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Ultrastructural localization of acid phosphatase in the rat lung

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INTRODUCTION

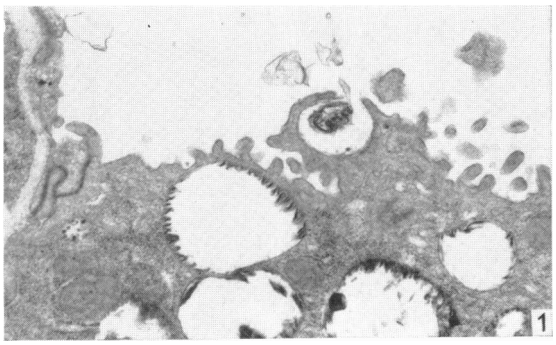
This study of acid phosphatase distribution in the lung was prompted by our interest in the pulmonary macrophage. It was assumed that a rich lysosomal component would be found in these phagocytic cells. At the same time attention was paid to the alveolar lining cells. One category of these, the type II pneumonocyte, contains laminated vacuoles believed by many workers (Buckingham & Avery, 1962; Klaus *et al.* 1962) to be concerned in the secretion of the surface active agent of the lung. Rather surprisingly, acid phosphatase has been reported in these vacuoles, suggesting that they are lysosomal in nature (Balis & Conen, 1964; Hatasa & Nakamura, 1965). This paper reports a study of acid phosphatase activity in all lung constituents but is chiefly concerned with the macrophage lysosomes and the laminated vacuoles of the type II pneumonocyte.

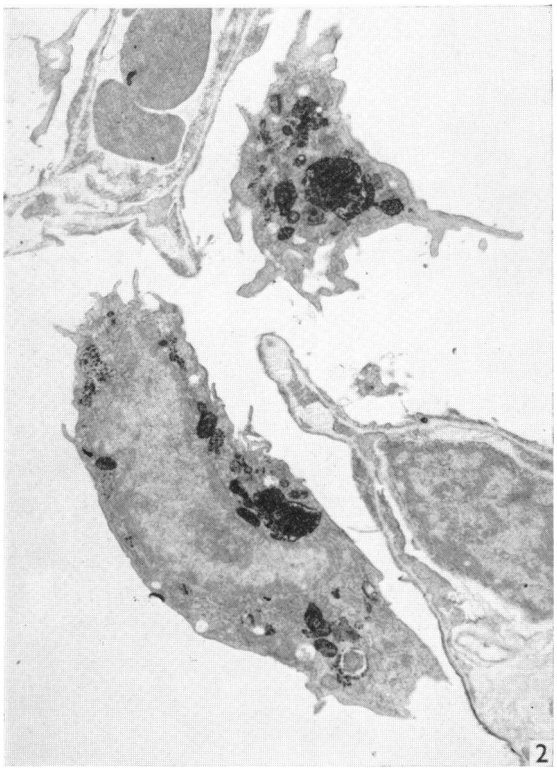
MATERIALS AND METHODS

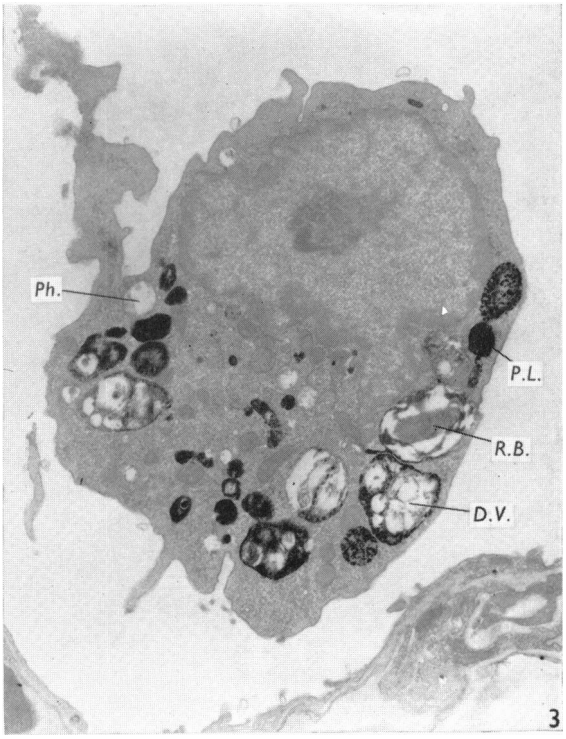
Young rats of about 150 g body weight were killed with coal gas and the thorax opened anteriorly. Fixative was gently instilled through the trachea into the lungs and these were quickly excised and small pieces immersed in fixative. The fixative used was glutaraldehyde 1 % in 0·1 M cacodylate buffer (pH 7·0) containing 3 % sucrose, as this was found by experiment to be optimum. Dilution of the aldehyde fixative to 1 % improved morphological preservation. After 2 h fixation at 4 °C, 0·5 mm free-hand frozen slices were processed by the Gomori technique as described by Miller & Palade (1964), and then post-fixed in cold (4 °C) osmium tetroxide-veronal acetate solution containing 1 % sucrose for 30–60 min. The tissues were embedded in Epon 812 and ultrathin sections mounted without a supporting film on copper grids. The sections were first examined unstained and again after staining with lead citrate and uranyl acetate. Technical points of importance include the use of hypertonic sucrose in the primary fixture as well as the incubating medium. This is thought to stabilise the lysosomes and minimizes leakage of the enzyme.

RESULTS

Ultrastructural detail was well preserved in the acid phosphatase preparations. The membranous or squamous type I pneumonocytes, the large granular or vacuolated type II pneumonocytes and the alveolar macrophages were all readily identified. The fine structure of the mammalian lung has been well described by other workers (Low, 1953; Karrer, 1956, 1958; Campiche, Gautier, Hernandez & Reymond, 1963)







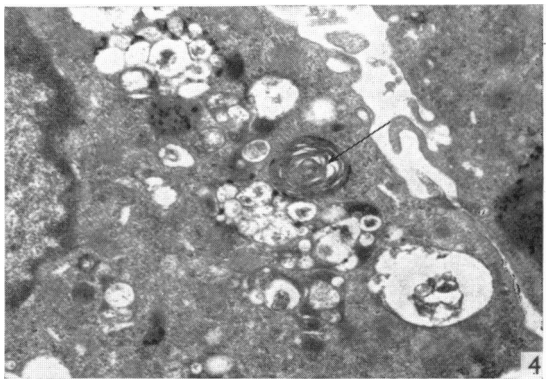
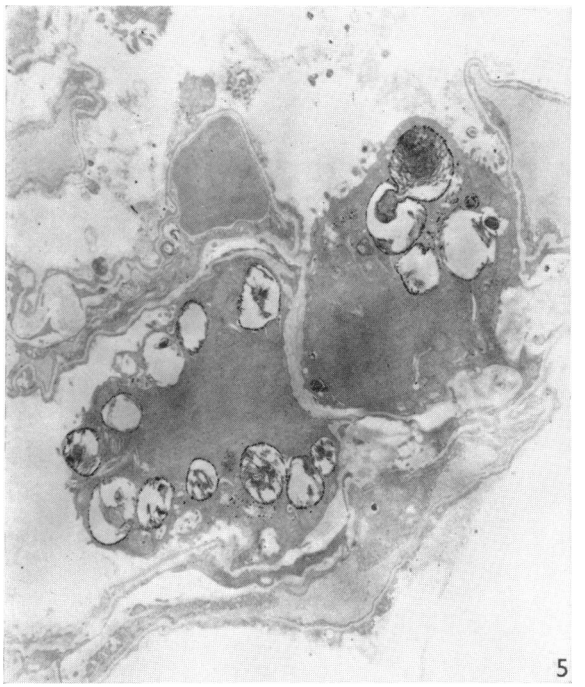


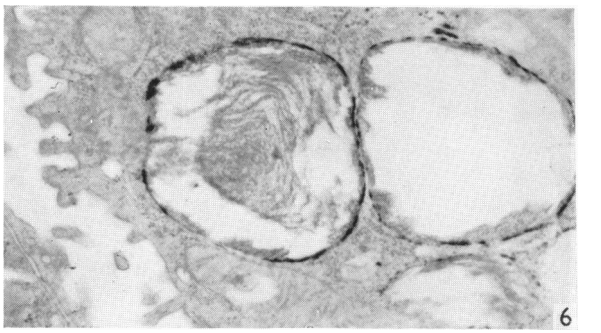
Fig. 1. Type II pneumonocyte with its characteristic microvilli and osmiophilic vacuoles. One such vacuole is seen discharging its contents into the alveolar lumen. An abundant rough-surfaced endoplasmic reticulum is also evident. Lead citrate and uranyl acetate, × 13000.

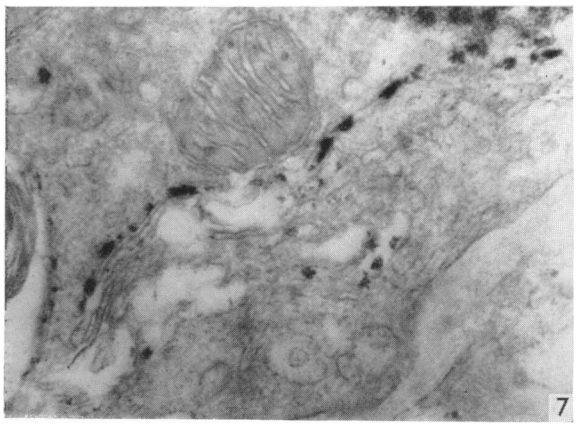
Fig. 2. Two alveolar macrophages are seen near a pore of Cohn. Both contain numerous acid phosphatase-rich lysosomes. Unstained acid phosphatase preparation, × 3800.

Fig. 3. An alveolar macrophage showing a variety of inclusions, many containing acid phosphatase. *P.L.*, Primary lysosome; *Ph.*, phagosome; *D.V.*, digestive vacuole; *R.B.*, residual body. Acid phosphatase/lead citrate and uranyl acetate, × 8000.

Fig. 4. Parts of two alveolar macrophages with a number of lysosomal storage and digestive vacuoles. A myelin figure-type of residual body (arrow) resembles the laminated type II cell inclusions, supporting the suggestion that the latter are of lysosomal derivation. Compare with Figs. 5 and 6. Acid phosphatase/lead citrate and uranyl acetate, × 15000.







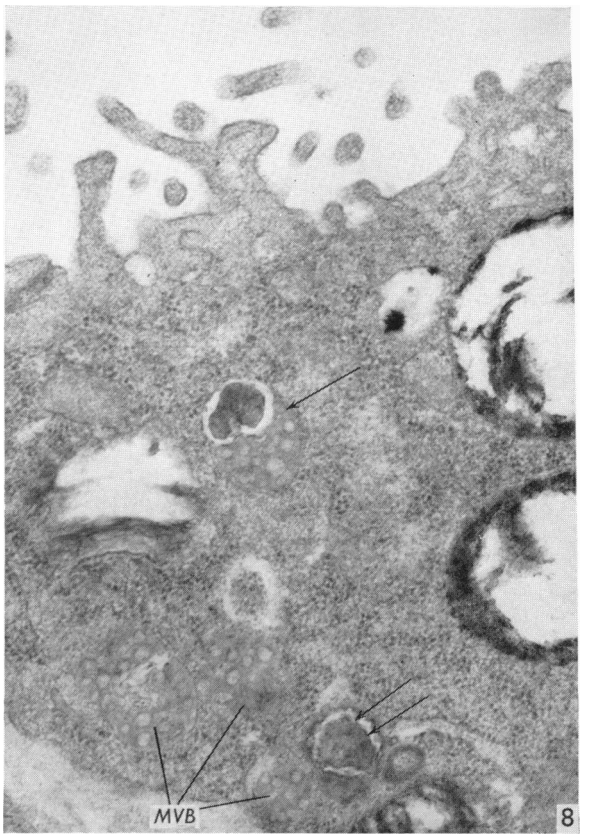


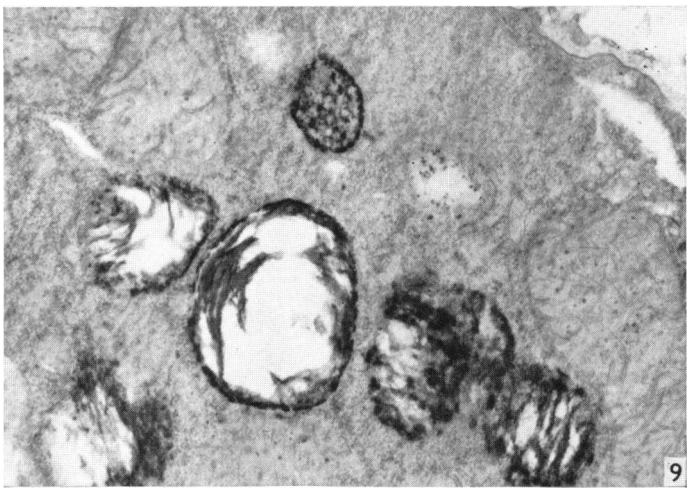
Fig. 5. Parts of three alveoli are shown. Characteristically nestling in the corners of the alveoli are two type II pneumonocytes. A thin rim of reaction product is seen about the periphery of almost all the laminated vacuoles. Unstained acid phosphatase preparation, × 5200.

Fig. 6. Part of a type II pneumonocyte recognizable by its microvilli and laminated cytosomes. The latter are bordered by a thin layer of acid phosphatase. Acid phosphatase/lead citrate, × 16000.

Fig. 7. Type II pneumonocyte. The endoplasmic reticulum contains scattered granules of acid phosphatase reaction product. Acid phosphatase/lead citrate, × 50000.

Fig. 8. Type II pneumonocyte. Several multivesicular bodies (*MVB*) are seen. In the centre a multivesicular body contains a clump of amorphous osmiophilic material (single arrow). Similar material is also seen in a membrane-bounded vacuole which is not obviously multivesicular (double arrows). Acid phosphatase/lead citrate, × 34000.

and will not be repeated here, but a few points are worthy of further comment. There was little similarity between the alveolar macrophages and the type I and II cells, so that if the latter were a source of free macrophages considerable structural alteration would be entailed. No intermediate cell forms were seen, and a vascular or interstitial source appears the most likely origin of alveolar macrophages. However, a recognizable macrophage was never observed breaking through the basement



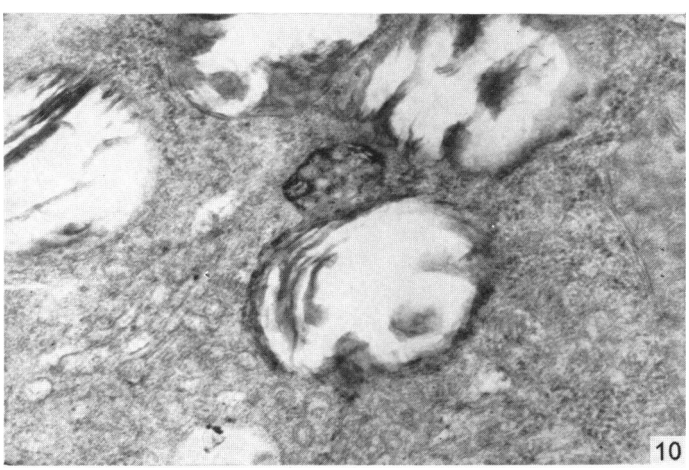


Fig. 9. Type II pneumonocyte. Acid phosphatase is seen in a multi-vesicular body as well as several laminated inclusions. Acid phosphatase/lead citrate, × 24000.

Fig. 10. Type II pneumonocyte. A multivesicular body is seen fusing with one of the laminated cytosomes. Acid phosphatase/lead citrate and uranyl acetate, × 40000.

membrane. As well as their characteristic laminated vacuoles, the type II cells were notable for a rich rough-surfaced endoplasmic reticulum, which is supportive evidence of a secretory function, and occasionally the contents of a laminated vacuole were seen discharging into the alveolus in the manner of an exocrine cell (Fig. 1).

Acid phosphatase activity was most obvious in the alveolar macrophages (Fig. 2). In all such cells many enzyme-rich bodies of a variety of shapes and sizes were present (Fig. 3). Compact structures uniformly filled with reaction product corresponded to the dense bodies seen in non-enzymic preparations and are regarded as the primary lysosomes or storage vacuoles. Other vacuoles, near the cell surface and devoid of the enzyme, were interpreted as phagosomes, whilst complex multilocular vacuoles with irregular enzyme distribution were thought to arise by fusion of primary lysosomes and phagosomes and to constitute the digestive vacuoles of the cell. Lipid is relatively indigestible and some acid phosphatase-containing vacuoles held residual bodies of laminated osmiophilic material, usually in the form of myelin figures (Fig. 4). These bore some resemblance to the laminated vacuoles of the type II alveolar lining cells but were far fewer and smaller.

Acid phosphatase was also found in the type II pneumonocytes, chiefly in the characteristic laminated vacuoles thought to be concerned in surfactant secretion. Not all such vacuoles nor indeed all such cells contained the enzyme; but in many, acid phosphatase reaction product formed a thin rim about the periphery of the vacuoles (Figs. 5, 6). The enzyme was not limited to the smaller of these cytosomes and was often present in the larger and presumably older vacuoles. Occasionally reaction product was aligned alongside or within channels of the endoplasmic reticulum (Fig. 7). The multivesicular bodies described by Sorokin (1966) and Goldenberg, Buckingham & Sommers (1967) (Fig. 8) generally contained acid phosphatase both at their periphery and between the individual vesicles (Fig. 9). Occasional structures intermediate in form between multivesicular bodies and the laminated vacuoles, as described by Sorokin, were observed. The sequence of events here appears to be the development of a solid clump of non-laminated osmiophilic material in a multivesicular body (Fig. 8), followed by its enlargement so that the multivesicular nature is obscured but a peripheral rim of acid phosphatase retained, and finally transformation of the amorphous lipid into the laminated or whorled structure characteristic of the typical type II cell vacuoles. Acid phosphatase is also incorporated into fully formed laminated vacuoles by their fusion with further multivesicular bodies (Fig. 10). The enzyme was never observed in mitochondria and no evidence of transition from mitochondria to lamellar bodies was found.

Acid phosphatase activity was very slight in the type I pneumocytes, only a very occasional primary lysosome being observed. In the interstitium an occasional tissue histiocyte with many enzyme-rich vacuoles was found. No acid phosphatase activity was observed in the vascular endothelium.

DISCUSSION

The distribution of acid phosphatase in the pulmonary macrophage was as expected; the enzyme was found in a variety of inclusion bodies, similar to those described in the alveolar macrophage by Karrer (1958) and in tissue histiocytes by Kajikawa (1964). The latter considered them to be concerned in the digestion of exogenous and endogenous materials, and our findings support this.

Acid phosphatase has previously been detected at the ultrastructural level in the type II pneumocyte by Balis & Conen (1964) and Hatasa & Nakamura (1965). The former obtained a positive reaction about the border of the laminated inclusions,

within the Golgi complex and in the multivesicular bodies. Much the same distribution has been found in the present study. The presence of acid phosphatase in the laminated vacuoles, together with their resemblances to myelin figures in macrophages, might be taken to support the view that they represent focal cytoplasmic degradation rather than secretory activity. However, other workers' evidence that the vacuoles are concerned with surfactant secretion (Klaus *et al.* 1962), although circumstantial, is strong and must be accepted. The presence of acid phosphatase indicates a lysosomal origin and Hatasa & Nakamura (1965) and Sorokin (1966) suggested that the secretory vacuoles represent modified lysosomal structures. Acid phosphatase has been found in the secretory granules of other cells but its significance here is unknown (Novikoff, 1963). The multivesicular bodies we regard as secondary lysosomes, whilst the presence of acid phosphatase in the endoplasmic reticulum probably signifies its site of production. A study of further lysosomal enzymes in the lung is obviously required and is at present being undertaken in this laboratory.

SUMMARY

Acid phosphatase was demonstrated in the normal rat lung in: (1) Alveolar macrophages, in a wealth and variety of lysosomal structures. (2) Type II pneumocytes in (*a*) the laminated surfactant-secreting vacuoles; (*b*) multivesicular bodies; (*c*) channels of the endoplasmic reticulum.

This work suggests that the secretion of the type II pneumocytes is formed in specialized lysosomal derivatives.

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