A COMPARISON OF MESOTHELIAL CELLS AND MACROPHAGES IN MICE AFTER THE INTRAPERITONEAL INOCULATION OF MELANIN GRANULES

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PLATES 40 AND 41

One facet of a series of studies which has been started in this laboratory and which involves the cells associated with the peritoneal cavity and those free in the peritoneal fluid, is a comparison of the reaction of mesothelial cells and macrophages to the presence of particulate matter in the peritoneal cavity. For this experiment melanin granules were used as a source of relatively inert particles native to the strain of mice chosen.

Materials and Methods

A group of 4½ month old DBA/2 males was inoculated intraperitoneally with 1 cc. of a heavy suspension of melanin granules in 0.85 per cent sodium chloride. The source of the melanin was an S91 Cloudman melanoma grown subcutaneously in a DBA/2 mouse. The melanin granule fraction was obtained by homogenizing the tumor in 8.5 per cent sucrose and centrifuging at 2000 R.P.M. for 10 minutes to remove the nuclei and unbroken cell fragments. The supernate, containing melanin granules, was centrifuged at 19,000 R.P.M. for 15 minutes after which it was discarded. The resulting sediment was washed twice in distilled water, and the top loosely packed gray layer, consisting chiefly of mitochondria and other cytoplasmic elements, was separated and discarded between washings. The final sediment was suspended in 0.85 per cent sodium chloride for inoculation. When the suspension was examined under the phase contrast microscope it was shown to consist chiefly of melanin granules ranging in size from about 0.25 μ to those barely discernible. Some clumping of the particles was evident.

6 days after inoculation the free cells from the peritoneal fluid of the melanin-injected mice as well as those from control mice of the same age were fixed for 20 minutes in chrome-osmium, pH 7.2 (1) and washed several times in 0.85 per cent sodium chloride by means of centrifugation and resuspension. An equal volume of warm 2 per cent agar containing 10 per cent formalin was added to the washed pellet of cells. The agar and cells were well mixed and kept in a water bath at approximately 47°C. for 10 minutes. The agar containing the cells was then allowed to harden. From this point on, the material was handled as tissue blocks, dehydrated, run through monomer, and embedded in partially polymerized plastic consisting of 1 part methyl methacrylate to 15 parts *n*-butyl methacrylate containing benzoyl peroxide as catalyst. Strips of the duodenum, small pieces from the tapering edges of the liver and spleen, and pieces of omentum from both the experimental and control mice were fixed in the chrome-osmium for 1 hour and washed in several changes of 0.85 per cent sodium chloride. The tissues were cut into sections of the desired size and shape in saline under a dissecting microscope. The sections were then placed in dishes containing warm 2 per cent plain agar for about 10 minutes. The agar was allowed to harden and cubes of the agar con-

J. BIOPHYSIC. AND BIOCHEM. CYTOL., 1956, Vol. 2, No. 4, Suppl.

taining the individual pieces of tissue were cut out. These were dehydrated and embedded in the partially polymerized plastic. Polymerization was completed with ultraviolet light or at 65°C. (2). The use of agar with the tissue was found to be advantageous not only in preserving the outermost layer of cells but also in making it easier to orient the sections in the capsule before final polymerization.

OBSERVATIONS

The macrophages free in the peritoneal fluid ingested the particles of melanin in a manner similar to that already described in a phase contrast microscope study (3). Large vacuoles containing only a few melanin granules in Brownian movement were rarely found. This was in contrast to the previous study in which such vacuoles were frequently encountered in the macrophages. It was felt that the absence of these vacuoles was due to the use of a melanin granule fraction relatively free of mitochondrial and other cytoplasmic remnants. The cells were harvested for study 6 days after melanin inoculation, at the peak of the macrophagic response and when large numbers of them contained appreciable amounts of melanin.

Macrophages from control peritoneal fluid, from the fluid of mice in which sarcoma 37 was growing as a free cell tumor, and from the melanin-inoculated mice were studied with the electron microscope. In all these cells neither the nuclear membrane nor the opposing cytoplasmic membrane was particularly prominent. The nucleus itself contained a diffuse, irregularly distributed material of medium electron-scattering power which usually showed a greater accumulation against the nuclear membrane. Nucleoli, when present in a section, were of more marked electron density but not as prominent as in mesothelial cells.

The cytoplasmic edges of the majority of macrophages showed a varying number of fine processes or thin cytoplasmic sheets. Frequently these processes were apparently fusing to form vacuoles and often a row of small vesicles could be seen along the plane of fusion (Fig. 1). That this behavior of the cell surface might be involved in the process of engulfing particles can be illustrated by the periphery of the lower melanin-containing macrophage in Fig. 2. Vacuoles containing particles are shown which have apparently been caught in the process of separation from the cell surface. Material can be seen which is clearly extracellular, in indentations between cell processes, and, in one area (arrows), the cell processes approximate each other. In addition to these there are a number of vacuoles which are obviously incorporated in the cytoplasm.

In control macrophages vacuoles were few and usually appeared electron-lucent with occasionally a few particles of electron-dense material within them. In macrophages which had ingested particulate material, as in this experiment with melanin granules, the particulate content of the vacuoles varied from a few discrete particles to such large numbers that the vacuole

was densely packed (Fig. 2). These densely packed vacuoles might measure as much as several microns in diameter.

Profiles of ergastoplasmic membranes with associated Palade granules, as well as unassociated Palade granules, were scattered irregularly throughout the cytoplasm in both control and experimental macrophages (Fig. 2). Mitochondria were numerous, had irregularly arranged cristae, and showed a marked variation in size within the same cell (Fig. 2). The Golgi apparatus was composed of parallel double membranes and small (400 A) vesicles and was usually situated near the nucleus. A few vesicles, similar in size and character to those associated with the Golgi zone, were usually found scattered in various parts of the cytoplasm. Dense bodies, larger than most mitochondria and as a rule smaller than lipide droplets, which seemed to have a limiting membrane and occasional faint internal structure, were to be seen in many macrophages. The presence of lipide droplets was almost always evident. They varied in number, probably depending upon the past history of the cell.

In control and experimental mice mesothelial cells from the visceral peritoneum covering the liver, spleen, and intestine, as well as from the omentum, were examined *in situ*. We were unable to distinguish any difference between mesothelial cells from control mice and those from mice exposed to the conditions described here. In the sections examined no evidence was found of the intracellular presence of melanin granules. Mesothelial cells free in the peritoneal fluid of the mice in this experiment, as well as under the majority of experimental conditions we have studied, were of such low concentration (0 to 1 per cent) from 3 days after inoculation, that to find one in a section would be extremely fortuitous. Upon observation of the peritoneal exudate with the phase contrast microscope the rare mesothelial cell seen after the 3rd day was found to be in a dead or dying condition, and no intracellular melanin was discernible.

The mesothelial cells from these various sites had many common characteristics. The nucleus was bounded by a well defined membrane (Figs. 3 and 4). Since the inner cytoplasmic membrane was also well defined, the resulting double membrane tended to be more prominent than in many cell types (Fig. 3). There was a loose, rather even distribution of material of medium electron density within the nucleus. Nucleoli were of marked electron density and were sharply relieved from the background (Fig. 4). The free surface of the cytoplasm exhibited a specialized border consisting of minute extensions of the cytoplasm similar to those of the striated border of the intestinal epithelium, except that they were fewer and more scattered (Figs. 3–5). Their distribution on the visceral mesothelium seemed to be scantier on the surface directly over the nucleus while on the mesothelial cells from the omentum they were few and widely dispersed. Since these structures gave every evidence of being permanent, in contrast to the fine processes at the periphery

of cells from the peritoneal fluid, white blood cells, and other cell types living in a fluid medium, it seemed that the term microvilli, already assigned to them, would be suitable. On the basal surface of the visceral mesothelial cells there was a basement membrane of low electron density (Figs. 3–5). It was about 45 to 65 mμ in width on the liver and spleen mesothelium and about 25 to 40 mμ on the intestinal mesothelium. The mesothelial cells from the omentum showed no evidence of a basement membrane. The fibrous layer of the serosa was very thin in the liver, intestine, and omentum. In the spleen it was thicker, denser, and more cellular.

In all the mesothelial cells from these locations the plasma membrane showed minute invaginations and small oval vesicles could be seen contiguous to the membrane or further in the cytoplasm (Figs. 3 and 5). The long axis of these ovals was usually perpendicular to the membrane and their size ranged from about 20 to 75 mμ across the short axis. It would appear that either the membranes pinched off to form small vesicles or that they represent profiles of tubules such as can be seen in the distal cytoplasm of cells from the proximal tubule of the kidney. This phenomenon seemed to be similar to that described by Palade in the endothelial cells of blood capillaries (4) and by Palade and Porter in mesothelial cells from the peritoneal surface of the rat intestine (5).

In the cytoplasm of the majority of mesothelial cells occasional small electron-lucent vacuoles were found but those from the area of the spleen displayed a considerable number of relatively large vacuoles. Also scattered ergastoplasmic membranes with associated Palade granules, mitochondria, and Golgi membranes and vesicles were present (Fig. 3). The plasma membranes of adjacent cells showed complicated interlocking patterns which in many instances were difficult to follow clearly (Fig. 5). The exception was in the omentum where there appeared to be a mere overlapping of tapering cell tips. Many characteristics of the visceral mesothelium described here have been shown in the mesothelium from the ovary of the rat by Odor (6).

CONCLUSIONS

Our results suggest that, a short interval after a single injection of native particulate matter into the peritoneal cavity of mice, the free macrophages actively ingest large amounts of the material while mesothelial cells *in situ* do not. Studies of the peritoneal fluid and of spread preparations of the omentum with the phase contrast microscope correlate well with these results, indicating that, with the exception of isolated, small particles, there was no evidence of phagocytic activity on the part of mesothelial cells comparable to that of macrophages, either *in situ* or free in the fluid. Odor (7) has reported the presence of particles in mesothelial cells *in situ* after the intraperitoneal injection of thorotrast. Among other possibilities, one explanation

of the difference in these findings might be particle size—melanin granules being many times larger. From long term experiments which involve repeated intraperitoneal injections of chemicals and relatively inert particulate matter now under way and planned, it is hoped that a more complete understanding of the capabilities and functional potentialities of the cells of the peritoneal cavity will be obtained.

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EXPLANATION OF PLATES

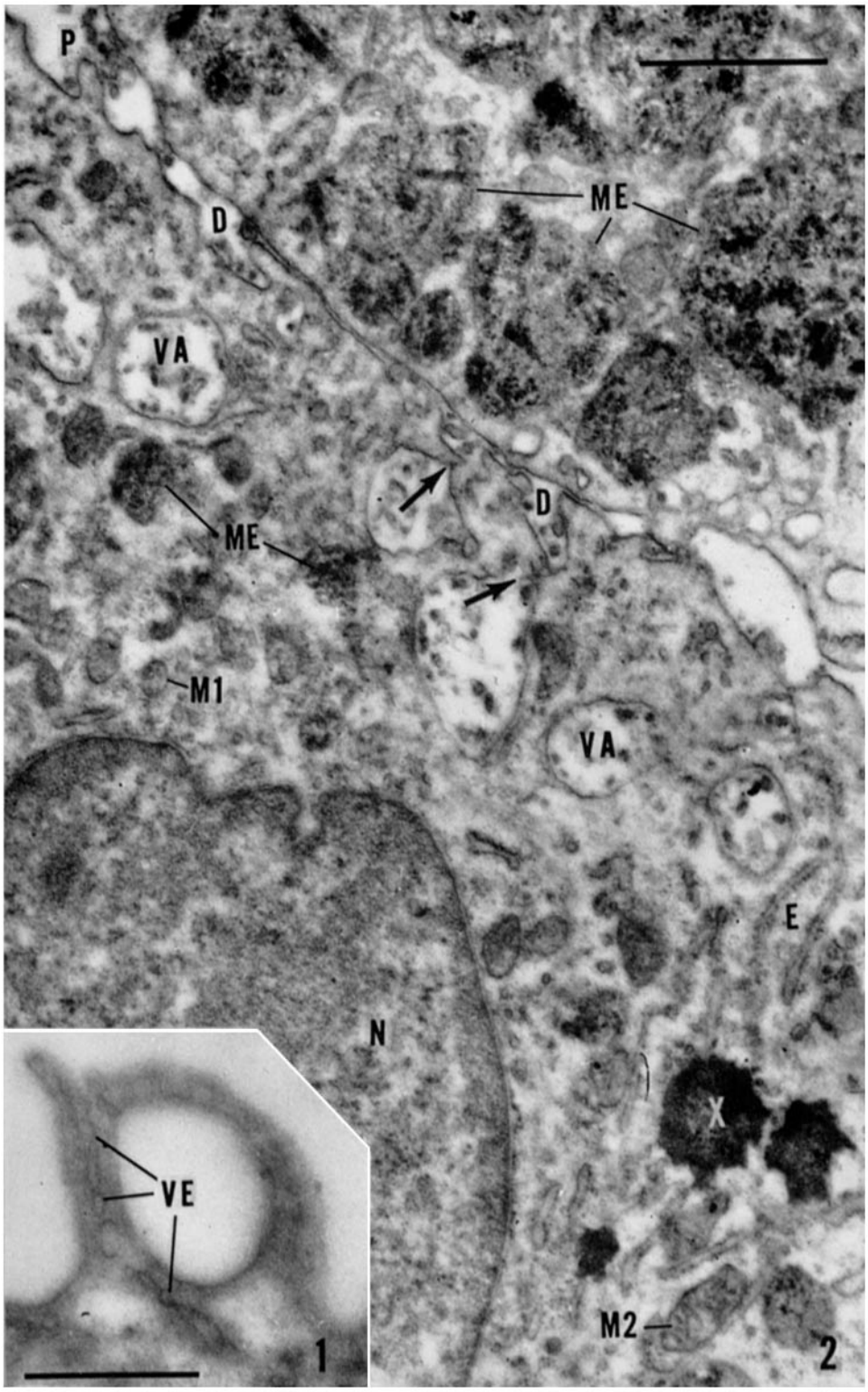
PLATE 40

Electron micrographs of macrophages from the peritoneal fluid of DBA/2 mice. The line on Fig. 1 represents 0.5 micron and on Fig. 2 1 micron.

The cells were fixed for 20 minutes in chrome-osmium (pH 7.2) followed by primary embedding in 2 per cent agar containing 10 per cent formalin. Final polymerization of the methacrylate embedding of the macrophage in Fig. 1 was completed with ultraviolet light, in Fig. 2 at 65°C.

FIG. 1. A small portion of the cytoplasmic edge of a macrophage from the peritoneal fluid of a DBA/2 mouse in which sarcoma 37 was growing as a free cell tumor. Cytoplasmic processes are shown apparently fusing to form a vacuole. Note the row of vesicles (*VE*) along the plane of fusion of two of the processes. × 50,500.

FIG. 2. A portion of two large macrophages whose cell membranes are in close approximation, from the peritoneal fluid of a DBA/2 mouse 6 days after the intraperitoneal inoculation of a suspension of melanin granules. The nucleus (*N*) shows a delicate double membrane and a diffuse, irregular distribution of material with a greater accumulation against the nuclear membrane as found in most of the macrophages in this study. At the periphery of the lower cell extracellular particles may be seen (*P*), others in indentations of the plasma membrane (*D*), and in two places, the cell processes seem to approximate each other (arrows). Several vacuoles are obviously incorporated within the cytoplasm (*VA*). Large vacuoles densely packed with melanin are near the periphery of the upper cell (*ME*) and smaller ones are scattered in the lower cell (*ME*). Prominent profiles of ergastoplasmic membranes with associated Palade granules are grouped to the right of the nucleus (*E*) as well as smaller profiles here and there throughout the cytoplasm. The marked variation in size of the mitochondria may be illustrated by the small one just above the nucleus (*M1*) and the large one at the lower right corner (*M2*). Two lipide droplets may also be seen at the lower right (*X*). × 28,000.



(Felix and Dalton: Comparison of mesothelial cells and macrophages)

PLATE 41

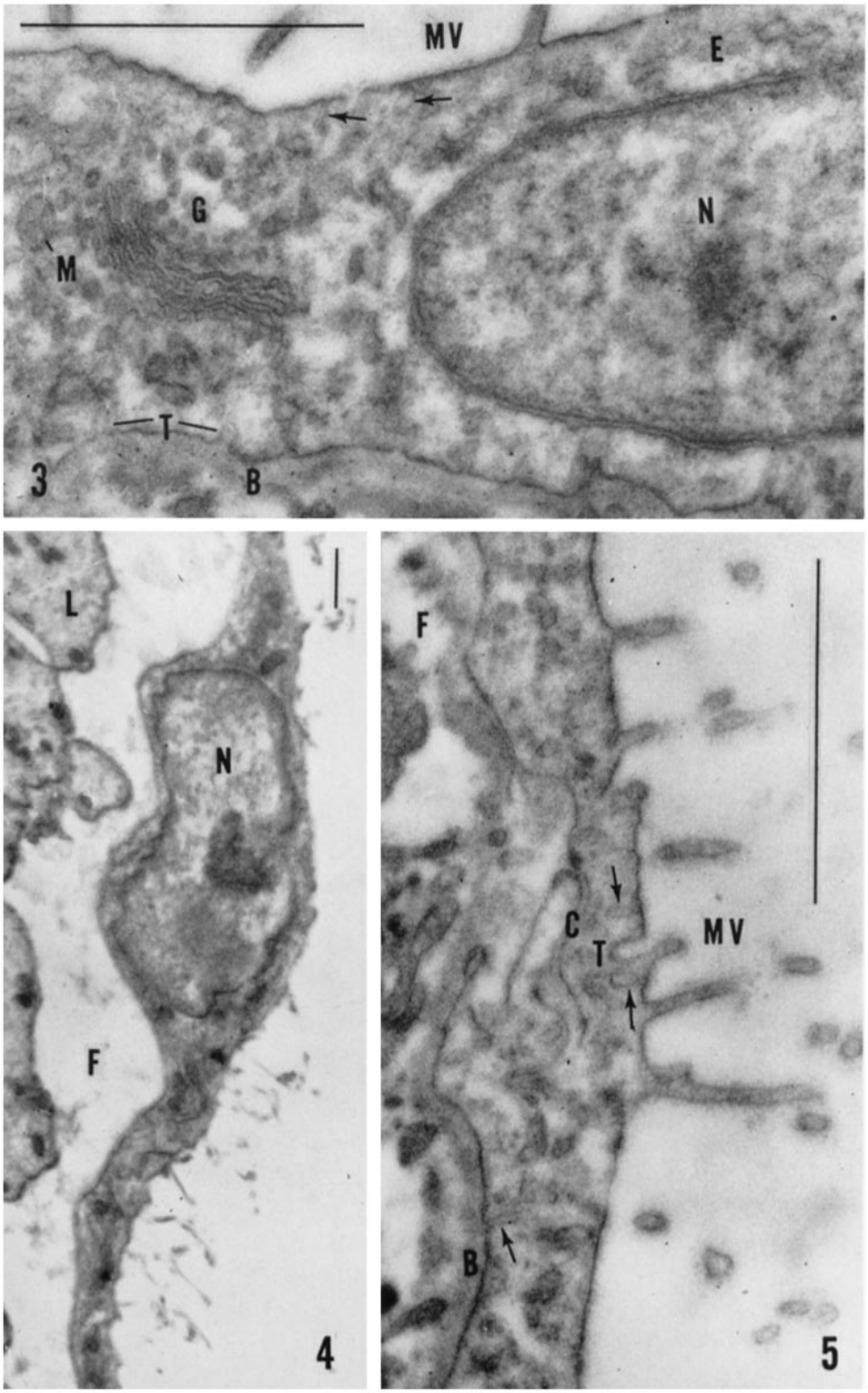
Electron micrographs of mesothelial cells from the peritoneal surface of the liver and duodenum of DBA/2 mice 6 days after the intraperitoneal inoculation of a suspension of melanin granules. The line on each figure represents 1 micron.

The tissues were fixed for 1 hour in chrome-osmium (pH 7.2) followed by primary embedding in 2 per cent plain agar. Final polymerization was at 65°C.

FIG. 3. A portion of a mesothelial cell from the peritoneal surface of the liver. The nucleus with its well defined membranes is at *N*. Three microvilli are in the field (*MV*), one showing continuity with the cytoplasm. A portion of the proximal surface of the cell with its basement membrane (*B*) is at the lower part of the figure. Two minute invaginations of the proximal plasma membrane (*T*) as well as a few oval vesicles contiguous to both the proximal and distal plasma membranes are evident (arrows). There is a well defined Golgi zone (*G*) and a few scattered ergastoplasmic profiles (*E*). One mitochondrion is in the field (*M*). × 50,000.

FIG. 4. A low magnification of part of a mesothelial cell from the peritoneal surface of the duodenum. Along the left side of the figure are the tips of cells from the longitudinal muscle layer (*L*). Situated between these and the mesothelial cell is the thin fibrous layer of the serosa (*F*). The nucleus (*N*) contains a prominent nucleolus. The scantier distribution of the microvilli over the nucleus is evident. × 9,000.

FIG. 5. The junction of two mesothelial cells from the peritoneal surface of the liver showing complicated interlocking of the adjacent plasma membranes (*C*). The thin fibrous layer of the serosa is at the left (*F*). A few liver cell processes are also in the field. The basement membrane (*B*) is clear in the lower half of the figure. Several microvilli (*MV*) can be seen in continuity with the cytoplasm and there is an invagination of the plasma membrane at the base of one of them (*T*). Several oval vesicles are contiguous with both the proximal and distal plasma membranes (arrows). × 50,000.



(Felix and Dalton: Comparison of mesothelial cells and macrophages)