MITOCHONDRIAL ARRANGEMENT IN ALVEOLAR EPICYTES AND FOAM CELLS OF MOUSE LUNGS, PARTICULARLY AS INDUCED BY THE VACUOLOIDS [1]

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Introduction

The problem of the causation of the design taken collectively by the mitochondria in a living or a fixed and stained cell has interested cytologists for many years. For instance, Lewis and Lewis (1915, p. 352), in studying the living cells of tissue cultures, ask: "What is it that governs the arrangement of the mitochondria? Is it the shape of the cell, the influence of the central body or of the nucleus, the internal structure of the cytoplasm, or do the metabolic activities of the cell govern the size, shape and arrangement of the mitochondria?" Full answers to these important questions have not yet been forthcoming, and the problem is a complicated one. No doubt each of the factors mentioned plays a part.

It seems clear, too, that the physical influence of mechanically inert bodies in the cytoplasm is a formative factor which may be predominant, for mitochondria occupy the general cytoplasm and not the special masses, living or dead, which may find a place in it; and hence anything which molds this general cytoplasm will incidentally establish the morphological pattern of the mitochondria in it. Thus Cowdry (1914) found that the mitochondria of large spinal ganglion cells occurred between the flakes of Nissl substance, and Thurlow (1917) observed that in nerve cells of the cranial nuclei the mitochondria avoided the canalicular apparatus. Similarly the Lewises (1915) showed, in their Figure 26, the mitochondria arranged in a network around the fat droplets of a tissue culture cell. Foreign body inclusions act likewise. This simple mechanical influence on mitochondrial arrangement is herein shown to be exerted by vacuoloids in the pulmonic alveolar epicytes and foam cells to such a degree that the ensuing picture is outstanding and characteristic.

Alveolar epicytes are the residual epithelial cells in the pulmonary alveolar walls (Macklin, 1946). They are also called "septal cells," "niche cells" and other names. Although in the marginal alveolar bases (Macklin, 1945) and other places they have but one air face and rest upon connective tissue, in the interalveolar partitions they frequently have two air faces. These are often of unequal size, the larger overlying the head of the cell and the smaller the foot. In silverwashed material the head and foot are each encircled by a line of silverized material which is part of the silver lineation of the alveolar walls and bronchioles (Macklin, 1938). At alveolar wall intersections the epicytes, as herein discussed, not seldom have three air faces (Fig. 5). This trifaciality is like that found in certain dust cells of mouse lungs

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treated with ammoniacal silver solution (Macklin, 1948). Epicytes may assume a phagocytic rôle (Macklin, 1946). Other functions have been ascribed to them (Hayek, 1942; Sjöstrand and Sjöstrand, 1938). That they may, on occasion, become malignant, so initiating a primary cancer of the lung, has been admitted as a possibility (Macklin, 1938). Their most prominent and characteristic feature is an array of vacuoloids which occupies much if not most of the cytoplasm (Macklin, 1947a). These are clear, round, discrete, non-lipoidal bodies averaging 0.5 μ to 0.75 μ in diameter, which do not take stains (Brodersen, 1933). They are relatively stable, indenting the nucleus in fixed and stained sections.

Alveolar foam cells of mammalian lungs have been described with special reference to their vacuoloids (Macklin, 1947b). They are found in ordinary histological sections, and some of them may be recovered by what has been termed the "gash-irrigation" technic, in which the fresh, collapsed lung is incised through a drop of physiological saline solution and the preparation inverted over a glass slide on which is received the emerging fluid carrying loose cells from the peripheral alveoli. This fluid is then spread and stained as for blood. Foam cells are regarded as originating from epicytes and possibly also from the diversifying epithelium of the bronchioles at the marginal zone adjoining the alveolar ducts, and as being developmental brothers of the dust cells. Difficulty may be encountered in distinguishing the smaller foam cells from well developed epicytes, in sections. The alveolar foam cells are thus of entodermal origin, and are not to be confused with the mesodermal "foam cells" of the pathological literature. As the name implies, they have a foamy appearance, the numerous vacuoloids accounting for the clear spots, which in sections often misleading seem to be merged. In dry smears the vacuoloid diameter may reach 1.5 μ.

There is no reason to suspect that the mitochondrial arrangements hereinafter described are peculiar to the alveolar epicytes and foam cells of the mouse. They are probably to be found in these cells throughout the mammalian class at least.

Material and Method

This short study is upon albino mouse lungs freed from as much blood as possible by hemorrhage and moderately distended by the prompt intratracheal injection of Regaud's fixing fluid. Paraffin sections, stained by Bensley's adaptation of the Altmann technic (Cowdry, 1943), reveal the mitochondria in brilliant ruby-red. Most of the mitochondria in epicytes and foam cells are of round or oval form. These are seen in all parts of the general cytoplasm. The ovals grade into short thick rods with rounded ends. Filaments, often beaded, occur. Mitochondria differ in size, the largest being conspicuous while the smallest are seen with difficulty. It is possible that the degree of differentiation with picric acid has something to do with the optical impression of size, which seems less in over- than in under-differentiated cases; but this factor can hardly be in operation when mitochondria in the same cell are being considered, for these have presumably been subjected to uniform technical action. It is the impression that the mitochondrial content of the well developed epicytes is more conspicuous than that of the simpler epicytes on the one hand and the larger foam cells on the other. These mature foam cells are lighter in color, lacking the marked rosy hue typical of the mature epicytes, and in them the mitochondria appear generally smaller and more weakly staining than in the smaller foam cells and epicytes, and are predominantly of coccoid form. Thus there is considerable variation in the relative prominence of the mitochondrial picture in the various cells examined.

Perivacuoloidal Grouping of Mitochondria

The design of groups of mitochondria in typical epicytes and foam cells, as seen in well stained thin sections, is dominated by the presence and spacial disposition of the vacuoloids and appears characteristically as a round-meshed sieve with circles of mitochondria, the perivacuoloidal clusters, bounding these clear spheroidal bodies. Mitochondria are never admitted to the interiors of the vacuoloids.

In Figure 2 is shown one of the smaller bifacial epicytes in which the structure is relatively simple, the vacuoloids being in only one layer. Perivacuoloidal groups *p v g* surround these bodies. Those mitochondria which lie just beneath the cell membrane may take part as well in the formation of the inframembranal group *i m g*, and similarly those which are immediately around the nucleus participate as well in the makeup of the perinucleal group *p n g*. This is a good example of an epicyte which goes right through the alveolar wall, and it shows a larger end, the head, well filled with vacuoloids, and a comparatively narrow shank or trunk with a small foot.

Where greater numbers of vacuoloids occur, the appearance is more complex. In the epicyte seen in Figure 3, there are many perivacuoloidal groups, mainly of ovals and spheroids. This cell appears lodged in a crotch of alveolar walls. It has a free air-face above, another to the left and a third to the right, represented in the small foot at the end of the narrow shank which is within a space of the alveolar wall like a pore. Above are rods just beneath and parallel with the free face *i m g*.

In the larger epicytes and foam cells the vacuoloids are in several layers, and hence the perivacuoloidal mitochondria comprise relatively much more of the total content. Figure 7 gives a good impression of this sievelike pattern. It is from a very thin slice of a foam cell at the side of the nucleus. The inframembranal layer is incomplete. In thick sections one can focus up and down through the numerous vacuoloids and find impressive numbers of mitochondria in the cytoplasm around them.

Figures 8, 9 and 10 show this perivacuoloidal arrangement in other foam cells. Under the oil immersion lens the overall picture is uniquely beautiful, and once seen is not forgotten. It is like chains of brilliant rubies festooned about large luminous pearls. Photographs at best give an inadequate representation. The numerous and often large mitochondria are mainly round or oval, and most of them are about the vacuoloids, with an incomplete layer under the cell membrane and another over the nuclear membrane.

Sometimes epicytes which appear to be underdeveloped are found, showing relatively few vacuoloids or mitochondria. In Figure 1, for instance, there is a single row of vacuoloids present only on the air surface. But one mitochondrion appears on the side next to the air, and it is between two vaculoids. This is an example of a very simple distribution of mitochondria. They are massed above and to the right, in the cytoplasm of the surface which rests on connective tissue.

It does not appear that the mitochondria are attracted by the vacuoloids, but rather that they occupy inertly the available space around them. By no means all of the vacuoloidal surface is contiguous to mitochondria. Most of the mitochondria around the vacuoloids are of the spheroid or ovoid type; but there

Plate I

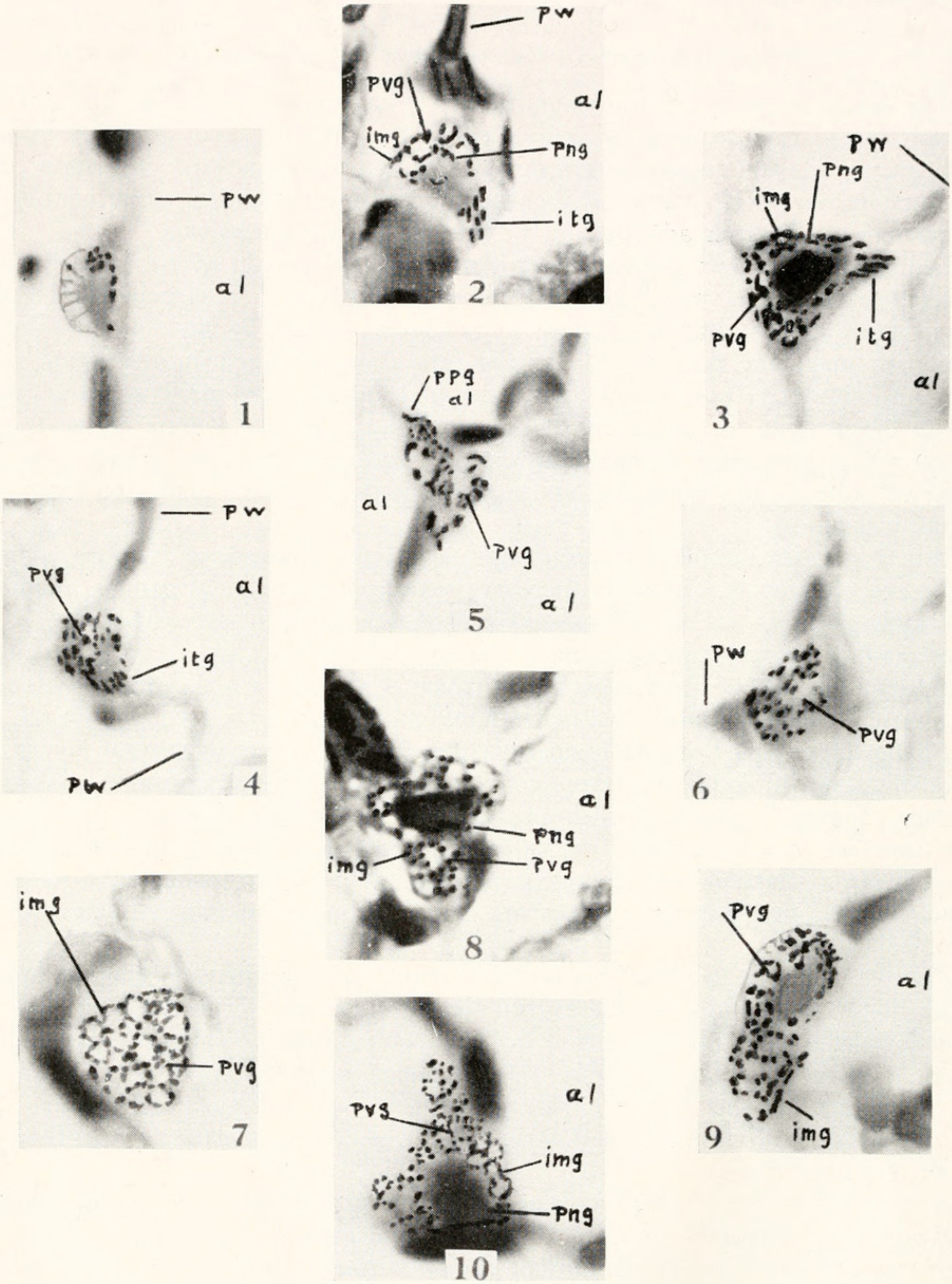

Plate I

The ten figures are photomicrographs at 1900 diameters made with a Bausch and Lomb 1.9 mm. 1.32 N.A. oil immersion fluorite system objective and 10 × ocular from 3 μ Altmann sections of mouse lungs prepared as described. On the prints the mitochondria were intensified with India ink applied with a fine pen on consultation of the original cell under the oil immersion lens. The first six figures are regarded as epicytes and the remainder as foam cells. Details of mitochondrial arrangement are given in the text.

are many short rods and these are found with the long axis lying tangentially to the vacuoloidal surface. There is no reason to suggest that mitochondria are in any way concerned in the formation of the vacuoloids or that they are influenced in form, size or any other way by contiguity with the vacuoloids. Experimental swelling and distortion of the vacuoloids is reflected in spreading and attenuation of mitochondrial arrangements around them.

Arrangements Not Determined by the Vacuoloids

When epicytes are so cut as to show the long axis of the cell approximately parallel with the optical plane, we may see mitochondrial rods of the intratruncal group *i t g* lying more or less parallel with one another in the trunk or shank and reaching to the foot (Figs. 2, 3). This region of the epicyte lies within the alveolar wall close to the capillaries. In cross sections of such shanks the now dotlike mitochondria are disposed in a circle. This arrangement suggests the shrunken staves of an empty barrel. When cut at a slant such a group appears as in Figure 4. No reason for this peculiar pattern is apparent.

Mitochondria have been noted in epicytes and foam cells lying close to the cell membrane *i m g*. As rods and filaments they often lie parallel with this membrane, and sometimes occur in a double row (Fig. 9). Another layer, which may be indefinite and typically composed of shorter forms, has been noted in the perinucleal cytoplasm *p n g* (Figs. 3, 8, 10). In the edges of the heads of epicytes, where the inframembranal cytoplasm underlying the air surface merges with that adjoining the connective tissue surface, the mitochondria of the peripheral group *p p g* (Fig. 5) in lateral view may have a curious pointed appearance like a pile of sticks, as in the profile of the supports of a North American Indian wigwam, which is difficult to photograph; while other groups simulate the downdrooping branches of the tops of balsam trees. Such clusters contain rounded and oval forms as well as rods. Sometimes mitochondria in these edges are packed in a dense triangular mass. Again, no explanation for these curious formations has been found.

Epicytes on marginal alveolar walls (those which rest on connective tissue) are well endowed with mitochondria. One of these is represented in Figure 6 in the angle between adjoining alveoli. To the left, a group of mitochondria juts into the partition *p w* separating the upper from the lower alveolar space. Perivacuoloidal formations are seen here as in other epicytes.

Substantially the same representation of mitochondrial arrangement in these cells was obtained after the use of Bensley's acid violet-safranin O (Bensley, 1911; Lillie, 1948, p. 98), and Regaud's modification of the iron hematoxylin method of Heidenhain (Cowdry, 1918), though hitherto, in the author's hands, less brilliantly.

Abbreviations:

a l	alveolus,
i m g	inframembranal group of mitochondria,
i t g	intratruncal group,
m w	marginal alveolar wall,
p n g	perinucleal group,
p p g	peripheral group,
p v g	perivacuoloidal group,
p w	partitional alveolar wall.

Summary

In epicytes and foam cells the combined perivacuoloidal groups of mitochondria present, in thin sections, an outstanding and characteristic lacelike picture based on the disposition and condition of the vacuoloids. Its determination is probably mechanical. Mitochondria are never found within the vacuoloids. An incomplete layer immediately underneath the cell membrane, and another around the nucleus, are found. In the former the rods often lie parallel with the membrane and sometimes in double rows.

In epicytes there is often a distinctive group, mostly of rods, which suggest the outer layer of the fasces, and lie in the long axis of the shank, arranged in a circle around a central area devoid of them. Bizarre angulated and branched arrangements are noted in the peripheries of the heads of epicytes.

In foam cells the mitochondrial content varies, often being abundant and conspicuous, and again, perhaps in older cells, relatively inconspicuous.

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