THE JOURNAL OF INVESTIGATIVE DERMATOLOGY

Vol. 58, No. 4
Printed in U.S.A.

ACETYLCHOLINESTERASE-POSITIVE LANGERHANS CELLS IN THE EPIDERMIS AND WOOL FOLLICLES OF THE SHEEP*

D. E. HOLLIS, F.A.I.M.L.T. AND A. G. LYNE, Ph.D.

ABSTRACT

Dendritic acetylcholinesterase-positive cells in the basal layer of the epidermis and in wool follicles of the sheep have been identified ultrastructurally as Langerhans cells using a copper ferrocyanide technique to demonstrate acetylcholinesterase. This enzyme is located on the outer surface of the plasma membranes of the Langerhans cells and in the intercellular spaces between these cells and the adjacent epithelial cells. No enzyme activity has been detected within Langerhans cells.

Langerhans cells in fetal and adult sheep are morphologically similar and no developmental stages have been detected. Ultrastructurally these cells are similar to those described in other mammals. However, in sheep epidermis they are restricted to the basal layer and are less frequent than in man or guinea pig where they are mainly found in the spinous layer. Langerhans cells in the sheep, which form a system in contact with all keratinocytes in the basal layer, may have a significant influence on the function of the epidermis.

Although acetylcholinesterase (AChE)-positive dendritic cells have been observed in the epidermis of the sheep with the light microscope (1) their identity has not previously been determined. Similar enzyme-reactive dendritic cells in the epidermis of other mammals (2, 3) have been identified as Langerhans cells using histochemical-electron microscope methods. The present study was undertaken to determine whether the AChE-positive cells in the sheep are in fact Langerhans cells.

MATERIALS AND METHODS

Twenty Merino sheep fetuses, ranging in age from 33 to 144 days of gestation (the gestation period is about 150 days), and six adult ewes (four white Merinos, one black Merino, and one Suffolk) were used in this study.

Samples of skin were taken from the midside of each animal. Trephine samples (0.5 or 1.0 cm in diameter) or rectangular samples (approximately 1.0 × 0.3 cm) were used for light microscopy. Samples for electron microscopy were removed by cutting vertically through the skin with two parallel razor blades set about 1 mm apart, or by taking superficial slices of skin either manually or with an electrodermatome.

To study normal ultrastructure, small samples of skin (approximately 1 mm^2) were fixed in 1.3% osmium tetroxide (buffered in 0.067M *s*-collidine at pH 7.2) or in 3% glutaraldehyde (buffered with 0.1M sodium cacodylate at pH 7.2) containing 1% (0.029 M) sucrose for 1.5 hr at 4° C, dehydrated in ethanol and embedded in Araldite.

Ultrastructural localization of AChE was studied using the method of Karnovsky (4) with the following modifications: glutaraldehyde (4%), formalin (4%) and a mixture of 4% formalin and 2% glutaraldehyde were used as primary fixatives. These fixatives were buffered at pH 7.6 with 0.075M phosphate buffer. Small samples of skin (approximately 1 mm^2) as well as frozen sections of skin (50 μ thick) were incubated. Sucrose concentrations of 0.44M or 0.22M were used in the incubation medium, washing solutions and primary fixatives. Incubation times ranged from 15 min to 2 hr at 4°C. Half of the small samples and frozen sections were treated with dilute ammonium sulfide following incubation. For control tests, samples were incubated in a medium without substrate and in a medium containing eserine (5×10^{-5}M). All samples were post-fixed in 1.3% osmium tetroxide buffered with 0.067M *s*-collidine at pH 7.2 for 1-2 hr at 4° C, dehydrated in ethanol and embedded in Araldite.

Sections of the Araldite blocks were stained sequentially with saturated aqueous uranyl acetate and lead citrate (5) and examined with a Hitachi HU 11C electron microscope.

The electron microscope method of Karnovsky (4) for the localization of AChE was modified for light microscopy as follows: samples of skin were fixed in 10% formalin (neutralized with calcium carbonate chips) for 4 hr at 2–4° C, either immediately after removal from the animal or following storage on solid CO_2. Frozen sections (30 and 50 μm thick), cut either vertical or parallel to the skin surface, were incubated for periods ranging from 15 min to 2 hr at 37° C. Following incubation the sections were washed in distilled water, treated with dilute ammonium sulfide for 4 min and washed again several times in distilled water. Permanent preparations were made by dehydrating the sections in ethanol, clearing in xylol and mounting in Piccolyte.

Counts of the number of Langerhans cells per unit area of skin were made on the frozen sections (50 μm thick), and later corrected for skin shrinkage. The sections used were parallel to the skin surface and treated for AChE using the Gomori method (6). A modification of this method for electron microscopy was found to be unsuitable, mainly because of the large particle size of the reaction product.

OBSERVATIONS

Langerhans cells are present in samples taken from all adult animals and in fetuses aged from

Received July 22, 1971; accepted for publication December 10, 1972.

* From the C.S.I.R.O. Division of Animal Physiology, Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W., Australia. (Reprint requests to P.O. Box 239, Blacktown, N.S.W., 2148 Australia.)

103 to 144 days gestation, but they are absent in the younger fetuses examined.

Electron microscopy. Langerhans cells, which are morphologically similar in animals of different ages, are always located in the basal layer of the epidermis (Fig. 1), or in the follicle wall between the epidermis and the level of the sebaceous glands (Fig. 2). Occasionally they are also present between the outer cells of the sebaceous glands. The Langerhans granules shown in Figure 3 are more numerous than usually encountered.

Although fine nerves appear to join Langerhans cells in light microscope preparations (Fig. 4), they are always separate from them when viewed with the electron microscope. However, nerves penetrate the basal lamina and at least some of them have endings in contact with the sensory Merkel cells (7). Epidermal nerves are more often seen in samples from fetuses at about 105 days than they are in the other fetuses examined.

Melanocytes are present in all samples of adult epidermis examined but are absent in the fetal epidermis. No pre-melanosomes are present in the epidermal cells other than in the melanocytes which are actively producing melanosomes.

Localization of acetylcholinesterase associated with Langerhans cells. Electron microscope observations indicate that the AChE activity observed in sheep epidermis by light microscopy (1) is in fact only associated with Langerhans cells. These cells have been identified by the presence of Langerhans granules, one of which is indicated in Figure 2.

The AChE activity, as judged by the reaction product, is located on the outer surface of the plasma membranes of Langerhans cells and in the intercellular spaces between these cells and the adjacent keratinocytes (Fig. 2). No reaction product occurs within Langerhans cells. The intensity of the reaction product frequently appears to be greater around the dendritic processes than around the perikarya of Langerhans cells.

Light microscopy. Figures 4–7 show AChE-positive Langerhans cells revealed by the Gomori method (6) and the modification of Karnovsky's method (4). In the former method the large reaction product crystals associated with Langerhans cells, also occur in the surrounding tissue (Fig. 5). In the latter method the reaction product crystals are much smaller and the basal cell nuclei are clearly visible (Fig. 7).

The dendritic processes of the Langerhans cells in the epidermis are mostly orientated in a plane parallel to the skin surface (Fig. 6). Camera lucida drawings of AChE-positive Langerhans cells in sections parallel to the skin surface show that these cells form a network which is in contact with all keratinocytes in the basal layer, even though the ratio of these basal cells to Langerhans cells is about 10:1.

Counts of the Langerhans cells in the epidermis of the fetuses range from 175 to 340 per mm^2 and in samples from the midside of adults from 277 to 433 per mm^2 (8). There is no consistent age change in the number of Langerhans cells per unit area between the fetuses or between them and adult sheep. However, the total number of Langerhans cells in adult sheep must be much greater than in the fetuses because of the increase in skin area. In the adult the skin area is approximately ten times that of a fetus at 107 days gestation, as calculated from the formulae of Lines and Peirce (9), which relate skin area to body weight.

In fetuses aged 103–105 days a complex network of AChE-positive nerves is present in the upper dermis, particularly in the region near the epidermis. Some of these nerves appear to enter the epidermis and join the Langerhans cells in the basal layer (Fig. 4). This nerve network is poorly developed in younger fetuses and less complex in older fetuses.

DISCUSSION

Electron microscope observations show that Langerhans cells are present in the epidermis of the sheep and that their ultrastructural features are similar to those of other mammals (10, 11, 12). While these cells are located in the spinous and basal layers in man (10), guinea pig (13) and mouse (14), in sheep they are found only in the basal layer. No indication of any outward migration of these cells towards the skin surface, as suggested by Riley (15) for other species, has been found in sheep epidermis.

The AChE-positive dendritic cells, observed in sheep epidermis by light microscopy (1), have now been identified as Langerhans cells. The ultrastructural localization of AChE around the Langerhans cells in sheep epidermis is similar to the localization of adenosine triphosphatase (ATPase) associated with Langerhans cells in human (2) and guinea pig (3) epidermis. In the guinea pig the enzyme is described as being localized within or at the surface of the plasma membrane. AChE is present at the surface of the plasma membrane of sheep Langerhans cells but not within it. In human epidermis (2) ATPase is distributed in the intercellular spaces between the epidermal keratinocytes and on the plasma membrane of melanocytes as well as Langerhans cells. Zelickson and Mottaz (2) suggest that the ATPase technique should not be used as a means of localizing Langerhans cells in human epidermis because of its non-specific staining, as regards cell type. In sheep epidermis the AChE reaction is specific only to Langerhans cells.

There appear to be fewer Langerhans cells per unit area of epidermis in sheep than in other mammals. For example, there are about 900 of these cells per mm^2 in the epidermis of the back of guinea pigs (13) and about 700 cells per mm^2 in adult human epidermis (16), compared with 175–340 cells per mm^2 in the epidermis of the sheep fetuses examined and 277–433 cells per mm^2 in the midside epidermis of adult sheep (8). However, the greater density in guinea pig and man

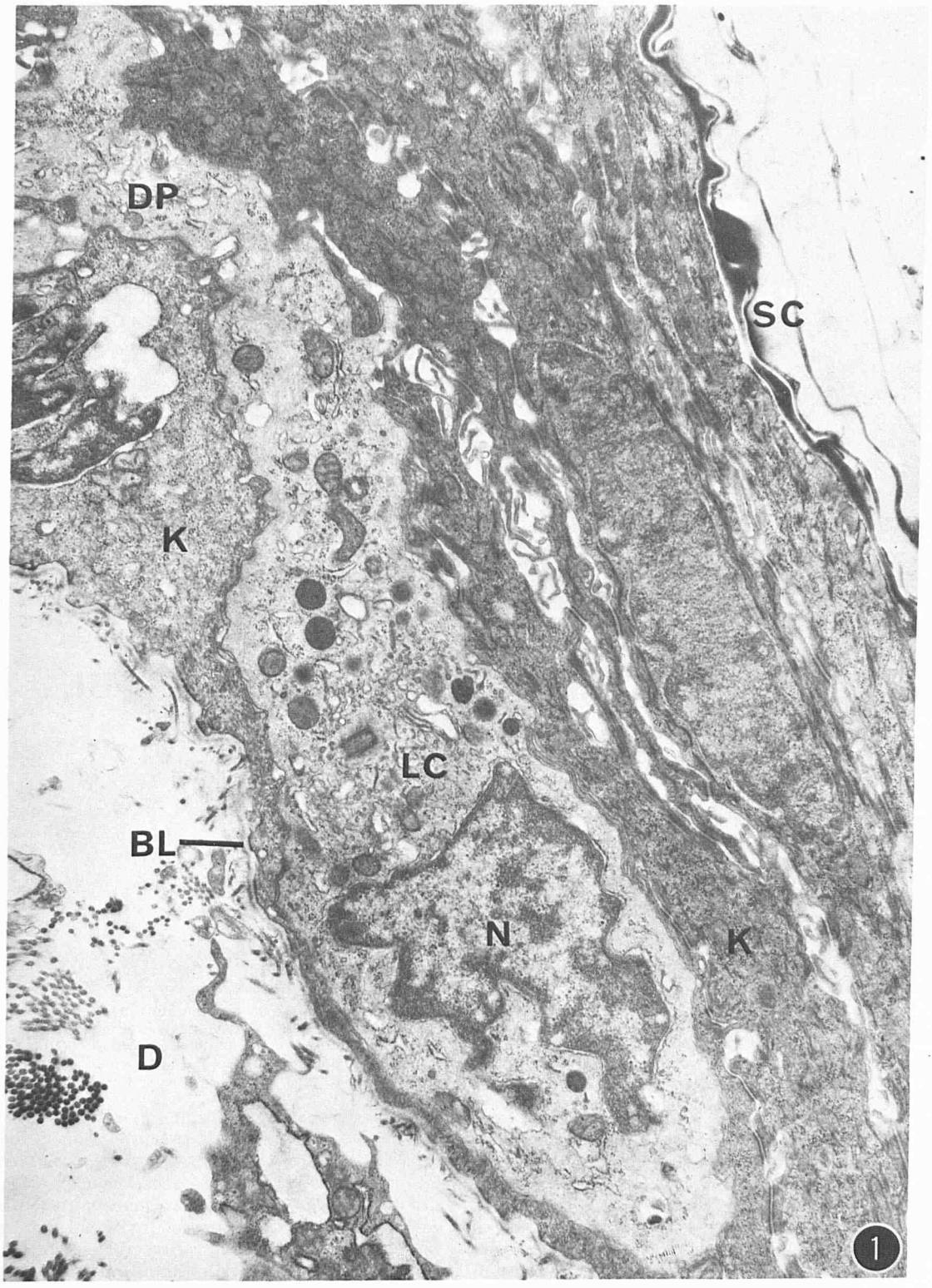


FIG. 1. Electron micrograph of a Langerhans cell (LC) in a vertical section of epidermis from a 131-day-old fetus. This cell is located in the basal layer of the epidermis and its cytoplasm is less electron-dense than the cytoplasm of the surrounding keratinocytes (K). BL, basal lamina; D, dermis; DP, dendritic process; N, lobate nucleus; SC, stratum corneum. Glutaraldehyde. × 16,500.

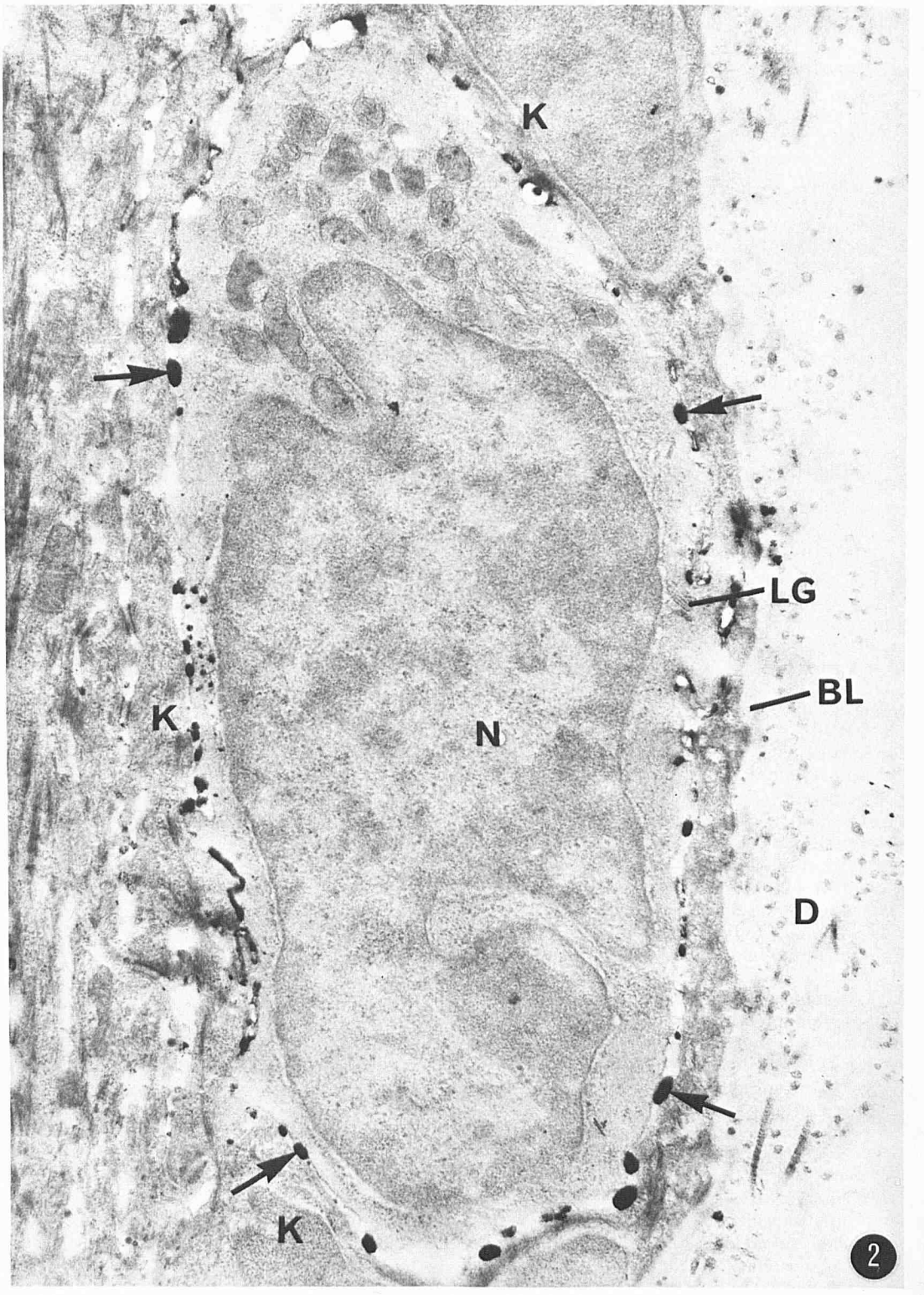


FIG. 2. Electron micrograph of an AChE-positive Langerhans cell in the wall of a follicle between the level of the sebaceous gland and the epidermis in an adult white Merino. Enzyme activity, as indicated by the electron-dense reaction product (arrows), is located in the intercellular space between the Langerhans cell and the adjacent keratinocytes (K). BL, basal lamina; D, dermis; LG, Langerhans granule; N, nucleus. AChE (Karnovsky). × 34,000.

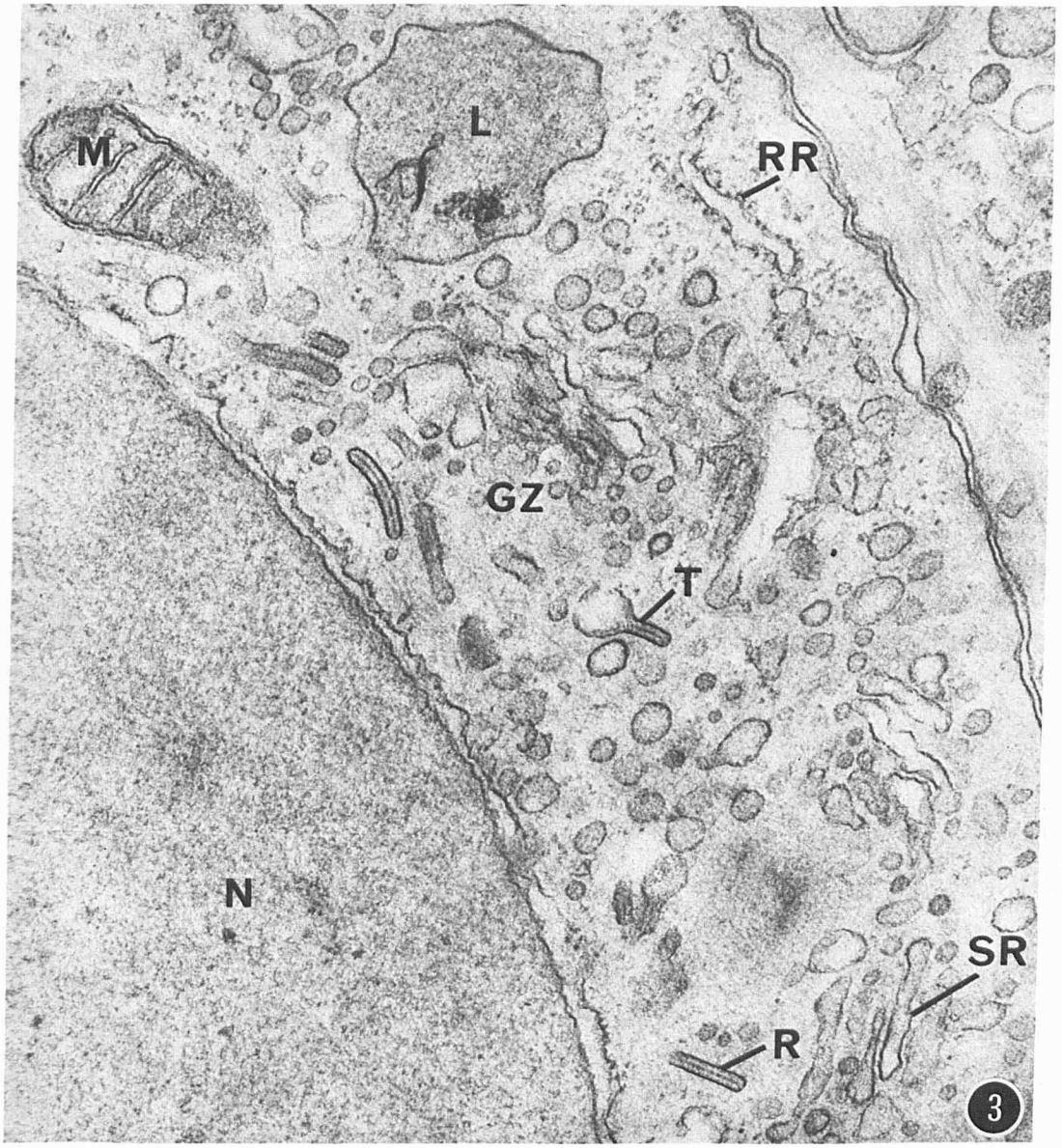
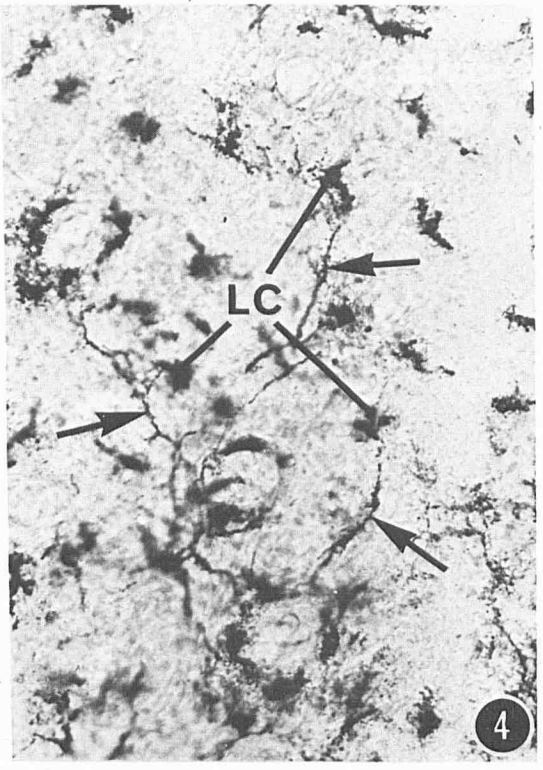


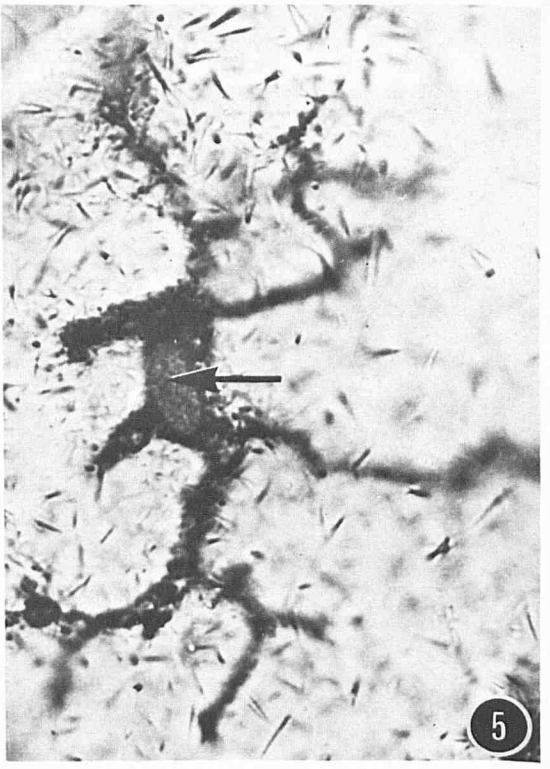
FIG. 3. Electron micrograph of part of a Langerhans cell in the epidermis of the same fetus as in Figure 1. Outlines of Langerhans granules-rod-shaped (R) and tennis-racquet-shaped (T)-are shown in the cytoplasm in close proximity to smooth endoplasmic reticulum (SR) and the Golgi zone (GZ). L, lysosome-like organelle; M, mitochondrion; N, nucleus; RR, rough endoplasmic reticulum. Osmium tetroxide. × 63,000.

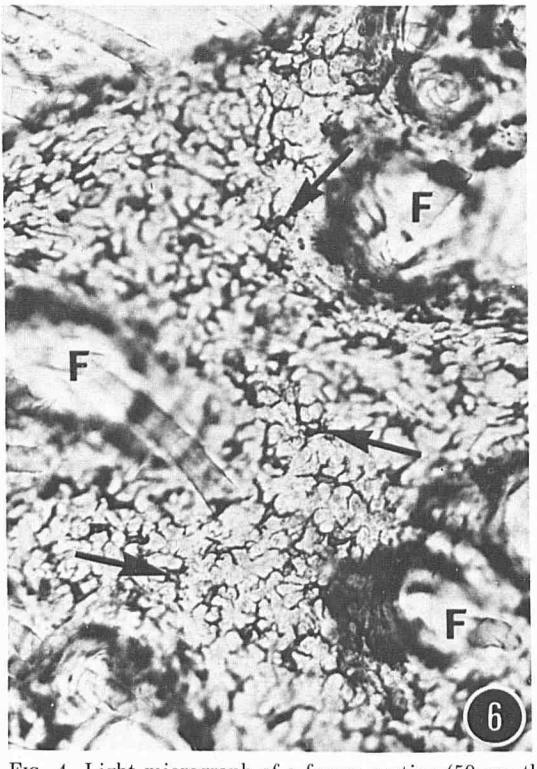
may in part be due to skin shrinkage during processing; no mention of this aspect is made by the previous authors.

The youngest sheep fetus in which Langerhans cells were present was 103 days old. At about this age the periderm is replaced by a keratinizing epithelium (17), suggesting that the Langerhans cells may have some influence on keratinization. The first observed Langerhans cells are fully differentiated and no developmental stages have been observed. Fully differentiated Langerhans cells have also been described in human fetal epidermis at about 100 days gestation (18). This suggests that Langerhans cells first appear in the epidermis at an earlier developmental stage in man than in the sheep, as the gestation period in man is approximately four months longer than it is in the sheep.

The present histochemical-electron microscope observations on the Langerhans cells in the epidermis of the sheep indicate that these cells, which constitute about 10 percent of the cells in the basal layer, are a system of active cells and, with their dendritic processes, are in contact with all basal cells. They are, therefore, in a position to impart a significant effect upon the keratinocytes.







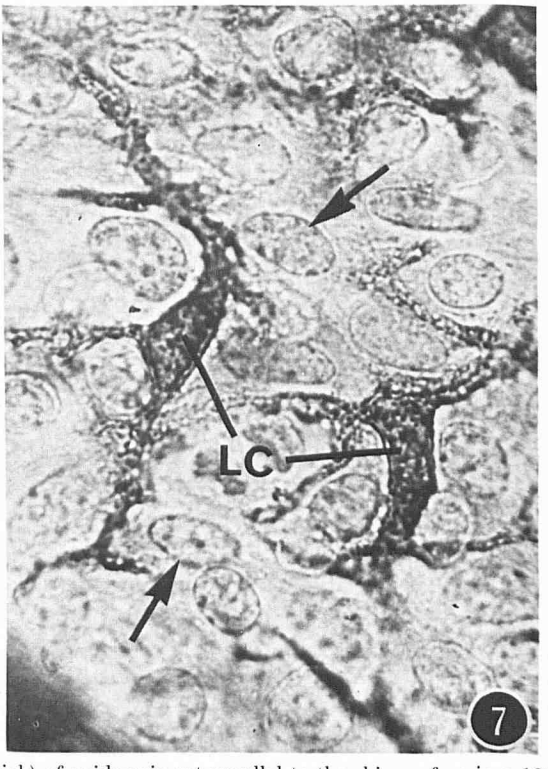


FIG. 4. Light micrograph of a frozen section (50 μm thick) of epidermis cut parallel to the skin surface in a 103-day-old fetus. Some AChE-positive nerves (arrows) appear to join the AChE-positive Langerhans cells (LC). AChE (Gomori). × 220.

FIG. 5. Light micrograph of an AChE-positive dendritic Langerhans cell in a frozen section (50 μm thick) of epidermis cut parallel to the skin surface in a 132-day-old fetus. Needle-like crystals of reaction product are present around the cell, and its nucleus (arrow) is visible. AChE (Gomori). × 1400.

FIG. 6. Light micrograph of AChE-positive, dendritic Langerhans cells (arrows) in a frozen section of epidermis from the same adult animal as in Figure 2, The section (50 μm thick) is cut parallel to the skin surface and includes the upper part of several follicles with fibers (F). AChE (Karnovsky). × 220.

FIG. 7. Light micrograph of two AChE-positive, dendritic Langerhans cells (LC) in a frozen section (50 μm thick) of epidermis from the same adult animal as in Figure 2. The reaction product is confined to the Langerhans cells, and the nuclei of the basal cells of the epidermis are clearly visible (arrows). AChE (Karnovsky). × 1400.

REFERENCES

1. Lyne, A. G. and Chase, H. B.: Branched cells in the epidermis of the sheep. Nature, *209:* 1357, 1966.
2. Zelickson, A. S. and Mottaz, J. H.: Localization of gold chloride and adenosine triphosphatase in human Langerhans cells. J. Invest. Derm., *51:* 265, 1968.
3. Wolff, K. and Winkelmann, R. K.: Ultrastructural localization of nucleoside triphosphatase in Langerhans cells. J. Invest. Derm., *48:* 50, 1967.
4. Karnovsky, M. J.: The localization of cholinesterase activity in rat cardiac muscle by electron microscopy. J. Cell. Biol., *23:* 217, 1964.
5. Reynolds, E. S.: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell. Biol., *17:* 208, 1963.
6. Gomori, G.: *Microscopic Histochemistry: Principles and Practice.* Univ. of Chicago Press, 1952.
7. Lyne, A. G. and Hollis, D. E.: Merkel cells in sheep epidermis during fetal development. J. Ultrastruct. Res., *34:* 464, 1971.
8. Lyne, A. G. and Hollis, D. E.: The skin of the sheep: a comparison of body regions. Aust. J. Biol. Sci., *21:* 499, 1968.
9. Lines, E. W. and Peirce, A. W.: The basal (standard) metabolism of the Australian Merino sheep. Bull. Coun. Scient. Ind. Res., Melbourne, No. 55, 1931.
10. Breathnach, A. S.: The cell of Langerhans. Intern. Rev. Cytol., *18:* 1, 1965.
11. Zelickson, A. S.: The Langerhans cell. J. Invest. Derm., *44:* 201, 1965.
12. Prunieras, M.: Interactions between keratinocytes and dendritic cells. J. Invest. Derm., *52:* 1, 1969.
13. Wolff, K. and Winkelmann, R. K.: Nonpigmentary enzymes of the melanocyte—Langerhans cell system. *Advances in Biology of the Skin*, Vol. 8. Eds., Montagna, W. and Hu, F. Pergamon Press, New York, 1967.
14. Breathnach, A. S., Silvers, W. K., Smith, J. and Heyner, S.: Langerhans cells in mouse skin experimentally deprived of its neural crest component. J. Invest. Derm., *50:* 147, 1968.
15. Riley, P. A.: A model of the relationship between melanocytes and Langerhans cells. Melanocyte population dynamics. Brit. J. Derm., *79:* 52, 1967.
16. Ferreira-Marques, J.: Systema sensitivum intraepidermicum. Die Langerhannschen Zellen als Rezeptoren des hellen Schmerzes: Doloriceptores. Arch. Derm. Syph., *193:* 191, 1951.
17. Lyne, A. G. and Hollis, D. E.: Ultrastructural changes in Merino sheep epidermis during foetal development. J. Anat., *108:* 211, 1971.
18. Breathnach, A. S. and Wyllie, L. M.: Electron microscopy of melanocytes and Langerhans cells in human fetal epidermis at fourteen weeks. J. Invest. Derm., *44:* 51, 1965.