An Ultrastructural Study of Bronchiolar Lesions in Rats Induced by 4-Ipomeanol, a Product From Mold-Damaged Sweet Potatoes

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Repeated intraperitoneal injections of 4-ipomeanol in rats resulted in extensive degeneration and necrosis of nonciliated (Clara) bronchiolar epithelial cells. Subsequently there was necrosis and detachment of ciliated cells from the bronchiolar basal lamina. The remaining nonciliated cells divided and differentiated into ciliated epithelial cells and mature Clara cells. The ability of Clara cells to metabolize certain xenobiotics suggests that they may play an important role in the pathogenesis of pulmonary disease and neoplasia. (Am J Pathol 1983, 111:56–61)

RECENT EXPERIMENTAL STUDIES have demonstrated that the lung, like the liver, contains enzyme systems capable of converting certain parenterally administered compounds into chemically reactive metabolites that may produce biochemical or structural damage.[1–4] Of importance is the mixed function oxidase system (MFO), which is contained in the microsomal fraction of lung cell homogenates[5] and is necessary for activation of certain environmental pollutants[6] and carcinogens.[7] Determination of the precise location of the MFO within individual cells has been difficult because of complexity and heterogenicity of pulmonary tissue. The use of radiolabeled 4-ipomeanol,[8] a naturally occurring furan isolated from mold-damage sweet potatoes (*Ipomoea batatas*),[9,10] has demonstrated that the cytochrome-P450–dependent MFO is present in the Clara (nonciliated) cells of rat bronchioles. Other histochemical[11–13] and morphologic studies[14–19] have suggested that Clara cells are metabolically active and may serve as progenitors of the bronchiolar epithelium.[20,21]

The hepatocellular toxicity[22–26] and carcinogenicity[7,27,28] of furans and related compounds are well documented, but little information is available on changes incurred in the lung as a result of furan metabolism. 4-Ipomeanol, a 3-substituted furan, is a potent lung edematogenic agent in laboratory animals[10] and cattle[29] and produces microscopic changes in the epithelium of terminal bronchioles. This investigation was designed for study of the ultrastructural changes in the bronchioles of young male rats after the intraperitoneal administration of 4-ipomeanol. Emphasis was placed on the morphologic alterations of Clara cells.

Materials and Methods

A group of 21 young male Sprague–Dawley rats weighing approximately 200 g each was injected intraperitoneally with 30 mg/kg of 4-ipomeanol dissolved in a solution of equal parts propylene glycol and distilled water. Seven rats were used as controls and received only the propylene glycol-water solution. Rats were given the dose at 24-hour intervals.

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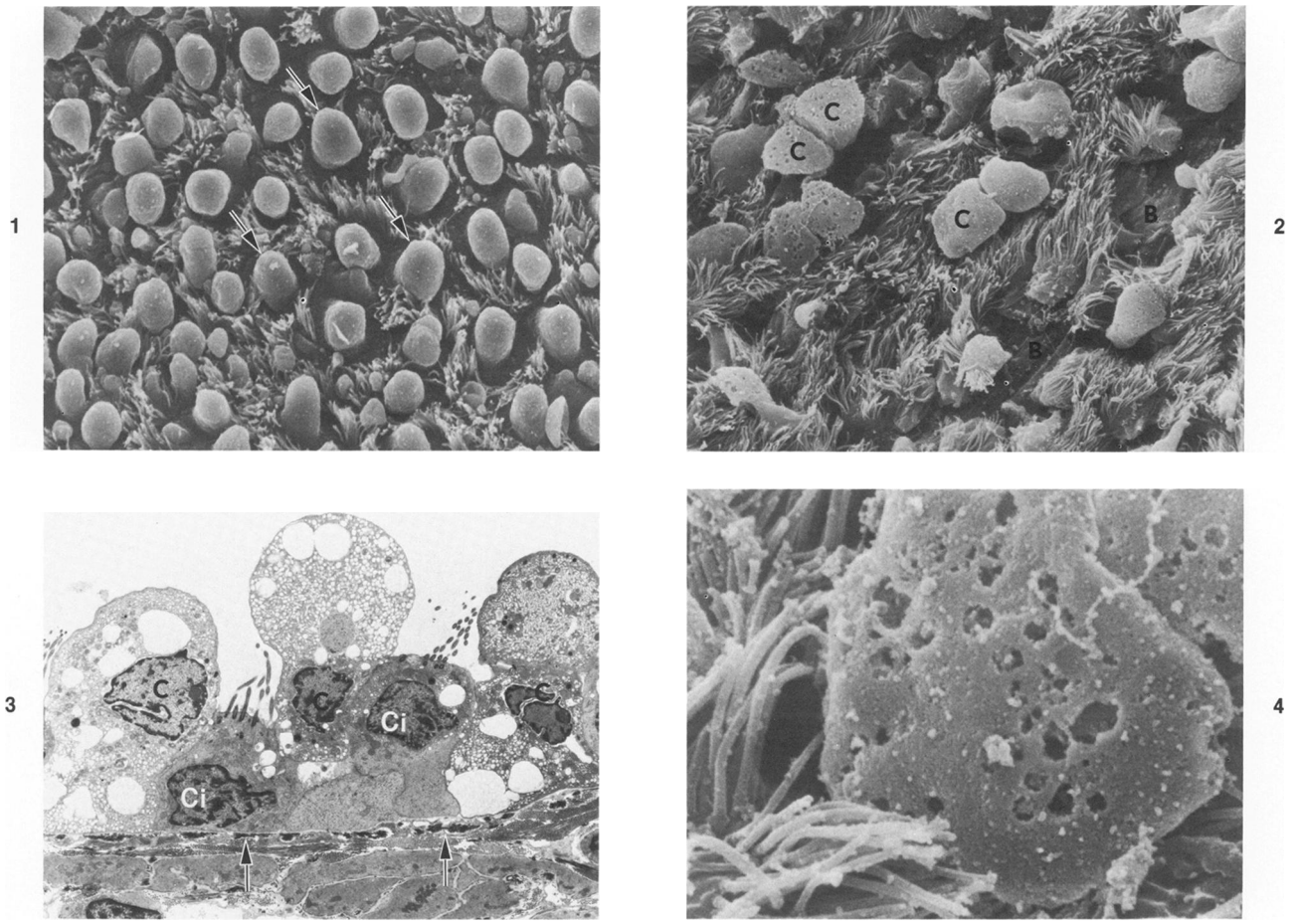


Figure 1—Terminal bronchiolar epithelium of control rat. Clara cells have smooth bulbous projections (*arrows*) which protrude above neighboring ciliated cells. (×1550) **Figure 2**—Terminal bronchiolar epithelium 24 hours after injection of 4-ipomeanol. Clara cells (*C*) are swollen and often partially obscure neighboring ciliated epithelial cells. Areas of naked bronchiolar basal lamina (*B*) are seen. (×1600) **Figure 3**—Terminal bronchiolar epithelium 24 hours after injection of 4-ipomeanol. Clara cells (*C*) have bulbous apical projections which contain dilated smooth endoplasmic reticulum and protrude far above neighboring ciliated cells (*Ci*). Scattered deposits of fibrin (*arrows*) are seen beneath the basal lamina. (×4700) **Figure 4**—An apical portion of a Clara cell 24 hours after injection of 4-ipomeanol. The cell is enlarged and has a "moth-eaten" appearance. (×12000) (All with a photographic reduction of 29%)

Three treated animals and 1 control were killed at 4, 12, and 24 hours after receiving the initial dose. Subsequent groups and controls were killed at 48, 72, and 96 hours and 7 days after receiving 2, 3, 4 and 7 doses, respectively.

The tracheas were rapidly exposed, and the lungs were fixed *in situ* by tracheal infusion of a solution of 4% paraformaldehyde and 5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. The tracheas were ligated, and the heart and lungs were excised and placed in fixative at 4 C for at least 48 hours.

Terminal bronchioles were dissected from adjacent tissue in longitudinal sections of lung with the aid of a stereomicroscope. These areas were minced into small blocks and washed overnight in 0.1 M cacodylate buffer, pH 7.4, with 5% sucrose. The blocks were further fixed for 1 hour in cacodylate-buffered 1% OsO_4, dehydrated in a series of graded acetone, and embedded in Spurr's medium.[30] Ultrathin sections were stained with uranyl acetate and lead citrate and examined with an RCA EMU-4A electron microscope at an accelerating voltage of 50 kv.

For scanning electron microscopy areas containing terminal bronchioles were selected with stereomicroscopy. Tissue blocks were washed in cacodylate buffer, pH 7.4, dehydrated in a series of graded ethanols, and critical-point-dried with the use of CO_2 The dried tissue was mounted on aluminum stubs with conductive silver paint, and a 200-Å layer of gold paladium was deposited with a Technics Hum-

mer V sputtering unit (Technics, Inc., Alexandria, Va). The tissue was examined with a JEOL JSM-35 scanning electron microscope (JEOL, Inc., Peabody, Mass) at an accelerating voltage of 15 kv.

Results

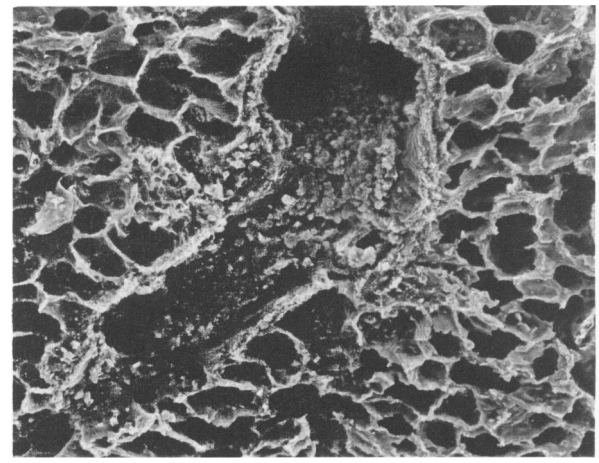
In control rats Clara cells were most numerous in the terminal and respiratory bronchioles and were situated among ciliated epithelial cells (Figure 1). Apical surfaces were covered with short microvilli, and most had a single smooth bulbous projection that protruded into the bronchiolar lumen.

No detectable changes were noted in the bronchiolar epithelium 4 hours after administration of 4-ipomeanol. However, beginning at 12 hours after injection specific changes were seen. Clara cells were swollen, apical cytoplasm projected above neighboring epithelial cells, endoplasmic reticulum was dilated, and there was loss of microvilli. By 24 hours these changes were much more accentuated. Clara cells were greatly enlarged and often obscured neighboring epithelial cells (Figures 2 and 3). Scattered deposits of fibrin were seen frequently beneath the epithelial basal lamina. Many Clara cells had a "moth-eaten" appearance due to the presence of many porelike structures on the surface (Figure 4). Occasionally these cells were fragmented indicating increased fragility and damage during processing.

Cilia of epithelial cells were clumped, often blunted, and had debris attached to their surface. Endoplasmic reticulum was often dilated. Necrosis and exfoliation of individual Clara cells were noted.

After 48 hours few recognizable Clara cells were seen in terminal bronchioles. Marked vacuolization of endoplasmic reticulum, disassociation of ribosomal aggregates, condensation of nuclear chromatin, and loss of cilia had occurred in ciliated cells. Diffuse necrosis of Clara cells and ciliated epithelial cells was noted. Bronchioles were clogged with cellular debris (Figures 5 and 6).

Seventy-two hours after 4-ipomeanol administration terminal bronchioles contained small groups of proliferating nonciliated epithelial cells. By 96 hours the bronchiolar epithelial lining was complete and composed of a single layer of nonciliated cuboidal cells (Figure 7). Apical cytoplasmic projections were not observed, but cilia and microvilli were seen on the surface of some cells. The apical cytoplasm of many cells contained abundant numbers of mitochondria, few profiles of rough endoplasmic reticulum, and no smooth endoplasmic reticulum (Figure 8). Ciliary basal bodies and cylindrical formations of microtubules arranged in singlets and doublets were occasionally seen (Figure 9). A second type of nonciliated cell could be identified at this time. It contained numerous mitochondria, polyribosomes, and spherical electron-dense granules but lacked smooth endoplasmic reticulum and ciliary basal bodies (Figure 10).



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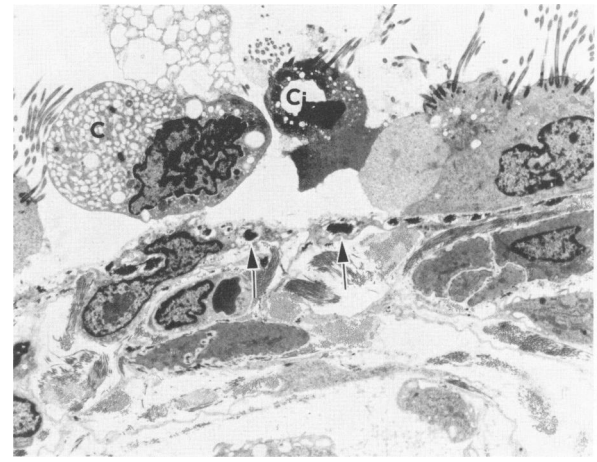

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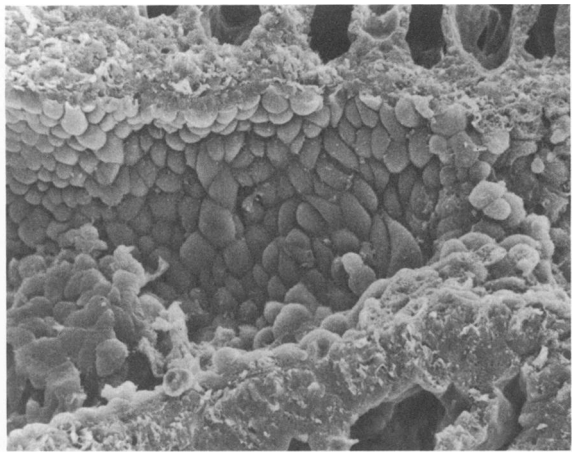
Figure 5 – Forty-eight hours after injection of 4-ipomeanol the lumen of a terminal bronchiole contains cellular debris and desquamated cells. (×200) **Figure 6** – Transmission electron micrograph of a bronchiole similar to that described in Figure 5. Exfoliation of Clara (*C*) and ciliated cells (*Ci*) occurred. The underlying muscular layer is edematous and deposits of fibrin (*arrows*) are seen. (×4700) (With a photographic reduction of 29%)

Seven days after the first injection of 4-ipomeanol the bronchiolar epithelium of treated rats was indistinguishable from that of controls. Clara cells were generally distributed in linear arrays, but occasionally small clusters were noted. Ciliated cells appeared normal.

Discussion

The morphologic appearance of the terminal bronchiolar epithelium in rats was selectively altered by



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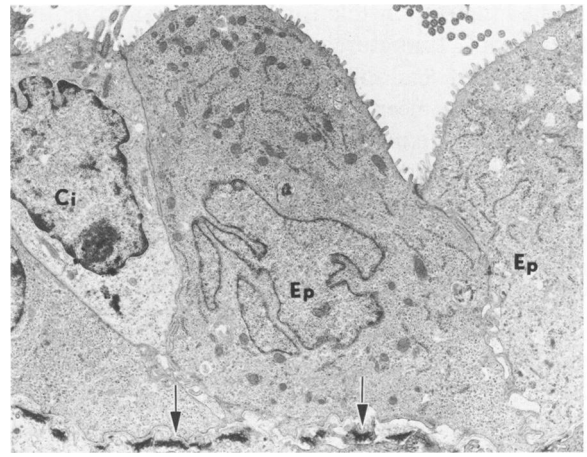

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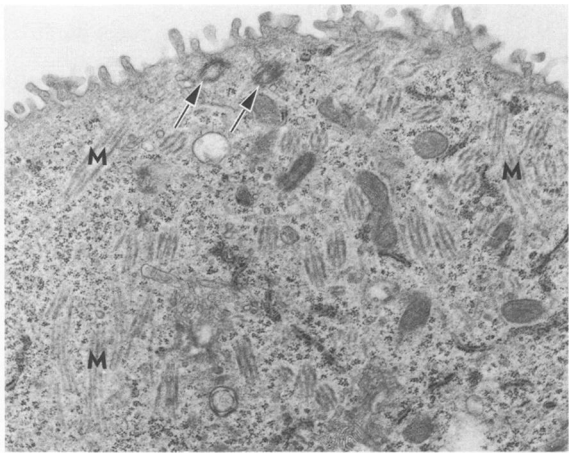

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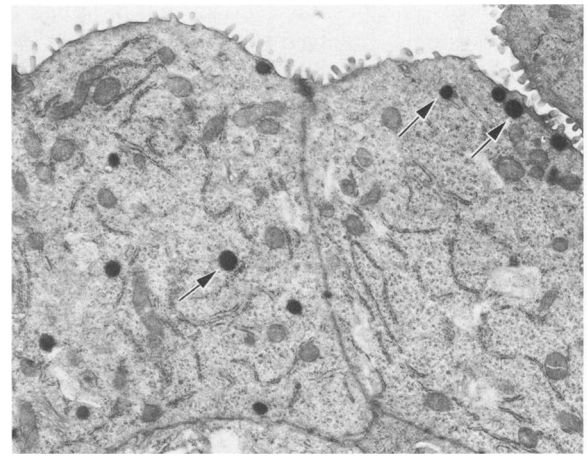

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Figure 7 — Terminal bronchiole 96 hours after injection of 4-ipomeanol. The bronchiole is lined entirely by cuboidal cells. (×550) **Figure 8** — Ninety-six hours after injection of 4-ipomeanol cuboidal cells (*Ep*) lining the terminal bronchiole have numerous short microvilli and no secretory granules. A single cell (*Ci*) with a few cilia is noted. Fibrin (*arrows*) is present beneath the basal lamina. (×12,700) **Figure 9** — Ninety-six hours after injection of 4-ipomeanol the apical cytoplasm of a regenerating cell contains numerous cylindrical arrays of microtubules (*M*) and developing basal bodies (*arrows*). (×27,750) **Figure 10** — Tangential section through two Clara cells 96 hours after injection of 4-ipomeanol. Numerous mitochondria, polyribosomes, and electron-dense secretory granules (*arrows*) are seen in the cytoplasm. (×8000) (All with a photographic reduction of 29%)

the administration of 4-ipomeanol. Initially degenerative changes were seen only in Clara cells and consisted of cell swelling, dilatation of the smooth endoplasmic reticulum, and loss of microvilli. As Clara cells increased in size, fragmentation and detachment of individual cells from the basal lamina was noted. Cilia of ciliated cells were clumped and often shortened. Debris was attached to the apical surface. By 48 hours much of the bronchiolar epithelium had sloughed. Segmental reepithelialization of the epithelial surface was noted at 72 hours, and by 96 hours the mucosal surface was completely intact.

During the phase of bronchiolar cell regeneration two different morphologic types of nonciliated epithelial cells were noted and could be classified as either Type A or Type B according to Evans et al.[20] The appearance of undifferentiated cells containing rudimentary structures consistent with immature Clara cells lends further support to the idea that the Clara cell may act as the progenitor of the bronchiolar epithelium.[20,31,32]

Degeneration and necrosis of ciliated epithelial cells occurred subsequent to that initially noted in Clara cells. 4-Ipomeanol has been shown to bind selectively to Clara cells.[8] However, it is possible that repeated injections of the drug caused induction of the MFO in ciliated bronchiolar cells and production of cytotoxicity.

Renewal of the bronchiolar epithelium was accomplished despite continuous administration of 4-ipomeanol, suggesting possible depletion of enzymes

in the lung responsible for activation and binding of the toxin. Immature Clara cells lack significant amounts of endoplasmic reticulum and therefore may have not been able to effectively metabolize and activate the toxin. The induction of enzyme systems in the liver and kidney may have also contributed significantly to the detoxification and elimination of 4-ipomeanol. Repeated sublethal doses of 4-ipomeanol have been shown to protect against challenge with a lethal dose.[10]

The lung is an important metabolic organ and through use of enzyme systems such as the MFO is capable of biotransformation and removal of foreign chemical compounds.[33] In some instances this xenobiotic activity may be detrimental and have serious consequences. Studies have shown that although the enzyme systems involved in xenobiosis are well coupled, production of highly reactive intermediate metabolites responsible for cytotoxicity and carcinogenesis by electrophilic attack on cellular proteins and nucleic acids may occur.[7,23]

Topographic location of Clara cells within pulmonary bronchioles and the presence of a well-developed MFO suggests that Clara cells may play an important role in the metabolism of environmental pollutants and pathogenesis of pulmonary disease and neoplasia. Diffuse bronchiolar necrosis is seen after inhalation of 3-methylfuran, a naturally occurring atmospheric contaminant,[6] or carbon tetrachloride.[34] Prolonged administration of nitrosamine derivatives to hamsters results in development of bronchogenic carcinomas composed of neoplastic Clara cells.[35,36] Identification of the specific cell of origin in bronchogenic neoplasms and characterization of its enzyme systems and biochemical properties may be important in therapy. Use of a bronchiolar alkylating agent such as 4-ipomeanol to cause cytotoxicity in a specific cell population capable of xenobiotic activity could prove to be a valuable adjunct in the chemotherapy of certain bronchogenic neoplasms.

References

1. Hook GER, Bend JR, Hoel D, Fouts JR, Gram TE: Preparation of lung microsomes and a comparison of the distribution of enzymes between subcellular fractions of rabbit lung and liver. J Pharmacol Exp Ther 1972, 182:474–490
2. Oppelt WW, Zange M, Ross WE, Remmer H: Comparison of drug hydroxylation in lung and liver of various species. Res Commun Chem Pathol Pharmacol 1970, 1:43–56
3. Mahvi D, Bank H, Harley R: Morphology of a naphthalene-induced bronchiolar lesion. Am J Pathol 1977, 86:559–572
4. Reid WD, Ilett KF, Glick JM, Krishna G: Metabolism and binding of aromatic hydrocarbons in the lung: Relationship to experimental bronchiolar necrosis. Am Rev Respir Dis 1973, 107:539–551
5. Gram TE: Comparative aspects of mixed function oxidation by lung and liver of rabbits. Drug Metab Rev 1973, 2:1–32
6. Boyd MR, Stratham CN, Franklin RB, Mitchell JR: Pulmonary bronchiolar alkylation and necrosis by 3-methylfuran, a naturally occurring potential atmospheric contaminant. Nature 1978, 272:270–271
7. Miller EC: Some current perspectives on chemical carcinogenesis in humans and experimental animals: Presidential address. Cancer Res 1978, 38:1479–1496
8. Boyd MR: Evidence of the Clara cell as a site of cytochrome P450-dependent mixed-function oxidase activity in the lung. Nature 1977, 269:713–715
9. Boyd MR, Burka LT, Harris TM, Wilson BJ: Lung-toxic furanoterpenoids produced by sweet potatoes (*Ipomoea batatas*) following microbial infection. Biochem Biophys Acta 1974, 337:184–195
10. Wilson BJ: Toxicity of mold-damaged sweet potatoes. Nutr Rev 1973, 31:73–78
11. Azzopardi A, Thurlbeck WM: The histochemistry of the nonciliated bronchiolar epithelial cell. Am Rev Respir Dis 1969, 99:516–525
12. Cutz E, Conen PE: Ultrastructure and cytochemistry of Clara cells. Am J Pathol 1971, 62:127–141
13. Yoneda K: Ultrastructural localization of phospholipases in the Clara cells of the rat bronchiole. Am J Pathol 1978, 93:745–752
14. Breeze RG, Wheeldon EG: The cells of the pulmonary airways. Am Rev Respir Dis 1977, 116:705–777
15. Jeffery PK, Reid L: New observations of rat airway epithelium: A quantitative and electron microscopic study. J Anat 1975, 120:295–320
16. Kuhn C, Callaway LA, Askin FB: The formation of granules in the bronchiolar Clara cells of the rat: I. Electron microscopy. J Ultrastruct Res 1974, 49:387–400
17. Smith P, Heath D, Moosavi H: The Clara cell. Thorax 1974, 29:147–163
18. Yoneda K: Pilocarpine stimulation of the bronchiolar Clara cell secretion. Lab Invest 1977, 37:447–452
19. Wang NS, Huang SN, Sheldon H, Thurlbeck WM: Ultrastructural changes of Clara and Type II alveolar cells in adrenalin-induced pulmonary edema in mice. Am J Pathol 1971, 62:237–252
20. Evans MJ, Cabral-Anderson LJ, Freeman G: Role of the Clara cell in renewal of the bronchiolar epithelium. Lab Invest 1978, 38:648–655
21. Evans MJ, Johnson LV, Stephens RJ, Freeman G: Renewal of the terminal bronchiolar epithelium in the rat following exposure to NO_2 or O_3. Lab Invest 1976, 35: 246–257
22. Allen JG, Seawright AA, Hrdlicka J: The toxicity of *Myoporum tetradrum* (Boobialla) and myoporaceous furanoid essential oils for ruminants. Aust Vet J 1978, 287–292
23. Guengerich FP: Studies on the activation of a model furan compound—toxicity and covalent binding of 2-(N-ethyl-carbamoylhydroxymethyl) furan. Biochem Pharmacol 1977, 26:1909–1915
24. McMurty RJ, Mitchell JR: Renal and hepatic necrosis after metabolic activation of 2-substituted furans and thiophenes, including furosemide and cephaloridine. Toxicol Appl Pharmacol 1977, 42:285–300
25. Mitchell JR, Nelson WL, Potter WZ, Sasame HA, Jollow DJ: Metabolic activation of furosemide to a chemically reactive hepatotoxic metabolite. J Pharmacol Exp Ther 1976, 199:41–52

26. Seawright AA, Mattocks AR: The toxicity of two synthetic 3-substituted furan carbamates. Experientia 1973, 29:1197–1200
27. Campbell TC, Hayes JR: The role of aflatoxin metabolism in its toxic lesion. Toxicol Appl Pharmacol 1976, 35:199–222
28. Miller JA, Miller EC: Carcinogens occurring naturally in foods. Fed Proc 1976, 35:1316–1321
29. Doster AR, Mitchell FE, Farrell RL, Wilson BJ: Effects of 4-ipomeanol, a product from mold-damaged sweet potatoes, on the bovine lung. Vet Pathol 1978, 15:367–375
30. Spurr AR: A low-viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 1969, 26:31–43
31. Evans JM, Stephens RJ, Freeman G: Renewal of pulmonary epithelium following oxidant injury, Lung Cells in Disease. Edited by A Bouhuys. Amsterdam, North Holland Publishing Company 1976, pp 165–183
32. Lum H, Schwartz WL, Dungworth DL, Tyler WS: Comparative study of cell renewal after exposure to ozone or oxygen. Am Rev Respir Dis 1978, 118:335–345
33. Said SI: The lung as a metabolic organ. N Engl J Med 1968, 279:1330–1334
34. Boyd MR, Statham CN, Longo NS: The pulmonary Clara cell as a target for toxic chemicals requiring metabolic activation. Studies with carbon tetrachloride. J Pharmacol Exp Ther 1980, 212:109–114
35. Reznik-Schüller H: Ultrastructural alterations of nonciliated cells after nitrosamine treatment and their significance for pulmonary carcinogenesis. Am J Pathol 1976, 85:549–554
36. Reznik-Schüller H: Ultrastructure of nitrosoheptamethyleneimine-induced lung tumors in European hamsters. Am J Pathol 1978, 93:45–52