J. Anat. (1976), **122**, 2, pp. 435–445
With 10 *figures*
Printed in Great Britain

Langerhans cells in the human oesophagus

TARIQ M. AL YASSIN AND PETER G. TONER
University Department of Pathology, Glasgow Royal Infirmary

(*Accepted* 30 *October* 1975)

INTRODUCTION

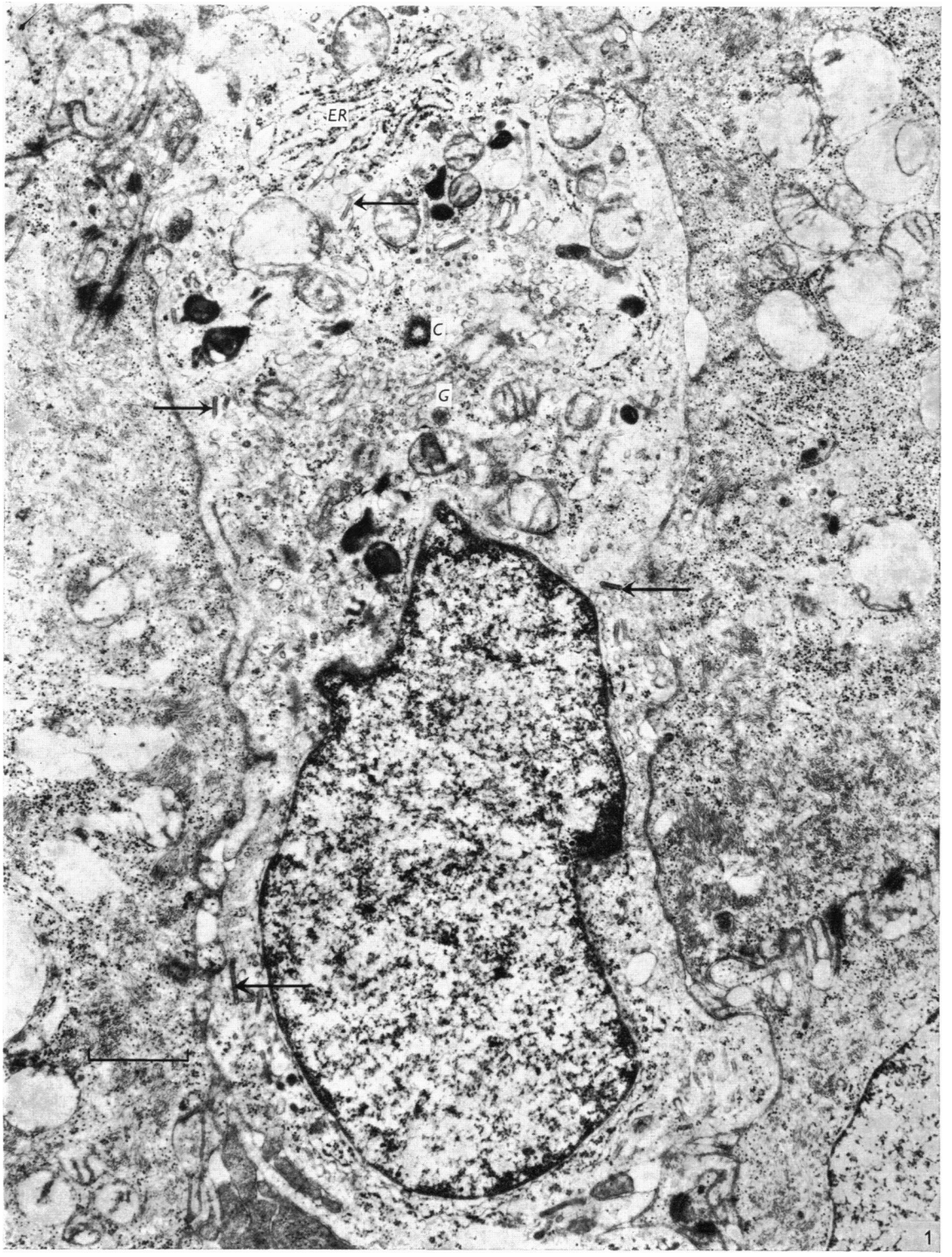
The Langerhans cell, first identified by histological techniques in the upper layers of the epidermis, is now best characterized electron microscopically by reason of the presence of distinctive cytoplasmic inclusions and by the absence of epithelial adhesion specializations and tonofilaments. Cells of this type have now been identified in various squamous epithelia, including skin (Birbeck, Breathnach & Everall, 1961; Zelickson, 1965), buccal mucosa (Waterhouse & Squire, 1967), the female genital tract (Younes, Robertson & Bencosme, 1968) the sheep rumen (Gemmell, 1973) and the upper alimentary tract of the mouse (Bock, 1974). Speculations as to the nature and function of Langerhans cells remains unresolved, although the earlier view that they were effete melanocytes (Masson, 1926, 1951) has now largely been discounted. This paper reports the occurrence of morphologically typical Langerhans cells in the normal human oesophagus and draws comparisons with the intra-epithelial lymphocyte.

MATERIALS AND METHODS

Ten specimens of normal human oesophageal mucosa were obtained from oesophageal biopsies and resections undertaken in the course of the diagnosis and treatment of various conditions. For electron microscopy, blocks from 0·5 to 1 mm in diameter were fixed in glutaraldehyde in phosphate buffer (pH 7·2) at 4 °C for 4–6 hours, washed in buffer, post-fixed in 1 % osmium tetroxide, dehydrated in ethanol and embedded in Epon. Tissues were also taken for diagnostic histopathology and were prepared for light microscopy by conventional methods. Sections 1 μm in thickness from the Epon blocks were examined to confirm tissue identity and orientation, and ultrathin sections were then cut from selected areas using the LKB Ultratome III. Thin sections were stained with uranyl acetate and lead citrate and were examined in the Philips EM 200 and EM 301 electron microscopes.

OBSERVATIONS

The oesophageal clear cells, which we have identified as Langerhans cells (Fig. 1), are found in the middle and superficial layers of the normal squamous mucosa and do not occur in the basal layer or beneath the basal lamina. They are often irregular in outline (Fig. 6) with cytoplasmic protrusions which extend out from the perikaryon between the surrounding squamous cells. As a result of this dendritic pattern not all sections pass through the perikaryon. In some fields the Langerhans cell is represented only by a small cytoplasmic island interpolated between the epithelial cells.



1

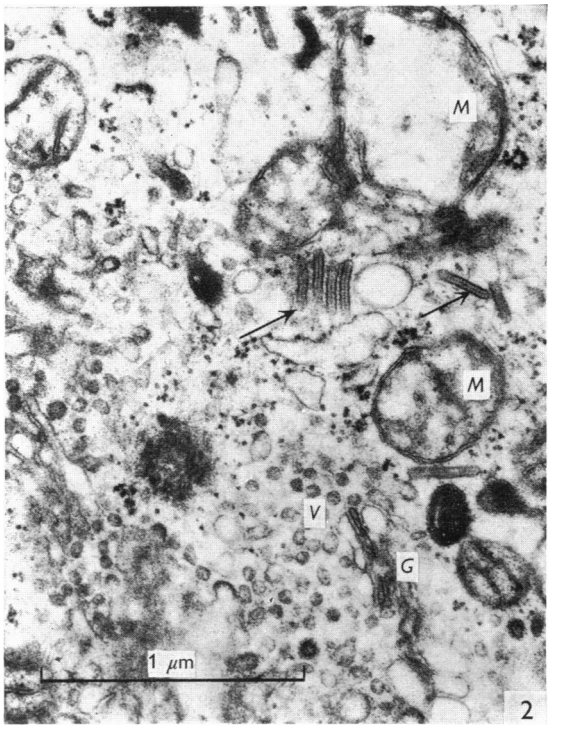
The relative pallor of the Langerhans cell cytoplasm is in contrast with the adjacent squamous cells. Langerhans cells are further distinguished (Fig. 1) by the complete absence of the desmosomes and tonofilaments which are a prominent feature of the normal squamous mucosa. Their cytoplasm (Figs. 2, 3) contains moderate numbers of free ribosomes, profiles of granular endoplasmic reticulum, a well developed Golgi apparatus with lamellae and vacuoles, often with a related contriole, and a few mitochondria, without particular structural distinction. The nucleus is irregular in contour, often with deep invaginations, and may contain one or, rarely, two nucleoli. However, not all Langerhans cells correspond to this pattern. We have observed cells such as that shown in Figure 7, which has typical Langerhans granules, but with much less elaborate cytoplasmic organization. It is, of course, difficult to exclude the possibility that such variations are simply the consequence of selection of different planes of section through different cells.

The single most distinctive feature of these cells is the typical Langerhans granule (Figs. 2, 3, 4), a rod-shaped inclusion up to 460 nm in length. A median section of a Langerhans cell in the oseophagus may display many of these granules. They have a characteristic linear laminated internal structure and in slightly tangential sections show an apparent periodic substructure. On occasion the rod-shaped Langerhans granule is seen to connect directly with the cell membrane (Fig. 5). Some granules have a rounded expansion at one end, measuring up to 150 nm in diameter (Fig. 2). These are the distinctive 'tennis racket' structures which are described in the epidermal Langerhans cell. In addition to these organelles there are moderate numbers of membrane-limited electron-dense bodies, possibly of lysosomal nature (Fig. 1).

A second clear cell found in the human oesophagus is the typical intra-epithelial lymphocyte (Fig. 8) which is similar in appearance to that found in the intestine (Toner & Ferguson, 1971). Like Langerhans cells these lymphocytes lack tonofilaments and desmosomes, but they differ from typical Langerhans cells in several respects. They do not have the characteristic Langerhans granules, their cytoplasmic organelles are, in general, less well organized, and their nuclear morphology tends to differ from that of the Langerhans cell in having a more prominent heterochromatin component. Nevertheless, it remains difficult in some cases to make a firm distinction on the basis of morphology between lymphocytes and Langerhans cells. Some cells containing unequivocal Langerhans granules (Figs. 7, 10) are in most other respects indistinguishable from lymphocytes (Fig. 8). Perhaps some of the cells we identify as lymphocytes (Fig. 9) are in fact Langerhans cells, their granules happening by chance not to be in the plane of section. There is no doubt that the well differentiated Langerhans cell (Fig. 1) and the typical lymphocyte (Fig. 8) are readily separable, but the borderline between the less classical examples of the these two cell types is at best indistinct. We did not identify any cells of melanocytic type in this study, despite a careful search for inclusions resembling pre-melanosomes.

All illustrations are from human oesophageal mucosa.

Fig. 1. A typical Langerhans cell in the human oesophageal mucosa. Desmosomes are seen between adjacent squamous cells but not between the Langerhans cell and its neighbours. The features of this cell include a centriole (*C*), Golgi complex (*G*), granular endoplasmic reticulum (*ER*), and various dense inclusions which resemble lysosomes. The typical Langerhans granules are just detectable at this magnification (arrows).



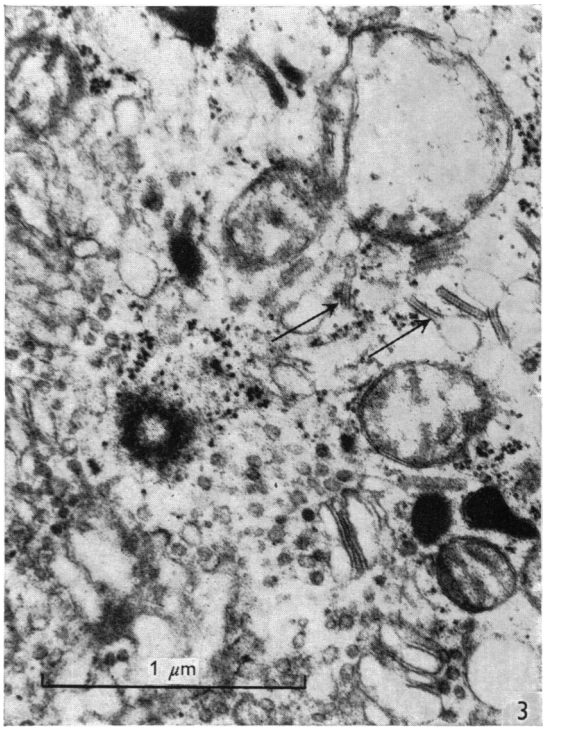


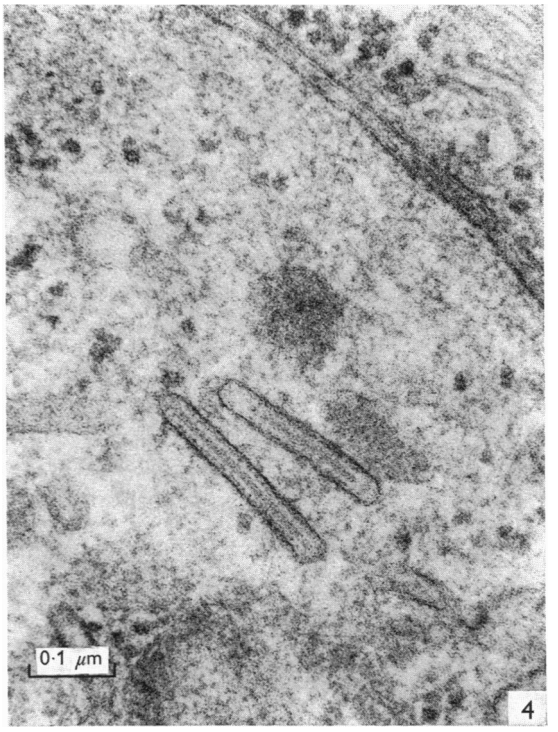
Fig. 2. Higher magnification micrograph of the centriolar region from the cell shown in Fig. 1. The Golgi complex (*G*) has numerous associated vesicles (*V*). The mitochondria (*M*), are undistinguished. Several Langerhans granules are present (arrows).

Fig. 3. This micrograph is of a deeper plane of section from the area shown in Fig. 2. Notice the variations in the disposition and shape of the Langerhans granules (arrowed). Some show the terminal vacuolar dilatations which give rise to 'tennis racket' structures.

DISCUSSION

The Langerhans cells of the human oesophagus as described above are morphologically identical to those previously reported in the epidermis (Birbeck *et al.* 1961; Zelickson, 1965) and in the squamous mucosa of the cervix (Younes *et al.* 1968). We know of no report of such intra-epithelial Langerhans cells being located in other than stratified squamous epithelium. The original view of Masson (1926, 1951) that the Langerhans cell represents an effete melanocyte has already been discredited by the observation that the Langerhans cells develop normally in animals experimentally deprived of the neural crest, from which the melanocytes are known to be derived (Breathnach, Silvers, Smith & Heyner, 1968). The absence of any identifiable melanocytes in the specimens of oesophagus which we have studied is perhaps circumstantial evidence in support of this experimental study. There is, in any case, little ultrastructural relationship between the Langerhans granule and the pre-melanosome or melanin granule.

The relatively common topographical relationship between the Langerhans granule and the Golgi apparatus has encouraged the view that this might be the site of its



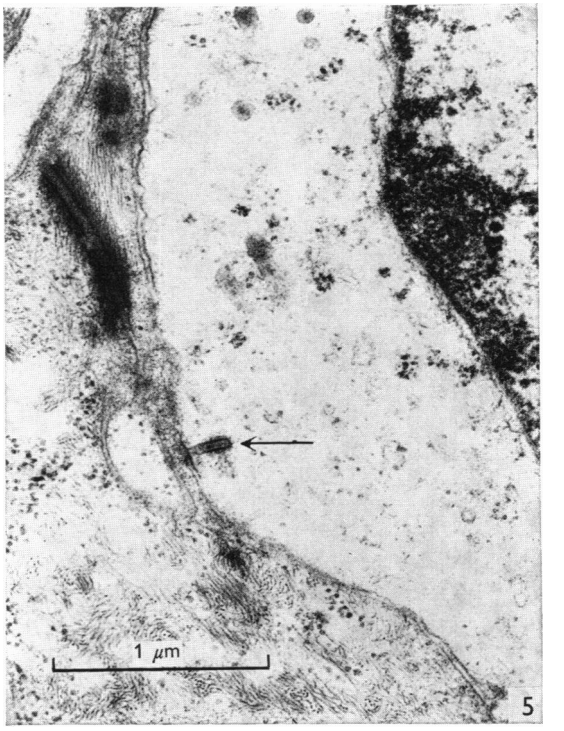


Fig. 4. High magnification micrograph of Langerhans cell granules, showing their characteristic internal structures.

Fig. 5. Part of a rather poorly differentiated Langerhans cell, showing the continuity of the characteristic granule (arrow) with the cell surface, suggesting an origin by invagination. The desmosome seen links adjacent squamous cells.

origin or synthesis (Breathnach, 1964; Niebauer, Krawczyk, Kidd & Willigram, 1969), the subsequent discharge of the granule being indicated by its commonly observed continuity with the cell surface. Equally persuasive, however, is the view that the granules form at the cell surface by a process of endocytosis (Hashimoto, 1971) akin to the specialized kind of micropinocytosis which is seen in macrophages, although tracer studies have so far failed to support an active phagocytic role (Wolff & Schreiner, 1970; Sagebiel, 1972).

Perhaps the most interesting of recent findings are reports of the identification of typical Langerhans granules in cells far removed from an epithelial location. Cells of Langerhans type have been described, for example, in a lymphatic vessel (Silberberg, Baer & Rosenthal, 1974); in lymph nodes, both human (Vernon *et al.* 1973; Shamoto, Kaplan & Katch, 1971) and rabbit (Konodo, 1969); and in the dermis (Kiistala & Mustakallio, 1968). Moreover, many reports have been published of typical Langerhans granules in the cells of histiocytic lesions (Tarnowski & Hashimoto, 1967; Hashimoto & Tarnowski, 1968; Watson & Swedo, 1968; Gianotti & Caputo, 1969; Morales, Fine, Horn & Watson, 1969; Imamura & Muroya, 1971; Hashimoto & Pritzker, 1973).

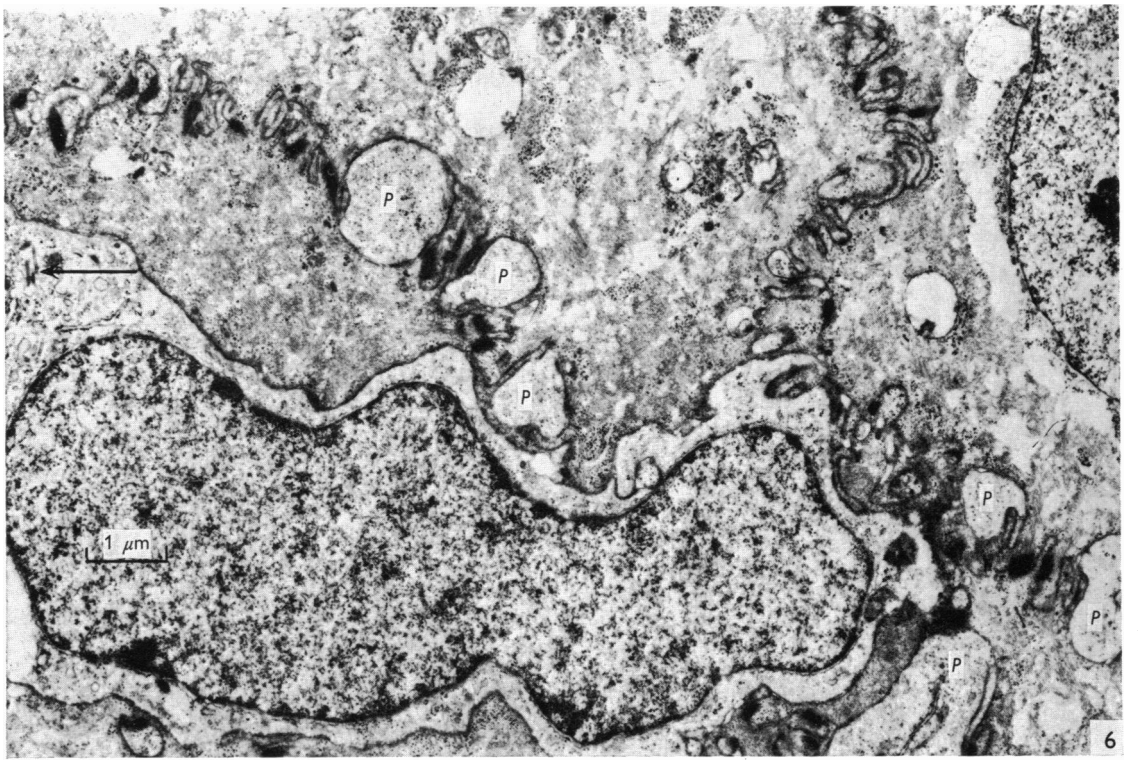


Fig. 6. The typical Langerhans cell granules are barely seen (arrow) at this low magnification, which serves, however, to emphasize the dendritic nature of the cell. There are several processes (*P*), which lie separate from the perikaryon in this plane of section.

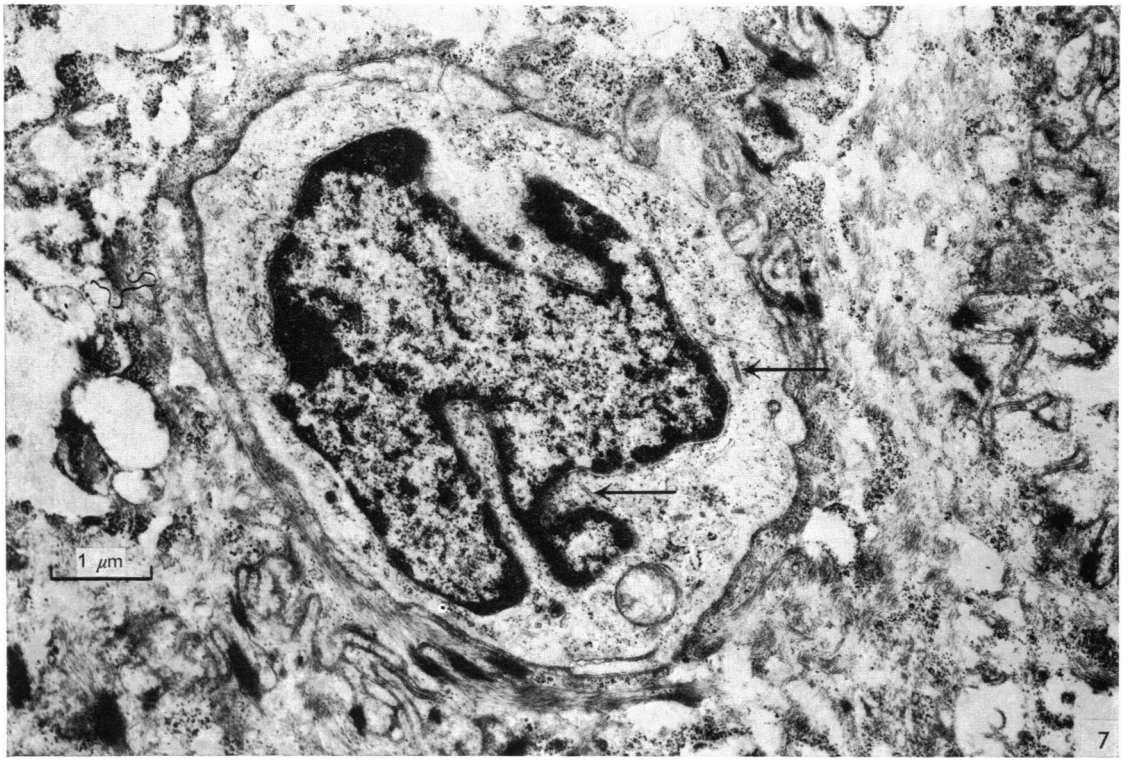


Fig. 7. A relatively poorly differentiated Langerhans cell with a single mitochondrial profile and little cytoplasmic organization. Two typical Langerhans granules are present (arrows).

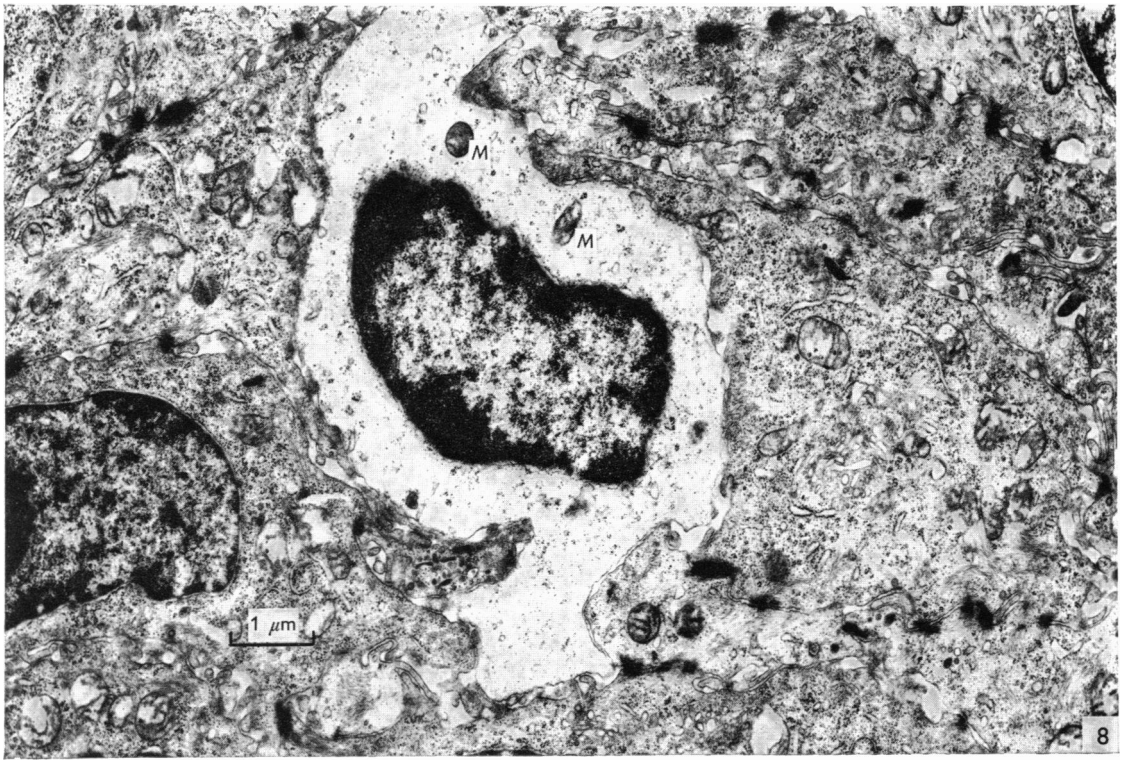


Fig. 8. A typical intraepithelial lymphocyte from the oesophageal mucosa, showing the characteristic lack of cytoplasmic organization. Two mitochondria (*M*) are the only formed structures.

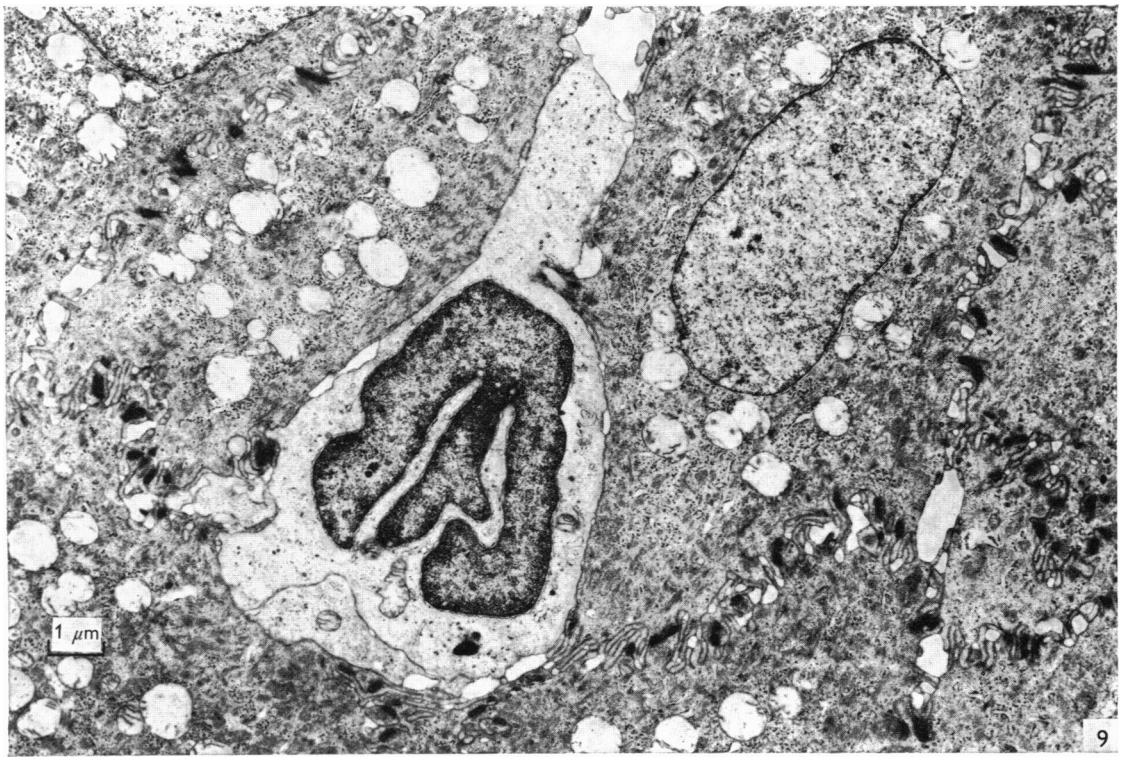
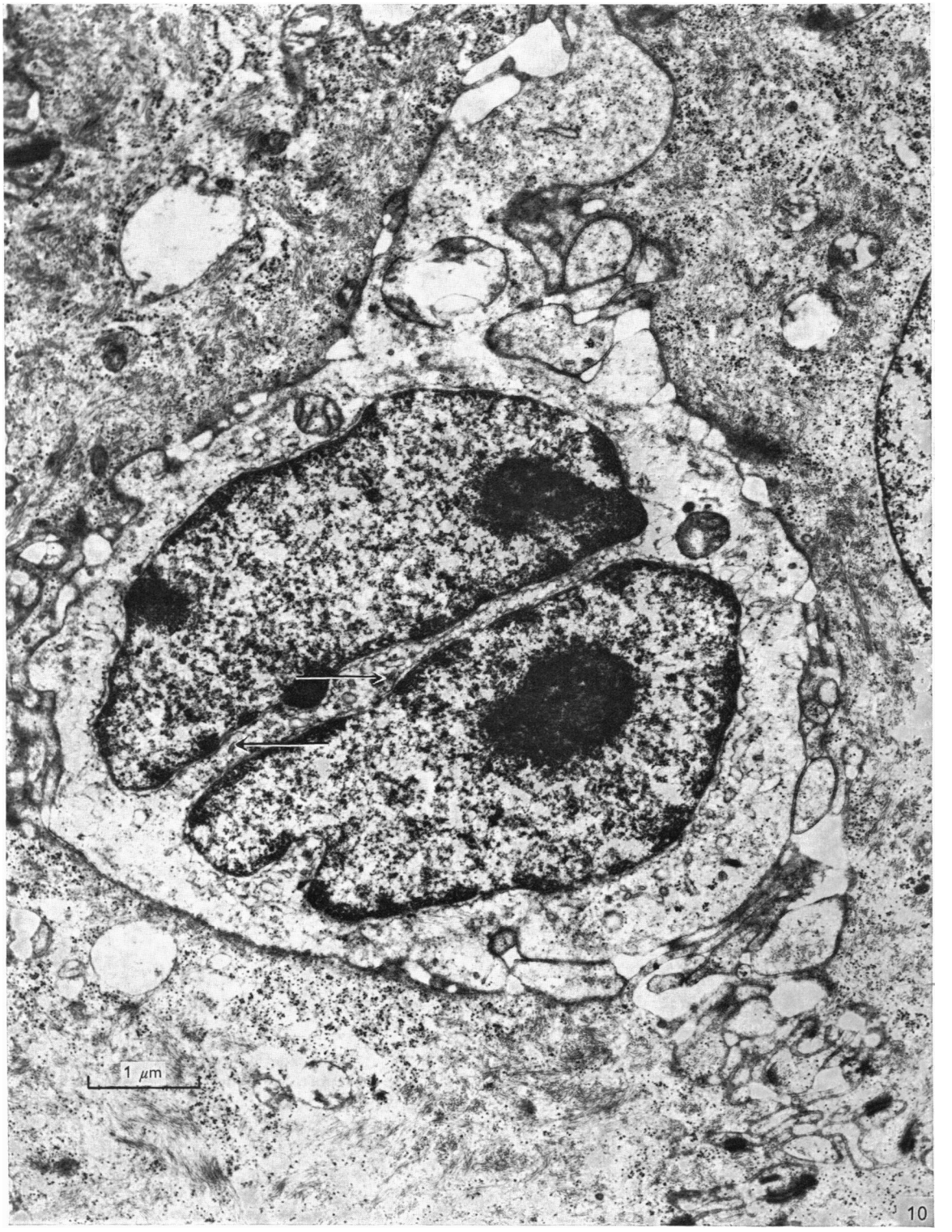


Fig. 9. In the absence of Langerhans granules the identity of this cell cannot be confidently established as either lymphocyte or Langerhans cell. The deep clefting of the nucleus might support the latter.


1 μm
10

These reports provide us with reasons for considering a 'mesenchymal' origin and function for the intraepithelial Langerhans cell. For example, a possible 'epidermoclast' role has been proposed by Prunieras (1969), along with the suggestion that the Langerhans cell might have a role in the capture of antigenic material in the skin. The observation by Silberberg (1973) of the juxtaposition of Langerhans cells and mononuclear cells at sites of contact-allergic reactions was taken by him to support such a role. The mononuclear branching cells of the sheep forestomach (Steven & Marshall, 1972), although not formally identified as Langerhans cells, were thought to serve a similar purpose. The ability of Langerhans cells to incorporate labelled thymidine (Giacometti & Montagna, 1967) and to divide within the epithelium suggests that they may well be more than transient migrants. Their behaviour recalls the similar proliferative capacity of the intra-epithelial lymphocyte of the intestinal tract.

This leads us to consider a further suggestion as to the origin of the intra-epithelial Langerhans cell. Billingham & Silvers (1965) have proposed the lymphocyte as a potential relative of the Langerhans cell. We have been impressed by the difficulty of distinguishing consistently between the simple intra-epithelial lymphocytes of the oesophagus, and the less well developed members of the Langerhans cell population. Some cells would certainly have qualified as lymphocytes, had we not seen a single typical Langerhans granule within the otherwise featureless cytoplasm. On this purely structural basis it seems possible that the Langerhans cell might be derived from the intra-epithelial lymphocyte. The regular occurrence of intra-epithelial lymphocytes in skin, oesophagus and other epithelia in which Langerhans cells are also present provides encouragement for this view, which has the merit of simplicity. However, it must be remembered that lymphocytes are virtually universal inhabitants of epithelial surfaces of all kinds, whereas the distinctive Langerhans specialization seems to be confined to stratified squamous surfaces.

Before leaving the histogenesis of the Langerhans cell we must mention the work of Reams (1973) which goes against the growing body of opinion in favour of a mesenchymal origin: his experiments suggest that the epidermal Langerhans cell in the mouse is of purely ectodermal origin.

Perhaps the 'Langerhans cells' which have been described in various situations in health and disease do not represent a single homogeneous population with a common histogenetic origin. Cells which exhibit a particular ultrastructural specialization do not necessarily have a common origin or identity. It may be that the Langerhans granule is a non-specific cellular inclusion derived from an activity shared by a variety of cells, normal and neoplastic, epithelial and mesenchymal, just as simple micropinocytotic vesicles or caveolae may be seen in sites as diverse as smooth muscle, endothelium, serosal mesothelium and some epithelial cells. Nevertheless, until this negative 'non-specific' hypothesis can be more fully substantiated, the Langerhans granule will remain as circumstantial evidence of a close relationship between those cells in which it is known to occur,

Fig. 10. The cleft nucleus is suggestive of a Langerhans cell. Careful examination of the narrow strip of cytoplasm between the nuclear profiles reveals two short Langerhans granules (arrows), sufficient to confirm its identity. Without these there are no specific features by which the cell can be distinguished from a lymphocyte. Perhaps the only difference between the cells shown in this and the preceding figure is the chance factor of the plane of section.

particularly those which are in association with squamous cells. One can only speculate about the role of this curious population of presumptive mesenchymal visitors in squamous epithelium.

SUMMARY

The dendritic cells of Langerhans, first identified in the epidermis, have now been observed in the middle and superficial layers of the normal human oesophageal mucosa. They exhibit typical Langerhans granules, but no desmosomes and tonofilaments. They often have irregular indented nuclei, with a relatively pale cytoplasm contrasting with that of the adjacent squamous cells. These cells are sometimes difficult to distinguish from intra-epithelial lymphocytes, which are also encountered in the oesophageal mucosa and which share certain ultrastructural characteristics with Langerhans cells.

REFERENCES

BILLINGHAM, R. E. & SILVER, W. K. (1965). Some unsolved problems in the biology of skin. In *Biology of the Skin and Hair Growth* (ed. A. G. Lyne and B. F. Shorts). Sydney: Angus Robertson.

BIRBECK, M. S., BREATHNACH, A. S. & EVERALL, J. D. (1961). An electron microscopic study of basal melanocytes and high level clear cells (Langerhans cells) in vitilligo. *Journal of Investigative Dermatology* **37**, 51–64.

BOCK, P. (1974). Fine structure of Langerhans cells in the stratified epithelia of the oesophagus and stomach of mice. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **147**, 237–247.

BREATHNACH, A. S. (1964). Observations on cytoplasmic organelles in Langerhans cells of human epidermis. *Journal of Anatomy* **98**, 265–270.

BREATHNACH, A. S., SILVERS, W. K., SMITH, J. & HEYNER, S. (1968). Langerhans cells in mouse skin experimentally deprived of its neural crest component. *Journal of Investigative Dermatology* **50**, 147–160.

GEMMELL, R. T. (1973). Langerhans cells in the ruminal epithelium of the sheep. *Journal of Ultrastructure Research* **43**, 256–259.

GIACOMETTI, L. & MONTAGNA, W. (1967). Langerhans cells: uptake of tritiated thymidine. *Science New York* **157**, 439–440.

GIANOTTI, F. & CAPUTO, R. (1969). Skin ultrastructure in Hand-Schuller-Christian disease: report on abnormal Langerhans cells. *Archives of Dermatology* **100**, 342–349.

HASHIMOTO, K. (1971). Langerhans cell granule. An endocytotic organelle. *Archives of Dermatology* **104**, 148–165.

HASHIMOTO, K. & PRITZKER, M. S. (1973). Electron microscopic study of reticulo-histiocytoma: An unusual case of congenital self-healing reticulo-histiocytosis. *Archives of Dermatology* **107**, 263–270.

HASHIMOTO, K. & TARNOWSKI, W. M. (1968). Some new aspects of Langerhans cells. *Archives of Dermatology* **97**, 450–464.

IMAMURA, M. & MUROYA, K. (1971). Lymph node ultrastructure in Hand-Schuller-Christian disease. *Cancer* **27**, 956–964.

KIISTALA, U. & MUSTAKALLIO, K. K. (1968). The presence of Langerhans cells in human dermis with special reference to their potential mesenchymal origin. *Acta dermato-venereologica* **48**, 115–122.

KONODO, Y, (1969). Macrophages containing Langerhans cell granules in lymph nodes of rabbit. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **98**, 506–511.

MASSON, P. (1926). Les Naevi Pigmentaries, tumeurs nerveuses. *Annales d'anatomie pathologique* **3**, 417–453.

MASSON, P. (1951). My conception of cellular nevi. *Cancer* **4**, 9–38.

MORALES, A. R., FINE, G., HORN, R. C. JR. & WATSON, J. H. L. (1969). Langerhans cells in localized lesions of the eosinophilic granuloma type. *Laboratory Investigation* **20**, 412–423.

NIEBAUER, G., KRAWCZYK, W. S., KIDD, L. & WILIGRAM, G. F. (1969). Osmium zinc iodide reactive sites in epidermal Langerhans cell. *Journal of Cell Biology* **43**, 80–89.

PRUNIERAS, M. (1969). Interactions between keratinocytes and dendritic cells. *Journal of Investigative Dermatology* **52**, 1–17.

REAMS, W. M. (1973). Ectodermal origin of epidermal Langerhans cells. *Anatomical Record* **175**, 421.

SAGEBIEL, R. W. (1972). *In vivo* and *in vitro* uptake of ferritin by Langerhans cells of the epidermis. *Journal of Investigative Dermatology* **58**, 47–54.

SHAMATO, M., KAPLAN, C. & KATCH, A. K. (1971). Langerhans cell granules in human hyperplastic lymph nodes. *Archives of Pathology* **92**, 46–52.

SILBERBERG, L. (1973). Apposition of mononuclear cells to Langerhans cells in contact allergic reactions. *Acta dermato-venereologica* **53**, 1–12.

SILBERBERG, I., BAER, R. L. & ROSENTHAL, S. (1974). Circulating Langerhans cells in a dermal vessel. *Acta dermato-venereologica* **54**, 81–86.

STEVEN, D. H. & MARSHALL, A. B. (1972). Branching mononuclear cells in the forestomach epithelium of the sheep. *Quarterly Journal of Experimental Physiology* **57**, 267–270.

TARNOWSKI, W. M. & HASHIMOTO, K. (1967). Langerhans cell granules in histiocytosis X. The epidermal Langerhans cells as macrophages. *Archives of Dermatology* **96**, 298–304.

TONER, P. G. & FERGUSON, A. (1971). Intraepithelial cells in human intestinal mucosa. *Journal of Ultrastructure Research* **34**, 329–344.

VERNON, M. L., FOUNTAIN, L., KREBS, M. M., HORTA-BARBOSA, L., FUCCILLO, D. A. & SEVER, J. L. (1973). Birbeck granules (Langerhans cell granules) in human lymph node. *American Journal of Clinical Pathology* **60**, 771–779.

WATERHOUSE, J. P. & SQUIRE, C. A. (1967). The Langerhans cell in human gingival epithelium. *Archives of Oral Biology* **12**, 341–348.

WATSON, J. H. L. & SWEDO, J. L. (1968). The Langerhans granule in histiocytoma. Its structure, location and possible genesis. *Journal of Cell Biology* **39**, 159a–160a.

WOLFF, K. & SCHREINER, E. (1970). Uptake of intracellular transport and degradation of exogenous protein by Langerhans cells. *Journal of Investigative Dermatology* **54**, 37–47.

YOUNES, M. S., ROBERTSON, E. M. & BENCOSME, S. A. (1968). Electron microscopic observation of Langerhans cells in the cervix. *American Journal of Obstetrics and Gynecology* **102**, 397–403.

ZELICKSON, A. S. (1965). The Langerhans cell. *Journal of Investigative Dermatology* **44**, 201–212.