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The Blood-Air Barrier in Desquamative Interstitial Pneumonia (D.I.P.)

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Der Blut-Luft-Weg bei desquamativer interstitieller Pneumonie

Zusammenfassung. Zwei als diffuse interstitielle Pneumonie (D.I.P.) diagnostizierte Lungenbiopsien wurden elektronenmikroskopisch untersucht zwecks Bestimmung der die Alveolarwand bekleidenden und der frei im Alveolarraum liegenden Zellen. Die Alveolarwände waren vorwiegend mit granulären Pneumocyten besetzt. Diese waren nach Diastaseeinwirkung PAS-negativ. Die frei im Alveolarraum liegenden Zellen waren hauptsächlich Diastase-resistente, PAS-positive phagocytäre Pneumocyten, untermischt mit abgelösten granulären Pneumocyten. Manchmal waren die phagocytären Pneumocyten durch verflochtene pseudopodienartige Fortsätze miteinander verbunden. Außer der zweifachen Zellreaktion (Hyperplasie und Ablösung von granulären Pneumocyten, Gruppen von phagocytären Pneumocyten in den Alveolarräumen), waren feinstrukturelle Veränderungen an den Capillaren nachweisbar sowie lymphocytäre, plasmacytäre und eosinophile Infiltrate im interstitiellen Gewebe der Alveolarwände. Die dadurch hervorgerufene Verbreiterung der Blut-Luftschranke scheint einer der für die in beiden Fällen nachgewiesene verringerte Lungendiffusionskapazität verantwortlichen Faktoren zu sein.

Summary. Two biopsies of lung diagnosed as D.I.P. were studied by electron microscopy to ascertain the type of cells lining the alveoli and of those lying free in the alveolar spaces. The alveoli were lined predominantly by granular pneumocytes that were P.A.S.-negative after diastase digestion. In contrast, the free intraluminal cells were mostly phagocytic pneumocytes mixed with desquamated granular pneumocytes and were diastase resistant P.A.S.-positive. Sometimes the phagocytic pneumocytes were joined by intertwining pseudopodial-like processes. In addition to that dual cellular reaction (hyperplasia and desquamation of granular pneumocytes, with groups of phagocytic pneumocytes in the alveolar spaces), there were ultrastructural changes in capillaries and infiltrates of lymphocytes, plasma cells and eosinophils in the interstitial tissue of the alveolar wall. We believe that these alterations in the blood-air barrier are one of the factors responsible for the reduced pulmonary diffusing capacity recorded in the two cases presented.

Liebow, Steer and Billingsley (1965) described a type of interstitial pneumonia characterized by the following light microscopic changes: 1. lining and filling of the lumina of thickened distal air spaces by masses of "large alveolar cells" containing P.A.S. positive granules; 2. minimal loss of tissue and absence of necrosis; 3. well circumscribed lymphoid follicles in septa and bronchiolar walls associated with interstitial infiltrates of lymphocytes, plasma cells and eosinophils; 4. thickened and narrowed arteries as a result of muscular hyperplasia in consolidated areas. At high magnification these authors noted that the prominent cells lining

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the alveolar spaces and the desquamated cells in the alveolar cavity were vacuolated and the vacuoles had a "stippled and even lamellated content". On the basis of this appearance together with some evidence obtained from electron micrographs of routinely fixed autopsy tissue, Liebow and associates (1965) suggested that these cells were granular pneumocytes.

In an effort to ascertain the nature of the alveolar lining cells and of the cells that filled the alveolar spaces in desquamative interstitial pneumonia, and to obtain additional morphologic data that may clarify the pathophysiology of this disease, and electron microscopic study of two pulmonary biopsies diagnosed as desquamative interstitial pneumonia (D.I.P.)[1] was carried out. The ultrastructural changes observed in these two cases form the subject of this report.

Materials and Methods

Some pertinent clinical and laboratory data on the two patients are recorded in the table. One of them (W.B.) was complaining of joint pains for the first time when he was admitted to the hospital; the latex fixation test was positive in this case. The other (E.S.) was asymptomatic except for a slight cough with withish sputum in the morning. A reduction in carbon monoxide diffusing capacity was recorded in both patients. There was neither cyanosis nor dyspnea on exertion. Roentgenograms of the chest revealed irregular densities at the base of both lungs for the first patient (W.B.). There were peribronchial densities associated with a finely reticulated appearance especially at the base of the left lung for the second case (E.S.).

Table. *Some clinical and laboratory data in two cases of D.I.P.*

	Case No. 1 (W.B.)	Case No 2 (E.S.)
Sex and age	♂, 66 years	♂, 38 years
Occupation	Mail carrier	Ambulance driver
Chief complaints	Joint pains[a]	None[b]
Clubbing of extremities	Present	Present
Rales	Present (at bases)	None
Hematocrit (%)	42	51
D_{CO}(ml/min mm Hg)	6.6 (33%)[c]	9.3 (34%)[c]
Arterial pCO_2 (mm Hg)	34.5	29
Arterial pO_2 (mm Hg)	83	74.1

D_{CO} = carbon monoxide diffusing capacity (single breath technique).
[a] X-ray of hands: nonspecific arthritis; latex fixation positive.
[b] Routine chest X-ray: abnormal.
[c] Percent of normal.

The lung biopsy in both cases was obtained by thoracotomy under general anesthesia. The specimen from the first patient (W.B.) was removed from the left upper lobe which, according to the surgeon, had an increased consistency. In the second case (E.S.) the biopsy was taken from the right middle lobe which seemed "normal" except for a "slight increased resistance" on section. The pleura of both specimen was smooth, gray and opaque. After fixation the porosity of both specimens was coarser than usual.

For light microscopic examination, part of the two biopsies was fixed in 10 percent solution of formalin in buffered saline. Sections from paraffin embedded pieces were stained with hematoxylin and eosin, Masson's or Mallory's trichrome stain, Weigert's resorcin fuchsin elastic

1. The diagnosis of D.I.P. was ascertained after consultation with Dr. Averill A. Liebow who reviewed the histologic sections.

stain, periodic acid-Schiff stain (P.A.S.) with and without diastase digestion, Gridley's and Gomori-Grocott's stain for fungi, Ziehl-Neelsen's stain for acid fast organisms, Gomori's iron reaction, and Gordon and Sweet's reticulum stain.

Preparation for electron microscopy was carried out as fellows: On removal, part of each biopsy was cut with sharp razor blades into 1 to 2 mm particles while immersed in 1 percent solution of osmium tetroxide buffered to pH 7.4 (Palade, 1952). The tiny fragments were rapidly transferred to a small vial of fixative which had been kept in ice. Fixation was continued for one hour at 2 to 5° C. The fixed particles were dehydrated through graded alcohols and propylene oxide and embedded in Epon 812 according to Luft (1961). Preliminary survey of the blocks for orientation and localization of alveolar septa was carried out by light microscopy on 1 μ thick sections stained with toluidine blue. Ultrathin sections were nade with diamond knives using an L.K.B. Ultratome. They were stained with uranyl acetate and lead citrate. They were examined and photographed with an R.C.A. EMU-3H electron microscope at magnifications ranging from 2,200 to 17,240. The electron microscopic plates were further enlarged on the prints.

Results

Light Microscopic Observations. The changes were similar to those described by Liebow and associates (1965). The first specimen (W.B.) showed extensive interstitial fibrosis associated with loss of alveolar spaces (Fig. 1 a, c). Slight thickening of alveolar walls without significant destruction was noted in the second specimen (E.S.) (Fig. 2). In both cases there were masses of cells in the alveolar spaces associated with focal hyperplasia of the alveolar lining epithelium. There was absence of fibrinous exudation or neutrophilic leukocytic reaction. Most of the cells lying in the alveolar spaces were large, round and had an accentric nucleus. They contained golden-brown cytoplasmic granules. These were diastase resistant P.A.S. positive, while the cuboidal cells lining the distal air spaces were P.A.S. negative after diastase digestion. The stain for iron was negative, except for a few cells with a diffuse faint blue cytoplasmic tinge. Other free cells were smaller than those described above and were cuboidal with a centrally located nucleus. Occasionally they were arranged in rows parallel to the alveolar wall. The thickened walls of distal air spaces in the first specimen contained fairly well demarcated lymphoid nodules and cellular infiltrates composed of scattered lymphocytes, plasma cells, eosinophils and a few mast cells. These were clearly identified in the epon embedded sections stained with toluidine blue. In the second specimen, lymphoid nodules were associated with bronchioles and the septa were infiltrated mainly by lymphocytes and plasma cells (Fig. 2). Vascular changes were prominent in the first specimen while inconspicuous in the second. These changes were characterized by marked reduction in the caliber of some small arteries (Fig. 1 c). Their thickened wall, which was formed by layers of crescent shaped muscle cells, was often infiltrated with plasma cells and lymphocytes. The walls of larger arteries showed marked muscular hyperplasia.

Electron Microscopic Observations. Although the lesions in the first case (W.B.) were more advanced than in the second (E.S.), in both cases the basic ultrastructural changes were similar. All the elements of the blood-air barrier were affected.

Epithelial lesions. Most of the alveolar spaces were lined predominantly by cells exhibiting the characteristics of granular pneumocytes (Type II cells) (Figs. 3, 8 and 9). These cells were large cuboidal with central nucleus. The lay directly on the basement membrane. Their free surface presented multiple short microvilli while their lateral surface was bound to that of adjacent cells by complex junctions. The

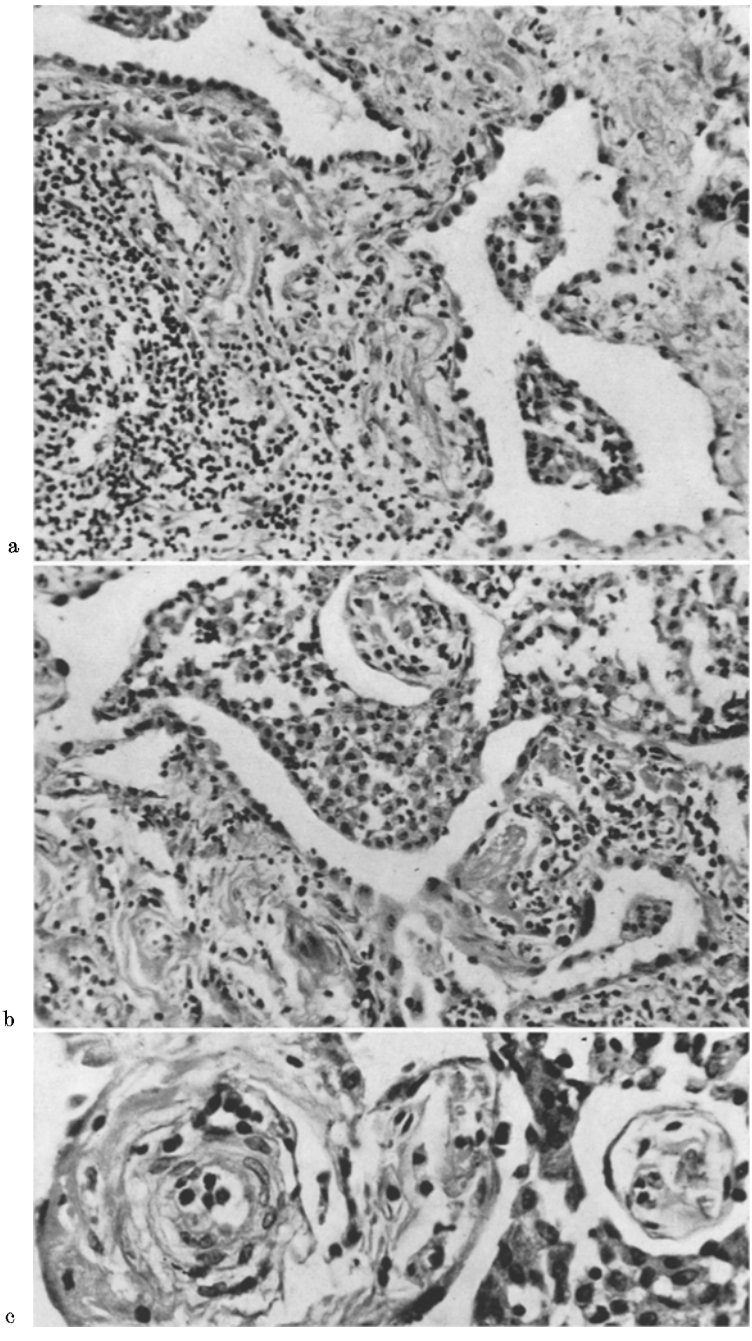

Fig. 1 a—c. (Case No. 1). a Loss of pulmonary alveoli with honeycombed appearance, part of a lymphoid nodule, and b scattered lymphocytes and plasma cells in the fibrosed walls of distal air spaces which contain masses of uniform cells with marginal epithelial-like arrangement. ×200. c Small artery with thickened wall infiltrated with a few lymphocytes and plasma cells. ×400. Hematoxylin eosin stain

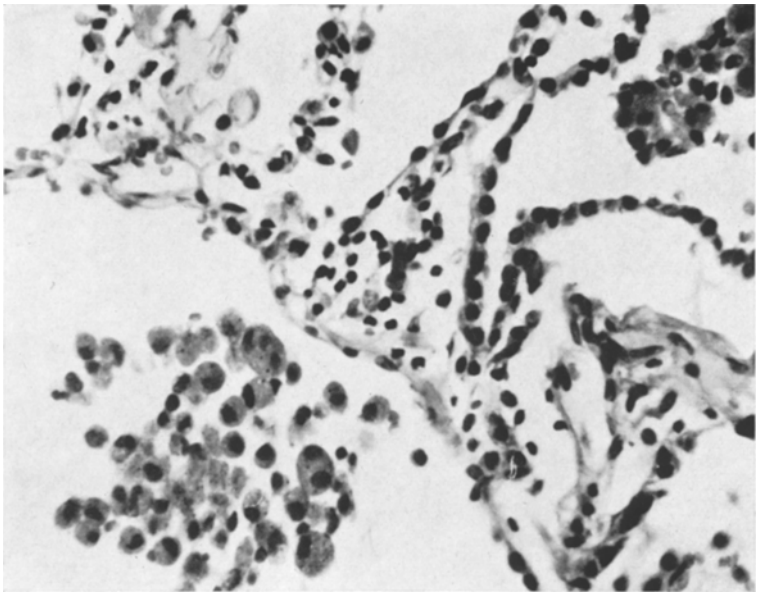

Fig. 2. (Case No. 2). Infiltrates of lymphocytes and plasma cells in slightly thickened walls of distal air spaces which are partly filled with large cells exhibiting a finely vacuolated and granular cytoplasm. Some of these spaces are lined by a cuboidal epithelium. ×325

attenuated cytoplasmic veil which characterizes membranous pneumocytes (Type I cells) was absent. The cytoplasm of these large cuboidal alveolar lining cells contained numerous whorled or lamellated intensely osmiophilic inclusions. Most of these inclusions were vacuolated; often they coalesced to form larger multilobed masses. They were enclosed by a single layered membrane. A few isolated inclusions were rounded and were very dense. Many granular pneumocytes showed dilatation of the cisternae of the endoplasmic reticulum and focal rupture of cytoplasmic membranes near the basement membrane (Fig. 3). The alveolar spaces contained a mixture of phagocytic pneumocytes (alveolar macrophages) and granular pneumocytes in various proportions. In some ultrathin sections approximately 50 percent of the free cells were granular pneumocytes. Some of these desquamated pneumocytes showed complex junctions with others that were still attached to the epithelial basement membrane (Figs. 4, 9). In many other sections, phagocytic pneumocytes formed approximately 90 percent of the intraalveolar cells (Fig. 5). Some were closely opposed to the cytoplasmic veil of membranous pneumocytes. Most of them presented electron lucent pseudopodial processes. Occasionally, spaces limited by fused pseudopodia contained engulfed myelin-like figures. In the cytoplasm of most of these phagocytic pneumocytes there were numerous inclusions with a heterogeneous morphology. Some inclusions contained lipid particles, masses of dense granular material and crystalloid lattice-like structure composed of dense lines with spacing of approximately 45 to 55 Å arranged in parallel or hexagonal pattern (Fig. 6, insert). This structure resembled that described by Karrer (1960) in pulmonary macrophages from mice. The epithelial basement membrane was indistinct in some areas; in others, it was pierced by cytoplasmic protrusions from epithelial

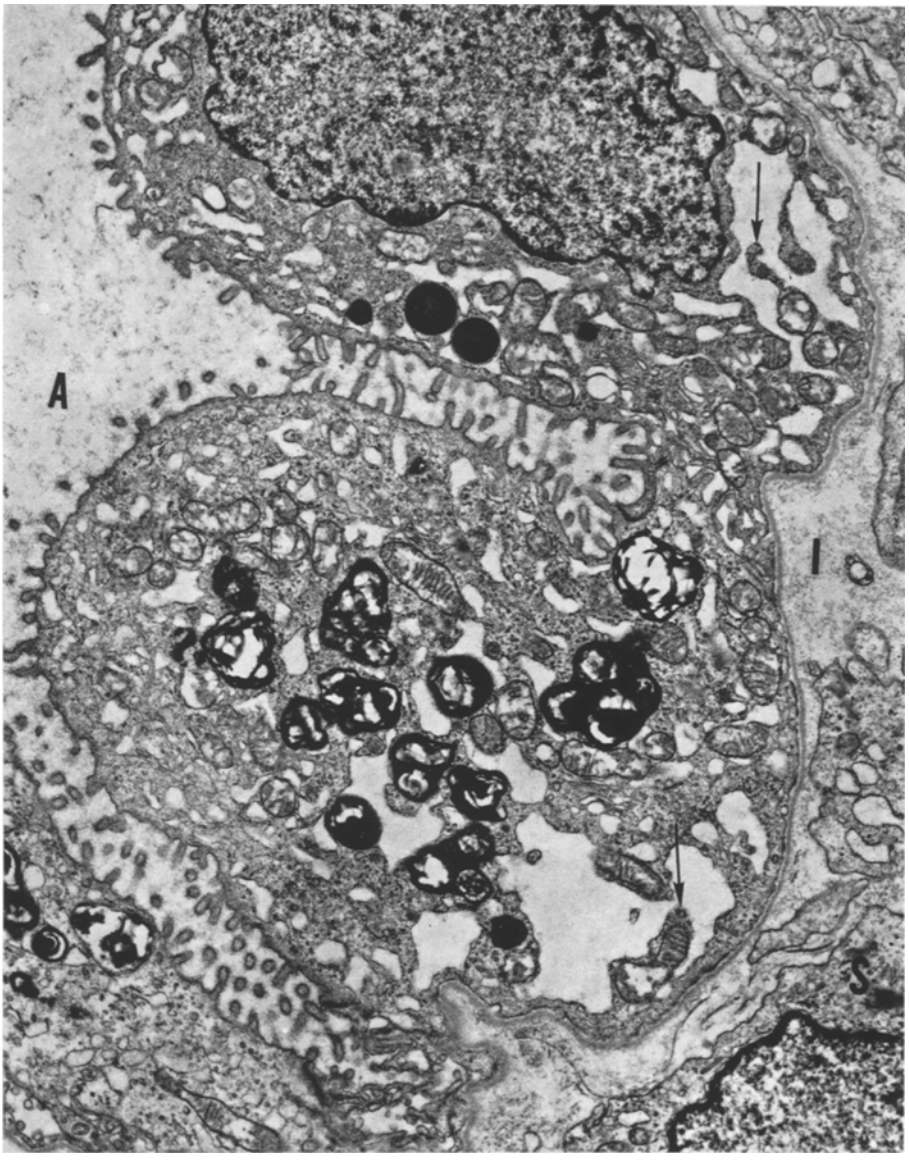

Fig. 3. Electron micrograph of part of an alveolus from the second patient (E. S.). Note the cuboidal cells with dilated cisternae of the endoplasmic reticulum, focal rupture of cytoplasmic membranes (arrow) and multilobed whorled, vacuolated osmiophilic cytoplasmic inclusions. In the edematous interstitium (*I*) there is a septal cell (*S*). Alveolar space (*A*). ×9,650.
Note: All sections for electron microscopic study were stained with uranyl acetate and lead citrate

cells or from cells located in the interstitium (Fig. 7). Occasionally protrusions from these interstitial cells contained vacuolated and lamellated osmiophilic masses similar to those usually seen in granular pneumocytes.

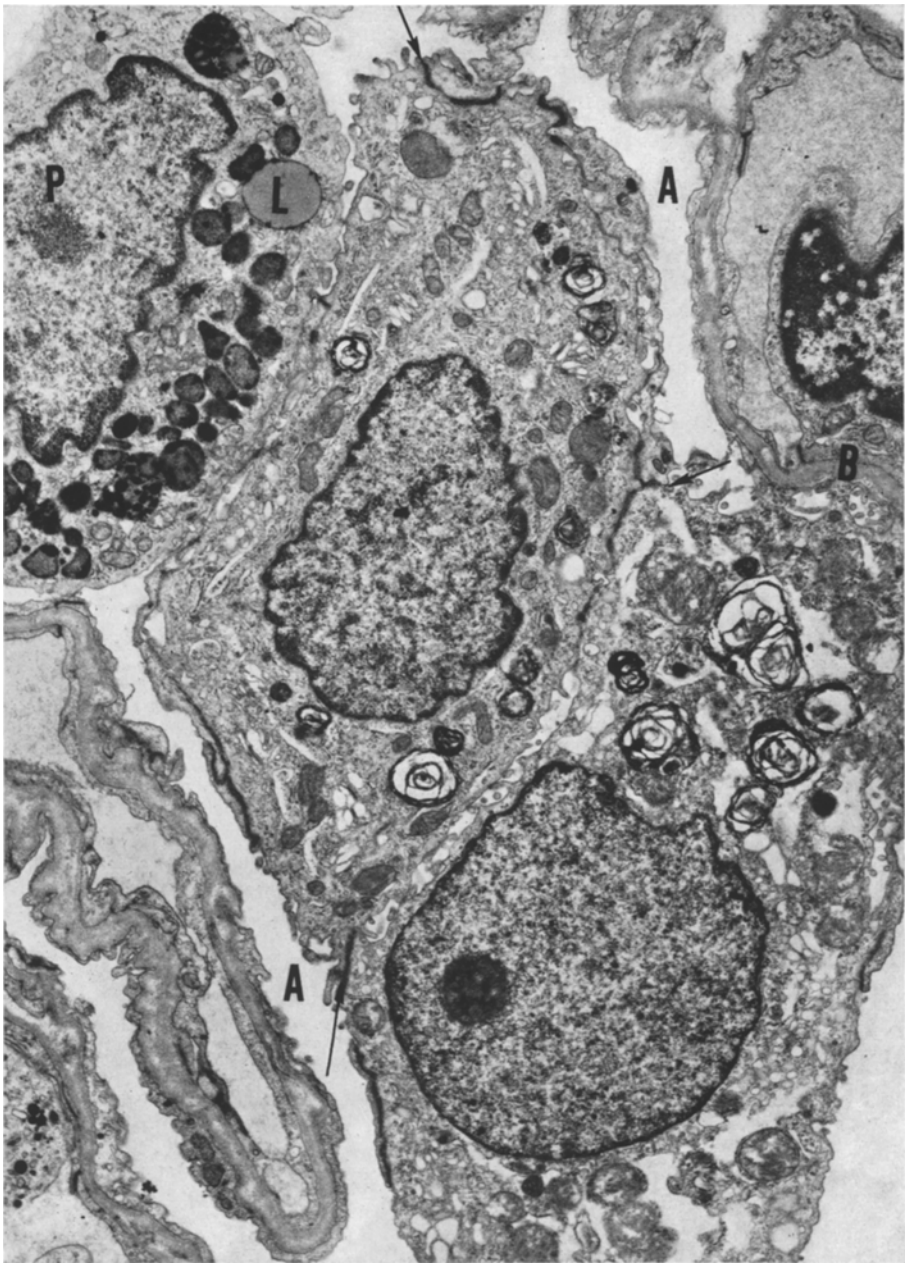

Fig. 4. Portion of an alveolar space from second patient (E. S.). It contains a phagocytic pneumocyte (*P*) exhibiting a lipid particle (*L*) and numerous dense heterogenous lysosomes contrasting with the whorled, vacuolated inclusions in the two granular pneumocytes; one of these pneumocytes is still attached to the epithelial basement membrane (*B*), the other seems to be free except for junctions (arrow) with adjacent cells. ×8,000

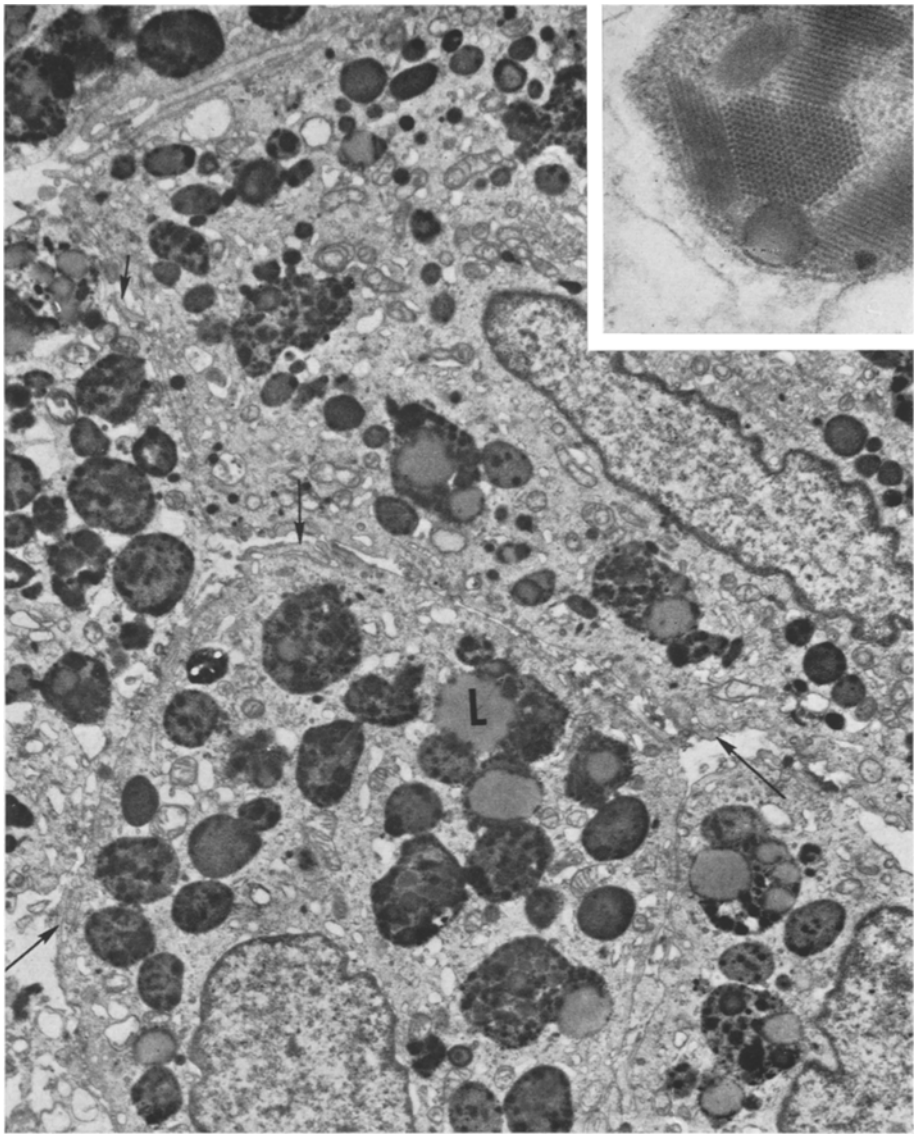

Fig. 6

Fig. 5

Fig. 5. Same specimen as for Fig. 4. Phagocytic pneumocytes with intertwining pseudopodial processes (arrow). Their cytoplasm contains many heterogenous lysosomes which vary from 0.15 to 2 μ. ×7.550

Fig. 6 (insert). Lysosome exhibiting masses of crystalline lattice structure with parallel or hexagonal pattern. ×68,000

Capillary Lesions. In the first biopsy the normal segmental protrusions of the capillary walls into the alveolar spaces were absent, most capillaries being embedded in the thickened interalveolar septa (Figs. 8, 9). The endothelium was irregular in thickness and some of the endothelial junctions were partly open. The capillary basement membrane was thickened. In some areas it was split into layers which

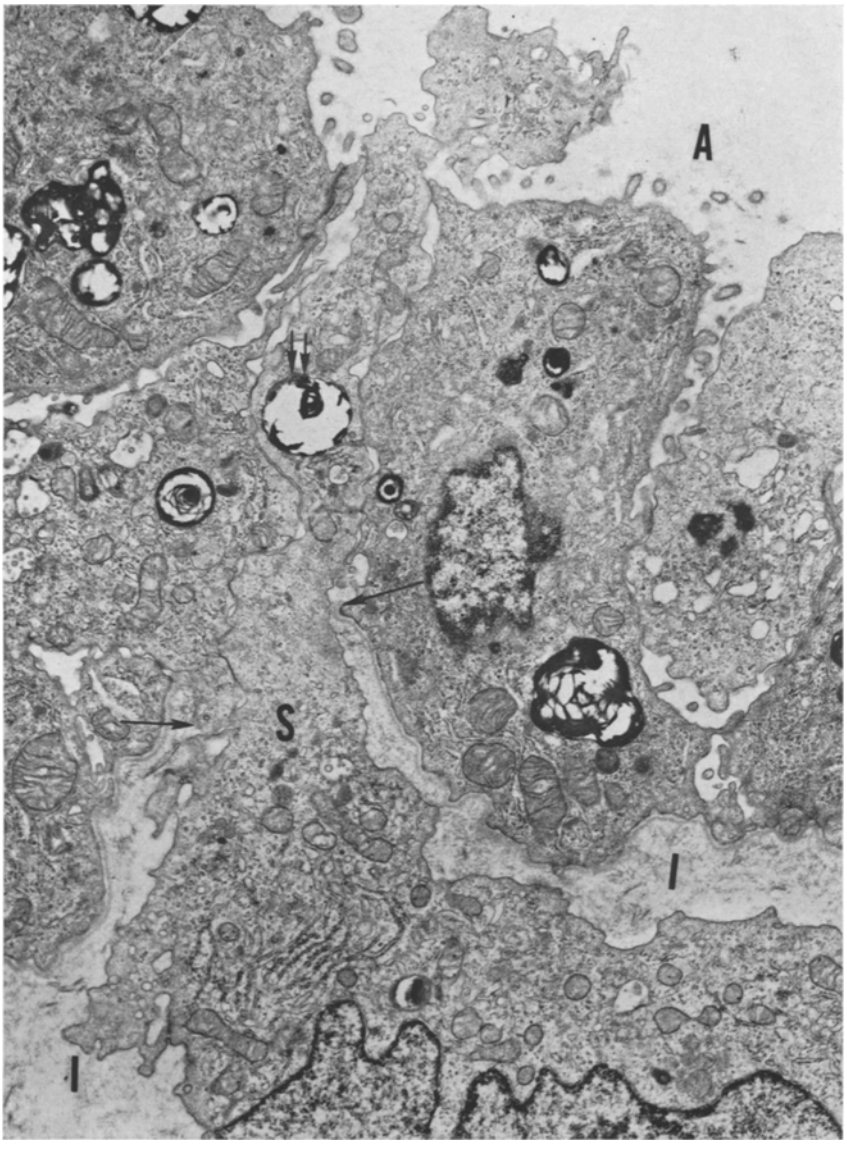

Fig. 7. Same sample as Fig. 5. A cytoplasmic projection from an interstitially located cell (*S*) pierces the epithelial basement membrane (arrow) and protrudes in the alveolar space (*A*). It contains a lamellar body (double arrow). The interstitium (*I*) is edematous. ×8,560

enclosed slender cytoplasmic endothelial projections; these were roughly parallel to the endothelial surface (Fig. 8). In the second biopsy the relationship between capillary walls and alveolar spaces appeared normal. However, some capillaries showed complex endothelial junctions and focal separation of the endothelium from the basement membrane which was irregularly thickened. The walls of other capillaries were normal.

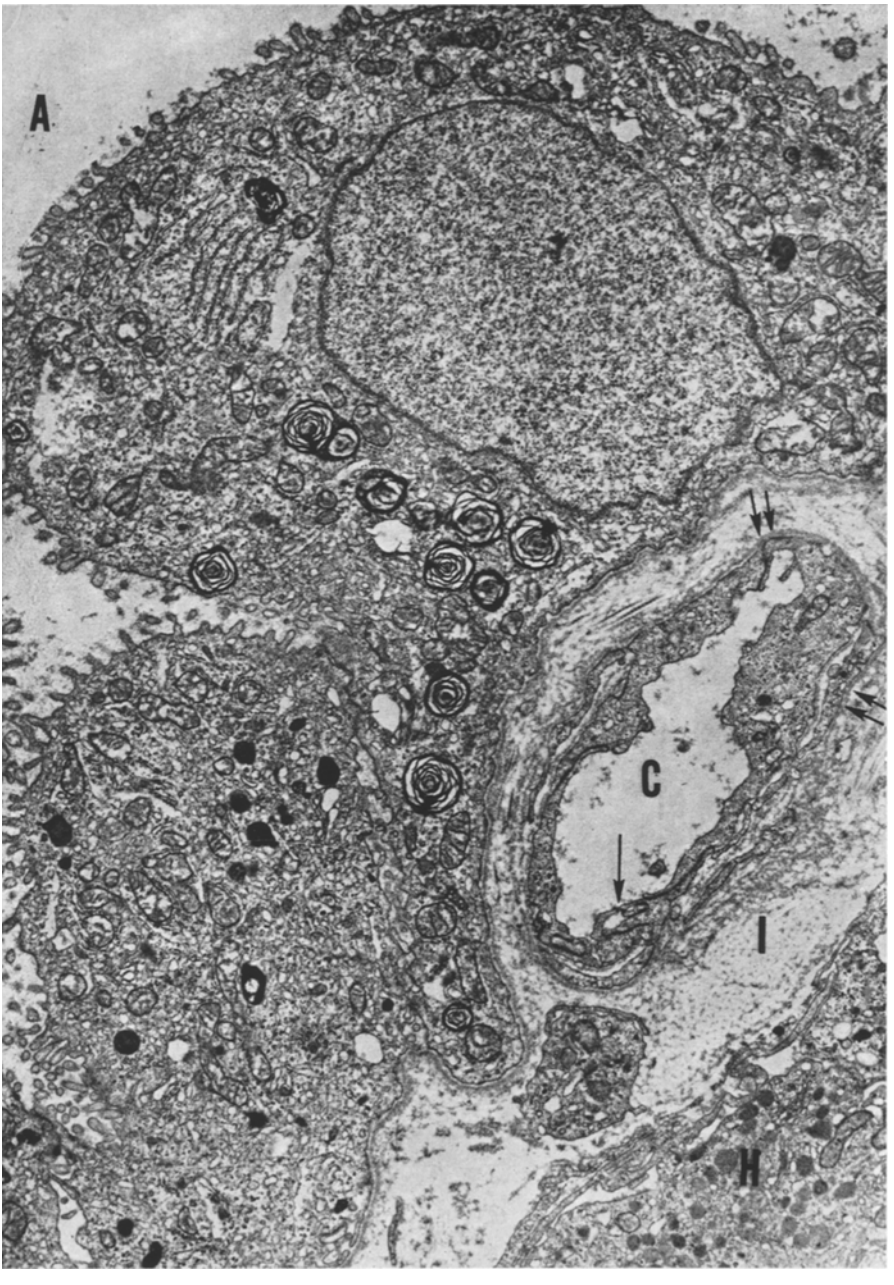

Fig. 8. Portion of an alveolus from first case (W.B.). The capillary (*C*) shows a complex endothelial junction which is partly open (single arrow). The basement membrane (double arrow) is split by slender cytoplasmic processes from the endothelium. The edematous interstitium (*I*) contains collagenous fibers and part of a histiocyte (*H*). The alveolar space (*A*) is lined by thick, cuboidal granular pneumocytes not suited for diffusion. ×8,000

Lesions of the Interstitium. In addition to inflammatory cells which had ultrastructural characteristics of lymphocytes and plasma cells, the edematous interstitium contained septal fibroblasts and cytoplasmic masses from unidentified cells.

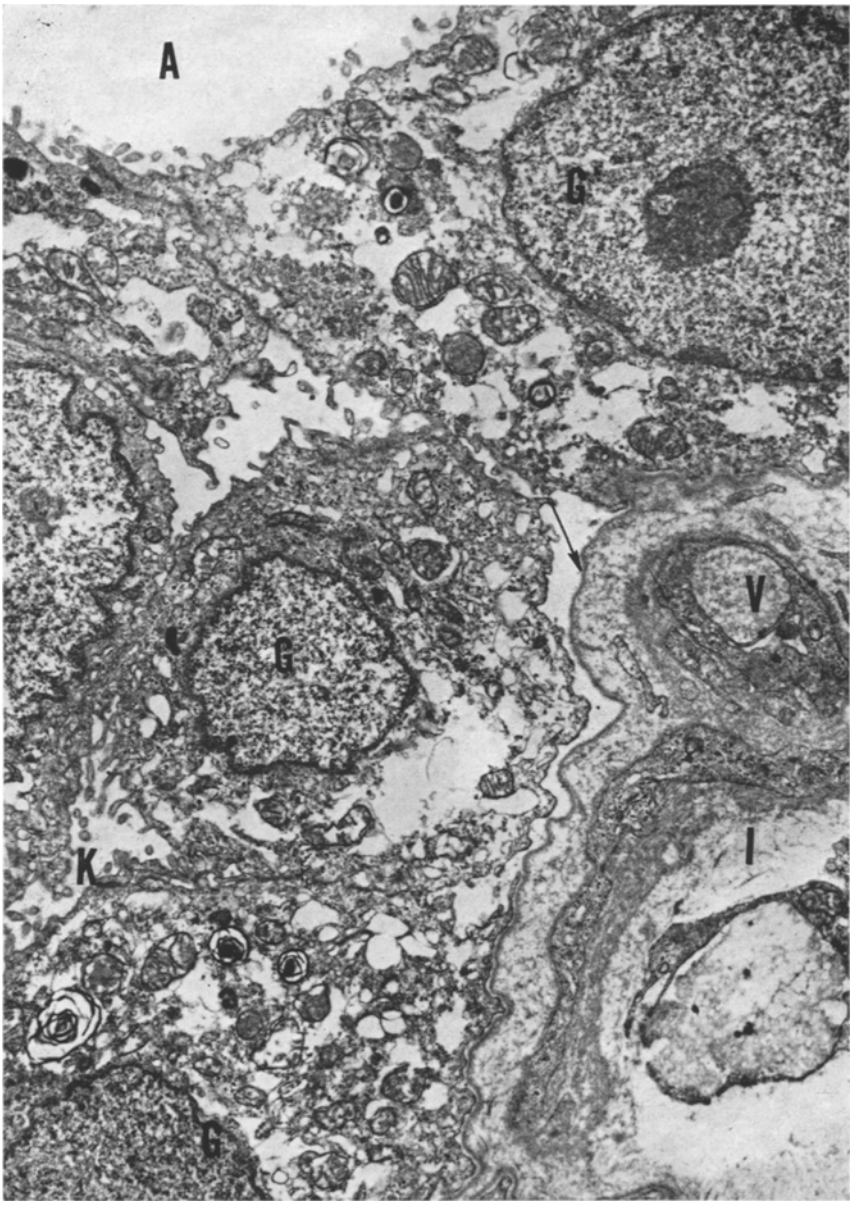

Fig. 9. Same specimen as for Fig. 8. One of the granular pneumocytes (*G*) is separated from the basement membrane (arrow). Its junction (*K*) with an adjacent fixed granular pneumocyte is intact. The edematous interstitium contains a capillary which is partly occluded by an endothelial vesicle (*V*). ×8,600

There was an increase of interstitial collagenous fibers especially in the first specimen. These fibers, when isolated, were surrounded by microfibrils. In some areas they were arranged in bundles (Fig. 8).

Discussion

On light microscopy, as stated by Liebow *et al.* (1965), it is difficult to be certain whether the large lining cells ("littoral cells") in D.I.P. overlay, or replaced the normal membranous pneumocytes. This electron microscopic study of two cases of D.I.P. reveals that the alveolar spaces are lined predominantly by granular pneumocytes instead of flattened membranous pneumocytes as in the normal lung. The cells lying in the alveolar lumina are phagocytic pneumocytes (alveolar macrophages) mixed with desquamated granular pneumocytes (Type II cells) in various proportions. Absence of neutrophilic polymorphonuclear reaction and fibrinous exudation is conspicuous. This together with the dual cellular exudate in the distal air spaces, differentiates D.I.P. from the usual type of interstitial pneumonia. The long, irregular, often fused pseudopodia indicative of phagocytic activity, the structural heterogeneity of the cytoplasmic inclusions which may reflect variable composition of phagocitized material as suggested by Karrer (1960), differentiate the phagocytic pneumocytes (alveolar macrophages) from the desquamated granular pneumocytes (Type II cells). These desquamated pneumocytes often show a few remaining complex junctions with other pneumocytes that are still attached to the epithelial basement membrane. Their microvilli are shorter, more narrow and more regular than the pseudopodia of the phagocytic pneumocytes. Usually no fusion of microvilli is observed. The whorl-like cytoplasmic inclusions of the granular pneumocytes may vary in shape and size. They are often vacuolated but structurally they are homogeneous. The question as to whether they are elaborated by the granular pneumocytes (Buckingham *et al.*, 1966) or whether they represent phagocytized phospholipids (Niden, 1967) cannot be elucidated by this study. The morphologic distinction between free phagocytic pneumocytes and desquamated granular pneumocytes by electron microscopy may not always be as evident as indicated above; pseudopodia are not constantly present in phagocytic pneumocytes and preserved junctions which indicate the epithelial nature of some desquamated granular pneumocytes may not be found in some sections. Moreover, it is possible that after exposure to some toxic agents, granular pneumocytes may show focal cytoplasmic degradation with formation of heterogeneous lysosome-like bodies similar to those reported by Swift *et al.* (1964) in other cells. Although such lesions were not observed in the fixed granular pneumocytes, the fate of desquamated granular pneumocytes is unknown. The possibility of their transformation into phagocytic pneumocytes has not been excluded in this study. The above considerations, together with the inherent limitations of electron microscopic techniques, made it difficult to evaluate with any degree of accuracy the proportion of desquamated granular pneumocytes in the alveolar spaces as compared with phagocytic pneumocytes. The minute samples are often inadequate and it is assumed that many free cells egress from the alveolar spaces during mincing, fixing and processing of the pulmonary biopsies.

The exact nature of the brown cytoplasmic granules in the free alveolar cells must await further cytochemical investigations. In the two cases of D.I.P. studied P.A.S.-positive diastase resistent granules are absent in the cuboidal alveolar lining cells. Subsequent electron microscopic examination reveals that these cells are predominantly granular pneumocytes. The question as to whether granular pneumocytes (Type II cells) remain P.A.S.-negative after their desquamation into the

alveolar spaces, or whether desquamated granular pneumocytes take up non-glycogen carbohydrate complexes from the environment, cannot be answered at present. Many of the free alveolar cells which contain P.A.S.-positive granules have ultrastructural characteristics of phagocytic pneumocytes filled with heterogeneous lysosomes. This suggests that the P.A.S.-positive granules are probably of lysosomal origin. The above suggestion is in accord with the findings of Hawrylko and Cohn (1968), who investigated the localization and origin of carbohydrate-protein complexes in alveolar macrophages of rabbit. These complexes were localized in the fraction of macrophages which contained lysosomal granules and mitochondria. Diastase resistant P.A.S.-positive substance has been demonstrated by Teilum (1956) in reticulo-endothelial cells of rabbits that were hyperimmunized with intravenous injections of formalin killed "Pfeiffer bacillus". Accumulation of P.A.S.-positive cells referred to as "pulmonary macrophages" has been observed by Deodhar and Bhagwat (1967) after intravenous injections of complete Freund's adjuvant to rabbits. In their report these authors did not record the reaction of the cells to the P.A.S. reagent after diastase digestion. The relationship between these experimentally induced lesions and the human D.I.P. is obscure. The exact cause of this form of interstitial pneumonia is unknown. The two patients were never exposed to any toxic fumes or chemicals. Repeated cultures of sputum in both cases and cultures of part of one of the biopsies (W.B.) were negative for pathogenic bacteria and fungi. These specimens were not cultured for viruses. Specific staining of histologic sections failed to reveal any organism. The presence of latex fixing globulins in the serum of one patient (W.B.) could suggest an interstitial pulmonary fibrosis associated with rheumatoid arthritis (Doctor *et al.*, 1962). However, no pulmonary rheumatoid nodule, as described by Christie (1954), was noted in the specimen submitted. Moreover, from the study of Gottlieb and associates (1965) it seems that latex fixing antibodies are not specific for any one disease.

Results of pulmonary function tests on most patients with D.I.P. (Gaensler *et al.*, 1966) indicate an "alveolocapillary block". While "alveolocapillary block" may be due to significant shunting of blood through poorly ventilated areas within the lung (Finley *et al.*, 1962), lesions of the blood-air barrier, such as those demonstrated in the two biopsies, may be one of the causes of the decreased carbon monoxide diffusing capacity recorded in these two cases. However, the small amount of tissue examined by electron microscopy in the present study makes it difficult to relate the lesions described to the lung as a whole.

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