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HETEROCHROMIA IRIDIS IN BUFFALOES

(With 5 Tables and 30 Figures)

By

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تباين اللون فى القزحية فى الجاموس

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أجريت هذه الدراسة على عدد ٥٩٣ حيوان بها تباين فى لون القزحية وكذلك على عدد ٤٥ عين طبيعية مستأصلة و ٨٥ عين مستأصلة بها تباين فى لون القزحية . وقد تمت مناقشة تقسيم تباين لون القزحية وكذلك الفحص الظاهرى والنسجى والتركيب الدقيق وكذلك التركيب المقطعى بالميكروسكوب الالكترونى فى هذه الدراسة . واتضح من النتائج أن نسبة تباين لون القزحية فى الجاموس كان ٧,٦٢٪ وكانت تحتوى على نوعين إما فى العينين أو فى عين واحدة وكان تباين لون القزحية فى الحالتين كاملا أو جزئيا . وقد تبين أن القزحية كانت صغيرة الحجم فى كل حالات تباين لون القزحية . وكان لون السطح الأمامى للقزحية الطبيعية مزدوج الالوان بينما كان فى الحالات التى بها تباين فى لون القزحية إما مزدوج الالوان أو ملونة بثلاثة أو أربعة الوان . وكان التركيب النسجى للقزحية الطبيعية والقزحية ذات التباين فى لونها متشابها فى التركيب ما عدا فى توزيع الصبغات .

SUMMARY

This study was carried out on a total number of 593 animals affected with heterochromia iridis, as well as 45 normal and 85 heterochromic enucleated eyeballs. Classification of heterochromia iridis, morphology of normal and heterochromic irises and histology, ultrastructure, and scanning electron microscopy were discussed in this study. The result of the present study indicated that, the incidence of heterochromia iridis in buffaloes was 7.62% including two types, bilateral and unilateral. Both of them were recorded in different forms of complete and partial heterochromia iridis. Iris hypoplasia was evident in all cases of heterochromia iridis. The color of the anterior

surface of normal iris was bicolored while in heterochromic iris was bicolored, tri-colored or tetracolored. The histological structure of the iris in normal and heterochromic cases was the same except in pigment distribution.

Key words: *Buffaloes - Eye - Heterochromia Iridis*

INTRODUCTION

Heterochromia iridis is one of the congenital affections of the iris. When multiple colors within one iris or between the two irides, the term heterochromia iridis is applicable (Vaughan, Cook and Asbury, 1967; Shively and Phemister, 1968; Vaughan and Asbury, 1974; Gelatt, 1981 and Gelatt, 1991). Heterochromia iridis in buffaloes was not stated in any of the available literatures.

The following study is dealing with heterochromia iridis in buffaloes taking in consideration its incidence, morphology, histology, ultrastructure and scanning electron microscopy in comparison with normal irises.

MATERIALS and METHODS

This study was carried out on a total number of 7782 buffaloes examined for presence of heterochromia iridis. A 593 animals were found affected with heterochromia iridis and were subjected to the present study. In addition 45 normal and 85 heterochromic enucleated eyeballs were used in this study. Eyeballs were collected from freshly slaughtered animals of both sexes and of different ages. Most of the collected eyeballs (40 normal and 80 heterochromic) were preserved in 10% formalin and the cornea was excised just before examination. The rest of eyeballs (5 normal and 5 heterochromic) were used for histological, ultrastructure and scanning electron microscopy. Irises were immediately excised from freshly enucleated eyeballs and specimens, 0.5 x 1.0 cm in diameter, were excised and immersed in Bouin's fluid for 24 hours, then dehydrated in graded alcohol series, cleared in methyl benzoate and embeded in paraplast. Serial sections were cut at 3-5 microns and stained with H & E for general histological examinatiion (Harris, 1898). For ultrastructure study

small tissue blocks were taken and fixed in paraformaldehyde glutaraldehyde as described by Karnovsky (1965). For scanning electron microscopy the specimens were fixed in paraformaldehyde glutaraldehyde solution, then washed in 0.1 M cacodylate buffer at 7.3, dehydrated in ascending graded ethanol, critical point-dried in liquid carbon dioxide, then examined using JSM-5400 LV scanning electron microscope.

The following points were discussed:

I - Classificatioin of heterochromia iridis:

a) Incidence of heterochromia iridis within 7782 examined buffaloes.
b) Number of bilateral and unilateral cases.
c) Number of eyeballs affected with complete and partial heterochromia iridis.
d) Different forms of bilateral and unilateral cases.
e) Different forms of partial heterochromia iridis taking in consideration the size of the affected segment.

II - Morphology of normal and heterochromic irises in buffaloes:

a) Shape of the eye pupil and its measurement.
b) Topography and color of the anterior and posterior surfaces of normal and heterochromic irises.
c) The total width of the iris as well as the width of the ciliary & pupillary zones.
d) The total iridial thickness and stromal thickness at the ciliary and pupillary zones.
e) Incidence, shape, length and thickness of the granula iridica.

III - Histology, ultrastructure and scanning electron microscopy of the irises:

a) Histological structure and ultrastructure of different layers of the irises.
b) Distribution of pigments in normal and heterochromic irises.
c) Scanning electron microscopy of the anterior and posterior surfaces of the normal and heterochromic irises.
d) Histology of granula iridica and its origin.

RESUTLS

I - Classification of heterochromia iridis: (Fig. 1 - 5)

a) Incidence of heterochromia iridis in buffaloes:
Heterochromia iridis was recorded in 593 animals out of 7782 with a percentage of incidence 7.62%.

b) Number of bilateral cases was 385 (64.92%) and unilateral cases was 208 (35.08%).

c) Number of affected eyeballs in bilateral cases was 770 (385 x 2) and in unilateral cases was 208 (208 x 1). The total number of affected eyeballs was 978. Number of eyeballs affected with partial heterochromia iridis was 436 (45%) and eyeballs affected with complete heterochromia iridis was 542 (55%).

d) Different forms of bilateral and unilateral heterochromia iridis were illustrated in Tables (1 & 2).

e) Different forms of partial heterochromia iridis according to the size of affected area were illustrated in Table (3)

II -Morphology of the normal and heterochromic irises: (Fig. 6, 7)

a) Shape of the eyepupil and its measurments:

The eyepupil in buffaloes in either normal or heterochromic eyeballs is elliptical in shape with slight constriction at the middle due to the presence of granula iridica giving the pupil dumbbell-shape appearance.

The average horizontal diameter of the eye pupil is 12.83 mm in normal iris and 14.95 mm in heterochromic iris. The average vertical diameter is 6.33 mm in normal iris and 7.1 mm in heterochromic iris. These results indicated that the eye pupil in average is wider in heterochromic irises than normal one.

b) Topography and color of the anterior and posterior surfaces of normal and heterochromic irises:

The normal iris is composed of central pupillary zone and peripheral ciliary zone. The demarcation between the two zones is a striking ridge encircling th pupillary zone in zig-zag fashion called the collarette. The surface of the ciliary zone is smoother than that of the pupillary zone. The latter appears thinner than the former. The edge of the pupil shows the pigmented ruff that is the extension of the posterior pigmented epithelium.

The color of the anterior surface of normal iris is bicoloured and ranges from brown to dark gray. The pupillary zone is usually darker than the ciliary zone. When the color of the ciliary zone is brown, the colour of the pupillary zone is dark brown and when the colour of the ciliary zone is gray, the color of the pupillary zone is dark gray.

The heterochromic irises are either complete or partial. In complete heterochromia iridis, the iris is bicolored and has a smooth wide white ciliary zone and relatively course, narrow bluish gray pupillary zone. The collarette appears as a zig-zag line between the two zones. The pigmented ruff appears black along the whole pupillary margin.

In partial heterochromia iridis the iris is either tricolored or tetracolored. The line of demarcation between different coloures is irrigular. The heterochromic segment may be present elsewhere along the whole circumference of the iris and differs according to its size. When the heterochromic patch is present at the ciliary zone only, the iris has three colors; white and brown (or gray) at the ciliary zone and dark brown (or dark gray) at the pupillary zone. When the heterochromic patch is present in the pupillary zone only, the iris has three colors, brown (or gray) at the ciliary zone and dark brown (or dark gray) and bluish gray colors at the pupillary zone. When the heterochromia iridis includes a full segment of the iris, the iris has four colors; brown (or gray) and white colors at the ciliary zone and dark brown (or dark gray) and bluish gray colors at the pupillary zone.

No difference can be detected in the topography and color of the posterior surface between the normal and heterochromic irises. In both cases, the posterior surface is heavily pigmented and appears black or dark brown in color. The ciliary zone contains radial folds while the pupillary zone has a smooth surface.

c) The total width of the iris as well as the width of the ciliary and pupillary zones at four direction in normal and heterochromic irises are illustrated in Table (4).

d) The total iridial thickness and stromal thickness at the ciliary and pupillary zones in normal and heterochromic irises are illustrated in Table (5).

The average total width and thickness of the iris in normal eyeballs are larger than that of heterochromic ones.

e) Incidence, shape, length and thickness of granula iridica in normal and heterochromic irises.

In normal eyeballs, granula iridica appears as black masses at the upper pupillary margin in all examined eyeballs and occupies 9.75 mm from the upper pupillary margin. They are 0.5 - 1.5 mm in thickness and not differentiated into separate masses. It is present in 30% of normal eyeballs at the lower pupillary margin. They occupy 3.51 mm from the lower pupillary margin and are smaller in size than that of the upper pupillary margin.

In heterochromic irises, granula iridica present in the upper pupillary margin of all examined eyeballs occupies an average of 9.1 mm from the pupillary margin. They are 0.5 - 1.5 mm in thickness and also could not be differentiated into separate masses. At the lower margin they are present in 30% of examined irises, and occupy 3.02 mm of the pupillary margin. They are smaller in size than that of the upper margin.

III- Histology, ultrastructure and scanning electron microscopy of the normal and heterochromic irises: (Fig. 8 - 30)

1- Histology and ultrastructure of the different layers of the iris:

The iris in buffalo is divided into three layers, rostral stromal or anterior border layer, stroma with sphincter muscle layer and posterior pigmented epithelium.

a) The rostral stromal layer:

In normal iris the rostral stromal or anterior border layer consists of two cell types; fibroblasts and melanocytes. The fibroblast cells form a discontinuous rostral layer and the cells are frequently separated from each other by a wide intercellular spaces. The melanocytes contain a considerable number of melanin granules. Most of these granules are rounded in shape and rarely ovoid.

In heterochromia iridis the anterior border layer is composed only of fibroblast cells while melanocytes are completely absent from this layer.

b) Stroma and sphincter muscle layer:

In normal iris the stroma comprises the bulk of the iridal substance and varies in thickness from one site to another but in general it is considerably thicker in the ciliary zone than in the pupillary zone. It consists mainly of collagenous fibers, melanocytes,

fibroblasts, blood vessels, nerves and smooth muscle fibers of the sphincter pupillae at the pupillary zone.

Considerable intercellular spaces are evident ultrastructurely. The fibroblast cells are scattered throughout the stroma however melanocytes are more prominent than fibroblasts but less in number and scattered all over the stroma. The melanocytes are branching cells whose processes are readily identifiable by melanin granules in their cytoplasm. The shape of melanin granules is almost rounded and rarely ovoid or elongated. The cytoplasm is considerably heavy laden by melanin granules.

Variable sized blood vessels are distributed throughout the stroma of the iris. They are larger in size near the basal portion of the iris than in the free portion.

The iridal stroma contains myelinated and nonmyelinated nerves. The myelinated nerve consists of a dense myelin sheath formed by Schwann cell that surrounds the axon inside the sheath. A few mitochondria appear inside the myelin space. The Schwann cell is surrounded by a basement membrane.

In heterochromic iris the stroma has the same structure as that of the normal iris except that melanocytes are absent or sometimes present as a solitary cell per one microscopic field.

c) Posterior pigmented epithelium:

In normal iris the posterior epithelium consists of a posterior pigmented layer of cells and an anterior layer of cells with a pigmented epithelial apical portion and a smooth muscle basilar portion. The smooth muscle portion of the anterior layer forms the dilator pupillae muscle of the iris that lies directly anterior to the pigmented layer of the iris. The apical portions of the anterior epithelial layer contain the cell nuclei and pigmented granules that are scattered in the muscular portion of the cell. The heavy pigmentation of the posterior epithelium precludes detailed examination with unbleached histological section.

In heterochromic iris the posterior pigmented epithelium has the same structure as that of the normal iris.

2- Distribution of pigments in normal and heterochromic irises:

a) Normal iris:

- Anterior border layer is heavily pigmented and the pigments are nearly equally distributed.

- The distribution of pigments in the stroma is less than the anterior border layer and at the same time increases toward the anterior border layer than toward the posterior epithelium of the iris.
- Posterior pigmented epithelium is heavily pigmented and the pigments are condensesd at the posterior 3/4 of the epithelium and are relatively less at the anterior 1/4 of the iridial epithelium.

b) Heterochromic iris:

- Anterior border layer; pigments are completely absent.
- Stroma; pigments are solitary in distribution.
- Posterior epithelium like that of the normal iris.

3- Scanning electron microscopy:

a) Anterior surface of the iris:

Scanning electron microscopy of the anterior surface of the iris in the normal and heterochromic irises in buffaloes is the same and revealed that, the anterior surface of the iris is not lined by a continuous layer of cells but contains small farrows or crypts of varying sizes. Crypts present in the ciliary zone are smaller in size than that of the pupillary zone. The latter has farrows of larger size and appears concentric with the periphery of the iris.

b) Posterior surface of the iris:

Scanning electron microscopy of the posterior surface of both normal and heterochromic irises is the same and revealed that the posterior pigmented epithelium is arranged in radial folds that extend to the base of the ciliary processes and end gradually at the junction between the ciliary and pupillary zones. The pigmented epithelial cells are oriented with their long-axis running circumferentially in the iris, thus giving rise to two patterns on the posterior surface. The radial folds are absent at the posterior surface of the pupillary zone.

4- The origin and histology of granula iridica in buffaloes:

Granula iridica originates only from the posterior pigmented epithelium of the iris. Histologically, they are cystic-like structures lined by a double layer of posterior pigmented epithelium. Cysts start to appear at the posterior surface of the pupillary zone and are smaller in size then they gradually increase in size towards the periphery of the granula iridica.

DISCUSSION

In the present study, the general incidence of heterochromia iridis within the examined animals was 7.62%. The available literatures lack any data about the incidence of heterochromia iridis in buffaloes and cattle. In equine and swine, Gelatt *et al.* (1973) stated that heterochromia iridis occured in 5-7%. In dogs, sporadic cases were stated by Shively and Phemister (1968). In addition, Huston *et al.* (1968) stated that heterochromia iridis in cattle was very rare. They added that a little number of calves suffering from changes in color of the iris from large number of dairy cattle were examined. From our point of view, the incidence of heterochromia iridis in buffaloes is the highest rate of incidence within domestic animals.

Two types of heterochromia iridis were stated; bilateral form in which both eyes are affected and unilateral form in which one eye is affected either right or left. The present study revealed that bilateral heterochromic case were nearly double in number that of unilateral cases. Bilateral cases were 64.92% while the unilateral cases were 35.08%. Again the bilateral cases were classified into bilateral complete, bilateral partial, bilateral right complete-left partial and bilateral right partial-left complete. The most common form was the bilateral complete (24.79%) while the bilateral partial occupies the second place (21.42%). Bilateral right complete-left partial occupies the third place and the last was the bilateral right partial-left complete (9.61% and 9.11% respectively).

Also unilateral heterochromic cases were classified into unilateral right complete, unilateral left complete, unilateral right partial and unilateral left partial. The most common one of unilateral cases was unilateral right complete (12.98%) then unilateral left complete (10.12%) and unilateral right partial occupies the third place (6.58%) and the last unilateral left partial (5.4%). When we are taking in consideration the affected eyeball faraway from the affected animals, complete heterochromia was diagnosed in 55% of total affected eyeballs (542 eyeballs), while partial heterochromia occurs in 45% (436 eyeballs). This means that complete form of heterochromia iridis either in bilateral cases or unilateral cases are more predominant than partial form.

In partial heterochromia, normal iris with small heterochromic patches was the most common form and represents 20.6% of all cases of partial heterochromia. The second prevalent form of partial heterochromia was found affecting 6/12 (half of the iris) and represents 16.74%. The affected segment was present elsewhere around the whole circumference of the iris. The third place was that the form occupies 1/3 or 1/4 of the iris. These segments of heterochromia may also occur at any place around the whole circumference of the iris. Heterochromic irises with normal patches were diagnosed in small number of cases with a percentage of 6.5% of total cases of partial heterochromia. Full pupillary zone heterochromia was detected very rare (4 eyeballs only with a percentage of 0.96%).

The shape of the eyepupil varies in domestic animals. Smythe (1958) stated that the shape of the pupil in ox and sheep is horizontally elliptical when constricted. He described also the shape of the pupil in different domestic animals except buffaloes. Gelatt (1991) described the shape of the pupil as oval in a horizontal plane in herbivores. The present study indicated that the shape of the pupil is nearly elliptical in a horizontal plane with a constriction at the middle due to presence of Granula iridica that give the pupil a dumbbell-shape appearance. In general, the shape of the pupil in buffaloes does not differ greatly from that stated in cattle by the aforementioned authors.

No statements were given in the available literatures concerning the dimensions of the eyepupil in buffaloes. Lavach (1990). mentioned that the horizontal diameter of the eyepupil in the horse is among 13 and 17 mm and the vertical diameter is among 3 and 5 mm. In the present study, the average horizontal diameter of the eyepupil in buffaloes is 12.83 mm in normal iris and 14.95 mm in heterochromic iris. The vertical diameter is 6.33 mm in normal iris and 7.1 mm in heterochromic iris. The large dimensions of the eyepupil in heterochromic irises than normal irises justify our opinion that the width of the iris in heterochromic eyeballs is shorter than that of normal eyeballs.

The anterior surface of the iris in normal and heterochromic irises is composed of a central pupillary zone and a peripheral ciliary zone. The demarcation between the two zones is in a form of a ridge encircling the pupil in a zig-zag fashion called the collarette. The

same results were stated by Hogan and Zimmerman (1968) and Gelatt (1991). The serrations of the collarette are more prominent adjacent to the medial and lateral canthi of the pupil. The ciliary zone is wider, lighter in color and smoother than that of the pupillary zone.

Iridal color varies considerably among various breeds and species of animals as well as among individuals. Gelatt (1991) stated that the color of the iris in domestic animals varies from dark brown to gold, blue and blue-green. The color of normal iris of buffaloes ranges from brown to dark gray. The color of the ciliary zone differs from that of the pupillary zone. The pupillary zone is usually darker than the ciliary zone. When the color of the ciliary zone is brown, the color of the pupillary zone is dark brown and when the color of the ciliary zone is gray, the color of the pupillary zone is dark gray. In complete heterochromia iridis, the iris is bicolored and has a smooth white ciliary zone and relatively coarse narrow and bluish-gray pupillary zone. The collarette again appears as a zig-zag line between the two zones and encircling the eyepupil. In spite of the bluish-gray coloration of the pupillary zone, the granula iridica still appeared as a black mass at the pupillary margin. Also the pigmented ruff appears black along the whole pupillary margin.

In partial heterochromia, the iris is tricolored or tetracolored. When the heterochromic patch is present either at the ciliary or pupillary zones, the iris has three colors and when the heterochromia iridis occupies a full segment of the iris the latter has four colors.

The available literatures lack any informations about different colors of the iris in heterochromia iridis in different domestic animals except the statement of Gelatt *et al.* (1973) who mentioned that the color of heterochromic iris in pigs is bicolored; white color at the ciliary zone and blue color at the pupillary zone.

The results of the present study indicated that the topography and color of the posterior surface of the iris is the same in normal and heterochromic irises. The posterior surface of the iris has no collarette but can be differentiated into ciliary and pupillary zones by the presence of radial folds at the former and its absence at the latter. The color of the posterior surface of the iris in normal and heterochromic eyeballs is black or dark brown. It is interesting to state here that, in spite of the white and bluish discoloration of the anterior surface of the iris in heterochromic eyeballs, its posterior surface remains black

and highly pigmented to keep the enterior of the eyeball in complete darkness and no light can pass except through the eyepupil.

Measurements of the total width and thickness of the iris in normal and heterochromic eyeballs indicated that heterochromic irises are smaller in width and lesser in thickness than normal irises. This fact supports our proposal that all cases of heterochromia iridis are accompanied by iris hypoplasia. Gelatt (1991) stated that heterochromia iridis as a component of multiple ocular anomalies may be accompanied by iridal anomalies such as iris hypoplasia and persistent pupillary membrane. In our study we can state that heterochromia iridis in buffaloes is accompanied by iris hypoplasia which can be explained by the smaller width and lesser thickness of the iris in heterochromic cases than in normal cases as well as the larger eyepupil in heterochromic cases than normal ones.

Many references stated that iridal granules occur along the pupillary margin of the iris in herbivores (Diesem, 1975; Dellmann and Brown, 1976; Anderson and Anderson, 1977; Gelatt, 1981; Martin and Anderson, 1981; Banks, 1993; and Barnett *et al.*, 1995). They mentioned that the Granula iridica of ruminants may be of equal size along the upper and lower margin of the pupil. Our results indicated that there is a great variation between the size, shape, length of the line of attachment and incidence of the granula iridica at the upper and lower pupillary margins. The incidence was 100% at the upper pupillary margin and 30% at the lower pupillary margin. In addition the size and length of the line of attachment are larger in normal iris than in heterochromic one.

The same authors stated that the granula iridica is a proliferative extension of the iridal stroma and posterior pigmented epithelium. The present results revealed that the Granula iridica is an extension of the posterior pigmented epithelium only.

Slatter (1990) mentioned that animals with horizontally elliptical pupil has a black mass suspended from the upper brim of the pupil to aid in control of light entering through constricted pupil. Our point of view, supports this statement as the sphincter muscles of the iris may act strongly in constricting the pupil at the lateral and medial canthi more than at the center of the pupil. Presence of Granula iridica at the upper and lower margins of the iris may aid in controlling the light entering the eye through the horizontally constricted eyepupil.

The iris in buffaloes, as irises in all other domestic animals, consists of three different layers; rostral stromal sheath (or anterior border layer), stroma with sphincter muscle layer and pigmented epithelium with dilator pupillae muscle layer.

The anterior border layer in normal iris, in buffaloes, consists of two types of cells; fibroblasts and melanocytes. In heterochromic iris it consists of one type of cell only (fibroblast cells). Rohen (1961) stated that in many species of mammals, the anterior border layer is continuously covered with endothelial cells. Dellmann and Brown (1976) also stated that the anterior border layer is lined with an endothelium that is continuous with the endothelium covering the trabicular network of the iris angle and consequently the corneal endothelium.

The second layer namely the stroma with sphincter muscle layer comprises the bulk of the iridial substance. It varies in thickness from site to site but condiderably thicker in the ciliary zone than in the pupillary zone.

In heterochromic irises, melanocytes in the stroma are nearly absent. Fibroblast cells are more prominent and extensively more in number than melanocytes. In normal iris, fibroblast cells are scattered throughout the stroma, while melanocytes are more prominent but less in number and scattered all over the stroma.

Smaller and larger vessels, myelinated and non-myelinated nerves and sphincter muscle of the iris were observed in the stromal layer in both normal and heterochromic irises.

The results of the present study are in agreement with that given by many authors that the posterior pigmented epithelial layer consists of two layers; posterior pigmented layer of cells, and anterior layer of cells with a pigmented epithelial apical portion and smooth muscle basilar portion. The structure of this layer is the same in normal and heterochromic irises.

The topography of the anterior and posterior surfaces of the iris is the same in normal and heterochromic irises when examined by scanning electron microscopy. Scanning electron microscopy of the iris is generally described by Gelatt (1991) who stated several features in dog as that stated in the present study in buffaloes.

LEGENDS OF FIGURES

Fig. 1: Bilateral heterochromia (A) and unilateral heterochromia (B) in buffaloes.

Fig. 2: Partial heterchromia iridis. Note that the heterochromic patch is located at the medial half of the iris.

Fig. 3: Complete heterochromia iridis. The ciliary zone is completely white and the pupillary zone is completely bluish gray.

Fig. 4: Different forms of partial heterochromia according to size of the affected area; 1/12 (A), 2/12 (B), 3/12 (C), 5/12 (D), 6/12 (E) and 7/12 (F).

Fig. 5: Heterochromic iris with normal patch (A) and normal iris with heterochromic patch (B).

Fig. 6: The collarette of heterochromic iris encircling the pupillary zone in zig-zag fashion. Note the pigmented ruff and granula iridica.

Fig. 7: Granula iridica in normal iris. At the upper margin (A), and the lower margin (B).

Fig. 8: Semithin section stained with toluidine blue showing the three layers of the normal iris. (A) Anterior border layer (S) Stroma and (PP) Posterior pigmented epithelium-dilator muscle layer. 40X.

Fig. 9: Semithin section stained with toluidine blue showing the anterior border layer of the normal iris. Fibroblast (F). Melanocyte (M). 1000 X.

Fig. 10: Transmission electron micrograph illustrating the anterior border layer of normal iris. Fibroblast cell (F). Melanocyte (M). Cell processes (CP). 8000 X.

Fig. 11: Semithin section stained with toluidine blue showing the anterior border layer of heterochromic iris. Fibroblast cell (F). Stroma (S). 1000 X.

Fig. 12: Transmission electron micrograph illustrating the anterior border layer of heterochromic iris. Fibroblast cell (F) Anterior chamber (AC). Nucleus (N). Rough endoplasmic reticulum (R). Stroma (S). 10000 X.

Fig. 13: Semithin section stained with toluidine blue showing the stroma (ciliary zone) of the normal iris. Melanocyte (M). Fibroblast (F). Collagen fibers (C). 1000 X.

Fig. 14: Semithin section stained with toluidine blue showing blood vessels of the stroma in normal iris. Small size blood vessel (A). Medium size blood vessel (B). Collagen fibers (C). 1000 X.

Fig. 15: Transmission electron micrograph illustrating stroma of normal iris with myelinated nerves. Schwann cell nucleus (S). Myelin sheath (M). Collagen fibers (C). 5000 X.

Fig. 16: Semithin section stained with toluidine blue showing stroma with myelinated and unmyelinated nerves in heterochromic iris. Myelinated nerve (MN). Nonmyelinated nerve (NN). Fibroblast cell (F). collagen fibers (C). 1000 X.

Fig. 17: Semithin section stained with toluidine blue showing the posterior pigmented epithelium of the normal iris at the ciliary zone. Posterior chamber (PC). Nucleus of the posterior pigmented cell layer (N1). Nucleus of the anterior pigmented myoepithelial cell layer (N2). 1000 X.

Fig. 18: Semithin section stained with toluidine blue showing the posterior pigmented epithelium of the normal iris at the pupillary zone. Posterior chamber (PC). Blood capillary (BC). Nucleus of the posterior pigmented epithelium (N). 1000X.

Fig. 19: Semithin section stained with toluidine blue showing the posterior pigmented epithelium and blood vessels at the stroma of heterochromic iris. Nucleus of the posterior layer of the iridial pigmented epithelium (N1). Nucleus of the anterior layer of the iridal pigmented epithelium (N2). Blood vessel (BV). Fibroblast cell (F). 1000 X.

Fig. 20: Transmission electron micrograph illustrating the posterior pigmented epithelium of heterochromic iris. Posterior chamber (PC). Nucleus (N). Melanin granules of variable sizes are usually rounded rarely ovoid (arrows). 5000 X.

Fig. 21: Scanning electron micrograph showing the anterior surface of the normal iris at the ciliary zone. Note the presence of crypts (arrows). 500 X.

Fig. 22: Scanning electron micrograph showing the anterior surface of the heterochromic iris at the ciliary zone. Note the presence of crypts (arrows). 500 X.

Fig. 23: Scanning electron micrograph showing the anterior surface of the normal iris at the pupillary zone.

Fig. 24: Scanning electron micrograph showing the anterior surface of the heterochromic iris at the pupillary zone.

Fig. 25: Scanning electron micrograph showing the posterior surface of the normal iris at the cilliary zone. Note the arrangement of posterior epithelium in the form of radial folds (arrows). 100 X.

Fig. 26: Scanning electron micrograph showing the posterior surface of the normal iris at the junction between ciliary and pupillary zones. 100 X.

Fig. 27: Scanning electron micrograph showing the posterior surface of the normal iris at the pupillary zone. Note the arrangement of posterior epithlium in the form of clusters (arrows). 100 X.

Fig. 28: Scanning electron micrograph showing the posterior surface of the heterochromic iris at the ciliary zone. Note the arrangement of posterior epithelium in the form of radial folds (arrows). 100 X.

Fig. 29: Scanning electron micrograph showing the posterior surface of the heterochromic iris at the junction between the ciliary and pupillary zones. 100X.

Fig. 30: Scanning electron micrograph showing the posterior surface of the heterochromic iris at the pupillary zone. Note the arrangement of posterior epithelium in the form of clusters (arrows). 100 X.

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Table 1: Illustrates different forms of bilateral heterochromia iridis in buffaloes.

Pattern of bilateral heterochromia	Number	%
Both complete	147	24.78
Both partial	127	21.42
Right complete-left partial	57	09.61
Right partial-left complete	54	09.11
Total	**385**	**64.92**

Table 2: Illustrates different forms of unilateral heterochromia iridis in buffaloes.

Pattern of unilateral heterochromia		Number	%
Complete	Right	77	12.98
	Left	60	10.12
Partial	Right	39	06.58
	Left	32	05.40
Total		**208**	**35.08**

Table 3: Illustrates different forms of partial heterochromia iridis according to size of affected area.

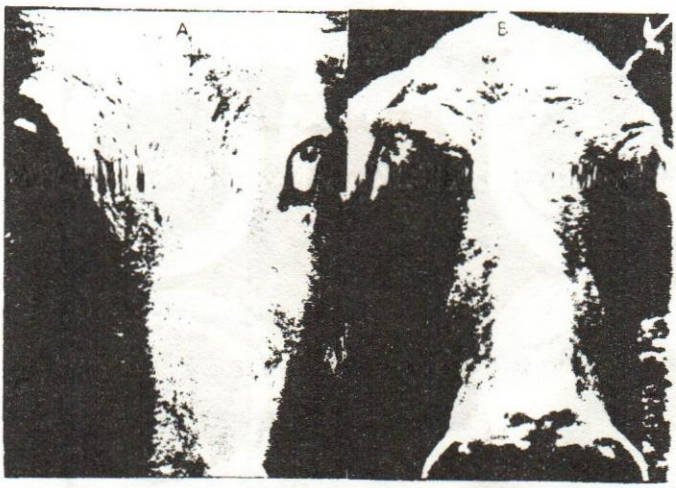
Size of affected area			Number	%
Less than 1/12 (Normal iris with heterochromic patch)	At ciliary zone only	26	86	19.72
	At pupillary zone only	38		
	At both zones	22		
1/12			35	08.03
2/12			26	05.96
3/12			48	11.02
4/12			48	11.02
5/12			42	09.63
6/12			73	16.74
7/12			18	04.13
8/12			11	02.52
9/12			10	02.29
10/12			8	01.83
11/12			0	00.00
More than 11/12 (heterochromic iris with normal patch)	At ciliary zone only	24	27	06.19
	At pupillary zone only	0		
	At both zones only	3		
Full pupillary zone heterochromia			4	00.92
Total			**436**	**100.00**

Table 4: Total width of the iris as well as the width of the ciliary and pupillary zones (at four directions) in normal and Heterochromic irises.

	Total width				Ciliary zone				Pupillary zone			
	Medial	Lateral	Superior	Inferior	Medial	Lateral	Superior	Inferior	Medial	Lateral	Superior	Inferior
Normal iris	9.35	12.35	11.20	12.00	6.2	9.50	6.80	7.50	3.15	2.85	4.4	4.50
Heterochromic iris	8.80	11.25	10.51	11.46	6.0	8.45	6.11	7.11	2.80	2.80	4.4	4.35
Difference between them	0.55	01.10	00.69	0.54	0.2	1.05	0.69	0.39	0.35	0.05	0.0	0.15

Table 5: Total iridial thickness and stromal thickness at the ciliary and pupillary zones in normal and Heterochromic iris (in μm).

	Total thickness of the iris		Stromal thickness of the iris	
	Ciliary zone	Pupillary zone	Ciliary zone	Pupillary zone
Normal iris	853 09	261.69	830.24	342.83
Heterochromic iris	803 00	238.50	768.10	190.60
Difference between them	050 09	023.19	062.14	052.23



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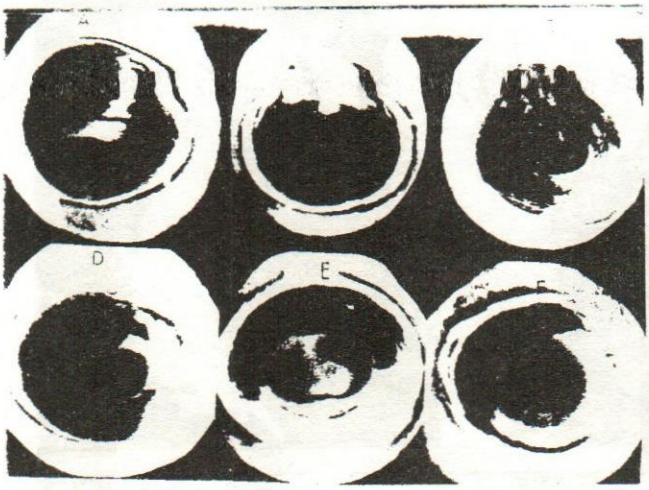

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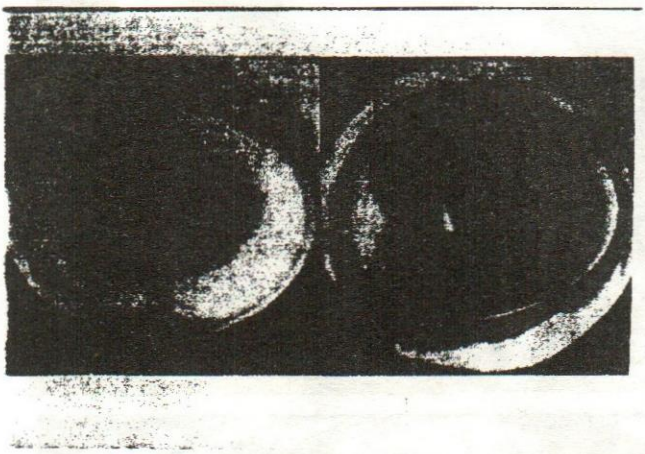

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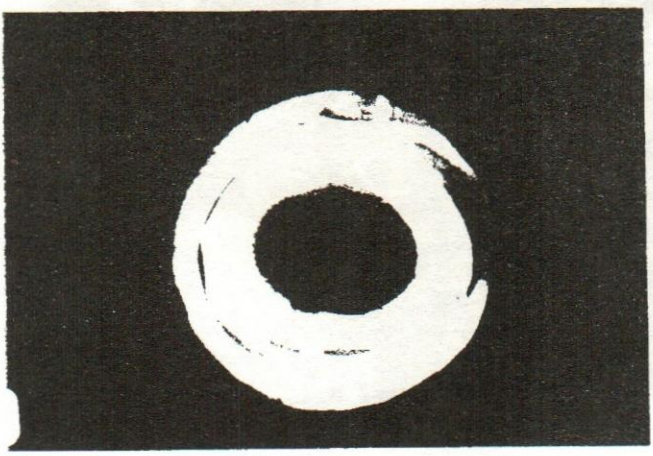

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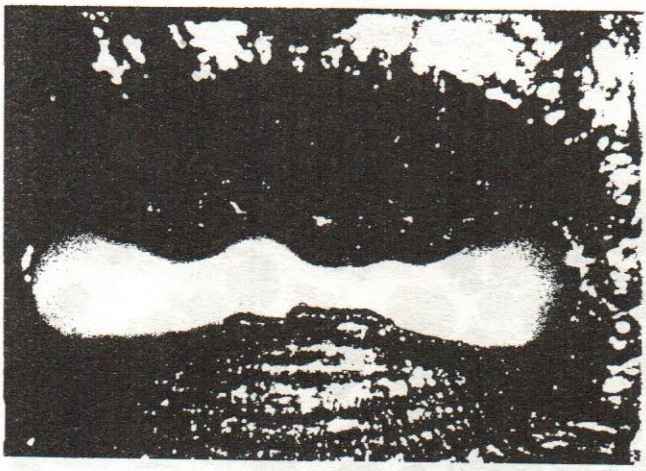

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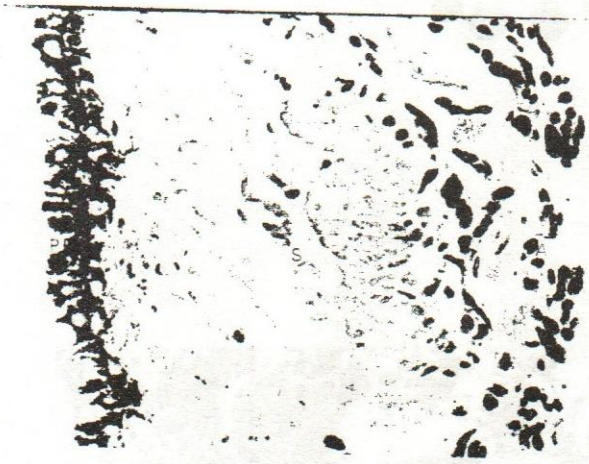

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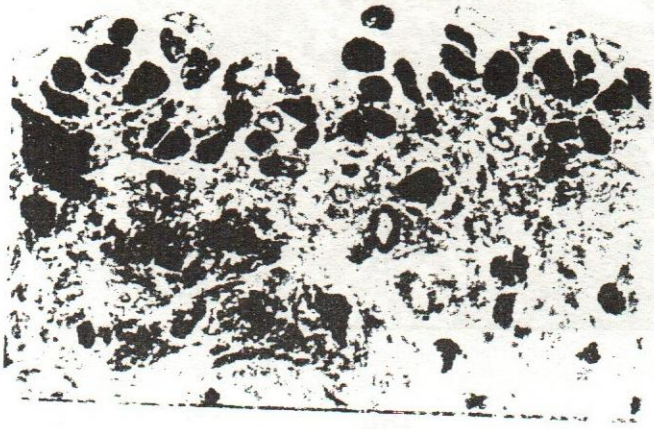

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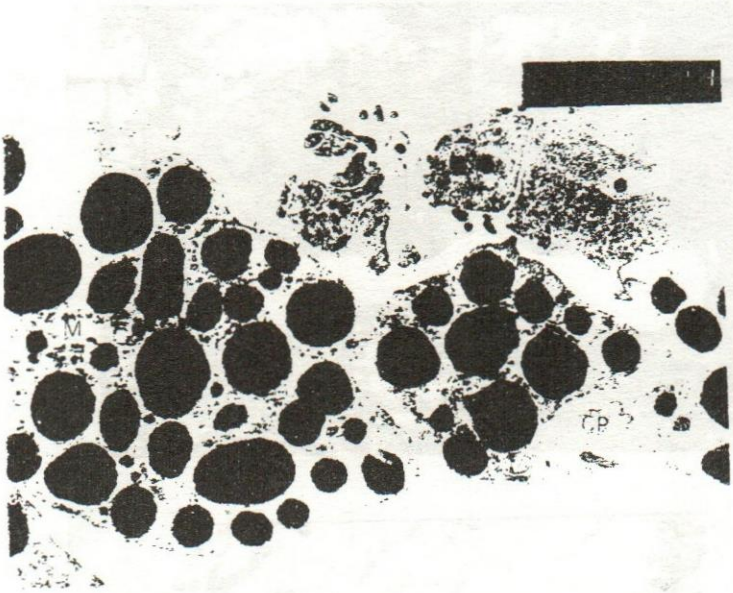

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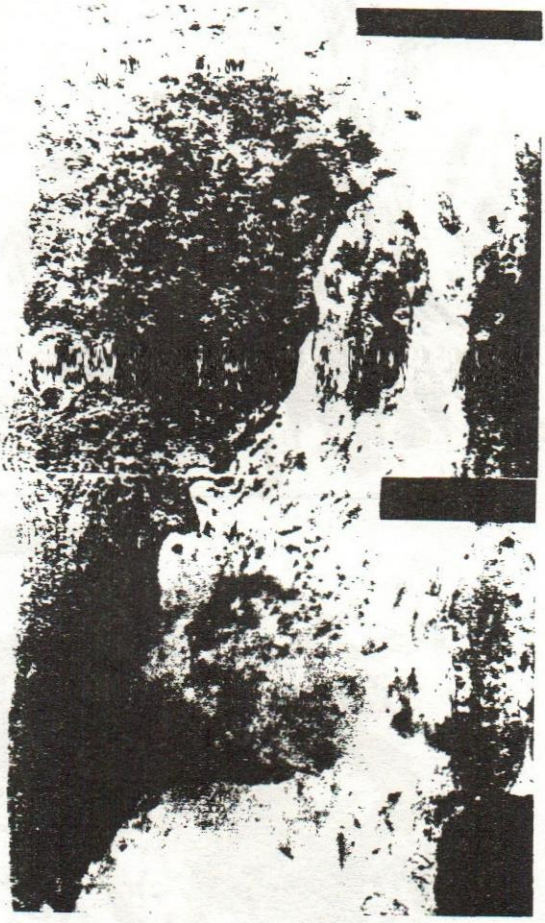

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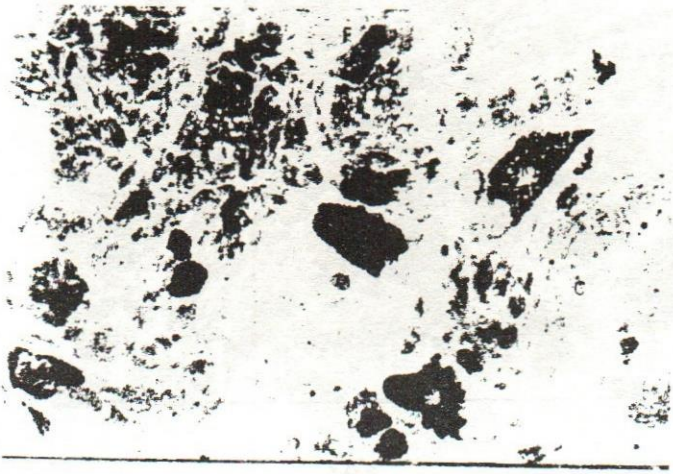

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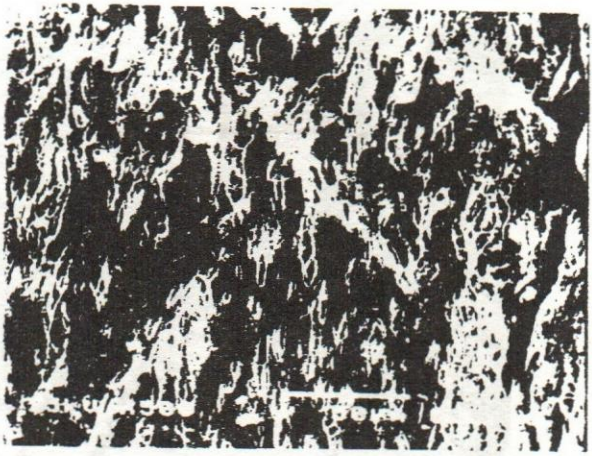

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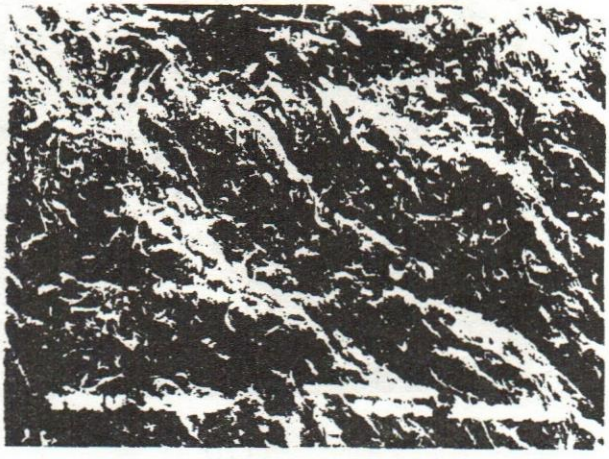

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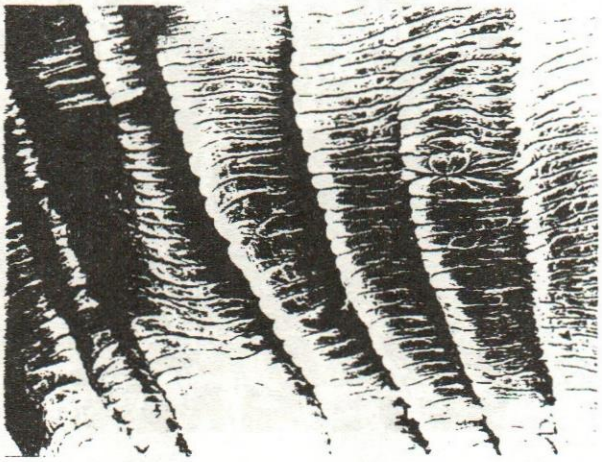

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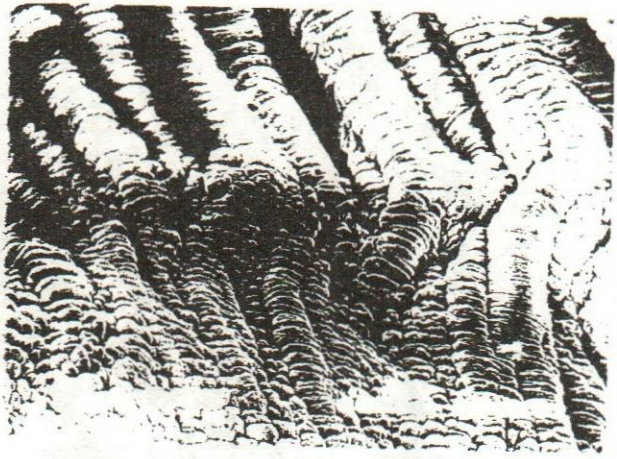

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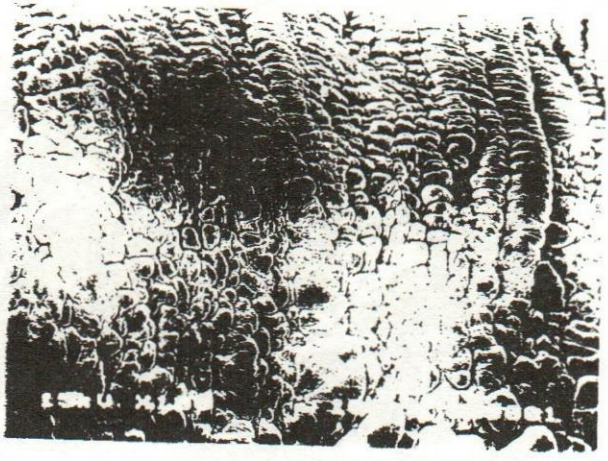

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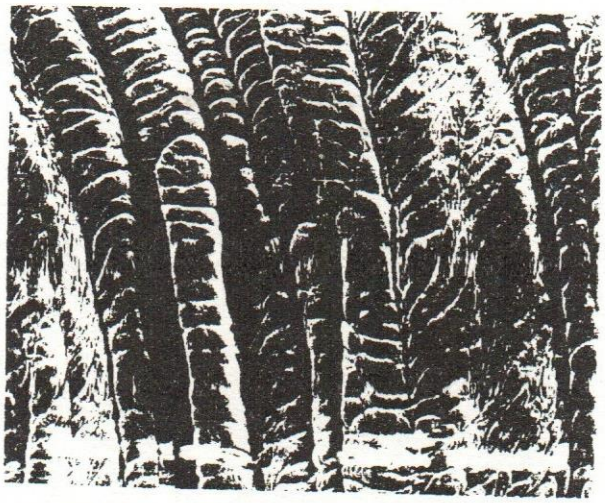

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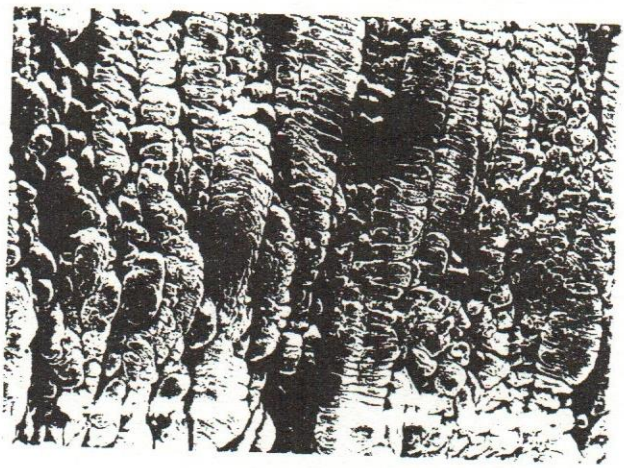

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