J. Anat. (1980), **131**, 1, pp. 145–155
With 12 *figures*
Printed in Great Britain

Merkel cell differentiation in the labial mucous epithelium of the rabbit

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(*Accepted* 19 *November* 1979)

INTRODUCTION

The Merkel cell, first described by Merkel in 1975, is a receptor-like cell widely distributed in the epidermis, in the oral epithelium and in the dermal sensory corpuscles of various vertebrates (reviews by Andres & Düring, 1973; Winkelmann & Breathnach, 1973; Halata, 1975; Munger, 1975, 1977). The origin and life history of the Merkel cell is obscure. Because of the synaptic contact with a nerve fibre, or the presence of an intranuclear rodlet in the cell, or from its assumed function, many investigators regard it as a neuronal cell (Iggo & Muir, 1969; Breathnach & Robins, 1970; Breathnach, 1971; Hashimoto, 1972*a*, *b*; Winkelmann & Breathnach, 1973; Fortman & Winkelmann, 1973; Straile, Tipnis, Mann & Clark, 1975; Fortman & Winkelmann, 1977; Winkelmann, 1977; Fox & Whitear, 1978). Recently, Fujita (1976) included the cell as a member of the paraneurons, which was a new concept proposed by Fujita & Kobayashi in 1975.

Although Breathnach & Robins (1970), Breathnach (1971) and Hashimoto (1972*b*), showed that the dermal Merkel cell is present in embryonic human skin and considered it to be a precursor of the epidermal Merkel cell, they did not demonstrate Merkel cells that were clearly immature.

On the other hand the presence of desmosomes and fine cytoplasmic filaments in the Merkel cell led some investigators to speculate that it might be derived from the epidermal cell (Munger, 1965; Kurosumi, Kurosumi & Suzuki, 1969; Smith, 1967; Lyne & Hollis, 1971; Nafstad, 1971*a*, *b*; Saxod, 1978). Iggo & Muir (1969) and Nafstad (1971*a*, *b*) demonstrated cells which showed a structure intermediate between that of the Merkel cell and keratinocyte. Nafstad (1971*a*, *b*), Budtz & Larsen (1975) and Saxod (1978) considered these 'transitional' cells to be differentiating Merkel cells and English (1974, 1977) thought that they might be either differentiating or degenerating because they occurred in both injured and denervated cutaneous Type I mechanoreceptors (Haarscheiben).

Tachibana (1979) showed that the immature Merkel cell occurred only in the epidermis of the labial ridge of the anuran tadpole. In the course of preliminary research on the distribution of Merkel cells in the oral mucosa of the rabbit the present authors noticed that the Merkel cell showed a tendency to increase in number with postnatal development (Tachibana, Nawa & Ishizeki, 1979); also, that transitional cells occurred more frequently in younger rabbits. The life history of the Merkel cell in the labial mucous epithelium was therefore examined in detail.

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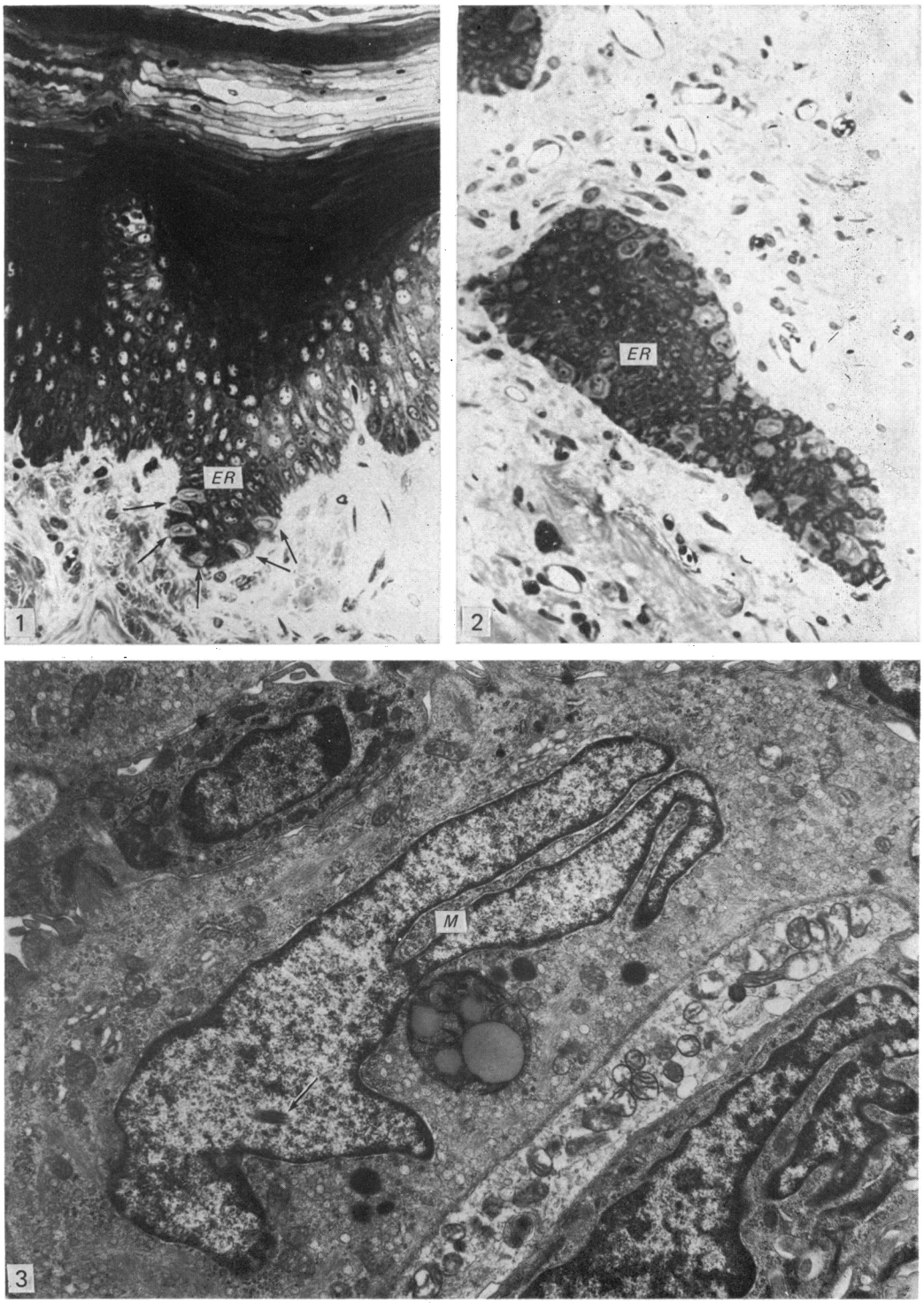


Fig. 1. Light micrograph of the labial mucosa of an adult rabbit. The Merkel cells (arrows) are seen in a cluster in the epithelial ridge (*ER*). ×600.

Fig. 2. Tangential section of the labial mucosa of an adult rabbit. The basal zone of the epithelial ridge (*ER*) is shown, with Merkel cells. ×800.

Fig. 3. Electron micrograph of a Merkel cell (*M*) in the labial mucous epithelium of an adult rabbit. The intranuclear rodlet (arrow) and a large lysosomal body can be seen. ×9400.

Table 1. *The distribution frequency of the Merkel cell and the transitional cell in the labial mucous epithelium of the rabbit*

Age of rabbits	Total length of scanned epithel. (μm)	Merkel cell count		Average count of Merkel cells per unit length of epithelium (/mm)	Transitional cell per total Merkel cell (%)
		Definitive	Transitional		
Infant (12 days)	11900	49	21	5·9 (4·1*)	30·0
Juvenile (6 weeks)	4500	70	14	18·7 (15·6*)	16·7
Adult	13300	96	1	7·3 (7·2*)	1·0

* Average count of definitive Merkel cells per unit length of epithelium

MATERIALS AND METHODS

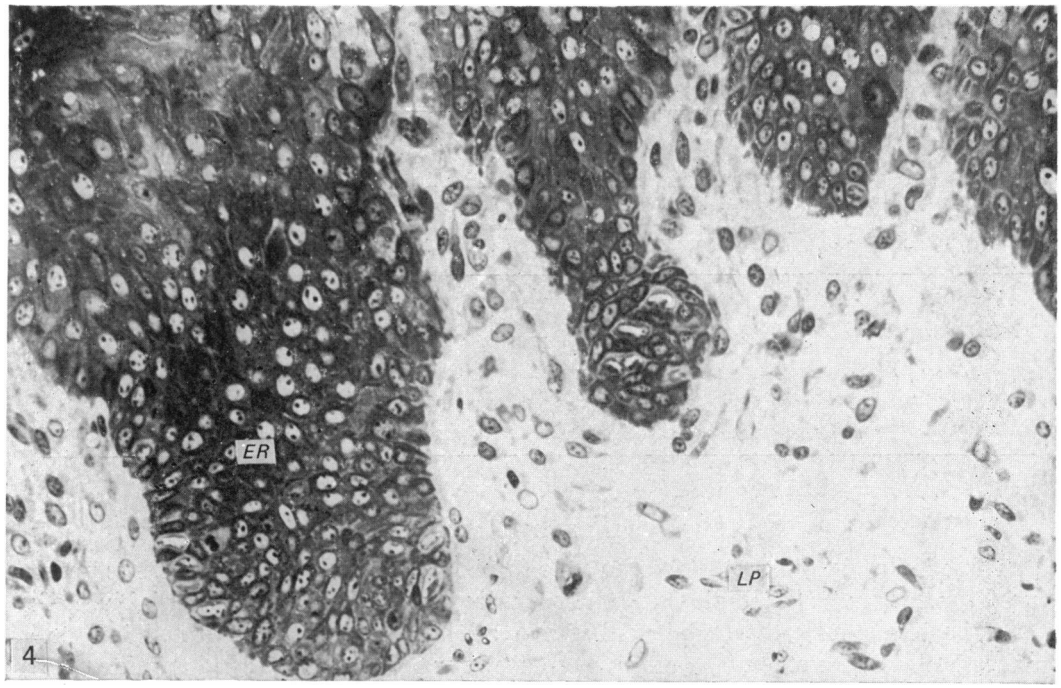
Three infant (12 days old), two juvenile (6 weeks old) and two adult rabbits were used for this study. Small pieces of mucosa were dissected from the lower labium of pentobarbitone-anaesthetized juveniles and adults and from decapitated infants, anaesthetized with ether. The pieces were fixed for 3 hours in 2·5 % glutaraldehyde buffered with cacodylate (pH 7·2), cut into small blocks, post-fixed in osmium tetroxide, and processed routinely for embedding in Epon 812. Semithin sections (stained with toluidine blue) and ultrathin sections (stained with uranyl acetate followed by lead citrate) were taken in the vertical and tangential planes. In order to estimate the frequency of different cell types, ultrathin sections were cut at 20–25 μm intervals, mounted on formvarcovered single slot grids and observed throughout. Serial ultrathin sections were carried out on four transitional cells from juvenile labial epithelium.

RESULTS

The definitive Merkel cell showed the following features. The irregularly ovoid cell contained specific cored granules, empty vesicles, and cytofilaments of moderate electron density scattered through the cytoplasm (Figs. 3, 5). Finger-shaped cytoplasmic projections, desmosomes, and synaptic contact with a nerve ending were found at the boundary of the cell (Fig. 3), and the intranuclear rodlet was of common occurrence (Figs. 3, 5).

Transitional cells, which partially fulfilled the criteria of definitive Merkel cells, were also found. Although the ultrastructure of the transitional cells differed slightly from one another, all cells had common inclusions, i.e., cored granules resembling the Merkel cell granules, intranuclear rodlets, and tonofilament-like fibrillar bundles which were located in the perinuclear region (Figs. 7–12). The transitional cells were easily distinguished from other cell types, including Langerhans cells and neutrophils, which were also present in the epithelium.

In the adult rabbits, Merkel cells and transitional cells were limited to the epithelial ridges. The perpendicular and horizontal sections showed the Merkel cells to be arranged in clusters at the apices of the ridges (Figs. 1, 2). Almost all Merkel cells were of the definitive type; when the mucous epithelium was scanned at 13300 μm in total length, 96 definitive Merkel cells and only one transitional cell were counted



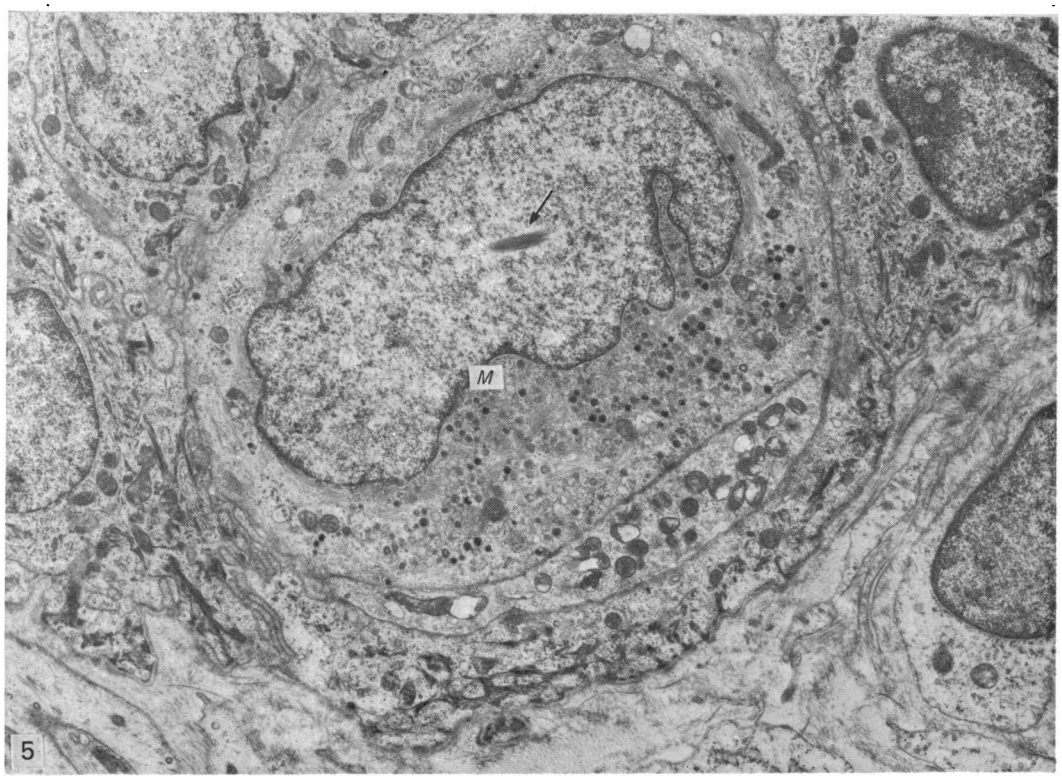
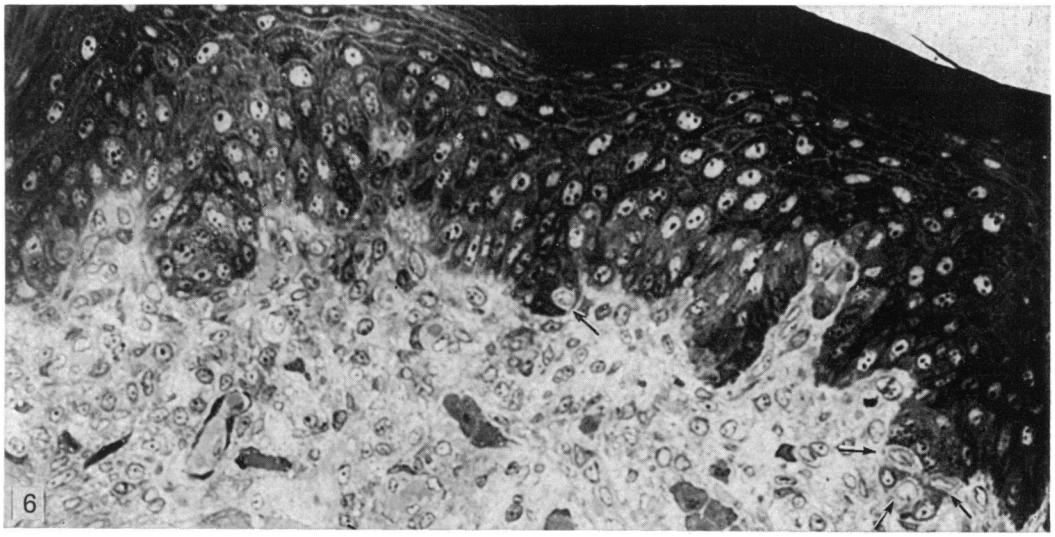


Fig. 4. Light micrograph of the labial mucosa of a juvenile rabbit. The Merkel cells are seen in the epithelial ridges (*ER*) in clusters. *LP*, lamina propria. × 800.

Fig. 5. Electron micrograph of a Merkel cell (*M*) in the labial mucous epithelium of a juvenile rabbit. Note the intranuclear rodlet (arrow). × 8000.



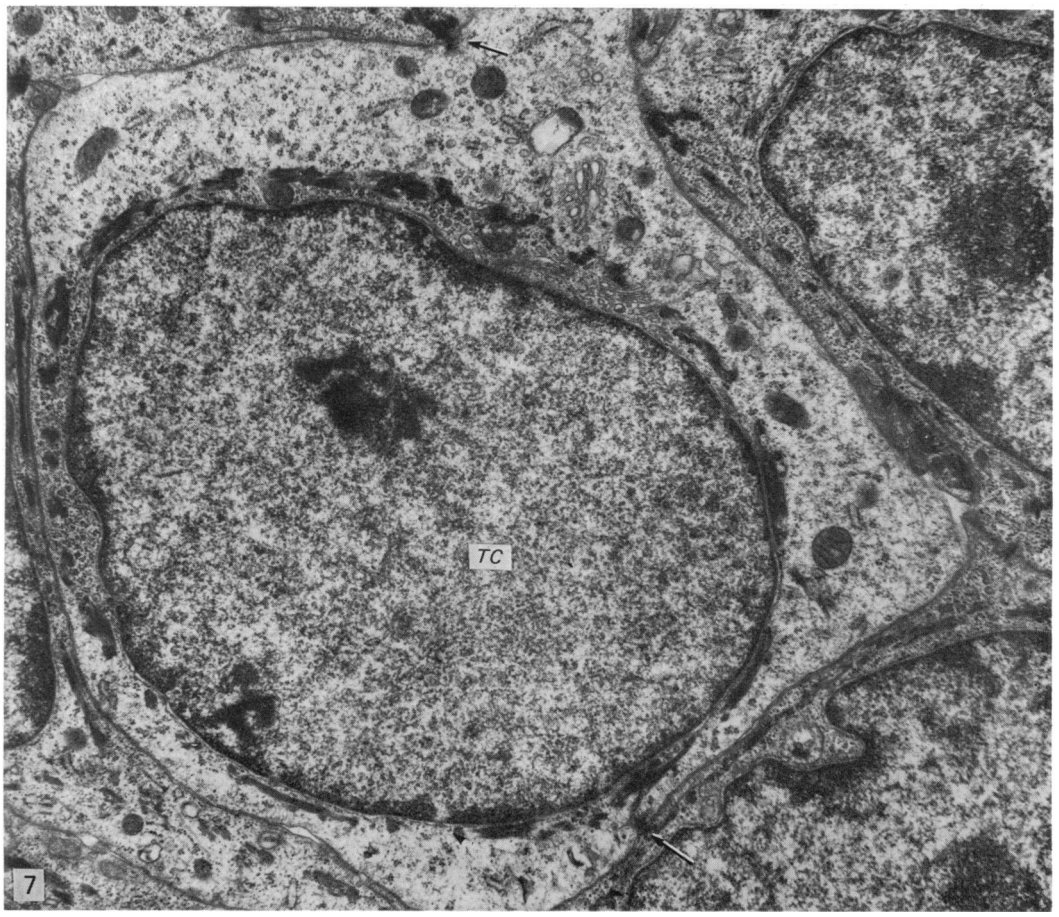


Fig. 6. Section of the labial mucosa of an infant rabbit. Arrows indicate Merkel cells. × 800.

Fig. 7. Electron micrograph of a transitional cell (*TC*). Note the clumped tonofilament bundles encircling the spherical nucleus. A few desmosomes (arrows) can be seen between the adjacent keratinocytes and the transitional cell. × 13400.

(Table 1). The average count of the definitive Merkel cells per unit length of the epithelium was about 7·2/mm. The percentage of transitional cells to Merkel cells was estimated to be about 1 %.

In the juvenile also, the Merkel cells were observed in clusters in the epithelial ridges (Fig. 4). Electron microscopy revealed that several transitional cells lay between the definitive Merkel cells, although they could not be distinguished by light microscopy (Fig. 10). Various types of transitional cells were observed, as will be described. When nine sections cut at 25 μm intervals were scanned with the electron microscope, 70 definitive Merkel cells and 14 transitional cells were counted within 4500 μm length of the epithelium (Table 1). The average of definitive Merkel cells per unit length of the epithelium was 15·6/mm. The transitional cell ratio to the sum of Merkel cells and transitional cells was about 16·7 %. In the lamina propria, although many Schwann cells and other similar cells were observed in association with unmyelinated nerve fibres or in isolation, no Merkel cells or transitional cells were identified.

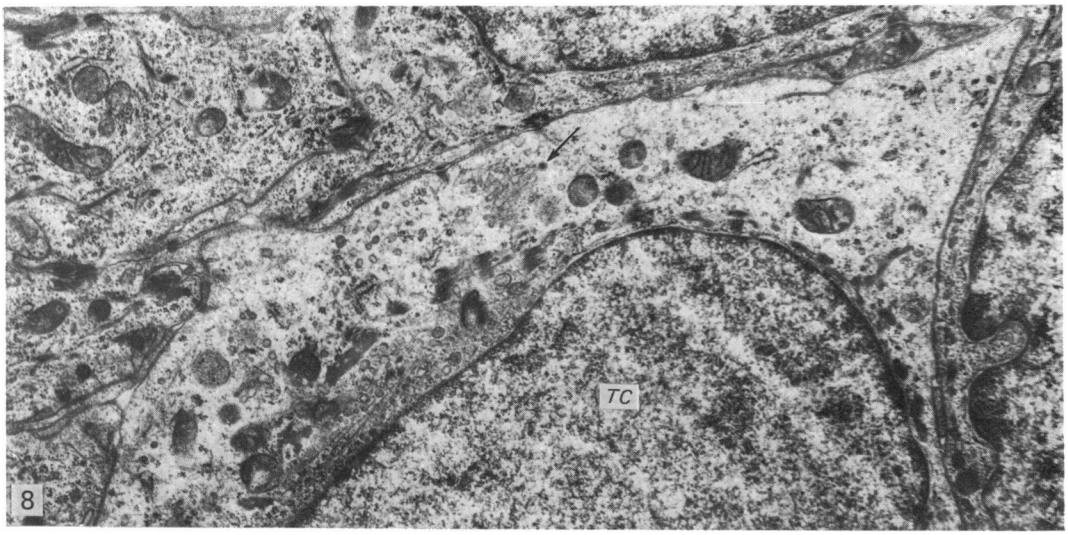
In the infants, the boundary between the epithelium and the lamina propria was slightly irregular, and the epithelial ridges and the papillae of the lamina propria were still poorly organized (Fig. 6). The Merkel cells and transitional cells were distributed in the small epithelial precursors of the future ridges (Fig. 6). In 14 sections cut at 20 μm intervals, 49 definitive Merkel cells and 21 transitional cells were observed within 11 900 μm length of the epithelium (Table 1). The average frequency of the definitive Merkel cells was 4·1/mm, and the ratio of transitional cells to the sum of Merkel and transitional cells was about 30 % (Table 1). No Merkel or transitional cells were identified in the lamina propria.

Of the four transitional cells studied in serial sections, two resembled immature keratinocytes in displaying abundant cytoplasmic fibrillar bundles resembling tonofilaments (Fig. 7). However, both cells showed smooth boundaries, unlike keratinocytes (Fig. 7). Furthermore, the tonofilament-like bundles were located only around the spherical nucleus (Fig. 7). Small Golgi complexes, oval mitochondria, and polysomes were scattered in the electron-lucent cytoplasm (Figs. 7–9). A number of cored granules resembling the Merkel cell granules, centrioles associated with microtubules, and small intranuclear rodlets were present (Figs. 8, 9). The serial sections also showed that these transitional cells lacked finger-shaped cytoplasmic projections and synaptic contacts with nerve fibres. On the other hand, a few short desmosomes connected them to neighbouring immature keratinocytes.

The other two transitional cells resembled definitive Merkel cells except that electron-dense fibrillar bundles persisted in places in the perinuclear region, and other fine filaments of moderate density were more widely dispersed (Fig. 10). The nuclei were irregular and the cell organelles, including cored granules, were numerous (Figs. 10, 12). Centrioles associated with microtubules, and intranuclear rodlets were also identified (Figs. 11, 12). Although connections between these cells and nerve fibres were observed in places, the contact areas were fairly narrow (Fig. 10). Several cytoplasmic projections of digital shape and short desmosomes were also observed at the cell boundaries (Fig. 10).

DISCUSSION

Of the two hypotheses concerning the origin of the Merkel cell, the neuroectodermal origin hypothesis has received more attention than the epithelial origin hypothesis. Certainly, the fine structure of the cell suggests that it belongs to the



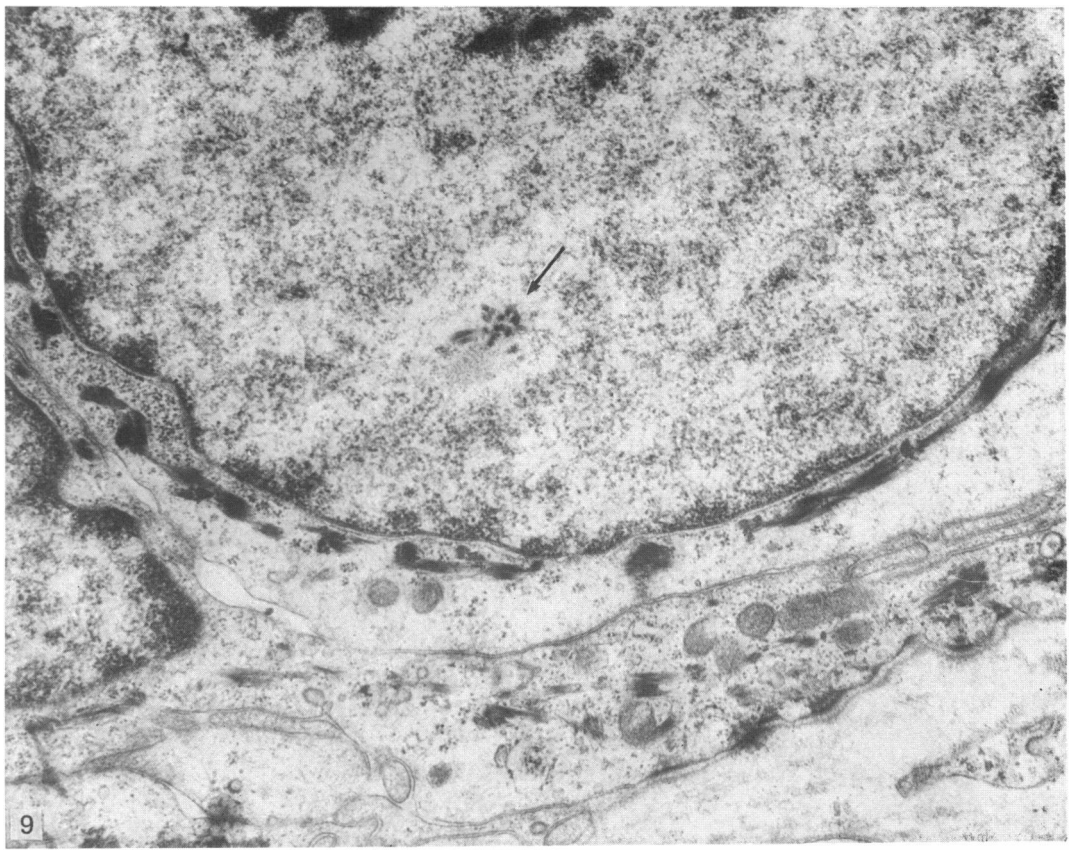
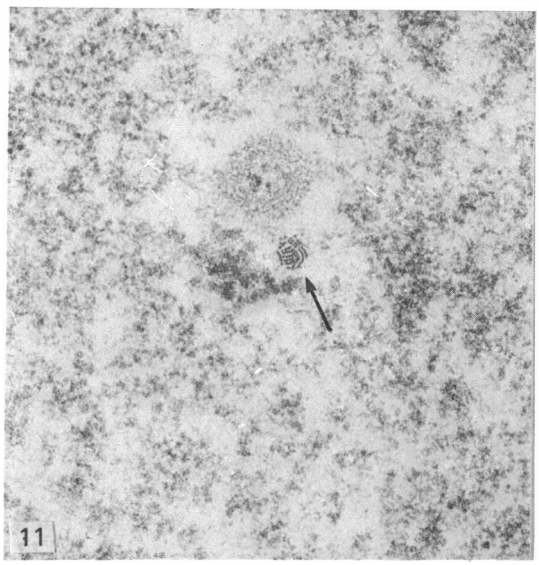


Fig. 8. Enlargement of part of the cell (*TC*) shown in Figure 7, in another serial section. A centriole, microtubules and small vesicles can be seen. Arrow indicates a Merkel cell granule. ×16800.

Fig. 9. Enlargement of the nucleus of the same transitional cell shown in Figure 7, in a further serial section. The small rodlet can be seen in the nucleus (arrow). ×21000.



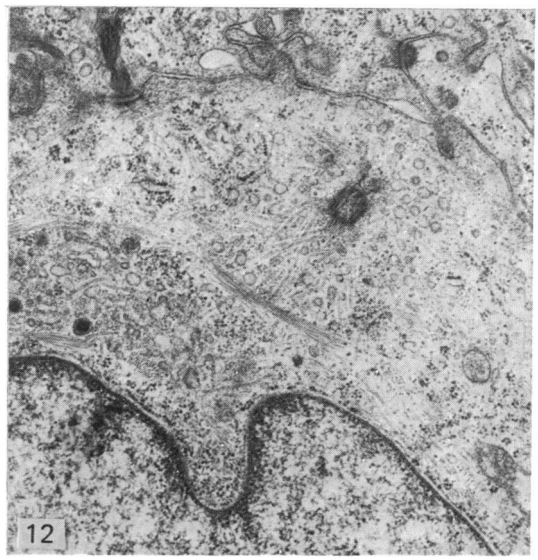


Fig. 10. Electron micrograph of a transitional cell (TC). The cell looks more mature than the cell shown in Figure 7. Some cytofilaments are in clumped bundles but others occur more widely dispersed. M, definitive Merkel cell: ×8700.

Fig. 11. An enlargement of the nucleus shown in Figure 10. The cross image of the intranuclear rodlet (arrow) is shown. ×32000.

Fig. 12. An enlargement of the cell shown in Figure 10, in a different serial section. The centriole and radiating microtubules are shown. ×18000.

neuronal cell line. For instance, its synaptic contacts with nerve endings and accumulation of the specific cored granules in the cytoplasm seem to indicate that the cell releases some neurotransmitter. According to Straile *et al.* (1975), the intranuclear rodlet of the Merkel cell, which was first described by Fortman & Winkelmann (1973), is similar to that of other neuronal cells. The finding of Merkel cells in fetal dermis (Breathnach & Robins, 1970; Breathnach, 1971; Hashimoto, 1972*b*) may support the concept that they could have migrated from the neural tube or crest during embryonic life.

However, the epidermal origin hypothesis has persisted as stated previously because of the existence of a cell showing features transitional between the Merkel cell and the keratinocyte. The transitional cell resembles the keratinocyte in having tonofilament bundles in the cytoplasm but it also resembles the Merkel cell in having the specific cored granules.

Different types of transitional cell, showing a gradual change between the keratinocyte and the definitive Merkel cell, were observed in the present investigation. When the distribution frequency of the definitive Merkel cell was compared, it was highest in the juvenile and lowest in the infant. On the contrary, the frequency of the transitional cell was highest in the infant and lowest in the adult. In view of the postnatal expansion of the oral mucosa, these results suggest that the Merkel cell increases in number during the postnatal development of the rabbit, and that the transitional cell is a differentiating Merkel cell. Nothing was seen to suggest that Merkel cells migrate from lamina propria to epithelium in the lip of the rabbit, agreeing with the condition found in developing anuran tadpoles (Tachibana, 1979).

The present evidence, although circumstantial, favours the differentiation of Merkel cells from precursors in the basal layer of the epithelium. The stimulus for differentiation is unknown: initiation does not appear to involve direct contact with nerve endings since the most immature transitional cells seen here were nerve-free. The presence of centrioles and microtubules in the transitional cells may indicate the possibility of Merkel cell production by means of transitional cell mitosis.

SUMMARY

To clarify the relation between the so-called 'transitional' cell and the Merkel cell, the frequency of these cells in the labial mucosal epithelium was compared between infant, juvenile and adult rabbits. The transitional cell was observed in serial ultrathin sections. Transitional cells were most numerous in the infant and juvenile, while the definitive Merkel cell showed a reciprocal increase in number. In the infant and juvenile, the transitional cells differed slightly from each other in their ultrastructure: some resembled immature keratinocytes, others resembled definitive Merkel cells. All transitional cells contained the Merkel cell granules and the intranuclear rodlet. It was interpreted that the Merkel cell in the labial mucous epithelium develops from the transitional cell. Neither definitive Merkel cells nor transitional cells were identified in the lamina propria. The present circumstantial evidence favours the hypothesis that Merkel cells differentiate from precursors in the basal layer of the epithelium.

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