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LIGHT AND SCANNING ELECTRON MICROSCOPICAL EXAMINATION OF DIFFERENT ANIMAL HAIR AND SKIN AS A MEDICOLEGAL TOOL FOR DETECTION OF MEAT ADULTERATION

(With 2 Tables and 9 Plates)

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فحص بالميكروسكوب الضوئي والإلكتروني الماسح لشعر وجلد الحيوانات المختلفة كوسيلة طبية شرعية للكشف عن غش اللحوم

منال عبد اللطيف عبد المحسن ، محمود عبد الناصر على

تم في هذه البحث دراسات مورفولوجية وهستولوجية لتحديد الاختلافات بين شعر و جلد الحيوانات المختلفة. ولقد أوضحت هذه الدراسة أنه يمكن اكتشاف حالات الغش التجاري في كل من اللحوم ومنتجاتها. تم تجميع خمس عينات من شعر و جلد الرأس (منطقة الجبهة) من كل من الجاموس والجمال والحمير والكلاب. تم إعداد عينات الشعر للفحص الميكروسكوبي وإجراء القياسات عليها. كذلك تم تحضير جزء من عينات الجلد من نفس المكان للفحص الهستولوجى والجزء الأخر للفحص بواسطة الماسح الإلكتروني. تبين من الدراسة وجود اختلاف بين الحيوانات المختلفة سواء في التركيب الهستولوجى للشعر والجلد أو باستخدام الماسح وقياسات الشعرة. سجلت الدراسة وجود اختلاف عند فحص سطح الجلد من حيث توزيع الشعر وشكل الطبقة الخارجية للشعرة (البشرة). فيخرج الشعر على هيئة مجموعات وكل شعرة منفصلة المنبع في الجمال والحمير ، بينما في الكلاب تخرج على هيئة مجموعات موحدة المنبع. أما في الجاموس فإنها تخرج منفصلة تماما. كما لوحظ أن خلايا البشرة غير منتظمة الترتيب في الجاموس والكلاب وتكون الخلايا على هيئة صفوف منتظمة في حالة الجمال والحمير. فحص الشعر ميكروسكوبيا يبين اختلاف في سمك الشعرة، حيث نجد شعر الجاموس أسمك الأنواع (٧٢,٢١ ± ٥,٠١ ميكرون) بينما نجد شعر الجمال أقلها سمكا (٣٣,٨٣ ± ٠,٤٥ ميكرون). ووجد أنه لا علاقة بين سمك الشعرة وسمك النخاع حيث أن النخاع يأخذ أكبر حيزا في شعر الحمار (٦٥,٦٦ % من سمك الشعرة) وأن أقل سمك للنخاع كان في شعر الكلاب (٤٤,٣٠% من سمك الشعرة). بالنسبة لسمك طبقات الجلد فكان أسمك جلد في الجمال (١٧١٥,٦ ± ٢٤٣,١ ميكرون) بينما أقل سمك كان في الحمار (٦٠٧,٠٤ ± ١١٨,٤ ميكرون). كذلك اختلاف في سمك باقي طبقات

الجلد بين أنواع الحيوانات المختلفة. وبالنسبة للغدد العرقية فكان أكثرها عمقا في الجمال يليها الكلاب ثم الجاموس وأخيرا الحمار. أثبتت الدراسة إمكانية استخدام ا لشعر و الجلد والذي قد تحتويه اللحوم المفرومة في الكشف عن مصادرها ومعرفة ما إذا كانت قد أضيف لها لحوم من حيوانات أقل قيمة من عدمه بغرض الغش التجاري.

SUMMARY

Morphological and histological examinations were carried out in the present work to find out the difference between animal species using hair and skin examination. This difference could be helpful in the detection of adulteration in meat and meat products. Hair and skin samples were collected from the frontal region of buffalo, camel, donkey and dog. Hair and skin samples were prepared and subjected to light as well as SEM examination and measurements. Results revealed a significant difference between hair and skin of different animals either in histological structures, measurements or scanning. The most characteristic difference between animal samples could be observed by using the scanning electron microscope either in hair or in skin. In camel, hair was arranged in bundles consists of single hair, while in dog the hair arranged as bundles arises from one origin. In buffalo and donkey, hair arranged separately, these findings confirmed by the histological examination. Light microscopical examination of hair revealed that the thickest one was in buffalo (72.21±5.01μ), while the thinnest one was in camel (33.83±0.45μ). The thickest medulla was in donkey (65.66%), and the thinnest was in dog (44.30%). Histological examination of the skin revealed difference in the thickness of dermis or epidermis or depth of the sweat glands according to the animal species. It could be concluded that differentiation between skin and hair of different animals is a significant tool in detection of meat adulteration.

Key words: *SEM, Hair, Skin, Medicolegal, Meat.*

INTRODUCTION

Hair as well as skin examination provides a very important clews in crimes investigations especially in cases of meat adulterations with less desirable or objectionable meat. Because hairs remain identifiable even in states of most advanced putrefaction, its examination offers a great help in the identification of animal species even in putrefied carcasses. Identification of animal hair have been studied by several

authors (Ibrahim, *et al.*, 1963; Gouda and Mosallam, 1983 and Abou-Salem *et al.*, 1999).

Meat and meat byproduct adulteration was recorded in the Egyptian market by many authors (El-Nawawi and Nouman, 1981; Refai, 1982; Eldaly and Ibrahime, 1987 and Morshdy *et al.*, 1987). Adulteration was recorded by meat of less quality as muscles of the head region and abdomen, Skin, tendons, fetuses, intestine and lymph nodes were recorded (Julini and Parisi, 1978). Meat of animals either not fit for human consumption or of law price was also added and recorded with the same authors.

Scanning Electron Microscope (SEM) technique can be most efficiently applied to show interesting morphological aspects of the hair and skin of domestic mammals. With SEM it is possible to supplement the knowledge available from conventional transmission electron microscope (TEM) and light microscope. Joshua and Cyril (1980) used the electron microscope for the more detailed morphology of hair. Meyer *et al.*, (1997) used a computer-assisted method for the determination of hair cuticle pattern and used it in species or group identification of mammals.

In this study we tried to find out the difference in the histological properties of hair and skin derived from the frontal regions of buffalo, camel, donkey and dog using light and scanning electron microscope. Histological features of hair structures as well as the cortico/medullary ratio was taken into consideration. Skin differentiation based on light microscopical examination of skin layers and skin thickness variation. Scanning electron microscope was used to differentiate between the surface structures of hair (cuticle) as well as hair arrangement on the surface of the skin.

MATERIALS and METHODS

Five groups of hair and five samples of skin were collected from the frontal region of each of normal healthy mature buffalo, camel, donkey and dog. These samples were prepared for morphological and histological examination using light and scanning electron microscopey in the following manners:

I- Examination of hair by light microscope:

Hair samples were collected from the same region of the head, cleaned using a mixture of alcohol and ether, and then cleared by xylol and subjected to examination.

II- Scanning Electron Microscope:

Samples were fixed in 5% glutaraldehyde in 0.1 M cacodylate buffer for 4 hours at 4°C. They were post-fixed in osmic acid for further 2 hours. After then, they were washed, dehydrated in ethanol, processed in amylacetate and critically-point dried with CO_2. They were sputtered with gold and examined with JEOL scanning electron microscope.

III- Histological examination of the skin:

Skin samples were collected in 10% formaline solution. The length of each piece of skin being parallel to the slope of the hair to provide a correct orientation for the vertical sections cutting. The skin specimens were hair clipped, then dehydrated using ethanol. Paraffin blocks were prepared and cutted into thin sections typically 7 μ thickness. Haematoxyline and eosin stains were used for general histological examination of the skin samples.

IV- Measurement of the hair and skin thickness:

Main computerized program (Image analysis system, Leica Q500) was used for measuring the total thickness of the hair, as well as the thickness of the cortex and medulla. Corticomedullary ratios were calculated mathematically. Total skin thickness as well as skin layers, depth of sweat glands as the length between the surface of the skin and last sweet gland detected in the section were measured using the same program.

Results were statistically analyzed by using SAS program (1990).

RESULTS

I- Morphological examination of hair sample by light microscope:

Buffalo hair looks the most thicker animal hair (72.21 ± 5.01 μ) compared with hair thickness of camel, donkey and dog, (33.83 ± 0.45; 66.52 ± 1.18 and 43.02 ± 1.22 μ, respectively, table 1 & plate 1). Medulla in dog looks the lest in thickness if compared with other animals where it takes about 44.30% of the total thickness of the hair. Donkey has the thickest medulla, where it takes 65.66% of the total thickness. In Camel and buffalo, medulla takes about half of the thickness, (51.09 and 48.60%, respectively). Ratio between medulla and cortex were 1.06:1; 1:1.05; 1:1.9 and 1:0.8 in buffalo, camel, donkey and dog, respectively.

Medulla in buffalo and donkey hair did not extend to the root, while in camel and dog medulla was observed directly after bulb of hair, the same observation was recorded by SEM examination (Plate 2 & 3).

Table 1: Measurements (μ) of hair of frontal region in different animals (Mean ± S.E.)

Animal Species	No. sample	Hair Thickness		Cortex		Medulla	Ratio	
		Mean	%	Mean	%	Mean	%	Med. : Cort.
Buffalo	150	72.21±5.01[a]	100	35.32± 2.76 [a]	48.91	37.51± 2.39 [b]	51.09	1.06 : 1
Camel	150	33.83± 0.45[c]	100	17.39± 0.28 [c]	51.40	16.77± 0.58 [c]	48.60	1: 1.05
Donkey	150	66.52± 1.18[a]	100	22.84± 0.41 [b]	34.34	43.57± 0.93 [a]	65.66	1 : 1.9
Dog	150	43.02± 1.22[b]	100	23.96± 0.56 [b]	55.70	18.9± 0.76 [c]	44.30	1 : 0.8

Means with the same letter are not significantly different ($p \leq 0.001$)

II- Scanning Electron Microscope of hair and skin:

Outer surface of the hair (cuticle) in buffalo and dog looks as irregular arrangement of scales allover the length of hair, while in camel and donkey, it looks as regular scales arranged in rows allover the hair length (Plate 4). Cross section on hair shaft showed that medulla composed of stacked and juxtaposed cells and of hair chambers (the aerial vesicles). The vesicles were surrounded by wall varied in shape from species to another. Medulla of buffalo composed of large vesicles with smooth and regular wall. In camel, medulla composed of small vesicles and irregular wall. Medulla in donkey was similar to buffalo but with rough and irregular wall thickness, while dog medulla has characteristic shape, where it arranged as similar disks (Plate 5).

The arrangement of hair follicles in camel and dog, was as bundles consists of group of hair follicles, each hair follicle originate separately in camel, while in dog, the group of hair bundles have same origin. In Buffalo and donkey, hair follicles distributed hemogenizally allover the surface of the skin (Plate 6). Histological sections confirm these findings (Plate 7).

III- Histological examination of the skin by light microscope:

Big variations between the total thickness of the skin in different examined animals were recorded. The total thickness was 923.1 ± 247.1, 1715.6 ± 243.1, 607.04 ± 118.4 and 1103.1 ± 282.9 μ for buffalo, camel, donkey and dog, respectively. The thickest skin was recorded in camel and the thinnest one was recorded in donkey (Table 2).

In buffalo general layers of epidermis was observed and it takes about 51.7 ± 8.8 μ thickness and stratum basal showed few pigmentation. The superficial layer (stratum corneum) appeared thin, as few threads and takes about 11.3 ± 2.8 μ, which considered the thinnest one compared with the other examined species (Table 2 & Figure 8a). The epidermal thickness in camel skin is about 83.5 ± 10.4 μ and the stratum basale composed of one layer rest on the basement membrane with dark melanin pigmentation. Stratum corneum in camel is very thick (56.6 ± 9.5 μ) and composed of several lamellated layers (Table 2 & Figure 8b).

In Donkey the thickness of the epidermis is about 37.7 ± 6.2 μ, the stratum basal composed of a single layer of high cuboidal type of cells with diffused melanin pigments in the cytoplasm. Stratum corneum composed of several layers of dead keratinized cornified flattened non-nucleated cells with average thickness of 15.03 ± 3.9 μ (Table 2 & Figure 8c). While in dog, the epidermis consists only of four layers, stratum basal, stratum spinosum, stratum granulosum and stratum corneum and its thickness is 41.7 ± 8.8 μ. The basal cell layer have nuclei and smaller amount of cytoplasm and more intensely stain than the superficial cells. Stratum corneum is thin layer at about 29.9 ± 5.9 μ, and consists of several layers of keratinized layers (Table 2 & Figure 8d).

The thickness of dermis in the same studied area was also varied according to animal species, where the thickest dermis was recorded in camel (1632.1 ± 123.8 μ), while the thinnest one was recorded in donkey (569.3 ± 98.3 μ). The thickness of the dermis in buffalo is about two times more than that in camel. This layer contain the hair follicles, sebaceous and sweat glands. In camel and dog the bundles of the hair follicles and the sweat glands extend more deeper in the dermis just above the region of subcutaneous tissue. The thickness of the dermis in buffalo and dog were 872.4±108.6 and 1061.4±99.7 μ, respectively.

Hair follicles in skin of frontal region of buffalo and donkey are evenly distributed in the superficial layer of the dermis as single hair. On the

other hand camel hair are more delicate than that of buffalo and distributed in-groups or bundles (Plate 9). In canine skin, hair follicles occur in groups each consists of one main hair and three to five accessory hairs, all of which arise out of a common hair bed with the supplemental hairs surrounding the main hair in a half circle.

In buffalo the sweat gland is simple tubular glands and its secretory portion has a large dilated lumen, While in camel the sweat gland is highly coiled simple tubular gland, lined with cuboidal to low columnar epithelium. In donkey sweat glands are large in number surrounded the hair follicle and occur relatively superficial. It consists of cuboidal to low columnar epithelium while, in dog, sweat glands are located deep in the dermis and one gland is associated with each hair follicle complex. The body of the gland is composed of widely dilated secretory tubules of single layer of columnar cells with basophilic cytoplasm containing pigment granules and spheroid nucleus. The depth of the sweat gland varies according to animal species and the physiological function of the skin. The sweat gland in buffalo and donkey is superficial and occur in depth about 870.3 ± 169.5 and 583.8 ± 76.5 μ, respectively, while in camel and dog are of no need of large sweating, the sweat gland occur very deep (1685.9±193.8 and 1078.9 ± 30.1 μ (Table 2 & Plate 8).

Table 2: Average measurements of skin (μ) of different animals

Animal species	Skin thickness	Thickness of corneum	Thickness of epidermis	Thickness of dermis	Depth of sweat gland
Buffalo	937.6± 247.1^{c}	11.3± 2.8^{d}	51.6± 8.8^{b}	886.1± 108.6^{c}	765.2± 93.4^{c}
Camel	1944.4± 243.1^{a}	56.6± 9.5^{a}	83.5± 10.4^{a}	1860.9± 123.8^{a}	1425.8± 123.2^{a}
Donkey	629.6± 118.4^{d}	15.5± 3.9^{c}	36.6± 6.5^{d}	593.0± 98.3^{d}	517.4± 82.7^{d}
Dog	1141.3± 148.2^{b}	30.6± 5.4^{b}	41.7± 8.8^{c}	1099.6± 99.7^{b}	1064.4± 46.4^{b}

Means with the different letter are significantly different ($p \leq 0.001$)

DISCUSSION

Hair and skin examination provides a very important clews to detect meat adulteration where adulteration of minced meat through addition of different kinds of meat and tissues of various origin in

various percentage to increase their gain was recorded by Refai (1982). Skin with hair has been recorded as one of the tissue that added for adulteration (Julini and Parisi, 1978).

Hair composed of cuticle, cortex and medulla, this general structure of hair was previously mentioned by Steven and Lowe (1977) and Froede *et al.* (1981). The results of this study revealed that there was a species variation in the histological structure of hair. Morphometric measurements of hair showed also great difference between hair from different animal species. Scales examination of hair and skin surfaces using scanning electron microscopes revealed a big difference from animal to another. Our results for hair measurements revealed similarity in the thickness of both medulla and cortex with slight difference where medulla occupy 51.09 % and 48.60 % of the total hair thickness in buffalo and camel, respectively. These results disagree with the results obtained by Dougmag and Berg (1983) as they mentioned that the hair medulla forms the great bulk of the hair in camels meanwhile, the cortex forms the great bulk in buffalo. They also recorded that the cortex in camel hair has no pigments and this result relatively disagree with our results, where we recorded cortical pigments in some camel hairs. Our results also revealed that donkey has the thickest medulla and it occupies 65.66 % of total hair thickness while dog has the thinnest medulla (44.30%). In the present work, the shape of hair cuticle was varied between animal species, this results was in agreement with Abou-Salem, *et al.* (1999).

Our results were in agreement with that of Dowling and Nay (1962) who mentioned that the hair follicles in camel formed groups, containing one primary follicle at the base, flanked by two or three secondary follicles and much tertiary ones and contradict to that of Nair and Benjamin (1965) who mentioned that there are no hair groups.

The hairs in canine skin occur in groups. Each group usually consists of one main hair and three to five accessory hairs, all of which arise out of a common hair bed with the supplemental hairs surrounding the main hair in a half circle. Jenkinson (1965) stated that hairs occurred singly in cattle and in groups in carnivores, swine and smaller ruminants. The hairs in canine skin occur in groups, each consists of one main hair and three to five accessory hairs, all of which arise out of a common hair bed with the supplemental hairs surrounding the main hair in a half circle. James and Getty (1957), were in agreement wit our results, where they found that the hair follicles are arranged in groups of two, three or four with the triad arrangement being the most numerous. They also

mentioned that the multiple hairs in the follicle diverge in the deeper layers of the dermis and branch out into separate follicles, in which the hair originate from individual hair bulb. The hair follicles and apocrine sweat glands extend into the subcutaneous adipose tissue. Epidermal papillae are normally present on the surface of the epidermis of the hairy skin of the dog.

Medulla appeared different from animal to another using SEM, where in camel it is rich in small vesicles, in donkey its large vesicles and its wall was rough and unequal thickness, while in buffalo it composed of large vesicles with smooth and regular wall's thickness. These results were in accordance with that recorded by Clement *et al.* (1981).

The results showed that the thickest skin was recorded in camel, and the thinnest was observed in donkey. While the thickest epidermis were in buffalo, followed by camel, donkey and dog respectively. These results disagree with that obtained by Talukdar (1972) as he found that the thickness of epidermis in cattle and horse obtained from same region was in the same thickness. The pigment in stratum basal in buffalo was few compared with donkey and camel, this result was in harmony with Shahjahan *et al.* (1997). The stratum corneum is considerably thicker in skin of occipital region of camel than that in the same region in buffalo, donkey and dog due to presence of heavy protective coat of hair of camel which prevent its desquamation and to accommodate with its function to minimize water evaporation.

Our results revealed that the sweat glands in donkey were large in number and superficially located around the hair bulb. These results were in harmony with that of Talukdar *et al.* (1970) who mentioned that the skin of horse is richly supplied with well developed sweat. In the skin areas, which contained glands, there was always one gland associated with each hair follicle, located either below the level of the follicle or surrounding it. In dog, Apocrine sweat glands are located deep in the dermis one gland is associated with each hair follicle complex. The body of the gland is composed of widely dilated secretory tubules of single layer of columnar cells with basophilic cytoplasm containing pigment granules and spheroid nucleus. Muller and Kirk (1976) recorded similar findings.

Finally it could be concluded that the use of scanning electron microscope in hair and skin surface examination is an advanced tool which is very useful in medicolegal differentiation of different animals as well as in the detection of meat adultration.

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LEGENDS

Plate 1: Different animals hair by light microscope a) Buffalo b) Camel c) Donkey d) Dog, showing the differences between cortex and medulla, and the special shape of medulla in dog X 40.

Plate 2: Distal end of the hair in different animals by light microscope a) Buffalo b) Camel c) Donkey d) Dog, showing disappearance of the medulla in the distal end of the hair in case of buffalo (X 6.3).

Plate 3: SEM of cross section near the bulb of different animals hair a) Buffalo b) Camel c) Donkey d) Dog, showing the medulla and cortex in camel and dog and only cortex in buffalo and donkey (X 500, 240, 1500 and 500, respectively).

Plate 4: SEM of different animals hair a) Buffalo b) Camel c) Donkey d) Dog, showing the outer layer of the hair (cuticle), X 1000, 1000, 1500 and 1000, respectively.

Plate 5: SEM of cross section of different animals hair a) Buffalo b) Camel c) Donkey d) Dog, showing shape of the medulla (X 750, 750, 750 and 1000, respectively).

Plate 6: SEM of different animals hair a) Buffalo b) Camel c) Donkey d) Dog, showing the arrangement of the hair in the skin (X 15, 50, 200 and 50, respectively).

Plate 7: Longitudinal section in skin of different animals a) Buffalo b) Camel c) Donkey d) Dog stained with H & E: showing the typical histological structure of the skin with its histological difference and arrangement of hair follicle (X 4, 4, 6.3 and 6.3, respectively).

Plate 8: Longitudinal paraffin section of skin of different animals hair a) Buffalo b) Camel c) Donkey d) Dog, stained with H & E: showing the epidermal structure and the keratinized layers (X 40, 40, 40 and 25, respectively).

Plate 9: Cross in paraffin section of skin of different animals hair a) Buffalo b) Camel c) Donkey d) Dog, stained with H & E: showing the structure of sweat glands (X 40, 40, 40 and 25, respectively).

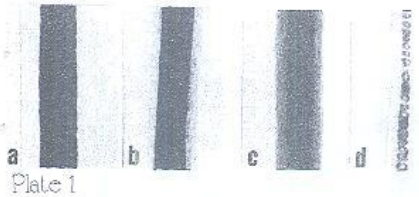

Plate 1

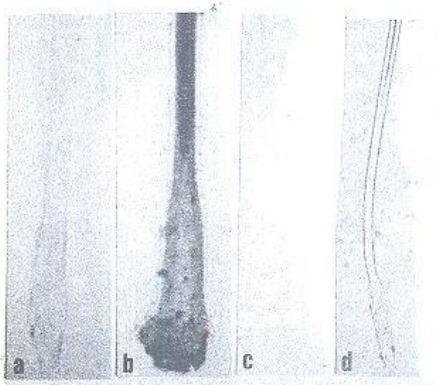

Plate 2

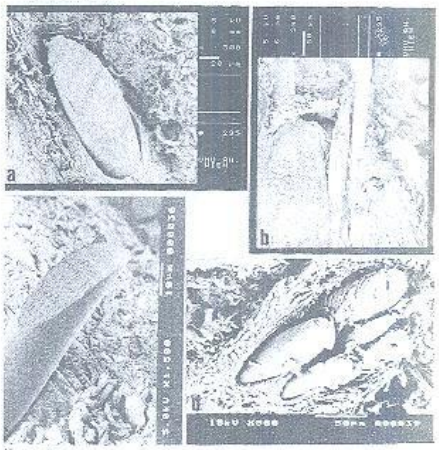

Plate 3

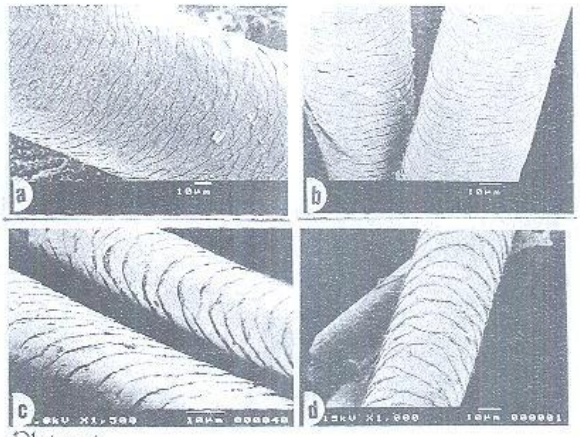

Plate 4

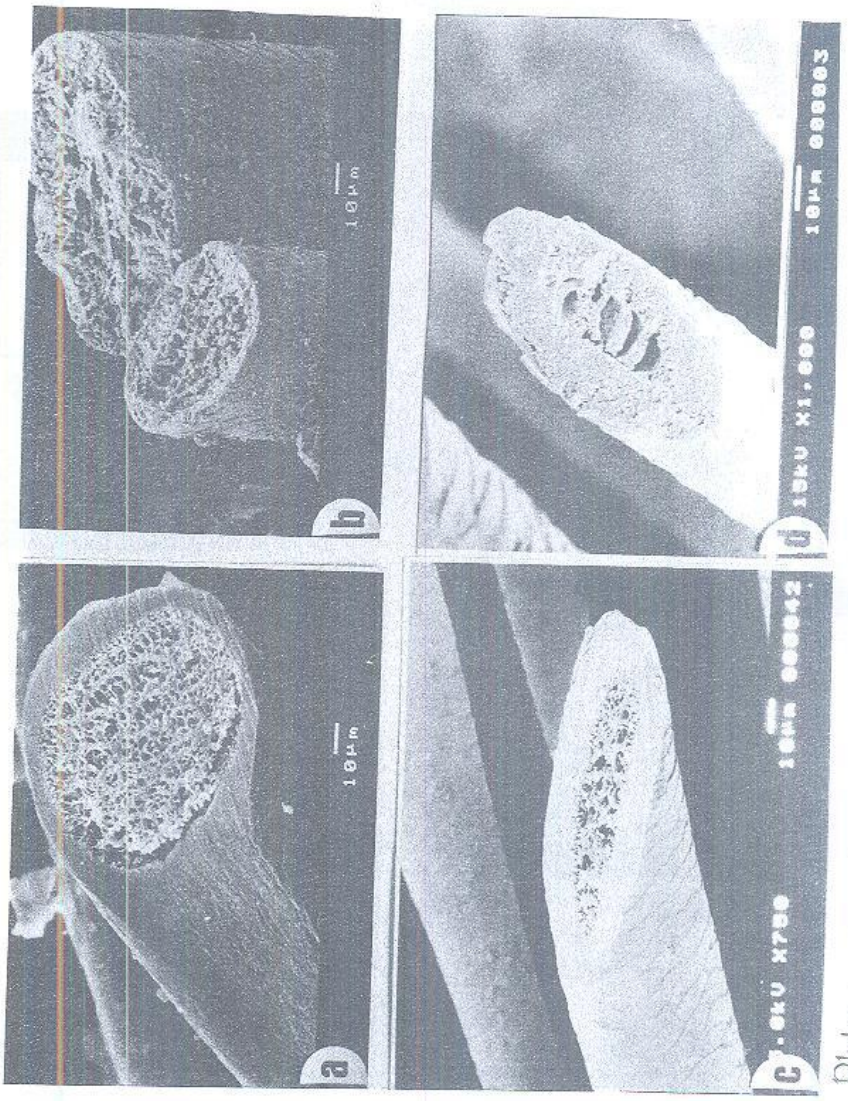

Plate 3

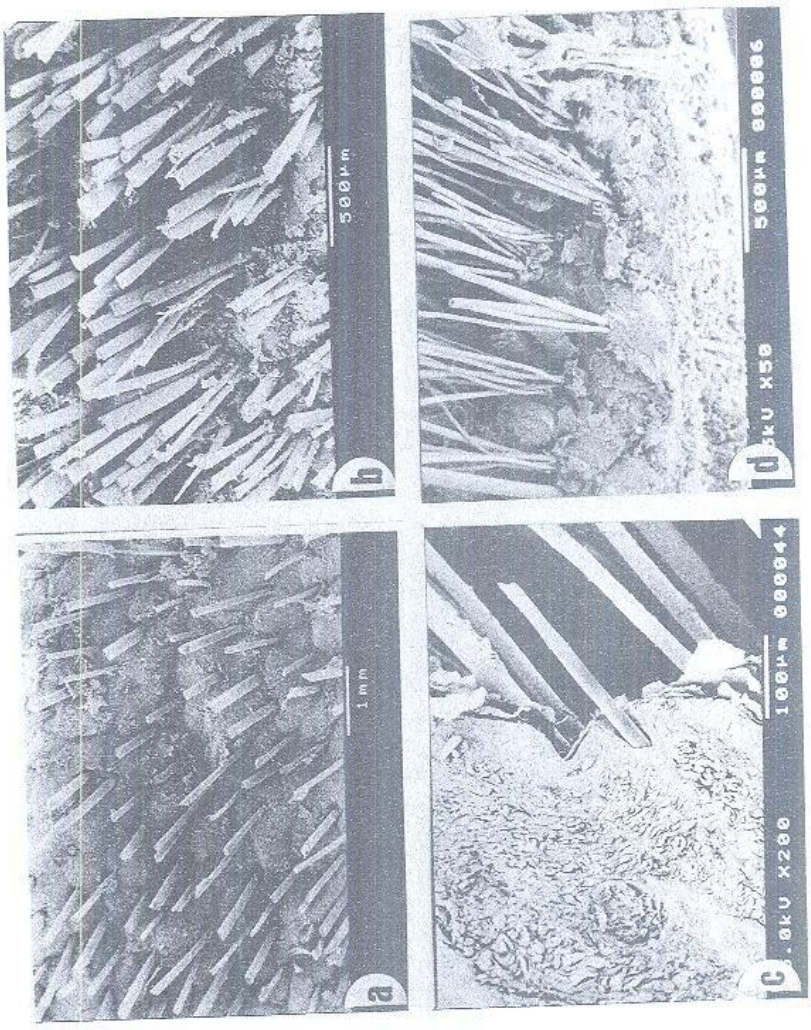
a
1mm
b
500µm
c
20.0kU X200 100µm 000044
d
20kU X50 500µm 000006

Plate 6

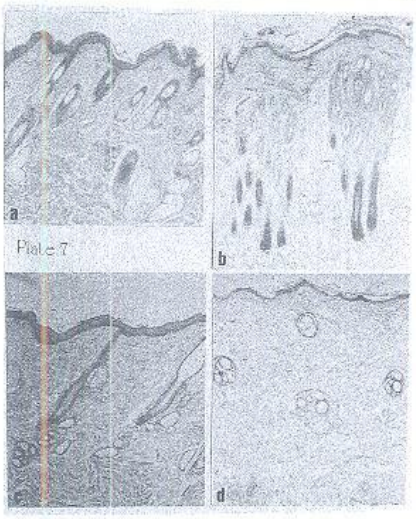

Plate 7

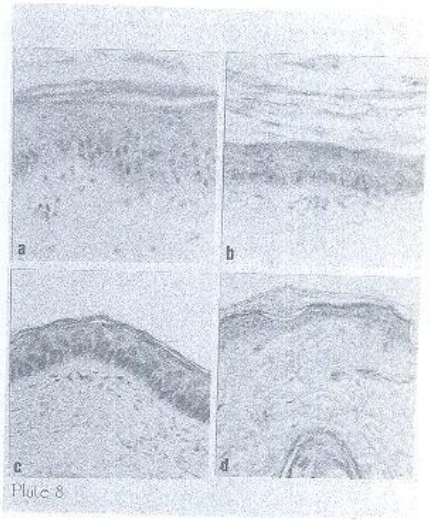

Plate 8

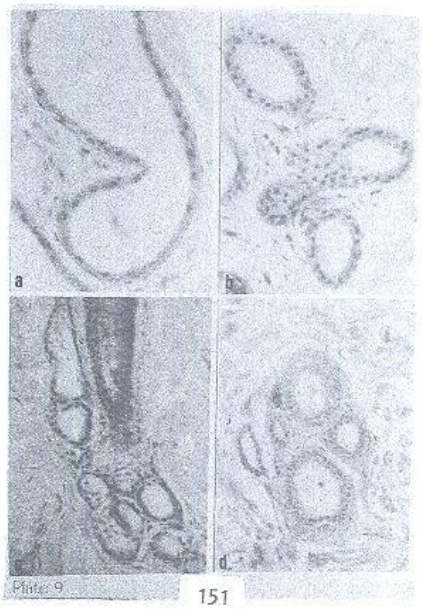

Plate 9