

ACUTE BRONCHITIS DUE TO COTTON PLANT POLYPHENOLS *

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The purpose of this brief paper is to present the concept that airways—particularly, distal airways in mammalian lung—are a site for the recognition of foreign materials and response by cell recruitment. This idea should not be alien to pathologists who study bronchopneumonia in man or to clinicians who observe profuse leukocytes in sputum from patients with exacerbations of bronchitis. However, it appears to be a new animal model for pulmonary response to organic materials and the preparation may be useful as a bioassay for inhaled toxic agents. The methods and typical results which have been published recently are summarized here.¹

Methods

Groups of hamsters (90–120 g) and of guinea pigs (200–300 g) were exposed for four hours to aerosols or dusts in 1.4 cubic foot plexiglass chambers. Aerosols contained 0.1 to 1 g sterile cotton cardroom trash extract or dialyzed extract in 100 ml of deionized water. Extracts of cotton dust, quercetin (a polyphenol plant flavone present in leaves and bracts of cotton, in tobacco leaves and other plants) and oxidized quercetin prepared by exposing quercetin solutions—at pH 9—to air or oxygen were evaporated on finely ground silica flour from ether or ethanol. This dust was dispersed by a Wright dust feed at concentrations of 60–110 mg/m³ of air in chambers. One group of hamsters were exposed to cotton extract four hours per day, five days per week for two weeks and one day and sacrificed after the 11th exposure.

Animals were anesthetized with sodium pentobarbital intraperitoneally and killed by intratracheal inflation fixation of the lungs with 1% osmium tetroxide dispersed in fluorocarbon (FC-80 3M Company).² Saggital sections 2 mm thick were cut after ½–1 hour, one of facing sections was dehydrated and embedded in paraffin, from the other 1–2 mm cubes containing airways were cut and dehydrated in increasing concentrations of ethanol. This was followed by propylene oxide, infiltration with plastic (Epon) and embedding. One

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micron thick sections were cut with glass knives, stained with Richardson's stain (methylene blue and azure II) and studied by light microscopy.

Areas were selected for ultrathin sections and these were cut using diamond knives on an ultra-microtome, stained with uranyl acetate and lead citrate and studied under an AEI EM6b electron microscope.

Results

The airways from trachea to distal bronchioles showed infiltration of epithelium within two hours after beginning exposure to aerosols of cotton trash extract, FIGURE 1. At two hours, many polymorphonuclear (PMN) leukocytes were beneath the interrupted elastin layer under the epithelium; fewer were located between ciliated cells and secretory cells and occasional PMN's were on the luminal surfaces. By four hours, the number of cells in subepithelial and interepithelial positions were similar, but masses of PMN's were on the luminal surfaces. The cells recruited after four hours of exposure were maximal at six hours, decreased to about the four-hour level at 12 hours and, at 24 hours, were about 10% of the six-hour level. Dust exposure showed the same profile of effect but effects were delayed to peak at 12 hours. Quercetin and oxidized quercetin dispersed in the aerosol generator as particles, in water or from the dust generator were also delayed and the time course resembled that of cotton trash dust. Control exposures which consisted of deionized water, ferric chloride in deionized water, saline, carbon dust 15 and 150 mg/m³, barium sulfate dust (micropaque) 65 mg/m³ and silica flour 60 and 130 mg/m³ produced no cell recruitment.¹

Leukocyte sieving and migration were studied on selected sections by electron microscopy. In addition to confirming the findings of light microscopy, these pictures showed PMN's passing through the basal lamina, FIGURE 2. The epithelial cells appeared normal—except for distortion due to intercellular PMN's. Longer exposure to agents which cause leukocyte recruitment may produce goblet cell hyperplasia, basal cell hyperplasia or other changes. The PMN's beneath the basal lamina appear normal; those in intercellular position have occasional vacuoles. In contrast, many other PMN's on the luminal surface have numerous clear cytoplasmic vacuoles. These are thought to indicate damage to leukocytes.³ The dark vacuoles containing electron dense crystals indicate phagocytosis of inhaled particles, probably oxidized quercetin from cotton trash.

Discussion

The mechanism by which PMN leukocytes penetrate the basal lamina of airways is unknown. However, it is inferred to be a proteolytic process.⁴ The similar question is how the desmosome at or near the apex of intercellular spaces is crossed by leukocytes. No descriptions are available to determine whether these cell junctions are maculae (spots) or zonulae (rings) of gap junctions. If they are maculae the PMN's could go around them but if they are zonulae they must be separated. *In vitro* experiments with PMN leukocyte chemotaxis show that a five-micron pore size is needed in demonstrate chemo-

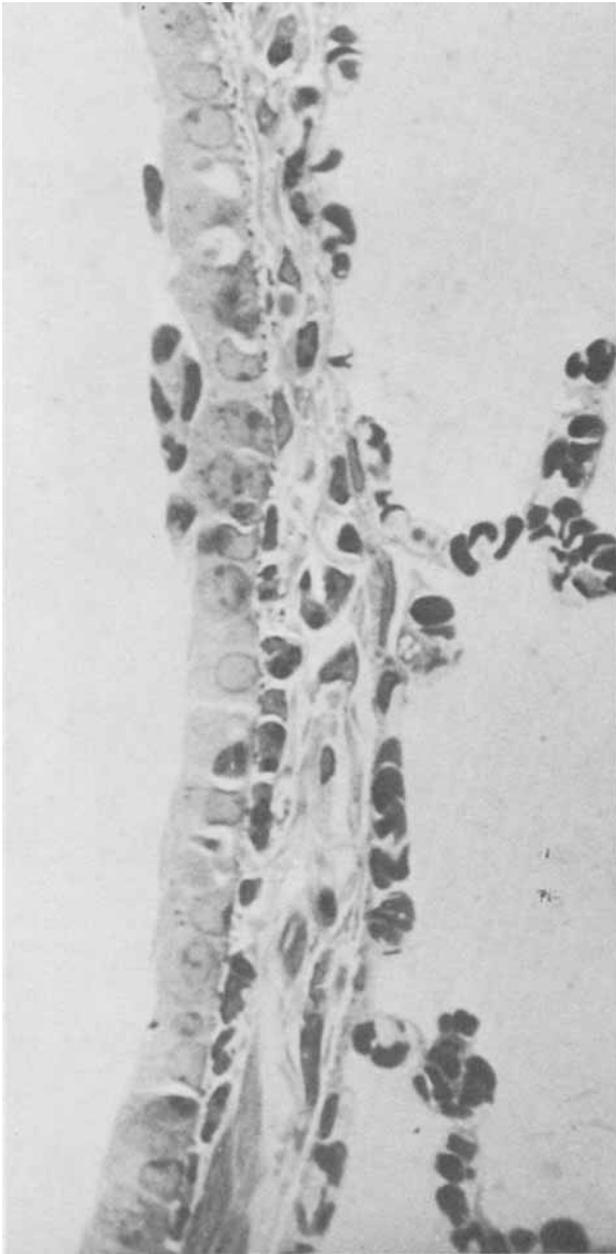


FIGURE 1. The upper lamina propria of this small airway contains a horizontal row of polymorphonuclear leukocytes and several scattered beneath the basal lamina. Several PMN's are in between ciliated and secretory cells and six are on the luminal surface. Osmium fluorocarbon fixation, Epon embedded Richardson's stain. Original magnification $\times 160$.

tropism.⁵ Although serial sections have not been examined the holes in basal lamina appear to be two or three microns in diameter rather than larger.

It is apparent from these experiments that the airways are a reactive zone of the lung to inhaled organic materials. The prime role which particles play in specifying the site and type of response which was shown previously for

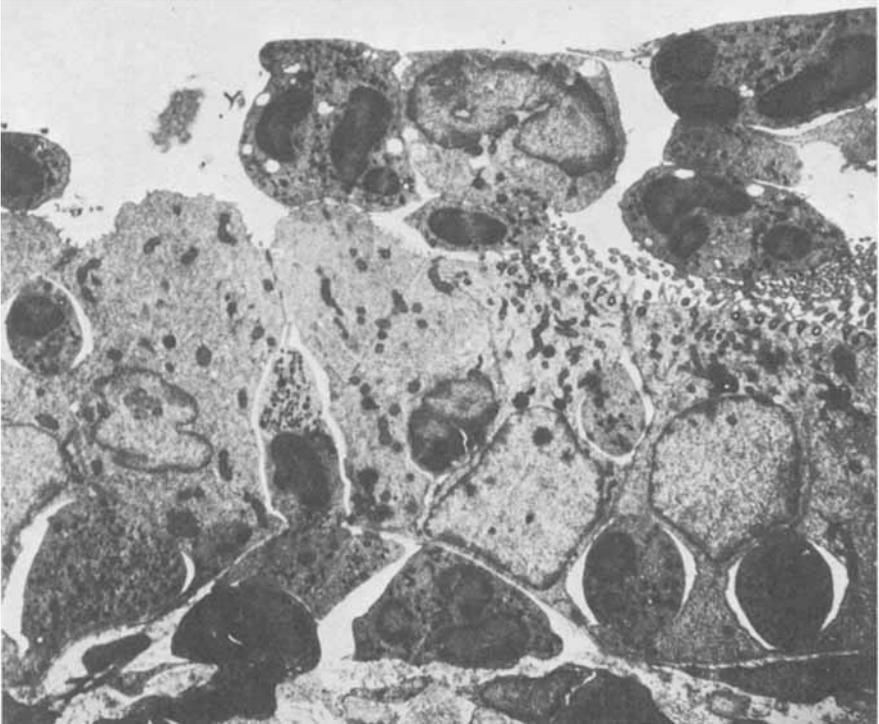


FIGURE 2. This low-power electron photomicrograph shows polymorphonuclear leukocytes beneath the basal lamina, one PMN with a shape like an arrowhead crossing the basal lamina, several PMN's in the intercellular spaces and several on the luminal surface in close proximity to cilia and apical cell membranes. The PMN's on the lumen contain cytoplasmic vacuoles. One mononuclear macrophage, right edge, contains phagocytized cotton material. Epithelial cells have intact mitochondria, normal apical desmosomes and other structures. Osmium-fluorocarbon fixation, sectioned with diamond knife, stained with uranyl acetate and lead citrate, original magnification $\times 1,500$, photograph magnification $\times 3,728$.

SO_2 and carbon particles is demonstrated again.⁶ Although all the steps in the mechanism of response are not known it is postulated that particles are phagocytized by PMN's or mononuclear cells on airway lumens. Such sentinel macrophages probably release a specific factor which is chemotactic for PMN's. This factor is of a small enough molecular weight to pass into the intercellular spaces, cross the basal lamina and diffuse to capillaries. This creates a con-

centration gradient which attracts PMN's to the lumen. The attracted cells may then phagocytize new foreign material and augment or enhance the effect.

Summary

Polyphenolic extracts from cotton trash and pure quercetin and oxidation-polymerization products of quercetin when administered as aerosols or on dust recruit PMN leukocytes on airways from trachea to terminal bronchioles in hamsters. It promises to model faithfully the time and phases of human responses to cotton trash inhalation or byssinosis. Further, it provides a method which should be applicable to appraise other toxic inhalants such as cigarette smoke, fungal spores or chemicals such as brass fumes or toluene diisocyanate.

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