differences in EA between neighboring hepatocytes over the zones of the lobule points to the absence of any constant spatial localization of hepatocyte populations, differing from each other the most in their EA, in the lobule. Consequently, if functional heterogeneity of hepatocytes (with respect to EA, for example) does exist it is dynamic in character both in space and in time.

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POSSIBILITY OF FORMATION OF BRUSH CELLS FROM TYPE II ALVEOLOCYTES IN RATS

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KEY WORDS: type II alveolycyte; brush alveolocyte; alveolocyte of intermediate type; differentiation.

Besides flat respiratory (type I) alveolocytes and the large type (II) which secrete lung surfactants, at present another third type of these cells is distinguished in the composition of the alveolar epithelium of many laboratory animals, namely "brush" alveolocytes (BA) [1, 5, 10, 14]. The ultrastructure of BA has been studied in detail by both transmission [1, 5, 10, 12, 13] and scanning [1, 9] electron microscopy, whereas the origin of these cells and the character of their interaction with other pneumocytes, including alveolocytes, has not been established.

In this investigation an attempt was made to study the character of intercellular interactions between alveolocytes in the solitary lung remaining in rats after left-sided pneumonectomy, in order to determine whether type II alveolocytes can differentiate into BA, the number of which in the alveoli rises sharply toward the end of the first week after the operation [6].

EXPERIMENTAL METHOD

The lungs of male rats weighing 140-200 g, some intact and others 5-7 days after left-sided pneumonectomy, were fixed by perfusion with 3.6% glutaraldehyde solution in 0.1M cacodylate buffer (pH 7.4) through the pulmonary artery followed by postfixation of pieces of tissue in 1% OsO_4 solution. Material for electron microscopy was processed by the usual method. Semithin sections 2μ thick, stained metachromatically with toluidine blue, were used for preliminary detection of BA under the light microscope and for trimming the blocks [5]. Ultrathin sections were examined in the JEM-100B electron microscope.

EXPERIMENTAL RESULTS

BA of the normal and hypertrophied rat lung were found most frequently in the region of alveolar nodes (junctions of 2-3 alveoli) or in thickened areas of the alveolar walls. The cells lay on a basement membrane which was common for all

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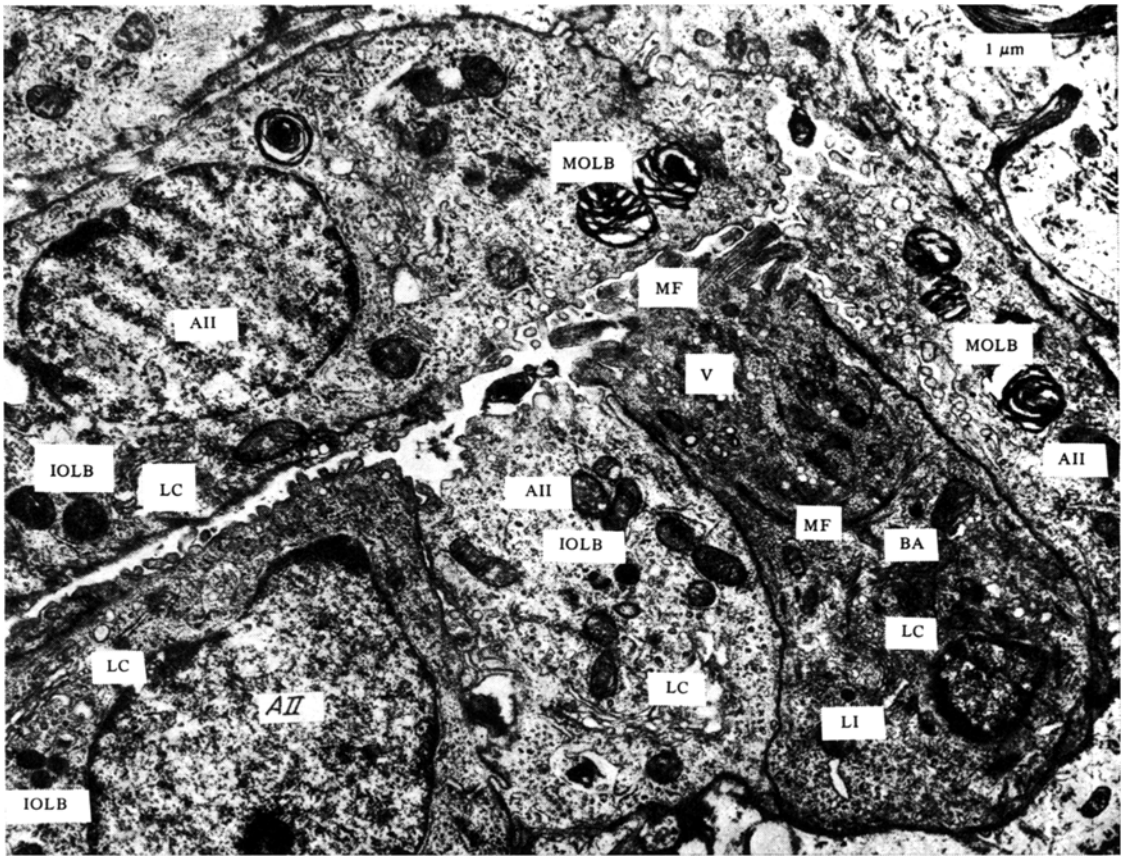

Fig. 1. BA in a cluster of AII with different degrees of maturity (5th day after pneumonectomy). Legend, here and in Figs. 2 and 3; MOLB) mature osmiophilic lamellar body; IOLB) immature osmiophilic lamellar body; LC) lamellar complex; MV) finger-shaped microvilli; MF) microfilbrils; V) vesicles; LI) lysosome-like inclusion; AL) alveolar lumen. Magnification 16,000 X.

epitheliocytes and they were intimately related to type I (AI) and type II (AII) alveolocytes.

The characteristic structural features distinguishing BA from other alveolocytes in semithin sections (especially AII), are the pyramidal shape of the body, the basal position of the nucleus, the higher density of the cytoplasm, and the presence of equal-sized blind microvilli in a longitudinal section through the cell, measuring up to 1.1μ in length and 0.2-0.3μ in width. Electron-microscopic analysis revealed numerous bundles of microfibrils penetrating the microvilli and passing into the cytoplasm in different directions, the presence of vesicles up to 0.3μ in diameter, bounded by a membrane and containing electron-translucent or floccular contents, beneath the apical plasmalemma, and single electron-dense lysosome-like inclusions (Fig. 1).

The AII were cubical, oval, or round in shape with irregularly arranged, thin microprojections of the apical plasmalemma up to 0.4μ long. In semi- and ultrathin sections these cells were easily identified by their characteristic secretory granules – osmiophilic lamellar bodies (Figs. 1 and 3a).

In the lungs of the intact rats BA were usually distributed singly. Much of the lateral and apical plasmalemma of the cell was covered on one or both sides by cytoplasmic outgrowths from AI. In 48% of cases BA were in contact with AI on one side and with AII on the other side. The cells made contact through the formation of zones of obliteration (zonula occludens) and desmosomes.

In the alveoli of the solitary remaining rat lung the number of BA was three to four times greater than normally. In this case 72% of cells were in contact with AII on one or even both sides. The AII could form groups in the alveolar epithelium containing four to eight cells in one plant of the section (Fig. 1). They were most frequently located in the region of the alveolar nodes, protruding a little into the underlying connective tissue. Most of the AII in these groups had a pale cytoplasm with many polysomes, a well-developed lamellar complex, and a few osmiophilic lamellar bodies, some of them immature, formed from multivesicular bodies (Fig. 1).

Besides AII of different degrees of maturity, the cell groups also contained one or two BA (Figs. 1 and 2a) and also alvelocytes with structural features of both cell types simultaneously (Fig. 2b, c, d). They were oval or cubical in shape,

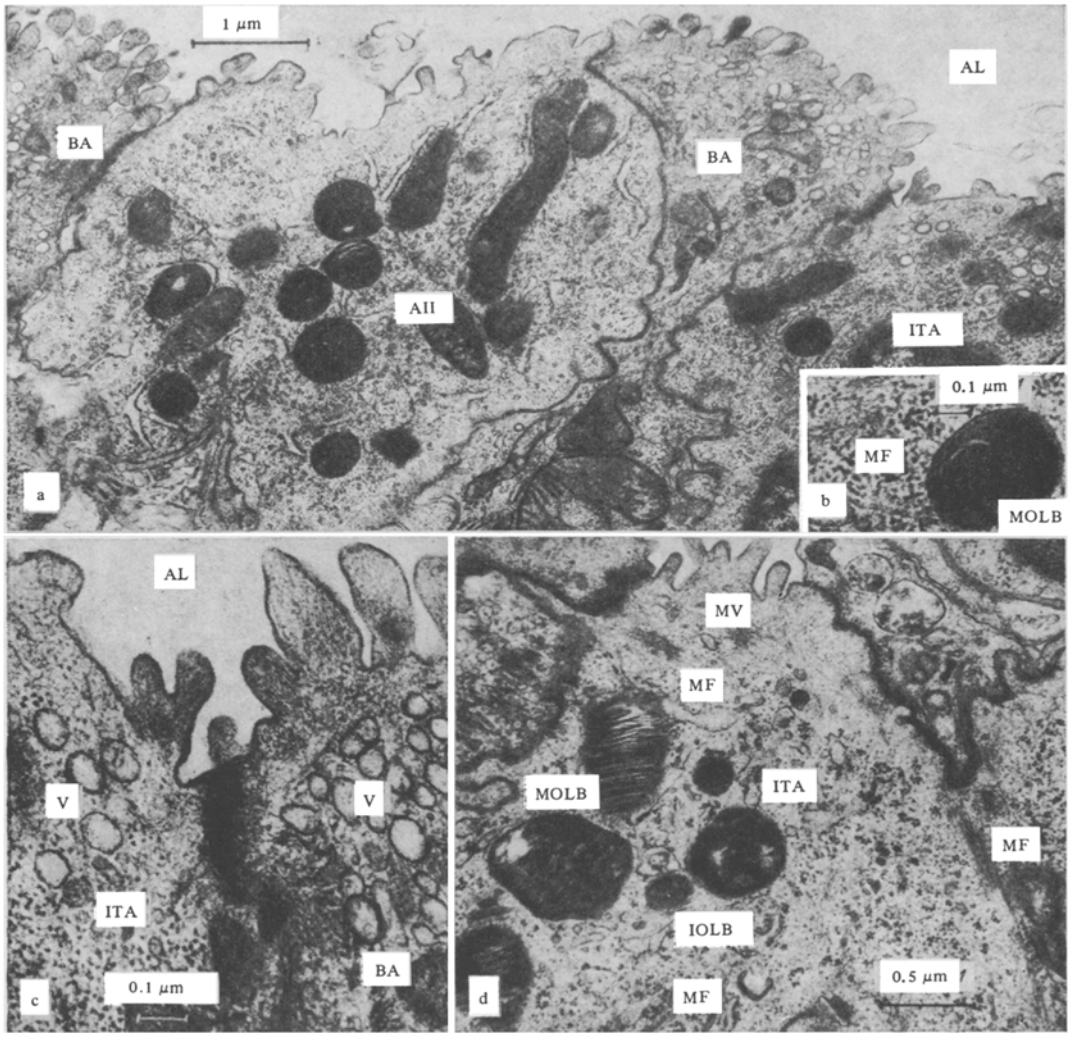

Fig. 2. Topography and ultrastructure of intermediate type of alveolocyte (ITA) during transformation of AII into BA. a) BA, AII, and ITA side by side (6th day after resection), 18,800 X; b) osmiophilic lamellar body with dense core and microfibrils in cytoplasm of ITA, 56,000 X; c) apical vesicles and single finger-shaped microvilli of ITA, 56,000 X; d) formation of finger-shaped microvilli on surface of alveolocyte of intermediate type, 29,400 X.

with a pale cytoplasm and many polysomes, and one to four osmiophilic lamellar bodies, and in these features they resembled immature AII. Meanwhile infrequent but clearly defined bundles of microfibrils, usually located in the perinuclear and (or) apical cytoplasm, and vesicles with electron-translucent contents, usual for BA, also were observed in these cells. Besides microprojections characteristic of AII, single finger-shaped microvilli containing microfibrils were found on the free, unevenly cut surface of these cells (Fig. 2c). In some cases the initial stages of formation of microvilli could be observed, when microfibrils located beneath the apical plasmalemma gradually grew into the finger-shaped evaginations of cytoplasm, giving them their characteristic shape and rigidity (Fig. 2d). The topography and the ultrastructural features of the alveolocytes with mixed signs described above suggest that they can be regarded as cells reflecting intermediate stages of differentiation of the young AII into BA.

Osmiophilic lamellar bodies with the typical structure were rare in the intermediate type of alveolocytes (ITA). Most frequently these structures lost their characteristic lamination and were converted into smaller, oval or round granules, 0.4-0.7μ in diameter, with homogeneous osmiophilic contents, resembling lysosomes. Evidence of this reorganization of the osmiophilic lamellar bodies was given by the presence of structures of intermediate type in the cells, with a homogeneous osmiophilic center and with the lamellar structure at the periphery (Fig. 2b).

The presence of osmiophilic lamellar bodies in the cytoplasm of BA, in the opinion of some workers, may be associated with the ability of the cells to ingest "osmiophilic bodies of immature surfactant" [4]. However, no direct proof

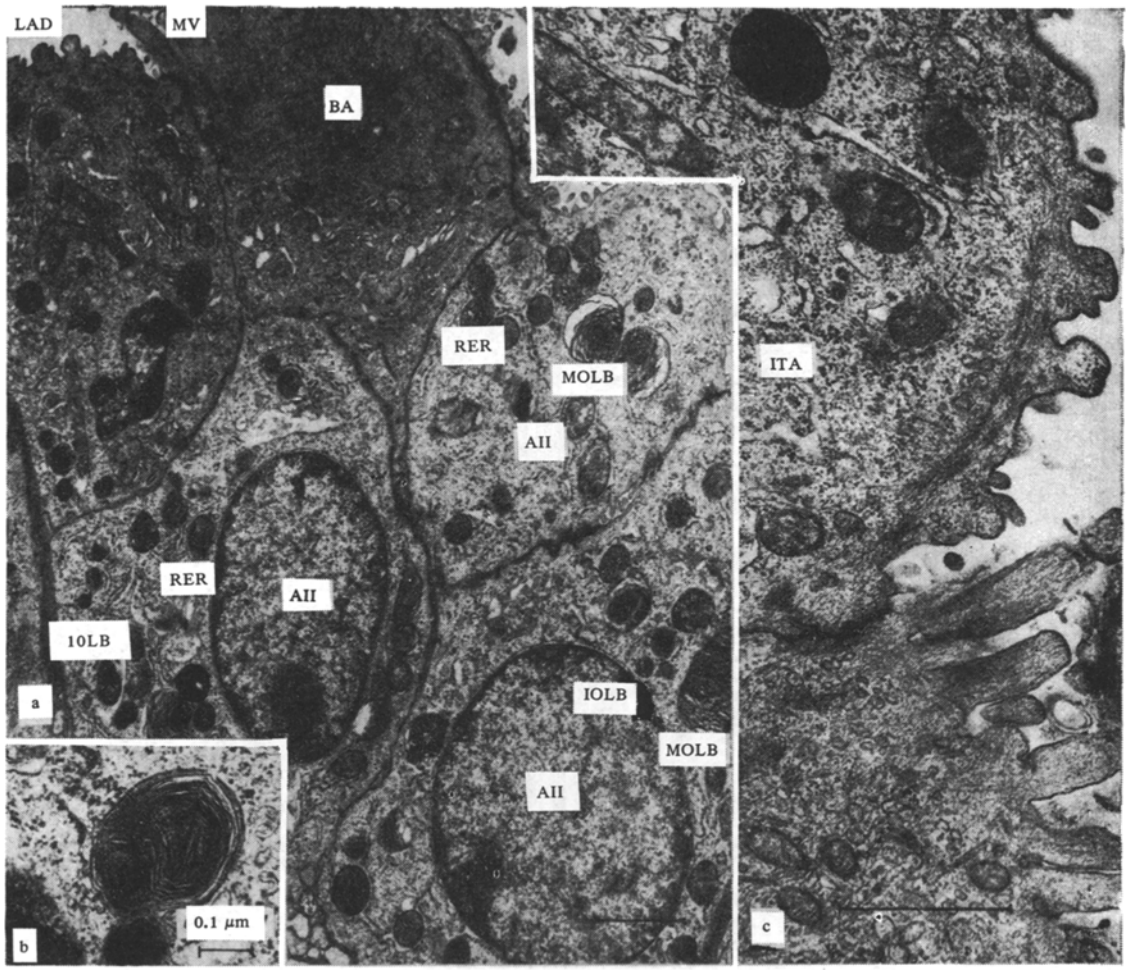

Fig. 3. Topography and ultrastructure of alveolocytes in group of cells in alveolar duct. a) Functionally active BA and ITA occupy the highest position in AII group facing lumen (6th day after resection), 12,600 X; b) mature osmiophilic lamellar body in cytoplasm of ITA, 60,200 X; c) bundles of microfibrils beneath apical plasmalemma and lysosome-like inclusion in ITA, 49,500 X. LAD) Lumen of alveolar duct; RER) rough endoplasmic reticulum.

of ingestion of any osmiophilic particles by "brush" cells could be found either in the accessible literature or in our own investigations. The presence of small invaginations of plasmalemma between the rigid microvilli and the formation of vesicles with a narrow opening and with electron-translucent contents indicate simply that these cells are able to absorb alveolar fluid [6, 10, 12].

In the present investigation the cell groups consisting of several AII and a mature BA and (or) ITA could be seen not only in the alveoli, but also in the epithelium lining the alveolar ducts. All alveolocytes were located on the basement membrane, in a depression in the wall of the duct (Fig. 3a). In this case, evidently, not all AII reach the lumen of the air passage, whereas the BA or ITA occupy the highest position in the group. Nearly the whole apical surface of BA under these circumstances is free and faces directly the lumen of the duct (Fig. 3c). The abundance of finger-shaped microvilli and the degree of development of the characteristic intracellular structures are evidence of the functional activity of the cell.

Besides lyosome-like inclusions and bundles of microfibrils (Fig. 3c), osmiophilic lamellar bodies could also be seen in the ITA (Fig. 3b).

The AII contained mature and immature forms of osmiophilic lamellar bodies and numerous tubules of the rough endoplasmic reticulum and lamellar complex, indicating that the production of surface-active substances were taking place in the cells (Fig. 3a).

Consequently, the increase in number of BA in the sole remaining rat lung may take place not only on account of the proliferation of these cells in hypertrophied alveoli [6], but also on account of their formation from AII. The latter are known to be the most resistant, actively dividing cells of the alveolar epithelium, responsible for the rapid restoration of its integrity after exposure to various destructive agents [7, 8]. The ability of AII to be transformed into AI has been

demonstrated, and cells of intermediate type reflecting the successive stages of this process have been discovered.

Enhancement of the mitotic activity of the alveocytes in the sole remaining rat lung has been found in the first week after left-sided pneumonectomy [2, 3]. Evidence of this in the present investigation was given by the presence of groups of AII of different degrees of maturity in the alveoli and alveolar ducts. The appearance of BA and (or) ITA, containing osmiophilic lamellar bodies, bundles of microfibrils, vesicles, and newly formed rigid microvilli all at the same time, in their composition point to the possibility of further differentiation of one of the daughter cells of a divided AII into BA.

Workers who have studied the respiratory epithelium of hamsters and horses by scanning electron microscopy state that BA can be formed from AII; depending on the character of the apical surface and the presence of microprojections and microvilli they also distinguished ITA [11].

Enhancement of the functional activity of BA in the sole remaining rat lung is evidently connected with the participation of these cells in regulation of secretory processes in AII. The increase in the number of BA during a period of sharp hypertrophy of the alveoli correlates with the increased secretion and augmentation of the reserves of surface-active substances in the sole remaining rat lung [6]. In the present investigation the appearance of brush cells with a well developed ultrastructure and a definite topography, in the composition of groups of functionally active AII, is also evidence of the regulatory role of BA in secretory processes in the acini, and in addition it reflects the genesis of these cells in the respiratory epithelium.

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