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Langerhans cells and the architecture of the epidermis in the chick and mouse

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LANGERHANS CELLS AND THE ARCHITECTURE OF THE EPIDERMIS IN THE CHICK AND MOUSE

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ABSTRACT

For more than a century, Langerhans cells have been known to occur in the mammalian epidermis. Experimental evidence has shown that their origin is ectodermal (Reams and Tompkins, 1973). Almost nothing is known of their function. Mackenzie (1972) has shown a good correlation between the arrangement of epidermal Langerhans cells in the mouse and the ordered structure of the epidermis into columns. He has suggested that Langerhans cells might serve as an active organizer of epidermal structure.

As chickens do not have epidermal Langerhans cells, the present histological study was undertaken to compare the epidermal architecture of chick and mouse skin. As anticipated, Langerhans cells showed a spacial relationship with the columns of cells in the upper epidermis of the mouse. As compared to the mouse, the epidermis of the chick was relatively thin. Although there was extensive dovetailing, especially in the stratum corneum, the cells of the stratum granulosum and stratum corneum were arranged in columns. It appears that in the chick the ordered structure of the upper layers of epidermis is not dependent upon the presence of Langerhans cells.

INTRODUCTION

In 1868 Paul Langerhans first described dendritic cells in the suprabasal layers of the epidermis which stained with an acid solution of gold chloride (Breathnach and Wyllie, 1966). These dendritic cells, Langerhans cells, have now been found not only in the basal and suprabasal layers of the epidermis (Breathnach, 1965; Prunieras, 1969) but also in nonepidermal tissue (Wong and Buck, 1971).

Although the identification of Langerhans cells with most light microscope staining methods is unreliable (Breathnach, 1965), they are stained specifically with the ATPase method (Wolff and Winklemann, 1967). However, the usefulness of the ATPase method is restricted to the epidermis.

Ultrastructurally, the Langerhans cells possess characteristics which provide a basis for their positive identification (Prunieras, 1969; Breathnach and Wyllie, 1966; Breathnach, 1965; et al). In low power electron micrographs the characteristic feature which distinguishes Langerhans cells from the surrounding keratinocytes is a markedly indented nucleus. The intranuclear material presents a finely granular appearance of varying density, and a nucleolus is present. The cytoplasm is free of tonofilaments and keratin granules. Also, the plasma membrane is

free of desmosomes. Typical Golgi membranes, centriolar structures, mitochondria, and endoplasmic reticulum are present.

Large vacoules are often seen in the cytoplasm. They contain ill-defined granular or reticular material. Single membrane bound structures with amorphous, granular or lamellated internal structures are frequently found. Morphologically, they are similar to lysosomes. However, the demonstration of hydrolases in these structures has not been given yet (Prunieras, 1969). Fully melanized melanosomes are sometimes found in lysosome-like vesicles (Breathnach and Wyllie, 1966; Mishima, 1966; Zelickson, 1965) but unequivocal premelanosomes have never been observed in the Langerhans cell (Prunieras, 1969).

Rod and racket-shaped profiles are present in Langerhans cells (Birbeck, 1961; Zelickson, 1967; Breathnach, 1965). These structures represent sectional profiles of disc shaped bodies containing four sheets of regularly spaced particles forming a three-dimensional lattice (Wolff, 1967). Three-dimensional models reconstructed after serial section reveal that these bodies are made up of a flattened or curved orthogonal net of particles which is bound externally by a limiting membrane (Sagebiel and Reed, 1968). They are referred to as Langerhans or Birbeck granules. Although Birbeck granules have been regarded as specific to Langerhans cells (Prunieras, 1969), they have been found in nonepidermal cells (Reams and

Tompkins, 1973). Most likely these granules are innocuous cytoplasmic inclusions resulting from incidental cytomembrane overfolding and adhesion (Reams and Tompkins, 1973).

Only recently has the origin of the Langerhans cells been ascertained. Most commonly Langerhans cells have been thought to be related to pigment cells but Reams and Tompkins (1973) have shown that they are not related. Pigment cells arise from the neural crest crest cells (Rawles, 1947) while Langerhans cells arise from ectoderm (Reams and Tompkins, 1973).

The function of the Langerhans cells has not yet been ascertained. Ultrastructural and histochemical evidence indicates that the Langerhans cell is an active functioning epidermal element. Since the Langerhans cell is in fully differentiated form in the human fetal epidermis as early as the fourteenth week, Breathnach and Wyllie (1966) have suggested that its most likely function is to influence somehow the surrounding keratinocytes.

Recently, the cells of the stratum corneum of mammalian skin have been shown to be aligned in regular columns (Christopher, 1971; Mackenzie, 1969). Mackenzie (1972) has shown that the Langerhans cells of the murine epidermis lie beneath the center of these columns. He has suggested that the Langerhans cell may have a function related to some aspect of the activity of keratinocytes during the establishment of

the columnar structures.

Experimental evidence has shown that chick skin does not contain Langerhans cells (Reams and Tompkins, 1973). Since there has been no architectural descriptions of the stratum corneum of the chick, a comparative study of the stratum corneum of the White Leghorn chicken and the PET/Wmr mouse was undertaken of determine if the architecture of the chick stratum corneum was arranged in columns like the stratum corneum of mammalian skin.

METHODS AND MATERIALS

In this study, White Leghorn chickens and mice of the PET/Wmr strain (Reams, 1967) were used. Fertile eggs were obtained from a flock of highly inbred White Leghorn chickens which were maintained at a local hatchery. These eggs were incubated in a David Bradley cabinet incubator which was maintained at 38 C with a constant level of humidity. After hatching, the chicks were kept and nurtured until they reached certain stages of development. Mice of the PET/Wmr strain which were maintained at the University of Richmond were used because of availability and the background of previous studies on their Langerhans cells.

In order to examine the architecture of the stratum corneum of the chick, representative skin samples of the saddle, abdomen, and thigh regions were obtained from young, juvenile, and adult chickens. Skin samples were obtained also from the abdomen and back of young and adult PET mice. As a cryostat, which is the instrument of choice, was not available, the skin samples with surface dimensions of 5 by 10 mm were prepared for study by the paraffin method.

After removing hair or feathers and subcutaneous fat, the skin samples were prepared for the paraffin method by placing them on squares of Whatman #1 paper which were slightly larger

than the samples. Then the samples were fixed in Bouins solution for at least 24 hours. After washing in distilled water, the samples were dehydrated with alcohol. Next, the alcohol was removed with xylene, and the samples were embedded in paraffin. Each sample of skin was then cut into ribbons at 4 microns with a microtome. The ribbons were placed on microscope slides and stained with Ehrlich's hematoxylin. Next, balsam and a cover slip were put on the slide, and the slides were examined microscopically.

Also, samples of skin from the mouse and chick were prepared by the histochemical method for ATPase (Wolff and Winklemann, 1967) to check for the presence of Langerhans cells. After removal of subcutaneous tissue and hair or feathers, the skin specimens that measured 1 by 1 cm were immersed with the dermal side adhering to Whatman #1 filter paper in a solution of 2 M sodium bromide for 45 minutes at 37 C. With the aid of a dissecting scope (X 20) and a pair of #5 Dumont forceps, the epidermis was carefully removed. These pure epidermal sheets were washed for five minutes in distilled water and fixed in 5% neutral formalin at 4 C for 20 minutes. After an additional rinse of 10 minutes in distilled water, the epidermal sheets were incubated free-floating for the demonstration of nucleoside triphosphate for 1½ hours at 37 C. Adenosine triphosphate was used as the substrate, and the incubation medium consisted of 10 ml of 125 mg per 100 ml of

solution of ATP disodium salt, 10 ml of 0.2 M (hydroxymethyl) aminomethane maleate (tris-maleate) buffer pH 7.2, 2.5 ml of 0.1 ml $MgSO_4$, 1.5 ml of 2% $Pb(NO_3)_2$, and 1.0 ml of distilled water. Great attention had to be given to the water. Only deionized, glass distilled, freshly boiled water would work because the amount of CO_2 and ion content greatly influenced the results. After the solution turned an opal color, it was filtered. Following incubation, the samples were rinsed three times in distilled water for a total of at least five minutes and developed in dilute ammonium sulfate for 10 minutes. Then they were rinsed in distilled water for sixty minutes and mounted in glycerin jelly with the dermis side up.

In order to determine the position of the Langerhans cells in relation to the squames of the stratum corneum, the mouse ear epidermis was used. Although the ATPase method was used, it was altered somewhat. After sulphiding, the samples were refixed in formalin for 20 minutes and stained in a solution of Sudan Black B in propylene glycol for 24 hours at 37 C. Then the skin samples were mounted flat in glycerin jelly on microscope slides with the dermis side down and were examined microscopically.

RESULTS

The epidermis of the mouse is composed of the following layers: the stratum basale, the stratum spinosum, the stratum granulosum, and the stratum corneum. The stratum basale is one cell thick; the stratum spinosum is approximately two cells thick; the stratum granulosum is approximately three cells thick; and the stratum corneum is approximately ten cells thick.

As previously shown by Mackenzie (1972), the cells (squames) of the stratum corneum of murine skin were found to be stacked one above the other to form columns. At the lateral edges of these columns, where adjacent cells met, a dovetail arrangement was found (Figure 1). This columnar arrangement existed not only in the stratum corneum but also extended down into the stratum granulosum. The regularity of the columnar arrangement seen in the vertical sections of the stratum corneum in the mouse skin depended on the thickness of the sections, on the degree of overlapping of squames of adjacent columns in proportion to their size, and on the angle of the plane of the section.

The most precisely ordered stacking of squames was seen in the murine ear epidermis. With the compound microscope, whole mounts of murine ear epidermis showed that the squames

were flattened discs with an irregularly polygonal, frequently hexagonal, outline (Figure 2). The regularity of the alignment of squames into columns and their degree of overlap was observed throughout the full thickness of the stratum corneum by changing the plane of focus with the microscope. Although there was some variation of polygonal outline from column to column, all the squames of a particular column were found to have a similar outline.

In addition, murine ear epidermal sheets from adult PET mice which were processed with the ATPase method and stained with Sudan Black B showed that after focusing through the thickness of the epidermis some of the Langerhans cells were centered beneath the columns (Figures 3 and 4). Other positions of Langerhans cells were observed also.

It can be seen in Figure 5 that chick epidermis is composed of the following layers: the stratum basale, the stratum granulosum, and the stratum corneum. While the stratum basale is only one cell thick, the stratum granulosum is approximately four cells thick, and the stratum corneum is approximately five cells thick.

Vertical sections of young and adult chick skin showed that the cells of the stratum corneum consisted of a number of layers of flattened keratinized cells or squames (Figures 6). These squames tended to be stacked, one above the other, to form a column of cells. Laterally, where the squames of

adjacent columns met, there was an overlap with the squames interdigitating with each other to produce a dovetail effect. This architectural pattern was seen also in the stratum granulosum of chick skin (Figure 7) from the thigh, saddle, and ventral abdomen.

A test with the ATPase method showed that chick skin does not contain Langerhans cells (Figure 8 and 9). In contrast, murine skin does contain ATPase-positive Langerhans cells in the basal and suprabasal layers of the epidermis (Figures 10 and 11).

DISCUSSION

In previous studies which examined the architecture of mammalian skin a cryostat was the instrument used. A cryostat allows one to use fresh, unfixed tissue which responds better to expansion of the squames. Thus the tissue can be studied with greater clarity. However, for the present study, as a cryostat was not available, various substitute methods were tried, and the paraffin method was found to be adequate but far from the most desirable method.

After treating cryostat sections of frozen unfixed skin from several mammalian species with sodium hydroxide which expands the squames, Mackenzie (1969) found a regular arrangement of the cells in the stratum corneum. These cells were shown to form vertical columns which ran from the stratum granulosum through the stratum corneum. Within each column, the squames were positioned one above the other, and at the lateral edges of the columns were two adjacent columns met, the cells interdigitated to form a dovetail pattern.

This arrangment of squames was found in the epidermis from man, dog, rabbit, rat, and mouse. However, there was some variation with respect to both species and site in the regularity of positioning of individual squames and in the

degree of their lateral interdigitation (Mackenzie, 1972). Certain specialized areas such as the palmar surfaces, the plantar surfaces, the aerola of the nipple, the vagina, the lip, and the oral mucosa of mammalian skin did not show this architectural pattern (Mackenzie, 1973). Moreover, primate epidermis seldom demonstrated an order as precise as that seen in rodents.

The most precise ordered stacking of squames has been seen in thin epidermis such as that covering the pinnae of rodents (Mackenzie, 1972). The stratum corneum of such tissues were found to form a closely united assembly of squames in which individual squames were positioned one above the other to form a column of cells running from the stratum granulosum to the surface. While most suprabasal cells were found to be flattened and aligned beneath overlying columns of squames, the basal cells showed no precise positioning. Laterally, where adjacent columns met, the cells were found to interdigitate and form a dovetail pattern.

It has been shown that after staining intact unsectioned sheets of mouse epidermis the squames were flattened discs with an irregularly polygonal, frequently hexagonal, outline (Mackenzie, 1972). Although some variation was found to occur from column to column, all the squames of a particular column formed a similar outline. It was found that the junctions between adjacent columns appeared as parallel bands running

around each column. Scanning electron micrographs of squames have shown this to be a step like depression (Menton and Eisen, 1971).

Upon examining sheets of mouse ear epidermis after incubating for ATPase and staining with Sudan Black, Mackenzie (1972) has shown that ATPase-positive dendritic Langerhans cells tend to lie beneath the center of overlying columns. However, in some fields of vision this positioning was found to be more precise than in others. Mackenzie (1972) stated that the Langerhans cell may have a function related to some aspect of the activity of keratinocytes during the establishment of columnar structure.

In this present study, a check of the stratum corneum of murine epidermis showed that the squames were aligned in columns (Figure 1). Also, the stratum granulosum was aligned in columns. At the edges of the columns where adjacent cells met, the squames interdigitated to form a dovetail effect. In addition, whole mounts of mouse ear epidermis showed that the squames had a polygonal, often hexagonal, outline (Figure 2). When mouse ear epidermis was processed with the ATPase method and stained with Sudan Black, the ATPase-positive Langerhans cells had a tendency to lie beneath the center of the overlying squames (Figures 3 and 4). However, this central position of the Langerhans cells was not found in all cases. Moreover, this present study confirms the

observations of Mackenzie (1969 and 1972).

Since the ATPase test showed that chick skin does not contain Langerhans cells (Figures 8 and 9), it was examined to see if the stratum corneum had a architectural pattern similar to that of the murine stratum corneum. Examination showed that chick epidermis is composed of the following layers: the stratum basale, the stratum granulosum, and the stratum corneum (Figure 5). The stratum corneum (Figure 6) and the stratum granulosum (Figure 7) were arranged in columns. In contrast to the stratum corneum of murine epidermis, the interdigitation of the chick's stratum corneum was much more extensive.

That the stratum corneum and stratum granulosum of chick skin were arranged in columns but did not contain Langerhans cells implies that Langerhans cells are not essential for the ordered structure of the epidermis. Although the present study would suggest that the Langerhans cells do not function as an active organizer in the chick epidermis, their function as an organizer in mammalian epidermis should not be ruled out.

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Figure 1. Cross section of adult murine ear epidermis. Squames of stratum corneum are arranged in columns, and the squames interdigitate to form a dovetail arrangement. ◄— Arrows show where the interdigitation of squames occur. X 450

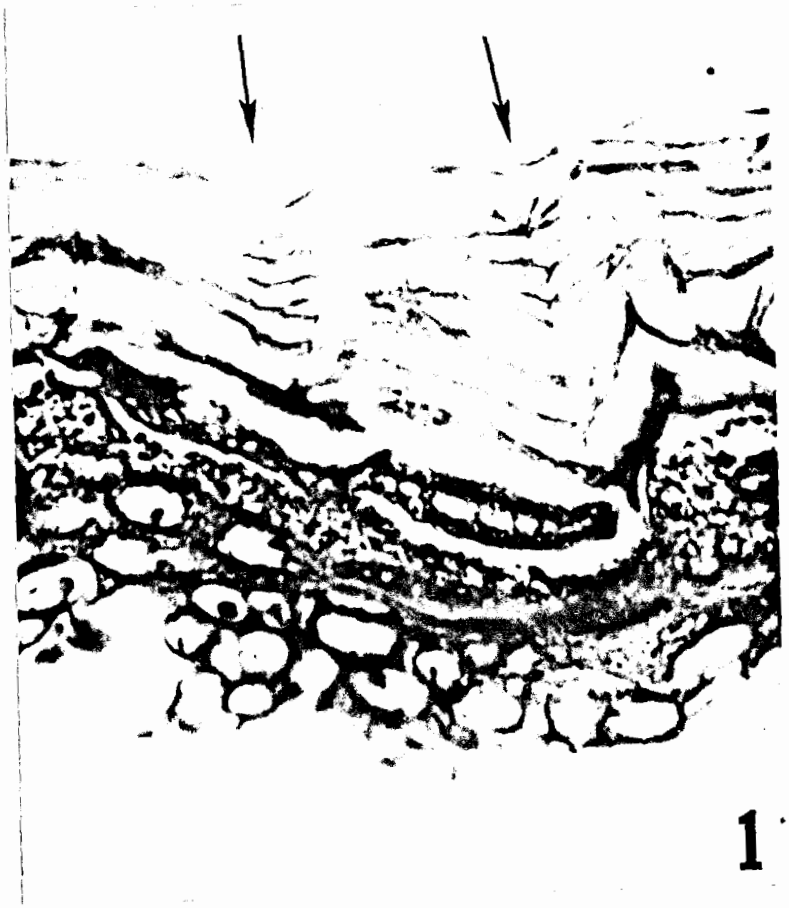

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Figure 2. Whole mount of adult murine ear epidermis. Surface view showing squames as flattened discs with irregularly polygonal outline. X 450

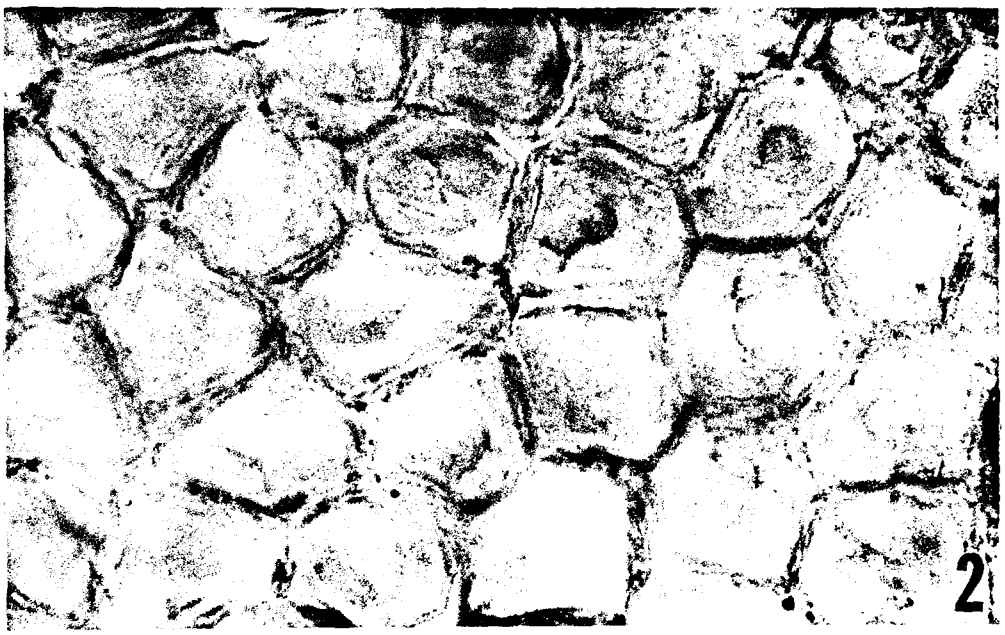
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Figure 3. Whole mount of adult murine ear epidermis. Showing ATPase-positive Langerhans cells in the basal layer. - Langerhans cells X 450

Figure 4. Surface view of above whole mount showing squames as they relate to positions of Langerhans cells below them. X 450


LC1
LC2
LC3
3


LC1
LC2
LC3
4

Figure 5. Cross section of young chick abdomen. ← Arrows indicate dovetailing edges of squames. X 450

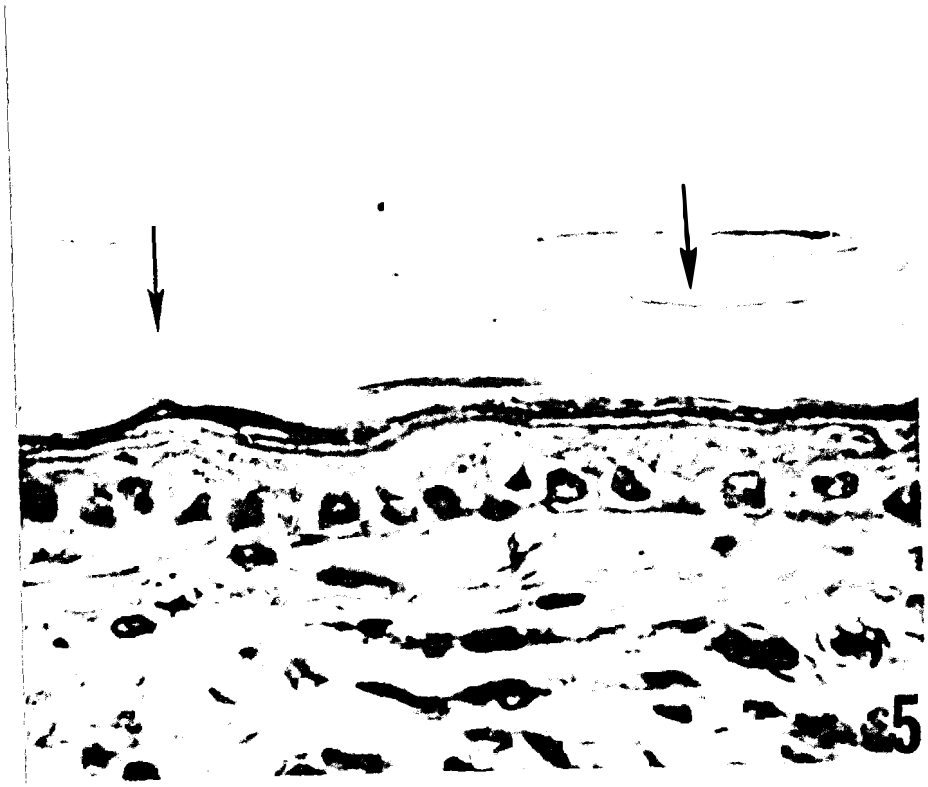
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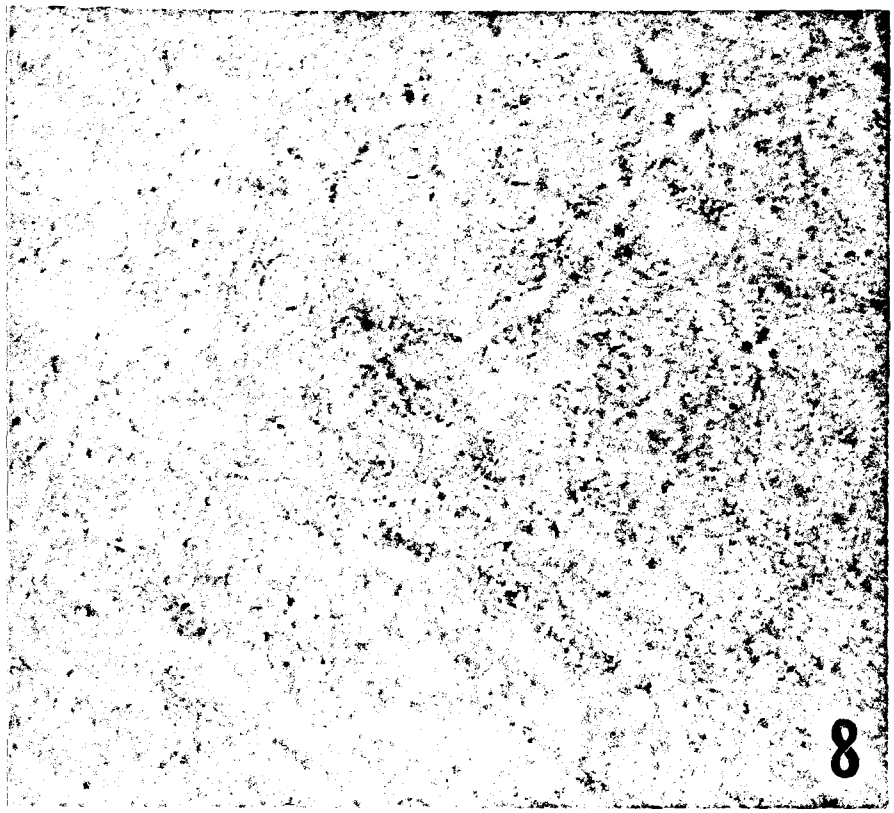
Figure 6. Cross section of young chick thigh.
◄—— Arrow indicates extensive dovetailing arrangement of cells in the stratum corneum.
X 450

Figure 7. Cross section of young chick thigh. Stratum granulosum (SG) is arranged in columns, and dovetailing is seen. X 450


SG
7

Figure 8. Whole mount of adult chick abdomen. ATPase negative. Suprabasal layer. No Langerhans cells present. X 450

Figure 9. Whole mount of adult chick abdomen. ATPase negative. Basal layer. No Langerhans cells present. X 450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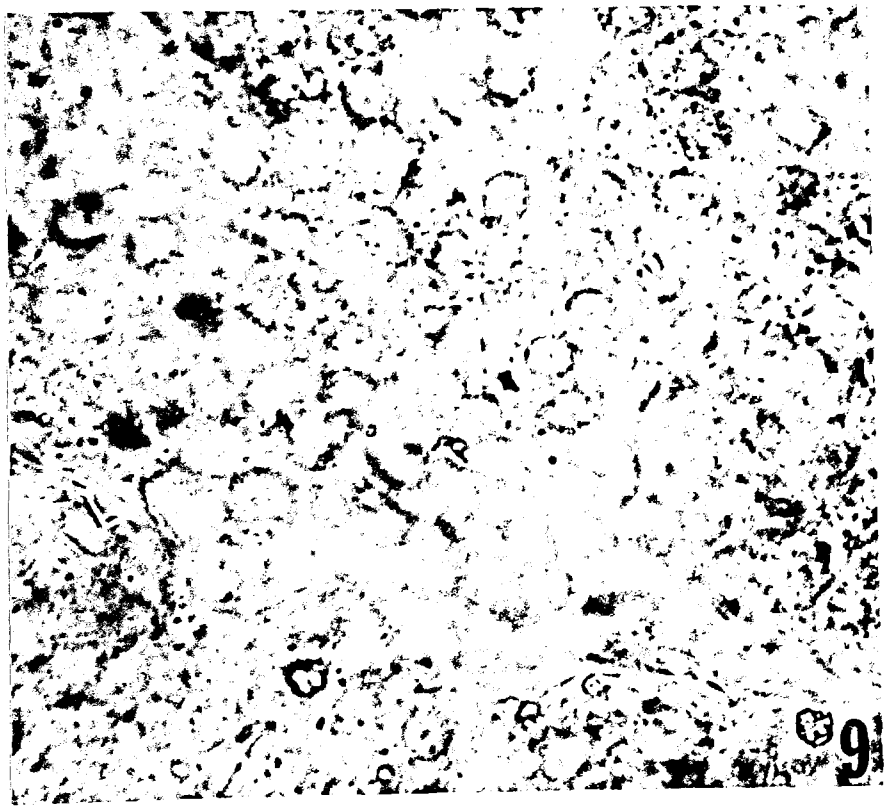
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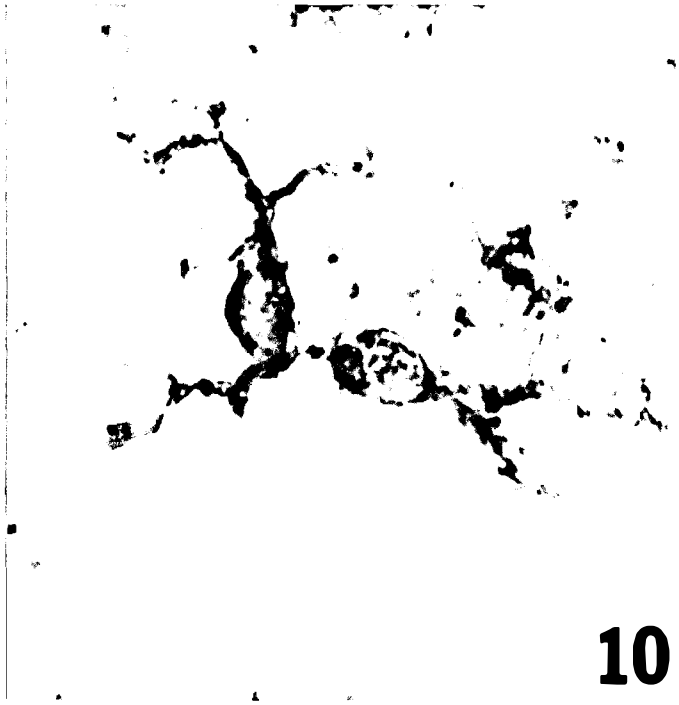
Figure 10. Whole mount of young murine back. ATPase-positive Langerhans cells are darkly stained. X 650

Figure 11. Whole mount of young murine back. ATPase-positive Langerhans cells are darkly stained. X 450



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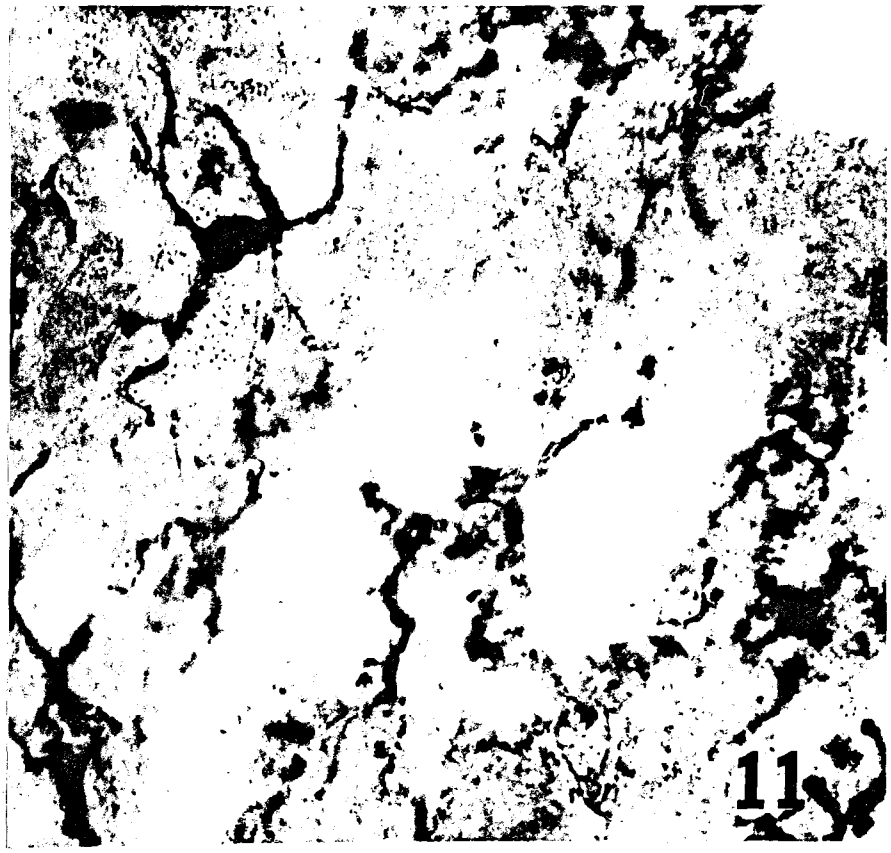

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VITA

William Clyde Williams III was born in Richmond, Virginia on September 19, 1949. He attended Central High School of Lunenburg and graduated in June, 1967. He entered Ferrum Junior College and graduated with an A. S. degree in Science in June, 1969. He entered the University of Richmond and graduated with a B. S. degree in Biology in June, 1971. In August, 1972 he entered the Graduate School of the University of Richmond in Biology. He received his Masters of Science in May, 1974. In August, 1974 he will enter the first year class in the school of medicine at the Medical College of Virginia.