Electron Microscopy of Melanophagocytosis in the Human Skin Window

TECHNIQUE AND CONFIRMATION OF THE NATURE OF THE PARTICLE

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SUMMARY

A modification of a tissue culture technique successfully applied to obtain preparations of the inflammatory response studied by the human skin window technique, is described. Previously described melanin granules in macrophages in Cape Coloured responses could be shown to consist of nests of melanosomes.

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In a series of earlier papers leucocytic melanin transport was described, and the events in the human skin window detailed.[1-5] The human skin window technique offers considerable technical problems for electron microscopy, and apart from a casting technique[6] we are unaware of any electron microscopic observations by this technique.

This communication describes our experience with a modification of a tissue culture technique[7] for the study of the human skin window preparation. Our main objective was to establish the nature of the granules, cytochemically identified as melanin granules.[2] Since our earlier studies, this became necessary because a histogenetic relationship between mast cells, melanocytes and melanophages was deduced from the morphological, enzymatic, and histochemical evidence of Okun and co-workers.[8,9]

TECHNIQUE

The skin window preparation is obtained by scraping away epidermis from the skin of the sterilised forearm, until minute bleeding points indicate that the corium has been reached. On this surface a drop of egg-white is placed to stimulate the inflammatory reaction against a non-immune antigen. This is covered by a sterile, chemically clean, glass coverslip, held in place by a sterile cardboard disc. The coverslips are removed at suitable intervals, and contain a single layer of cells adherent to the coverslip.

The problem was to place this single layer in a resin block for sectioning with an ultramicrotome before the cell layer became air-dried. The inflammatory reaction was induced and monitored on glass coverslips removed at 2-hourly intervals and stained with May-Grunewald-Giemsa stain. For harvesting the cell population for electron microscopy, a sterile, chemically clean, mica coverslip was introduced at the selected time. The mica coverslip was then removed and immediately covered with phosphate buffer at pH 7,4 for 1 minute. It was placed at the bottom of a Sykes-Moore tissue culture chamber, a rubber ring spacer was introduced, and the chamber covered with a glass coverslip and closing metal ring.

From this stage onward all reagents were introduced through the porthole in the chamber by injection through the rubber ring. (A fine needle was introduced through another porthole to serve as an air vent.)

The phosphate buffer was replaced by glutaraldehyde for 1 hour at 4°C. This was withdrawn and washed away with 2 washings of phosphate buffer, of 5 minutes each. Osmium tetroxide was then introduced at 4°C for 30 minutes, and then followed 3 washings with distilled water. The preparation was dehydrated for 10 minutes in each of a series of alcohols (30, 50, 75, 95 and 2 × 100%).

The chamber was next filled for infiltration with a 50 : 50 mixture of 100% alcohol and Spurr's resin, for 1 hour. After removal, infiltration with resin for 30 minutes followed. The chamber was then opened and the resin replaced for 30 min at 60°C followed by another replacement of resin for 1 hour at 60°C, when the resin was cured for 24 hours at 60°C. (To remove the mica coverslip, the resin is plunged into a bath of iced water when the coverslip splits off by shrinkage.)

The resin block was then examined under phase contrast microscopy to locate cells containing yellowish granules, and cells showing connections with each other. Such areas were marked with a diamond marker in a circle of 1 mm diameter. This was cut out from the resin block, and sectioned on a LKB Ultratome III.

Very little tolerance for orientation of the sectioning plane existed, as this was a single layer of cells flush with the surface of the block. In several blocks cells were lost at this stage. We obtained satisfactory preparations in about half of the blocks prepared. Sections were transferred to grids and studied on a Zeiss EM 952 electron microscope.

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RESULTS

In our initial studies Black patients were studied exclusively because it is known from previous experience that the 'melanin granules' are observed consistently in all preparations. Furthermore, the melanosomes are fully melanised in the Black population groups. No granules resembling mast cell or basophil granules[10] were seen on any of the preparations. Figs 1 and 2 illustrate a few fields that were studied.

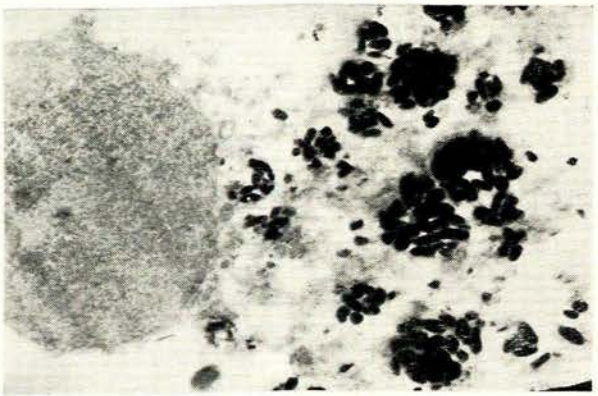

Fig. 1. Macrophage containing completely melanised melanosomes (× 38 000).

DISCUSSION

This study showed that satisfactory preparations for electron microscopy can be obtained by this technique. The 'granules' consisted of fully melanised melanosomes in lysosomes. The size, form, and electron density conformed to melanosomes studied in tissue sections and tissue culture preparations.

In none of the Black patients studied could incompletely melanised granules be found. We are at present studying exceptionally dark White and Cape Coloured patients to search for incompletely melanised melanosomes which could exhibit the typical scroll-like appearance and cross-linked fibres of the early stages of melanisation.

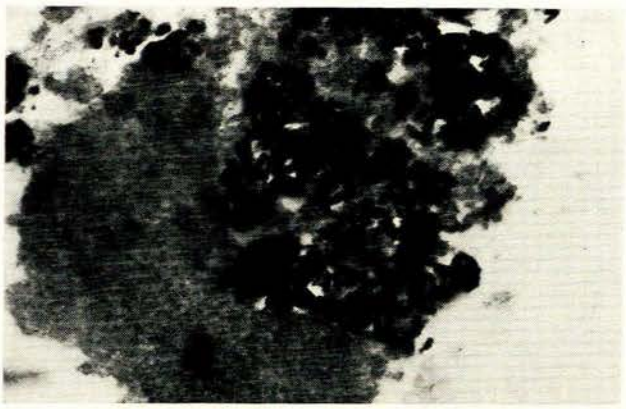

Fig. 2. Macrophage with several melanosomes grouped together. Under light and phase microscopy each nest appears as a single, large 'melanin granule' (× 38 000).

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