Dept. of Anatomy & Histology,
Faculty of Vet. Med., Assiut University,
Head of Dept. Prof. Dr. A. Hifny.

HISTOGENESIS OF THE FOETAL SKIN IN BOUSCAT RABBIT

(With 1 Table & 10 Fig.)

By

M.N.K. MOUSTAFA and M.A. GABR*

(Received at 25/2/1992)

تنسج الغطاء الجلدي في أجنة الأرانب البوسكـــات

محمد نبيل كامـل ، محمد عبد المغنـي

تم دراسة التغيرات الهستولوجية التي تحدث في جلد أجنة الأرانب التي بلغـــت أعمارها ما بين ١٤ ـ ٢٨ يوم من تاريخ الحمل · تكونت البشرة في الأجنة التي بلغـــت أعمارها ما بين ١٤ ـ ١٦ يوم من طبقة أو طبقتين من الخلايا · ثم زادت صفوف هـــذه الخلايا باستمرار الحمل إلى أن تميزت البشرة إلى أربع طبقات رئيسية هي الطبقـــة القاعدية والطبقة الدبوسية والطبقة المحببة والطبقة القرنية في الأجنة التي بلغـــت أعمارها ٢٤ يوم · تم ظهور بشائر الأغماد الشعرية في جلد أجنة الأرانب التي تتـــراوح أعمارها ٢٠ يوم بينما ظهرت بشائر الغدد الأنيبية والغدد الدهنية في نهاية مدة الحمل ·

SUMMARY

The histomorphological changes occuring in the foetal skin of Bouscat rabbit (lepus Caniculus) ranging from 14 to 28 days old were studied.

In embryos of 14 to 16 days old, the epidermis was composed of one to two cell layers. The epidermis increased in thickness throughout the intrauterine life until it was differentiated into 4 cell layers; stratum basale, stratum spinosum, stratum granulosum and stratum corneum in 24 days old embryos.

The anlagen of hair follicles could be demonstrated in 20 days old embryos while the primary elements of both sebaceous and tubular glands appeared in embryos of 26-28 days old.

* Dept of Anatomy, Faculty of Medicine, Assiut University.

INTRODUCTION

The histogenesis of the skin in various animal species has been investigated by several authors (LOVELL, 1955; MORGOLENA, 1959; BAKER, 1966; EL-SAKHAWY, 1973; DOUGBAG and BERG, 1983; MOUSTAFA, 1986; KELANY, 1988 and MOUKHTAR, et al. 1989). Moreover, KONSOWA (1990) studied the morphogenesis of the skin of the rabbit.

The present work was carried out to give more information on the histogenesis of the fetal skin in Bouscat rabbit.

MATERIAL and METHODS

The material employed in the present study originated from skin specimens of 20 Bouscat rabbit embryos at various periods of the intrauterine life (Table 1).

Table 1: Material available for study

No. of embryos	2	4	3	2	2	2	2	3
Age (day)	14	16	18	20	22	24	26	28

The embryos were removed shortly after evisceration and fixed in 10% neutral buffer formalin. Skin specimens could be obtained from the fore-head, back and belly regions of 24, 26, 28 days old embryos and refixed in Bouin's fluid. After proper fixation, the materials were dehydrated, cleared and embedded in paraffin wax. Serial vertical and horizontal sections were cut at about 7 Um and stained with Haematoxylin and Eosin, Van Gieson's stain, Crossmon's trichrome and PAS technique (DRURY and WALLINGTON, 1980).

RESULTS

In rabbit embryos of 14-16 old days, the epidermis of the covering skin (3.3 to 6.6 Um) was consisted of one to two cell layers. The single layered epidermis (Fig. 1) was composed of one row of low cuboidal cells resting on a distinct corrugated PAS positive basal lamina. The cells were lightly stained with ill-distinct cell boundaries and their nuclei were large, oval, rounded and deeply stained. Several mitotic divisions could be observed between the cells of this layers.

The bilayered epidermis (Fig. 2 a, b & c) was formed of a basal layer of cuboidal cells and an outer periderm. The basal layer cells simulated the beforementioned cells of the single layered epidermis. Several cells of this layer contained large oval or rounded vesicular nuclei with distinct nucleoli and defined cell boundaries. The periderm was composed of few elongated cells with deeply stained oval or flattened nuclei, forming discontinuous layer. Each periderm cell covered 2-3 cuboidal cells of the basal layer. Mitotic divisions could be demonstrated between the cells of the periderm (Fig. 2 b).

The dermis was composed of mesenchymal tissue (Fig. 1 and 2). The mesenchymal cellular elements were more aggregated in the outer surface of the dermis while these elements were loose in arrangement in most of the dermis (Fig. 2 a, b & c). Mitotic divisions could be observed among the cellular elements of the connective tissue. Blood islets with nucleated blood elements could be observed in the dermis (Fig. 2 a, b & c). Mesenchymal cells were showed to arrange themselves around the blood islets to form the primitive endothelium.

In 18 days old, embryos the epidermis (8.2 Um) was composed of 2 cell layers (Fig. 3 a & b). The basal layer was consisted of cuboidal cells having large rounded or oval vesicular nuclei with distinct nucleoli. The periderm cells increased in number. Few polyhedral cells were occasionally observed inbetween the basal layer and the periderm representing the intense mitotic divisons of the basal layer.

The dermis was distinguished into 2 layers; an outer thin cellular layer and an inner thick layer presenting few fine fibrillar elements of collagen (Fig. 3 a).

The blood elements contained pyknotic periplerally situated nuclei and were surrounded by more regular primitive endotnelium than those observed at the previous age (Fig. 3 b).

In embryos of 20-22 days age, the epidermis ranged from (9.1 to 13.2 Um in thickness). It was composed of 3 layers; basal layer, an intermediate layer and periderm (Fig. 4, 5). The basal layer was formed of a single row of columnar cells having large oval vesicular nuclei with distinct nucleoli. The intermediate cell layer was composed of one row of irregularly arranged polygonal cells. These cells increased in number towards the end of this age (Fig. 5). The periderm was composed of fusiform cells with oval or rod shaped nuclei and defined acidophilic cell boundaries. These cells became flattened with deeply stained flattened nuclei and acidophilic cell boundaries representing the primordia of stratum corneum towards the end of this stage of development (Fig. 5).

Most of the cellular elements changed to fibroblast cells at the end of this age. Moreover, the dermis showed more fibrillar elements of the collagenous variety. The blood elements lost its nuclei and thin walled blood vessels with wide lumina were demonstrated (Fig. 4 a).

The basal layer of the epidermis invaginated within the underlying dermis at different intervals forming the primitive hair germ (Fig. 4). The cells of the basal layer, at these regions, were of the columnar variety with large oval vesicular nuclei and distinct nucleoli. They arranged themselves in a regular manner on a distinct basal lamina where they were surrounded by a condensation of 2-3 layers of fibroblast cells. These invaginations increased in depth towards the end of the this stage of development (Fig. 5).

In embryos of 24 days age, the epidermis increased in thickness (26.4 Um). It could be differentiated into 4 cell layers (Fig. 6). The stratum basale was composed of one row of columnar cells resting on a distinct PAS-positive basal lamina. These cells contained large oval vesicular nuclei with distinct nucleoli. The stratum spinosum was formed of 2-3 rows of irregularly arranged polygonal cells. These cells contained large rounded vesicular nuclei, faintly acidophilic cytoplasm and distinct cell boundaries. The superficial cells of this stratum became fusiform in shape with large oval centrally located nuclei. Few flattened cells with deeply-stained flattened nuclei and very fine basophilic granules especially at the openings of the primordium hair follicles could be observed representing the primary elements of stratum granulosum. The stratum corneum was represented by one to two layers of deeply acidophilic flattened cells with no nuclei and no cell boundaries.

The dermis presented more cellular and fibrillar elements than those observed at the aforementioned age (Fig. 6).

The primordium of the hair follicles increased substantially in amount and depth at this stage and were represented by follicle plugs. These could be differentiated into large and small follicles (Fig. 7 a & b). The cells in the follicle plugs were arranged either regularly around the periphery, where they continued with the cells of the basal layer of the epidermis, or irregularly where they were scattered within the follicle plugs. Intense mitotic divisions could be observed between the cells of the follicle plugs. The were surrounded by 2-3 layers of fibroblast cells.

The rate of development and density of follicle plugs differed alongside the covering skin of rabbit embryos at this stage of development. They were more developed in the belly region (Fig. 8) than at the back region (Fig. 7 a). Some of the largest follicles at the belly region, extended into the deep surface of the dermis and showed hair matrix and elements of inner root sheath.

In embryos of 26-28 days age, the epidermis (Fig. 9) decreased slightly in thickness (16.5 Um). The stratum basale was composed of low cuboidal cells with acidophilic cytoplasm and rounded or oval nuclei. The stratum spinosum was formed of 2-3 layers of irregularly arranged polygonal cells with acidophilic cytoplasm and rounded nuclei.

The stratum granulosum was composed of one to two layers of fusiform cells with basophilic granules. These granules increased in amount towards the end of this age. The stratum corneum increased in thickness than those observed at the latter age.

The cellular elements of the dermis (Fig. 9 a) decreased and the fibrillar elements increased than at the previous age. Both the collagen fibers and fibroblast cells appeared disposing parallel to the epidermis.

The primordium of the hair follicles increased in depth and amount than those observed in the latter age. Most of the largest follicles showed hair papilla, hair matrix, inner root sheath, outer root sheath and primordium of keratinized hairs (Fig. 9). These follicles increased in size, depth and amount towards the end of this stage (full-term embryos). In the frontal region, the largest hair follicles were more or less blended in the dermis to the extent that they were disposed parallel to the epidermal surface (Fig. 9).

The primary elements of the sebaceous glands could be demonstrated at the outer third on one or both sides of the large hair follicles. They appeared as 1-3 large faintly stained cells with large vesicular rounded nuclei and distinct centrally located nucleoli. They were surnounded by a layer of flattened cells, with depply staiend oval nuclei, extending from the basal layer of outer root sheath. (Fig. 9 & 10).

The primordium of the tubular glands (Fig. 9 & 10) appeared as cord-lik 2-3 thick structure of basophilic cells with deeply stained nuclei. They lied above the sebaceous glands. This basophilic structure increased in depth towards the end of this age.

DISCUSSION

The present investigation revealed that, the cells of the basal layer of the epidermis showed several mitotic activity during the earlier periods of intrauterine life (14 to 16 days old embryos). This phenomenon discusses the first step in the life cycle of every epidermal cell (keratinocyte) where new epidermal cells are continuously formed by the germinal layer to compensate for those which exfoliate at the surface (MONTAGNA and PARAKKAL, 1974). Also in this vicinity, the variation in thickness and number of cell layers of the epidermis in the present work from one to two cell layers at 14 to 16 days-old embryos to 4 distinguished cell layers at 24 days-old embryos complete life cycle of keratinocyte (2nd step: differentiation and 3rd step: exfoliation).

The present work revealed that the epidermis was covered by periderm in 16 to 22 days old embryos, then it was replaced by layers of stratum corneum. The periderm undergoes partial keratinization before it is shed into the amniotic cavity and

the cells of the stratum corneum and periderm are basically similar (MONTAGNA and PARAKKAL, 1974). The periderm may do more than act as a protective covering for the developing epidermis. It participates in the exchange of material between the fetus and the amniotic fluid (HOYES, 1968 and WOLF, 1967 c). In addition, the stratum corneum not only retards water loss from the inner hydrated layer but also prevents the enterance of most toxic agents. Moreover, it protect the animal against the environmental damage and maintains the internal milieu (ROTHBERG, et al. 1961).

The present work revealed that, the dermis was composed of mesenchymal connective tissue at 14 to 16 days-old embryos then the dermis was distinguished into 2 layers; an outer thin layer more cellular and an inner thick layer less cellular with few fine fibrillar elements of collagen at 18 days-old rabbit embryos. The fibrillar elements were increased with the advancement of age as well as most of the cellular elements changed into fibroblasts. The dermal tissue plays a major role in protection and acts as a barrier to infection. Another important function of the dermis is its role in inductive interaction with the epidermis as well as ion exchange, water binding and fibrillogenesis (MONTAGNA and PARAKKAL, 1974). The latter authors mentioned that the specific nature and function of the dermis in any given area of the body are closely related; that is, what it is depends primarily on what it does.

The present investigation revealed that, the primordium of hair follicles appeared at 20 days old rabbit embryos as primitive hair germ. Similar observations were obtained by KONSOWA (1990) in the face region of 15 days old rabbit embryos, while the primitive hair germ appeared in 50-60 mm CVR length dog foetuses (MOUSTAFA, 1986). On the other hand, AHMED, et al. (1985) recorded that the anlagen of hair follicles appeared around the 78th days of foetal life in sheep. From these latter results, we showed that the appearance and also the differentiation of the hair follicles differ in domestic animals. This difference was dependent on the physiologic and histologic ability of the skin to intiate and support such development and growth (MARGOLENA, 1959). Also this difference could be attributed to the time of gestation period in different domestic animals.

This study revealed that the largest hair follicles in the frontal region were more or less blended in the dermis to the extent that they run parallel to the epidermis. This phenomenon could be attributed to the narrow of the dermal tissue.

The anlagen of both sebaceous and tubular glands were demonstrated in the present work at 26-28 days-old embryos. Similar findings were observed at 130 mm CVR length dog foetuses (MOUSTAFA, 1986) and in sheep foetuses of 78th days old (AHMED, et al. 1985). Moreover, sweat gland buds appeared on the primary hair plugs at 42 cm CVR camel foetal length and at 50 cm CVR, the sebaceous gland rudiment was first recognized (MOUKHTAR, et al. 1989).

In coculsion, a complete study of the developmental changes occuring within the foetal skin of rabbit must involve further investigation both in the early post-natal life and throughout the prepubertal age.

REFERENCES

Ahmed, M.A.A.; R. Schwarz and M.R. Fath El-Bab (1985): Micromorphological studies on the epidermis, hair follicles and skin glands of sheep during prenatal life. Assiut Vet. Med. J., Vol. 14, No. 28: 21-26.

Baker, K.P. (1966): Postnatal development of dog's skin Br. Vet. J., 122: 344.

Dougbag, A.S.A.M. and R. Berg (1983): The development of the sweat and sebaceous glands in the skin of the one-humped camel (Camelus dromedarius). Z. mikrosk. Anat. Forsch., Lepipzig 97, 5, S. 894-902.

Drury, R.A. and E.A. Wallington (1980): Carleton's histological technique. 4th ed. Oxford Univ. press New York. Tornto.

El-Sakhawy, M.A. (1973): The prenatal development of skin and hair in Egyptian buffaloes. Thesis. Fac. of Vet. Med., Cairo University.

Hoyes, A.D. (1968): Fine structure of human aminotic epithelium in early pregnancy. J. Obstet. Gynaecol. Brit. Commonw. 75: 949-962.

Kelany, A.H.M. (1987): Histogenesis of the skin of the goat with special reference to its mode of vascularization. Ph.D. Thesis, Dept. of Anatomy & Histology, Faculty of Vet. Med., Assiut University.

Konsowa, M.M.H. (1990): Morphogenesis of the skin of rabbit with reference to its vasculature. Thesis. Dept. of Anatomy & Histology, Faculty of Vet. Med., Zagazig University.

Lovell, J.E. (1955): Histological and Histochemical studies of growth changes of canine skin. Thesis. Dept. of Anatomy, School of Vet. Med., Iowa State College, Ames.

Morgolena (1959): Skin and hair follicles development in dairy goats. Virginia J. Sci., 10: 1.

Moukhtar, H.G.M.; M.S. Nada and El-Sayed, M.Mosallam (1989): Histogenesis of the skin of the one-humped camel (Camelus-dromedarius) the New Egypt. J. Med., 3, No. 3, July, 831-842.

Moustafa, M.N.K. (1986): Studies on the histogenesis of the skin of the dog with special reference to its vasculature. Ph.D. Thesis. Dept. of Anatomy & Histology. Faculty of Vet. Med., Assiut University.

Muller, G.H. and R.W. Kirk (1976): Small animal dermatology. W.B. Saunders Company. Philadelphia, London, Toronto.

Rothberg, S.; R.G. Crounse and J.C. Lee (1961): Glycine C^{14} incorporation into the proteins of normal stratum corneum and the abnormal stratum corneum of psoriasis. J. Invest. Dermatol., 37: 497-505.

Wolf, J. (1967 c): The relationship of the periderm to the amniotic epithelium. Folia Morphol., 15: 384-391.

LEGENDS

Fig. 1: Skin of 16 days old rabbit embryo showing:
E: single layered epidermis, D: dermis. Mitotic division (arrow) (X 400).

Fig. 2: **(a, b & c):** Skin of 16 days old rabbit embryo showing: basal layer (b) periderm (P) dermis (D) Mitotic division within periderm cells (arrow), blood islet with nucleated blood cells (c) (X 400).

Fig. 3: **(a & b):** Skin of 18 days old rabbit embryo showing: Epidermis (E), dermis (D) primitive endothelium (arrow) (X 400).

Fig. 4: **(a & b):** Skin of 20 days old rabbit embryo showing: epidermis (E), dermis (D), Thin walled, wide lumen blood vessel (V), primordia of hair follicle (arrow) (a: X 250, b: X 400).

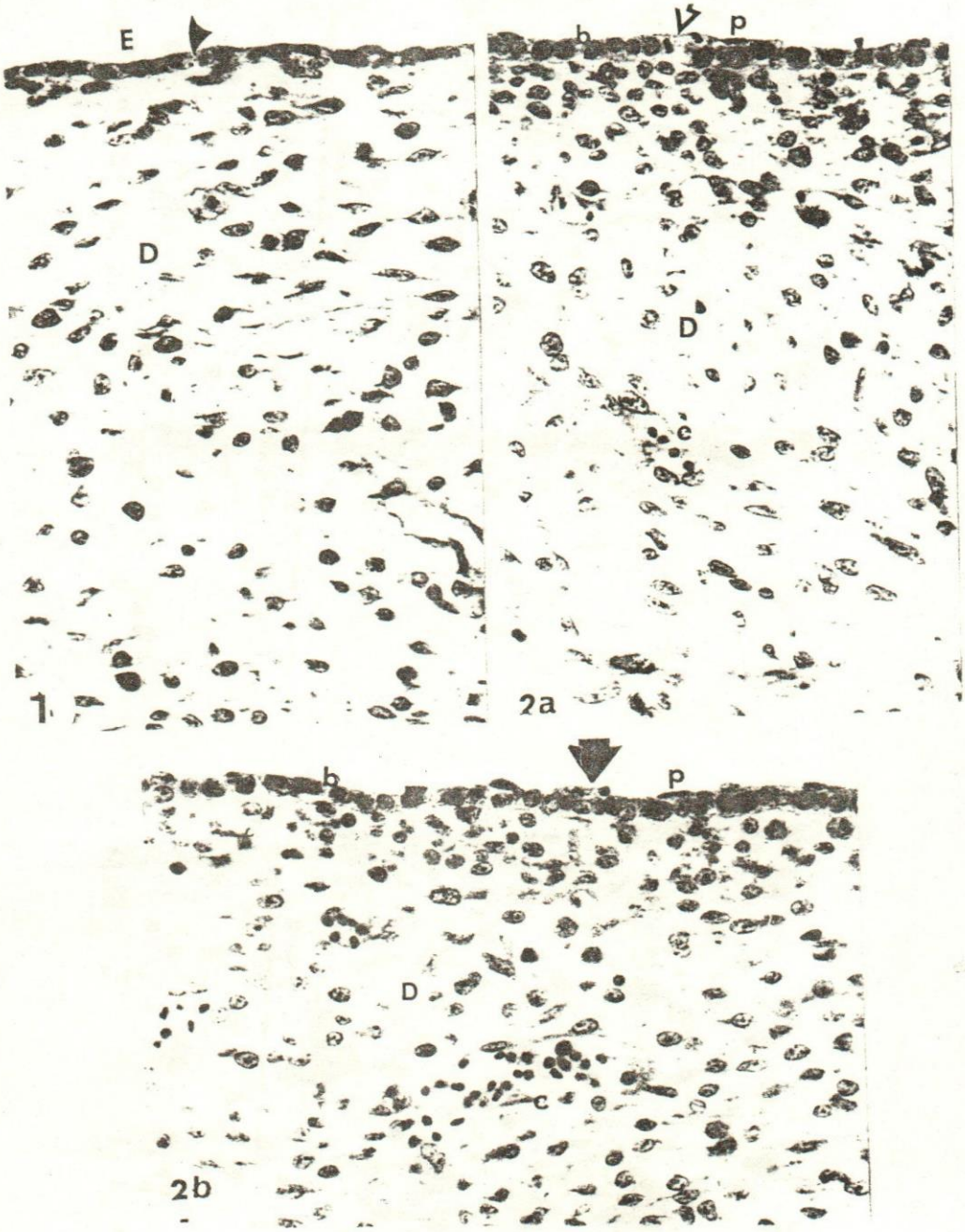
Fig. 5: **(a & b):** Skin of 22 days old rabbit embryo showing: E: epidermis, D: dermis, primordia of hair follicle (arrow) a: X 250, b: X 400).

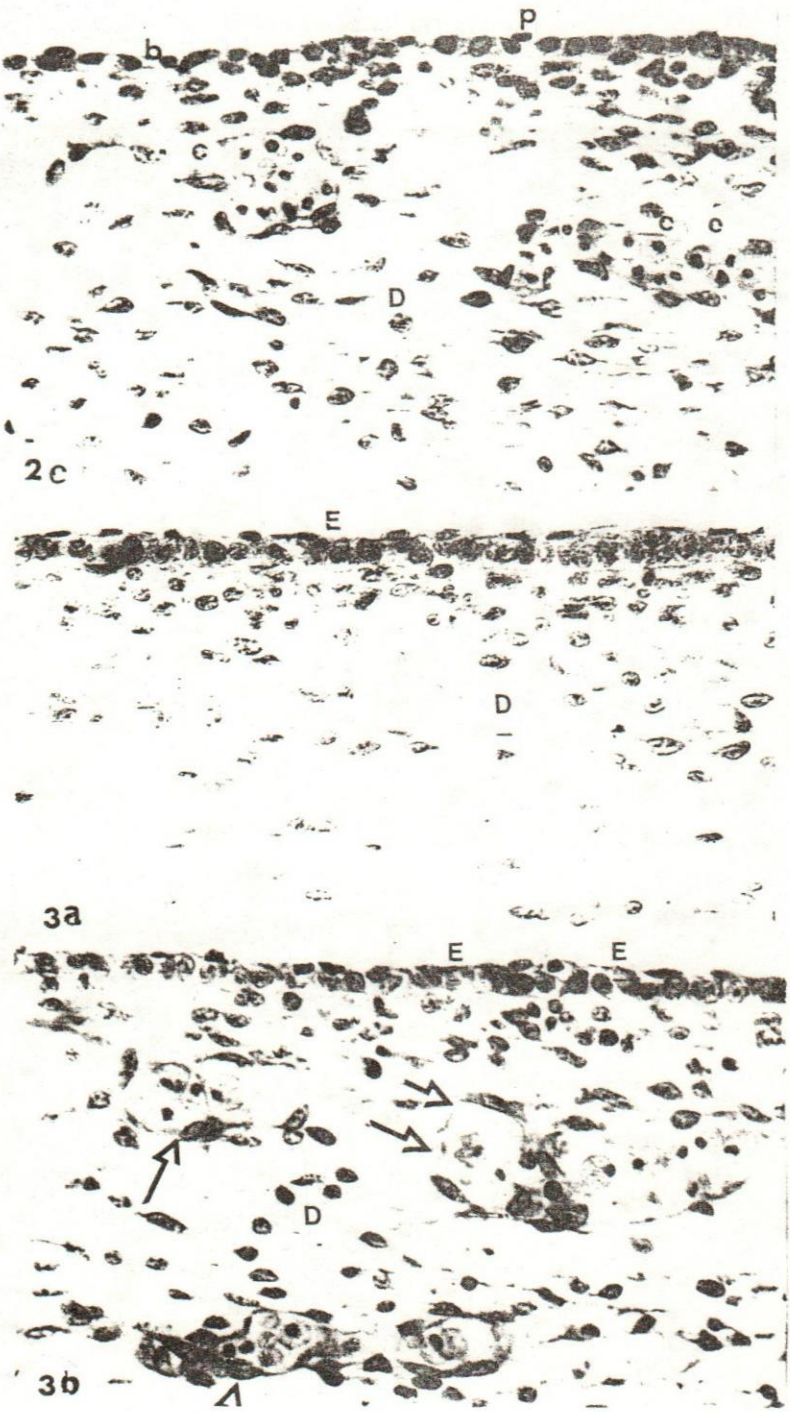
Fig. 6: Skin of 24 days old rabbit embryo showing: E: epidermis, D: dermis, primary elements of stratum corneum (arrow) (X 400).

Fig. 7: **(a & b):** Skin of 24 days old rabbit embryo at the back region showing: small follicle plug (s), large follicle plug (g), mitotic division within follicle plug (arrow), (a X 250 and b X 400).

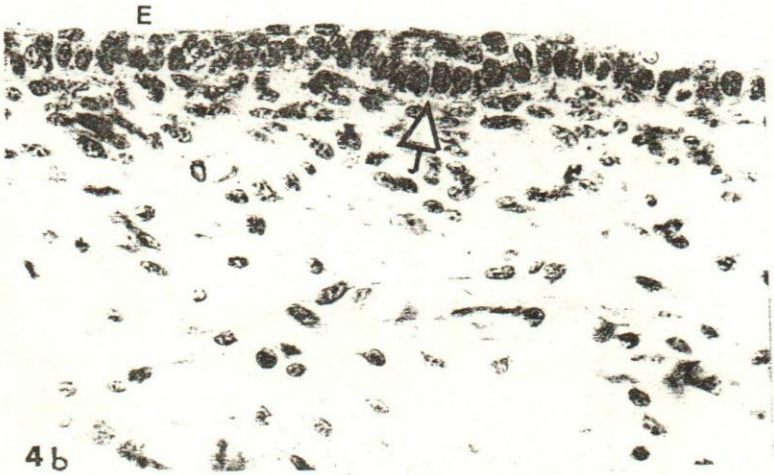
Fig. 8: Skin of 24 days old rabbit embryo at the belly region showing: inner root sheat (arrow) (X 250).

Fig. 9 & 10: Skin of 28 days old rabbit embryo at the frontal region showing: primordium of sebaceous gland (s), primordium of tubular gland (t), outer root sheath (o), inner root sheath (I), hair matrix (m), hair papilla (p) (9: X 400 and 10 X 400).

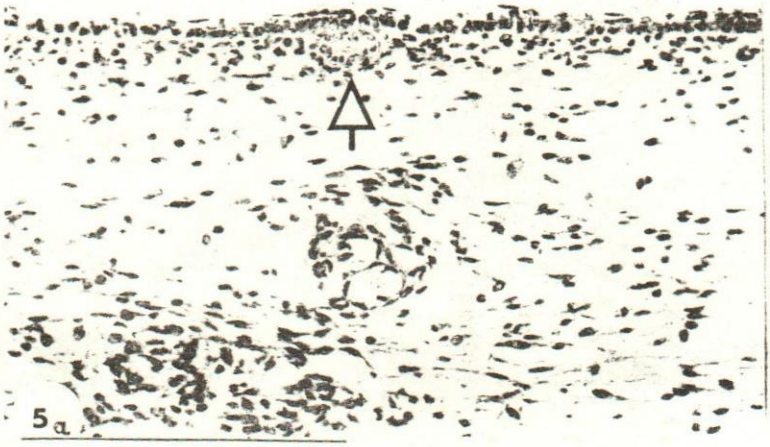

E
D
1
b
p
D
c
2a
b
p
D
c
2b


P
b
c
c
c
D
2c
E
D
3a
E
E
D
3b

4a

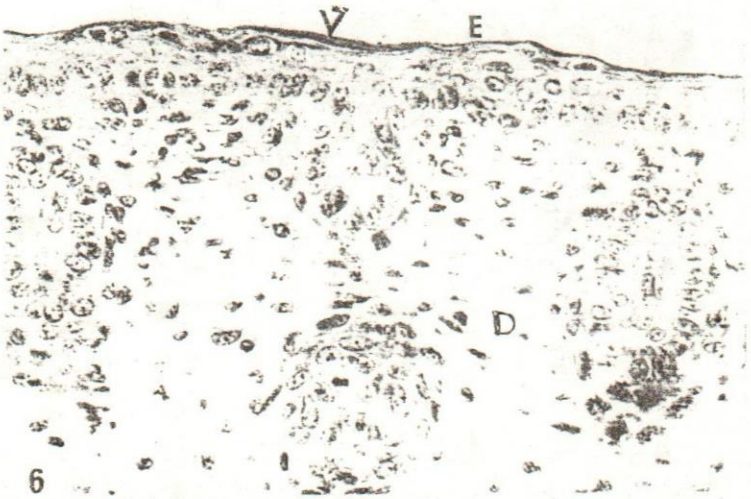


4b



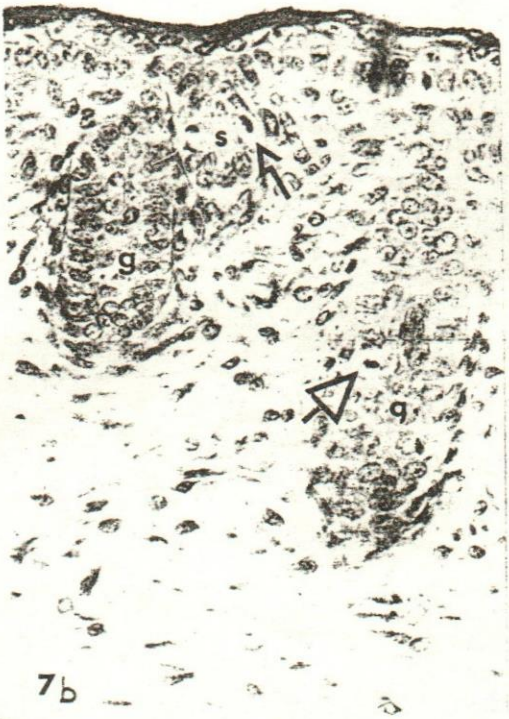
5a

5b

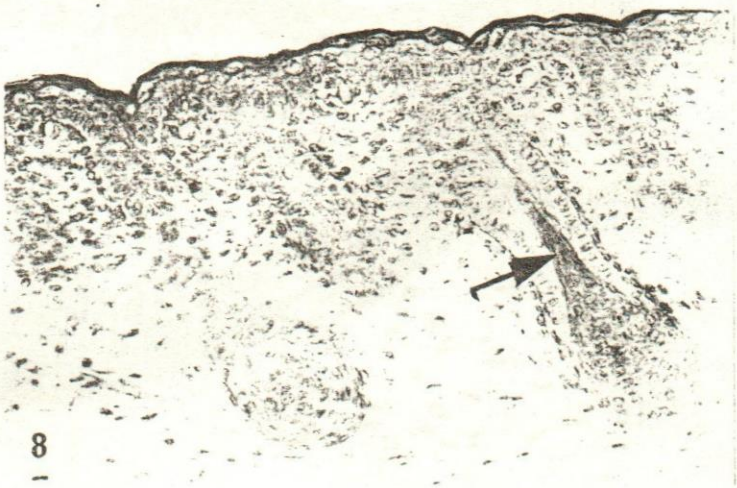


6

7a

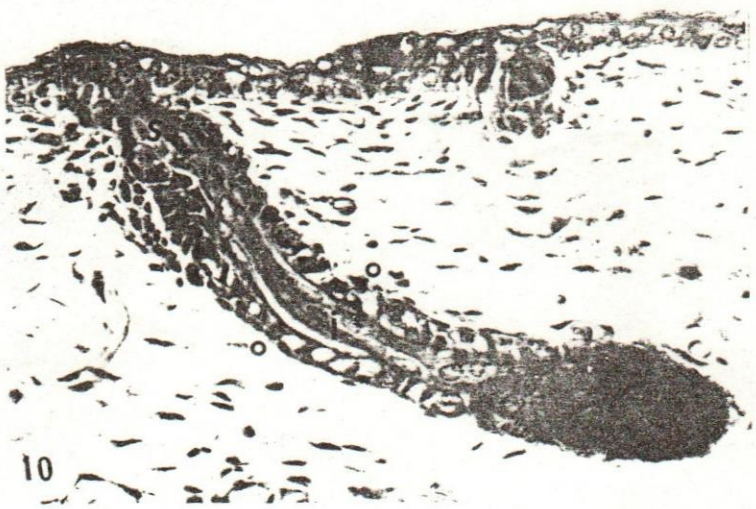


7b



8

9



10