

Preliminary and Short Report

**OSMIUM IODIDE POSITIVE GRANULES IN SPINOUS AND
GRANULAR LAYERS OF GUINEA PIG EPIDERMIS***

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Small lamellated granules about 0.1-0.3 μ in diameter have been observed by several investigators in the upper spinous and lower granular layers of various keratinizing epithelia, and there has been some speculation concerning their chemical constitution and probable function (1-3). In a recent electron microscopic study (4) of guinea pig epidermis stained by the technic of Champy and Coujard, granules similar in size and location were observed to exhibit a strongly positive reaction to osmium iodide. So intense was the reaction, in fact, that the granules appeared uniformly electron-opaque, and since nothing could be seen of the internal structure, their identification remained uncertain. Subsequent examination of reacting granules in similar material exposed to the staining solution for a shorter period, has, however, as reported here, revealed structural details sufficient to enable one to compare them for purposes of identification with the above mentioned granules seen in epidermis not stained with osmium-iodide (1-3). In so far as any identity can be established, the observations could be of significance in relation to the chemical nature of the latter.

RESULTS

Pieces (3 x 3 mm) of white, *i.e.* non-melanin containing, skin of spotted guinea pigs were immersed for 12-24 hours in an osmium-tetroxide/sodium iodide mixture following the procedure described by Mishima and Miller-Milinska (5), and were then processed for electron microscopy. Preservation of fine tissue structure is not the best with this technic, but background detail adequate for the present purpose was discernible. Figure 1 shows the location and distribution of osmium-iodide positive granules within the cells of the upper spinous and granular layers. The most superficial cell contains relatively few granules, but in other micrographs they were present in slightly greater number in this situation. Granules were most heavily concentrated in the region of the cell adjoining that part of the plasma membrane facing the epithelial surface (Figs. 1 and 2). The granules were oval or rounded in section, the longer diameter varying between 0.13 μ and 0.24 μ , and the shorter between 0.12 μ and 0.2 μ . Granules were not seen in the stratum

corneum, but occasional blebs of osmium-iodide positive material were present in the intercellular space at this level.

At higher magnifications (Figs. 1, 2) the majority of granules presented a lamellated internal structure due to the alternation of parallel dense and light zones with a spacing of about 70-80 Å, and within some, several sectors exhibiting different main orientation of the lamellae could be discerned. A few granules exhibited faint traces of a limiting membrane, but with the majority this feature was not evident.

DISCUSSION

The close resemblance in size, structure, and location between the granules described above, and granules figured by previous authors in epidermis not stained with osmium-iodide (1-3) leaves little doubt about their identity, as a comparison of micrographs will confirm. The fact that such granules exhibit a strongly positive reaction to osmium iodide is presumably related to their chemical composition. It is unfortunate therefore that the histochemical significance of the reaction remains somewhat obscure, and that it lacks specificity (4, 5). However, lipid substances, and in particular the myelin sheaths of nerves, which have a high phospholipid content, are known to exhibit a strong reaction. Frithiof and Wersäll (3) drew attention to the fact that the fine structure of the granules in question closely resembles that of synthetic phospholipid preparations, and assumed this similarity to be due to a chemical relationship. The present observations lend some support to this assumption.

SUMMARY

The structure and distribution of osmium-iodide positive granules in cells of the spinous and granular layers of the epidermis is described. They are similar in every respect to granules described by previous authors in material not stained by the osmium-iodide technic, and the assumption that these latter granules are phospholipid in nature is augmented by the present observations.

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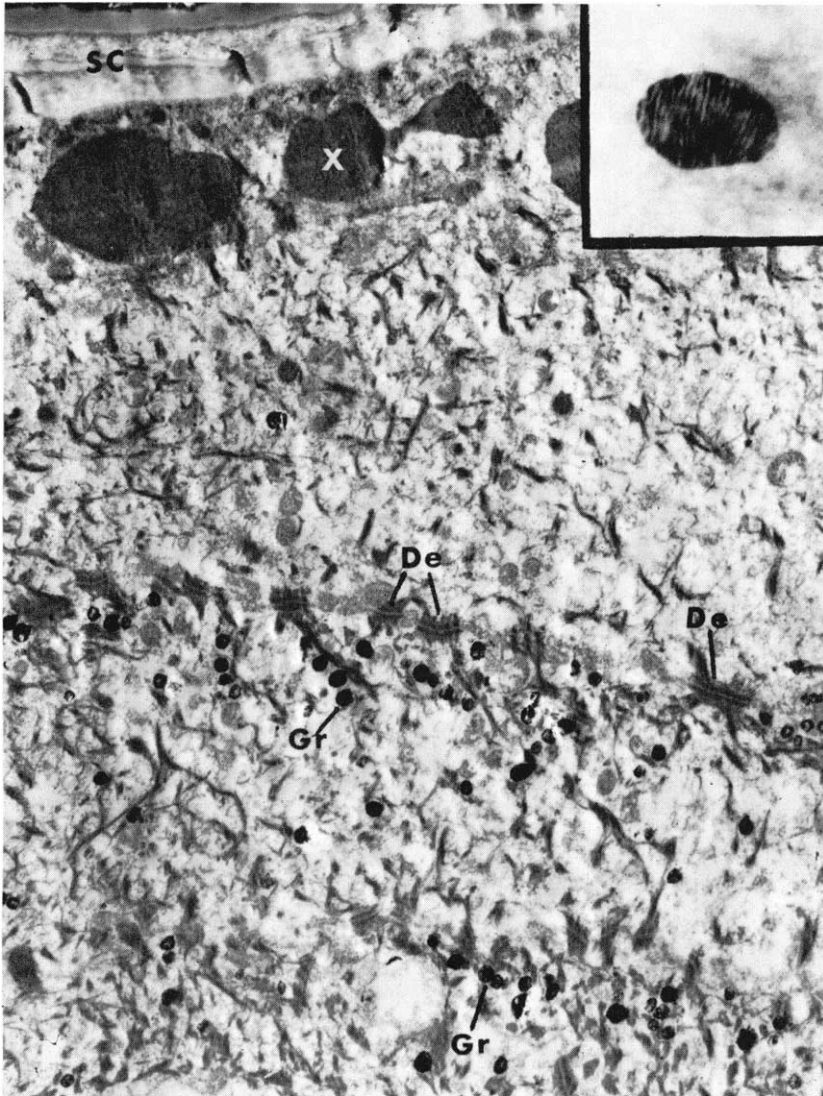


Fig. 1. Upper spinous and granular layers of epidermis to show distribution of osmium-iodide positive granules (Gr). De., desmosomes, indicating line of plasma membrane; X, keratohyalin; S.C., stratum corneum. $\times 12,000$. *Inset*: a granule at higher magnification to show lamellar internal structure. $\times 96,000$.

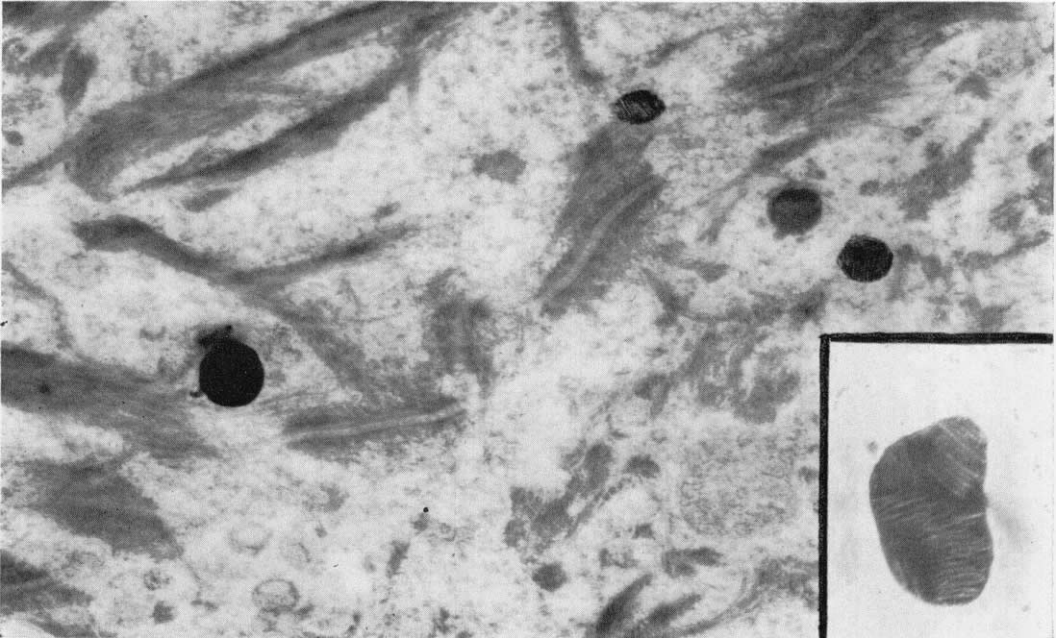


FIG. 2. Osmium-iodide positive granules in the neighbourhood of desmosomes connecting adjacent spinous layer cells. $\times 48,000$. *Inset*: granule with several sectors exhibiting different main orientation of lamellae. $\times 96,000$.