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Ultrastructural and morphometric study of the Langerhans cell in the normal human exocervix

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INTRODUCTION

The 'biography' of the Langerhans cell has recently been reviewed by Shelley & Juhlin (1978), with special emphasis on the methodological difficulties involved in the study of this cell type and on its probable functional role(s). It is only during the last few years that the Langerhans cell has been considered to belong to the monocyte-macrophage-histiocyte family (Shelley & Juhlin, 1978) and Stingl *et al.* (1977) have suggested that it may represent the most peripheral outpost of the afferent limb of the immune system.

Although the Langerhans cells localized in the epidermis have been the most thoroughly studied, they have also been found in other stratified epithelia. Two publications report the presence of Langerhans cells in the human uterine exocervix, as revealed with conventional electron microscopic techniques (Younes, Robertson & Bencosme, 1968; Hackemann, Grubb & Hill, 1968).

The lack of information with respect to cell density and distribution of the Langerhans cells in the normal human exocervix and the paucity of information concerning their morphological characteristics at both light and electron microscopical levels led to the present investigation. The study was made by applying two standardized zinc-iodide-osmium procedures which selectively stain the Langerhans cells (Rodríguez & Caorsi, 1978); the fact that the reaction product is light- and electron-dense, makes possible the visualization of the cell, and especially of its granules, in both light and electron microscopy.

MATERIALS AND METHODS

Three uterine exocervices were obtained from women undergoing total hysterectomy due to the presence of uterine miomas. Cervices A, B and C were obtained from women 37, 32 and 38 years of age respectively and were regarded as normal on the following grounds: (*a*) the normal macroscopical appearance, (*b*) the absence of signs of inflammation, displasia or other alterations that can be revealed under the light and electron microscope. Other cervices of normal macroscopical appearance but which displayed some microscopical alterations were not included in the present study.

By making a sagittal and a horizontal cut, each exocervix was divided into four segments of roughly the same dimensions. One of the resulting quadrants was used to study the density and distribution of the Langerhans cells in total preparation of epithelial sheets; the second quadrant was used to visualize the Langerhans cells in histological sections. From the remaining two quadrants, tissue samples were

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obtained and processed either for conventional electron microscopy or according to two zinc-iodide-osmium procedures.

Zinc-iodide-osmium procedures (ZIO). The zinc iodide was prepared in two ways. 2·4 g of zinc powder and 1 g of iodine bisublimate were dissolved either in 40 ml of 0·2 M citric-acid-disodium phosphate buffer, pH 4·4 (C.4.4-ZI) or in 40 ml of 0·2 M veronal sodium-HCl buffer, pH 7·4 (V.7.4-ZI). After the solutions had been shaken for 10 minutes, they were immediately filtered. Then each solution was mixed with 2% OsO_4 at a ratio 3:1, to obtain the ZIO mixtures (C.4.4-ZIO and V.7.4-ZIO).

Light microscopy

Preparation of epithelial laminae and staining with ZIO

One of the quadrants was divided into several fragments ranging in size between 6 and 12 mm^2. These fragments were incubated in a 20 mM PBS-EDTA solution, pH 7·4, for 2 hours at 37 °C (Scaletta & MacCallum, 1972). This treatment resulted in the detachment of the epithelial layer from the connective tissue lying beneath. The epithelial sheets thus obtained were incubated in V.7.4-ZIO for 18 hours at 4 °C with continuous agitation (V.7.4-ZIO-4 °C-18 hours; Rodríguez & Caorsi, 1978). The tissues were then thoroughly washed in distilled water, dehydrated in a graded series of ethanol and mounted on microscope slides. In these preparations the whole Langerhans cell was clearly visualized and, by adjusting the plane of focussing, even its smallest branches could be traced. In each epithelial sheet the population of Langerhans cells per square millimetre was determined by using a microscope with a graticule in the eyepiece. The position of each epithelial sheet was projected in a schematic drawing of the corresponding quadrant. This made it possible to establish the distribution of the Langerhans cells in one quadrant of the exocervix. This procedure was applied to each one of the three exocervices studied.

Paraplast-embedded material

Tissue samples from a second quadrant were incubated in V.7.4-ZIO-4 °C-18 hours. The blocks of tissue were then dehydrated in a graded series of ethanol and embedded in paraplast. Serial sections, 10 μm thick, were obtained.

Electron microscopy

Random samples from the third and fourth quadrants were processed for either of the following methods.

Conventional electron microscopy

The samples were fixed for 2 hours in a threefold aldehyde mixture containing 2·5% glutaraldehyde, 4% formaldehyde and 2% acrolein buffered to pH 7·4 with 0·2 M phosphate (Rodríguez, 1969). After three rinses in 0·1 M phosphate buffer the tissue samples were fixed in 1% OsO_4 for 2 hours. Dehydration was in a graded series of ethanol and pure acetone and was followed by embedding in a mixture of Epon and Araldite (Richardson, Jarrett & Finke, 1960). The ultrathin sections were stained with uranyl acetate and lead citrate.

V.7.4-ZIO-4 °C-18 hours

The blocks of tissue were incubated in V.7.4-ZIO for 18 hours at 4 °C. After washing in distilled water they were dehydrated and embedded as for conventional electron microscopy.

K.P.7.4-C.4.4-ZIO-4 °C-18 hours

The tissue samples were fixed with gluteraldehyde-formaldehyde fixative (Karnovsky, 1965) buffered to pH 7·4 with 0·2 M phosphate for 2 hours at room temperature. The blocks were then thoroughly washed with the same buffer (diluted to 0·1 M) and incubated in C.4.4-ZIO for 18 hours at 4 °C with continuous agitation. Ultrathin sections obtained from material processed with either of the ZIO procedures were stained with lead citrate only.

RESULTS

Light microscopy

Epithelial sheets

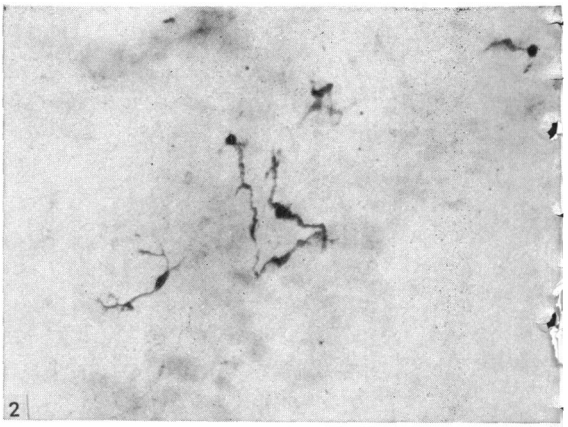
The examination of the epithelial sheets (*in toto* preparation) processed with V.7.4-ZIO made possible a detailed study of the gross morphology of the Langerhans cells as well as a quantitative and a topographical analysis (Figs. 1, 2).

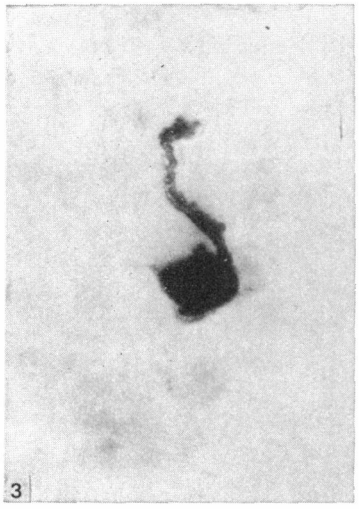
Gross morphology. The combination of both methodologies (epithelial sheets and V.7.4-ZIO) allowed, for the first time, the visualization, in a single preparation, of the whole profile of the Langerhans cells. According to the number of cell processes and the degree of branching of these processes, the Langerhans cells of the normal human exocervix were classified into five types. Type I was characterized by presenting only one unbranched process (Fig. 3); Type II also had one process, but it divided into branches (Fig. 4); Type III displayed two processes (Fig. 5); Types IV and V presented three or more processes, but the latter type also had several collaterals which, in turn, branched dichotomously (Figs. 6, 7).

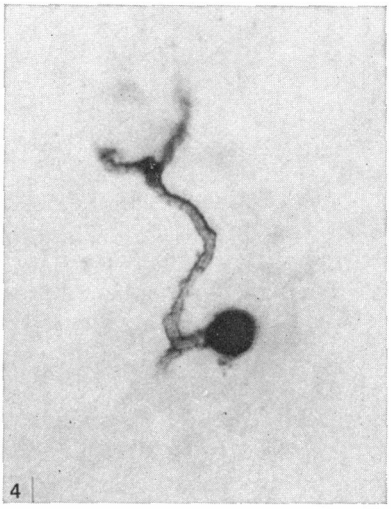
Quantitative analysis. The estimation of the total number of Langerhans cells in one quadrant of the human exocervix resulted in the following figures for the three cases studied: exocervix A 1441 cells distributed in a surface of 168·5 mm^2; exocervix B 1610 cells in a surface of 158 mm^2; exocervix C 793 cells in a surface of 126 mm^2. As shown in Table 1 the cell densities for the three cases were 8·55 cells/mm^2, 10·19 cells/mm^2 and 6.29 cells/mm^2 respectively..

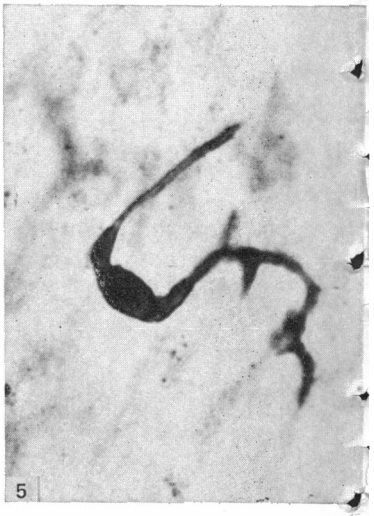
The cell density was also estimated for each one of the five types of Langerhans cell (Table 1). In the three cases studied, Type III cells were the most numerous whereas those of Type V were the least frequent. The relative frequency of each cell type is shown in Table 1.

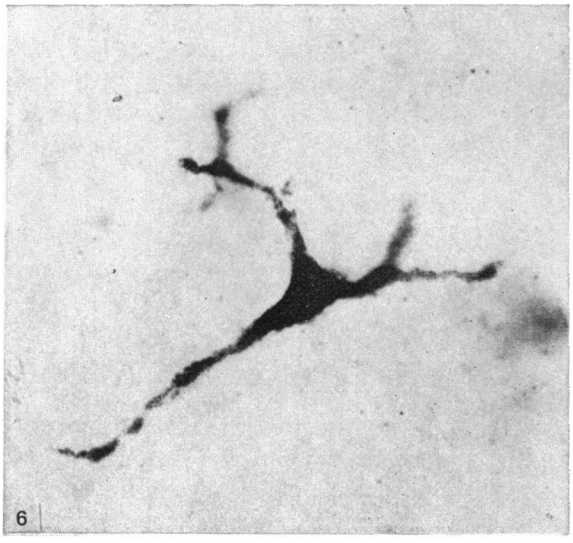
Distribution. The distribution of the different types of Langerhans cells in one quadrant of one normal human exocervix is shown in Figure 8. The other two cases followed roughly the same pattern of distribution. There seemed to be a random distribution of the cells but there were areas with a relatively high cell density alternating with others virtually devoid of Langerhans cells. The relative frequency of the different cell types was constant in areas with high density as well as in those with low density. Another consistent feature was the high cell density in the vicinity of the external os.

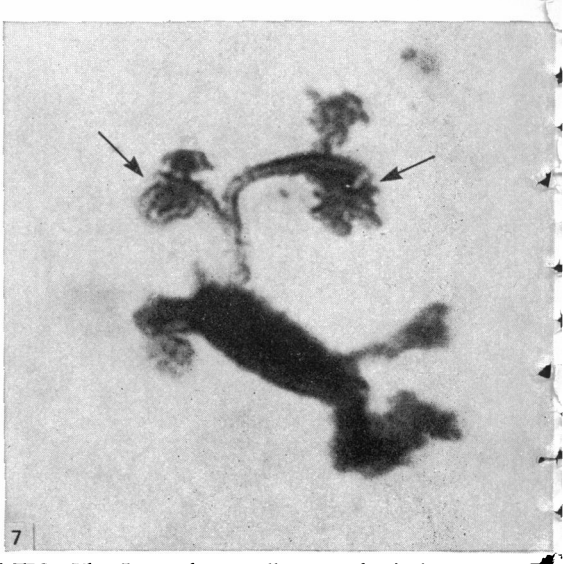












Figs. 1–7. Epithelial sheets stained with V.7.4-ZIO. The Langerhans cells are selectively stained whereas the exocervical epithelium appears as a light background. Figs. 1 and 2 represent two regions of the exocervix with different cell densities. ×170. Fig. 3. Type I. ×690. Fig. 4. Type II. ×600. Fig. 5. Type III. ×640. Fig. 6. Type IV. ×700. Fig 7. Type V. The arrows point to bundles of small branches emerging from two cell processes. ×960.

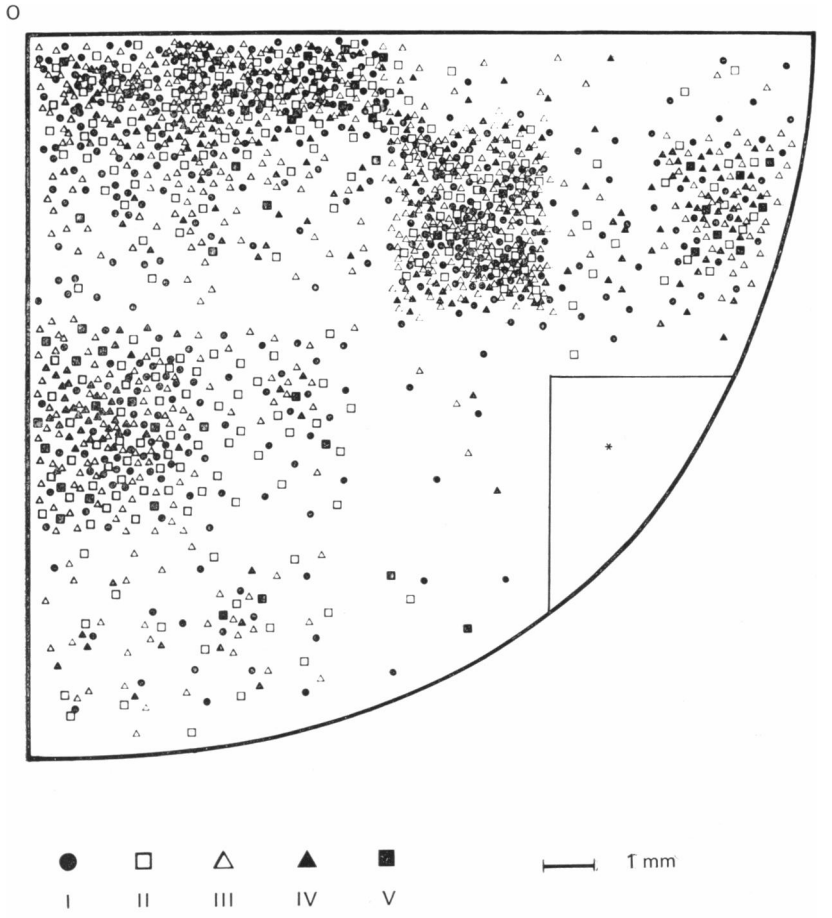


Fig. 8. Schematic representation of the distribution of *all* the Langerhans cells found in a quadrant of a normal human exocervix (case B) using epithelial sheets stained with V.7.4-ZIO. The distribution of each type of Langerhans cell (I to V) has also been represented. O, external os. The area indicated by an asterisk represents a region where it was not possible to obtain the epithelial sheet.

Table 1. *Langerhans cell density in three normal human exocervices* (Expressed as cells/mm²)

Cell types	A	B	C	$\overline{X}$	S.E.
I	2·47	2·60	1·36	2·14	0·32
II	1·32	1·42	0·63	1·12	0·20
III	2·53	3·44	1·90	2·62	0·37
IV	1·16	1·59	1·52	1·42	0·11
V	0·20	0·32	0·21	0·24	0·03
UC	0·87	0·82	0·67		
Total	8·55	10·19	6·29	8·34	0·92

A, B and C represent the three exocervices studied, $\overline{X}$, average; S.E., standard error; UC, Langerhans cells that because of their deep location in the epithelial sheet could not be clearly characterized, i.e. were unclassified.

Fig. 9. Section, several microns thick, from an Epon-embedded exocervix processed with V.7.4-ZIO. The preferential location of the Langerhans cells in the intermediate layers of the epithelium is shown. × 230.

Fig. 10. Paraplast section. V.7.4-ZIO procedure. Langerhans cell processes projecting toward the free surface of the epithelium are shown. × 560.

Paraplast sections

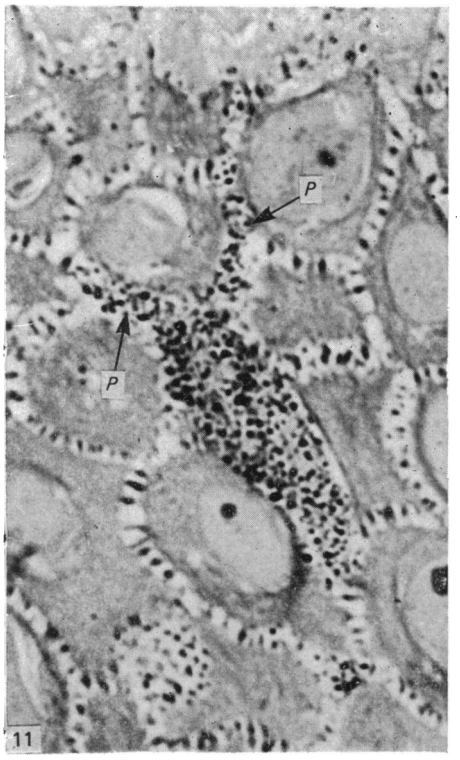
Although Langerhans cells were found at all levels of the exocervical epithelium, they appeared to be preferentially located to the intermediate and superficial layers (Fig. 9). Most of their branches projected toward the free surface of the epithelium (Fig. 10).

Semithick plastic sections

One micron sections of material processed with V.7.4-ZIO and embedded in Epon clearly revealed that the ZIO-positive reaction was confined to numerous granules distributed in the perikaryon as well as in the processes of the Langerhans cell (Fig. 11). By contrast, other methods currently used to stain these cells, e.g. the ATPase reaction, reveal only the cell membrane. Since the V.7.4-ZIO procedure stains the specific structure of the cell, the Langerhans cell granule, this technique appears to be the method of choice for demonstration of this cell type.

Conventional electron microscopy

This method revealed the typical fine structure of the Langerhans cell, as previously reported for other epithelia (Fig. 13). The cell processes, including those lying close to the free surface of the epithelium, contained the characteristic granules



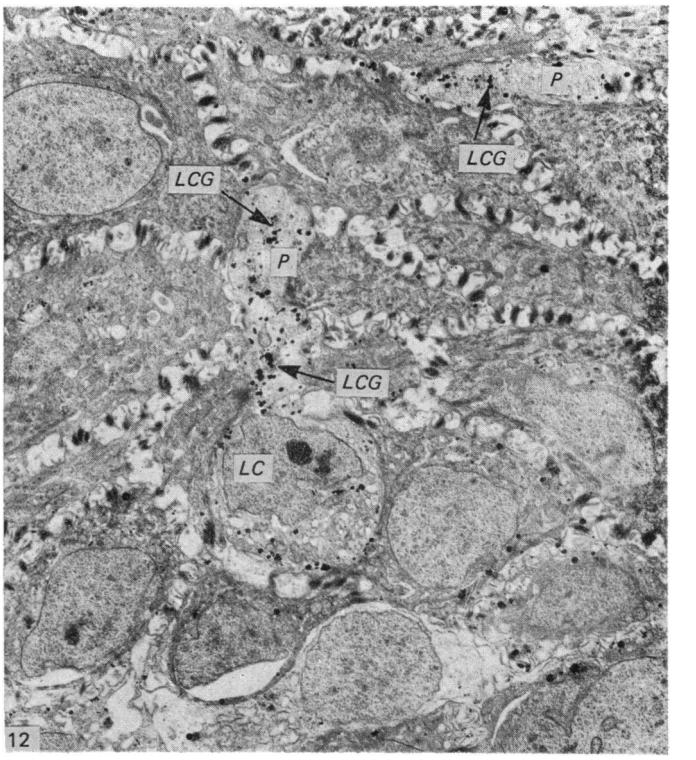


Fig. 11. One micron section from Epon-embedded material stained with toluidine blue. V.7.4-ZIO procedure. Numerous ZIO-positive granules appear in the perikaryon and cell processes (P) of a Langerhans cell. × 2000.

Fig. 12. Low power electron micrograph. V.7.4-ZIO procedure. A Langerhans cell (LC) and its processes (P) are observed. The arrows point to Langerhans cell granules (LCG) which appear as ZIO-positive dots. × 3000.

and other cytoplasmic components, including Golgi complexes. Cell junctions between Langerhans cells and the neighbouring epithelial cells were not observed. Surface protrusions of the Langerhans cells interdigitated with similar formations projected by the epithelial cells (Fig. 13).

Zinc-iodide-osmium procedures

The two ZIO procedures applied stained the Langerhans cell granules. This facilitated the visualization under the electron microscope of the perikaryon and of the finest branches of the Langerhans cells (Figs. 12 & 15) which, in material processed for conventional electron microscopy, might frequently be overlooked. The exocervical Langerhans cells reacted in essentially the same way as the epidermal Langerhans cells after treatment with the two ZIO mixtures (Rodríguez & Caorsi, 1978). Therefore, the results obtained with the application of these two methods to the normal human exocervix will be described briefly.

V.7.4-ZIO-4 °C-18 hours. Both components of the Langerhans cell granules, the disc-shaped formation (LCD) and the vacuole (LCV), appeared stained with this method (Figs. 14, 15). The lumen of the LCV was completely filled with a V.7.4-ZIO-positive material. In the LCD, the presence of graticules of orderly arrayed particles, similar to those described by Rodríguez & Caorsi (1978) in the epidermal Langerhans cell, was observed (Figs. 14*A*, *B*). This procedure also stained vesicles of the Golgi

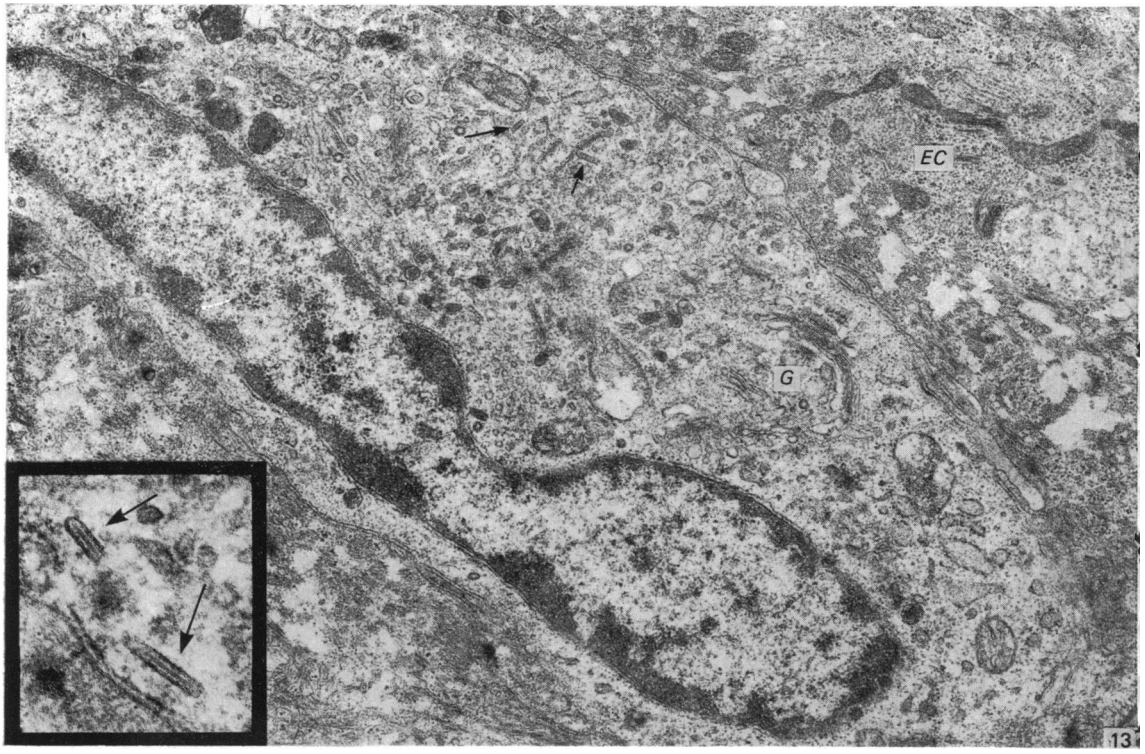
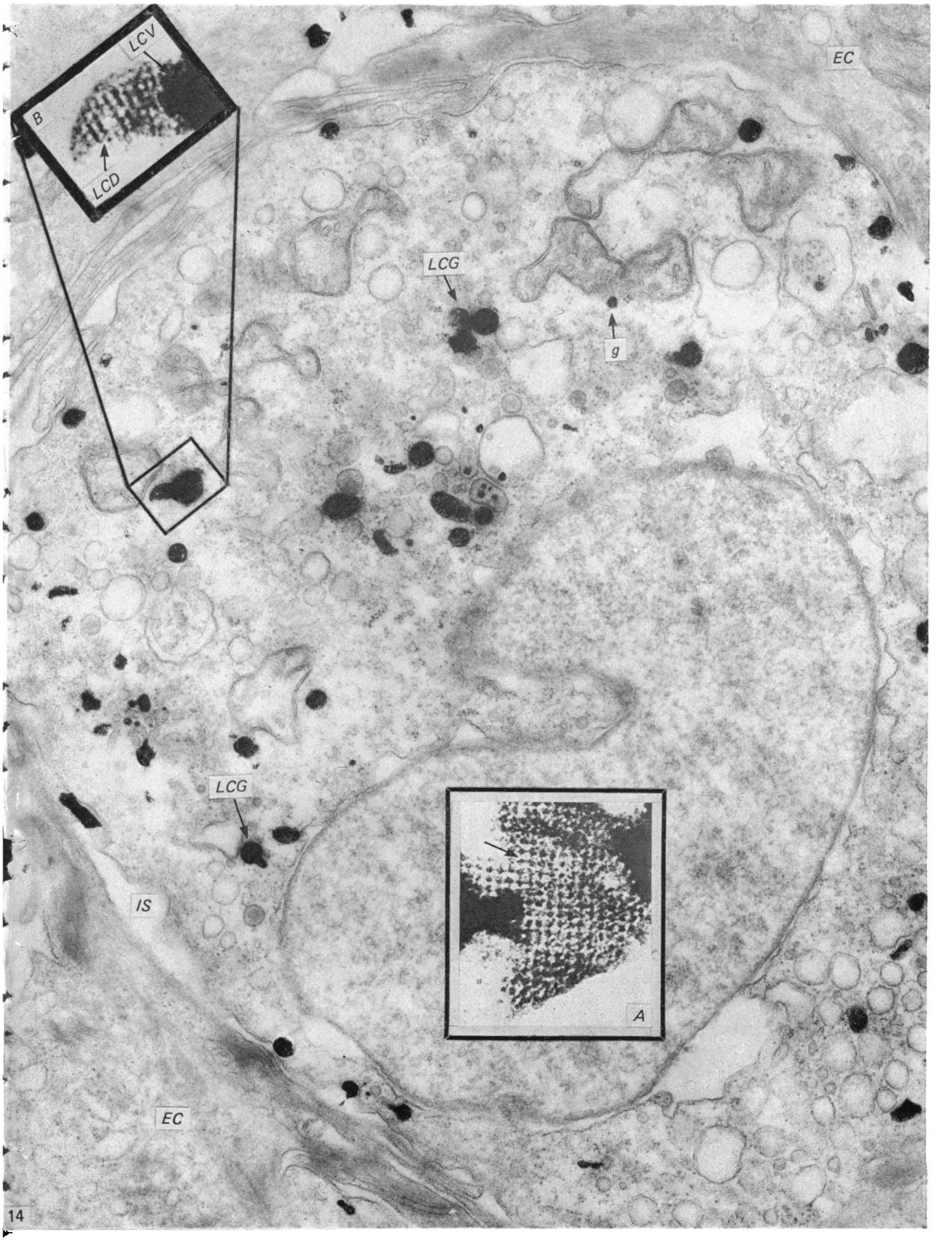


Fig. 13. Conventional microscopy. A Langerhans cell with its characteristic ultrastructure is shown. G, Golgi apparatus. The arrows indicate Langerhans cell granules. EC, epithelial cell. × 18000. Insert: The typical appearance of the disc component of two Langerhans cell granules is shown (arrows). × 48000.

apparatus and tubular formations which most probably corresponded with the smooth endoplasmic reticulum.

*K.P.*7.4-*C.*4.4-*ZIO*-4 °*C*-18 *hours*. The only structure stained by this procedure was the LCG (Fig. 16). The LCD presented exactly the same structure as that revealed by the V.7.4-ZIO procedure. The LCV, however, did behave in a different way, since only a peripheral ring was ZIO-positive whereas most of the lumen of the vacuole was unreactive (Fig. 16). Sections perpendicular to the surface of the LCD clearly showed that the ZIO-positive material was located in three different regions of the disc, i.e. in the internal leaflet of the unit membrane limiting the disc, in a layer along the internal surface of the limiting membrane and in the core of the disc.

Fig. 14. Langerhans cell processed with V.7.4-ZIO shows the selective staining of the Langerhans cell granules (LCG) and some Golgi vesicles (g). IS, intercellular space; EC, epithelial cell. × 23000. *Insert A*. Langerhans cell granule cut *en face*, showing the ZIO-positive grid formed by orderly arrayed particles (arrow). This grid is present only in the disc component of the Langerhans cell granule, not in the vacuolar component. × 120000. *Insert B*. Langerhans cell granule slightly rotated along its longitudinal axis and cut *en face*. The summation of the images of the particles gives the disc (LCD) a striated appearance. The vacuole (LCV) appears to be filled with V.7.4-ZIO-positive material. × 96000.


LCV
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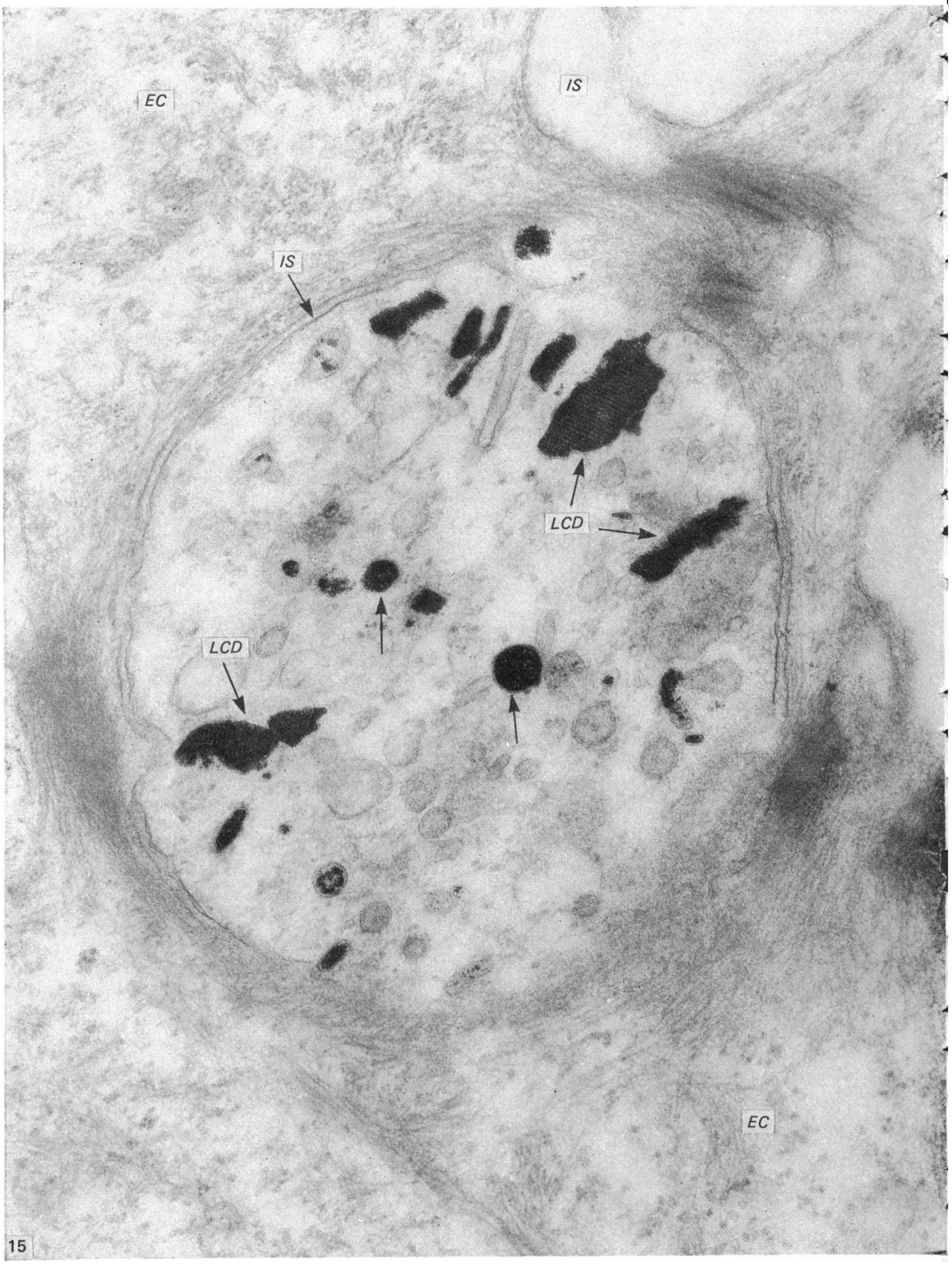
EC
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15

DISCUSSION

The use of the two modifications to the ZIO method, introduced by Rodríguez & Caorsi (1978) for the analysis of the Langerhans cell in the epidermis, has led to the demonstration that this cell is a constant component of the normal human exocervix. Furthermore, the application of the V.7.4-ZIO procedure to epithelial sheets allowed, for the first time, the visualization of the whole profile of the Langerhans cell and the demonstration of large variations in the number and branching of the cells' processes in the normal human exocervix. For descriptive purposes the cells were tentatively grouped into five types, Type I and Type V representing those cells with the minimum and the maximum number of cell processes, respectively. Since even the smallest processes contain all the organelles found in the perikaryon, including Golgi complexes and the Langerhans cell granules it seems reasonable to assume that between Type I and Type V there is a gradient of cell activity and that this rather arbitrary classification of the Langerhans cells, together with a quantitative analysis, might be a useful tool to evaluate the degree of activity of this cell type.

During recent years a large body of evidence has been presented which clearly indicates that the Langerhans cell belongs to the monocyte-macrophage-histiocyte family (Shelley & Juhlin, 1978). Thus, it has been demonstrated that there are Fc-IgG and C_3 receptors on the surface of the Langerhans cell (Stingl *et al.* 1977). Furthermore, it has also been shown that the Langerhans cell is capable of producing a surface glycoprotein (Ia) associated to the immune response (Rowden, Lewis & Sullivan, 1977; Klareskog *et al.* 1977; Stingl *et al.* 1978), sharing this capacity with macrophages (Stingl *et al.* 1978).

There is also evidence strongly suggesting that the Langerhans cells are of mesenchymal origin and that they derive from a mobile pool located in the bone marrow from whence they migrate into stratified epithelia, to function as antigen-binding and presenting cells (Katz, Tamaki & Sachs, 1979).

Following this line of reasoning, it may be suggested that the different types of Langerhans cells found in the normal human exocervix represent different degrees of antigenic stimulation of the cells at the moment of fixation. Those with the largest number of processes, and therefore with the largest surface, might contain a large number of surface receptors and so display a high antigen-binding activity. However, the possibility that the number of surface receptors associated to the immune response is not related to the extent of the cell surface should be borne in mind. It is interesting to note that in the normal human exocervix the most frequent types of Langerhans cells are those with few processes, whereas Type V cells are rare. According to preliminary observations, this situation seems to change drastically in neoplastic human exocervices (Caorsi & Figueroa, unpublished observations). Nevertheless, more experimental data and different methodological approaches are needed to decide whether or not this functional interpretation of the present morphological findings is correct.

The use of epithelial sheets made it possible accurately to establish the density

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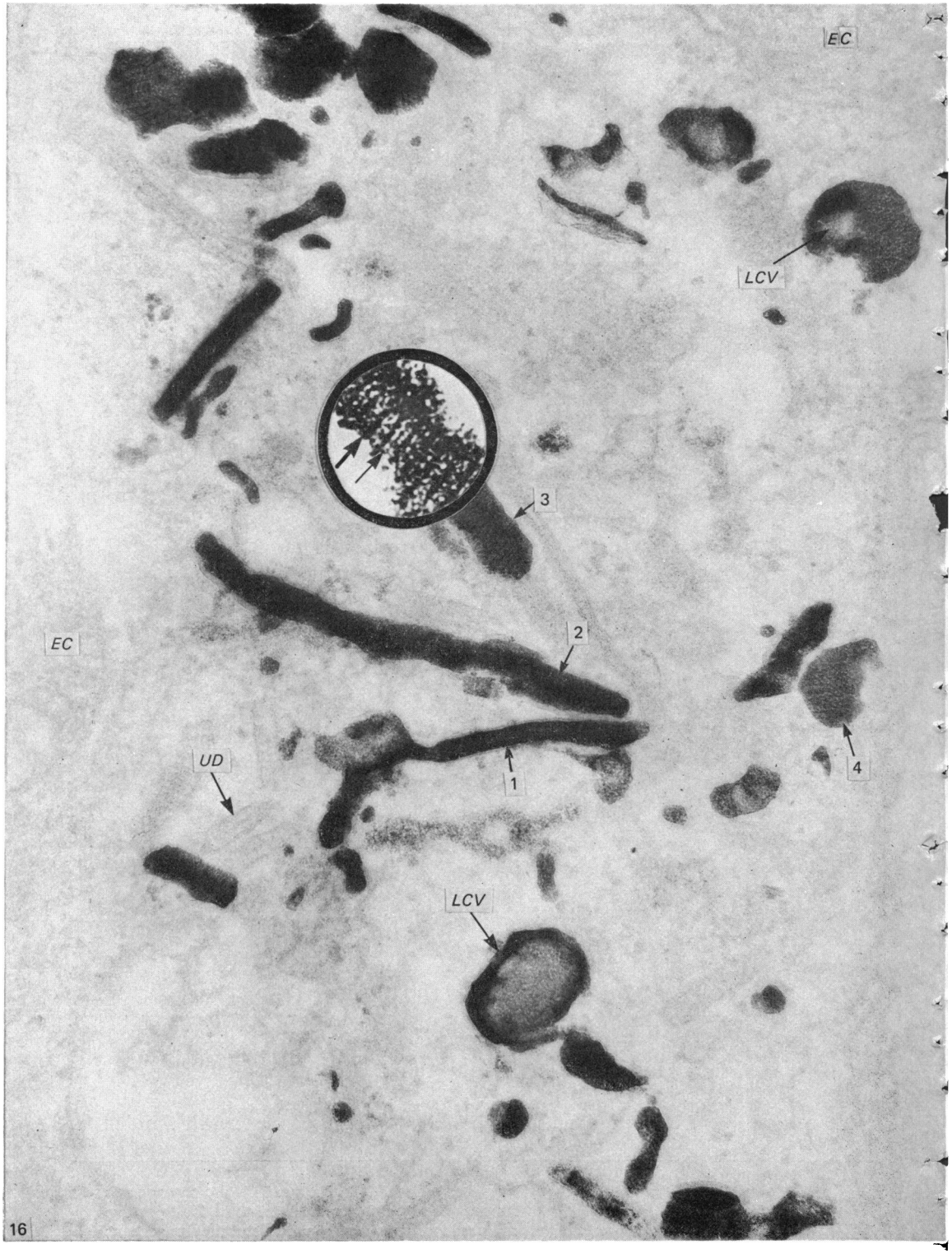
Fig. 15. Cross section of a Langerhans cell process, V.7.4-ZIO procedure. The disc components (LCD) of several Langerhans cell granules are clearly visualized. The round ZIO-positive (arrows), however, may correspond either to Golgi vesicles or to the vacuole of the Langerhans cell granule. EC, epithelial cell; IS, intercellular space. × 50000.


EC
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and distribution of the Langerhans cells in the exocervical epithelium. In the three cases studied, the average cell density was 8·3 LC/mm^2. This density is much lower than that found in the normal human epidermis (Brown, Winkelmann & Wolff, 1967) and could be interpreted as an indication that the exocervix is less exposed to antigens than is the epidermis.

The distribution of the Langerhans cells within the exocervix is uneven and to some extent, apparently, random except for the fact that in the three cases studied the cells appeared to be preferentially located in the vicinity of the external os.

The present ultrastructural findings, especially those referring to the reactivity of the cell components to the ZIO procedures, and to the macromolecular arrangement of the Langerhans cell granule, confirm those described for the epidermal Langerhans cell by Rodríguez & Caorsi (1978). The appearance of the granule in different planes of section conforms to the three dimensional model proposed by Rodríguez & Caorsi (1978).

Although all the recent findings place the Langerhans cell within the monocyte-macrophage-histiocyte family, they do not point to a specific function for it, since the presence of Fc and C_3 receptors, the capacity to express immune response-associated antigens (Ia) and the ability to bind and present antigens are all features shared by other cells such as macrophages. However, the Langerhans cell does have a distinct structural feature, its granule, not shared with any other known cell type. The origin, nature and functional role of this highly organized structure continue to be an enigma, the solution of which will probably clarify the specific role of the Langerhans cell.

It seems likely that the present results, indicating that the Langerhans cell is a constant component of the normal human exocervix, may open new avenues in the investigation of certain pathological processes of the exocervix, especially those where the immune response plays an important role. Furthermore, the accessibility of the human exocervix to clinical and morphological studies may also contribute to clarification of involvement of the Langerhans cell in both physiological and pathological processes.

SUMMARY

The gross morphology, density, distribution and ultrastructure of the Langerhans cell of the normal human exocervix were investigated. Two standardized zinc-iodide-osmium (ZIO) procedures were applied to epithelial sheets as well as to tissue samples processed for both light and electron microscopy. Conventional electron microscopical techniques were also used.

The epithelial sheet preparation allowed the vizualization of the whole profile of the Langerhans cell. It was found that the number of cell processes and the degree of their branching varied greatly from one cell to another. The cells were tentatively

Fig. 16. Process of a Langerhans cell, K.P.7.4-C.4.4-ZIO procedure. The disc of the Langerhans cell granules presents the same appearance as that observed with V.7.4-ZIO. They appear cut in different planes. The numbers (1–4) indicate a progression from the vertical (perpendicular to the surface) to the horizontal plane of section (90° rotation). The vacuole (LCV) of the Langerhans cell granule presents a distinct reactivity since the K.P.7.4-C.4.4-ZIO-positive material appears confined to its periphery. Some unreactive discs (UD) can be seen. EC, epithelial cell. × 60000. *Insert.* LCD slightly rotated along its longitudinal axis showing a thin (thin arrow) and a thick (thick arrow) striation given by the two types of particles forming the grid of the disc. × 120000.

grouped into five types and it is suggested that they represent different degrees of cell activity. The cells appeared unevenly distributed, but with a preferential location around the external os. In the three cases studied the cell density averaged 8·3 LC/mm^2.

The ultrastructural study revealed the classical fine structure of the Langerhans cell. The cell processes and their branches contained all the organelles found in the perikaryon, including Golgi complexes and Langerhans cell granules. The two ZIO procedures revealed that the complex inner organization of the granule does not differ from that in the epidermal Langerhans cell. It is concluded that the Langerhans cell is a constant component of the normal human exocervix.

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