (Figures 78, 79). Anteriorly, the stromal surface of the epithelium is molded and contoured by large stromal blood vessels which also extend centrally over the anterior surface of the developing sphincter (Figures 78, 79).

The developing sphincter is a distinct knob-like or egg-shaped mass of light staining, elongated and centrally tapering cells (Figures 77-79). It is the pupillary (central) expansion of the anterior epithelium of the iris. The cells may be randomly arranged or they may be oriented towards the center of the cell mass. The developing muscle cells are closely packed together. Individual cell outlines are not visible. The nuclei, like the cells, are elongated and smaller than those of the adjacent anterior epithelium. The cytoplasm is sometimes slightly finely vesiculated. The central and anterior surfaces of the developing sphincter presents a smooth outline to the overlying and overhanging stroma. A small but quite easily discernible intercellular space separates the smooth surface of the developing muscle tissue from the iris stroma (Figure 79). Over the rest of the iris there is usually no intercellular space between the stroma and

the anterior epithelium (Figure 77).

The iris stroma is highly vascular. In fact, sometimes one large blood vessel with its patent lumen oriented parallel to the length of the iris occupies most of the extent of the stroma (Figures 78, 79). Unlike other large blood vessels in other locations in the body, or even some of the blood vessels in the adult iris stroma, these blood vessels have only an endothelial lining and no other supporting structures. As before mentioned, the endothelium closely lines the stromal surface of the anterior epithelium. The few stromal cells that are present are small, fibroblast-like with dark nuclei. They are usually arranged parallel to each other and to the posterior iris surface. These stromal cells can also come into very close contact with the anterior epithelium, where the latter is not lined by endothelium. The stroma overhangs the extreme pupillary edge of the developing sphincter and the stromal elements and the blood vessels seem to stream centrally presumably to contribute to the make-up of the pupillary membrane (Figures 77-79).

The anterior surface of the iris is convex anteriorly so that there is more stromal tissue in the middle of the iris. This surface is relatively smooth except for an occasional bubble-like cell (Figures 78, 79).

Figure 77 19 Days Fetal (LM)

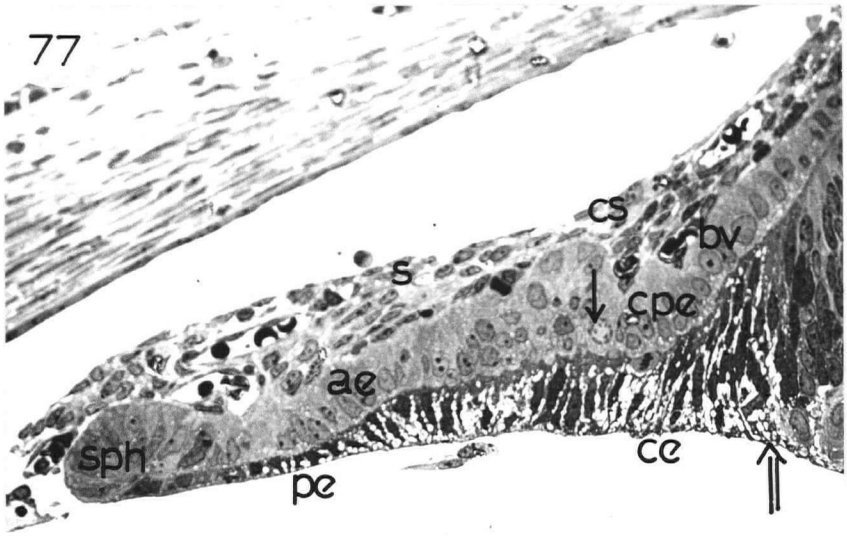
The retina, developing ciliary body and iris are not grossly well separated from each other. Cytologically, there is a distinct line of demarcation between the cells differentiating into the retina and those differentiating into ciliary epithelium (double arrow). The ciliary epithelium (ce) consists of a stratified mass of cells. The cytoplasm is highly broken up except for a thin strip along the boundary with the ciliary pigment epithelium (cpe). The ciliary pigment epithelium is a relatively straight, light staining, high columnar layer. A cell is seen in a mitosis (arrow). The posterior surface of the ciliary pigment epithelium is closely adherent to the ciliary epithelium. The contour of the stromal surface is being carved out by the blood vessels (bv). The posterior epithelium (pe) of the iris decreases in height from the ciliary junction towards the pupillary margin. As with the ciliary epithelium, the cytoplasm is broken up except for a thin strip between the anterior (ae) and posterior epithelium. The anterior epithelium is a uniformly high columnar pale staining layer. The developing sphincter (sph), a knob-like structure, consists of closely packed cells oriented centripetally. Nucleoli are seen in many of the cells of the ciliary pigment epithelium, the anterior epithelium of the iris and the developing sphincter. The ciliary stroma (cs) and iris stroma (s) are in continuity. It consists of small blood vessels and stromal cells. The stromal cells are closely adherent to the stromal surface of the anterior epithelium but there is a visible gap between the stroma and the anterior boundary of the sphincter.

The anterior and posterior surfaces of the iris and ciliary body is relatively smooth. x 220

Figure 78 19 Days Fetal (LM)

The developing ciliary body forms a slightly more prominent bulge between the iris and the retina. The appearance of the ciliary epithelium (ce), posterior epithelium (pe), anterior epithelium (ae) and developing sphincter (sph) is similar to that in Figure 77. However, the ciliary pigment epithelium (cpe) is bent into a shallow V with the trough of the V being filled with blood vessels. The endothelium of the blood vessel (bv) in the stroma (cs) is closely apposed to the ciliary pigment epithelium (arrow). The blood vessel of the ciliary stroma extends forwards as the blood vessel lining the anterior surface of the anterior epithelium of the iris (ae).

Blood vessels are seen along the posterior surface of the ciliary body (bv1) and past the pupillary tip of the sphincter (bv2) x 220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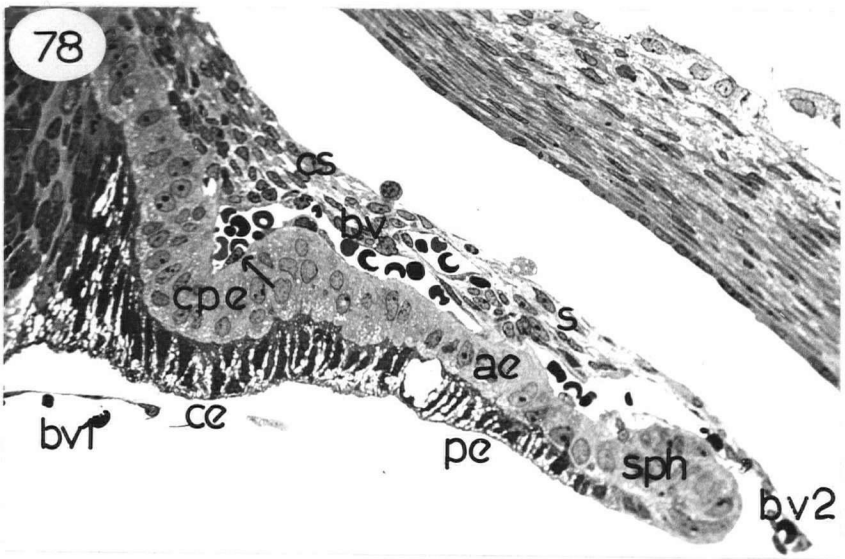
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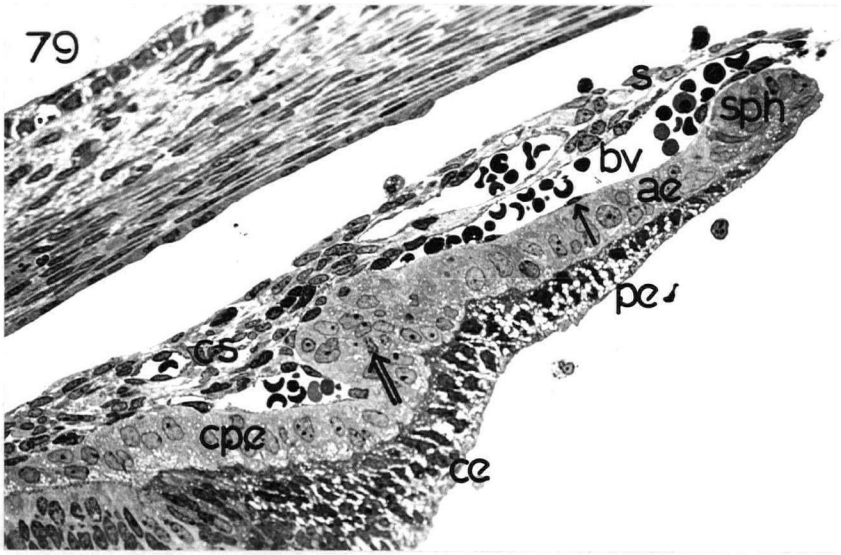
Figure 79 19 Days Fetal (LM)

The histological features of the ciliary epithelium (ce), posterior epithelium (pe), anterior epithelium (ae) and sphincter (sph) are similar to those in Figure 77. The ciliary epithelium (cpe) is bent into a V with a blood vessel occupying the trough (bv). At the crest of the fold of the ciliary pigment epithelium, there is a pile-up of cells. One of the cells of the ciliary pigment epithelium is dividing (double arrow). The cells of the ciliary stroma (cs) stream past into the iris stroma (s). Large blood vessels (bv) with the lumens parallel to the length of the iris are present. A large blood vessel lines the whole of the stromal surface of the anterior epithelium and of the sphincter. The endothelium (arrow) is adherent to the stromal surface of the anterior epithelium but there is a space between it and the sphincter (*). x 220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2. 20-21 Days Fetal (Figures 80-82)

At 20 and 21 days fetal the appearance of the ciliary body and iris is not too different from that at 19 days. Grossly, the ciliary body may be seen as a slightly larger bulge posteriorly better delineated from the retina peripherally and the iris centrally (Figures 80, 82). Fetal rats from the same litter do show differences in the stage of development of the tissues under study. Thus in some instances what is observed at 20 days fetal is very similar to what is observed at 19 days fetal.

Histologically, some features which are not as apparent at 19 days fetal are slightly accentuated at 20 days fetal. In the ciliary body the appearance of the posterior ciliary surface and of the ciliary epithelium is much like that at 19 days fetal. However, the anterior (apical) outline of the ciliary epithelium may bulge anteriorly so that the ciliary epithelium looks like little hillocks directed towards the pigment epithelium. A thin strip of darkly staining cytoplasm of the ciliary epithelium contrasts remarkably with the lighter staining vesiculated apical cytoplasm of the ciliary pigment epithelium.

The pigment epithelium is beginning to buckle and shows the earliest signs of folding of the whole epithelium (Figure 80). The stromal surface of the epithelium may be deeply folded so that only a small cleft separates the epithelial surfaces (Figure 82). The cleft is normally entirely occupied by capillaries with thin walled squamous endothelium. On the other hand, the epithelium may show a couple of shallow folds (Figure 80). Blood vessels and stromal cells from the ciliary stroma tumble down to fill the troughs of the folds (Figures 80, 82). Most often, the capillaries are found in the depths of the folds. The darker staining endothelial cell nuclei can be seen protruding into the lumen. The peaks of these folds are usually intimately associated with the stromal cells.

The pigment epithelium may be uneven in its thickness. In most places it is a light staining, simple, high columnar epithelium with oval to polygonal nuclei containing nucleoli. However, particularly at the crests of the folds there may be instead a thick mass of cells (Figure 80).

In the anterior half of the ciliary stroma the cells seem to stream past the ciliary body on to the iris, whereas, in the posterior half of the stroma the cells cascade down into the folds of the pigment epithelium (Figures 80, 82).

The iris has not grown considerably in length as compared to that at 19 days fetal. The sphincter appears slightly more pronounced (Figure 81). The developing muscle cell mass is an egg-shaped structure. The cells are elongated, centrally tapering and centripetally oriented. In terms of its stainability with toluidine blue, the developing sphincter muscle cells stain similar to, or at the very most, very slightly different from that of the anterior epithelium. The developing sphincter is essentially the pupillary expansion of the anterior epithelium.

At this stage many of the cells are actively dividing. Mitotic figures are not confined to any particular region of the iris or ciliary body so that it is not possible to speak of a zone of growth. Many of the mitotic figures are found in the ciliary stroma (Figure 80), ciliary epithelium (especially near the retinal junction) and the iris anterior epithelium (Figure 82). Some of the developing sphincter muscle cells are also dividing (Figure 82). Since no chemical agents, like colchicine, are used to arrest mitosis at metaphase, the number of mitotic figures present is only a mere indication of the extent of cell division that might be taking place.

Figure 80 20-21 Days Fetal (LM)

The histological characteristics of the posterior epithelium (pe), anterior epithelium (ae) and ciliary epithelium (ce) are much like those at 19 days fetal (Figures 77-79). The sphincter muscle cells are closely packed together (sph). The ciliary pigment epithelium (cpe) is beginning to buckle giving rise to a series of shallow folds, the depths of which are filled with capillaries containing red blood cells (bv). Mitoses are seen in all parts of the ciliary (cs) and iris (s) stroma and in the ciliary pigment epithelium (arrows). The cells and blood vessels (bvl) of the iris stroma stream past the pupillary tip of the sphincter. x 220

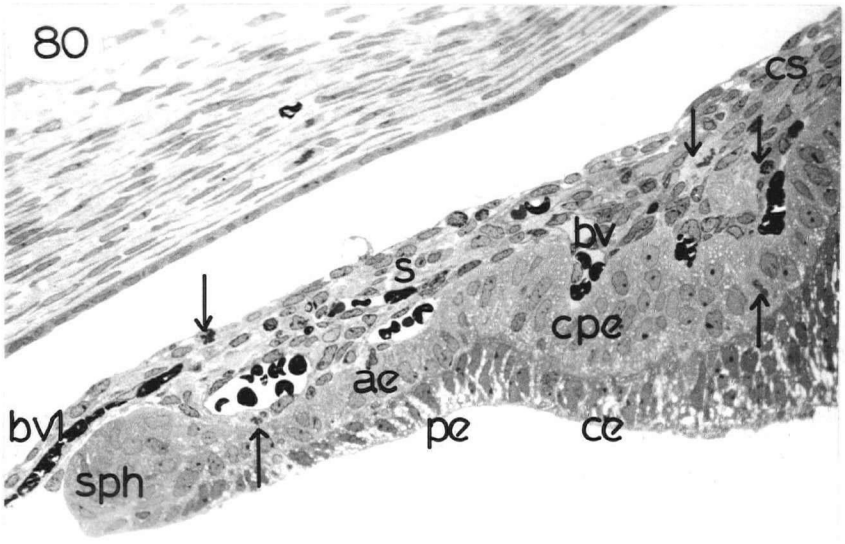
Figure 81 20-21 Days Fetal (LM)

The developing sphincter (sph) is clearly seen as the pupillary expansion of the anterior epithelium (ae) of the iris. The nuclei are large, pale and irregularly shaped. The developing muscle cells are packed close together. A few vesicles are present. There is a small but perceptible gap (arrow) between the anterior surface of the sphincter and the overlying stromal (s) blood vessel (bv).

The posterior epithelium (pe) decreases in height till the peripheral extent of the sphincter where the cells become trapezoidal as they line the posterior surface of the sphincter. The stroma consists of a mass of small cells mainly oriented parallel to the iris posterior surface. A large blood vessel of the iris stroma is seen following the contours of the stromal surface of the anterior epithelium (ae), the anterior surface of the sphincter, turning round at the pupillary tip of the sphincter and appear to be headed posteriorly. The anterior and posterior surfaces of the iris is smooth.
x 350

Figure 82 20-21 Days Fetal (LM)

Mitotic figures are seen in the ciliary epithelium (ce), anterior epithelium (ae) and in the developing sphincter (sph) (arrows). The iris stroma (s) continues centrally as part of the pupillary membrane (pm). x 220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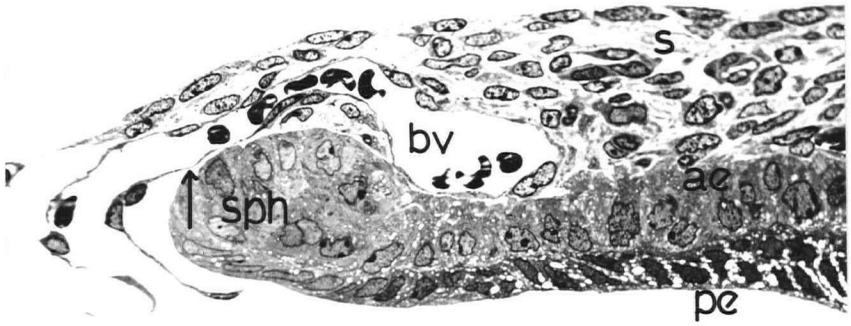
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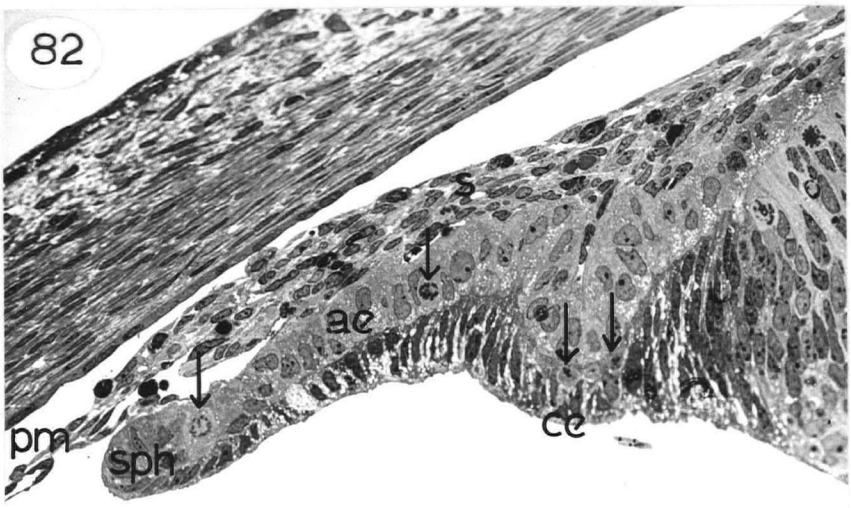
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3. 1-4 Days Post-natal (Figures 83-86)

In the first few days after birth, certain changes are seen in the ciliary body as well as in the iris. In the first day, the ciliary body forms a prominent bulge into the posterior chamber (Figure 83). The demarcation between retina, ciliary body and iris is quite distinct. The ciliary body is seen as one large bulky process, unlike the delicate ciliary processes in the adult eye. However, by the third and fourth days, the beginnings of the division of the ciliary body into ciliary processes is observed (Figure 84). At birth, the ciliary pigment epithelium is folded, with blood vessels occupying the clefts of the folds. Now, the ciliary epithelium follows suit and begins to fold (Figure 84). The tip of the ciliary body bifurcates. Initially, the bifurcation is only seen in the pigment epithelium, but gradually the ciliary epithelium also bifurcates. In certain specimens, the ciliary body is divided into two sets of processes, one being directed centrally and the other more laterally. These ciliary processes are short and bulky. Or, there may be more than two ciliary processes. Differences are quite common, and is the rule rather than the exception. As a whole, the ciliary body is still attached to the retina by a broad base, but it is not as broad as in younger eyes.

The iris grows in length although not to a considerable extent. The ratio of the bulk of the sphincter to the total length of the iris is an indication of the rate of growth. The bulk of the sphincter remains relatively constant. Initially, the sphincter makes up about half of the iris length. By four days after birth, the sphincter may comprise one third to one fifth of the iris length (Figure 83).

The ciliary epithelium (Figures 83, 84) shows different configurations according to its location. Near the ciliary-retinal junction, the cells are elongated and are closely packed together. The darkly staining

nuclei are long and thin and tapering at both ends. There may be fine specks of heterochromatin. Since the cells are narrow, one gets the impression of a series of closely aligned dark nuclei. The ciliary epithelial cells over most of the ciliary body are columnar, especially at the tip of the ciliary body. The nuclei are elongated, polygonal or irregularly oval. They may show indentations of the nuclear envelope. If ciliary processes are distinguishable, then the cells at the tip of the ciliary process are slightly higher than those at the base of the ciliary process. The change is gradual. Towards the root of the iris, the ciliary epithelial cells alter in form from columnar to cuboidal. The nuclei are dark and polygonal. The overall appearance of the ctyoplasm is not consistent. It is usually vesiculated, more so posteriorly. The cytoplasm is a little denser at the boundary zone with the pigment epithelium (Figure 83). The cytoplasm may even acquire a lace-like effect at the tip of the developing ciliary processes (Figure 84). Some of the epithelial cells are seen in mitotic division. The posterior surface of the epithelium is relatively smooth, or at times, it might have a frayed look to it (Figures 83, 84).

The pigment epithelium of the ciliary body is always more deeply and more extensively folded than the ciliary epithelium. The posterior surface follows the contour of the ciliary epithelium but the anterior surface outline is much more exaggerated than the contour of the posterior surface. The cells are columnar, in differing degrees (Figure 84). The epithelium consists of a single layer of columnar cells except where the folds occur. Here, there is a pile up of cells. It appears that some rearrangement of the cells in this layer is taking place. The nuclei are large, polygonal and light staining (Figure 83). They are usually placed in the center of the cells. Nucleoli are often present. The cytoplasm

is finely vesiculated in most cells. Sometimes, though, one cell is vesiculated while its neighbor is not. Overall, the cytoplasm appears pale. There are fewer vesicles towards the stromal poles of the cells, so that the cytoplasm is darker, whereas towards the apical poles of the cells, the cytoplasm at times appears frothy. The boundary between the pigment epithelium and the stroma is barely visible except in isolated spots.

The ciliary stroma is not wide (Figure 83). It consists of small, closely-packed stromal cells and some blood vessels. The stromal elements are closely associated with the anterior surface of the pigment epithelium. They squeeze in to occupy the narrow grooves formed by the pigment epithelial folds (Figure 83). In general, blood vessels (mainly capillaries) invaginate themselves into these grooves accompanied only by a few stromal cells. The stromal cells towards the anterior and more superficial surface of the ciliary stroma are oriented differently from those deeper in the stroma. They are parallel to the iris, whereas the deeper cells change direction by ninety degrees to enter the clefts of the developing ciliary processes (Figure 83).

Dividing cells are not only encountered in the ciliary epithelium but also in the pigment epithelium and in the stroma. The formation of the ciliary processes would entail not only a shifting and repositioning of cells but also an increase in the number of cells. Hence, the number of mitoses that are present.

The iris is short and stubby at first but grows a little by 4 days post-natal. The pupillary membrane is still seen extending centrally past the developing sphincter.

The posterior epithelium as a whole is diminishing in height (Figures 83-86). The posterior epithelial cells are cuboidal or brick-shaped cells over most of the iris posterior surface. At the junction with the

ciliary body, they may be slightly higher (Figure 83). Towards the pupillary margin, the posterior epithelium becomes squamous or very low cuboidal where it lines the posterior surface of the sphincter (Figures 85, 86). The nuclei are small, irregularly polygonal and occupy most of the cell volume. Where the cells are squamous, the nuclei are flattened parallel to the cell length. The cytoplasm stains darkly and is vesiculated. The posterior iris surface is relatively smooth (Figure 83) or may be slightly wavy or frayed (Figure 86).

The anterior epithelium is a high columnar epithelium throughout its extent. In most instances, the anterior epithelium is two to three times the height of the posterior epithelium. Towards the iris root, the epithelium may appear stratified with two rows of nuclei being observed (Figure 84). The epithelium, as a whole, stains lighter than the posterior epithelium. The nuclei are pale and are usually round or oval in shape, possessing an occasional nucleolus. When the nucleus is oval, the long axis of the oval is perpendicular to the length of the iris. The nuclei are quite regularly spaced out along the epithelium, indicating the number of cells in the epithelium. However, the boundaries between adjacent cells are not easily seen. The cytoplasm is light staining. Tiny vesicles are seen in the cytoplasm usually towards the posterior halves of the cells at the boundary zone with the iris posterior epithelium (Figure 83). Initially, the anterior half of the cytoplasm stains uniformly. However, by the third or fourth day after birth, patches of uniformly darker staining areas are seen along the sides of the nuclei and especially more so at the anterior stromal parts of the anterior epithelium. Oftentimes, there are more such dense patches towards the periphery of the iris. These dense patches, visible at the light microscopic level at this stage in the development of the iris, are possibly patches of myofibrils of the future dilator muscle.

The anterior surface of the anterior epithelium is very closely associated with the stromal elements (Figures 83, 85). It is difficult to separate out the two layers since the cytoplasm of the stromal cells stains almost the same intensity as the cytoplasm of the anterior epithelial cells. It almost seems as if the cytoplasm of both layers merge together. The outline of the anterior surface of the epithelium is fashioned out by the stromal elements. It may have a scalloped or undulating appearance (Figure 83).

The anterior epithelium decreases in height and becomes flattened at the peripheral edge of the developing sphincter (Figure 85). It invaginates itself between the posterior epithelium and the sphincter cells at the peripheral half of the sphincter mass (Figure 85). Then, centrally, the anterior epithelium seems to flare out, as it were, to give rise to the sphincter cells. There is no intercellular space between the anterior surface of the squamous epithelial cells and the sphincter cells.

Rarely, there is a space towards the tip of the iris between the anterior and posterior epithelia. It is the once so-called "margin sinus" (Mann, 1964). However, recent data suggest that the marginal sinus is only an artifact of fixation in the human (Hvidberg-Hansen, 1970) and monkey (Tamura and Smelser, 1973) developing iris. Hence, in the rat, any such space, which is rarely encountered, is probably also an artifact.

The sphincter, in meridional sections, is variously shaped (Figures 83, 85, 86). It may be a large oval (Figure 83) or sausage-shaped mass of cells (Figure 85). It is sometimes slipper-shaped like a Paramecium. The pupillary and peripheral border of the sphincter is smooth and rounded. Sometimes, though, a bit of the sphincter may extend peripherally much like a cliff over the anterior epithelium, with stromal tissue occupying the intervening space (Figure 86). On the first day after birth, the

anterior outline of the developing sphincter is relatively smooth (Figure 83). But by the third or fourth day post-natal, it has acquired a scalloped appearance, as if the whole sphincter muscle is being divided into a number of large bundles of fibers (Figures 85, 86). The sphincter, unlike the rest of the anterior epithelium, is distinctly separated off from the stroma by an intercellular space (Figures 83, 85, 86).

On the first post-natal day, the developing sphincter cells are usually elongated or wedge-shaped and they are arranged centripetally. The cytoplasm is uniformly staining, much like that of the anterior epithelium. The cells are packed closely together so that the cell boundaries are not apparent. The nuclei are small, elongated or irregularly shaped. From the first to the fourth post-natal day, a few changes are observed. There are more sphincter cells. This is a result of mitosis among the sphincter cells. An occasional mitotic figure is seen. The cells are small and are still quite close together. However, some small intercellular spaces are beginning to appear in between the developing sphincter cells, thus giving the sphincter a crackled glass effect (Figure 86). The nuclei are small, have irregular nuclear outlines and are unevenly placed among the cells. The cytoplasm may be uniformly densely staining for all of the cells. Most commonly, though, the individual cells show varying staining intensities. This perhaps indicates that the muscle cells do not all attain the same degree of differentiation at the same time. This is logically to be expected if some of the cells are still dividing.

The iris stroma is both highly cellular and highly vascularised (Figures 83, 85, 86). Overall the stroma is not very thick. At the iris root, the stroma is thin but its width increases slightly as one moves towards the pupillary margin. At the iris root, the stromal cells are small and are closely packed so that there are no, or few, intercellular spaces.

At the root, unmyelinated nerves are sometimes discernible. In other parts of the stroma, the cells are more loosely spaced. The cells may be haphazardly arranged although in general they are oriented parallel to the posterior iris surface. There are many blood vessels of various sizes in the stroma. Capillaries are seen throughout the stroma but are especially concentrated in the sparse stroma over the sphincter. These capillaries and some stromal cells extend beyond the pupillary margin of the sphincter muscle and overhang it (Figure 83). The capillaries continue on as, presumably, part of the pupillary membrane. The capillaries of the pupillary membrane are interconnected to the iris stroma proper by fine connective tissue strands. Large or small blood vessels usually follow the curve of the anterior epithelium and of the sphincter (Figure 83). Sometimes a single large blood vessel enters the root of the iris and extends all the way to the pupillary margin with its lumen parallel to the iris length. These blood vessels, though large, consist only of a simple endothelial lining. The endothelium often abuts directly onto the anterior epithelium. Sometimes, a stromal cell or two may be interposed between the endothelium and the anterior epithelium. As mentioned previously, the stroma is almost adhesive to the anterior surface of the anterior epithelium (Figures 83, 85), but is distinctly separated from the sphincter (Figure 83). Whether this is of any functional significance is not known.

The anterior surface of the iris stroma is smooth (Figures 83, 85, 86). It almost seems to be lined by a continuous endothelium. Occasionally, some of the anterior endothelial cells show fine microvilli projecting into the anterior chamber. Mitoses are seen among these endothelial cells as well as in the stroma.

Figure 83 1-4 Days Post-natal (LM)

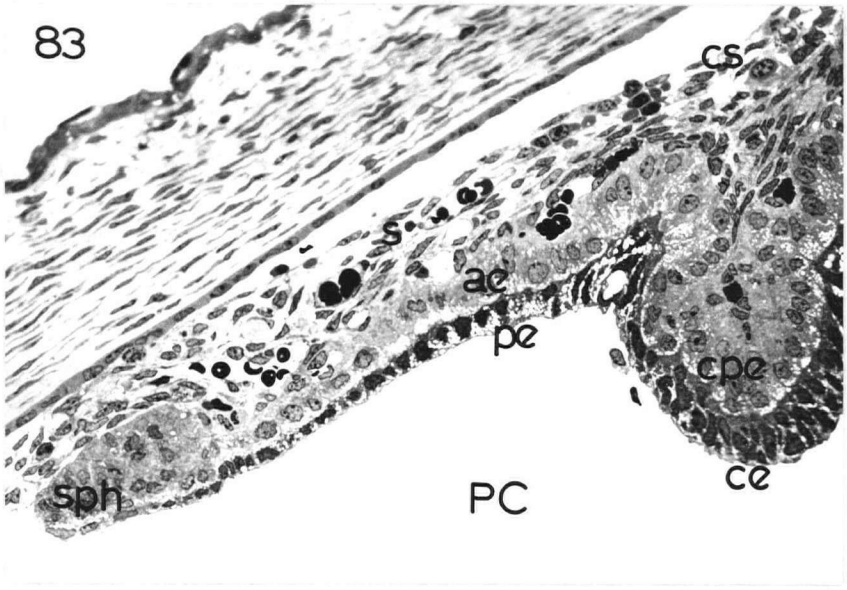
At 1 day post-natal, the iris is still short. The ciliary body forms a prominent bulge into the posterior chamber (PC). The ciliary epithelium (ce) is a dark-staining layer. The ciliary pigment epithelium (cpe) is light staining and deeply folded. Blood vessels and stromal cells tumble in from the ciliary stroma (cs) to occupy the narrow clefts of the ciliary pigment epithelium. At the boundary with the ciliary epithelium, the cytoplasm of the ciliary pigment epithelium is vesiculated. The posterior epithelium of the iris (pe) is not as high as in younger eyes. The cells are more or less cuboidal or brick-shaped with very dark staining nuclei. At the posterior surface of the sphincter (sph), the posterior epithelial cells are more flattened with lighter staining nuclei. The anterior epithelium (ae) is a pale, high columnar layer. The boundary between the anterior epithelium and the stroma (s) is not distinct. The stromal surface of the anterior epithelium is being molded by the stromal elements.

The stroma of the ciliary body and iris is filled with small stromal cells and numerous thin walled capillaries.

The anterior outline of the sphincter is relatively smooth. The sphincter is oval in shape and consists of a mass of small cells which appear to stain darker than the rest of the anterior epithelium. Small intercellular spaces are seen in between some of the sphincter muscle cells. x 220

Figure 84 1-4 Days Post-natal (LM)

At 4 days post-natal, a number of large, bulky ciliary processes are formed by the foldings of both the ciliary pigment (cpe) and the ciliary epithelium (ce). Stromal cells and blood vessels insinuate in between the spaces formed by the folds of the epithelia. The cytoplasm of the ciliary epithelial cells at the tip of the developing ciliary process has a lace-like effect. x 220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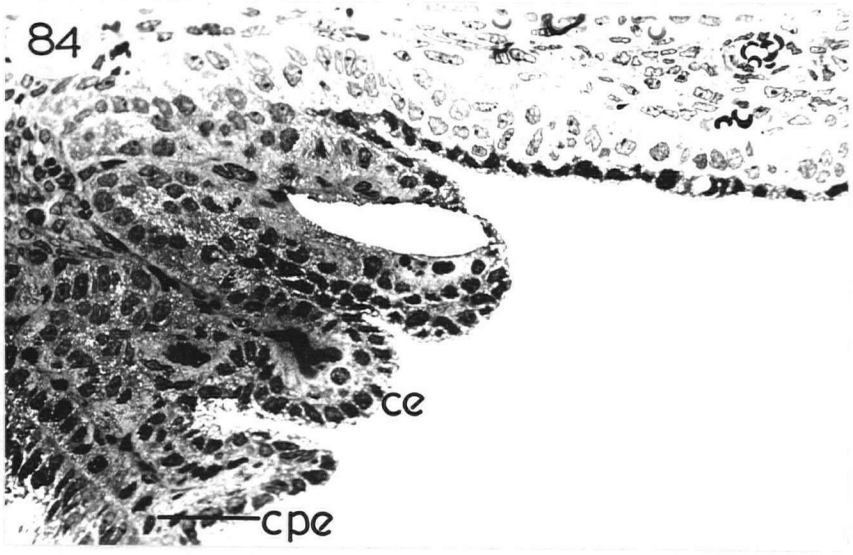
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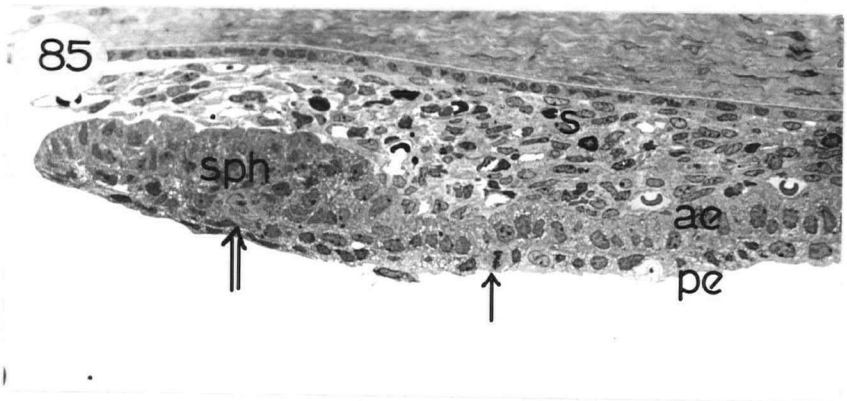
Figure 85 1-4 Days Post-natal (LM)

The posterior epithelium (pe) of the iris is a layer of cuboidal cells which become squamous along the posterior surface of the sphincter (sph). One of the posterior epithelial cells is dividing (arrow). The anterior epithelium (ae) is just very slightly higher than the posterior epithelium. At the peripheral posterior half of the sphincter, the anterior epithelial cells become squamous and invaginate themselves in between the posterior epithelium and the sphincter (double arrows). The sausage-shaped sphincter has acquired a slightly scalloped appearance anteriorly. Small intercellular spaces are seen in between some of the cells. x 220

Figure 86 1-4 Days Post-natal (LM)

The anterior (ae) and posterior (pe) epithelium of the iris are as in Figure 85. The sphincter (sph) consists of groups of muscle cells separated by intercellular spaces giving the sphincter a crackled glass effect. The anterior surface of the sphincter is highly scalloped. At the peripheral extent of the sphincter, a portion of the sphincter extends over the anterior and posterior epithelium and the intervening gap is filled with stromal elements (arrow). x 220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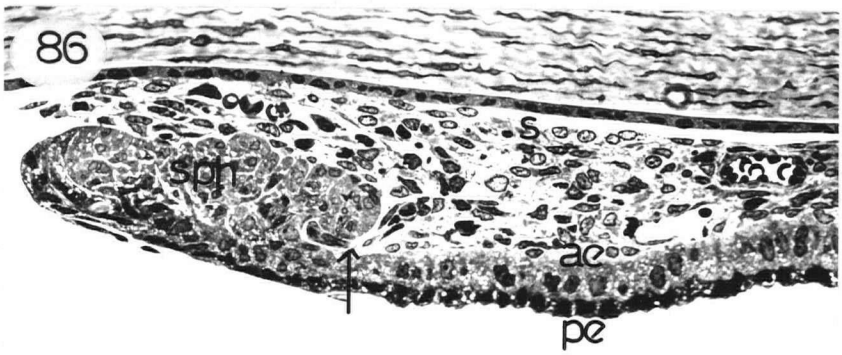
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4. 5-10 Days Post-natal (Figures 87-90)

Between the fifth and tenth days after birth, the iris and ciliary body are slowly acquiring characteristics typical of the adult form. The ciliary body at first consists of one or two large, bulky and short processes. But by the tenth day they have grown a little (Figure 87). They are still relatively short and are by no means anywhere like the delicate finger-like processes of the adult. The ciliary processes are not held close to the retina but are positioned more towards the iris. The iris has grown in length so that by the tenth day, the sphincter forms only one fifth or less of the length of the iris. Overall, the iris appears quite long and thin and has somewhat the configuration of the adult iris in pupillary constriction.

When the ciliary processes are short and bulky, as at 5 days post-natal, the ciliary epithelium is much like that at 4 days post-natal. By the seventh to tenth days after birth, the ciliary processes are a little longer. Differences are seen among the cells of the ciliary epithelium according to their location (Figure 87). The ciliary epithelium near the retina consists of columnar cells which may have a stratified appearance. The nuclei are darkly staining and elongated. The cytoplasm is highly vesiculated except for a small strip of cytoplasm adjacent to the pigment epithelium which is devoid of vesicles.

The ciliary epithelium along the sides of the developing ciliary processes is low columnar or cuboidal. The nucleus, which is variously shaped, is in the center of the cell and occupies most of the cell volume. The cytoplasm is quite dark staining with a few vesicles towards the posterior surface. At the apical poles of the ciliary epithelial cells the cytoplasm is uniformly darkly staining in contrast to the lighter staining, generally vesiculated cytoplasm of the pigment epithelium. The

posterior surface of the ciliary epithelium lining the sides of the ciliary processes is relatively smooth or it may be just very slightly irregular.

The ciliary epithelial cells at the tip of the developing ciliary processes are high columnar cells (Figure 87). The nuclei are longer and are oriented along the length of the cells. They have a tendency to be placed towards the apical poles of the cells, that is, towards the pigment epithelium. The apical cytoplasm is uniformly darkly staining whereas the basal cytoplasm appears finely vesiculated all over and has a lacy appearance. There seems to be many invaginations and interdigitations, thus imparting to the posterior surface of the tips of the ciliary processes a highly irregular outline.

As the ciliary epithelium continues towards the posterior epithelium of the iris, there is a gradual transition from low columnar to low cuboidal cells to almost squamous cells (Figure 87). The nuclei are large and dense and fill up almost all of the cell. The posterior surface of the epithelium facing the posterior chamber is smooth.

No significant changes are seen in the pigment epithelium or stroma of the ciliary body. Both are similar to what has been previously observed and described. As before, dividing cells are present in the ciliary epithelium, pigment epithelium and in the stroma.

By the tenth day after birth, the iris is quite long and distinct. The posterior epithelium consists of a single row of very low cells. They may be trapezoidal in shape. At other times, the whole epithelium consists of a row of brick-like cells, or the cells may be flattened (Figures 87-90). In all cases, the posterior epithelium becomes flattened when it lines the posterior surface of the developing sphincter (Figure 90). The nuclei are small and are usually flattened antero-posteriorly. They may occur regularly along the whole epithelium or there may be large gaps between the

nuclei. This is probably due to the plane of sectioning.

The posterior surface of the iris is usually smooth although occasionally it may be a little wavy (Figure 90). By the tenth day after birth, there are still some posterior epithelial cells which are dividing to form more epithelial cells.

The anterior epithelium is of a uniform width from the root to the lateral edge of the sphincter. The shape of the cells range from columnar (Figures 88, 90) to rectangular (Figure 89). Initially, the anterior epithelial cells are two to three times the height of the posterior epithelial cells. But gradually, it almost seems as if the anterior epithelium is being stretched out such that by the tenth day post-natal the anterior epithelium is oftentimes the same height as the posterior epithelium (Figure 89). When the anterior epithelium is columnar, the nuclei are large and round or oval with some nuclear indentations and are placed in the center of the cells. The cytoplasm is slightly vesiculated at its apical poles (Figure 88). In the cytoplasm at the basal (or stromal) parts of the anterior epithelium there are dark patches of the developing dilator muscle. When the anterior epithelium is about the same height as the posterior epithelium, the cells are cuboidal or rectangular (Figure 89). The nuclei, which may be irregularly shaped, are generally oriented parallel to the posterior iris surface.

The sphincter is a large, relatively tightly packed mass of small cells bounded anteriorly by a small amount of stroma and posteriorly by low epithelium (Figure 90). The anterior surface of the sphincter is scalloped in appearance and is separated from the stroma by an intercellular space. The developing muscle cells are small with small, variously irregularly shaped nuclei. Groups of muscle cells seem to associate together in groups and are separated from other muscle groups by distinctly

visible intercellular spaces (Figure 90). However, the individual muscle cells may be interconnected with each other. The muscle cells generally stain darker than the anterior epithelial cells. The cytoplasm is uniformly darkly staining for any one cell but staining intensities vary for the individual cells. In the midst of this mass of differentiating muscle cells, an occasional mitotic figure is encountered.

The iris stroma is not extensive. It is thin at the iris root (Figures 87, 89) and increases only slightly in width towards the pupillary margin. Blood vessels, nerves, stromal cells and connective tissue are present. In the first few days after birth, the stroma still overhangs the pupillary edge of the sphincter. However, by the tenth day, either the stroma has regressed or the neuroectodermal elements of the iris have stretched centrally. Some blood vessels of the stroma curve around the pupillary edge of the sphincter. Also, there are still some vessels which continue past the pupillary border of the iris into the pupil itself. These small capillaries are usually strung out in a line with connective tissue strands interconnecting the individual capillaries. It could be that even at this late stage of development (10 days postnatal), remnants of the pupillary membrane are still apparent. Whether the vessels are still functional cannot be determined from our present studies.

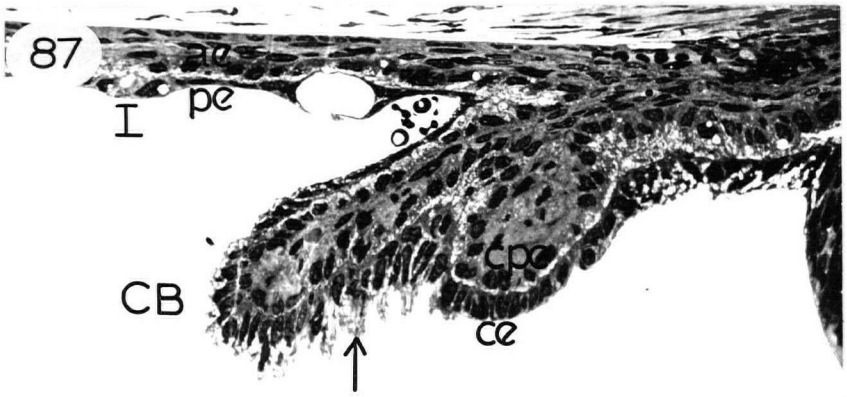
Figure 87 5-10 Days Post-natal (LM)

The iris (I) and ciliary body (CB) are acquiring characteristics of the adult. Distinct ciliary processes are seen although they are still not long. The ciliary epithelium (ce) varies in height in different locations on the ciliary process, being higher at the tip. At the tip of the ciliary process, the cytoplasm of the cells is vesiculated and invaginated giving the cells a highly irregular outline (arrow). The ciliary pigment epithelium (cpe) follows the ciliary epithelium closely. Both the epithelia fold to form a hair-pin loop with the narrow intervening space being filled with stromal elements.

The iris is thin. The posterior (pe) and anterior (ae) epithelium are low. x 220

Figure 88 5-10 Days Post-natal (LM)

The posterior epithelium of the iris (pe) is made up of squamous or cuboidal-type cells. There is slight wavy appearance to the posterior surface of the iris created by the bulging out of the cells. The anterior epithelium (ae) is relatively high and is very closely associated with the iris stroma (s). x 220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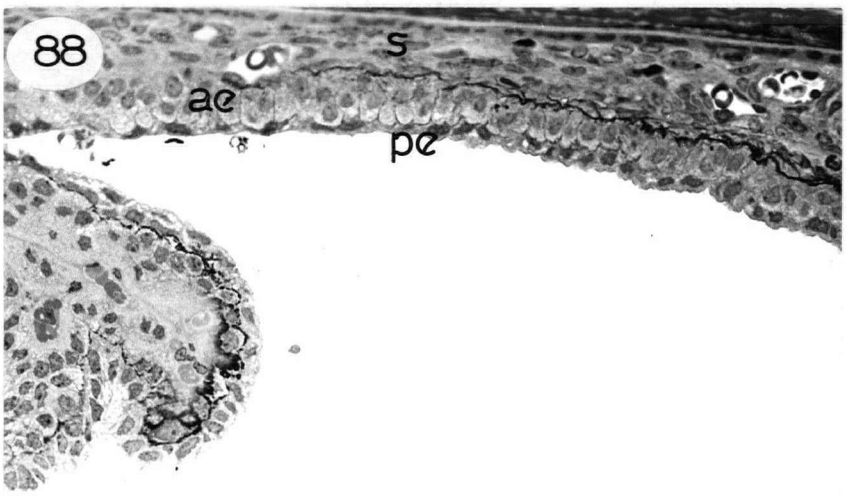
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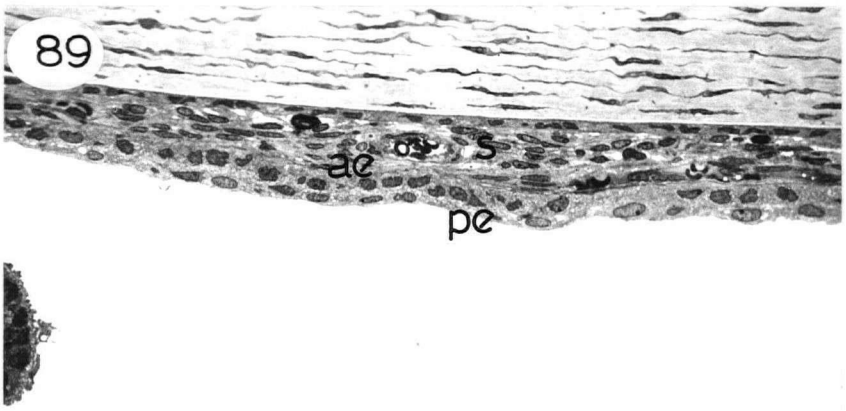
Figure 89 5-10 Days Post-natal (LM)

The anterior (ae) and posterior (pe) epithelium of the iris are of about the same height. The posterior epithelium consists primarily of squamous cells with flattened nuclei, whereas the anterior epithelium consists of cuboidal or rectangular cells. The two layers are closely apposed. The cells of the stroma (s) are all arranged parallel to the length of the iris and they abut onto the anterior surface of the anterior epithelium. x 220

Figure 90 5-10 Days Post-natal (LM)

The sphincter (sph) is a cluster of small cells which appear to be divided into large muscle groups by intercellular spaces (arrows). Spaces are also found in between the individual muscle cells. x 220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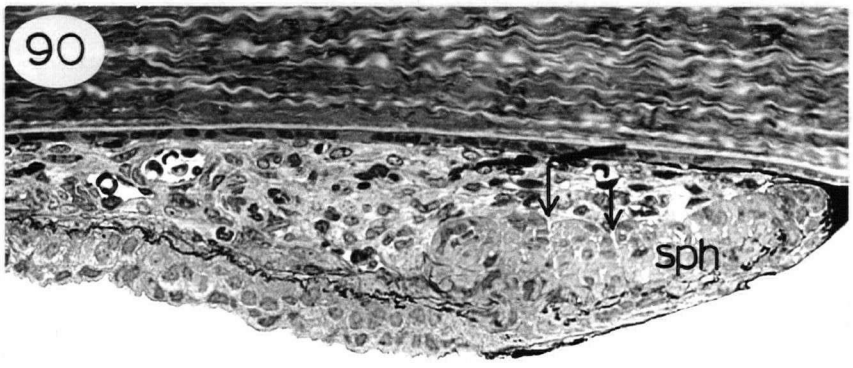
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5. From 11 Days Post-natal On (Figures 91-95)

At about two weeks after birth, the rat eyes open. It would seem then that by the 14th or 15th day post-natal the iris would be fully functional in order to cope with the environment. Structurally, the iris and the ciliary body would have attained the adult form. This is found to be so.

By the 12th day after birth, there are numerous long, thin and finger-like ciliary processes, much as in the adult rat eye (Figure 91). Each ciliary process is essentially a vascular structure. The ciliary epithelium and the pigment epithelium of the ciliary processes are closely adherent to each other. They make a long thin hair-pin loop, the bend of the loop being the tip of the ciliary process. The pigment epithelium of both arms of the loop are quite close together and are only separated by the stroma of the ciliary body. There are few stromal cells. Normally a large blood vessel completely occupies the cleft between the folds of the pigment epithelium such that the endothelium is closely associated with the ciliary pigment epithelium. Occasionally there may be a few stromal cells present. Sometimes, the incoming blood vessel expands and becomes sac-like at the tip of the ciliary process. Thus the tip of the ciliary process appears knob-like externally (Figure 91).

The iris is long and thin and assumes the configuration of the adult iris in pupillary constriction. The antero-posterior width of the iris increases slightly from the periphery towards the pupil. This increase is due to the increase in the width of the stroma up to the peripheral edge of the sphincter.

The posterior epithelium of the iris consists of a single row of thick or brick-like cells (Figure 92). The nuclei are elongated along the length of the cells. The posterior surface of the iris is still relatively

smooth because of the shape and arrangement of the posterior epithelial cells. The cell boundaries are not distinctly visible. By the 15th day after birth, parts of the posterior surface of the iris becomes finely irregular (Figure 93). Some of the nuclei of the squamous cells may bulge out a little posteriorly, thus contributing to the wavy appearance of the posterior iris surface. In some specimens at 15 days post-natal, the characteristic features of the posterior epithelium are as follows. The epithelial cells often appear hemispherical in shape (Figure 94). They are attached in a row to the anterior epithelium, with the curved portions of the hemispheres directed posteriorly into the posterior chamber. The sides of the hemispherical cells do not touch each other so that each individual epithelial cell is well demarcated from its neighbor. The arrangement of the hemispherical epithelial cells gives to the posterior iris surface a regularly scalloped appearance. The plasma membrane of the individual epithelial cells is not smooth but shows irregularities as well. The small dark nuclei are situated in the center of the cells. Some of the posterior epithelial cells are still dividing two weeks after birth.

The anterior epithelium is about the same width as the posterior epithelium by the 15th day (Figures 92-94). It might even at times be a little narrower than the posterior epithelium. The nuclei are generally elongated parallel to the iris posterior surface. They are not regularly spaced out along this layer. The cytoplasm is relatively uniformly staining. At the anterior stromal surface of the anterior epithelium, there is a smooth, dark, dense line which clearly sets off the anterior epithelium from the stroma. This is the dilator muscle layer of the anterior epithelium. The anterior epithelium is a myoepithelial layer. The nucleus and cell organelles are in the apical portion of the cell while the contractile elements are in the basal portion of the cell. In general, the

boundary zone between the anterior epithelium and the stroma is a smooth straight line. At times, individual epithelial cells, or groups of cells, may bulge out into the stroma.

The sphincter is still a large mass of small tightly packed cells which are darkly staining (Figure 95). Cell division is still taking place within the sphincter region. Besides the muscle cells, capillaries and nerves are also detected within the sphincter.

In the stroma, there is an increasing number of capillaries. Larger blood vessels have their lumens parallel to the posterior surface of the iris (Figures 93, 94). Some of the large blood vessels consist of only an endothelial lining, whereas others may have, in addition, a pericyte layer external to the endothelium. In young eyes, the stromal cells and blood vessel endothelium is always very closely associated with the anterior epithelium. In fact, at times, the two layers seem to be fused together. However, in development, the stromal elements slowly move away from the anterior surface of the anterior epithelium. It is very rare to see a stromal cell abutting closely onto the anterior epithelium. The nuclei of the stromal cells are kept at a distance from the anterior epithelium. Cytoplasmic processes of the stromal cells occupy the region between the anterior epithelium and the stromal cell nuclei. The nuclei are large structures and would probably interfere mechanically with the mobility of the iris if they are placed too close to the anterior epithelium. The anterior surface of the iris stroma is relatively smooth although at times it appears that there are gaps in the lining endothelium.

Figure 91 From 11 Days Post-natal On (LM)

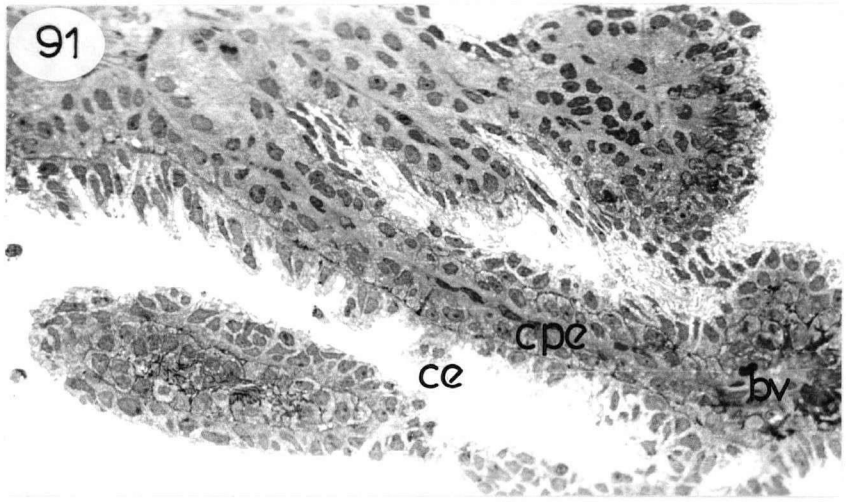
The ciliary processes are vascular structures. The ciliary epithelium (ce) and ciliary pigment epithelium (cpe) make hair-pin loops enclosing capillaries and stromal cells. At the tip of the ciliary process, the blood vessel (bv) may expand to give a knob-like appearance to the tip of the ciliary process. The ciliary epithelial cells possess a highly irregular outline. The ciliary pigment epithelial cells are cuboidal or very low columnar. x 220

Figure 92 From 11 Days Post-natal On (LM)

The posterior (pe) and anterior (ae) epithelium consists of two rows of brick-shaped cells. The posterior epithelial cells are arranged end to end giving the posterior surface of the iris a smooth contour. The cytoplasm of the anterior epithelial cells stains relatively dark so that the boundary between the stroma (s) and the anterior epithelium is discernible. The stromal cells and blood vessels (bv) are all arranged parallel to the posterior surface of the iris. x 220

Figure 93 From 11 Days Post-natal On (LM)

The posterior surface of the iris is finely irregular (compare with Figure 92). The individual cells of the posterior epithelium (pe) may bulge out a little. The anterior epithelium (ae) or dilator is a dense staining layer distinctly separated from the stroma (s). The stromal elements do not come right next to the anterior surface of the anterior epithelium, as in younger eyes. x 220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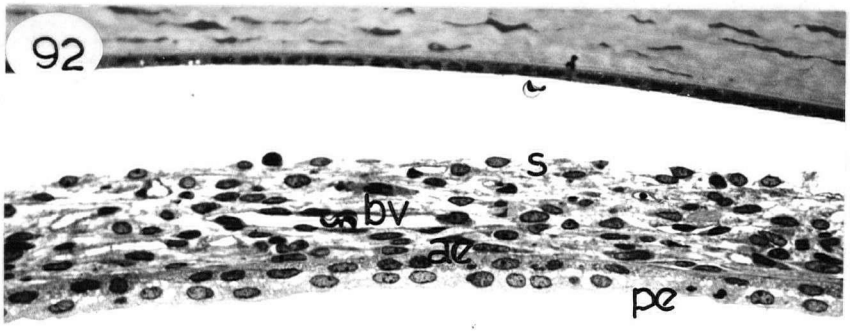
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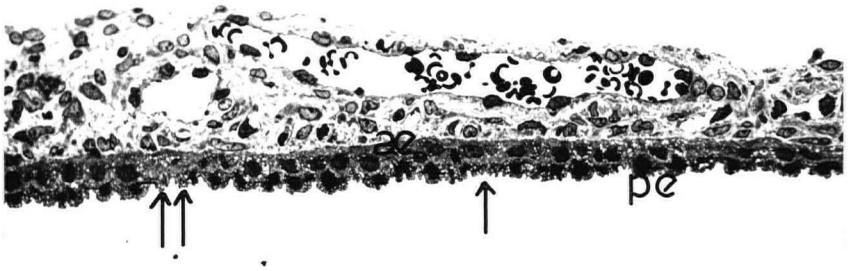
Figure 94 From 11 Days Post-natal On (LM)

The posterior surface of the iris has a regularly scalloped appearance. The posterior epithelial cells (pe) are hemispherical in shape. The cell cytoplasm is vesiculated and cell processes are beginning to be seen (arrows). The anterior epithelium or dilator (ae) is a distinct layer. The cells in the stroma are kept away from the dilator. x 220

Figure 95 From 11 Days Post-natal On (LM)

The sphincter (sph) muscle cells are cut in cross-section. There is not such a clear well-defined space between the sphincter and the stroma (s) as is regularly seen in younger eyes. Capillaries are present in the loose iris stroma. A small part of the iris stroma still overhangs the pupillary edge of the sphincter and a blood vessel (bv) appears to be headed into the pupillary aperture. x 220

94



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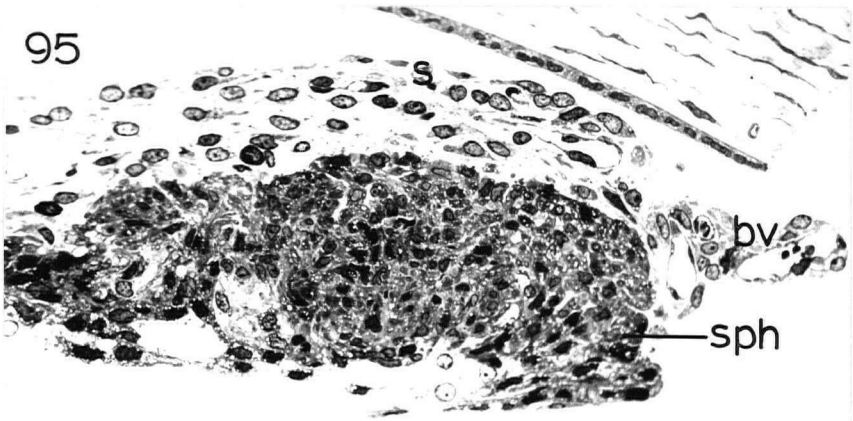


F. A Scanning Electron Microscopic Study of the Posterior Surface of the Developing Rat Iris

1. 17 Days Fetal - Term (Figures 96-105)

The fetal iris in a scanning electron micrograph is a narrow rim of tissue surrounding a very large pupil. The posterior surface of the iris may be covered by an amorphous, sheet-like substance (Figure 96). It is sometimes pitted and pock-marked and appears capable of being flaked off (Figure 96). At times, this amorphous layer is partially removed from the posterior surface of the iris during the processing of the tissues (Figure 97). It is then seen as a thin sheet adherent to the iris. Near the pupil, its surface appears relatively smooth except for bits of fibrillar material. Superficial to but closely associated with this amorphous layer are numerous blood vessels containing red blood cells (Figures 96, 97). The lining layer of the blood vessels is normally torn off when the surrounding tissues, such as the lens, are removed (Figure 96). More often than not, both the superficial blood vessels and the amorphous layer are removed during the preparation of the tissues to expose the basement membrane covering the posterior epithelium of the developing iris (Figure 98). The posterior surface of the iris near the pupillary margin is relatively smooth. There may be a few dents and grooves, which presumably, are made by the superficial blood vessels. The more peripheral portion of the posterior surface of the iris has a very slightly more rugged contour (Figures 98, 99). A fine network of fibrillar material in varying amounts (Figures 97, 99, 100) covers all of the posterior surface of the iris. From meridional sections of the developing rat iris, it is observed that the posterior surface of the iris is lined by a layer of epithelial cells. However, the boundaries of the posterior epithelial cells are not visible

in these scanning electron micrographs. The overlying basement membrane of the posterior epithelial cells and the adherent fibrillar material would obscure any cell boundaries that may be present. At this early stage in the development of the rat iris, there is no indication at all from scanning electron microscopy that the iris posterior epithelium is in actuality made up of a layer of discrete cells. The nuclei of the posterior epithelial cells do not bulge out to indicate that this is so.

There are some capillary systems associated with the fetal iris (Figure 101); a large annular vessel of the hyaloid system located at the region of the developing ciliary body (Figure 102), capillaries arising from the annular vessel and traversing the posterior surface of the iris (Figures 101, 102) and capillaries of the pupillary membrane (Figures 101, 103).

The annular vessel is relatively large in comparison to the feeder capillaries (Figure 102). It is usually torn off during specimen preparation, but when left intact, it is seen overlying the developing ciliary body. A group of interconnecting capillaries of the retinal portion of the hyaloid vascular system meet the annular vessel (Figure 102). These capillaries have relatively thick walls although the red blood cells within the lumens can be perceived (Figure 104). The highly branched capillaries are held together and possibly to the surface of the retina by a dense meshwork of fibrils (Figures 102, 104). The fibrils are of varying diameters (Figure 104). Capillaries also radiate out from the annular vessel over the posterior surface of the iris (Figures 101, 102). They are usually more delicate looking than the retinal hyaloid vessels. They form a complicated pattern on the posterior surface of the iris and are seen to reach the pupillary margin (Figure 101).

The pupillary membrane (Figures 97, 98, 101, 103) extends centrally

from the anterior surface of the iris. The pupillary membrane has not been observed to cover all of the pupillary aperture but extends from the pupillary margin to varying degrees (Figures 98, 101, 103). The capillaries of the pupillary membrane (Figure 105) have thinner walls than those of the retinal hyaloid system (Figure 104). They are almost transparent so that the red blood cells within the capillary lumens impart to the capillaries a bead-like appearance. The capillaries pursue a tortuous course and are complexly interconnected with each other (Figures 101, 103, 105). The pupillary membrane blood vessels are held in the pupillary aperture by a scaffolding network of connective tissue fibers (Figures 103, 105). Rarely, some cells are seen within the connective tissue network.

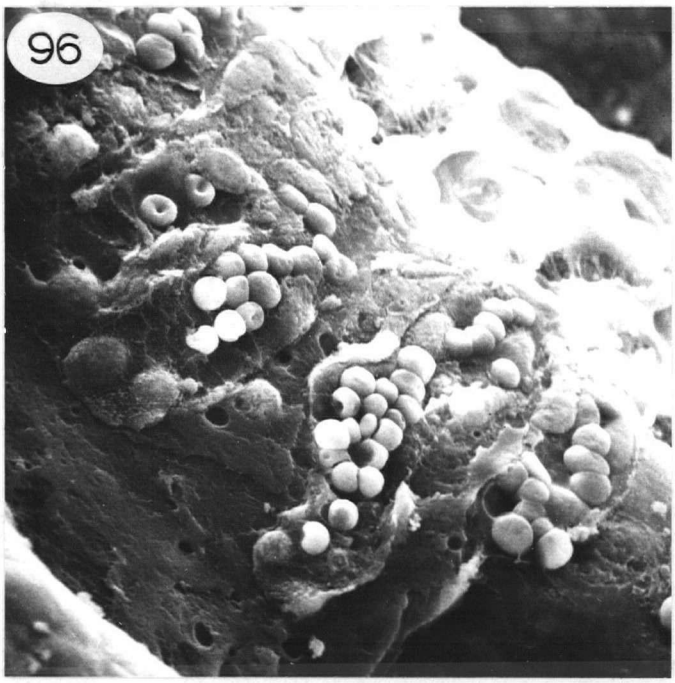
Some of the capillaries from the anterior portion of the iris do not extend centrally to form the pupillary membrane. Instead, at the pupillary margin, they turn around and traverse the posterior surface of the iris (Figures 97, 103).

Figure 96 17 Days Fetal - Term (SEM)

The posterior surface of the iris is covered by an amorphous, sheet-like substance. It may be pitted. Parts of the walls of the blood vessels on the posterior surface of the iris have been removed to expose the contents, the erythrocytes.
x 820

Figure 97 17 Days Fetal - Term (SEM)

Most of the superficial flaky amorphous substance is removed to reveal the underlying basement membrane covering the posterior surface of the iris. Parts of the walls of two blood vessels remain. These continue past the pupillary margin and are continuous with the blood vessels from the anterior surface of the iris. x 1,530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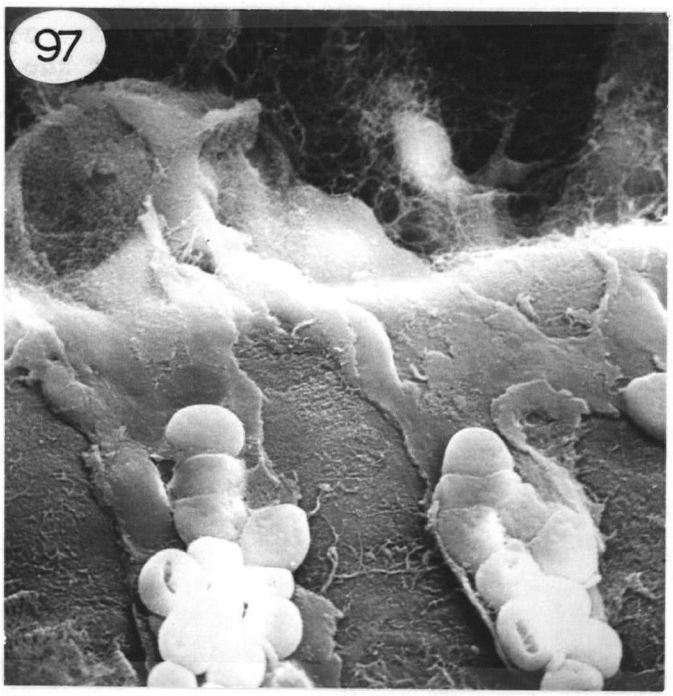
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Figure 98 17 Days Fetal - Term (SEM)

The narrow zone around the pupil is relatively smooth with a few shallow grooves and low bulges. Peripheral to that, the posterior surface is covered with a finely fibrillar material. At the pupillary margin, the lumens of many blood vessels, suspended in a dense connective tissue framework, are seen. They have all come from the anterior surface of the iris. One blood vessel appears to be changing in direction at the pupillary margin. x 700

Figure 99 17 Days Fetal - Term (SEM)

A high magnification scanning electron micrograph of the smooth and fibrillar parts of the posterior surface of the iris. x 3,800

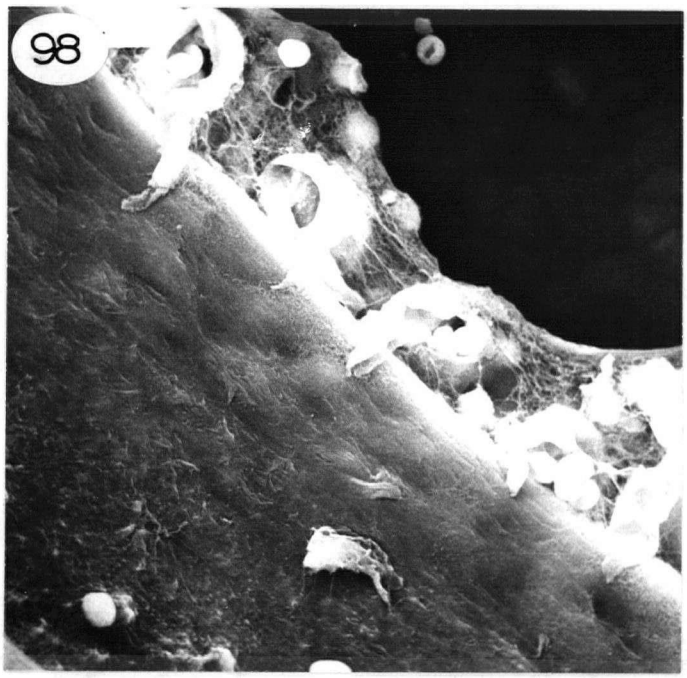
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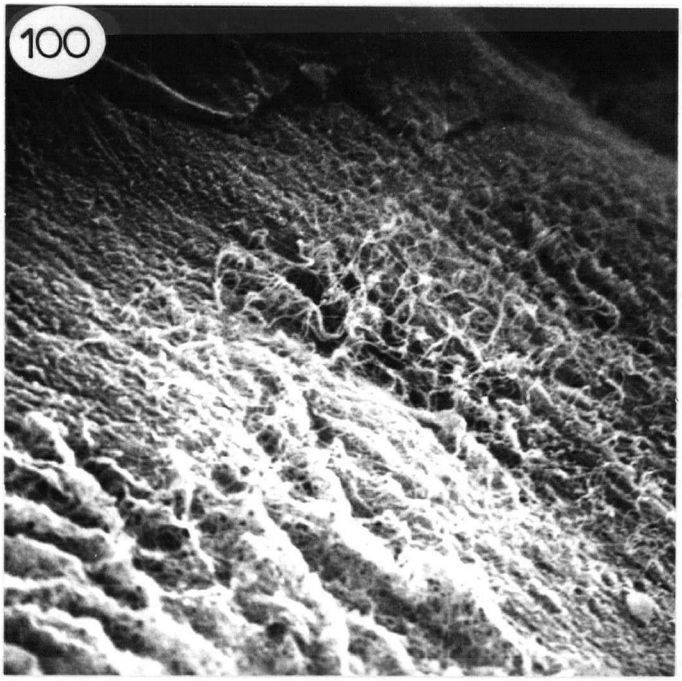
Figure 100 17 Days Fetal - Term (SEM)

Occasionally, there is a large amount of fibrillar material on the posterior surface of the iris. x 4,400

Figure 101 17 Days Fetal - Term (SEM)

Three networks of blood vessels are seen in close association with each other, the blood vessels extending from the pupillary margin, the blood vessels on the posterior surface of the iris and the blood vessels on the inner surface of the retina. Most of the blood vessels on the posterior surface of the iris have been removed during specimen preparation. The developing iris is a narrow strip of tissue with a relatively smooth surface. x 150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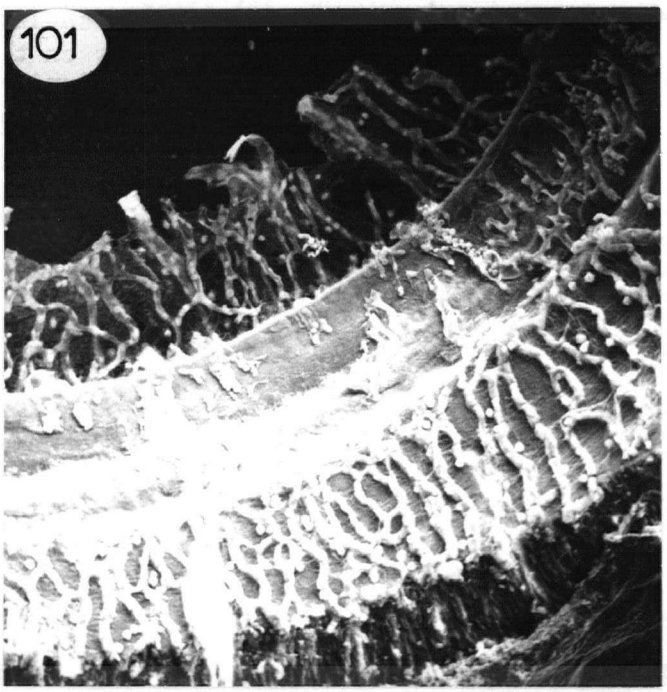
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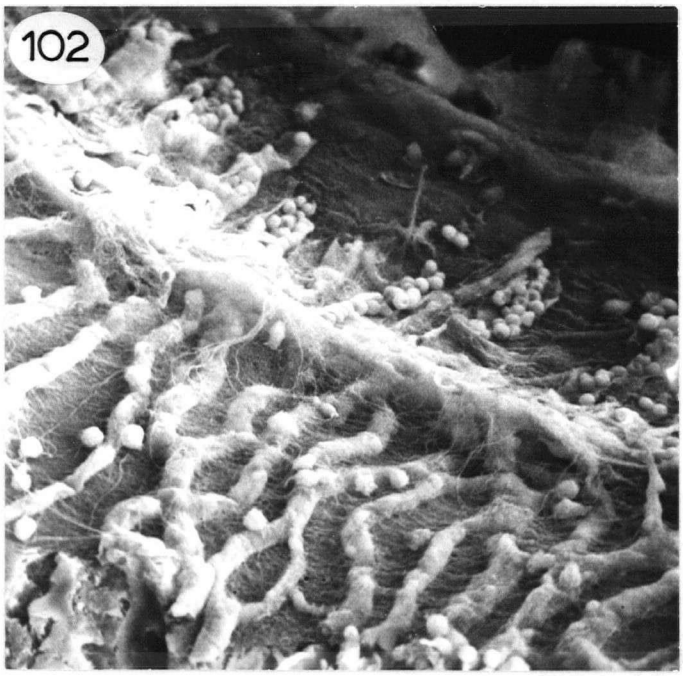
Figure 102 17 Days Fetal - Term (SEM)

At a high magnification, a large annular vessel is seen at the region of the developing ciliary body. Blood vessels on the inner surface of the retina are held together by a dense connective tissue network. These capillaries link up with the annular vessel. At the opposite pole of the annular vessel, another series of capillaries (on the posterior surface of the iris) are connected into it. Most of these have been broken off. x 380

Figure 103 17 Days Fetal - Term (SEM)

The posterior surface of the iris is smooth except for a few pieces of amorphous material. The pupillary membrane extends for varying distances into the pupil from the anterior surface of the iris. The pupillary membrane consists of a network of thin-walled capillaries suspended in a fibrillar connective tissue framework. x 380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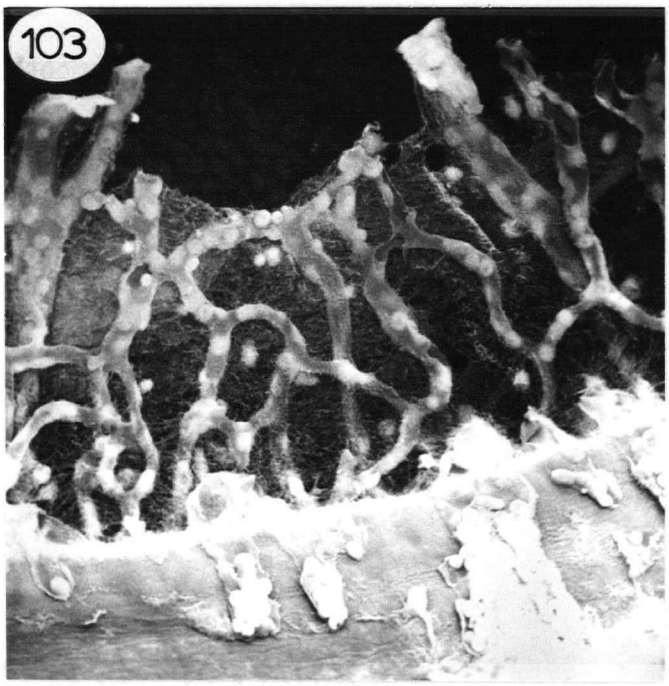
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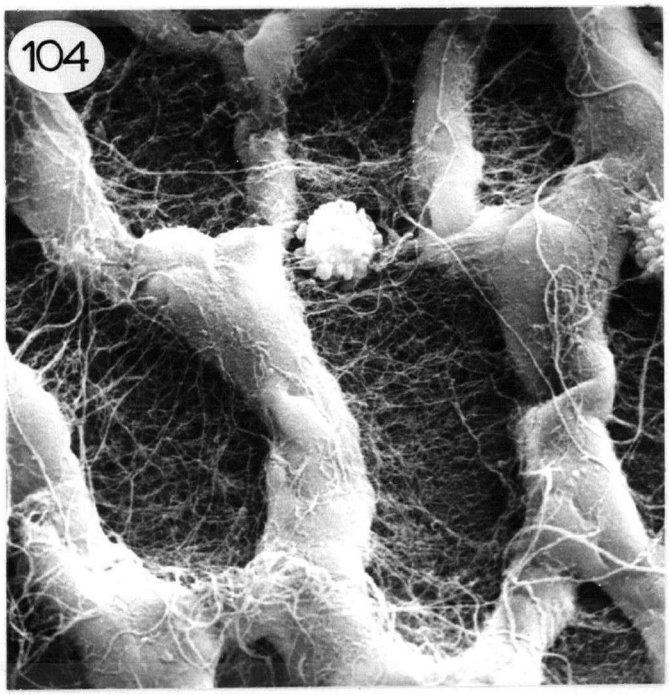
Figure 104 17 Days Fetal - Term (SEM)

The capillaries on the inner surface of the retina have relatively thick walls. An occasional blood cell may be perceived. The capillaries branch and are quite extensively interconnected. The capillaries are held together by a very dense fibrillar meshwork. The fibrils are of varying diameters. x 1,530

Figure 105 17 Days Fetal - Term (SEM)

The capillaries of the pupillary membrane are highly branched, They have thin, almost transparent walls so that the red blood cells readily show through. A framework of mainly fine connective tissue fibers hold the capillaries in their relative positions. x 1,530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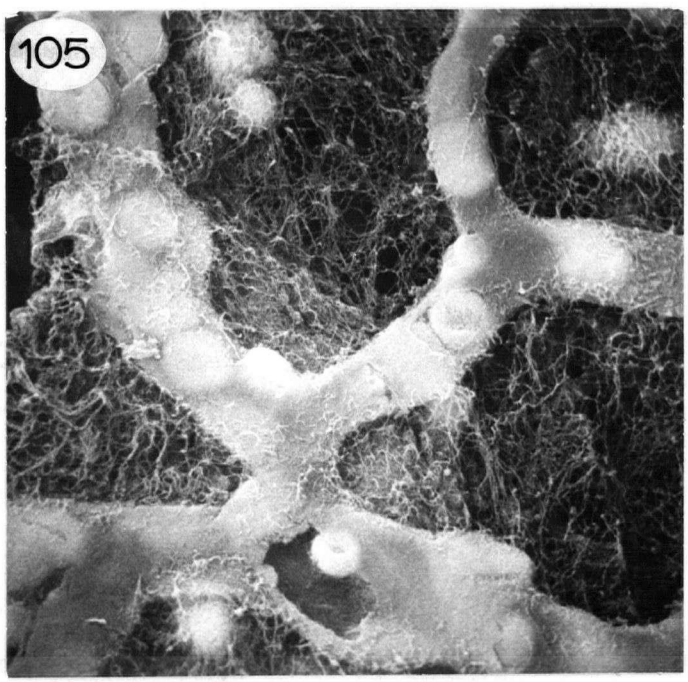
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2. 1-4 Days Post-natal (Figures 106-115)

During the first four days after birth, certain changes are observed in the posterior surface of the iris. Other features, present in the fetal iris, are observed in more explicit detail, especially pertaining to the blood vessels of the pupillary membrane and the blood vessels on the posterior surface of the iris.

In a low magnification scanning electron micrograph, it is seen that the posterior surface of the iris can be divided into two regions, a central or pupillary and a peripheral region (Figure 106). The pupillary region makes up about one third of the length of the iris. It is covered by a layer of amorphous material, the basement membrane. It is oftentimes extensively and deeply grooved so that this region has a corrugated appearance (Figures 106, 107). In certain eyes, this characteristic is not as clearly observable and the two regions are not as distinctly delineated (Figure 108). The grooves are radially disposed (Figures 106, 107). The grooves are almost all of the same width and depth. They may follow a straight course peripherally, or they may branch as they do so (Figure 107). The peripheral two thirds of the posterior surface of the iris is beginning to show some contouring (Figure 106) as compared to the fetal iris. It seems to be made up of a myriad little elevations (Figure 106), which at a higher magnification, are revealed to be the nuclei of the posterior epithelial cells. Superimposed on the posterior surface of the iris are impressions. They are generally radially oriented, they may branch and they are of about the same width as, but shallower than, the grooves on the pupillary zone of the posterior surface of the iris.

At a higher magnification, the contours of the posterior surface of the iris are seen in a little greater detail. In the pupillary region, relatively large, more or less oval elevations are present (Figures 108,

109). The basement membrane covering the pupillary portion of the iris posterior epithelial cells is quite smooth save for a few fine grooves and ridges. Tiny holes may be present (Figure 109). Over the peripheral portion of the posterior surface of the iris there is a mosaic of small round to oval forms, separated by shallow depressions, which bulge ever so slightly towards the posterior chamber (Figures 109-111). These bulges, most probably representing the nuclei of the posterior epithelial cells, are not oriented in any particular fashion either in their relationships with each other or with respect to the iris as a whole. Occasionally, crater-like formations (Figure 111) are seen within this mosaic arrangement of epithelial cells. The basement membrane covers all of the cell surfaces. Particulate material, probably proteinaceous, is present on the basement membrane (Figure 111).

The pupillary membrane is still as extensive four days after birth as it is in the fetal eye (Figure 112). Here, it is observed that the capillaries arising from the annular vessel and traversing the posterior surface of the iris, and the capillaries of the pupillary membrane are apparently closely inter-related (Figures 112-114). The capillaries from the annular vessel are morphologically similar to those of the pupillary membrane. They have thin walls so that their content of red blood cells shows through. The capillaries tend to have a radial course with respect to the iris, and they are linked to each other by sometimes smaller interconnecting vessels (Figure 114). They are also supported by a dense network of connective tissue fibers which appear to intermingle with the fibers of the pupillary membrane (Figures 112-115). These capillaries sometimes appear to link up with those of the pupillary membrane (Figure 114), although the image obtained in our scanning electron micrograph may actually be due to the superimposition of one set of capillaries on another.

The pupillary membrane blood vessels, as in fetal eyes, radiate from the pupillary margin held in its connective tissue framework. They branch, join, form arcades and loops. The width of the pupillary membrane as a whole differs for each part of the iris, being quite extensive in parts and practically non-existent in others. Most times, the capillaries hugging the pupillary margin are broken off. However, they are usually broken off in such a way as to suggest that they are turning around at the pupillary margin and apparently going for the posterior surface of the iris (Figures 113, 114). Or, a part of a blood vessel may actually be seen making this turn and becoming adherent to the posterior surface of the iris (Figure 113). Occasionally, a capillary from the anterior iris stroma encircles the pupillary margin and appears to become continuous with or as a capillary arising from the annular vessel of the hyaloid system (Figure 113).

Figure 106 1-4 Days Post-natal (SEM)

The posterior surface of the iris is distinctly divided into a central pupillary region which is deeply radially grooved, and a peripheral region which is speckled and appears to be made up of a mosaic of tiny elevations. Superimposed on the peripheral region are some radially oriented impressions.
x 150

Figure 107 1-4 Days Post-natal (SEM)

At a higher magnification, the radial grooves in the pupillary region are deep and wide. They all are of about the same widths. Sometimes they may bifurcate. x 760

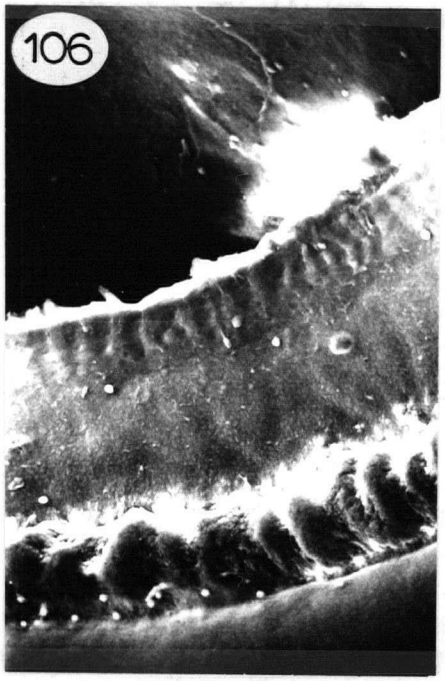
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Figure 108 1-4 Days Post-natal (SEM)

The pupillary membrane extends past the pupillary margin. The pupillary region is relatively smooth with hints of a few elevations. The peripheral region consists of smaller polygonal elevations. x 790

Figure 109 1-4 Days Post-natal (SEM)

The basement membrane covering the large, oval, low elevations of the pupillary region is relatively smooth. In the peripheral region, the basement membrane shows wrinkles. A mosaic of polygonal forms are barely perceptible. Some tiny holes are present. x 1,530


108

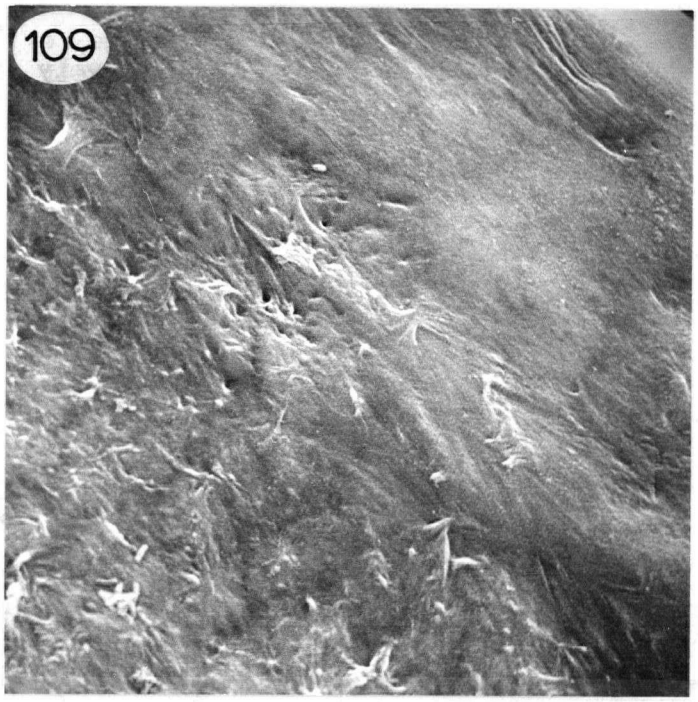
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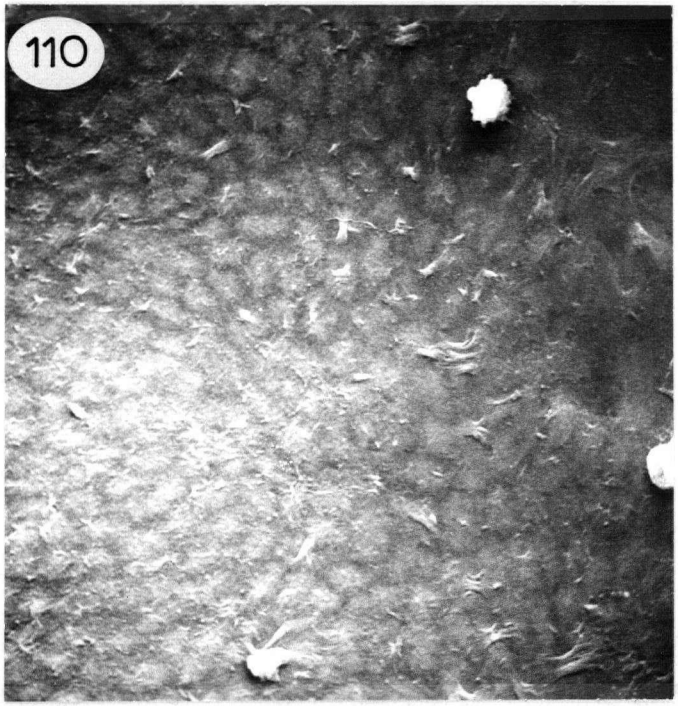
Figure 110 1-4 Days Post-natal (SEM)

Most of the posterior surface of the iris is a mosaic of polygonal forms distinctly separated from each other by shallow depressions. These forms bulge out ever so slightly. They are not arranged in any particular relationship with each other. x 760

Figure 111 1-4 Days Post-natal (SEM)

A high magnification of Figure 110. A crater-like formation is also seen. x 1,530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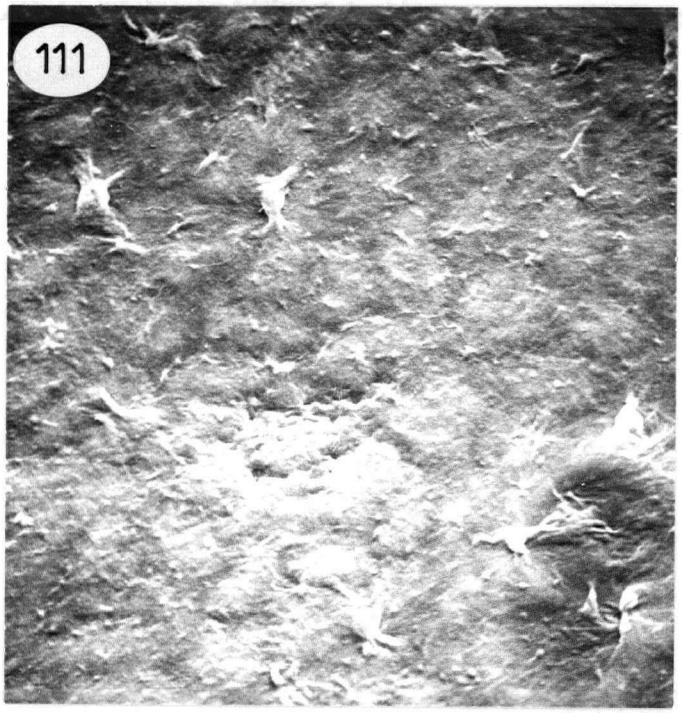
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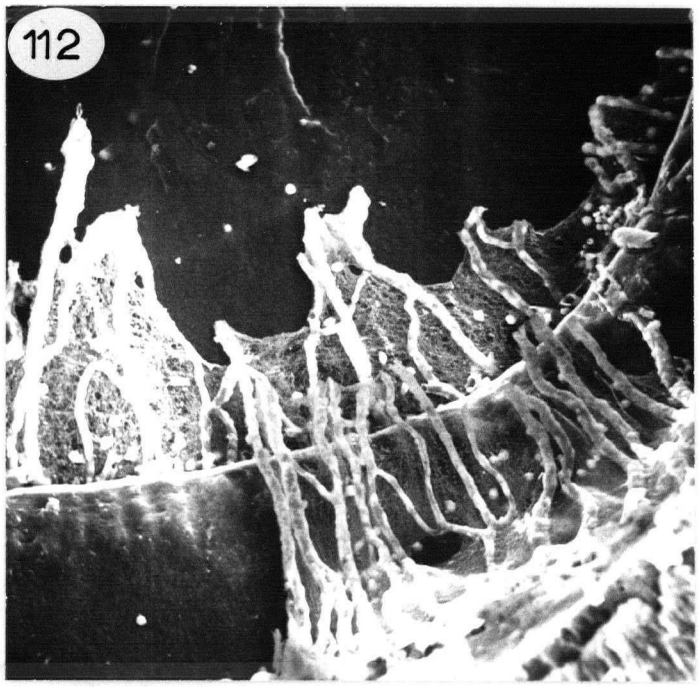
Figure 112 1-4 Days Post-natal (SEM)

The capillaries arising from the annular vessel appear to be intimately associated with the those of the pupillary membrane. These capillaries lie on the posterior surface of the iris, being held together by a connective tissue network. These capillaries are very similar to those of the pupillary membrane. They have thin walls so that the red blood cells show through.
x 180

Figure 113 1-4 Days Post-natal (SEM)

Not all of the blood vessels from the anterior surface of the iris extend centrally to become part of the pupillary membrane. Many of these blood vessels appear to turn at the pupillary margin and be headed posteriorly. Rarely, a capillary from the anterior surface of the iris turns around at the pupillary margin and becomes continuous with one of the capillaries arising from the annular vessel. x 450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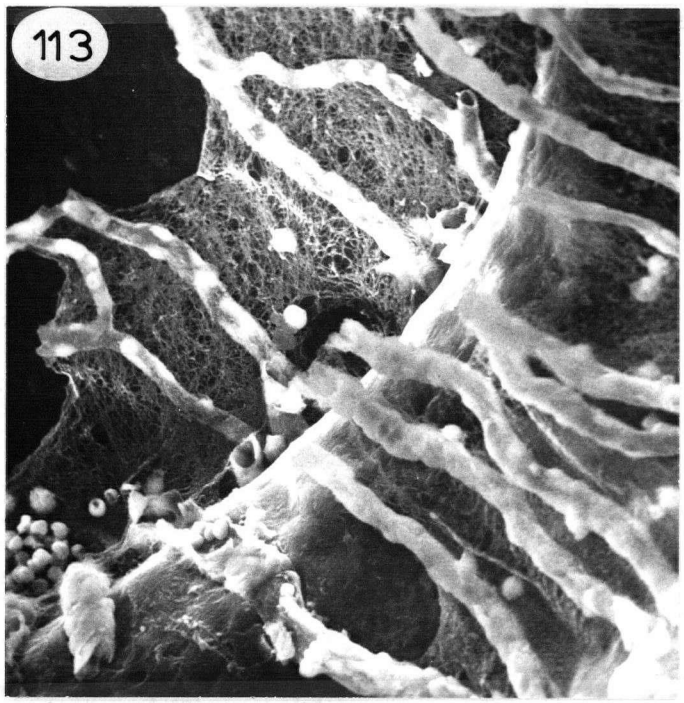
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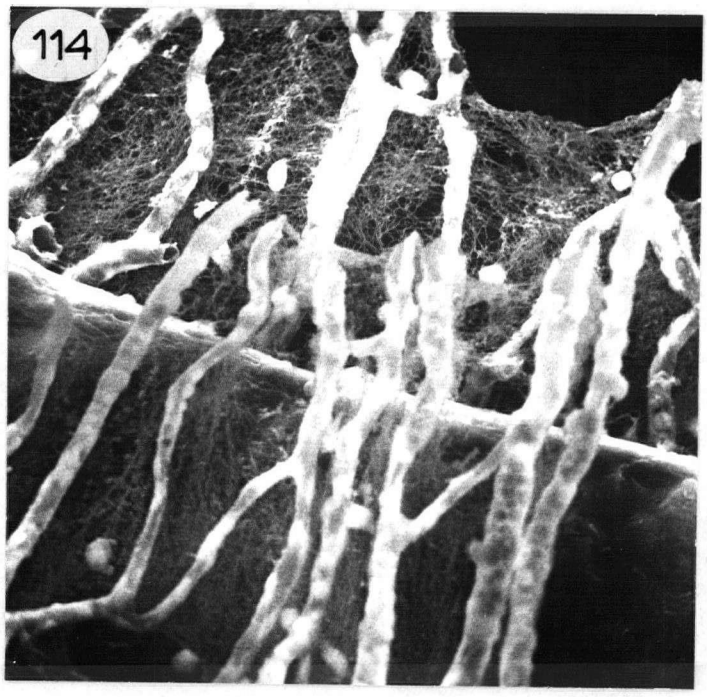
Figure 114 1-4 Days Post-natal (SEM)

The capillaries on the posterior surface of the iris arising from the annular vessel extend past the pupillary margin to join the capillaries of the pupillary membrane arising from the anterior surface of the iris. Both capillary systems are held in a fine fibrillar connective tissue framework.
x 450

Figure 115 1-4 Days Post-natal (SEM)

The connective tissue fibers of the pupillary membrane and of the capillaries on the posterior surface of the iris appear to merge and intermingle. x 900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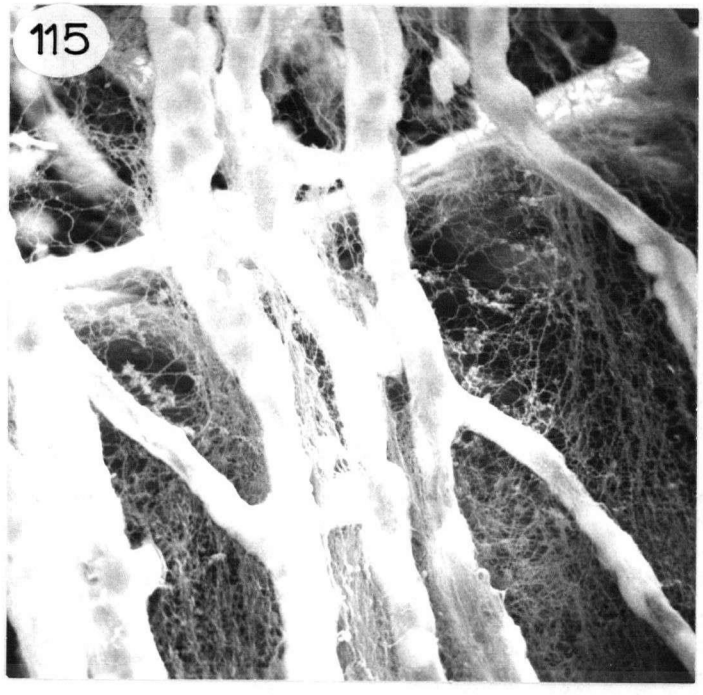
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3. 5-10 Days Post-natal (Figures 116-127)

The rate of development of the rat iris varies from one litter of rats to another and also among the members of any one litter of animals. This is seen in our studies where the irises from a litter of rats aged 6 days post-natal have a more mature form than those from a litter of rats aged 8 days post-natal.

From the fifth to tenth days after birth, the rat iris is slowly acquiring characteristics of the adult iris, as exemplified by the posterior surface. Initially, the pupil is still large but the iris has grown. Around the pupillary margin, as previously, there is a region which is different in appearance from the rest of the posterior surface of the iris. This pupillary zone makes up only about one fifth or less of the total iris length. The pupillary region assumes different forms. It may be covered by a continuous layer of amorphous, smooth and sheet-like material, or parts of this material may have flaked off to reveal the underlying epithelium (Figures 116, 117). This layer is not of a uniform thickness throughout its extent. Right at the pupillary margin, it appears to be relatively thick but as one moves along it peripherally, this amorphous layer seems to thin out and becomes continuous with the basement membrane covering the posterior surfaces of all of the epithelial cells (Figure 117). Occasionally, there may be a quite sharp transition between this thick amorphous layer and the basement membrane of the posterior epithelial cells (Figure 116). Blood vessels are oftentimes embedded within this amorphous layer (Figure 118).at the pupillary zone but they become free and lie superficial to the posterior epithelium at the peripheral extent of this layer. These blood vessels are still interconnected by a few wisps of connective tissue fibers, reminescent of the connective tissue framework holding together all of the capillaries on the posterior surface of the

iris which have come from the annular vessel. These capillaries may run all the way peripherally to the region of the ciliary body, or they may stop short midway on the iris.

This amorphous sheet is sometimes not only confined to the pupillary region of the iris but extends into the pupil to varying degrees (Figure 119). Capillaries from the anterior portion of the iris are seen completely embedded in this amorphous substance (Figure 119).

The posterior epithelial cells are now quite clearly visible over the rest of the posterior surface of the iris (Figures 116-118, 120). In a low magnification scanning electron micrograph, the posterior surface of the iris has a raspberry-like appearance (Figure 116). Numerous, generally round elevations are disposed throughout the posterior surface of the iris. They are relatively equally spaced from each other by shallow depressions. This is seen more clearly at a higher magnification (Figure 120). Each rounded protuberance represents the nucleus of a posterior epithelial cell. The nuclei are round and they are all of the same size. A basement membrane covers the posterior surfaces of the epithelial cells so that there are no distinct cell boundaries although the extent of each cell is perceptible.

A little further along in development, some changes are seen. The amorphous plaque-like material is still present along the pupillary zone, thicker at the pupillary border and diminishing in thickness peripherally (Figure 121). Radially oriented blood vessels are still enmeshed within this substance. If these capillaries are removed in the preparation of the specimens, deep grooves are present all along the pupillary region to suggest where these capillaries had been (Figure 122). When the capillaries are removed, part of the amorphous layer usually goes with them. However, a thin layer still remains. Sometimes, an interesting configuration of

the amorphous layer in the pupillary region is observed (Figure 123). The peripheral extent of the layer is quite regularly scalloped in its outline so that a series of V-shaped structures are seen. Each V is occupied by a blood vessel or a groove suggesting that a blood vessel had been in position before. In between the blood vessels, the amorphous layer has apparently retracted towards the pupillary margin. As has been previously noted, there is most often no really sharp transition between the amorphous layer and the basement membrane of the posterior epithelial cells. As the amorphous layer retracts, it exposes the underlying posterior epithelial cells with their basement membrane to varying degrees (Figure 123).

The posterior epithelial cells are very distinctly visualised (Figures 123-126). Each cell with its centrally located nucleus bulges quite prominently into the posterior chamber. Relatively deeper depressions delineate the confines of each cell. However, unlike in the adult where the posterior epithelial cells are arranged in ridges depending on the degree of pupillary dilation or constriction, here in the early post-natal iris, the posterior epithelial cells just cover all of the posterior surface of the iris without being arranged in any particular way. Occasionally, though, deeper grooves are present which separate out groups of epithelial cells (Figure 124). This may be the beginnings of the epithelial ridges and grooves. Scattered along the posterior surface of the iris are some protruding structures (Figures 122, 125, 126). They are crater-like in appearance and are elevated to different heights above the posterior surface of the iris. They are many times larger than the surrounding posterior epithelial cells. They are in some ways similar to the bulbous structures on the posterior surface of the adult rat iris in extreme pupillary constriction.

A basement membrane covers all of the posterior surfaces of the

epithelial cells and bulbous structures. Unlike the relatively smooth basement membrane in younger eyes, the basement membrane now shows wrinkles and crinkles (Figures 125, 126) as it follows the contours of the posterior epithelial cells. Over the crater-like structures, the basement membrane appears to be a little smoother than it is over the rest of the posterior surface of the iris (Figure 126).

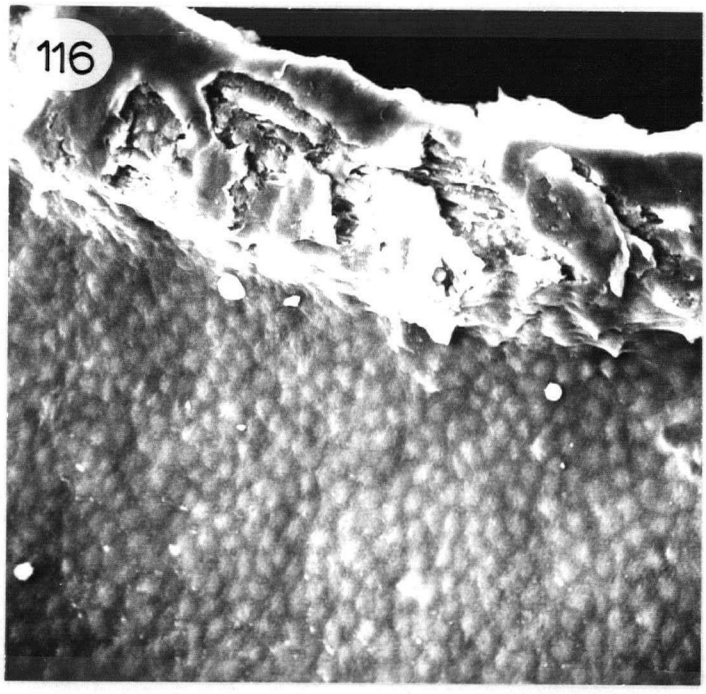
The pupillary membrane, which is still prominent in the first few days after birth, has in large measure disappeared. A few capillaries supported by its connective tissue fibrillar network are seen extending centrally from isolated parts of the pupillary border (Figure 127). The capillaries are still filled with red blood cells. In most instances, the capillaries protrude just slightly from the pupillary margin.

Figure 116 5-10 Days Post-natal (SEM)

The pupillary region is covered by an amorphous material which has flaked off in parts. There is a sharp transition line between the pupillary and peripheral regions. The peripheral region consists of a mosaic of round to oval elevations separated by shallow depressions. x 380

Figure 117 5-10 Days Post-natal (SEM)

The amorphous material is thicker at the pupillary region. It seems to thin out peripherally and becomes continuous with the basement membrane covering the rest of the posterior surface of the iris. Where the amorphous material has been removed, the underlying cells are seen. x 610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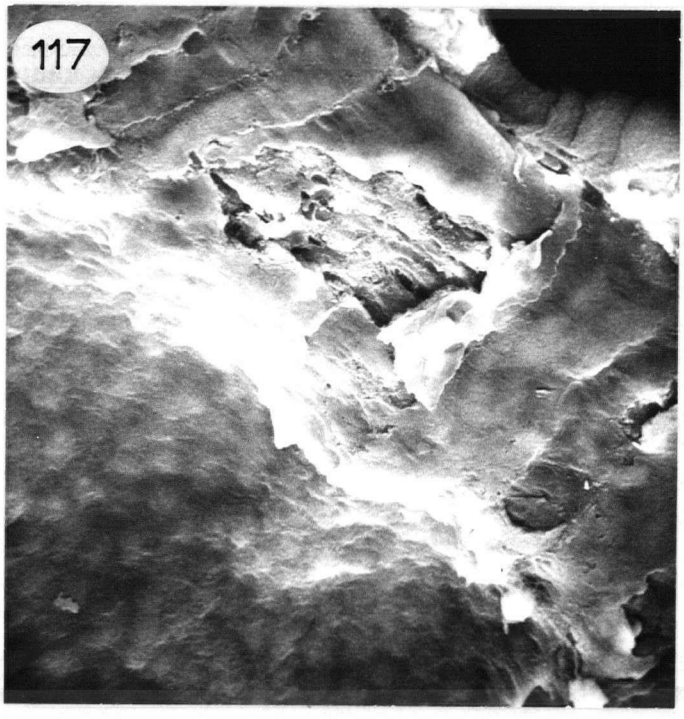
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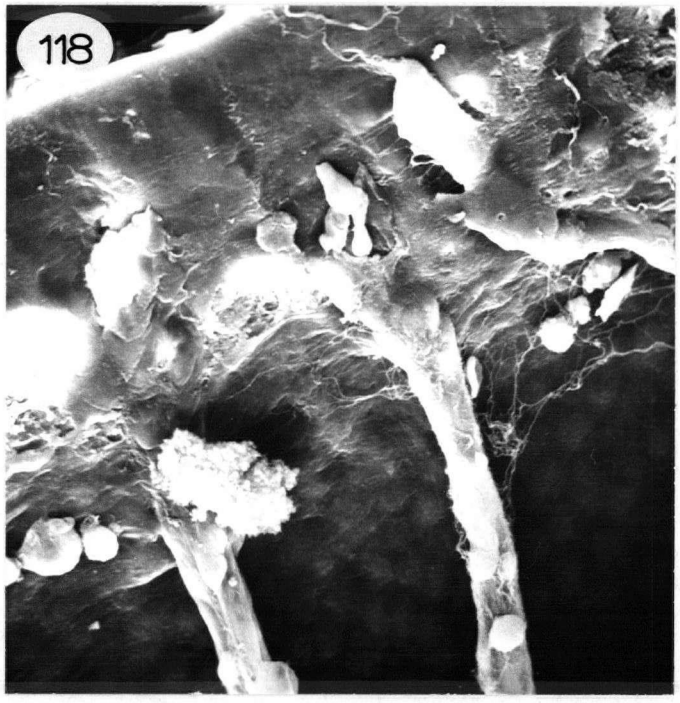
Figure 118 5-10 Days Post-natal (SEM)

Some blood vessels are embedded in the amorphous material at the pupillary margin, but they become free peripherally. Wisps of connective tissue fibrils interconnect these blood vessels. Some of these blood vessels extend all the way to the ciliary region. x 760

Figure 119 5-10 Days Post-natal (SEM)

Blood vessels from the anterior surface of the iris are embedded in amorphous material. Remnants of the walls of the blood vessels suggest that these anterior stromal blood vessels make a turn at the pupillary margin to traverse the posterior surface of the iris. x 1,220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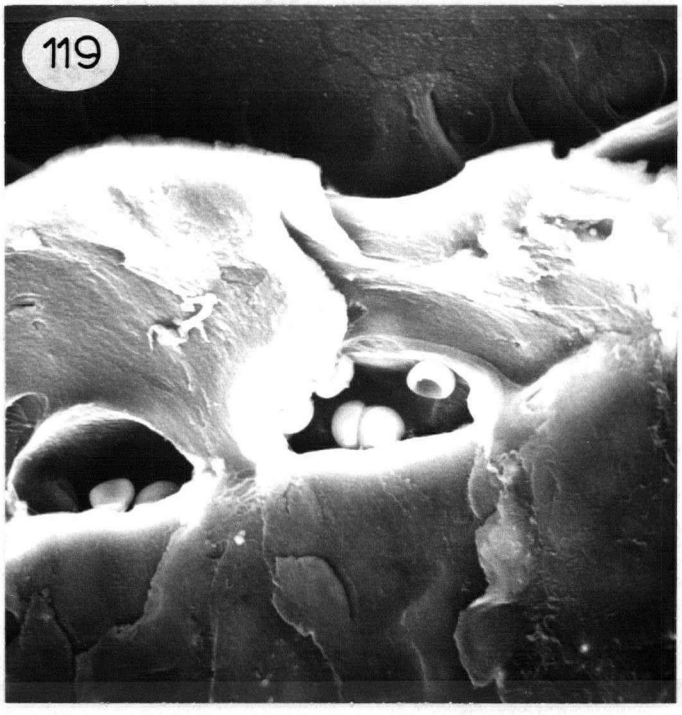
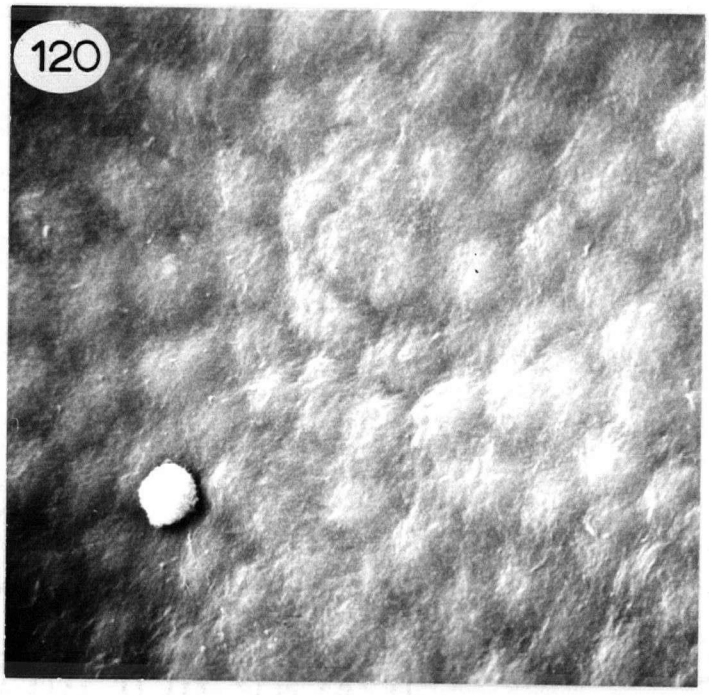

119

Figure 120 5-10 Days Post-natal (SEM)

The posterior epithelial cells, covered with a basement membrane, possess round nuclei. The cells are equally spaced from each other by shallow depressions. Cell boundaries are not evident. x 1,530

Figure 121 5-10 Days Post-natal (SEM)

Radially oriented blood vessels are embedded in the amorphous material at the pupillary margin but they become free more peripherally. These blood vessels occasionally branch. The amorphous material is thick at the pupillary region but thins out and merges with the basement membrane over the rest of the iris surface. x 390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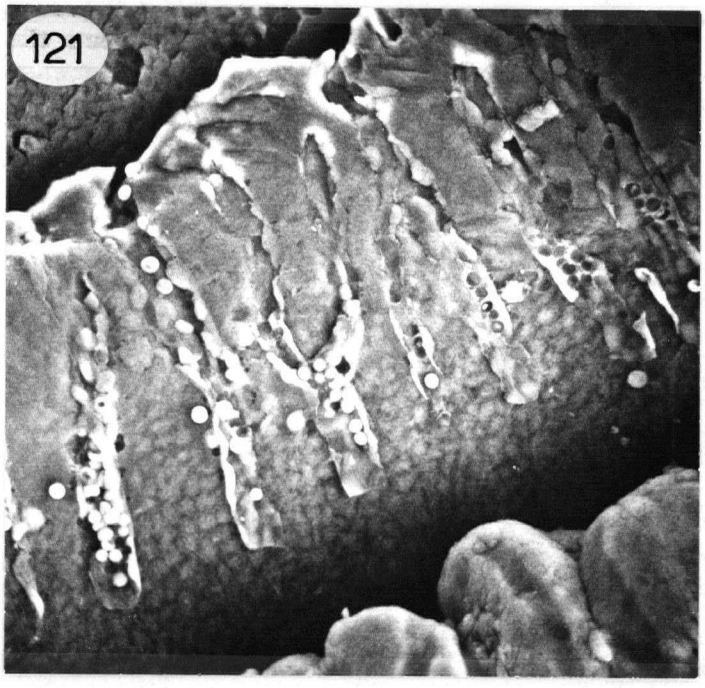
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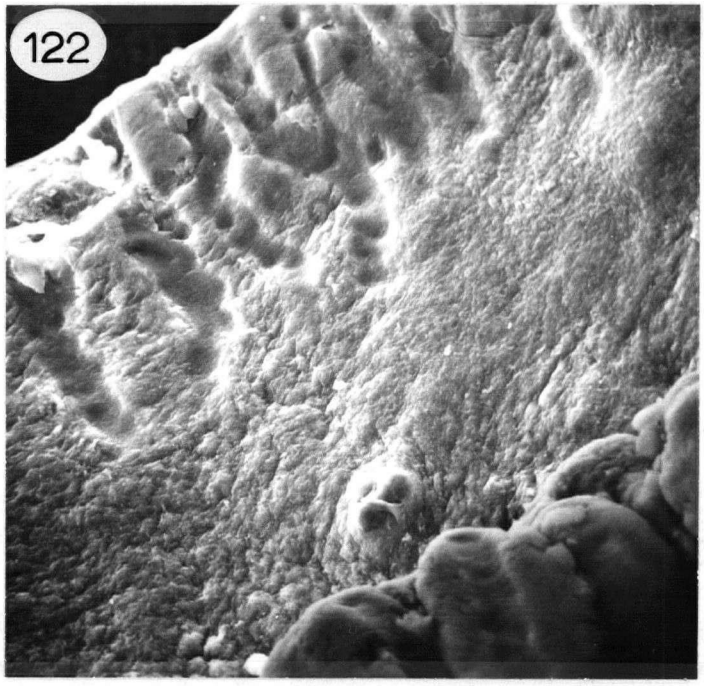
Figure 122 5-10 Days Post-natal (SEM)

The blood vessels on the posterior surface of the iris have been removed but deep impressions made by the blood vessels *in situ* remain to mark their course *in vivo*. A large crater-like structure is seen in the midst of the posterior epithelial cells. x 410

Figure 123 5-10 Days Post-natal (SEM)

The amorphous material appears to be continuous with the basement membrane in some parts and to be completely separate in other parts. It appears to be retracting towards the pupillary margin. A large blood vessel is adherent to the posterior surface of the iris only at the pupillary region. The posterior epithelial cells are distinctly seen. x 760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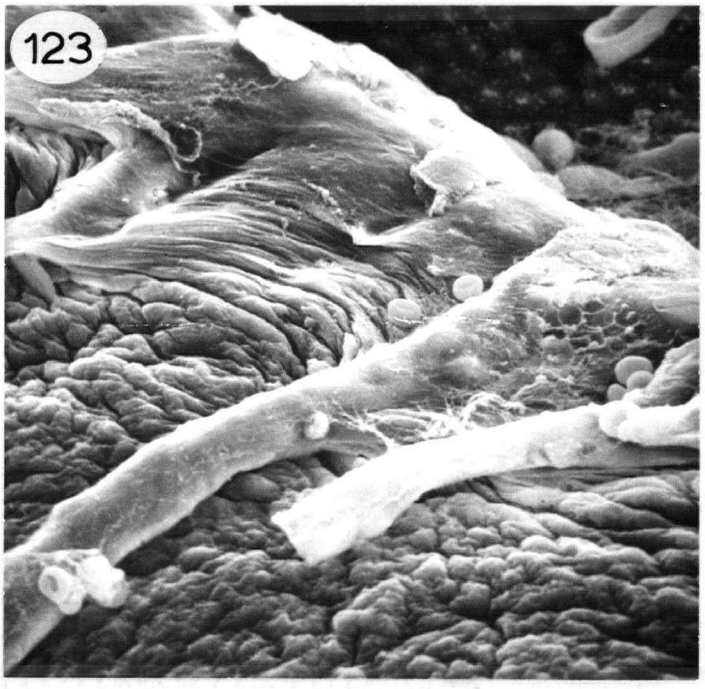
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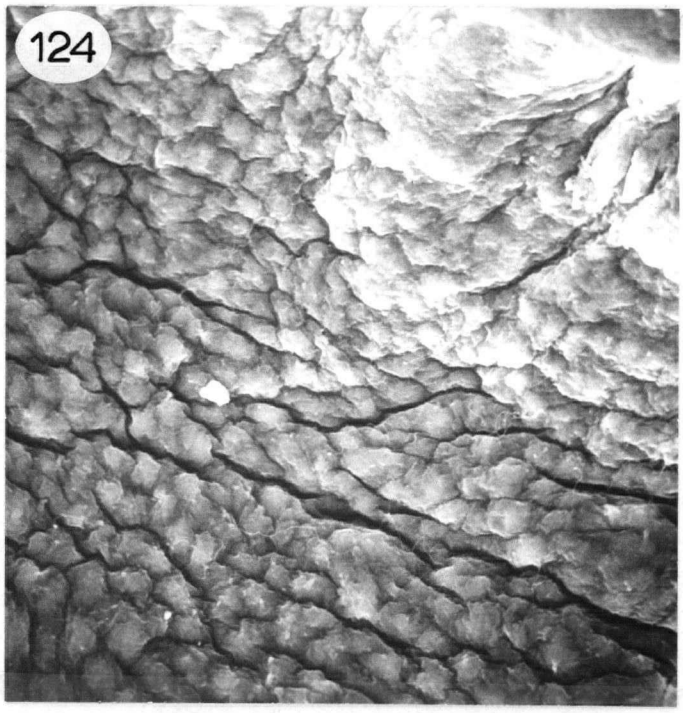
Figure 124 5-10 Days Post-natal (SEM)

The posterior epithelial cells are relatively closely packed together being separated only by shallow depressions. Occasionally, deeper grooves are seen separating out groups of rows of epithelial cells. x 820

Figure 125 5-10 Days Post-natal (SEM)

The posterior epithelial cells are covered by a basement membrane, which shows many crinkles, which are less prominent over the bulging nuclear region. A crater-like structure is present. It is elevated above the posterior surface of the iris. The basement membrane covering it is continuous with the basement membrane covering the other posterior epithelial cells. The basement membrane also shows some crinkles. x 1,630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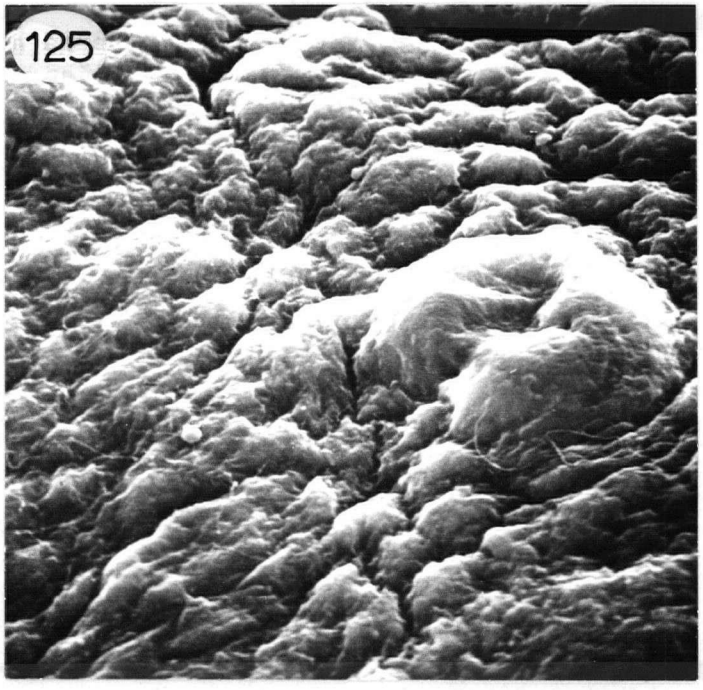
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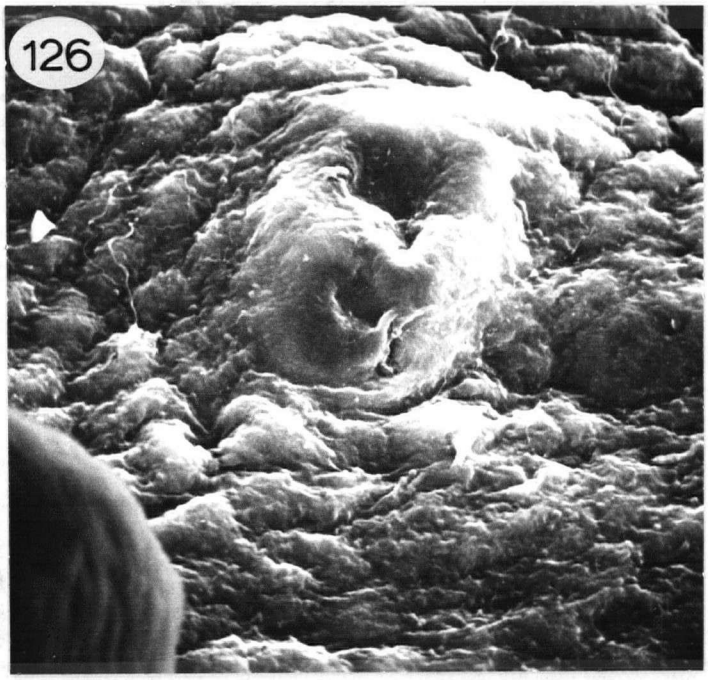
Figure 126 5-10 Days Post-natal (SEM)

This crater-like structure appears high above the plane of the other epithelial cells. It is generally bloated. It is collapsed in the middle. The covering basement membrane layer is relatively smooth. x 1,630

Figure 127 5-10 Days Post-natal (SEM)

The pupillary membrane is still present in parts of the iris, but it is not as extensive as in younger eyes. Other blood vessels on the posterior surface of the iris appear to be continuous with those of the anterior surface. x 390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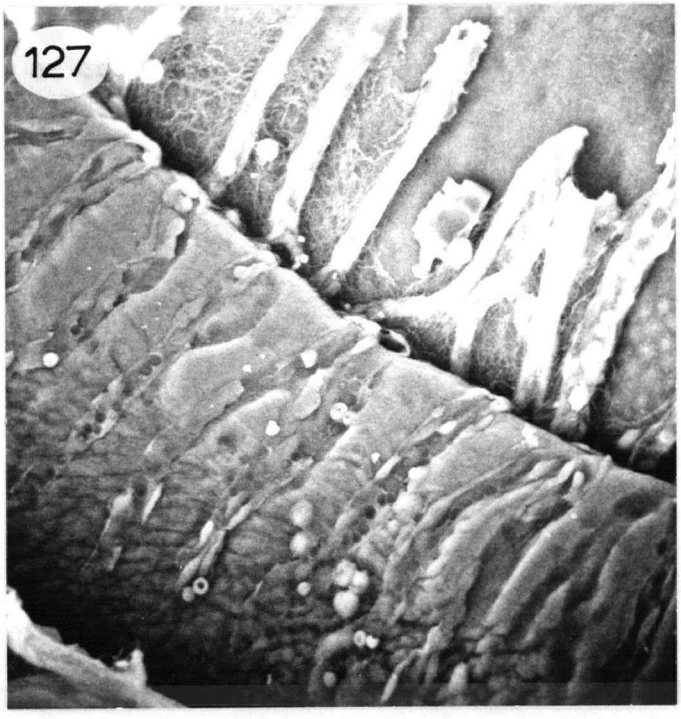

127

4. From 11 Days Post-natal On (Figures 128-137)

By about two weeks after birth, the rat iris has attained the adult form in terms of the morphology of the posterior surface. There are a few remnants of the fetal and early post-natal vascular system still present and these will be alluded to. They are lost or present in only a limited extent in the adult rat iris.

The iris is usually observed in some degree of pupillary constriction (Figures 128, 129). The posterior surface of the iris can be divided into a pupillary and a peripheral region. In a low magnification scanning electron micrograph, the pupillary margin is not smooth. In the pupillary region there are numerous blood vessels embedded in an amorphous material (Figures 128, 129). Some of the blood vessels remain only in the pupillary region while others stretch out towards the ciliary body. The number of these long blood vessels varies from one eye to the next (Figures 128, 129). The rest of the posterior surface of the iris appears relatively smooth at a low magnification. Faint circumferential lines may be seen (Figure 128). Along the periphery of the iris at the region of the ciliary body, there are some barely visible radial ridges (Figure 128). These are short and peter out almost immediately. Occasionally, wisps of fibrillar material are seen stretching across the pupillary aperture. These are probably remnants of the connective tissue network of the pupillary membrane, which has at this point virtually disappeared (Figure 129).

At a slightly higher magnification, the differences between the pupillary and the peripheral regions are clearly seen (Figure 130). Large, rounded, relatively thin-walled capillaries stretch from the pupillary margin to the periphery. They are filled with red blood cells and appear functional. When the blood vessel is slightly displaced from its position, it is observed that the vessel makes a radially oriented impression on the

circumferentially arranged rows of posterior epithelial cells. Many of the blood vessels, though, seem to end at the pupillary region. It is difficult to say whether this is artifactual, that is, the capillaries have been removed in the handling of the tissues, or whether this is a true indication of the regression of the capillaries taking place.

A closer examination of the pupillary region reveals that most of the capillaries turn around and hug the pupillary margin itself (Figure 131). A scanning electron micrograph of the pupillary margin viewed edge on (Figure 132) shows that these blood vessels come from the iris stroma and make a complete turn at the pupillary margin. Most of the capillaries lie superficial to the underlying amorphous layer although a few are still embedded in it (Figure 131). Most of the capillaries that are confined to the pupillary region, are no longer round tubes containing red blood cells. Instead, many of them show bulges on the sides (Figure 133). It appears as though the endothelial cell walls are thinned out so that any red blood cells in the lumens can push outwards. Many of the capillaries appear in a state of atrophy (Figure 134). The cells present do not look normal. Highly coiled fibrillar material and cellular debris are common (Figures 133, 134). Beneath the atrophied capillaries, the posterior surface of the iris is smooth, except for a few striations. This is very similar to what is boserved in the adult sphincter region (Figure 134).

The posterior epithelial cells are arranged in ridges which are in general circumferentially oriented (Figures 135, 136). It is difficult to trace the entirety of a row of epithelial cells as the epithelial ridges merge and branch in a complicated way (Figures 135, 136). The epithelial ridges are separated by grooves of varying depths. The individual posterior epithelial cells can be recognised as their nuclei bulge a little more than the rest of the cell cytoplasm. The basement membrane covering the

posterior epithelial cells is highly wrinkled but to a lesser extent over the nuclei. The crinkles over the cytoplasmic portions of the cells probably suggest that there are cytoplasmic cell processes underlying the basement membrane.

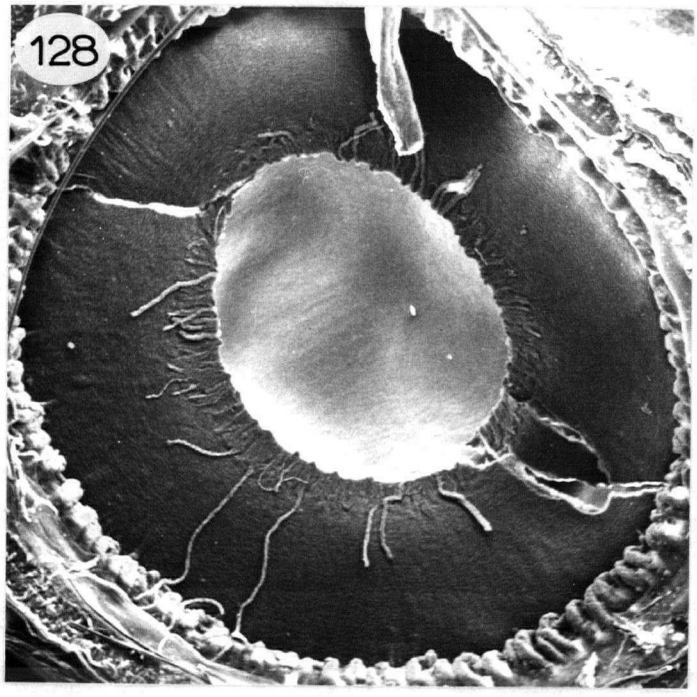
Near the ciliary body, the beginnings of the adult ciliary-iris processes are observed (Figure 137). These radial ridges are not as prominent as those in the adult but they show the general characteristics of the adult ciliary-iris processes. It is a peripherally located, radially oriented ridge, being higher at the peripheral end and going deep into the iris tissue at the pupillary end (Figure 137). There is a break in the continuity of the posterior epithelial ridges over the highest point of the ciliary-iris process. The other epithelial ridges tend to skirt around it to some degree. Some epithelial ridges are seen going over the lower portions of the ciliary-iris process. These ridges are not as high nor are the grooves as deep as those of the rest of the posterior surface of the iris.

Figure 128 From 11 Days Post-natal On (SEM)

The pupil is in partial constriction. The posterior surface of the iris is relatively smooth. Some faint circumferential striations are barely perceptible. At the periphery of the iris in the region of the ciliary body, a few small, short radial ridges are seen. The pupillary margin is slightly corrugated. Right around the pupillary margin, blood vessels of varying lengths are embedded in an amorphous layer. Some of the blood vessels terminate at the pupillary region while a few traverse the posterior surface of the iris to varying degrees. x 40

Figure 129 From 11 Days Post-natal On (SEM)

The pupil is much constricted. The pupil is small and wisps of connective tissue are seen across the pupillary aperture. The posterior surface of the iris is smooth except for a small zone around the pupil. An enormous number of blood vessels are embedded in the amorphous material around the pupil, but they are free peripherally. Many of these blood vessels traverse the entire length of the iris to the ciliary region. x 40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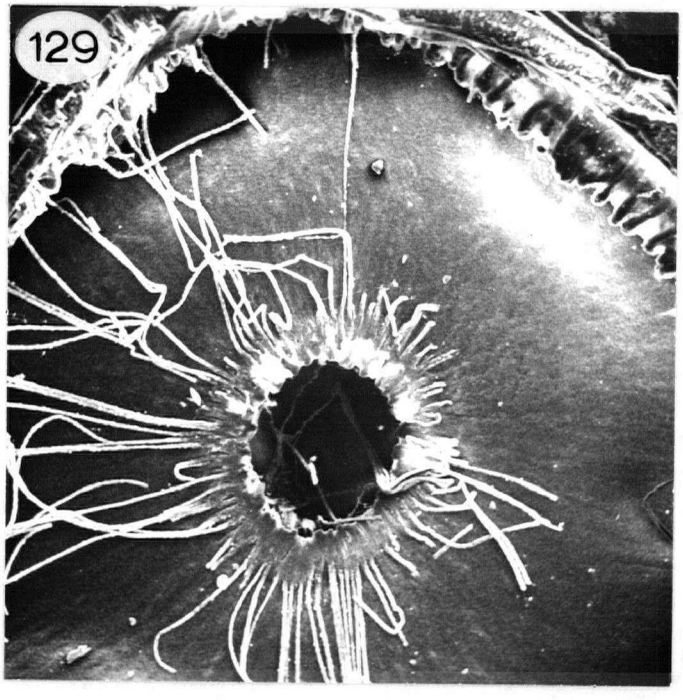
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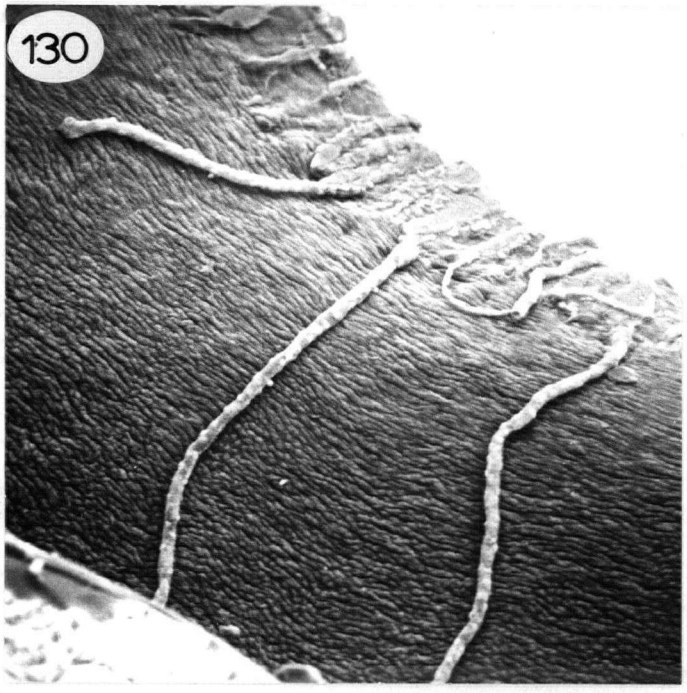
Figure 130 From 11 Days Post-natal On (SEM)

Circumferential rows of posterior epithelial cells are present over most of the posterior surface of the iris. Most of the blood vessels are limited to the pupillary region. A few stretch between the pupillary edge and the ciliary body. These blood vessels have thin walls so that the red blood cells within the lumens give them a beaded appearance. When a blood vessel has been displaced from its original position, an impression of its path is observed running across the circumferential epithelial ridges.
x 160

Figure 131 From 11 Days Post-natal On (SEM)

All of the blood vessels on the posterior surface of the iris turn around and hug the pupillary margin itself. A small amount of amorphous material and some connective tissue fibers are still present in the pupillary region. The posterior epithelial cells are arranged in circumferential rows separated by narrow grooves. The individual nuclei of the epithelial cells can be seen bulging outwards. x 410



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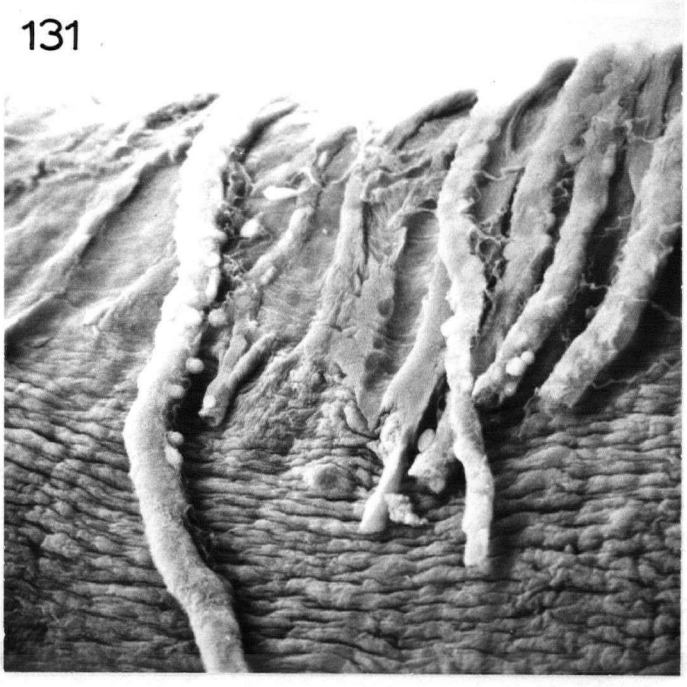
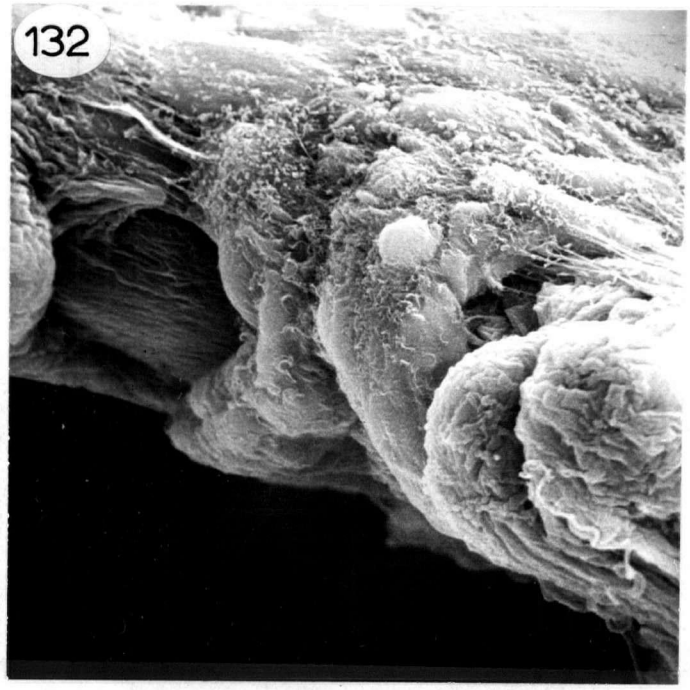


Figure 132 From 11 Days Post-natal On (SEM)

An edge on view of the pupillary margin shows that the blood vessels from the anterior iris stroma turn around at the pupillary margin and apparently go posteriorly and peripherally. x 1,630

Figure 133 From 11 Days Post-natal On (SEM)

Atrophying blood vessels are seen in the pupillary region together with some connective tissue remnants. The blood vessel walls bulge out in many places. x 820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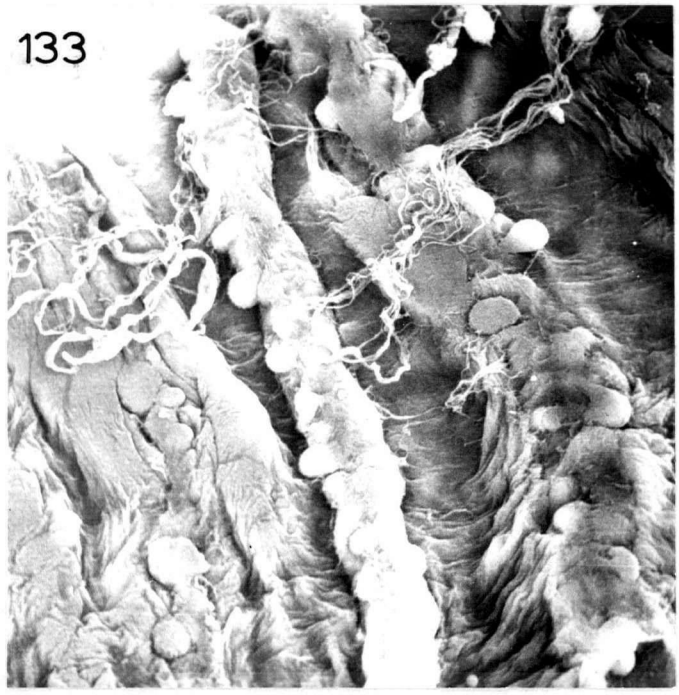
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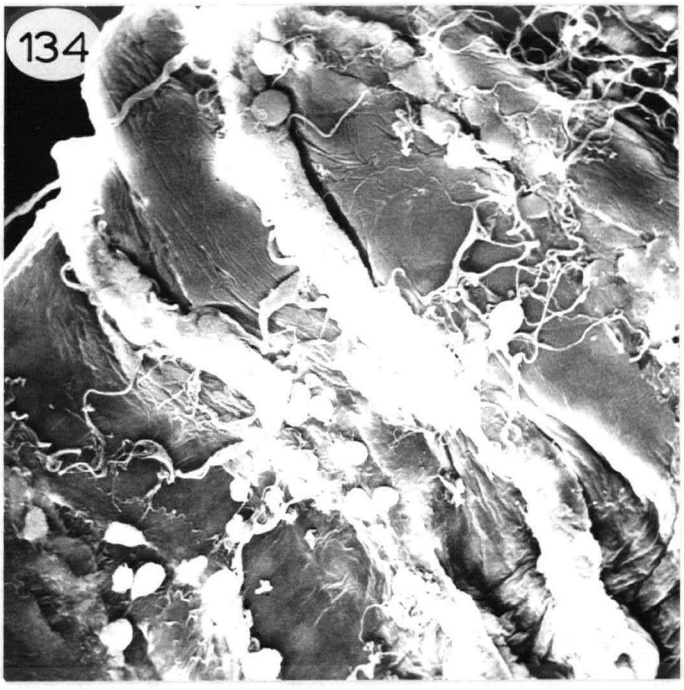
Figure 134 From 11 Days Post-natal On (SEM)

Only a few atrophied blood vessels and some connective tissue fibers are present. A few fine striations are seen in the pupillary region. x 700

Figure 135 From 11 Days Post-natal On (SEM)

The circumferential rows of posterior epithelial cells are clearly visible. The individual cells in each epithelial ridge is demarcated by the bulging nuclei. Deep narrow grooves separate the epithelial ridges. x 820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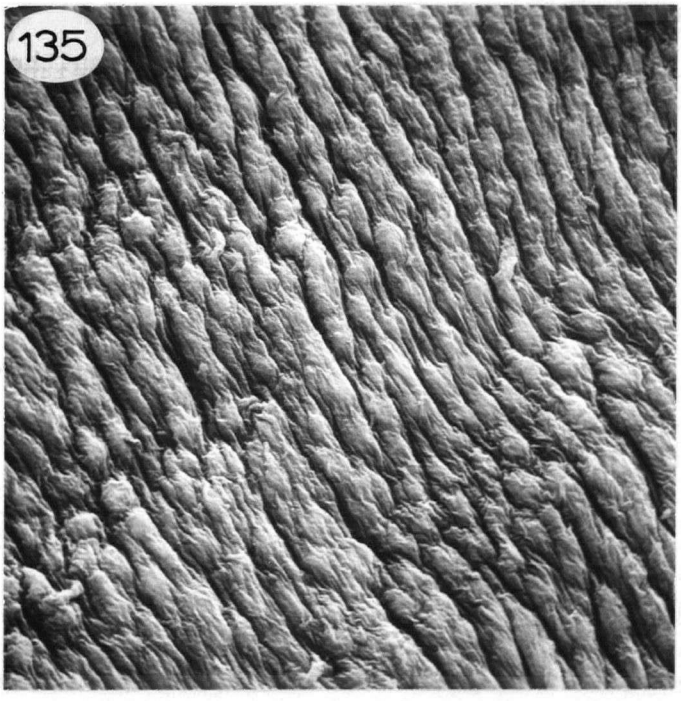
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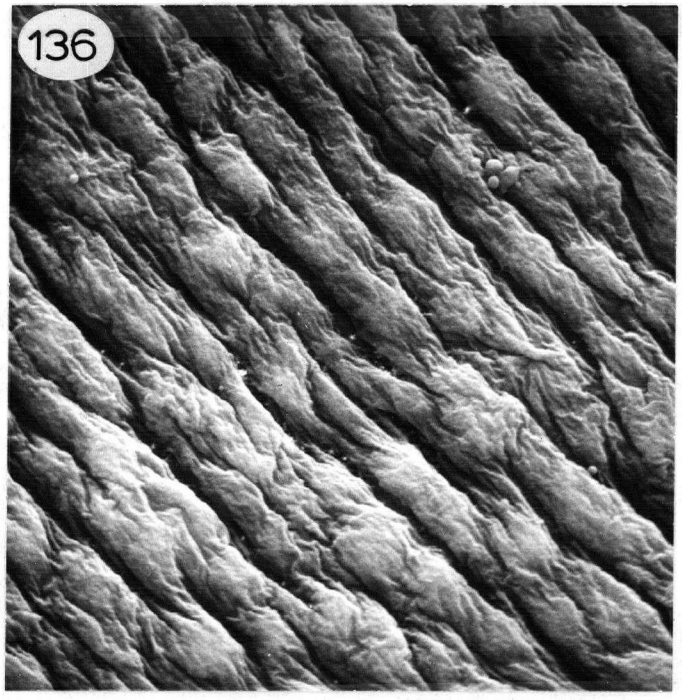
Figure 136 From 11 Days Post-natal On (SEM)

A higher magnification of Figure 135. The epithelial ridges bifurcate, taper down or blend with other epithelial ridges. The basement membrane covering the epithelial cells is wrinkled, but slightly less so over the bulging nuclear regions. x 1,630

Figure 137 From 11 Days Post-natal On (SEM)

The beginning of the ciliary-iris process is shown here. It is a low radial ridge. The circumferential epithelial ridges either skirt around the ciliary-iris process, or there is a break in the continuity of the epithelial ridges. If the epithelial ridges go over the radial ridge, they seem to be flattened or stretched out. x 1,000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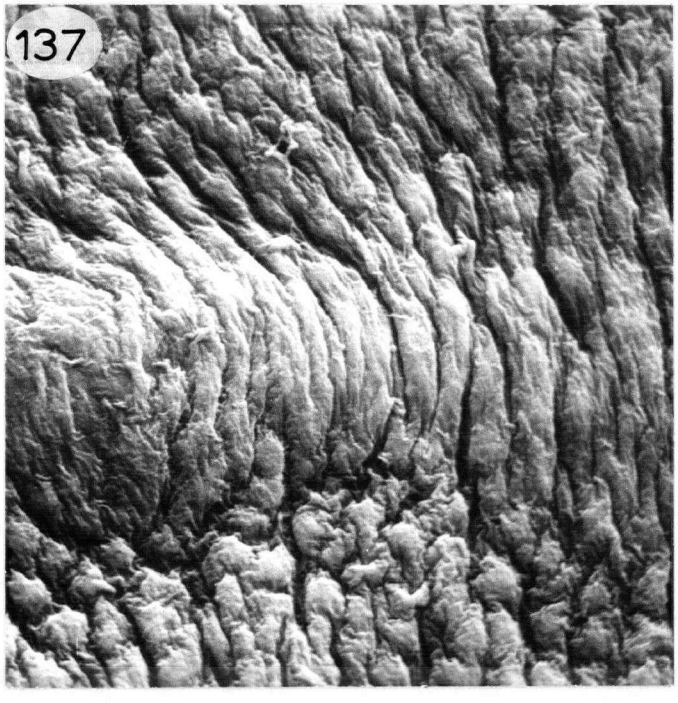

137

G. Horse-radish Peroxidase (HRP) Studies of the Iris in Fetal, Post-natal and Adult Rats

The glutaraldehyde-fixed iris tissue is incubated according to the method set out by Karnovsky (1967). The reaction product, a brownish to black precipitate, indicating the location of the HRP, is observed with the light microscope. In these studies, it is found that neither the type of anesthesia used nor the route of the intravascular infusion of the HRP affected the localisation of the reaction product. The eyes were removed at different time intervals, from 1 to 45 minutes, following the injection of the HRP. It is observed that there is no qualitative difference in the localisation of the reaction product dependent on time. Occasionally, there are quantitative differences in the intensity of the reaction between the eyes that are removed soon after the injection of the HRP and those that are removed 45 minutes later. Smith (1971) in his studies on the mouse, observed that there is a gradual increase in the amount of tracer present with time. However, in our investigation, this is not consistently observed.

In fetal eyes, (Figures 138, 139) the tracer HRP is seen in the developing ciliary body and iris as early as 1 minute following the infusion of the HRP. The overall intensity of the reaction in the tissues varies. Only occasionally, but not consistently, does this variation in reaction intensity appear to be time dependent, that is, there is an increase in the intensity of the reaction with time.

When the reaction is light, the reaction product is present both in the ciliary body and in the iris. In the ciliary body, the reaction precipitate is found along the stromal (basal) surfaces of the ciliary pigment epithelium. In the developing iris, the reaction product lines the stromal

surfaces of the anterior epithelium (Figure 138).

When the reaction is intense (Figure 139) the reaction precipitate is found along the basal surfaces of the ciliary pigment epithelium, in between the individual pigment epithelial cells in varying degrees, and at times in the space between the ciliary and pigment epithelia. The stromal surfaces of the pigment epithelial cells are often coated with the precipitate, especially if they are abutting directly against a blood vessel in the stroma. The precipitate may be seen only between the basal halves of the pigment epithelial cells, or it may be found all along the lateral surfaces of the cells. Some of the stromal cells in the ciliary stroma are also covered with precipitate. In the developing fetal iris, the reaction product is seen along the anterior surfaces of the anterior epithelium and occasionally in between some of the anterior epithelial cells. As in the ciliary body, the reaction is always more intense if a blood vessel is immediately adjacent to the anterior epithelium. Some of the iris stromal cells overlying the anterior epithelium also show reaction product along their cell surfaces. Some precipitate is seen along the anterior and pupillary borders of the developing sphincter muscles. Sometimes, reaction precipitate may outline the individual cells of the developing sphincter.

Some precipitate is occasionally seen along the posterior surfaces of the iris posterior epithelium and of the ciliary epithelium facing the posterior chamber of the eye.

In young post-natal rat eyes, up to 12 days after birth (Figures 140-149), the localisation of the HRP reaction product is essentially similar to that observed in the fetal eyes. In the ciliary body, the reaction precipitate is found along the stromal surfaces of the pigment epithelial cells, in between the individual pigment epithelial cells and also in between the ciliary and pigment epithelia (Figures 141, 143, 145, 147,

149). During this period of time, the ciliary body is developing morphologically. Ciliary processes are beginning to form. It is observed that whenever ciliary processes are present, the reaction precipitate is either more intense or is only localised at the tip of the developing ciliary processes (Figure 149). Here, the ciliary blood vessel is usually very intimately associated with the pigment epithelium without any intervening stromal cells. The reaction precipitate may surround all of the surfaces of the pigment epithelial cells, or the precipitate is found only between the pigment epithelial cells and between the ciliary and pigment epithelia. Occasionally, reaction product is found within the pigment cells themselves, as has been noted in the mouse (Smith, 1971). This reaction precipitate is usually concentrated in the anterior basal poles of the cells. As one moves away from the tip of the developing ciliary process, the reaction either diminishes or disappears. The precipitate is then usually located between the ciliary and pigment epithelia (Figures 147, 149).

In the iris, the reaction precipitate is always found along the stromal surfaces of the anterior epithelium, thus demarcating the boundary zone between the anterior epithelium and the stroma (Figures 140-144, 146-148). Some precipitate is also found in between the individual anterior epithelial cells and in between the iris anterior and posterior epithelia (Figures 140, 141, 143, 144, 146-148). This is more prominently evident in the peripheral portion of the iris towards the root. Usually some precipitate surrounds the pupillary and anterior borders of the iris sphincter (Figures 140, 144, 146). Reaction precipitate may be found in between all of the muscle cells of the developing sphincter. Most often, the reaction precipitate is found amongst the cells in the posterior half of the sphincter (Figures 140, 142, 144, 146). Some precipitate is occasionally seen in the blood vessel overhanging the sphincter (Figure 140) and among

some of the stromal cells (Figures 147, 148).

With further development and growth of the rat, some changes are seen in terms of the localisation of the HRP reaction product. From 15 to 22 days after birth, the sites of the HRP precipitate in the ciliary body are similar to those observed previously and also to those observed in the adult. In the ciliary body, the reaction precipitate is more definitively localised at the tips of the ciliary processes (Figure 150). However, in the iris some changes occur. At 15 days after birth, there is some, but very little, reaction precipitate seen in the iris tissue itself. It is usually localised at the very root of the iris near to the ciliary body. By 22 days after birth, the reaction in the iris disappears. As in the adult rat eye, reaction precipitate for HRP is found only in the ciliary processes but not in the iris.

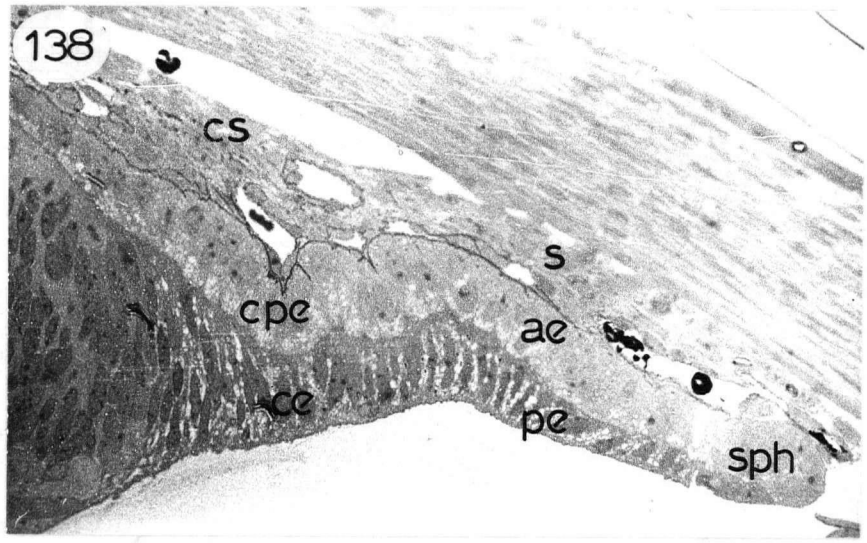
Figure 138 20-21 Days Fetal (HRP)

The overall reaction for HRP is light. The precipitate is seen lining the stromal surfaces of the ciliary pigment epithelium (cpe) and of the anterior epithelium (ae) of the iris. No reaction precipitate is seen in the ciliary epithelium (ce), posterior epithelium of the iris (pe) or in the sphincter (sph). There is a small amount of precipitate in the ciliary stroma (cs) but not in the iris stroma (s). x 250

Figure 139 20-21 Days Fetal (HRP)

The reaction for HRP is strong. In the developing ciliary body, reaction precipitate is found all along the basal (stromal) surface of the ciliary pigment epithelium (cpe), outlining the individual cells of the ciliary pigment epithelium and in between the ciliary epithelium (ce) and the ciliary pigment epithelium.

In the developing iris, the reaction is intense all along the stromal surface of the anterior epithelium (ae) and this continues on to line the anterior and pupillary borders of the developing sphincter (sph). Some precipitate permeates in between some of the sphincter muscle cells. At the root of the developing iris, some precipitate is seen delineating the boundaries of the anterior epithelial cells. There is some precipitate in between some of the cells at the ciliary-iris junction (arrow). Some precipitate is present both in the ciliary (cs) and iris (s) stroma. x 220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cs
s
cpe
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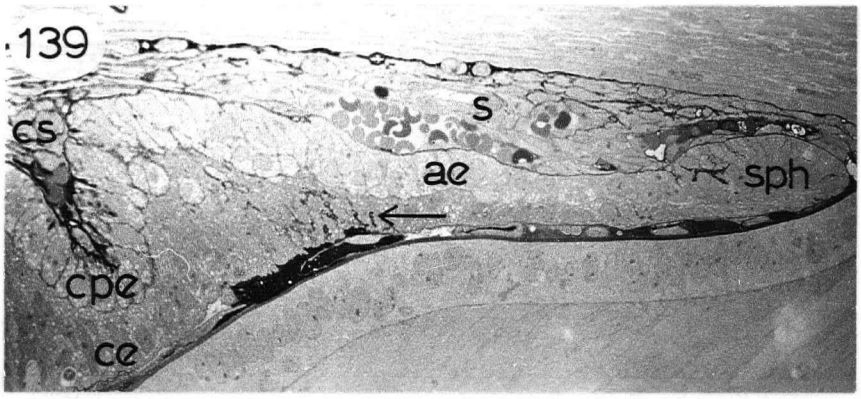
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cs
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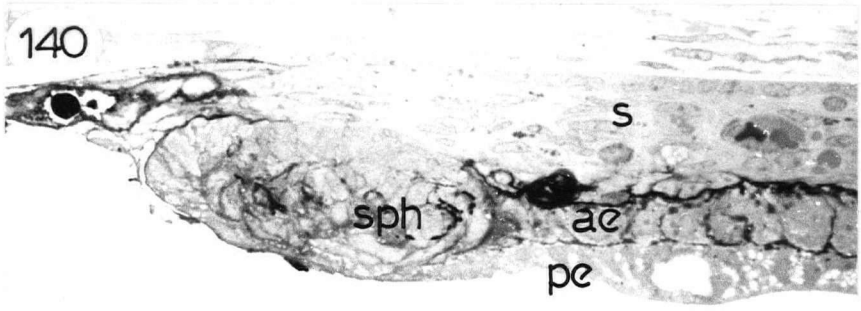
Figure 140 1 Day Post-natal (HRP)

In the developing sphincter (sph), the reaction precipitate is found in between almost all of the muscle cells. Some precipitate is present along the pupillary margin of the sphincter. There is a relatively intense reaction in the stroma (s) overhanging the pupillary margin of the sphincter and along the stromal surface of the anterior epithelium (ae). Reaction precipitate is seen in between the cells of the anterior epithelium and along the boundary between the anterior epithelium and the posterior epithelium (pe). x 350

Figure 141 1 Day Post-natal (HRP)

In the developing ciliary body, the reaction is most intense along the stromal surface of the ciliary pigment epithelium (cpe), especially where it is right next to a blood vessel (bv) of the ciliary stroma (cs). The reaction precipitate lines all of the surfaces of the ciliary pigment epithelial cells. At the bases of some of the ciliary pigment epithelial cells, some precipitate appears to be within the cell cytoplasm. x 350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s
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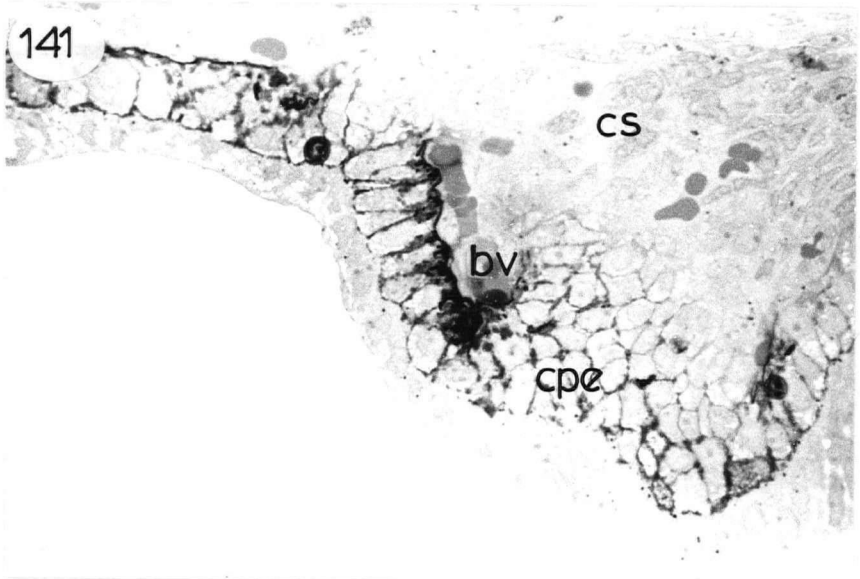
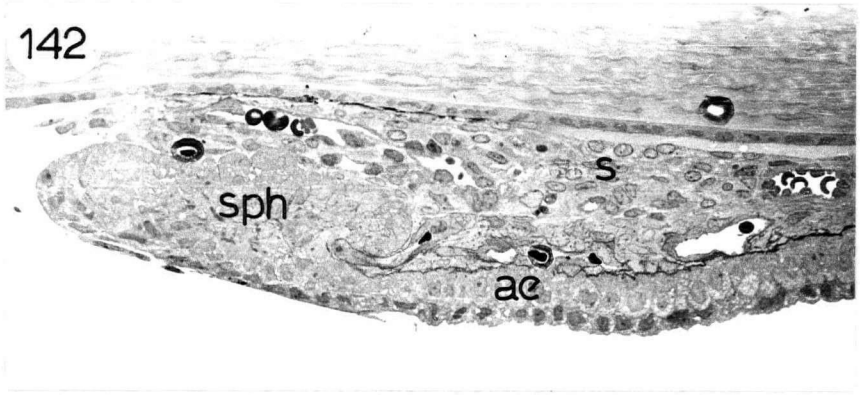

141
cs
bv
cpe

Figure 142 4 Days Post-natal (HRP)

There is much reaction precipitate all along the stromal surface of the anterior epithelium (ae). There is some precipitate in the stroma (s) at the peripheral part of the developing sphincter (sph). A very faint reaction is seen in between some of the developing sphincter muscle cells.
x 220

Figure 143 4 Days Post-natal (HRP)

In the developing iris, reaction precipitate coats the stromal surface of the anterior epithelial cells (ae). Some precipitate is also found between the individual anterior epithelial cells, between the anterior and posterior epithelium (pe), especially towards the root of the iris. The ciliary body is beginning to form ciliary processes. The reaction precipitate is found primarily in between the ciliary epithelium (ce) and the ciliary pigment epithelium (cpe) and in between the individual cells of the ciliary pigment epithelium. Some precipitate is also found along the stromal surface of the ciliary pigment epithelial cells towards the tip of the developing ciliary process. x 220


142
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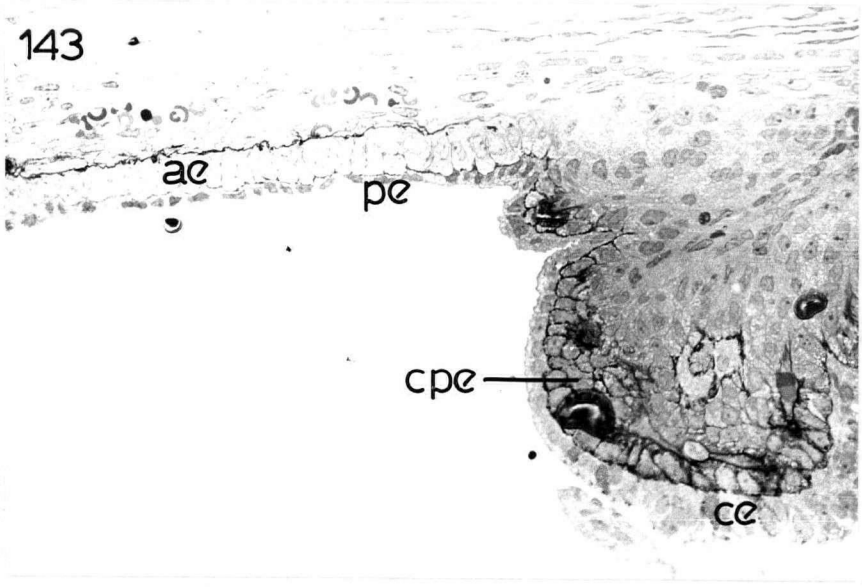
143
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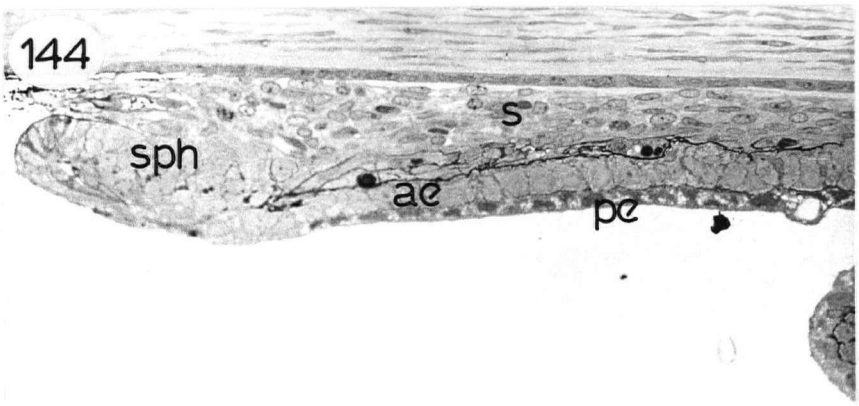
Figure 144 5 Days Post-natal (HRP)

Reaction precipitate for HRP is seen all along the pupillary margin of the sphincter (sph), in between the muscle cells, along the stromal surface of the anterior epithelium (ae), in between the anterior epithelial cells and in between the anterior and the posterior epithelium (pe). In the iris stroma (s) immediately adjacent to the anterior epithelium and in the stroma overlying the tip of the sphincter, there is also some reaction. x 220

Figure 145 5 Days Post-natal (HRP)

The ciliary body consists of one large ciliary process which is beginning to branch. The reaction appears more pronounced towards the tip of the ciliary process, where the precipitate lines all of the surfaces of the ciliary pigment epithelial cells (cpe). Some precipitate may be within the basal poles of the cells. Towards the base of the ciliary process, the precipitate is found mainly between the ciliary epithelium (ce) and the ciliary pigment epithelium. There is also some precipitate in between a few of the cells of the ciliary epithelium. x 220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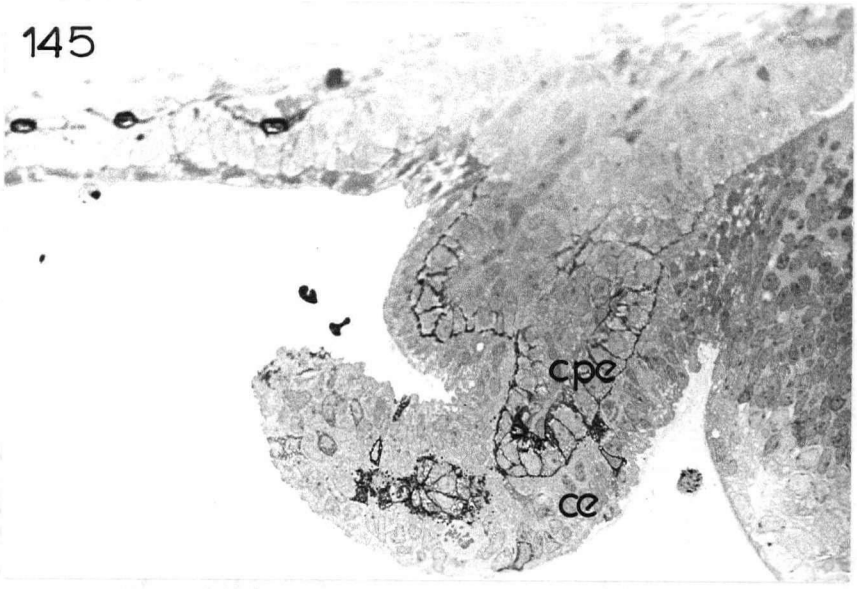
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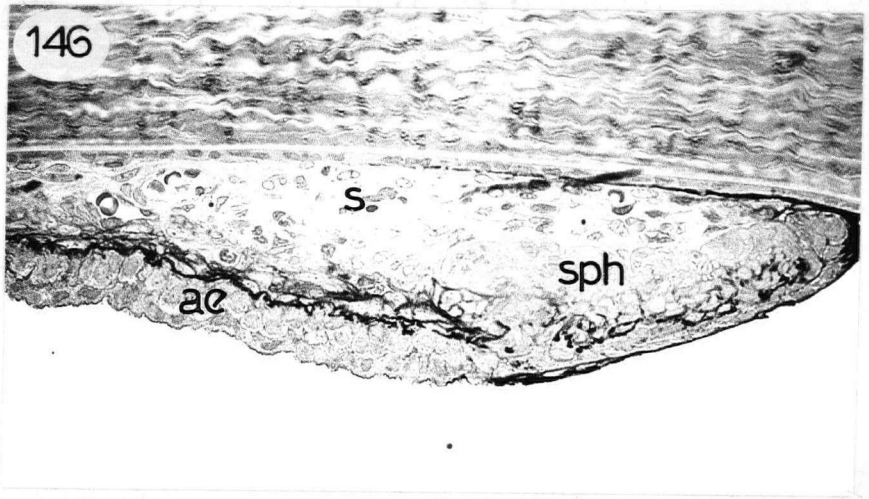
Figure 146 7 Days Post-natal (HRP)

In the developing sphincter (sph) there is reaction precipitate only among the muscle cells in the posterior half of the sphincter. Some precipitate is seen coating the anterior, pupillary and posterior borders of the sphincter. Reaction is abundantly present along the stromal surface of the anterior epithelium (ae) and in the immediately adjacent stroma (s). There is a hint of some precipitate along the posterior surface of the iris. x 220

Figure 147 7 Days Post-natal (HRP)

In the developing iris, the reaction precipitate clearly demarcates the extent of each anterior epithelial cell (ae). However, the reaction peters out towards the root of the iris. In the developing ciliary process, the reaction is more intense towards the tip of the ciliary process and gradually diminishes away from the tip. At the tip of the ciliary process, the precipitate lines all of the surfaces of the ciliary pigment epithelial cells (cpe). At the base of the ciliary process, the precipitate is mainly seen in between the ciliary pigment epithelium and the ciliary epithelium (ce). x 220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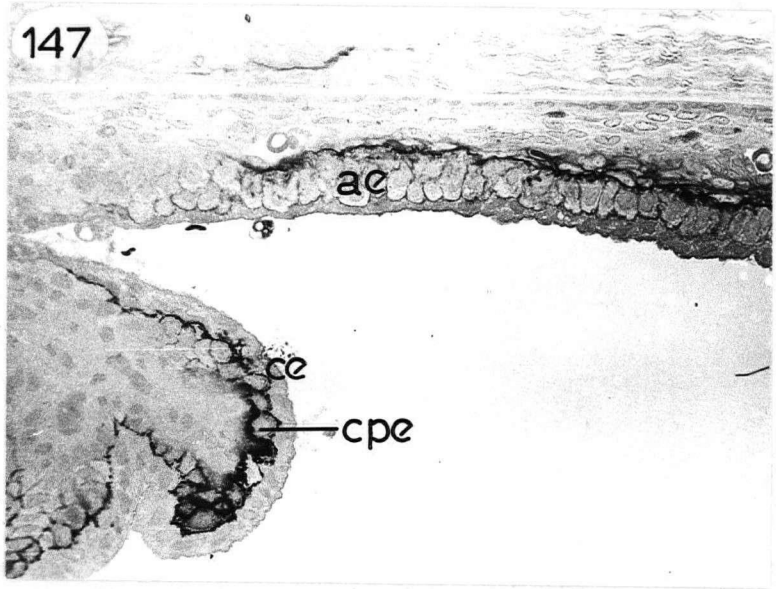
147
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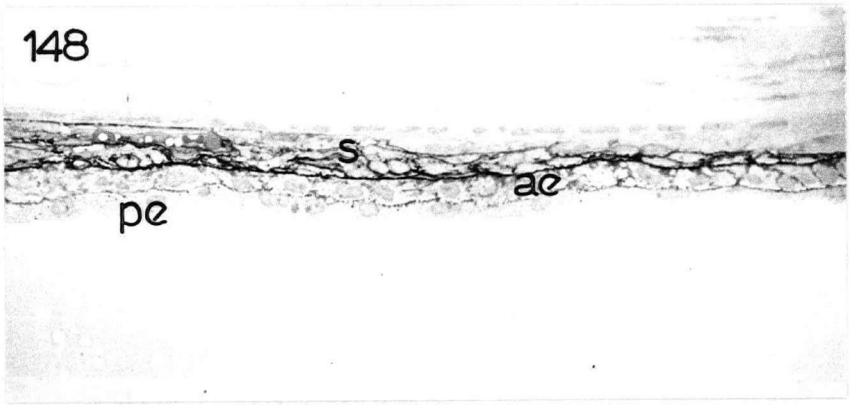
Figure 148 12 Days Post-natal (HRP)

The reaction precipitate is observed mainly in the iris stroma (s) and along the stromal surface of the anterior epithelium (ae). There is a faint reaction in between the anterior epithelial cells and in between the anterior and posterior epithelium (pe). x 220

Figure 149 12 Days Post-natal (HRP)

Long ciliary processes are observed. The HRP reaction precipitate is more intense at the tips of the ciliary processes, especially where the blood vessels (bv) expand. At the tips of the ciliary processes, the reaction is very intense along the stromal surface of the ciliary pigment epithelial cells (cpe) and less so in between the individual pigment epithelial cells and in between the ciliary pigment epithelium and the ciliary epithelium (ce). Some precipitate is also found within the stromal poles of the ciliary pigment epithelial cells. As one moves towards the root of the ciliary process, the precipitate along the stromal surface of the ciliary pigment epithelium disappears leaving some precipitate in between the individual ciliary pigment epithelial cells and in between the ciliary pigment epithelium and the ciliary epithelium. x 220


148
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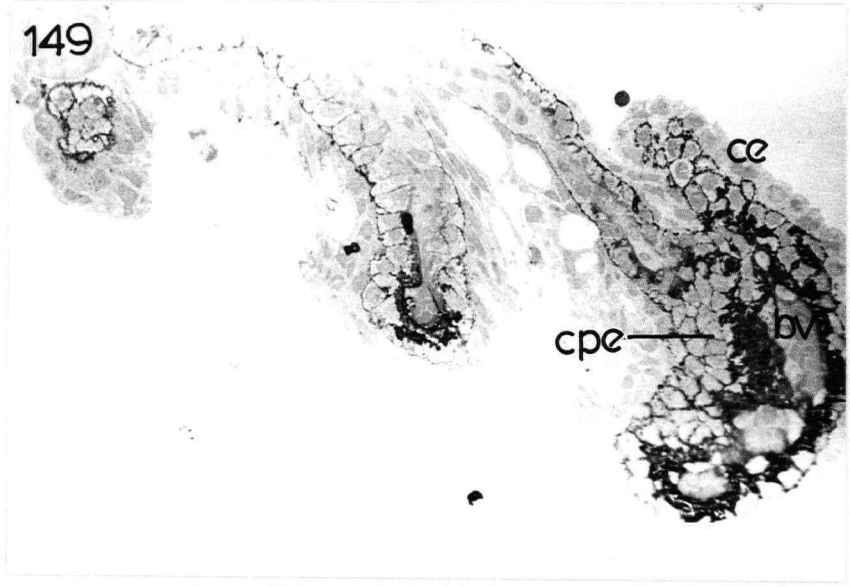
149
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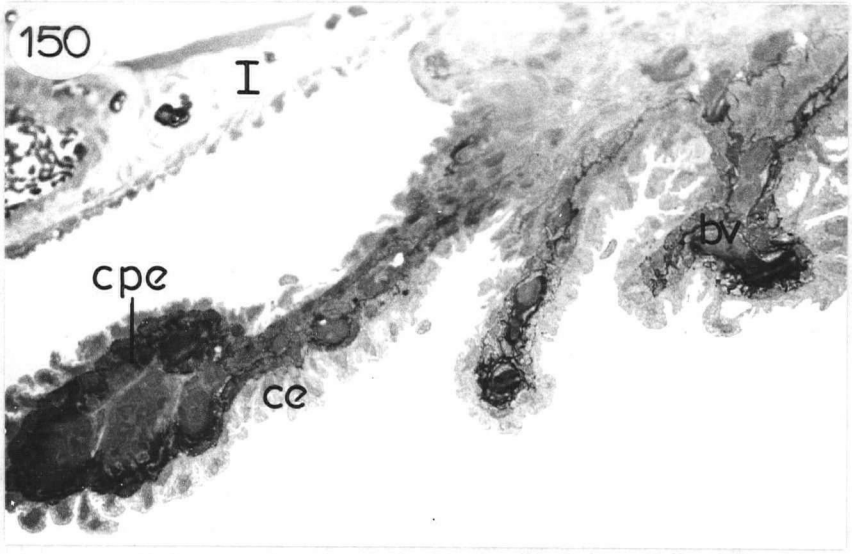
Figure 150 Adult (HRP)

The localisation of the reaction precipitate for HRP in the ciliary process is similar to that in Figure 149. HRP reaction precipitate is only found in the ciliary process and not in the iris (I). x 220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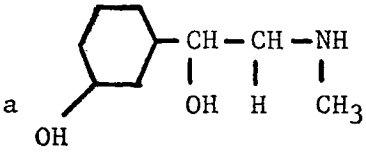
IV. DISCUSSION AND SUMMARY OF THE RESULTS

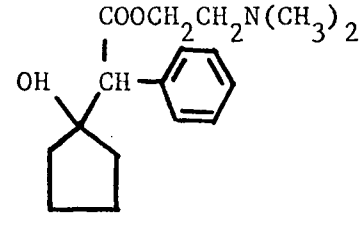
A. A Light and Transmission Electron Microscopic Study of the Rat Iris in Pupillary Dilation and Constriction

1. General

The rat pupil is capable of extensive excursions in response to changing light conditions. This can be quite easily observed by either shining a bright light onto the eyes, at which point the pupils constrict to oftentimes a pinhole, or observing the eyes in a slightly shaded condition, when the pupils dilate. In normal laboratory conditions, the rat pupils are in some degree of pupillary constriction. In order to study the structural changes of the iris that must necessarily occur as the pupil constricts or dilates, we must be able to maintain the iris in a fixed state of dilation or constriction. In our investigations this is done with the aid of chemical mediators. A mixture of phenylephrine hydrochloride and cyclopentolate and echothiophate iodide are regularly used to dilate and constrict the pupils, respectively. Phenylephrine hydrochloride (Goodman and Gilman, 1967) with the chemical structure

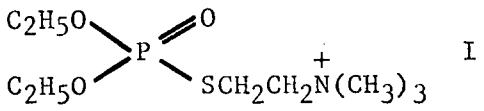


is a sympathomimetic drug. It is used as a mydriatic in ophthalmology. Dilation of the pupil occurs without any increase in intraocular pressure. Phenylephrine hydrochloride is used together with cyclopentolate, (Goodman and Gilman, 1967) an antimuscarinic agent. Cyclopentolate,



blocks the action of acetylcholine on the iris sphincter muscle, thus resulting in mydriasis.

Echothiophate iodide (Goodman and Gilman, 1967) is a potent derivative of choline. It is a powerful anti-cholinesterase agent. When it is applied to the eye, it causes miosis within a few minutes. Concomitant with miosis there is usually a fall in the intraocular pressure and thus it is also used in the treatment of glaucoma.



I

The mixture of phenylephrine hydrochloride and cyclopentolate, and echothiophate iodide are applied locally and the pupils reach maximal dilation or constriction after about 20 minutes. Usually both dilation and constriction is observed from the same animal as one eye is dilated while the other is constricted. The rat is placed under anesthesia while enucleation occurs.

The irises are fixed both by immersion and by perfusion in various fixatives. When the irises are fixed by immersion, a few difficulties are sometimes encountered. As soon as enucleation occurs, dilation of the pupil tends to take place unless it is transferred almost immediately into the fixative and chemical fixation is almost instantaneous. Any delay would result in the pupil being in partial constriction rather than in extreme miosis. If the pupil is already dilated, then there is no tendency for constriction to occur. This phenomenon of "death dilation" has been commonly observed but not understood (Alphen, 1963; Kelly and Arnold, 1972). To prevent pupillary changes from taking place after the eyes are removed from the animal, Alphen (1963) froze the monkey eyes in situ, while Kelly and Arnold (1972) perfused the rats with first a salt solution which is then followed by solutions of osmium tetroxide or glutaraldehyde. Both methods are equally effective for maintaining the pupils in extreme miosis or mydriasis.

In our investigation, it is found that with speed and care the irises

can be maintained in pupillary dilation or constriction using the method of immersion fixation. For light microscopic studies, the iris tissue appears relatively adequately fixed. There are some vesicles in the posterior and anterior epithelial layers but at the magnification and resolution obtainable with the light microscope it cannot be determined if these vesicles are artifactual or real. However, the requirements for the preservation of ultrastructural details of the tissue are much more stringent for transmission electron microscopy than for light microscopy. Immersion fixation does preserve the overall configurations and structural characteristics of the cellular components of the iris but there is some intracellular tissue disruption. The mitochondria are primarily affected. The cristae break up so that many vesicles with cellular debris are found. This would contribute in part to the vesiculated nature of some of the posterior and anterior epithelial cells as seen light microscopically. To circumvent this, perfusion fixation is used. As the rat is being perfused with the fixative, the pupils remain in the same degree of miosis or mydriasis suggesting that chemical fixation has happened almost instantaneously. The presence of both thick and thin filaments in the sphincter and dilator muscles is one criterion that fixation is adequate (Kelly and Arnold, 1972). These are observed in our perfused specimens. However, contrary to Kelly and Arnold's findings, perfusion with a salt solution prior to the administration of the fixative is not essential for preserving the thick and thin filaments. Perfusion with the fixative alone is sufficient. Even when the iris as a whole appears well-fixed, some tissue disruption is still present in isolated parts. This is especially noticeable in the posterior epithelium. The posterior epithelial cells appear to be particularly sensitive to any external changes. Perhaps, the posterior epithelial cells are held in such a fine balance with the surrounding aqueous humor that even the slightest

change in the milieu, like the presence of a chemical fixative, immediately evokes disruptive changes within the cell cytoplasm.

Perhaps it will be questioned whether the histological and ultrastructural changes of the iris tissues that are seen in extreme pupillary dilation or constriction as induced by the local administration of drugs are truly indicative of the changes that normally occur in response to changing light conditions without the intervention of an external agent. From present studies this question remains unresolved. Further studies will have to be carried out to compare the histological characteristics of the iris in pupillary dilation and constriction during its normal responses to changing light conditions and also during its responses to different types of drugs.

2. The Histological and Ultrastructural Features of the Iris in Pupillary Dilation and Constriction

The most striking differences between the histology of the iris when it is in pupillary dilation from when it is in pupillary constriction are primarily noted in the posterior neuroectodermally derived layers. Thus our attention and interest is focussed here. Observations made on the light microscope will be correlated with observations made on the transmission electron microscope.

When the pupil is dilated, the iris is short and plump while when the pupil is constricted, the iris is thin, delicate and tenuous. There is an apparent drastic change in the total exposed surface area of the iris. This is very clearly shown by scanning electron microscopy. The shapes of the posterior epithelial and dilator cells, and their relationships to each other alter in response to changes in pupillary size. We are interested in

probing into what these changes are and what are the characteristics of these layers which allow them to so very efficiently accommodate themselves to changes in pupillary diameter.

The posterior epithelial cells form a layer covering the posterior surface of the iris. In pupillary dilation, the posterior surface of the iris is most often deeply convoluted as the result of the rows of peg-like epithelial cells which are discretely separated from each other. Occasionally, the posterior epithelial cells are very close together without any intervening gaps, as seen light microscopically. The posterior surface of the rat iris in pupillary dilation is not arranged in arcades as in the monkey iris where each arch is made up of eight to ten cells (Alphen, 1963). Rather, the posterior epithelial cells are arranged individually in rows. With the transmission electron microscope, the arrangement of the posterior epithelial cells with respect to each other, as seen with the light microscope, is verified. In addition, the transmission electron microscope reveals that a typical basement membrane is associated with the cell membranes of the posterior epithelial cells. There is always a clear zone between the cell membranes and the electron-dense basement membrane. Perhaps, this space allows some freedom of movement to the epithelial cells. A closely adherent membrane might be a mechanical disadvantage. Light microscopically, the posterior epithelial cells show large cell processes which are devoid of nuclei. Electron microscopically, these processes consist essentially of a mass of cell infoldings with some organelles. In fact, cell infoldings are extensively found on all the surfaces of the posterior epithelial cells leaving only a small rim of cytoplasm around the nucleus. The cell infoldings are highly complex and they interdigitate with each other in a three dimensional meshwork. The basement membrane does not follow the outlines of the cell infoldings. Rather, it covers the posterior surface

of the iris like a loose sheet.

In pupillary constriction, the configuration of the posterior epithelial cells is changed markedly. From being high columnar cells in pupillary dilation, the epithelial cells are flattened out so that they appear as a thin layer. The gaps in between the individual cells are no longer apparent. The posterior surface of the iris is relatively flat in extreme miosis with only an occasional bulge. Each epithelial cell appears closely associated with its neighbour. In the rat iris, there are no observable tight junctions between adjacent posterior epithelial cells, whereas tight junctions are present in the human iris (Hogan, Alvarado and Weddell, 1971). This would probably account for the observation that the rat iris can be more extensively dilated or constricted than the human iris (Newsome and Loewenfeld, 1971). The cell infoldings are still present. It is impossible to say whether some of the cell infoldings have opened out, as it were, to provide more cell membrane to cover the posterior surface of the iris. They still interdigitate complexly with each other. The thickness of the basement membrane is not measured in pupillary dilation. It may perhaps also thin out when the pupil is extremely constricted. In some of our scanning electron micrographs of pupillary constriction, the basement membrane appears to have been thinned out to reveal the underlying cell interdigitations. The basement membrane may have been artificially removed in the specimen preparation rather than being thinned out as a result of pupillary constriction.

Within the substance of the posterior epithelial cells, certain changes are also seen, especially with respect to the shape and orientation of the nuclei and the disposition of the intracellular filaments. In pupillary dilation, the nucleus of the posterior epithelial cell has a very irregular outline. The length of the nucleus lies along the long axis of

the cells. In electron micrographs, the nuclei show deep indentations of the nuclear membrane. These indentations are occupied by tongues of uniformly staining cytoplasm devoid of cell organelles. There is much dense heterochromatin associated with the nuclear envelope and within the nuclear substance itself. It seems that there is always a clear space between the outer and inner nuclear membranes. This is seen in well-fixed material so that this space is unlikely to be artifactual. From pupillary dilation to pupillary constriction, the nuclei of the posterior epithelial cells change in shape and in orientation. The nuclei become oval or elongated in shape and are oriented parallel to the posterior surface of the iris, as are the epithelial cells. The nuclear outline is relatively smooth. There are very rarely any nuclear indentations. The nucleus of the posterior epithelial cells, though large in relation to the cell volume, is highly pliable and readily alters its shape from pupillary dilation to pupillary constriction. Perhaps, the looseness of the enveloping nuclear membranes facilitates, or at least, does not hinder, the alteration of the nuclear configuration.

The usual cell organelles are found in the cytoplasm. In addition, there are bundles of intracellular filaments. Such filaments have not been described by other investigators. It is not known whether they are present only in the posterior epithelial cells of the rat iris. The other cell organelles are not oriented in any particular way within the cell cytoplasm. These intracellular filaments, however, change in their orientation within the cell in miosis and mydriasis. The filaments are usually found quite close to the nucleus. This may not be of any significance since there is only a thin rim of cytoplasm around the nucleus and the rest of the cell volume is made up of cell infoldings. In pupillary dilation, bundles of filaments seem to cascade around the nucleus, primarily along the lateral

and posterior regions. There are occasionally some filaments in the anterior cytoplasm. These bundles of filaments may be quite large as is fortuitously seen in a cross-section. On the whole, the filaments appear to form a loose 'hammock' around the nucleus. In pupillary constriction, the filaments are mainly found in the posterior half of the epithelial cells. The filaments run in bundles along the long axes of the cells and parallel to the posterior surface of the iris. They form an intermeshing network, or they may form a discrete bundle. Occasionally, a bundle of filaments appear to attach to dense areas of the cell membranes. We can only speculate as to the possible nature and function of these filaments. Perhaps, they are only a structural component of the epithelial cells. They may have a certain elasticity to them. But any elasticity would seem to be functional only if the filaments are anchored in position at certain points. We cannot tell whether this is so from our results. There is only a hint that some of the filaments are attached to the cell membranes.

The posterior epithelium and dilator are intimately related to each other. In pupillary dilation, the dilator muscle layer is readily seen both with the light and transmission electron microscopes. It may be as thick as the epithelial cells are high. In mydriasis, the boundary between the posterior epithelium and the dilator is not easily traced. The posterior epithelial cells interdigitate with the dilator cells in different planes so that the extent of each cell is not well-delineated. In miosis, however, the boundary between the two layers is a relatively smooth, undulating line. Microvillous processes from both layers interdigitate loosely with large gaps in between the interdigitations. The two layers are bound together by cell junctions. Tight junctions are the most common, although some junctions occasionally show some semblance to desmosomes, but these are rare. In the excursions of the iris, these cell junctions would play a

role in maintaining the integrity of the two layers in their relationships to each other.

The individual dilator cells also interdigitate extensively with each other. This accounts for the light microscopic observation that the boundaries of the dilator cells are not apparent. In pupillary dilation, the dilator muscle cells interdigitate with each other in a more three dimensional manner whereas in pupillary constriction, there appear to be fewer interdigitations which relate with each other in a somewhat two dimensional fashion.

The nuclei of the dilator muscle cells behave similarly to those of the posterior epithelial cells. In pupillary dilation, the nuclear outlines of the dilator muscle cells are more highly indented than those of the posterior epithelial cells. Again, cytoplasmic protrusions occupy the depths of the indentations. In pupillary constriction, the nuclear outlines smooth out, generally to give a long cigar-shaped nucleus. Occasionally, a deep indentation is seen but this would be oriented parallel to the posterior surface of the iris.

The most striking changes are seen along the boundary zone between the dilator muscle fibers and the stroma. In pupillary dilation, numerous arborescent processes are observed all along the boundary zone. They stain very darkly with toluidine blue and are also very electron-dense. The sarcoplasm is highly concentrated here so that the myofilaments are not readily visible in electron micrographs. These dilator processes may be simple protrusions of the dilator into the stroma, or they may arise from a hillock of dilator material, in which case, they branch and spread out in a fan-like manner. Occasionally not only dilator processes but groups of dilator cells encroach on the stroma as muscle spurs. This is not as commonly seen as the dilator processes. Since the dilator layer is attached

to the posterior epithelium, which is itself a bulky structure, in extreme pupillary dilation, there is not enough room to accommodate all of the dilator cells. Parts of the cells have to be squeezed outwards and this is only possible anteriorly into the relatively loose stroma. It would be interesting to speculate that there are certain well-defined regions all along the stromal surfaces of the dilator muscle cells where dilator processes can protrude outwards. The dilator processes do appear to be well structured components. They do not seem to have been formed haphazardly. Perhaps, the dilator cells are arranged in contractile units. At least in the sphincter, there is some indication that the muscle cells are arranged in functional groups (Hogan, Alvarado and Weddell, 1971). This may be the case with the dilator as well. The force of contraction for each functional unit of cells may be concentrated at certain points. Here, the dilator material would bulge out as a hillock from which would arise a series of profusely branched dilator processes.

In pupillary constriction, the dilator layer is barely visible light microscopically. The nuclei are large and occupy most of the cell volume. The contractile portion is confined to a small strip along the boundary zone with the stroma, which appears as a dense line with the light microscope. With the electron microscope, the myofilaments are quite readily discernible as the sarcoplasm is not as dense as in pupillary dilation. The stromal surface of the dilator is usually smooth. The dilator processes, so numerous and striking in pupillary dilation, are missing in pupillary constriction. Very rarely, there may be a few short dilator processes jutting into the stroma. The whole anterior surface of the dilator layer has been flattened. In pupillary constriction, all of the dilator cells can be accommodated within one layer and there is no necessity for protrusions of the dilator to occur.

A basement membrane lines the anterior surface of the dilator cells. As with the basement membrane of the posterior epithelial cells, the basement membrane of the dilator drapes loosely the dilator and its processes in pupillary dilation. The basement membrane has to be relatively malleable so that the dilator processes can protrude into the stroma without any difficulty. In pupillary constriction, the basement membrane appears to be qualitatively thinner, almost as if it had been stretched.

The various types of cells in the stroma are not identified as the main concern here is in the overall arrangement of the cells and connective tissue framework in the stroma. In mydriasis, the stromal cells and their processes appear to be disposed in vertical columns extending from the anterior surface of the iris to the boundary zone with the dilator. The spaces in between the cells accentuate this impression of vertical linearity. Only the processes of the stromal cells, but not the nuclei, come relatively close to the dilator processes, so as not to impede the conformational changes of the dilator. The basement membrane of the dilator cells and the surrounding collagen fibers around the dilator processes impart a 'halo' to them which is free of cells. In miosis, the stromal cells and the associated intercellular spaces are no longer perpendicular but parallel to the posterior surface of the iris. With a special stain for collagen, Mallory's Trichrome Stain, it is seen that the collagen network within the iris stroma follows closely the changes in orientation of the stromal cells. The collagen fibers are parallel to the posterior surface of the iris in pupillary constriction but they are perpendicular to the posterior surface of the iris in pupillary dilation. Thus between the two extremes of pupillary size, the stromal components apparently go through a shift in position. In some studies (Tousimis and Fine, 1959b) the collagen appears to surround the stromal cells and to delineate out various intrastromal tissue channels

which are quite different and separate from the readily identifiable blood vessels (Gregersen, 1958a, 1958b, 1959b; Francois, Neetens and Collette, 1960). Perhaps the whole collagen network in the stroma is interconnected and it serves to maintain the stromal cells in their positions relative to each other, as well as to keep open the tissue spaces or channels. Thus the functional integrity of the stroma is maintained through the extreme changes in structural orientation that occur in pupillary dilation and constriction.

B. A Scanning Electron Microscopic Study of the Rat Iris in Pupillary Dilation and Constriction

1. Comments on the Methodology

The scanning electron microscope is used in our present investigation to study the ultrastructure of the anterior and posterior surfaces of the rat iris. In light microscopic studies of meridionally sectioned rat iris, it is observed that the posterior surface of the iris is covered by a layer of posterior epithelial cells while the anterior surface of the iris is covered by a layer of squamous endothelial cells. Our main interest is focussed on the configurations of these surface cells and their relationships to each other. In addition, we know that the iris is not a static structure but it is constantly changing its total surface area, thus regulating the size of the pupil, in response to external changing light conditions. In our light microscopic studies, the shapes of the posterior epithelial cells likewise change to accommodate to these excursions of the iris tissue in pupillary dilation and constriction. It is not possible, however, to tell whether the shapes of the anterior endothelial cells alter with pupillary dilation and constriction although it is observed that the overall contour of the anterior iridial surface is different when the iris is

in pupillary dilation than when it is in pupillary constriction. Thus it was deemed useful to employ the scanning electron microscope as a tool to compare the ultrastructural features of the anterior and posterior surfaces of the rat iris in pupillary dilation and constriction, as such a study has not been carried out before.

The pupil constricts or dilates not only in response to light conditions but also in response to certain drugs, the miotics and mydriatics. In our studies a mixture of phenylephrine hydrochloride and cyclopentolate, and echothiophate iodide are regularly used to respectively dilate and constrict the pupils. The eyes are removed immediately and fixed by immersion. Hansson (1970), in his investigations of the ultrastructure of the surface of the rat iris, mentions that by using fixation by immersion, the tissues become twisted and curled. However, this problem is not encountered in our studies. The iris tissues do occasionally curl a little, but this is the result of later steps in the preparative procedures, namely, during the drying of the tissues. Such slight twisting or curling of the iris tissue does not affect the morphological characteristics as examined with the scanning electron microscope.

The iris tissues are fixed in glutaraldehyde, or in glutaraldehyde followed by post-fixation in osmium tetroxide. It was thought that perhaps the incorporation of a heavy metal into the tissue itself would give better resolution, or allow the tissues to stand up better under the electron beam. In our investigation, there appears to be no difference between the tissues that are fixed in glutaraldehyde alone and those which had a double fixation.

Freeze-drying is commonly used to prepare biological specimens for examination with the scanning electron microscope. Freeze-drying is the method used by Hansson (1970). However, freeze-drying has certain dis-

advantages. During the initial freezing process, ice-crystals inevitably form and disrupt the tissue components. Ice-crystal artifacts can be reduced, but not eliminated entirely, by numerous methods (Boyde and Wood, 1969). To circumvent these problems, two other methods have been introduced for drying the tissues, the camphene method (Watters and Buck, 1971) and the critical point drying method (Boyde and Wood, 1969; Smith and Finke, 1972). These two methods are utilised in our studies to prepare the tissues for scanning electron microscopy. Both are as effective in giving a consistent image of the posterior and anterior surfaces of the rat iris in pupillary dilation and constriction.

Following the camphene method (Watters and Buck, 1971), the fixed tissues are first dehydrated in a graded alcohol series and then transferred into propylene oxide. The tissue is infiltrated with liquid camphene at 45°C. When brought to room temperature, the camphene solidifies. It is sublimed from the tissue in a vacuum evaporator leaving a dried specimen. The advantage of this method is that a fluid-air interphase is eliminated and with it any damages or distortion which can occur as a result of surface tension forces.

The critical point drying method (Boyde and Wood, 1969; Smith and Finke, 1972; Hollenberg and Erikson, 1973) of dehydration of tissues gives consistently good results with the scanning electron microscope. The water content in the tissues is replaced in a series of steps by alcohol, amyl acetate and liquid carbon dioxide. The tissues, immersed in liquid carbon dioxide, is enclosed in a pressurized 'bomb' which is then heated to above the critical point of the carbon dioxide. At this critical point, the liquid and gaseous phases of the carbon dioxide are in equilibrium, so that there is no surface tension on the tissues as the carbon dioxide is removed from the specimens. Distortions that occur as a result of phase boundaries

are eliminated.

Certain differences are perceived between the results of Hansson's studies (1970) and our studies. These will be alluded to in the appropriate parts of the discussion. The most likely explanation lies in the differences in methodology for drying of the iris tissues.

The differences in morphology of the posterior and anterior surfaces of the rat iris in pupillary dilation and constriction is of special interest to us. Observations made with the scanning electron microscope are correlated with observations made with the light microscope on plastic sections of the rat iris which have been cut meridionally.

The changes in the contours of both the posterior and anterior surfaces of the iris from pupillary dilation to pupillary constriction are quite dramatic. A hint of these surface changes is given by studying meridional sections of the rat iris with the light microscope. However, the total view of the surfaces is not possible, due partly to the limited extent of the sampling of the tissues in sections and also partly to the fact that the posterior and anterior surfaces are only seen in two dimensions. The scanning electron microscope has these two advantages over the light microscope. A large area of the tissue surface can be viewed concurrently and what is perceived is a three dimensional rather than a two dimensional image. In addition, new information on the surface structures of the iris is also obtained.

2. The Posterior Surface of the Iris in Pupillary Dilation and Constriction

In pupillary dilation, the posterior surface of the rat iris, as viewed light microscopically, is lined by a row of large, peg-like, columnar epithelial cells from the root of the iris to the peripheral extent of the

sphincter. The heights of the epithelial cells vary. The grooves in between the individual posterior epithelial cells also vary in width and depth depending on the degree of pupillary dilation. In general, the posterior surface has a highly convoluted appearance up to the periphery of the sphincter. The posterior surface of the sphincter is lined by a squamous epithelium and this presents a smooth outline. With the light microscope, it is not possible to visualise the basement membrane which covers the posterior surfaces of all the epithelial cells but with the transmission electron microscope, the basement membrane is readily apparent.

A scanning electron micrograph of the posterior surface of the iris in pupillary dilation shows that it is deeply grooved. Circumferentially oriented ridges are separated by grooves over most of the iris posterior surface. These ridges represent rows of posterior epithelial cells. The height of these ridges vary corresponding to the differing heights of the epithelial cells in varying degrees of pupillary dilation. The whole posterior surface is covered by an amorphous basement membrane so that the outlines between individual cells in a single epithelial ridge are not discernible. The posterior epithelial cells are arranged in circumferential but not concentric rows. Neighboring rows of cells may come together, or a row of cells may bifurcate to form two rows which then blend with other rows of cells. This aspect of the arrangement of the posterior epithelial cells in the whole iris is not evident in light microscopic studies. According to Fine and Yanoff (1972), in the human iris, the posterior epithelial cells within any one ridge are arranged in a staggered fashion. This observation is also most probably applicable to the rat posterior epithelium, although we are not able to make this observation ourselves owing to the superficial layer of basement membrane covering the posterior iridial surface. This staggered arrangement of the epithelial cells in each row would explain what

is seen in a meridional section of the rat iris. The widths of the epithelial cells are not always similar and the nuclei are not regularly observed within each and every one of the cells from the root of the iris to the periphery of the sphincter.

Light microscopic studies show that the individual posterior epithelial cells have a highly irregular outline as a result of variously sized cytoplasmic cell processes. With the scanning electron microscope, it is observed that the basement membrane follows the overall contours of the cells but it does not extend into the cells to cover all of the smaller cytoplasmic cell processes. This is verified by the scanning electron microscopic picture. The basement membrane is uneven over the ridge of epithelial cells and shows bumps and corrugations. The basement membrane extends deeply into the grooves between the epithelial ridges but it does not extend into the row of cells, that is, into each cell.

At the pupillary margin, the posterior surface of the iris in pupillary dilation is relatively smooth as viewed with the scanning electron microscope. It corresponds to the flattened epithelium which lines the posterior surface of the sphincter.

Quite distinct differences are seen between the posterior surface of the iris in pupillary dilation and the posterior surface of the iris in pupillary constriction. Unlike the extensively ridged iridial posterior surface seen in pupillary dilation, in pupillary constriction the posterior surface is relatively smooth. The appearance of the iridial posterior surface varies slightly depending on whether the pupil is partially or extremely constricted.

In less extreme states of pupillary constriction, referred to here as simply pupillary constriction (refer to the results), the posterior epithelial cells are low cuboidal, as seen with the light microscope. They may

bulge a little, thus giving the posterior surface a very slightly scalloped appearance in meridional sections. In a scanning electron micrograph, the iris posterior surface in pupillary constriction exhibits slight corrugations and bulges. Each bulge presumably represents a single posterior epithelial cell which is polygonal or slightly spindle-shaped, as has been previously observed (Hansson, 1970). The circumferential arrangement of the epithelial cells in rows in pupillary dilation is lost over most of the posterior surface of the iris. Occasionally, the epithelial cells may appear to be disposed in just perceptible circumferential rows but the deep grooves and high ridges have been flattened out as the iris goes from pupillary dilation to pupillary constriction. The basement membrane still covers the posterior cell surface and obscures the outlines of the individual cells. However, the limits of each cell can be vaguely made out by assuming that each bulge represents the nucleus of a posterior epithelial cell. A line drawn midway between the nuclei would give us an idea of the size of the posterior epithelial cells at a particular stage of pupillary constriction. Such measurements of cell size are not made in our investigation. Occasionally the basement membrane is thinned out or removed so that what appears to be cytoplasmic processes of the epithelial cells are seen.

In extreme pupillary constriction, the posterior epithelial cells are flattened, in light microscopic studies. The posterior surface appears as a smooth line. The sphincter consists of a bundle of closely packed muscle cells located at the pupillary margin. In scanning electron microscopy, the posterior iridial surface is smooth and quite non-descript over most of the iris, except for a small zone around the pupil. This difference is not observed in plastic sections probably because of the sampling size. The posterior epithelial cells are completely flattened out. Only slight variations in the contours of the posterior surface suggest that the

posterior surface is actually made up of numerous epithelial cells.

Around the pupillary edge, the sphincter muscle is gathered very tightly together as a series of overlapping humps. These are covered by a smooth surface layer, the basement membrane, which overlies the flat cells lining the posterior surface of the sphincter. Peripheral to the sphincter region, the posterior epithelial cells are arranged in rows. Unlike in pupillary dilation, these rows are oriented radially rather than circumferentially. Thus, the posterior epithelial cells are capable of being lined up either circumferentially (in pupillary dilation), or radially (in pupillary constriction). There are also ridges of epithelial cells separated by grooves of varying depths and widths. The epithelial ridges also bifurcate, taper down and blend with adjacent ridges. Nearer the pupil, the epithelial cells are packed closer together but as one moves peripherally the grooves and ridges become shallower and lower, respectively, until the posterior surface is seen as a smooth sheet. Within this zone of epithelial ridges and grooves, large bulbous structures are seen. They may be puffed up or partially collapsed. They are of differing sizes and shapes and are covered with a basement membrane similar to that covering the rest of the iris surface. It is suggested that these bulbous structures represent posterior epithelial cells which have been crowded out of their normal position because of the extreme pupillary constriction. The equivalent in meridional sections has not been observed.

In addition to what has been presented, two new features of the posterior surface of the rat iris, which have only been made visible by scanning electron microscopy, are described here.

At the periphery of the rat iris near the ciliary body there are numerous, large, radial processes connecting the ciliary body to the iris. Thus, they are named here as ciliary-iris processes. The number of these

ciliary-iris processes varies from one iris to the next. They are of slightly differing lengths in an individual iris. They act, as it were, to anchor the iris to the ciliary body and thus stabilising the root of the iris during the extensive excursions of the iris in pupillary dilation and constriction. In pupillary constriction, the posterior epithelium and basement membrane covering the ciliary-iris process is similar to what is observed over the rest of the iridial posterior surface. However, in pupillary dilation, the posterior epithelial cells over the ciliary-iris process do not form ridges or grooves, which are very prominently seen over the rest of the iris posterior surface. Instead, there is a break in the continuity of the epithelial ridges over the body of the ciliary-iris process. At the pupillary end of the ciliary-iris process where it dives deeply into the iris tissue itself, the other epithelial ridges skirt around it, as though the ciliary-iris process were a relatively solid obstruction.

Superficial to the posterior surface of the iris are capillaries which radiate from the pupillary margin to the region of the ciliary body. These capillaries vary in number and size in different irises. Unfortunately, the beginning and termination of these blood vessels are not well defined. At the pupillary margin, the blood vessel appears to have come from the iris stroma. It then turns around at the pupillary border and traverses the posterior surface of the iris. We speculate that these are connected to the iris stromal capillaries and that they are functional in the adult, otherwise they would have atrophied. Possibly, they are remnants of the fetal circulatory system.

3. The Anterior Surface of the Iris in Pupillary Dilation and Constriction

The anterior surface of the adult iris is covered by an anterior

endothelium with intervening crypts and pores (Vrabec, 1952; Gregersen, 1958a, 1958b, 1959, 1961; Coulombre, 1961; Klika and Kloucek, 1962; Purtscher, 1962; Newsome and Loewenfeld, 1971). However, the nature of these large-sized crypts and smaller-sized pores can only be adequately studied by scanning electron microscopy.

In meridional sections, it is seen that the contour of the anterior iridial surface in pupillary dilation is highly scalloped due to large iris stromal blood vessels which bulge out anteriorly. The larger blood vessels are present in most of the iris stroma whereas capillary-sized blood vessels are predominant over the sphincter region. Using the scanning electron microscope on the rat iris in pupillary dilation, it is seen that the anterior iridial surface is indeed covered by a mass of blood vessels of varying sizes which are closely packed together in a generally circumferential direction. The blood vessels show different configurations. They may bifurcate or branch, they may join, they may travel superficially or dive deep into the iris stroma. In the region around the pupillary margin, the blood vessels are smaller and do not bulge as much anteriorly. These represent the capillary system over the sphincter. In extreme pupillary dilation, this capillary region becomes telescoped into the more peripheral region so that it is no longer apparent.

The morphology of the anterior surface of the rat iris and the course of the blood vessels is best studied when the pupil is constricted, and with scanning electron microscopy. In the constricted pupillary condition, the blood vessels are spread out and the anterior endothelial cells are well exposed and the morphology of the iris crypts and pores are displayed to their best advantage. Some crypts, though, are visible in the pupillary region in the dilated pupil.

The iris blood vessels are believed to pursue a slight corkscrew

course in the iris (Hogan, Alvarado and Weddell, 1972). In our investigation, it appears that the larger blood vessels are arranged in a more planar fashion, much like the pleats of an accordion. They zig-zag from the periphery of the iris to the pupillary margin. Branches are given off at each external bend of the parent blood vessel and these change direction to go deep into the iris stroma. At the pupillary margin, the capillaries are more tortuous and inter-relate with each other in a complicated pattern. At times, these capillaries form arcades which hug the pupillary margin itself. Some blood vessels seem to go over the pupillary edge. Perhaps, such blood vessels do indeed turn posteriorly to traverse the posterior surface of the iris to the ciliary region.

The detailed anterior surface structure of the rat iris is seen in high magnification scanning electron micrographs. In our investigation, the morphology of the anterior endothelial cells and of the iridic crypts and pores is much more well-defined and seen in finer detail than that presented by Hansson (1970). The structural differences observed are probably a result of the difference in methodology employed in preparing the iris tissues for scanning electron microscopy, especially with regards to the drying process. Hansson (1970) used fixed or unfixed tissues which are frozen in isopentan-propane and then freeze-dried. The tissues prepared by this method are very susceptible to ice crystal and phase-boundary artifacts. Distortions then generally occur. In our case, the tissues are dried by the camphene (Watters and Buck, 1971) and critical point drying (Boyde and Wood, 1969; Smith and Finke, 1972) methods, which have been proven to be superior to the freeze-drying method for preserving the ultrastructural details of tissues for examination with the scanning electron microscope.

The anterior surface of the rat iris is covered incompletely by a

layer of endothelial cells, substantiating what has been previously observed (Vrabec, 1952). The anterior endothelial cells are variously polygonal in shape. The oval nucleus is located approximately in the center of each anterior endothelial cell and bulges slightly into the anterior chamber. The rat anterior endothelial cells do not show microvilli in our study, unlike what is described by Hansson (1970). In fact, the anterior endothelial cell surface is quite smooth. There are occasionally some blebs on the cell surface which in no way resemble microvilli. Intercellular gaps occur in between the anterior endothelial cells but these gaps are bridged by numerous cytoplasmic processes of the surrounding endothelial cells. The resulting defects are probably equivalent to the iris pores of light microscopy. In addition, there are larger holes or crypts, in contrast to the smaller pores. The crypts are of varying sizes and are found throughout the anterior surface of the iris. A number of crypts may be grouped together at a specific location, oftentimes around a blood vessel. The crypts are variously round or oval in shape with well-defined borders. The crypt is not an open hole. The openings are spanned by cytoplasmic processes of the anterior endothelial cells and of the underlying stromal cells, and by fibrillar material, presumably, belonging to the collagen connective tissue network in the iris stroma.

C. A Light Microscopic Study of the Development of the Rat Iris Using Toluidine Blue Stained Plastic Sections

The developing rat iris, like that of the adult, is very susceptible to the effects of chemical fixation. This is particularly evident in the posterior epithelium. Different fixatives combined with different buffers were tried but all apparently still produce some degree of tissue disruption.

In this study, the development of both the iris and the ciliary

body are observed as they are so closely associated. The bulky immature ciliary body, through a process of mitotic division, differentiation and reorganisation of the cells, is transformed into a series of delicate, finger-like ciliary processes. The iris grows in length and in complexity. The anterior neuroectodermal layer develops into the sphincter and dilator muscles by a gradual process. All this takes place in the latter pre-natal days and during the first two weeks after birth, so that by the end of the second week the adult form is attained. The rate of growth and differentiation of the ciliary body and iris varies from one rat to the next and between different litters of rats.

Mitotic figures are often encountered in all parts of the developing rat iris and ciliary body. There do not appear to be foci of cell division. Cell division is still taking place in the posterior epithelium of the iris and in the developing sphincter as late as two weeks after birth. According to Imaizumi and Kuwabara (1971) the neuroepithelium ceases to divide actively by the 18th pre-natal day. This is not upheld by our observations.

At 19 days fetal, the iris and ciliary body are initially co-extensive with each other and with the retina. The ciliary body is seen only as a mere bulge interposed between the retina and the iris. However, cytologically the ciliary epithelium can be demarcated from the retina by virtue of its staining characteristics with toluidine blue. At 19 days fetal, the ciliary epithelium consists of a thick, stratified mass of cells, contributing to the bulkiness of the ciliary body. At term, the ciliary body as a whole becomes more well delineated. In the first few days after birth, the beginnings of the division of the ciliary body into ciliary processes are observed. The ciliary epithelial cells alter both in their arrangement and form. Instead of a stratified mass of cells, there is now usually a single layer of cells which are elongated and closely packed to-

gether. As ciliary processes become distinguishable, through the folding of both the ciliary epithelium and the ciliary pigment epithelium, the ciliary epithelial cells at the tips of the developing ciliary processes are higher than those at the bases of the processes. From the 5th to the 10th days after birth the ciliary processes grow in length. The ciliary epithelial cells differ in their appearance according to their locations along the ciliary processes. At the tips of the developing ciliary processes, the cells are high columnar but they are lower along the sides of the processes.

Concomitant with the changes seen in the ciliary epithelium are the changes in the ciliary pigment epithelium. In the pre-natal eye, the ciliary pigment epithelium is a light staining layer of high columnar cells. Its posterior surface closely follows the contours of the ciliary epithelium whereas its anterior surface is molded by the blood vessels and cells of the ciliary stroma. In development, the height of the epithelium is decreased. As well, extensive folding of the pigment epithelium occurs as ciliary processes are being formed. In fact, the foldings of the ciliary pigment epithelium always precedes and are more extensive than the foldings of the ciliary epithelium. The troughs in between the folds of the pigment epithelium are filled with blood vessels and some stromal cells.

By the 14th or 15th post-natal day, the ciliary processes are long and finger-like. Each ciliary process consists of two closely adherent layers, the ciliary epithelium and pigment epithelium making a hair-pin loop enclosing many capillaries and a few stromal cells within the loop. It is essentially a vascular structure. The blood vessels at the tip of the ciliary process may expand to give a knob-like appearance to the tip of the ciliary process.

The iris is relatively undeveloped at birth. It is short and stubby. In our observations, most of the growth in length of the iris occurs prim-

arily after the 5th post-natal day. This growth is probably the result of both mitotic division and the postulated stretching of the epithelial layers (Imaizumi and Kuwabara, 1971). The ratio of the bulk of the developing sphincter to the total length of the iris is a good indication of the rate of growth. The bulk of the sphincter remains relatively constant although the number of cells in the sphincter increases with growth. Initially, the sphincter makes up half to one third of the iris length at 19 days fetal, but in the first few days after birth, it makes up only one quarter or one fifth of the iris length.

The posterior and anterior epithelia are neuroectodermal in origin. The posterior epithelium retains its epithelial character while the anterior epithelium differentiates into smooth muscle, namely, the sphincter, and into myoepithelium, namely, the dilator.

The posterior epithelium in the pre-natal iris is not of uniform height, being columnar near to the ciliary junction and gradually decreasing in height to the peripheral extent of the developing sphincter. Gradually, with development, the posterior surface of the iris becomes lined by a layer of brick-like cells. The posterior surface of the iris is smooth until relatively late in development. This is more clearly visualised in scanning electron micrgoraphs of the posterior surface of the developing iris. The posterior epithelial cells are formed into rows separated from each other so as to impart a regularly scalloped appearance to the posterior surface of the iris.

The development of the anterior epithelium has been an area of much interest. It has been debated whether the sphincter muscle, in particular, is really of neuroectodermal rather than of mesodermal origin. Our observations corroborate those of other investigators that both the sphincter and dilator muscles arise through differentiation of the anterior epithelium

(Imaizumi and Kuwabara, 1971; Lai, 1972a, 1972b; Tamura and Smelser, 1973). By 19 days fetal, the developing sphincter is seen as a distinct knob-like mass of elongated, centripetally directed cells at the pupillary tip of the anterior epithelium. The cells are relatively large and closely packed together. The developing sphincter is distinctly separated from the iris stroma by an intercellular space. In terms of its stainability with toluidine blue, these cells do not stain differently from those of the rest of the anterior epithelium. By 4 days post-natal, the number of cells have increased. Small intercellular spaces appear in between the developing muscle cells giving the sphincter a crackled glass effect. The developing muscle cells show different staining intensities between individual cells, but the cytoplasm is uniformly dense within any one cell. This is the first indication, light microscopically, that these cells are cytologically different from those of the rest of the anterior epithelium. Presumably, the bundles of myofilaments stain more intensely with toluidine blue. With the transmission electron microscope, myofilaments are observed in the developing muscle cells as early as 19 days fetal (Lai, 1972a), or at the latest by term (Imaizumi and Kuwabara, 1971). However, the toluidine blue stain for light microscopy is probably not able to differentiate out the myofilaments till they are present in large amounts. Within the developing sphincter, the cells are in different stages of differentiation. Some cells are still dividing while others have acquired a full complement of myofilaments. This mosaic characteristic of the developing sphincter is not as readily apparent in electron microscopic studies. With development, there is a decrease in the diameter of the individual cells, as has been previously observed (Lai, 1972a), so that by the second week after birth the sphincter consists of a mass of small cells. They are usually quite closely packed together although small intercellular spaces are found in between the cells.

In addition, groups of muscle cells seem to associate together and be separated from other muscle groups by intercellular spaces. This is probably the beginning of the division of the sphincter into functional units. In the human sphincter, the muscle cells are linked in groups which function as a unit (Hogan, Alvarado and Weddell, 1971). Capillaries and nerves insinuate themselves in between the muscle cells during development.

The anterior epithelium is usually a uniformly high columnar epithelium throughout its extent. Up to the first few days after birth, the anterior epithelium is two to three times the height of the posterior epithelium. But by 10 days post-natal, the anterior epithelium appears to have been stretched out, as it were, so that it is about the same height of the posterior epithelium. The development of the muscular portion of the anterior epithelium, the dilator, is more tardy than that of the sphincter. In transmission electron microscopic studies, there is a discrepancy as to the first appearance of myofilaments in the developing dilator cells of rats. According to Lai (1972b) myofilaments appear as early as 20 days fetal, whereas, according to Imaizumi and Kuwabara (1971) myofilaments are seen two days after birth. In light microscopic studies, patches of densely staining material, presumably the myofilaments, are seen in the basal cytoplasm of the anterior epithelium only by 3 or 4 days post-natal. The dilator has begun to develop. By three weeks after birth, the dilator appears asa dense line at the stromal boundary, much as in the adult.

The stroma of the ciliary body and of the iris are considered together as they are in continuity. Stromal cells and blood vessels make up the stroma. Large blood vessels are often seen extending from the developing ciliary stroma to the iris. Their lumens are always patent and parallel to the length of the iris. In the ciliary body, some of the blood vessels and cells change direction to tumble into the clefts of the developing

ciliary processes. In both the iris and the ciliary body, the endothelium of the blood vessels is characteristically very intimately associated with the anterior surface of the dilator or pigment epithelium, respectively. There is no perceivable gap as seen with the light microscope, between the dilator cells or the ciliary pigment epithelium and the endothelium of the stromal blood vessels. Rarely are there cells interposed between the blood vessels and the dilator or ciliary pigment epithelium. In the adult iris, blood vessels are never seen in such close apposition with the dilator. Thus with development, there must be a slight reorientation of the stromal elements with respect to the posterior epithelial layers. In the adult eyes, the proximity of blood vessels or stromal cells to the dilator would be mechanically disadvantageous in the movements of the iris tissue and the conformational changes that occur at the anterior surface of the dilator. In the very young developing eyes, this would seem to be of no consequence. Although tests were not made to determine when the sphincter and dilator become functional, it would seem logical to postulate that this does not happen till quite late in development. Structurally, the sphincter and dilator muscles are relatively undeveloped at birth. Besides, the rat eyes are not opened or exposed to varying light conditions till about two weeks after birth, thus not necessitating an early functional iris.

The blood vessels and cells of the iris stroma do not end at the pupillary margin, as has been reported (Imaizumi and Kuwabara, 1971). In fact, in fetal and young post-natal eyes, the iris stroma always overhangs the pupillary tip of the iris. With development, the relative position of the stroma with respect to the iris shifts so that in the adult, the iris stroma does not extend beyond the pupillary margin. Capillaries of the iris stroma are often seen streaming out past the pupillary margin to form the pupillary membrane. These capillaries are strung together by connective

tissue strands. Some iris stromal capillaries also turn around at the pupillary margin and appear to be going posteriorly. Capillaries are quite often seen along the posterior surface of the iris. All these features of the fetal vasculature are better observed and appreciated in scanning electron micrographs and have been described. The possible inter-relationships of these blood vessels are speculated upon in scanning electron microscopic studies of the developing rat iris.

D. A Scanning Electron Microscopic Study of the Posterior Surface of the Developing Rat Iris

1. General

The scanning electron microscopic studies of the posterior surface of the fetal and young post-natal rat iris are carried out to complement the light microscopic studies of the development of the rat iris as observed on toluidine blue stained plastic sections which have been meridionally cut. Owing to the limited sampling size and the two dimensional nature of tissue sections, very little information can be gleaned from a study of tissue sections pertaining to the development of the surface configurations of the posterior epithelial cells, the development of the ciliary-iris processes, the topography of the pupillary membrane, and the relationships of other associated capillary networks to the pupillary membrane. A wealth of information is obtained from our scanning electron microscopic studies.

Much as it would have been desired to carry out a concomitant study on the anterior surface of the iris in fetal and young post-natal rats, to elucidate the development of the iridic crypts and pores, this is not done at the present time due to technical difficulties. The developing iris is short and fragile. In order to study the anterior surface of the iris, the

cornea would have to be dissected away. This was tried but without the support of the cornea, the iris becomes highly curled and distorted during specimen preparation and is not easily visualised by scanning electron microscopy.

2. The Posterior Surface of the Developing Rat Iris

In the fetal iris, the posterior surface is often covered by an amorphous sheet-like layer which is capable of being flaked off during the preparative procedures for scanning electron microscopy. Blood vessels are often embedded in this material. This amorphous layer may be removed completely to expose the basement membrane covering the posterior epithelial cells. In the first few days after birth, this layer is still present but is usually confined to the pupillary region. It may extend somewhat into the pupil itself. This amorphous layer is usually associated with the blood vessels found all along the pupillary region. We can only speculate on the nature of this amorphous layer. It is perhaps a modification of the basement membrane, which acts as an adhesive to hold the capillaries of the pupillary margin onto the posterior surface of the iris. Peripheral to this layer, the capillaries become free on the surface of the iris and can be dislocated from their positions during the handling of the tissue. Their original paths are suggested by impressions left on the posterior surface of the iris. In addition, this amorphous layer merges into the basement membrane covering the posterior epithelial cells. Very often, there is no sharp transition between this amorphous layer and the basement membrane. It almost seems as if the amorphous layer thins out and becomes the basement membrane of the posterior epithelial cells. By two weeks after birth, when most of the blood vessels at the pupillary margin are atrophying, much of this amorphous layer is no longer present. In parts, only the basement

membrane covering the squamous epithelial cells of the sphincter region is visible.

The basement membrane always covers the posterior surface of the epithelial cells. It is so very closely related with the epithelial cells that it is rarely removed during specimen preparation. The surface characteristics of the basement membrane change with the development of the posterior epithelial cells. In fetal eyes, the posterior surface of the iris is smooth. From scanning electron microscopy, it is not possible to say that the posterior surface of the iris is lined by a layer of epithelial cells, as is readily seen in light microscopic studies. The pupillary region is usually smoother than the peripheral region where there might be a few irregularities on the posterior surface of the iris. The basement membrane is also smooth. Occasionally, there is a mass of fine filamentous material on the iris surface. The source and nature of these filaments is not known.

In the first four days after birth, some contouring of the posterior surface of the iris is seen, primarily in the peripheral two thirds of the iris. These are the first superficial signs that the posterior surface of the iris is indeed made up of myriads of cells. In a low magnification scanning electron micrograph, the posterior surface of the iris has a speckled appearance. At a higher magnification, it is observed that the posterior surface of the iris consists of a mosaic of round to oval slight elevations separated by barely discernible depressions. The elevations represent the nuclei of the posterior epithelial cells. The basement membrane covering the posterior epithelial cells is still relatively smooth except for a few irregularities. The posterior surface of the pupillary region does not show these elevations and depressions but is smooth. The basement membrane may show a few fine striations. This is the epithelium

which underlies the developing sphincter muscle.

Up to 10 days post-natal, the posterior surface of the iris still appears smooth in light microscopic studies, although occasionally it may show some waviness. With scanning electron microscopy, certain changes in the contours of the posterior surface of the iris are observed. The individual posterior epithelial cells slowly become more well-defined. Initially, the posterior surface of the iris has a raspberry-like appearance. The posterior epithelial cells, covered by a smooth basement membrane, bulge a little more into the posterior chamber. With further development, the posterior epithelial cells become almost similar to those of the adult. They bulge out quite prominently. However, they are still not arranged in any particular way. Sometimes, a few grooves are seen parcelling out groups of posterior epithelial cells. This would be the beginnings of the epithelial ridges and grooves of the adult posterior surface of the iris. The basement membrane is no longer smooth but shows many crinkles. Some bulbous structures are seen scattered throughout the posterior surface of the iris. They remind us of the bulbous structures seen on the posterior surface of the adult rat iris when it is in extreme pupillary constriction. In light microscopic studies of the developing rat iris, it is observed that mitotic figures are often found in the posterior epithelium of young post-natal rats. Perhaps, these bulbous structures, as seen by scanning electron microscopy, represent foci of cell division in the posterior epithelium of the iris. These newly formed cells have not as yet been fitted, as it were, into the posterior epithelium as a whole. Thus, they bulge outwards much as the posterior epithelial cells of the adult iris are crowded out of position during extreme pupillary constriction.

By two weeks after birth, the posterior epithelium has practically attained the adult form as seen by both light and scanning electron micro-

scopy. The individual posterior epithelial cells are more readily apparent than in the adult iris. The epithelial cells are arranged in circumferential rows or ridges separated by grooves of varying widths and depths. The branching and merging of the epithelial ridges appears to be a little more profuse than in the adult. The basement membrane covers the posterior epithelial cells and dips deep into the grooves between the epithelial ridges. The basement membrane is highly crinkled as in the adult, suggesting the numerous cytoplasmic processes underneath, as revealed by light microscopy.

At this time too, the beginnings of the ciliary-iris processes are observed. As opposed to the circumferential posterior epithelial ridges, the ciliary-iris processes are much larger radial ridges located only towards the periphery of the iris. The developing ciliary-iris process interferes with the continuity of the posterior epithelial ridges, much as in the adult, but to a lesser extent.

3. The Peri-natal Vascular System of the Rat Iris

In our investigation of the developing rat iris, and in the investigations of Matsuo and Smelser (1971) of the rabbit, the pupillary membrane is present not only in the fetal animals but persists in young post-natal animals. Unlike in humans (Mann, 1964) where the pupillary membrane is a transient fetal structure, in the rabbit and in the rat, it is more correctly thought of as being a transient peri-natal structure. The pupillary membrane is presumably still functional after birth, as suggested by the large number of red blood cells that are always present in the lumens of the thin-walled capillaries.

In the rat, the pupillary membrane is detected as early as 17 days fetal when the iris is still relatively undeveloped. It may be present even earlier, but from our results we are not able to say when the pupillary

membrane begins to form. The pupillary membrane is still seen up to 10 days after birth. At this time, it is usually not as extensive as in younger eyes and is seen only in some specimens. By two weeks after birth, the pupillary membrane is virtually non-existent. Sometimes strands of fine connective tissue fibrils are left and they span the pupillary aperture like a cobweb. In our investigation, the pupillary membrane has not been observed to extend completely across the pupil, as has been observed with the slit lamp in the rabbit (Matsuo and Smelser, 1971). The pupillary membrane is a very fragile structure. The most likely explanation is technical. When the lens is removed, part of the pupillary membrane is most probably removed with it. Thus the topography of the rat pupillary membrane is not examined in its totality.

The rat pupillary membrane consists mainly of a series of thin-walled capillaries which issue from the iris stroma. The capillaries interconnect with each other by branches. They form a complex pattern of capillary arcades and loops. All of the capillaries are held together by a scaffolding of dense connective tissue fibers. The fibers are of varying diameters and they form a complicated meshwork around and in between the capillaries. These fibers are probably collagen fibers. They serve perhaps to hold the capillaries of the pupillary membrane in a relatively stable configuration with respect to each other and with respect to the lens so as to ensure that blood flow through the capillaries is not impeded. Since the lens has been removed, it is impossible to say whether these fibers also anchor the pupillary membrane capillaries to the capsule of the lens. In light microscopic studies of the developing rat iris, it is observed that the iris stromal blood vessels extend centrally into the pupil, being strung together by connective tissue strands. This is well substantiated by scanning electron microscopy. It is very rare to find cells associated with the

capillaries, or enmeshed in the connective tissue skeleton of the pupillary membrane. In transmission electron microscopic studies of the rabbit pupillary membrane (Matsuo and Smelser, 1971), fibroblasts and macrophages are also present. This may be a species difference.

The iris stromal capillaries, besides extending forward as the pupillary membrane, also turn around at the pupillary margin, change direction and travel towards the periphery of the iris. In light microscopic studies of the developing rat iris, an occasional iris stromal blood vessel is observed to encircle the pupillary border. These capillaries are closely applied to the posterior surface of the iris, especially in the pupillary region. In the pupillary region of fetal and young post-natal irises, these capillaries are embedded to a certain extent in an amorphous layer. More often than not, these capillaries are removed during specimen preparation. However, they leave quite readily visible impressions on the posterior surface of the iris which are especially concentrated in the pupillary region. The capillaries are radially oriented. They may branch and link up with each other.

At the region of the ciliary body, there is a large annular vessel. This is connected to the hyaloid system (Hollenberg and Dickson, 1971). From the optic nerve head, the hyaloid vessels separate into two groups. One group of vessels runs directly to the inner surface of the lens while the other group radiates out onto the inner surface of the retina. The hyaloid vessels on the retina are seen in some of our specimens. Much like the pupillary membrane, the hyaloid vessels on the retina form a complicated pattern. The capillaries are also held together by a connective tissue framework. These capillaries are connected to the annular vessel located in the region of the developing ciliary body.

Another system of capillaries arises from the other side of the

annular vessel. These capillaries have all the characteristics of the capillaries in the pupillary membrane. They are thin-walled so that the red blood cells show through the almost transparent walls. The capillaries form an interconnecting vascular network. The capillaries are enmeshed in a fibrillar connective tissue framework. Oftentimes, these capillaries from the annular vessel are seen extending into the region of the pupillary membrane and may even appear to be incorporated into some of the iridial vessels of the pupillary membrane. Rarely, a capillary from the annular vessel runs along the posterior surface of the iris and becomes continuous with a capillary which has come from the iris stroma and has made a turn at the pupillary margin.

From our rather limited scanning electron microscopic data on the peri-natal vasculature of the rat iris, we cannot say with absolute certainty that the hyaloid vessels are connected to the iris stromal vessels to any considerable extent. But the data we have at the present moment suggest that this is a possibility.

The rat pupillary membrane consists mainly of capillaries from the iris stroma. However, it may receive a contribution from the branches of the annular vessel.

In the developing rat iris, there are numerous capillaries traversing its posterior surface. Many of the iris stromal vessels turn at the pupillary margin to lie on the posterior surface of the iris. There are also branches of the annular vessel which lie on the posterior surface of the iris and seemingly extends into the pupillary membrane. The question here is whether the two systems of capillaries, from the iris stroma and from the annular vessel, are interconnected. There is a hint that at least some of the iris stromal capillaries are continuous with the capillaries of the annular vessel, thus linking the hyaloid vessels to the iris stromal

blood vessels. The capillaries from the annular vessel atrophy and disappear during development, much like the capillaries of the rest of the hyaloid system. The iris stromal capillaries on the posterior surface of the iris atrophy to varying degrees, so that even in the adult iris, there are a few such capillaries remaining on the posterior surface of the iris. It is problematic as to the termination of these capillaries, which is unfortunately not observable in our present investigation.

The pupillary membrane and the capillaries on the posterior surface of the iris are part of the transitory vascular system of the eye, which also includes the hyaloid vessels. The underlying mechanisms of the atrophy of these blood vessels is obscure. In the regressing pupillary membrane of the rabbit, Matsuo and Smelser (1971) hypothesise that cessation of blood flow through the pupillary membrane capillaries, due perhaps to mechanical and/or physiological factors, precedes the actual atrophy of the capillaries. This is followed by an accelerated synthetic activity of an increased number of fibroblasts in the pupillary membrane. The fibroblasts and the collagen fibrils degenerate, perhaps releasing some enzymes which have a destructive effect on the junctions of the endothelial cells. The atrophied capillaries are then slowly removed by phagocytic activity.

E. Horse-radish Peroxidase (HRP) Studies of the Iris in Fetal, Post-natal and Adult Rats

The iris is bathed in aqueous humor of the anterior and posterior chambers of the eye. The aqueous humor is not a static component but it is constantly being produced and removed from the eye. Its composition is presumably modified in its passage from the posterior to the anterior chamber by the surrounding tissues, namely, the iris and the lens (Kinsey and Palm, 1955). Morphologically, the iris tissue is intimately associated

with the surrounding aqueous humor. There is a system of tissue spaces within the iris stroma which communicates with the aqueous humor by means of the iridic crypts and pores (Vrabec, 1952; Gregersen, 1958a, 1958b, 1959, 1961; Klika and Kloucek, 1962; Purtscher, 1962; Coulombre, 1961; Newsome and Loewenfeld, 1971). Substances injected into the anterior chamber of the eye make their way into the iris stroma via these iridic crypts and pores (Gregersen, 1958a, 1958b, 1961). Conversely, it is conceivable that substances from the iris tissues also enter the anterior chamber in a similar manner. The iris may, to a certain extent, modify the composition of the aqueous humor. The permeability of the iris blood vessels to small molecules would give some indication of the role the iris might play in the modification of the aqueous humor in the eye.

Horse-radish Peroxidase (HRP) is a protein with a low molecular weight of about 40,000 and a molecular diameter of 44-47Å (Vegge, 1971b). It has a lower molecular weight than many serum proteins. The HRP tracer technique was originally developed by Karnovsky (1967) for studying the ultrastructural basis of capillary permeability. Since then, HRP has also been used as a tracer substance in other diverse studies (Webber and Blackbourn, 1971). The reaction product is easily visualised both with the light and electron microscopes as a brownish-black or electron-dense precipitate, respectively. The sites of the reaction precipitate indicate the location of the HRP. Thus the pathways of the HRP can be followed.

In the present investigation, the permeability of the ciliary and iris capillaries to HRP is studied in fetal, post-natal and adult rats at the light microscopic level. At different time intervals after an intravascular injection of HRP, the eyes are removed and fixed in glutaraldehyde. The eyes are then appropriately incubated to demonstrate the sites of deposition of the colored reaction precipitate.

In the adult rat, the ciliary capillaries are readily permeable to the HRP, as has also been observed in the mouse (Smith, 1971) and in the Vervet monkey (Vegge, 1971a). In the ciliary processes, the capillaries at the tip appear to be more permeable to the HRP than those towards the base of the ciliary processes, and in the main mass of the ciliary body. Once the HRP leaves the ciliary capillaries, it makes its way outwards towards the posterior chamber. The HRP reaction product is seen all along the stromal surfaces of the pigment epithelial cells. The precipitate also permeates in between the individual pigment cells and accumulates all along the boundary zone between the ciliary and the pigment epithelia. This region is sometimes known as the apical extracellular space (Smith, 1971). There is no precipitate in the intercellular spaces of the ciliary epithelial cells. There is thus an apparent barrier to the outward movement of the HRP from the apical extracellular space between the ciliary and pigment epithelia to the posterior chamber of the eye. This barrier is located at the apices of the ciliary epithelial cells. Electron microscopic studies, using HRP as an ultrastructural cytochemical tracer, have been performed on the mouse (Smith, 1971) and on the Vervet monkey (Vegge, 1971a). Their findings are consistent with our observations. At the electron microscopical level, it is seen that there is an epithelial blood-aqueous barrier to HRP in the ciliary processes (Vegge, 1971a). A tight junction is always found at the apex of the ciliary epithelial cells. This would structurally exclude the HRP reaction precipitate in the apical extracellular space from entering the intercellular spaces of the ciliary epithelial cells. In the formation of aqueous humor in the ciliary processes, a filtrate of blood leaves the ciliary capillaries to enter the ciliary stroma. However, the composition of the fluid in the ciliary stroma (the potential aqueous humor) and that in the posterior chamber is somewhat different, especially with

regards to the amount of proteins present. The aqueous humor has a low protein content whereas quite a considerable amount of proteins leave the ciliary capillaries (Vegge, 1971a). In our system, the ciliary capillaries are readily permeable to HRP, a low molecular weight protein. But not all of the proteins that leave the ciliary capillaries enter the posterior chamber and contribute to the make-up of the aqueous humor. There is a structural blood-aqueous barrier to proteins (in this case, HRP), which has been demonstrated to be located at the apices of the ciliary epithelial cells (Smith, 1971; Vegge, 1971a). Perhaps, not only proteins but other anions and non-electrolytes are also partially held back from entering the posterior chamber. It is suggested that the apical extracellular space may be the site where there is selective absorption of the proteins and other substances which readily filter out of the ciliary capillaries (Smith, 1971). Coupled with this, there may be some selective secretion as well.

In comparison with the ciliary capillaries, the iris capillaries of the adult rat, mouse (Smith, 1971) and Vervet monkey (Vegge, 1971b) are highly impermeable to HRP. In our studies, no HRP reaction precipitate is found within the iris tissue of adult rats. The HRP reaction product is frequently observable only within the lumens of the iris capillaries. In the adult rat, proteins, as exemplified by HRP, do not leave the iris capillaries to enter the iris stroma. In the iris, the blood-aqueous barrier to proteins lies in the walls of the iris blood vessels, whereas in the ciliary processes the blood-aqueous barrier lies not in the ciliary capillary walls but in the ciliary epithelium. In the adult rat, mouse and monkey, it appears that the iris capillaries do not at any rate contribute to the protein composition of the aqueous humor whereas the ciliary capillaries do, although to a very limited extent. In the mouse, Smith (1971) found that some of the HRP does reach the posterior chamber. But the HRP enters the

posterior chamber probably not by an extracellular route because of the tight junctions at the apices of the ciliary epithelial cells, but by an intracellular route, that is, by traversing the cytoplasm of the ciliary epithelial cells. These observations made with protein tracers do not, however, totally exclude the possibility that the iris capillaries might contribute other substances, which are not demonstrable histologically with our present day techniques, to the aqueous humor of the anterior chamber of the eye. This suggestion is elicited by the observation that there are two kinds of aqueous humor in the eye (Kinsey and Palm, 1955). The aqueous humor in the posterior chamber of the eye is different in composition from that in the anterior chamber. Since the fluid in the iris stroma is in open communication with the aqueous humor of the anterior chamber, it seems logical to postulate that there is an interchange of materials between the iris and the aqueous humor. One highly possible pathway where such an interchange of substances can take place is across the iris capillary walls. Kinsey and Palm (1955) found that thiocyanate and sodium enter the anterior chamber of the eye partly by flow from the posterior chamber and partly by diffusion through the iris vessels.

HRP tracer studies have not, as far as is known, been carried out in fetal and young post-natal rats. This is done in our present investigation to compare the permeability of the ciliary and iris capillaries to HRP in the adult and in the developing eye. As in the adults, in the fetal and post-natal rats, the HRP is introduced intravascularly, via the umbilical veins in fetal rats, and via the retro-orbital, jugular or saphenous veins in the post-natal rats. The eyes are removed, fixed and incubated for HRP in exactly the same manner as for the adult eyes.

In fetal eyes, the ciliary body is perceptible only as a bulge interposed between the retina and the developing iris. Even at this early

stage, the ciliary capillaries are very similar to those in the adult rat with respect to their permeability to HRP. The HRP leaves the ciliary capillaries and the reaction precipitate is localised on the stromal surfaces of the pigment epithelial cells, in between the individual pigment epithelial cells and along the space between the ciliary epithelium and the pigment epithelium. Although the ciliary body in the fetal rat is morphologically quite different from the fine, long, finger-like processes which make up the adult ciliary body, the locations of the reaction precipitate of HRP are qualitatively similar. As in the adult, the fetal ciliary capillaries are permeable to proteins, in this instance, HRP. The exit barrier to proteins into the posterior chamber is epithelial and lies at the apices of the ciliary epithelial cells. Very rarely, some HRP reaction product lines the posterior surface of the ciliary body. It is not known if this HRP reaction product has reached the posterior chamber following an extracellular or intracellular route across the ciliary epithelium. It could possibly be that in the fetal ciliary body, the epithelial blood-aqueous barrier is not as well established so that some HRP occasionally leaks through extracellularly.

In the ensuing days after birth, the ciliary body gradually develops and acquires the characteristics of the adult form, that is, the single bulky ciliary body is divided up into numerous long thin ciliary processes. Initially, the reaction precipitate is present in the locations mentioned in all of the ciliary body but as the ciliary processes develop, the reaction precipitate is concentrated at the tips of the ciliary processes, as in the adult.

The fetal rat iris is short and quite undeveloped when compared with the adult rat iris. However, very early in development, the component parts of the mature iris are easily perceptible. The fetal rat iris consists of a

posterior and anterior epithelium, a highly vascular stroma and a developing sphincter muscle bundle at the pupillary end. In the first two weeks after birth, the iris grows in length and histological changes occur within the iris tissue itself. The adult form is attained after this time. The iris capillaries in the fetal and young post-natal rats, up to 14-15 days after birth, are quite different from the iris capillaries in the adult rat in terms of their permeability to intravascularly injected HRP. Unlike the adult iris capillaries, these capillaries are readily permeable to HRP. In younger eyes, the iris blood vessels are always closely associated with the anterior epithelium, much as the ciliary capillaries are associated with the pigment epithelium. HRP reaction product is usually found along the stromal surfaces of the anterior epithelium, in between the anterior epithelial cells and between the anterior and posterior epithelia. The analogy with the observations on the ciliary body is unmistakeable. There is also some reaction precipitate in the stroma immediately adjacent to the anterior epithelium as well as some precipitate in amongst the cells of the developing sphincter. Thus in young eyes, both sets of capillaries, those of the ciliary body and those of the iris, are permeable to HRP. In the ciliary body, a blood-aqueous barrier exists at the ciliary epithelium, whereas in the young iris the blood-aqueous barrier exists at the posterior epithelium. However, it must be borne in mind that any HRP that permeates out of the iris capillaries into the iris tissue can conceivably enter the aqueous humor by an anterior route, that is, through the anterior surface of the iris. But it is not known in the rat whether the iridic crypts and pores are formed at this stage, so that an anterior endothelial barrier may exist. Thus, in young eyes, proteins readily leave the iris capillaries but to what extent these proteins contribute towards the composition of the aqueous humor is not known. Since the iris capillaries are capable of leaking out

proteins, other substances might leak out as well. The developing iris may be a partial source of aqueous humor in the eye.

The iris capillaries are only permeable to HRP up to about two weeks after birth. Then the capillaries become like those in the adult and are impermeable to HRP. There is a change in the permeability of the iris capillaries with developmental age.

Cotran and Karnovsky (1967) have cautioned us that HRP may artificially increase the permeability of small blood vessels and induce vascular leakage so that the observations with HRP tracer studies do not give a true indication of the actual permeability of the blood vessels under study. This increase in permeability is apparently due to the release of histamine. However, in our investigation, artificially increased permeability of the capillaries can be considered an unlikely possibility for the following reasons. In the adult rat, the ciliary capillaries are permeable to HRP whereas the iris capillaries are not. If histamine is released into the blood stream and capillary permeability is increased artificially, it seems highly unlikely that it should only be limited in its effect and be confined to the ciliary capillaries, and even then, only to those capillaries at the tip of the ciliary processes, and not affect the iris capillaries at all. In young eyes, both the ciliary and iris capillaries are permeable to HRP. But the iris capillary permeability is lost quite abruptly at about two weeks after birth whereas the ciliary capillary permeability is retained throughout life. The only variable here is the developmental age of the eyes. Again it does not seem likely that an artificially HRP-induced vascular leakage is age dependent for the iris and not for the ciliary body. For these reasons, we contend that in our present investigation, the permeability or impermeability of the capillaries to HRP is a true reflection of its natural condition.

V. CONCLUDING REMARKS

The present studies (1) have given us new information on the structure of the adult and developing rat iris, and (2) have shown us the dynamic, rapidly altering nature of the histology of the adult rat iris in pupillary dilation and constriction. These aspects of the iris will be mentioned in brief here as they have been adequately described in the appropriate sections of the discussion and summary of the results.

1. The new information on the structure of the iris is as follows:

(a) In the adult rat, varying numbers of blood vessels are present on the posterior surface of the iris, as observed on scanning electron micrographs. These come from the iris stroma, turn around at the pupillary margin and traverse the whole extent of the posterior surface of the iris to the region of the ciliary body. These blood vessels lie superficial to the posterior epithelium. These blood vessels are remnants of the fetal and early post-natal circulatory system associated with the development of the iris. In the developing iris, most of the blood vessels from the iris stroma extend centrally as the pupillary membrane. Other blood vessels turn around at the pupillary margin to lie on the posterior surface of the iris. At times, these blood vessels on the posterior surface of the iris appear to be connected to the hyaloid system via the large annular vessel. With development many of these blood vessels on the posterior surface of the iris regress, so that in the adult rat iris only a few such blood vessels are observed. A number of questions are immediately raised as to:

i. the function of these blood vessels on the posterior surface of the iris,

ii. the continuity of these vessels in the region of the ciliary body with the overall vascular system of the eye,

iii. the direction of blood flow in the vessels, and

iv. the extent of the presence of these blood vessels in other adult vertebrate eyes.

In the developing rat eye, both the blood vessels on the posterior surface of the iris and the blood vessels of the tunica vasculosa lentis may be important for the nutrition of the lens. However, in the adult, these blood vessels would represent the closest source of nutrients and also a means of removing metabolic waste products from the lens. The answers to the other questions have as yet to be explored.

(b) At the periphery of the adult iris in the region of the ciliary body there is a series of ciliary-iris processes. These are relatively large radial ridges, distinctly separate from but associated with the ciliary processes. They are high close to the ciliary processes but they then go deeply into the iris tissue more centrally. The ciliary-iris processes are covered by the posterior epithelial cells and the overlying basement membrane much like the rest of the posterior surface of the iris. However, the posterior epithelial cells over the ciliary-iris processes are lower than those lining the posterior surface of the iris. These ciliary-iris processes appear to be relatively solid structures. Possibly they serve to stabilize the root of the iris during the extensive excursions of the pupil in dilation and constriction.

In fetal and early post-natal eyes, the ciliary-iris processes are absent. At about two weeks after birth, the beginnings of the development of the ciliary-iris processes are observed. At first, they are relatively low radial ridges but these develop in height and bulk with age. It is significant that these ciliary-iris processes are only beginning to develop at two weeks after birth, that is at the time when the rat eyes open and presumably when the irises start to function. At present it is not known

whether such ciliary-iris processes are found in all vertebrate irises.

(c) Early light microscopic studies have shown that there are crypts and pores on the anterior surface of the iris allowing an intercommunication of the aqueous humor and the ground substance of the iris stroma (Vrabec, 1952; Gregersen, 1958a, 1958b, 1959, 1961; Coulombre, 1961; Klika and Kloucek, 1962; Purtscher, 1962; Newsome and Lowenfeld, 1971). However, the morphology of the iridic crypts and pores is not well established owing to the inherent limitations posed by the two dimensional nature of tissue sections. An initial attempt was made by Hansson (1970) who applied scanning electron microscopy to the study of the anterior surface of the iris. However, the tissues were inadequately preserved. In our studies we have been able to take advantage of the improvements in the preparative procedures for scanning electron microscopy.

The iridic crypts are oval or round, vary in size and have well defined borders. Occasionally cytoplasmic processes of the anterior endothelial cells making up the crypt border may span the opening of the crypt. A network of the cytoplasmic processes of the underlying stromal cells as well as a network of fine fibrillar material are seen occupying the crypt openings. The smaller iridic pores are formed as a result of intercellular gaps between the cells lining the anterior surface of the iris.

(d) In transmission electron micrographs of the posterior epithelial cells of the adult rat iris, the usual organelles are present, as has been observed by other invesitgators. In addition there are bundles of intracellular filaments which may be relatively large which have not been previously noted. The filaments are most often associated with the nucleus. Occasionally, a bundle of intracellular filaments appears to insert into dense areas of the basal plasma membranes of the posterior epithelial cells. Unlike the other cell organelles, the intracellular filaments appear to

change in orientation during pupillary dilation and constriction. In pupillary dilation, the filaments cascade around and form a hammock for the nucleus. In pupillary constriction, the filaments are oriented parallel to the length of the cells. From our present studies, it is not possible to say whether the filaments are contractile, or whether they may have some degree of elasticity, or whether they are just a structural component of the posterior epithelial cells.

(e) Light microscopic studies of the development of the rat iris have given us an overall view of the changes that take place in different parts of the iris tissue. A time sequence for the development of the iris is established, which can thus serve as a reference for developmental histochemical and physiological studies. The changes of the posterior and anterior neuro-ectodermal layers into the posterior epithelium and the dilator and sphincter muscles and the relationships of these layers to the stroma have been described and will not be reiterated.

(f) From scanning electron microscopic studies, it is observed that in the adult iris the posterior epithelial cells are arranged in rows. These may be circumferentially oriented as in pupillary dilation, or they may be radially oriented around the pupil as in pupillary constriction. The shape of the individual posterior epithelial cells and the arrangement of the cells with respect to one another contribute to the surface configuration of the posterior surface of the iris. The contour of the posterior surface of the adult iris is constantly changing with changes in pupillary size.

In the fetal iris, the individual posterior epithelial cells are not discernible in scanning electron micrographs. With development, the posterior epithelial cells first appear as a mosaic of little elevations on the iris surface. The cells are arranged in a haphazard fashion with re-

spect to each other. Later, the bulging outwards of the posterior epithelial cells becomes more prominent. The overlying basement membrane, initially smooth in fetal and early post-natal irises, becomes wrinkled. Grooves begin to appear in between groups and rows of posterior epithelial cells. By the end of the second week after birth, the posterior epithelial cells are arranged in rows which bifurcate, taper down or blend with adjacent rows of cells, as on the posterior surface of the adult rat iris. By two weeks after birth, the posterior epithelial cells are structurally adapted to accommodate the changes in total surface area of the iris in dilation and constriction.

(g) The architecture of the pupillary membrane is well illustrated in the scanning electron microscopic studies of the developing rat iris. Most of the blood vessels of the pupillary membrane come from the iris stroma, with some contribution, perhaps, from the hyaloid system. The blood vessels of the pupillary membrane form an interconnecting network supported by a fine fibrillar connective tissue meshwork. The pupillary membrane blood vessels have thin, almost transparent, walls. In the rat, the pupillary membrane is not only present in the fetal eye but also in the early post-natal eye.

(h) Changes in the permeability of the iris capillaries to an intravenously injected tracer substance, Horse-radish Peroxidase (HRP) occur with development. In fetal and young post-natal eyes, up to two weeks after birth, the iris capillaries are permeable to HRP, an average sized protein molecule. However, there is an epithelial barrier to the outflow of the reaction product for HRP to the posterior chamber of the eye, as in the ciliary processes (Vegge, 1971a). It is not known whether there is an outflow of the HRP reaction product directly to the anterior chamber through the anterior endothelial layer. In the adult rat iris, the iris

capillaries are impermeable to HRP.

2. These studies have given us an insight into the changes in the morphology of the iris associated with its function of dilating and constricting the pupil. These studies demonstrate clearly that the morphology of the iris must always be considered in relation to the pupillary size. The following features are characteristic of the iris in pupillary dilation:

(a) The posterior epithelial cells throughout the iris are arranged in circumferential rows or ridges with grooves of varying depths in between the epithelial ridges.

(b) The individual posterior epithelial cells are discretely separated from the adjacent cells. The nuclei show indentations of the nuclear envelope. The intracellular filaments form a loop around the nucleus.

(c) The dilator muscle layer is thick. Dilator hillocks and dilator processes are the prominent structures associated with pupillary dilation. The nuclei of the dilator muscle cells also show indentations.

(d) The stromal cells and the intercellular connective tissue network appear to be oriented in columns perpendicular to the posterior surface of the iris.

The following features are characteristic of the iris in pupillary constriction:

(a) Most of the posterior surface of the iris is smooth. The circumferential epithelial ridges seen in pupillary dilation have been flattened out. In extreme pupillary constriction, the posterior epithelial cells around the pupil are disposed radially. These decrease in height and peter out peripherally. Large, bulbous structures are sometimes seen in amongst the radial epithelial ridges. These may represent sites where eversions of the posterior epithelium occur.

(b) The posterior epithelial cells are generally low. They form a continuous layer over the posterior surface of the iris. The elongated nuclei have smooth nuclear outlines. The length of the nucleus lies along the length of the cells. The intracellular filaments are seen as longitudinal bundles parallel to the length of the cells and situated mainly in the posterior portions of the epithelial cells.

(c) The dilator muscle layer is low. Dilator hillocks are absent. There may be an occasional simple dilator process breaking the smooth, straight contour of the stromal surface of the dilator muscle layer. The nuclei generally have smooth outlines and they are oriented parallel to the posterior surface of the iris.

(d) The stromal components are oriented parallel to the posterior surface of the iris.

In our studies, chemical mediators were used to maintain the pupil in dilation or constriction. Similar changes in the histology of the iris in pupillary dilation and constriction are also observed without the intervention of drugs. It is uncertain however whether the very extreme form of pupillary constriction is obtainable as a natural response to intense light conditions.

3. A number of experiments may be carried out as an extension of the present study.

(a) Comparative studies: Vertebrates possess not only round but also slit and variously shaped pupils (see Introduction). Comparative scanning electron microscopic studies of the posterior surface of the iris would reveal the arrangement of the posterior epithelial cells during pupillary dilation and constriction, the presence or absence of blood vessels along the posterior surface of the iris, and the presence or absence

of the ciliary-iris processes. The blood vessels on the posterior surface of the iris come around the pupillary margin from the anterior iris stroma to traverse the posterior surface of the iris. At the present time it is not known how these blood vessels relate to the overall vascular system of the eye. Further scanning electron microscopic studies may reveal this. It would also be interesting to see if there is a correlation between the number of ciliary-iris processes present and the mobility of the iris tissue. This would give some indication as to the function of the ciliary-iris processes.

(b) Developmental studies: There is some indication that iridic crypts and pores are absent in young eyes and that the anterior surface of the iris is covered by a continuous layer of cells. With development, crypts and pores are formed (Vrabec, 1952). Scanning electron microscopic studies of the anterior surface of the developing iris would show how the formation of the crypts and pores take place.

Rat irises from their earliest fetal stages to the first few days after birth could be specifically prepared for scanning electron microscopy to study the formation and loss of the pupillary membrane.

(c) Functional studies: At birth the rat eyes are closed. The iris is immature histologically. By two weeks after birth, the rat eyes open and the iris has acquired the adult form. It would be interesting to find out when the iris can first respond to external chemical mediators and whether this response can be correlated with the histological structure of the iris.

(d) Permeability studies: With the light microscope it has been found that the iris capillaries in the developing iris are permeable to HRP, whereas those in the adult are not. Examination with the transmission electron microscope would reveal

i. the exit pathways of the HRP at the ultrastructural level, and

ii. the ultrastructure of the endothelial layer of the iris blood vessels both in the adult and in the developing rat iris.

In addition, other electron microscopic tracers, for example, ferritin and lanthanum, could also be used.

(e) Miscellaneous studies: In some vertebrates, for example, the newts, if the lens is removed, a new lens is formed from the posterior epithelial cells of the iris. In other vertebrates, for example, man, lens regeneration does not take place. A scanning electron microscopic study of the iris at different times after lens removal would elucidate the changes that occur in the iris tissue in response to lens extirpation.

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