

Morphogenesis of Alveolar Bronchiolization

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So-called bronchiolization of alveoli was observed in mice chronically exposed to synthetic smog or CaCrO_4 dust. The epithelial cells lining the affected alveoli were identified by electron microscopy as typical bronchiolar cells. Basement membrane pores were often found in the bronchiolar walls of exposed mice, laterally connecting the bronchioles with adjacent alveoli. In animals exposed to synthetic smog, these openings were always occupied by cells resembling bronchiolar epithelium. In CaCrO_4 -exposed animals with bronchiolar epithelial hyperplasia, the pores appeared to have developed into small bronchiolar-alveolar channels with a patent lumen and a length of two to three cell diameters. Bronchiolar epithelialization was seen only occasionally in age-matched control animals; openings in the basement membrane have not yet been identified in controls. We propose that colonization of alveoli with bronchiolar cells via bronchiolar-alveolar pores is one morphogenetic mechanism of alveolar bronchiolization.

Additional key words: Alveolar metaplasia, Alveolar epithelialization, Basement membrane pores, Synthetic smog, CaCrO_4 dust.

The various regions of the respiratory tract differ markedly in their response to injurious agents. This was recently demonstrated in a cytokinetic study performed on NO_2 -exposed animals, in which special attention was given to the response of the preterminal, terminal, and respiratory bronchioles.⁸ Because of the close anatomical relationship between the most distal bronchioles and the alveolar ducts and alveoli, the exact origin of epithelial changes following injury of this part of the respiratory tract is often difficult to determine. A typical example is the so-called epithelialization or bronchiolization of alveoli, which can be observed after a variety of insults such as respiratory infection,^{1, 6, 9, 11} exposure to chemical irritants^{8, 19} and carcinogens,^{15, 20} and even circulatory disturbances involving the lung.² Whether the cuboidal and often columnar cells lining the alveoli resemble typical bronchiolar epithelium in all instances and whether the histogenesis of the alveolar "epithelialization" is the same in the various pathologic conditions is uncertain.

The study reported here is part of an effort to determine the effects of different air pollutants on the respiratory tract of mice.¹²⁻¹⁴ Specifically, we have investigated the morphologic changes occurring in the lower respiratory tract as a result of chronic low level injury. Since the animals used were germ-free-derived, specific pathogen-free mice, we were not faced with the problem of intercurrent respiratory infection, which so often complicates the interpretation of morphologic changes developing after inhalation exposure to noxious chemicals. Characteristic changes appearing in the small airways and alveoli after chronic exposure to synthetic smog and CaCrO_4 dust are described. From these observations a possible morphogenetic mechanism for the "bronchiolization" of alveoli is proposed.

MATERIALS AND METHODS

Germ-free-derived, specific pathogen-free mice of the C57BL/6 inbred line were exposed to filtered air, ozonized gasoline fumes (synthetic smog), or calcium chromate dust (CaCrO_4) for 5½ hours a day, 5 days a week. The synthetic smog was created by evaporating a straight run, unleaded gasoline into a stream of oxygen and ozone, resulting from the exposure of pure oxygen to ultraviolet light. The maximal concentrations maintained were 1 p.p.m. of ozone and 24 to 30 p.p.m. of gasoline. The mean geometric diameter of calcium chromate particulates was 0.2 μ . The dust was dispersed into the inlet air by a Wright dust feed at a concentration of 13 mg. per cu. m. Details of the exposure facility and conditions have been reported previously.^{12, 13} Eighteen months after the start of the exposure, 15 mice were removed from each chamber and used for bacteriologic, virologic, and parasitologic testing and for histopathologic investigation. Electron microscopic studies were performed only on tracheas and lungs from animals exposed to either artificial smog or filtered air.

For electron microscopy, mouse lungs were prefixed by intratracheal injection of 1.75 per cent Tyrode-buffered glutaraldehyde at 4° C. for several weeks. After glutaraldehyde fixation, the tissues were washed in phosphate-buffered sucrose (pH 7.4) for a period of 1 week at 4° C. They were postfixed in phosphate-buffered osmium tetroxide (pH 7.4) for 90 minutes and later dehydrated and embedded in Epon. Bronchioles were identified on 1- μ thick Epon sections stained with a mixture of methylene blue and azure II. Blocks containing the desired segments of bronchioles were sectioned for electron microscopic examination on an LKB Ultratome III. Serial sections were mounted on grids that had a single 1- by 2-mm. opening and were coated with Formvar and carbon. This

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allowed construction of serial montages of the electron microscopic images of bronchioles after double staining with uranyl acetate and lead citrate. The sections were examined with an HU-11 Hitachi electron microscope.²¹

RESULTS

No consistent abnormalities were detected in the trachea and large bronchi of animals exposed to artificial smog. Upon examination of paraffin-embedded lung sections, preterminal and terminal bronchioles were remarkably conspicuous and irregularly shaped. Closer inspection revealed that these changes were frequently due to epithelialization of alveoli lying either alongside the bronchioles or at their distal end (Fig. 1*a*) rather than to hyperplasia of the epithelium within the bronchioles themselves. The epithelialization of alveoli was even more pronounced in CaCrO₄-exposed animals (Fig. 1*b* and *c*). The lining of the large and medium bronchi in the latter group of animals showed pathologic changes ranging from epithelial necrosis to marked hyperplasia. The histopathologic findings have previously been reported in detail.¹⁴

With ordinary hematoxylin- and eosin-stained paraffin sections, it was difficult to determine whether the cells lining the bronchiolized alveoli were hypertrophic type II alveolar cells or whether they resembled bronchiolar epithelial cells. However, 1- μ sections of Epon-embedded material (and ultrathin sections examined with the electron microscope, see below) revealed that these cells had the same morphologic characteristics as bronchiolar cells (Fig. 2*a* and *b*). Occasionally, ciliated cells could be identified. In approximately one-third of the lung sections obtained from numerous animals exposed to CaCrO₄, we observed openings and small channels in the bronchiolar walls, laterally connecting small bronchioles with adjacent alveoli. These channels were usually two to three cell rows deep and 1 to 2 μ wide and ran through the increased thickness of the bronchiolar-alveolar wall (Fig. 1*b*). However, the possibility of an artifact could not be ruled out entirely.

Numerous serial 1- μ sections of plastic-embedded lung tissue from animals exposed to artificial smog and air were examined to determine whether they contained bronchioles with openings similar to those found in the lungs of animals exposed to CaCrO₄. (Tissues fixed in glutaraldehyde, postfixed in osmium, and embedded in Epon are less subject to shrinkage and tearing than are formalin-fixed and paraffin-embedded tissues.) Fifteen blocks of Epon-embedded lung tissue from different mice contained adequate segments of terminal bronchioles to warrant further sectioning and investigation. In five instances, light microscopic examination of the bronchiolar basement membrane revealed one or more basement membrane pores that were occupied by cells resembling bronchiolar epithelial cells (Fig. 2*a* and *b*). These pores were much smaller than the openings seen in the paraffin-embedded material of mice exposed to CaCrO₄. The alveolus itself was partially lined by cells of bronchiolar type, which sometimes formed an acinar structure (see arrows in Figs. 1*c* and 2*c*). Bronchiolization of alveoli was seen occasionally in mice exposed for 18 months to

filtered air, but, so far, we have failed to demonstrate bronchioloalveolar pores in these control animals. In order to identify unequivocally the cell type involved and to demonstrate more clearly the basement membrane pores, we prepared serial ultrathin sections (sectioning through the entire region of the pore) from the tissue of several animals and examined them under the electron microscope. Micrographs of the three most informative sections are depicted in Figures 3 to 5. In most instances the pores are bordered by capillaries and are wide enough to allow the apparent passage of secretory or ciliated cells into adjacent alveoli (Figs. 3 to 5). The basement membranes of alveoli, which are "in communication" with the lumen of terminal bronchioles through the above mentioned pores, are frequently lined by bronchiolar type secretory cells with ultrastructural characteristics identical with those of the secretory cells lining the bronchiolar wall (secretory granules and sparsity of mitochondria; compare cells on the bronchiolar side with those on the alveolar side of the basement membrane in Figs. 3 to 5). Thus, these cells bear no resemblance to the type II alveolar cells, which are easily identified by their multivesiculated bodies and osmiophilic lamellated bodies.

To facilitate description and visualization of a terminal bronchiole region in which there is an apparent "migration" of epithelial cells to alveoli, a three-dimensional reconstruction of the basement membrane, illustrating its pores and associated capillary network, is presented in Figure 6. The model shows two bronchioloalveolar openings and two interalveolar pores in the basement membrane of the terminal bronchiole and an interalveolar septum, respectively. The planes of spaced sections used for the reconstruction of this region are indicated by lines *S1* through *S5* on the drawing; the electron micrographs of three of the five sections (*S2*, *S3*, and *S4*) are pictured in succession as Figures 3, 4, and 5. Arrows *A*, *B*, and *C* in Figure 6 show possible routes of cell migration or intercommunications of the illustrated lumina. In reality, and as shown on the montages (Figs. 3 to 5), the illustrated bronchiolar basement membrane is covered with secretory and ciliated epithelial cells, some of which obstruct the pores in the basement membrane through which arrows *A* and *B* are threaded. The alveolar surfaces of the basement membrane are covered by the thin extensions of alveolar epithelial cells or by bronchiolar secretory or ciliated cells that have grown into the alveolus, presumably through the bronchiolar-alveolar pores (Figs. 3 to 5). These cells are in intimate contact with alveolar epithelial cells and are connected to them by desmosomes. The space between capillaries is filled not only with the homogeneous matrix of the basement membrane but also with bundles of reticular fibers and the processes, or cell bodies, of fibrocytes. The inner surface of the basement membrane of the capillaries illustrated on the drawing is covered by the thin extension of endothelial cells as shown on the three montages of electron micrographs.

DISCUSSION

Recent studies have drawn attention to the epithelial changes occurring in the peripheral segments of the

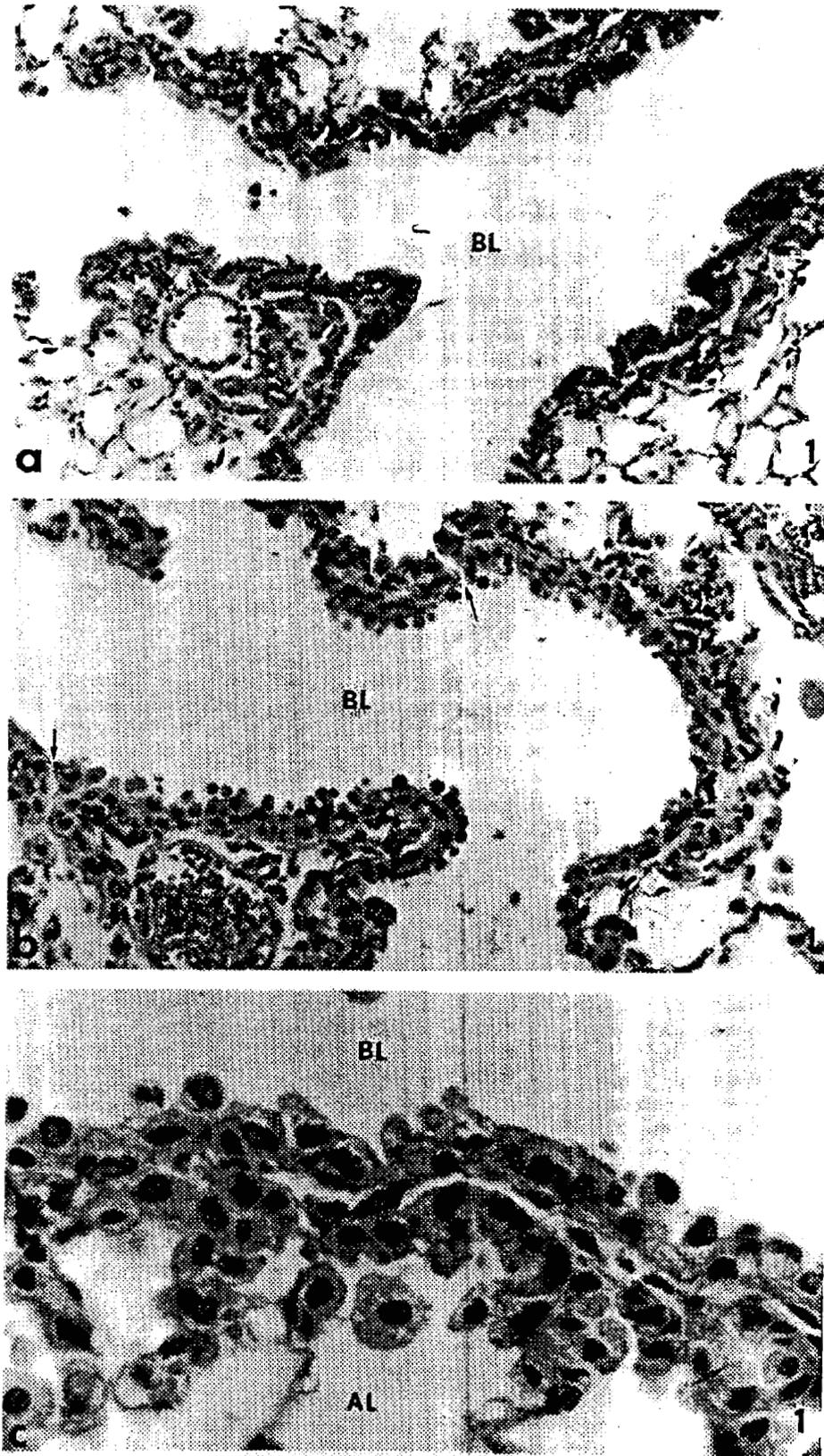


FIG. 1. "Bronchiolization" of alveoli bordering on preterminal and terminal bronchioles. *BL*, Bronchiolar lumen; *AL*, alveolar lumen. *a*, After 18 months exposure to ozonized gasoline; *b*, after 18 months exposure to CaCrO_4 dust; note communications between bronchiole and alveoli (arrows); *c*, after 18 months exposure to CaCrO_4 dust; note "acinar" structure (arrow). Hematoxylin and eosin stain; Figure 1*a*, $\times 250$; *b*, $\times 410$; *c*, $\times 850$.

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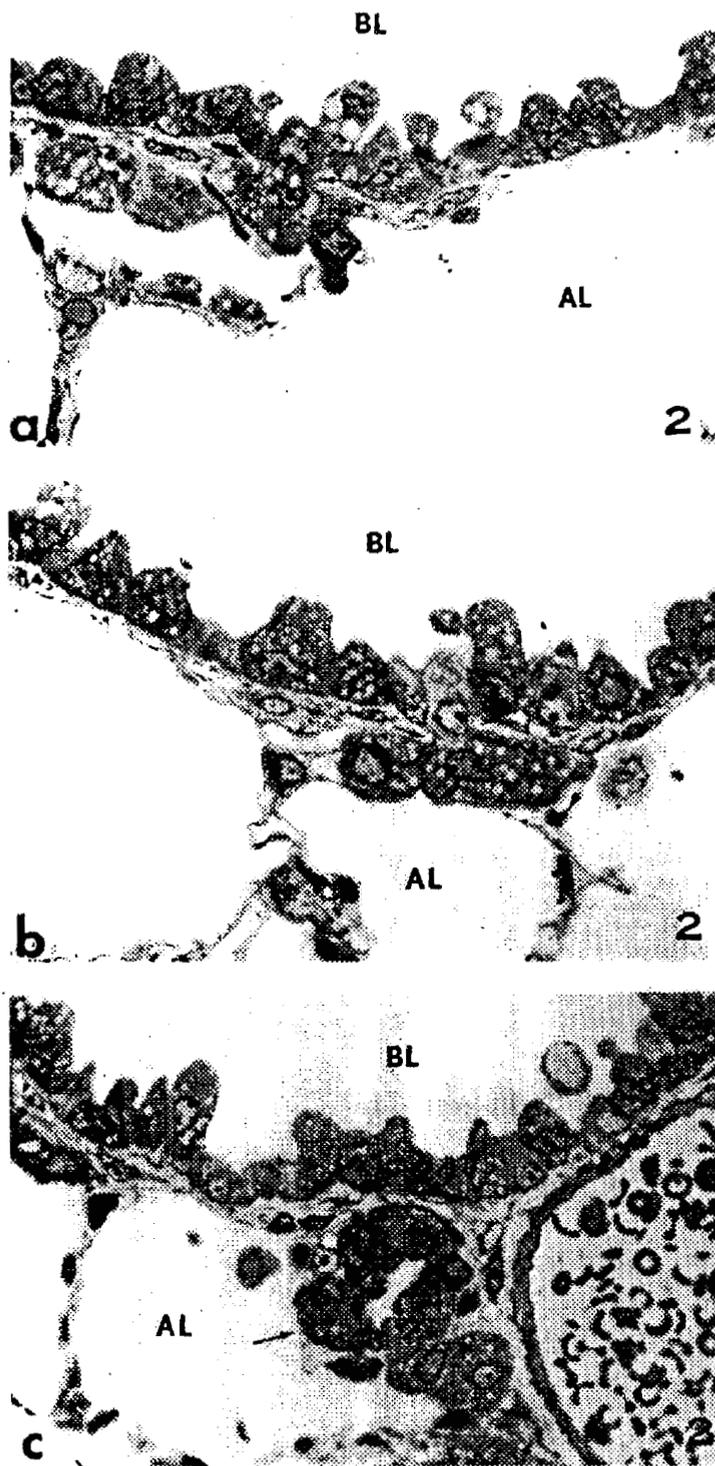


FIG. 2. Examples of bronchioalveolar pores and epithelialization of alveoli in artificial smog-exposed animals as seen in 1- μ sections of Epon-embedded material. *BL*, Bronchiolar lumen; *AL*, alveolar lumen. Note gap in basement membrane (arrows) occupied by (a) secretory cell, (b) ciliated cell, and (c) alveolar acinar formation of secretory cells (arrow). Methylene blue stain; Figure 2a, $\times 950$; b, $\times 1,100$; c, $\times 950$.

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conducting airways of laboratory animals exposed to irritant gases.^{4, 5, 8} Our own histologic and electron microscopic findings emphasize the intimate relationship between the cellular response of the most peripheral bronchioles and the alveoli, following chronic low level injury.

Bronchiolization of alveoli (i.e., lining of alveolar walls by cells resembling, but not necessarily identical with, bronchial epithelium) occurs in a great variety of unrelated pathologic conditions. It can involve large parts of lung parenchyma and may or may not be accompanied by interstitial fibrosis or scarring (e.g., in-

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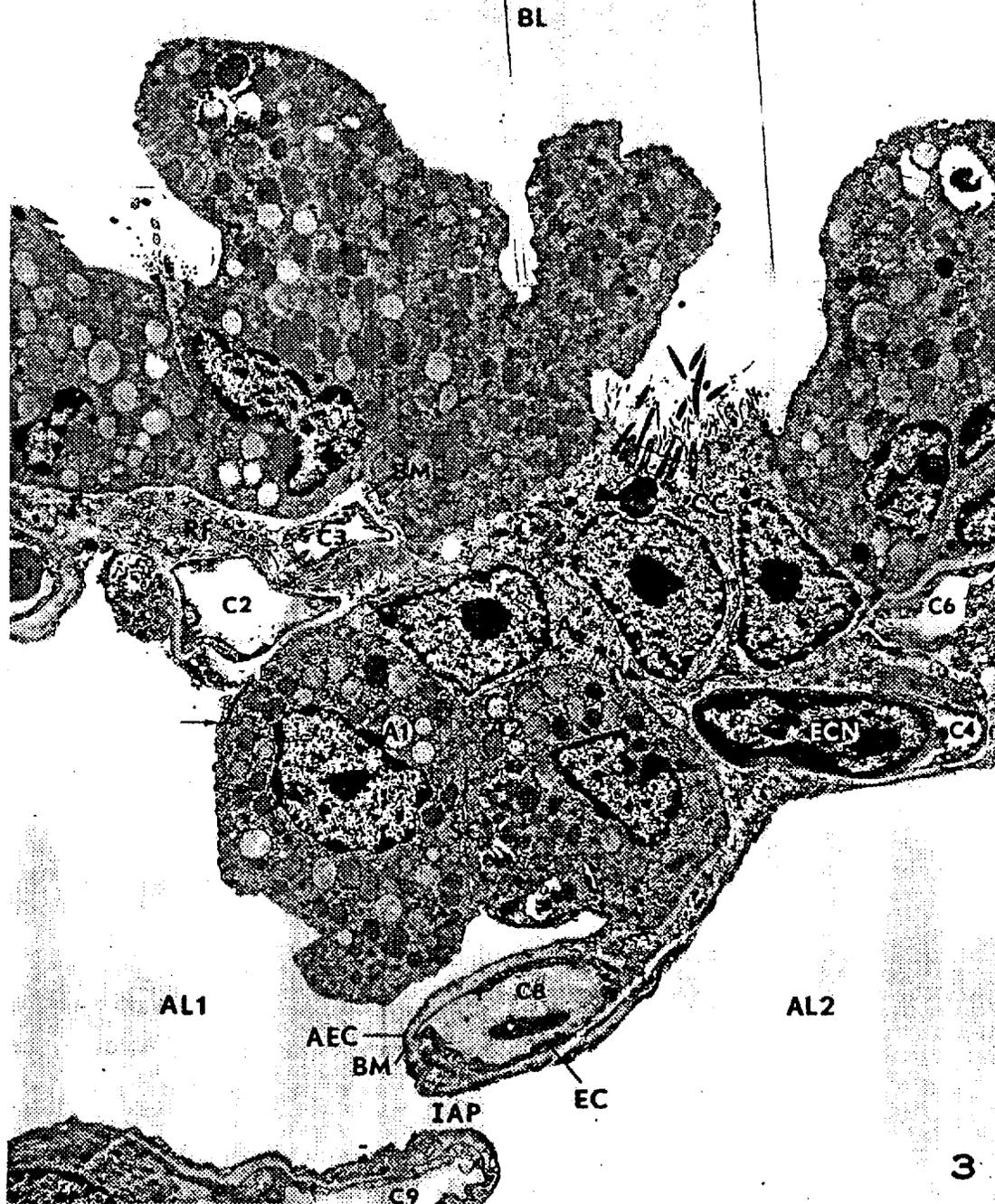


FIG. 3. A montage of electron micrographs illustrating a bronchiolar-alveolar pore of the terminal bronchiole at the S2 level in Figure 6. The micrograph shows pore A delineated by the basement membrane (BM) and capillaries C3 and C4. A1 to A2 are bronchiolar epithelial cells that apparently have migrated through the pore and

are connected to alveolar epithelial cells (AEC) by desmosomes (arrows). BL, Bronchiolar lumen; AL1, AL2, alveolar lumen; RF, reticular fibers; C1 to C9, capillaries; CC, ciliated cell; arrowheads, electron-dense inclusions; ECN, endothelial cell nucleus; SC, secretory cell; IAP, interalveolar pore; EC, endothelial cell. $\times 6,500$.

are connected to alveolar epithelial cells (AEC) by desmosomes (arrows). BL, Bronchiolar lumen; AL1, AL2, alveolar lumen; RF, reticular fibers; C1 to C9, capillaries; CC, ciliated cell; arrowheads, electron-dense inclusions; ECN, endothelial cell nucleus; SC, secretory cell; IAP, interalveolar pore; EC, endothelial cell. $\times 6,500$.

Bronchiolization may, on the other hand, involve only a small group of alveoli, as is the case in our own experiments with chronic low level exposure to artificial smog and CaCrO_4 dust. It should be mentioned that

FIG. 3. A montage of electron micrographs illustrating a bronchiolar-alveolar pore of the terminal bronchiole at the S2 level in Figure 6. The micrograph shows pore A delineated by the basement membrane (BM) and capillaries C3 and C4. A1 to A2 are bronchiolar epithelial cells that apparently have migrated through the pore and are connected to alveolar epithelial cells (AEC) by desmosomes (arrows). BL, Bronchiolar lumen; AL1, AL2, alveolar lumen; RF, reticular fibers; C1 to C9, capillaries; CC, ciliated cell; arrowheads, electron-dense inclusions; ECN, endothelial cell nucleus; SC, secretory cell; IAP, interalveolar pore; EC, endothelial cell. $\times 6,500$.

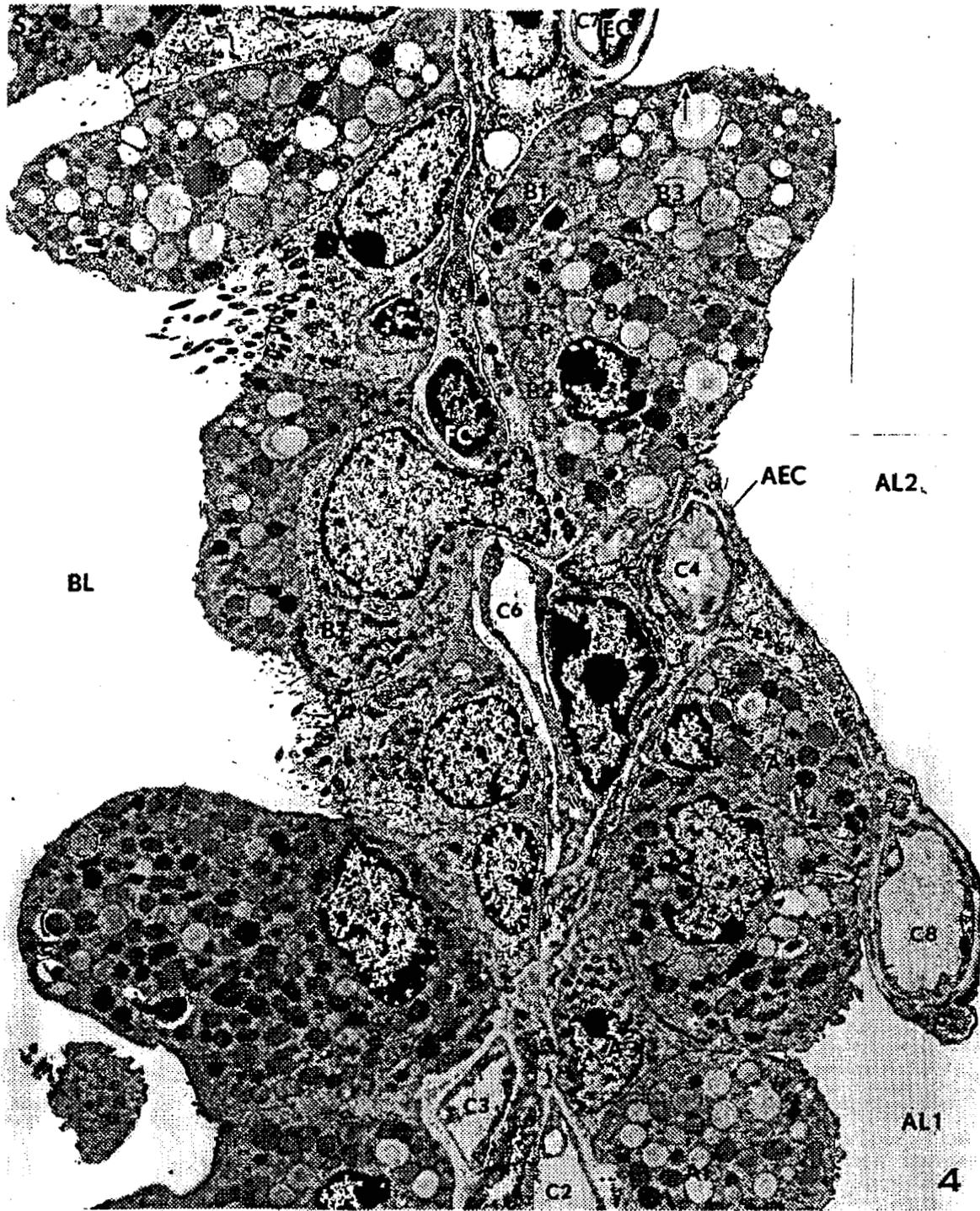


FIG. 4. A montage of electron micrographs illustrating a bronchiolar-alveolar pore of the terminal bronchiole at the S3 level in Figure 6. The section shows pore B delineated by basement membrane (BM) around a capillary (C6) and a fibrocyte (FC). A1 to A4, cells that appear to have migrated through pore A (see Fig. 3), are here separated by the basement membrane from the epithelium of the terminal bronchiole. B7 is a ciliated cell apparently in the process

of migrating from the bronchiolar epithelium to the alveolar lumen (AL2) surface of the basement membrane through pore B, as judged from the shape of its nucleus and the cell process (CP) attached to the alveolar side of the basement membrane. B1 to B4 are secretory cells that have already migrated. EC, Endothelial cell; arrows, desmosome between migrated and alveolar epithelial cells (AEC); BL, bronchiolar lumen; C1 to C8, capillaries. $\times 6,500$.

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the extent of the lesion seems to be related to the dose of the injurious agent, since we observed widespread bronchiolization of lung parenchyma following repeated

intratracheal injection of rather large amounts of CaCrO_4 .¹⁴

The histogenesis of bronchiolization of alveoli has

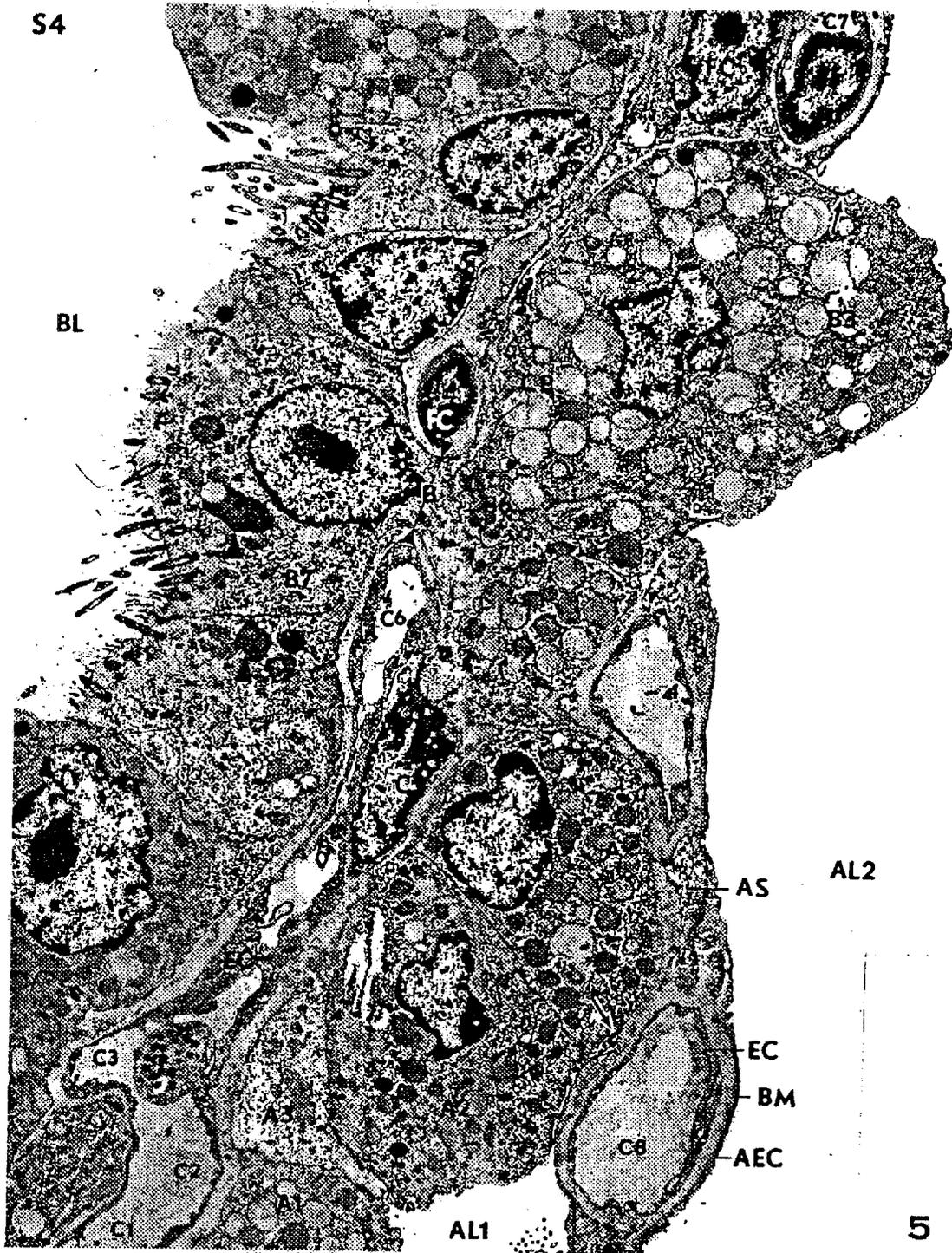


FIG. 5. A montage illustrating a bronchiolar-alveolar pore of the terminal bronchiole at the *S4* level in Figure 6. The micrograph shows essentially the same cells as in Figure 4 but a different level of sectioning. Pore *B* is bordered by the capillary formed from the union of capillaries (*C1* to *C3* and *C6*) on the *left*, and by the fibrocyte (*FC*) on its *right*. *B7*, a ciliated cell, appears to be migrating through the pore. *A1* to *A7* are cells which appear to have migrated

to the *left* of the alveolar septum (*AS*) via pore *A* (see Fig. 3) and cells *B3* to *B6* which seem to have migrated to the *right* side of the septum through pore *B*. *BL*, Bronchiolar lumen; *arrows*, desmosome between migrated and alveolar epithelial cells (*AEC*); *CP*, cell process; *arrowheads*, electron-dense inclusions; *AL1*, *AL2*, alveolar lumen; *EC*, endothelial cell; *BM*, basement membrane. $\times 6,500$.

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FIG. 6. A montage illustrating a bronchiolar-alveolar pore of a mouse terminal bronchiole at the *S1* level.

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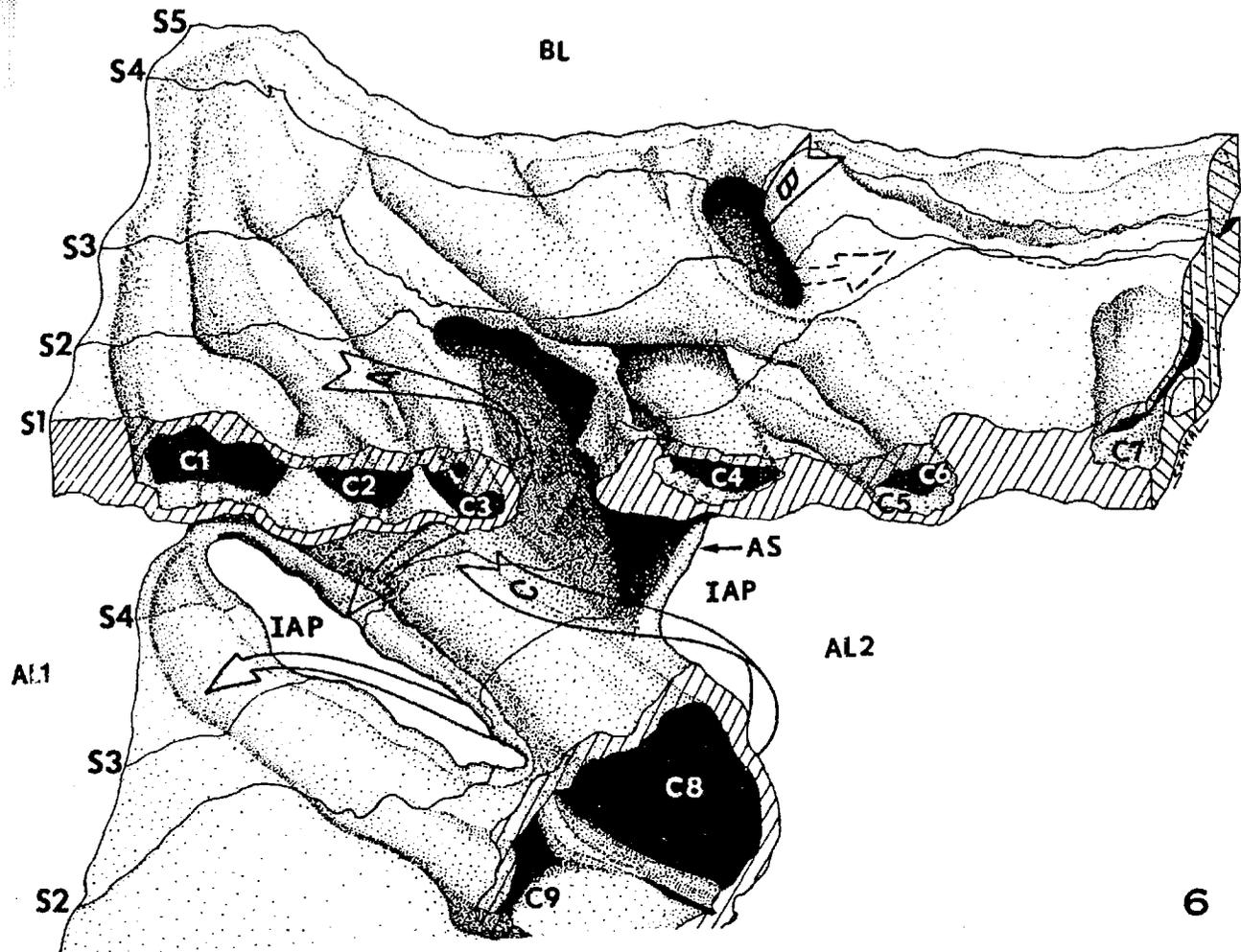


FIG. 6. A three dimensional reconstruction of the basement membrane of a terminal bronchiole and the adjacent alveolar wall from a mouse exposed to artificial smog. The model illustrates a transparent basement membrane with its bronchioloalveolar pores (arrows A and B), associated capillary network (C1 to C9), and alveolar sep-

tum (AS) with its interalveolar pore (IAP and arrow C). S1 to S5 represent levels of sections used for the reconstruction. S2 to S4 are illustrated in Figures 3 to 5. BL, Bronchiolar lumen; AL1, AL2, alveolar lumen. $\times 4,400$.

been a controversial issue for many years: some investigators feel that it is a metaplasia (or "transformation") of autochthonous alveolar epithelium.^{1-3, 6, 16} In most cases, however, neither opinion is supported by sufficient evidence on either the exact morphologic identity or the origin of the abnormal alveolar lining. In our own study, a very mild form of alveolar bronchiolization was observed as a result of chronic low level exposure to air contaminants. Histologic and electron microscopic investigation of the altered peripheral airways showed epithelial cells in alveoli morphologically identical with the secretory and ciliated cells of the adjacent bronchioles. Evidence is presented which strongly suggests that these cells are derived from bronchioles and that they reach the alveoli through pores, laterally connecting bronchioles and alveoli. It is conceivable that the minute gaps in the bronchiolar-alveolar basement membranes are a normal feature of the peripheral airways of small

rodents, which become more conspicuous and easier to locate under pathologic conditions. They may be related to the much larger open connections between bronchioles and alveoli described in rabbits and humans.¹⁰ The migration of cells through these pores with subsequent "colonization" of alveolar walls might also normally occur at a very slow rate, since occasional alveolar bronchiolization can be seen in aging mice not exposed to any obvious injury. It is not clear whether the rather conspicuous bronchiolar-alveolar channels in the CaCrO_4 -exposed mice are but an exaggerated form of the small pores seen in the smog-exposed animals or whether they are a separate morphologic entity.

It should be stressed that we are not implying that the same histogenetic mechanism applies to all forms of alveolar metaplasia. In other instances, bronchiolar epithelium may extend distally into the alveoli and alveolar ducts, as is suggested by findings such as that presented

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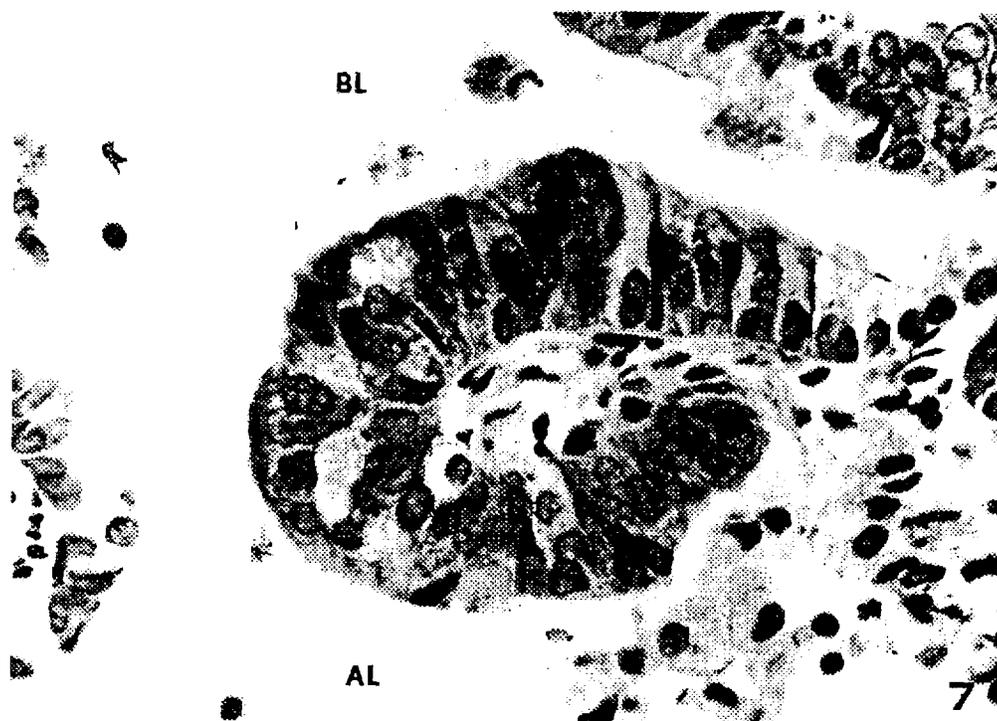


FIG. 7. Extension of epithelium of terminal bronchiole into alveolus in a rat given repeated intratracheal methylcholanthrene injections. BL, Bronchiolar lumen; AL, alveolar lumen. Hematoxylin and eosin stain; $\times 930$.

in Figure 7, which is taken from a carcinogen-treated rat lung,¹⁷ or the metaplasia of lung parenchyma may actually be derived from autochthonous alveolar epithelium.

Our findings also raise an interesting possibility pertinent to the morphogenesis and pathogenesis of bronchioloalveolar neoplasms. Bronchiolization of alveoli is a common sequela of injury affecting lower respiratory tract tissues. If such modified alveolar tissues are exposed to carcinogens, tumors with the morphologic and perhaps biologic characteristics of bronchiolar carcinomas could develop. These would topographically be of alveolar, but histogenetically of bronchiolar, origin. While these considerations are presently rather speculative, they emphasize the possible role of "cofactors" in respiratory carcinogenesis, which may significantly modify the target tissues and thereby alter their response to carcinogens.

Date of acceptance: October 13, 1971.

This work was supported jointly by the National Cancer Institute and by the United States Atomic Energy Commission under contract with the Union Carbide Corporation.

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