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ULTRASTRUCTURAL AND CYTOCHEMICAL STUDIES OF MERKEL CELL GRANULES IN THE MOUSE HAARSCHEIBE

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ABSTRACT

Chung-Faye Chao and Hsu-Mu Liang (1975). *Ultrastructural and Cytochemical Studies of Merkel Cell Granules in the Mouse Haarscheibe.* Bull. Inst. Zool., Academia Sinica 14(2): 71-78. (1975) The formation, release and chemical nature of dense core granules of Merkel cells of the Haarscheibe (hair-disc) were studied in adult NIH white mice by electron microscopy and cytochemistry. It was found that the granules are formed from the Golgi apparatus and their content released into the synaptic cleft by fusion with the synaptic membrane and that the content of the granules is monoamine in nature but not catecholamine.

The Merkel cell was discovered by Merkel in 1875. It is found in cats, dogs and primates including men[1~3,6,7,9~13]. Many authors believe that the Merkel cell is a slowly adapting touch receptor[7,9,11~13]. The cell posesses various structural characteristics, among which the presence of cytoplasmic dense core granules is the most outstanding feature because the granules are suggested to correlate closely with the function of the cell as a touch receptor. However, the formation, release and chemical nature of the specific granules have not been clearly known. In order to elucidate these points the Merkel cells in Haarscheibe (hair-disc) of the mouse skin were studied in th present work with electron microscopy and cytochemistry.

MATERIALS AND METHODS

Adult NIH white mice were used in this study. The mice were sacrificed by neck dislocation. Specimens of the middorsal skin were obtained by corneoscleral punch.

The skin specimens were cut into 1 mm^2 pieces and prefixed in Karnovsky's fixative for one and half hours, rinsed in sodium carcodylate buffer (0.1 M, pH 7.2) and postfixed in 1% osmic acid for two hours. They were then dehydrated in rising concentrations of ethanol, treated with propylene oxide and embedded in Epon 812. Thin sections were cut in a Reichart OmU2 ultramicrotome, placed on Formvor coated single hole copper grids and stained with uranyl acetate and lead citrate. The sections were observed with JOEL 100B electron microscope for morphological purpose.

The cytochemical techniques used are as following:

1. Ammoniacal silver[8] test for catecholamine:

It is known that the granules of adrenal medulla cells contain catecholamine. Therefore

we used the adrenal gland as an indicator for the presence of this compound. The skin specimens and adrenal glands were treated simultaneously with the same procedure. The specimens were fixed in 6.5% glutaraldehyde, rinsed in Milloning buffer and incubated in ammoniacal silver solution for 20, 40 and 60 seconds respectively. They were then rinsed in 1% sodium thiosulfate, dehydrated in rising concentrations of ethanol, treated with propylene oxide and embedded in Epon 812. Thin sections were observed without staining. The control specimens of skin and adrenal glands were incubated in the medium without silver nitrate.

2. Potassium dichromate test for catecholamine[14]:

Adrenal glands were also used as the reference standard. Both skin specimens and adrenal glands were fixed in 3% glutaraldehyde, incubated in 2.5% potassium dichromate solution for 24 hours at pH 4.1, 4°C, dehydrated in rising concentrations of ethanol, treated with propylene oxide and embedded in Epon 812. Thin sections were observed without staining. The control tissues were incubated in the medium without potassium dichromate.

3. Potassium permanganate test for monoamine[4]:

Potassium permanganate reacts with different types of biogenic monoamines resulting in the formation of precipitates. This may be an oxidation-reduction reaction in which $KMnO_4$ is reduced to MnO_2 by hydroxyl groups of the amine. Therefore the monoamine storing granules can be identified by $KMnO_4$ fixative under electron microscopical level.

Skin specimens and adrenal glands were fixed in 3% $KMnO_4$ (Kerb-Ringer phosphate buffer pH 7.0, at 4°C.) for 15–30 min, dehydrated in rising concentrations of ethanol, treated with propylene oxide and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate.

4. Reserpine inhibition test for monoamine:

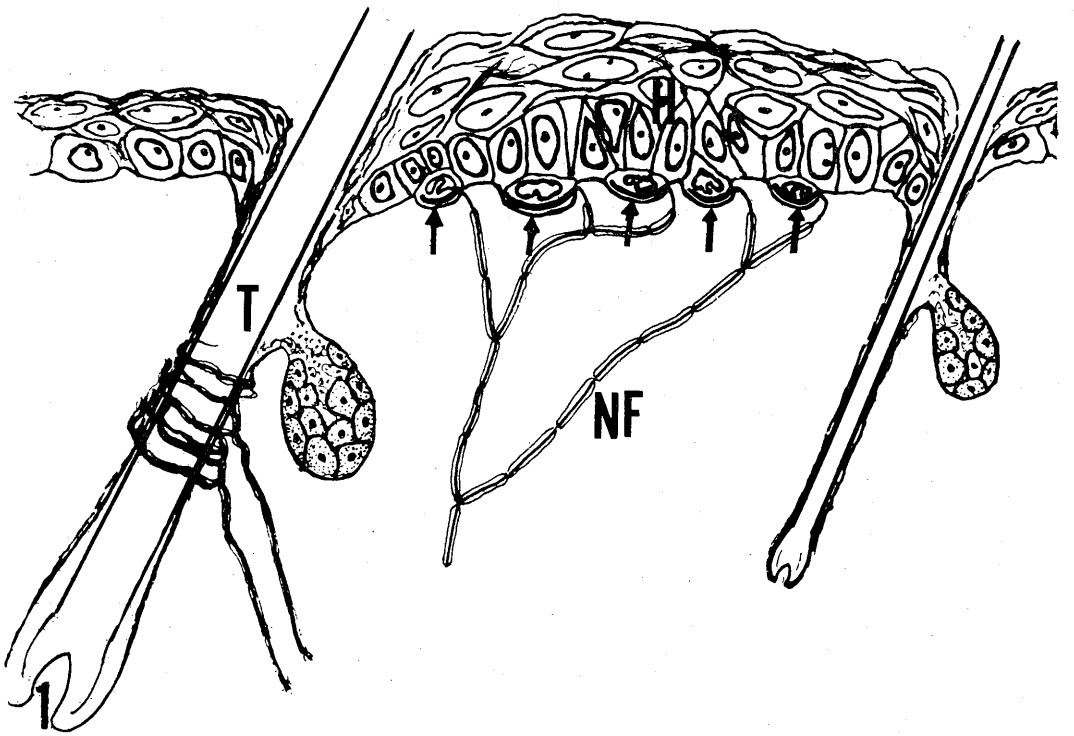
Six mice were equally divided into two groups. Two of the first group were injected (i. p.) daily with 5 mg/kg of reserpine for 2 days, the remaining one injected with distilled water as the control. Two of the other group were injected with the same dosage of reserpine for 4 days, and one mouse again as the control. The skin specimens and adrenal glands were excised for morphological and cytochemical examinations.

RESULTS AND DISCUSSION

1. Morphology of the Merkel cell (Figs. 1 and 2):

Merkel cells (arrows, Fig. 1) observed in this study are situated in the basal layer of the epidermis arranged in a row of about 6-cells under a dome-shaped elevation of the epidermis known as the Haarscheibe (H, Fig. 1). Most of the Merkel cells are oblong in shape with their major axes parallel to the epithelial-dermal border. Its superficial part is embedded in the epidermis and its deep part bulging toward the dermis. The Merkel cell is attached to adjacent keratinocytes by desmosomes (D, Fig. 2) and short rod-like processes (RP, Fig. 2) protruding to the surrounding keratinocytes but not into the intercellular space. The nerve end plate (NEP, Fig. 2), closely associated with the dermal face of the Merkel cell, is embraced by thin lamellae (TL, Fig. 2) of the Merkel cell.

The other characteristics of the Merkel cell are clear cytoplasm, lobulated nucleus and specific granules. Each granule (g, Fig. 2) is composed of an electron-dense core bounded with a membrane about 800–2000 Å in diameter. Few organelles and granules are observed in the projecting lamellae of the Merkel cell, numerous pinocytotic vesicles are found in the lamellae. The rough endoplasmic reticulum is usually poorly developed but free ribosomes are numerous. The centriole (C, Fig. 2) if present, is found in the upper part of the cell. The mitochondrin are elongated. The Golgi apparatus (G, Fig. 2) is often located at the upper part of the cell. Vesicles and multivesicular bodies are



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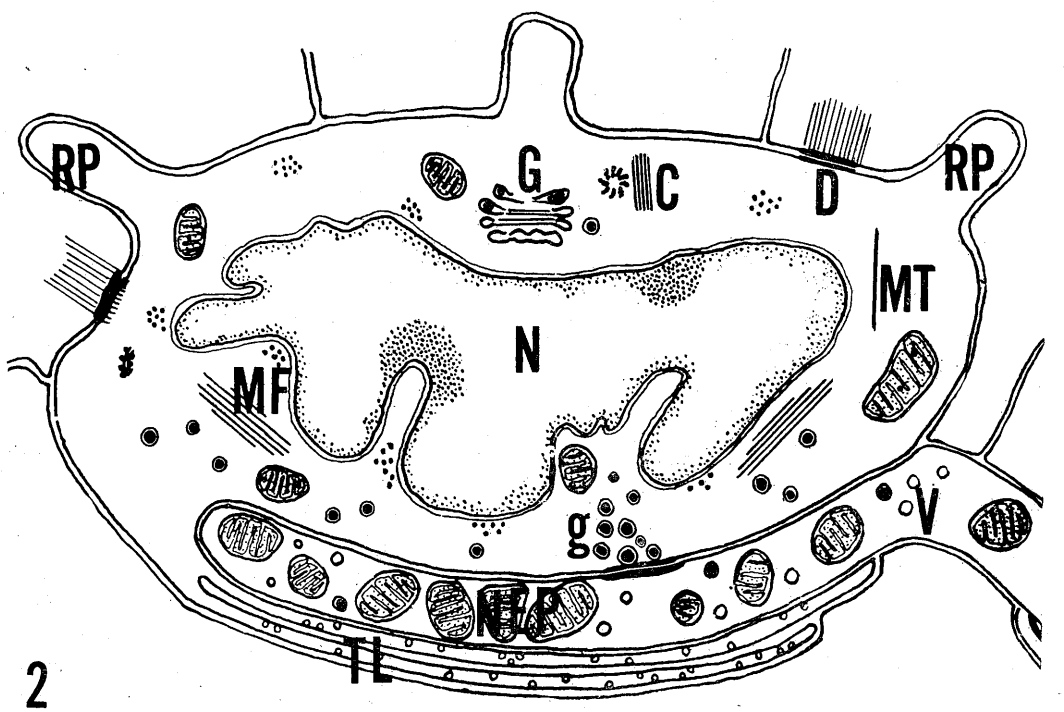

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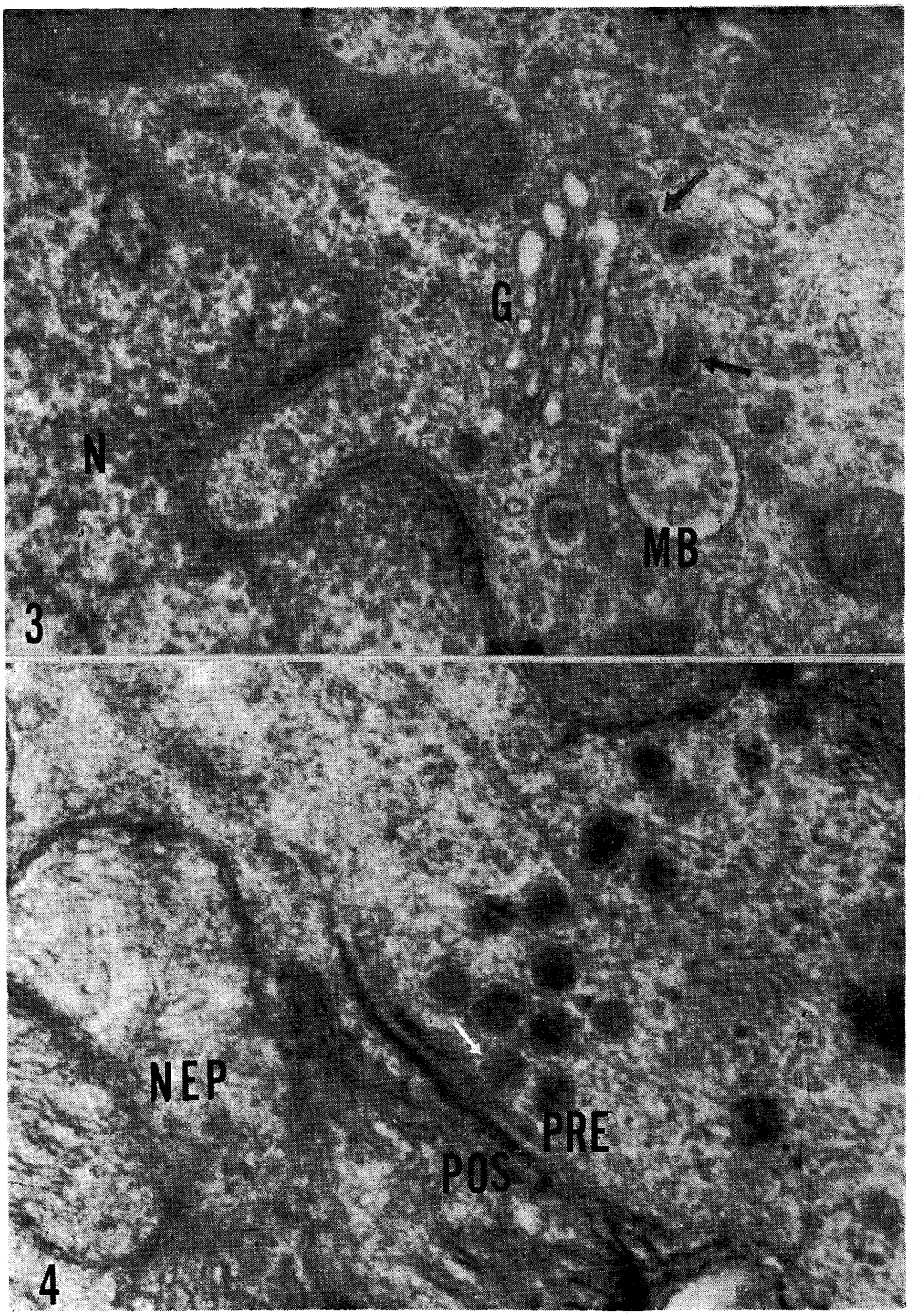
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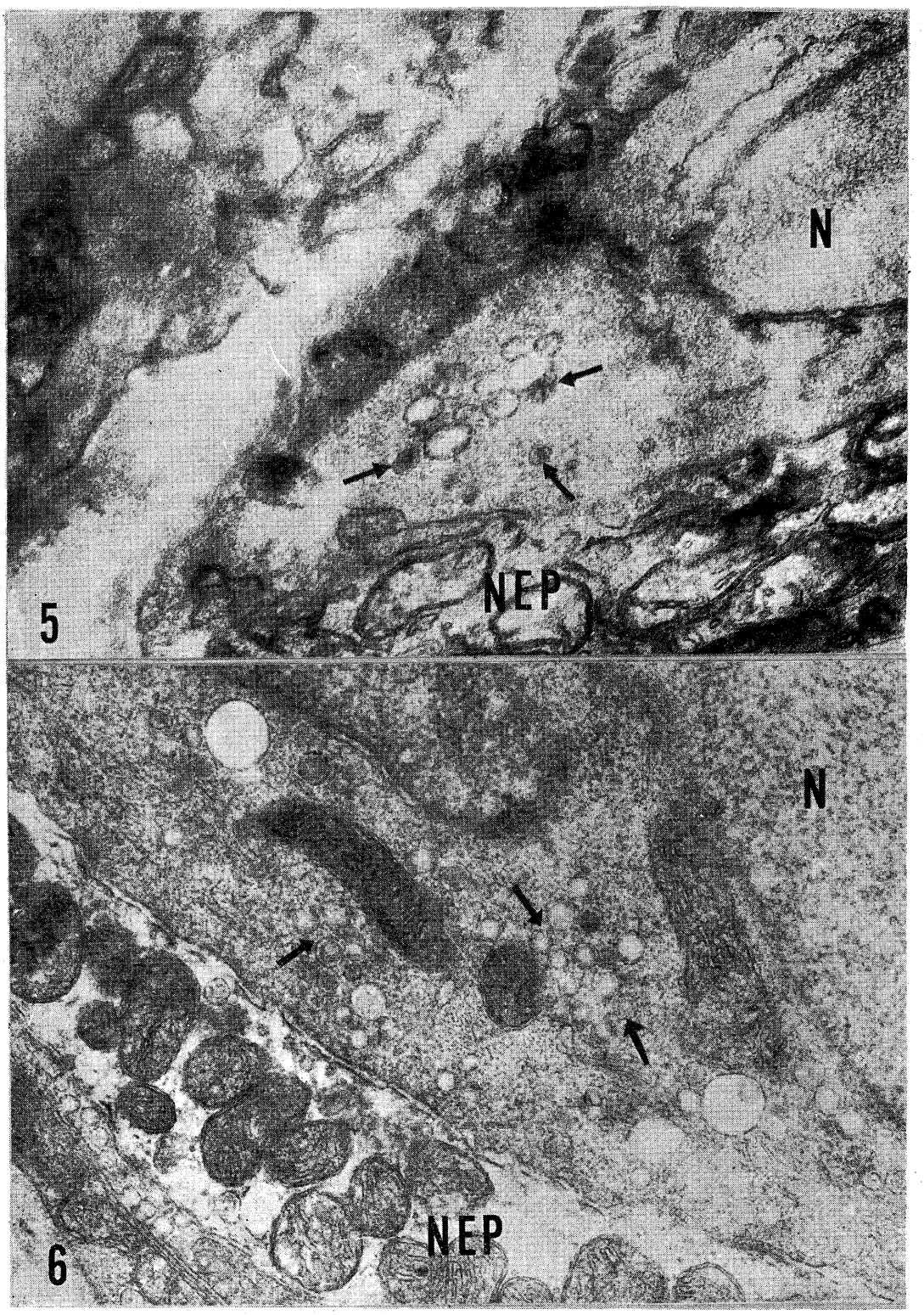
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also present. The nucleolus is not prominent.

The dermal face of the Merkel cell is innervated by a nerve end plate that is derived from the myelinated nerve fiber. The nerve fiber (NF, Fig. 1) loses its myelin sheath close to the epidermis. The dense core granules, vsicles (V, Fig. 2) and numerous mitochondria are present in the nerve end plate.

Our observations on the ultrastructures of the Merkel cell confirm well the findings of previous workers on this subject[3,6,7,9,12,13].

2. Formation and release of the dense core granules:

Figure 3 indicates that the dense core granules are formed from the Golgi apparatus. The material of the core is produced in the Golgi saccules and packaged within the Golgi membrane to form the dense core granules. Iggo and Muir[7] first proposed that the granules of the Merkel cell were formed from the Golgi apparatus; Hashimoto[6], Chen[3] and Winkelmann[13] made the same proposition. However, there was no direct proof. The present result shows the evidence that the granules are definitely formed from the Golgi apparatus.

The granules were found in the lower part of the cytoplasm and aggregate near the synaptic-like membrane (Fig. 4). This membrane is the thickened part of the plasma membrane of both the Merkel cell and nerve end plate. Chen[3] noted that the membrane structure was comparable to that of the adrenergic synapse of the nervous system. Consequently, the dense core granules observed in this study could simulate morphologically the dense core of the synaptic vesicles.

Some of the Merkel cell granules were observed to fuse with the synaptic membrane (Fig. 4), suggesting the release of the content of the granules into the cleft between the Merkel cell and the nerve end plate. This observation agrees with that of Chen[3].

3. Cytochemistry of the dense core granules:

Cytochemical test for catecholamine showed a positive reaction in the adrenal gland while negative result was obtained in the Merkel cell granules. However, test for monoamine gave a positive reaction (Fig. 5). The results indicate that the content of the Merkel cell granules is monomine in nature but not catecholamine. This concept is further supported by the result of depletion of the granular content after the injection of reserpine (Fig. 6) wihch is known to inhibit the synthesis of monoamine and to decrease the uptake of monoamine into the granules[5].

In our reserpine experiment, the dose injected was 5 mg/kg 4 times per day whereas Iggo and Muir[7] delivered the injections as 0.3–0.5 mg/kg also 4 times per day. However, their result was negative. The discrepancy could be due to dosage difference.

FIGURES

Fig. 1. A diagram showing Merkel cells (arrows) and their associated tissues. H, Haarscheibe (hair disc); T, tylotrich hair; NF, nerve fiber.

Fig. 2. A diagram showing the fine structure of the Merkel cell and its associated nerve end plate (NEP). N, nucleus; G, Golgi apparatus; C, centriole; RP, rodlike process; D, desmosome; g, dense core granule; MF, microfilament; MT, microtubule; TL, thin lamellae; V, vesicle.

Fig. 3. A portion of the Merkel cell showing that the dense core granules (arrows) are formed from the Golgi (G) saccules. N, nucleus; MB, multivesicular body. 60,000×

Fig. 4. A part of the Merkel cell with its adjacent nerve end plate (NEP) showing that the dense core granules aggregate near the presynaptic membrane (PRE) and one of the granules (arrow) is fused with it. POS, postsynaptic membrane. 60,000×

Fig. 5. A part of the Merkel cell with its adjacent nerve end plate (NEP). Arrows indicate positive reaction for monoamine test in the dense core granules after $KMnO_4$ incubation. N, nucleus. 60,000×

Fig. 6. A part of the Merkel cell with its adjacent nerve end plate (NEP) showing depletion of the dense core granules (arrows) after reserpine injection. N, nucleus. 15,000×

The chemical nature of the Merkel cell granules poses a perplexing problem. Various suggestions have been proposed primarily based on morphological resemblance between the Merkel cell granules and those with known chemical content. Thus, the granules of the Merkel cell were suggested to contain monoamine[1,7,9] because the granules closely resemble the dense core granules of the chemoreceptor in the carotid body and the argentaffine cells in the gastrointestinal mucosa. The granules of the latter two kinds of cells contain monoamine. Catecholamine was also proposed as a chemical content in the Merkel cell granules[4,18] because the granules appear similar to the catecholamine-containing dense core granules of the cells in the adrenal medulla and in the sympathetic ganglion. A third possibility of the nature of the Merkel cell granules was serotonin[3]. The proposition was based on the fact that serotonin is found in the dense core granules of the neuroepithelial cells in the rabbit respiratory mucosa and that both the neuroepithelial and Merkel cells possess indented nuclei and dense core granules and both form synapses with the nerve fiber.

However, the aforementioned three proposals have not been evaluated experimentally. The present cytochemical study indicates clearly that the Merkel cell granules contain monoamine but not catecholamine. The exact chemical nature awaits further investigation.

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小鼠毛盤中麥克氏細胞顆粒之電子鏡學及細胞化學之研究

趙壯飛　梁序穆

本實驗應用電子鏡學及細胞化學以探究 NIH 小白鼠皮膚毛盤中麥克氏細胞顆粒之形成、釋放及其化學含物。結果顯示該顆粒係由高基氏器所形成，藉與聯會前膜 (pre-synaptic membrane) 融合，而將含物釋出，其含物之性質屬單胺類，但非磷苯二酚胺。