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THE PRENATAL DEVELOPMENT OF THE EYELIDS OF THE ONE-HUMPED CAMEL (*CAMELUS DROMEDARIUS*)

(With 29 Figures)

By

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(Received at 22/12/2003)

التطور الجنيني لجفون العين في الجمل وحيد السنام

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لقد تم في هذه الدراسة إستخدام ٥٥ جنين جمل تتراوح في الطول من ٢,٨ سم - ١١٠ سم (أجنة كاملة النمو)، إستخدم منها ٣٥ جنينا لدراسة الشكل العيني و ٢٠ جنينا للميكروسكوب الضوئي. وقد لوحظ أن براعم الجفنين العلوى والسفلى تبدأ في الظهور في أجنة بطول ٢,٨ سم وتتمو حتى تتقابل وتلتحم عند طول جنيني ٨,٤ سم. ويستمر هذا الإلتحام في الأجنة حتى طول ٥٨ سم، ثم تبدأ الجفون في الإفتصال في أجنة ذات طول ٦٦ سم. هذا وقد أظهرت الدراسة أن جلد الجفون والملتحمة الجفنية يمران بتغيرات واضحة خلال فترة ما قبل الولادة، حيث لوحظ أول ظهور لبصيلات الشعر في أجنة ذات طول ٨,٤ سم في حين أن أول ظهور للغدد العرقية والغدد الدهنية يبدأ في أجنة بطول ٣٦,٥ سم، أما إكمال ظهور جلد متقرن بتركيبه المتكاملة من أهداب العين وشعر حسي فقد لوحظ في أجنة طولها ٦٦ سم. هذا وقد لوحظ وجود بصيلات شعر وغدد دهنية على الملتحمة الجفنية قرب الموق الأنسي فقط وفي أجنة بطول ٣٦,٥ سم. وتم ملاحظة أن هذا الجزء من الملتحمة الجفنية ظهر به شعر مزود بمجموعة كبيرة من الغدد الدهنية في الأجنة كاملة النمو.

SUMMARY

Fifty-five camel fetuses ranging from 2.8 to 110cm crown vertebral rump length (CVRL) were used in this study. Thirty-five fetuses were used for gross morphology and the other twenty for paraffin sectioning and light microscopy. The upper and lower eyelids appear as two ectodermal folds at 2.8cm CVRL, then grow until they meet and fuse at 8.4cm CVRL. Fusion lasts until 58cm CVRL, the lids then start to reopen at 66cm CVRL. The palpebral skin and conjunctiva display striking changes throughout the prenatal life. The first indication of hair follicles is seen at 8.4cm CVRL, while sweat and sebaceous glands are

first demonstrated in fetuses of 36.5cm CVRL. A cornified palpebral skin with complete cutaneous structures including eyelashes and tactile hairs is demonstrated in fetuses of 66cm CVRL. The one-humped camel lacks tarsal glands. However, hair follicles are demonstrated on the conjunctival side of the eyelids, only near the medial canthus, in fetuses of 36.5cm CVRL. At 110cm CVRL (full-term fetuses), this part of the palpebral conjunctiva appears hairy and associated with well-developed sebaceous glands.

Key word: *Eyelids – development - camel.*

INTRODUCTION

The ontogeny of the eye has been described in the human and some other mammals (Barber, 1955; Mann, 1964; Pei and Rhoden, 1970; Aguirre *et al.*, 1972; Bistner *et al.*, 1973). The development of the eyelids has been studied briefly in human (Haines and Mohiuddin, 1970; Patten and Bruce, 1974; Vaughan *et al.*, 1992; England, 1996); in canines (Aguirre *et al.*, 1972) and in bovines (Bistner *et al.*, 1973). A detailed developmental study, on the eyelids of the camel, is lacking in the available literature. The aim of this investigation is to throw light on the prenatal development of the eyelids of the one-humped camel both grossly and light microscopically.

MATERIALS and METHODS

Fifty-five camel fetuses ranging from 2.8cm CVRL to 110cm CVRL (full term) were used in this study. Thirty-five fetuses were used for gross morphology and for examination with stereomicroscope, the other twenty were processed for light microscopy. The young fetuses (2.4-8.4cm CVRL) were wholly perfused using 4% neutral buffer formalin, washed, dehydrated in ascending grades of ethanol, cleared in methyl benzoate and embedded in paraffin wax. In fetuses of 9-88cm CVRL, the eye and eyelids were extracted and processed for paraffin embedding as described above. The prepared sections of 3-5 μ m thickness were stained with H & E (Bancroft and Stevens, 1977) as well as Masson's trichrome (Crossman, 1937) stains, examined and photographed using the light microscope. The epithelial heights (both on the skin and conjunctival side) were measured using an image analysis system (Leica Q500).

RESULTS

The first indication of the eyelids could be demonstrated, by stereo- and light microscopes, in fetuses of 2.4cm CVRL in the form of upper and lower ectodermal folds (eyelid buds) in front of the eyeball. The lips of the above mentioned folds grow toward each other until they meet and fuse in fetuses of 8.4cm CVRL (Figs. 1, 2, 7-13). The fusion lasts until 58cm CVR (Figs.2-4, 13, 14). The lids reopen in fetuses of 66cm CVRL and stay reopened until full term stage (110cm CVRL) as shown in figs. 5, 6, 15, 16.

In paraffin sections the fused eyelids closes a well-defined conjunctival sac. The fusion includes only the superficial layers of the epidermis which appear as a bridge of pale polyhedral cells flanked superiorly and inferiorly by two well defined layers of basal cell layers with darkly stained and crowded nuclei (Fig.13). At 58cm CVRL, the eyelids are still fused, but the signs of separation start in the form of breakdown in the occluding-bridge between the upper and lower eyelids (Fig.14). Complete separation is seen in fetuses of 66cm CVRL (Fig. 15), eyelashes are demonstrated at 88cm CVRL (Fig. 16).

The covering skin:

Until 8.4cm CVRL, the ectoderm covering the eyelids (future epidermis) is formed of 2-3 layers of epithelial cells. In 9cm CVRL fetuses, the covering epithelium measures about 12 μ m in thickness and is formed of 3-4 layers of epithelial cells, melanin pigments are seen scattered in between these cells (Fig.17). By advancement of age, the epidermis shows an increase of its thickness, which reaches about 56 μ m at the age of 66cm CVRL. Signs of keratinization start to appear at 36.5cm CVRL, become clearly obvious at 41cm CVRL and become fully keratinized in 66cm fetuses (Figs18-21).

In 66cm CVRL, the epidermis is characterized by darkly-stained basal layer, with many melanocytes in between. The polyhedral cell layer (Stratum spinosum) is very thin, while the Stratum granulosum, Stratum lucidum and Stratum corneum constitute most of the epidermis (Fig. 21). A decrease in the thickness of the epidermis starts in 77cm CVRL fetuses where it measures about 52 μ m. It progressively decreases until it becomes about 22 μ m in near- and full term fetuses (88-110cm CVRL), the epidermis is now formed of Stratum basalis, about two layers of Stratum spinosum and a very thin Stratum corneum (Fig.22).

Hair follicles:

The first indication of hair follicles is seen in fetuses of 8.4cm CVRL in the form of minute proliferations and condensations of the epidermal cells (hair plugs). In 9cm CVRL fetuses some of these plugs start to be pushed deeper in the dermis (Fig.17). At 19cm CVRL, the growing follicles form cylindrical cords, always angled with the epidermal surface. The peripheral cells of these cords are columnar and continuous with the basal cell layer of the epidermis while the central cells are polyhedral and lightly stained. Several melanocytes are scattered in between the peripheral cells of the follicles. Addition of new follicles continues until 41cm CVRL. Tactile hair follicles start to be formed as early as 24cm CVRL, but blood spaces are first recognized in fetuses of 41cm CVRL (Fig. 19). These tactile follicles are larger than normal hair follicles. In near-term fetuses (88cm CVRL), they appear surrounded by large blood sinuses, which are interposed in a collagenous fibrous capsule (Fig.22).

In 66cm CVRL up to full term fetuses, most of the hair follicles appear in groups of 3-5 follicles in a superficial position in the dermis (Fig.20), but larger single follicles extend deeper into the dermis. The hair follicles do not penetrate to the level of the M. Orbicularis oculi, which form a more or less continuous layer in a mid-position between the epidermis and palpebral conjunctiva.

Sweat and sebaceous glands:

The primordia of sweat and sebaceous glands are demonstrated in fetuses of 36.5cm CVRL (Fig.18). The sweat glands appear in the form of side solid tubes connected to the upper third of the growing hair follicles. The sebaceous glands, however appear in the form of budding from the hair follicles at a level below that of the sweat glands. The tactile hairs are characterized by small sebaceous glands that are located at the upper borders of the blood sinuses.

Palpebral conjunctiva:

In younger fetuses (up to 41cm CVRL), the conjunctival epithelium resembles that of the epidermis (Fig.23). By advancement of age, more layers and goblet cells as well as melanocytes are seen in the conjunctival epithelium. The thickness of the conjunctival epithelium displayed changes similar to that of the epidermis. It increases in thickness from about 4 μ m in 9cm CVRL to reach about 19 μ m in 66cm CVRL then decreases again to reach about 10 μ m in full term fetuses.

In fetuses of 19cm CVRL, the palpebral conjunctiva displayed local thickenings in the epithelium near the medial canthus of the eye,

that later on start to penetrate into the subepithelial tissue forming hair follicles (Fig.23). In 36.5cm CVRL, sebaceous gland buds are associated with these follicles (Fig.24). In near- and full term fetuses (88-110cm CVRL), the palpebral conjunctiva, at the medial canthus of the eye, shows scattered minute hair follicles. Each hair follicle is surrounded by huge multilobular sebaceous gland (Figs.25, 26).

The conjunctival epithelium varies in type in different regions. It is stratified squamous non-cornified near the free border of the eyelids, transitional mid-way between the conjunctival fornix and the free border, but it is stratified columnar to pseudostratified columnar near the fornix. Many goblet cells and melanocytes are seen among the conjunctival epithelium, the former cells are more encountered near the conjunctival fornix (Figs.27-29).

The elements of the orbicularis oculi are first seen at 19cm CVRL, start to form bundles in fetuses of 36.5cm CVRL and become fully developed at 66cm CVRL (Fig.20). In this stage the muscle appears in the form of bundles of circularly arranged skeletal muscle fibres forming a continuous layer mid-way between the epidermis and the palpebral conjunctiva.

DISCUSSION

The present results are in a general agreement with the previous studies in human being and some domestic animals that the eyelids originate from lid buds in the form of two mesodermal folds covered by ectoderm in front of the future cornea. These two buds grow over the cornea, fuse and reopen later on (Haines and Mohiuddin, 1970; Aguirre *et al.*, 1972; Bistner *et al.*, 1973; Patten and Carlson, 1974; England, 1996).

Due to the differences in the gestational period between different domestic animals and human being, the timetable of the above-mentioned events is quite different from species to another. Because of the difficulty to determine the exact gestational age in the camel, the technique of measuring the CVRL is applied to trace the prenatal development of the eyelids. The present investigation reveals that the lid buds appear at 2.4cm CVRL stage, fuse at 8.4cm CVRL and reopen at 66cm CVRL. In bovines, a species with a relatively short gestational period in comparison with the camel, the lid buds appear and fuse at 1.5cm and 4cm CVRL respectively, but reopen at birth (Bistner *et al.*, 1973). In the dog however, the lids reopen about two weeks after birth (Aguirre *et al.*, 1972), possibly due to the very short gestational period.

In human being, on the other hand, the lids reopen as early as the seventh month of gestational age (Patten and Carlson, 1974; England, 1996). From these findings one could conclude that the differences in the timetable of the developmental events may be explained in two directions. As the gestation period prolongs, which is the case in the camel, the lid buds appear and fuse later than in canines and bovines (with shorter gestational period), but reopen quietly distant from birth. This explanation will be argued by the case in human beings, but some authors noticed that the events in human eye development start very early and progress at a higher rhythm than in some domestic animals (Aguirre *et al.*, 1972).

The covering skin of the eyelids of the camel experiences prenatal changes, that fall in three stages. The first stage is characterized by increase in the thickness due to addition of more layers and ends at the 58cm CVRL where keratinization is clearly evident. The second stage is accompanied by addition of a very thick keratin layer during the period between 58 and 66cm CVRL. The simultaneous occurrence of reopening of the eyelids with the keratinization of the skin is recorded by Williams and Warwick (1980) in the human being. The third stage extends from 66cm CVRL until birth and is characterized by loss of the Stratum granulosum and Stratum lucidum, while the Stratum corneum becomes extremely thin. This thinning seems to be logic prerequisite to an easy movement of the newly reopened eyelids.

The first indication of the hair follicles is noticed at 8.4cm CVRL, while addition of new follicles lasts until 41cm CVRL. These finding disagree with Dougbag and Berg (1983a), in an earlier study on the prenatal development of the hair follicles in the camel, who mentioned that the development of the hair follicles starts in the prescapular region of CRL 15cm fetuses as follicular plugs and addition of new follicles last until 50cm CRL fetuses. The present results disagree also with the observations of Winter *et al.* (1942) in cattle fetuses and El-Sakhawy (1973) in buffalo fetuses, who recorded a later onset of hair follicle formation. These authors have not included the skin of the eyelids in their material. One can conclude that eyelids may be the first place in the body in which the initiation of hair follicles occurs.

The present results agree with the statement of Dougbag and Berg (1983a) that the hair follicles in camel fetuses tend to form groups, on the other hand Hafez *et al.* (1955) in cattle and buffalo; Lyne *et al.* (1959) and Nair and Benjamin (1965) in cattle mentioned that there are no hair groups.

In the present study, the sweat and sebaceous glands appear for the first time at 36.5cm CVRL originating from the upper third of the hair follicles, but the sebaceous glands are located at a lower level. Dougbag and Berg (1983b), also in camel fetuses, reported the same onset for the sweat glands, however they noticed the sebaceous glands at a later stage (45cm CRL). In the same connection, Lyne and Heideman (1959) in cattle and El-sakhawy (1973) in buffalo recorded a much earlier appearance of the primordia of sweat glands (at 24cm CRL). The present study supports the previous findings in buffalo (Prasad and Sinha, 1979; Hifny *et al.*, 1985) that the tactile hairs are associated by small sebaceous glands, but disaccords the observation of Last (1961) which denied the presence of sebaceous glands with the tactile hairs.

Tarsal glands (Meibomian glands) are recorded in many domestic animals (Prasad and Sinha, 1979; Hifny *et al.*, 1985) and man (Patten and Carlson, 1974; England, 1996). The present work supports the previous observation of Ibrahim (1990) that the camel has no tarsal glands. However, well-developed collections of sebaceous glands associating fine hair follicles are demonstrated in the palpebral conjunctiva near the medial canthus. These gland collections may substitute the absence of tarsal glands in the camel.

The conjunctival epithelium experiences more or less similar developmental events as the epidermis concerning the changes in the thickness. In near- and full-term fetuses the palpebral conjunctival epithelium varies from stratified squamous non-cornified near the free border of the eyelids to transitional epithelium mid-way between this free end and the fornix and finally stratified columnar to pseudostratified columnar with goblet cells toward the fornix. These local differences of the conjunctival epithelium have been recorded in many domestic animals (Calhoun and Stinson, 1976; Hifny *et al.*, 1985). The presence of transitional epithelium may allow an easy stretching of the eyelids, while the goblet cells produce mucous lubricant that minimize friction between the palpebral and bulbar parts of conjunctiva.

LEGENDS

Figs. 1-6: Eyelids of the camel in different prenatal stages. Note the lid buds (arrows) above and below the future cornea (C) at 3.5cm CVRL. They appear fused at 10.5cm CVRL (Fig. 2), 45cm CVRL (Fig. 3) and 58cm CVRL (Fig. 4). Reopened eyelids are demonstrated in fetuses ranging from 88cm CVRL (Fig.5) to

110cm CVRL (Fig.6), where the third eyelid (arrow), eyelashes and hairs are recognized on their skin.

- Figs 7-13:** Paraffin sections stained with H & E showing the early formation of the eyelids of the camel. They start as lid buds (arrow heads), grow until being fused in front of the future cornea (C) closing the conjunctival sac (asterisk). The fusion includes only the superficial layers of the epidermis (arrow). Notice the lens vesicle (LV), lens (L) and optic cup (OC). X10.
- Figs 14-16:** H & E stained paraffin sections showing parts of the upper and lower eyelids in mid- and later stages of prenatal life. Fusion lasts until 58cm CVRL (Fig. 14, X63), the lids then reopen at 66cm CVRL when their skin is fully keratinized (Fig. 15, X25). In near-term fetus (88cm CVRL), the eyelids are characterized by the presence of eyelashes (arrowhead), hairy skin (thick arrow) and thin epidermis (double arrow). Note the signs of separation (arrow) in Fig. 14 and cornea (C) posterior to the eyelids in Fig. 16 (X25).
- Figs. 17-22:** H & E stained paraffin sections of the eyelid skin in different prenatal stages.
- Fig. 17:** The initiation of hair follicles in the form of hair plugs (arrows). X400.
- Fig. 18:** The skin and associated hair follicles and glands. Note the primordia of sweat glands (SG), sebaceous glands (arrow) arising from a growing hair follicle. X160.
- Fig. 19:** A thick tactile hair follicle showing the first indication of blood sinuses (arrows). X160.
- Fig. 20:** Fully keratinized skin (E). Note the hair follicles groups (arrows), tactile hair (TH) and M. orbicularis oculi (OM). X63.
- Fig. 21:** Magnification from Fig. 20 showing different layers of the epidermis: basal layer (B), polyhedral layer (R), granular layer (G) and cornified layer (C). X40.
- Fig. 22:** A trichrome stained section showing a tactile hair with large blood sinuses (BS) and small sebaceous glands (arrow). Note the thin epidermis (arrow head). X63.
- Figs. 23-26:** H & E stained paraffin sections of parts of the palpebral conjunctiva near the medial canthus of the eye showing the development of hairs in this part.
- Fig. 23:** Local thickenings in the palpebral conjunctiva in 19cm CVRL (arrows). X40.

- Fig. 24:** Many hair follicles (arrows) and their associated sebaceous gland buds (arrow heads) in the palpebral conjunctiva in 36.5cm CVRL. Note the third eye lid (TL) below the palpebral conjunctiva, the epidermis on the outer surface of the eyelid (E) and the accompanying hair follicles (HF). X40.
- Figs. 25, 26:** Well-developed collections of sebaceous glands associating hair follicles (arrows) in full term fetuses. Note the epithelial lining of the palpebral conjunctiva (arrow heads). X50.
- Figs. 27-29:** Paraffin sections demonstrating the regional differences in the type of the conjunctival epithelium in near-term fetuses (88cm CVRL). It is transitional mid-way between the free and attached borders (Fig.27), stratified squamous non-cornified toward the free border (Fig.28) and pseudostratified columnar with goblet cells toward the conjunctival fornix (Fig.29). Note the melanocytes (arrow) X250.

ACKNOWLEDGMENT

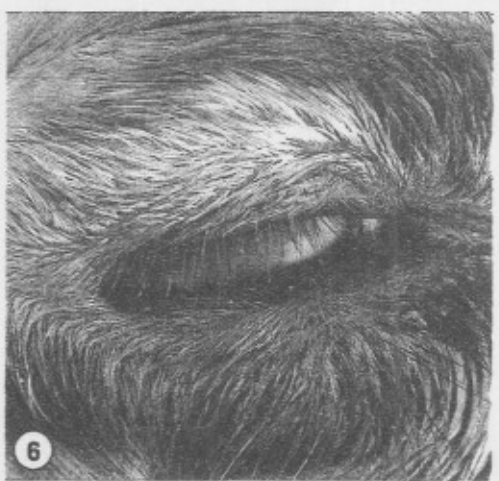
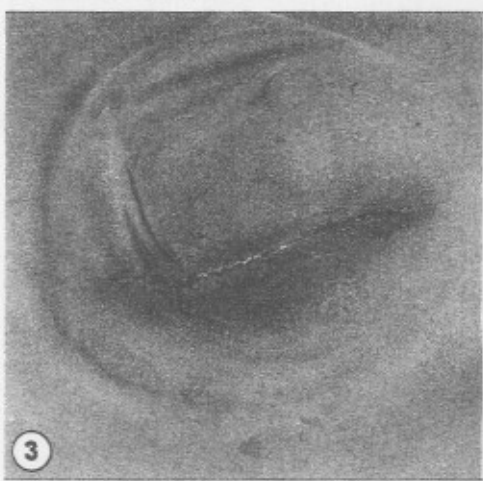
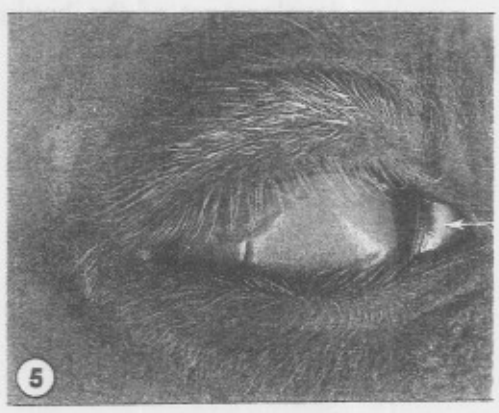
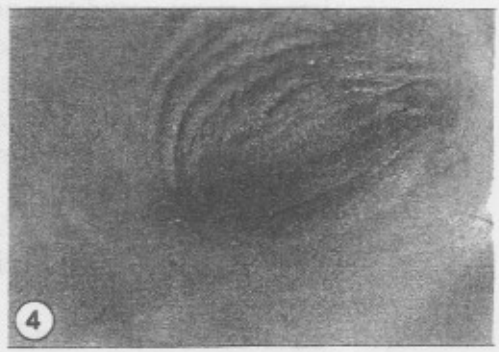
The author wishes to thank *Prof. Dr. M.E. Abdel-Moniem, professor of Anatomy* for his help in the collection of the materials of this study.

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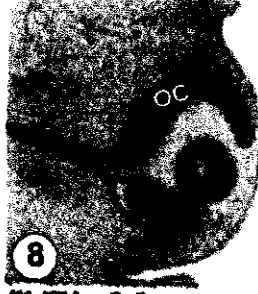
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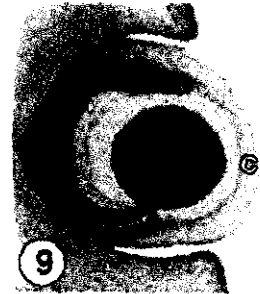




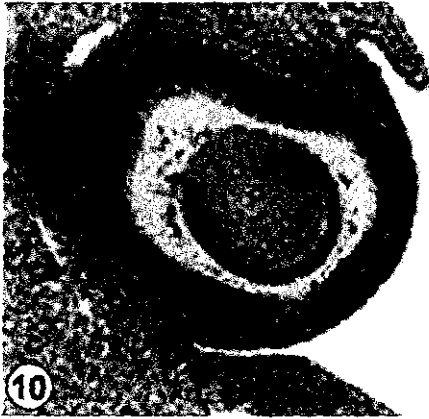
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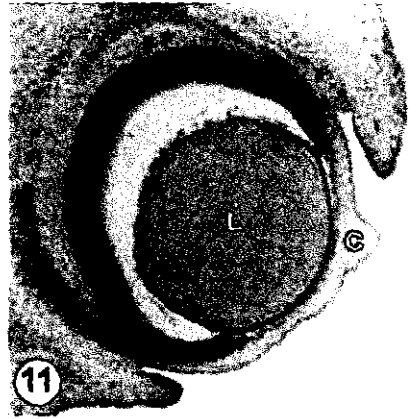
CVRL 2.8 cm



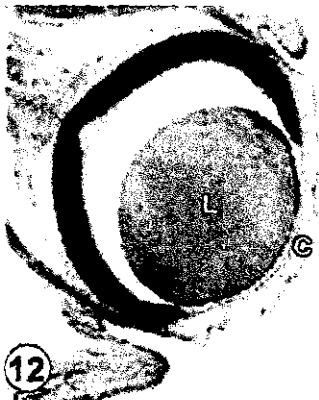
CVRL 3.6 cm



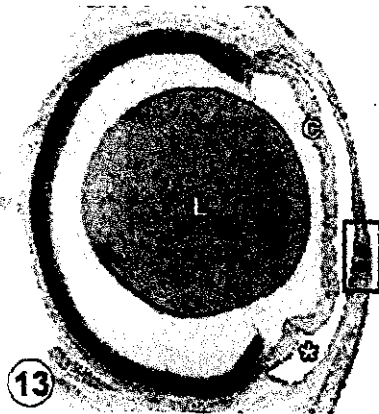
CVRL 3.8 cm



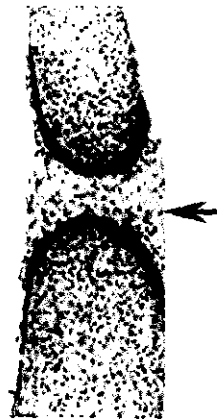
CVRL 5.3 cm

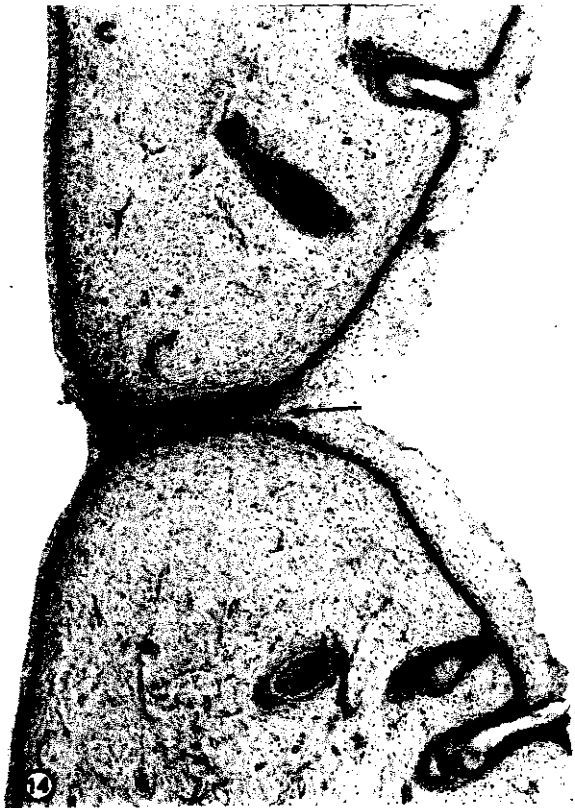


CVRL 5.8 cm



CVRL 8.4 cm

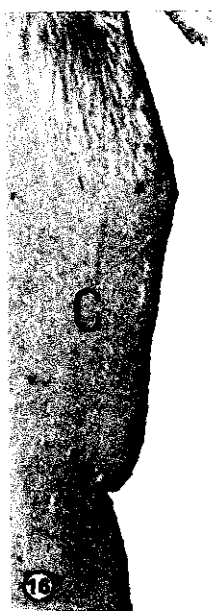




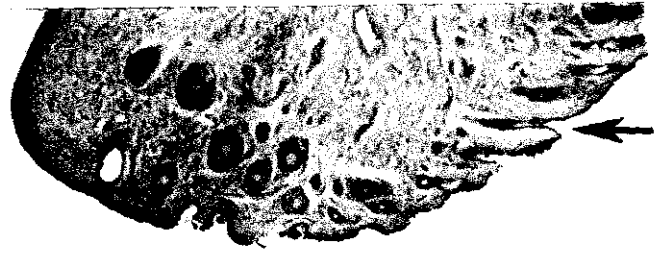
14
CVRL 58 cm

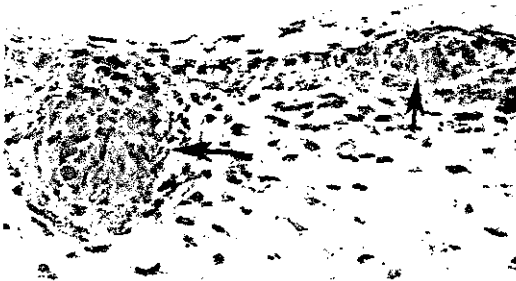


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CVRL 66 cm

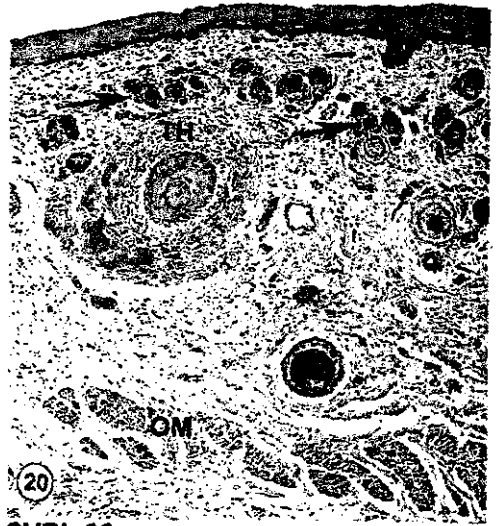


16
CVRL 88 cm

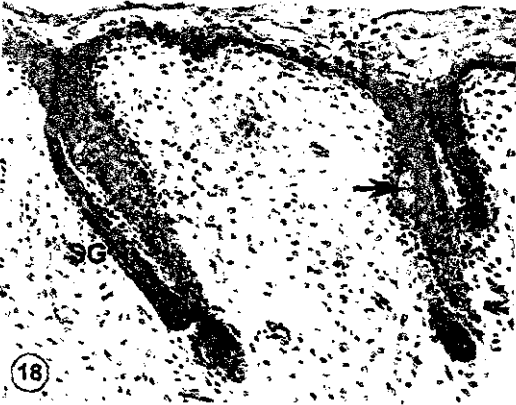




17
CVRL 9 cm



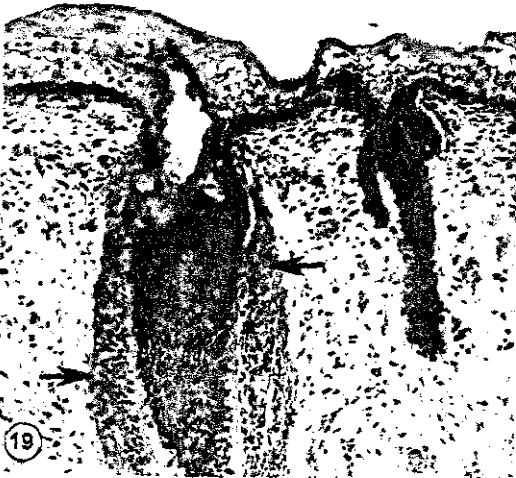
20
CVRL 66 cm



18
CVRL 36.5 cm



21
CVRL 66 cm



19
CVRL 41 cm



22
CVRL 68 cm

