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Pulmonary Compliance: Alteration during Infection

Abstract. *Lungs excised from rats infected with* Mycoplasma pulmonis *are more difficult to inflate with air than those from uninfected animals; they show no significant differences from controls inflated with saline. The altered pulmonary function in lungs from infected rats is attributed to an increase in surface forces, implying disruption of the lung surfactant system.*

The cause of impaired respiration during pulmonary infection is not clear. Microbial infection may directly affect the lung tissues or indirectly affect function by altering the lung surfactant (*1*), a phospholipid that decreases the surface tension of the alveoli. Without surfactant, higher pressure would be required to inflate the lung, and lung stability would be impaired. Nothing is known regarding the status of surfactant in pneumonias of microbial origin.

We have used the rat as an animal model to show the effects of *Mycoplasma pulmonis* on pulmonary function. Respiratory function was assessed by the ability of freshly excised lungs to expand. Air and saline fillings were used to separate tissue effects from changes in surface tension.

Mycoplasma-free rats (*2*) were subdued by intramuscular injections of Innovar (*3*) prior to intranasal inoculation with 0.1 ml of a concentrated suspension of *M. pulmonis* (*4*). Control animals were similarly inoculated with growth medium (*5*). One to 5 days later, *M. pulmonis* was isolated from the throats of 90 percent of the animals inoculated with organisms. Throat cultures collected daily showed that, once animals became infected with *M. pulmonis*, they remained so until killed. Some infected animals exhibited either upper respiratory symptoms or middle ear involvement (manifested as labyrinthitis) or both. One rat had a sanguineous nasal exudate. However, most animals were asymptomatic until killed, and no animal died as a result of the infection.

Before they were killed with an overdose of sodium pentabarbital (*6*), infected and control animals were matched according to weight. The lungs were removed before cessation of heartbeat. A 5-mm section was sliced from the apical lobe, and the remaining portion was ligated. The section was minced and inoculated into mycoplasma culture media, cell cultures (*7*), and blood agar plates. Mycoplasmas were isolated from lung material as early as 8 days and recovered from one rat killed 39 days after inoculation. No mycoplasmas were isolated from lungs of control animals nor were bacteria or viruses recovered from the lungs of either the control or infected group.

Lung function was assessed by obtaining continuous pressure-volume (*P-V*) curves. By measuring the pressure needed to expand the freshly excised lungs with air, we calculated total compliance (C_L), the ratio of the change in volume to the change in pressure. The C_L consists of at least two components: (i) the contribution of tissue compliance (C_{tis}) due to elasticity and (ii) the surface compliance (C_{surf}) due to surface tension at the gas-liquid interface in the alveoli. To distinguish these factors, we first inflated and deflated the lungs with air to calculate C_L. An estimation of the tissue forces was determined from a *P-V* curve obtained with the use of saline instead of air, because the gas-liquid interface is presumably eliminated by filling with liquid. The net difference between C_L and C_{tis} is attributed to C_{surf} and was estimated by the relationship

$$\frac{1}{C_L} = \frac{1}{C_{tis}} + \frac{1}{C_{surf}}$$

The *P-V* technique was essentially as described by Beckman and Weiss (*8*). Lungs were attached to an infusion pump by a tracheal cannula (*9*) and inflated and deflated with air at a slow rate of 9.9 ml/min to a maximum pressure of 20 cm-H_2O. After the air cycles, lungs were degassed under vacuum, and the procedure was repeated with saline at 2.5 ml/min (*10*). Filling with saline proceeded to a volume at least equal to that achieved with air at 20 cm-H_2O. Volume and pressure changes were recorded automatically on an XY recorder, thus providing a continuous *P-V* curve.

In the comparison of compliance between infected and uninfected animals (Table 1), the criteria for infection were as follows: recovery of mycoplasmas from the lungs, or recovery from the throat along with serologic evidence of infection (*11*). Mycoplasmas were isolated from the throats of all 12 animals of the infected group. We needed to maintain lung integrity for *P-V* studies. Therefore, only a small segment (5 mm or less) from a single lobe was available for isolation of mycoplasmas from lungs. Even so, mycoplasmas were cultured from the lung biopsies from 6 of the 12 animals included in the infected group.

An analysis of the infected and comparable control group (Table 1) shows that infected animals tended to be smaller. Their lungs were heavier and contained less retained gas. However, none of these differences, including the higher lung weight/body weight ratio, were statistically significant ($P > .05$). Thus, while *M. pulmonis* may have slowed growth of the animals and caused slightly more pulmonary edema

and atelectasis, these effects were not great enough to complicate the direct comparison of lung compliances.

A typical *P-V* curve from the control series is shown in Fig. 1. One filling-emptying air cycle required about 45 seconds. The virtually coincident third and fourth air curves were averaged to provide total lung compliance values. For the saline curves, a shift to the right in the *X*-axis was made to simplify registering the negative pressures on emptying. Three saline *P-V* curves were run; the second and third curves (B and C) were nearly coincident and were averaged. In contrast to the air curves, the inflation and deflation limbs are very similar. After *P-V* curves were obtained, lungs were sectioned and examined histologically.

Composite air and saline *P-V* curves for control and infected animals are shown in Fig. 2. To reduce error due to edema or atelectasis, the *Y*-axis was calibrated in terms of total lung volume instead of the filling volume. The total lung volume was obtained as follows. To each volume change due to filling or emptying, a volume representing the initial lung weight (assuming a density of one) and a volume representing retained gas (measured as buoyancy in 0.9 percent saline) were added. The resultant curves fall within similar volume ranges and, therefore, are directly comparable.

Except for pressures of 0 and 5 cm-H_2O during inflation, all points on the air curve, both inflation and deflation, were significantly lower for the infected animals ($P < .05$) than for the controls.

Table 1. Physical and microbiological characteristics of rats used in lung compliance measurements. Retained volume and body and lung weights are expressed as averages ± standard error of mean.

Item	Control	Infected
Animals (No.)	12	12
Mycoplasma from throats (No.)	0	12
Mycoplasma from lungs (No.)	0	6
Developing antibody to *M. pulmonis* (No.)	0	11
Body weight (g)	339.5 ± 30.1	331.3 ± 25.5
Lung weight (g)	1.93 ± 0.17	2.09 ± 0.19
Lung weight/body weight (g/kg)	5.75 ± 0.28	6.28 ± 0.24
Retained volume (ml)	0.38 ± 0.04	0.34 ± 0.02

Table 2. Effect of *M. pulmonis* infection on the compliance of rat lungs. Values are averages, (in milliliters per centimeter of water) of animals in each group ± standard error of the mean. The C_L is measured as the volume changes between the start (zero pressure) and end (20 cm-H_2O pressure) of filling. The C_{tis} was measured as the slope of the saline curve over a volume range similar to that found with the air curve. Inflation and deflation limbs were averaged.

Parameter (ml/cm-H_2O)	Controls	Infected
C_L (0–20)	0.22 ± 0.02	0.15 ± 0.01*
C_{tis}	1.33 ± 0.10	1.17 ± 0.09
C_{surf}	0.27 ± 0.03	0.17 ± 0.02*
Slope of air curves between 5 and 15 cm-H_2O		
Inflation C_L (5–15)	0.19 ± 0.02	0.14 ± 0.01*
Deflation C_L (5–15)	0.20 ± 0.02	0.13 ± 0.01†
Slope of saline curves		
Inflation C_{tis}	1.43 ± 0.11	1.25 ± 0.10
Deflation C_{tis}	1.23 ± 0.10	1.09 ± 0.10

* $P < .05$. † $P < .01$.

However, slopes of saline curves, determined as the average of inflation and deflation, of lungs from infected animals do not differ significantly from those of controls. These data indicate that infected lungs are more difficult to inflate with air and have less volume at any given pressure. The similarity between the saline curves indicates that the high resistance to filling does not reside within the tissue elasticity of the lung.

There was no histologic evidence of an acute inflammatory process in lungs, and we could not find significant pathologic changes even in rats where mycoplasmas could be isolated from the lungs (*12*). Rat lungs subjected to *P-V* studies did show some alveolar disruption, probably due to manipulations needed to obtain the *P-V* curves.

Total lung compliance was 33 percent lower ($P < .05$) in infected animals than in the control group (Table 2),

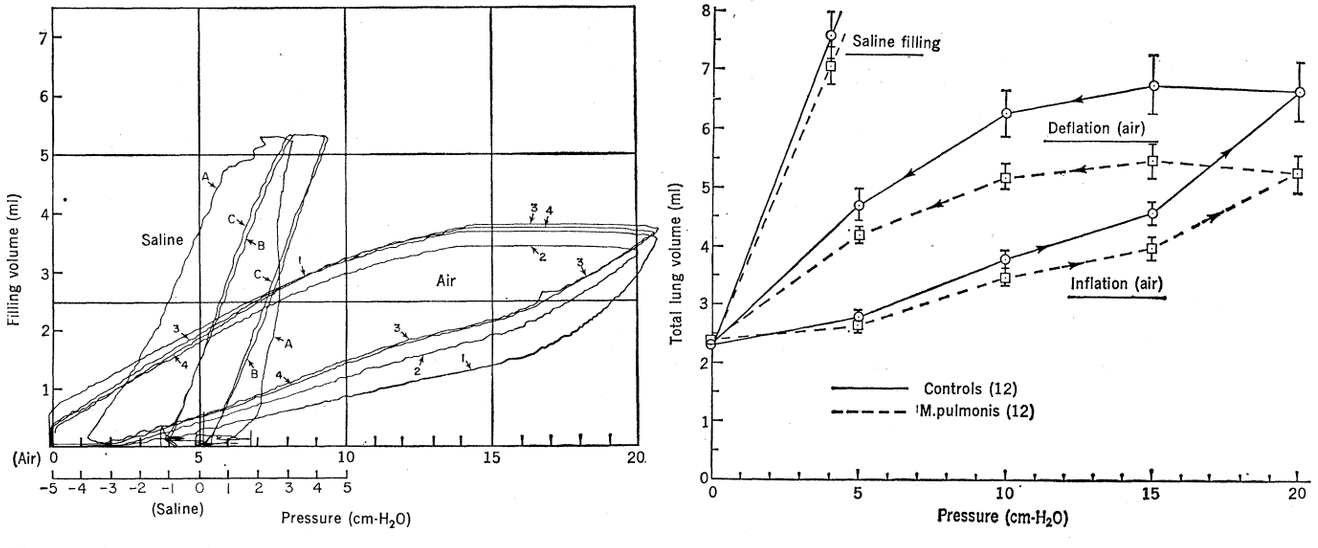

Fig. 1 (left). Compliance curves on an excised rat lung. Cycles of air inflation and deflation are numbered; saline cycles are lettered. Fig. 2 (right). Average pressure-volume (compliance) curves from excised rat lungs. The slopes of saline curves are averages of inflation and deflation limbs.

whereas compliance due to tissue forces was not significantly different ($P > .05$). The calculated C_{surf} was very significantly lower, 37 percent ($P < .01$), in infected rats. Within control and infected groups, the inflation and deflation limbs of the air *P-V* curves had similar slopes. This indicates that opening pressure was not the explanation of the lower compliance.

Our evidence indicates that altered pulmonary function in rat lungs infected with *M. pulmonis* is due predominantly to an increase in surface forces (*13*), implying disruption of the surfactant system.

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2. Since mycoplasmas are indigenous in most rat colonies, we purchased gnotobiotic rodents (CD-R stock males, Charles River) or we used CD-F derived rats which had been raised under gnotobiotic conditions at Ohio State. Rats were then housed individually in disposable plastic cages (Maryland Plastics) and raised in a conventional manner. Cages were covered with a filter frame and fiberglass-plastic filter (Isocap, Carworth). Protection against airborne cross-contamination was effective, since we could not culture mycoplasmas from throat swabs taken from animals before inoculation or from control animals. Rats were isolated under these conditions at least 1 week before the start of experiments. All appeared to be free from previous experience with *M. pulmonis*, since, by a fluorescence test, their serums did not show antibody against this organism.
3. Innovar Injection (McNeil Lab, Fort Washington, Pa.) contains, per milliliter, 0.05 mg of fentanyl, a narcotic analgesic, and 2.5 mg droperidol, a narcoleptic agent. Rats received 0.075 to 0.1 ml, depending on their age and weight.
4. *Mycoplasma pulmonis* strain N3 had been obtained from J. Nelson of Rockefeller University. In our laboratory, it was passaged through rats to enhance its virulence, and the isolate used in these experiments had been recovered from the lungs of one of the animals. The organism was cultured and concentrated according to the method described by N. L. Somerson, W. D. James, B. E. Walls, R. M. Chanock, *Ann. N.Y. Acad. Sci.* **143**, 348 (1967). Suspensions of organisms inoculated into rats contained at least 10^{10} mycoplasma colony-forming units.
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6. The time of killing was arbitrarily selected. Infected animals were killed within a period of 8 to 49 days after inoculation.
7. Minced lung tissue was inoculated into roller-tube cultures of rat embryonic kidney, WI-38, HeLa cells, and primary green monkey kidney cells. Two cultures of each type of cell were incubated at 37°C, and one at 33°C. Cell cultures were held at least 7 days, examined for cytopathic effects, and subjected to two additional passages.
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9. All cardiac tissue had been removed from the lungs. Lungs were tested for leaks by inflating them with air while submerged in saline; small leaks were repaired with Eastman 910 adhesive.
10. Buoyancy in 0.9 percent saline after the air *P-V* curves was used to estimate trapped or retained gas.
11. Antibody to *M. pulmonis* was measured in a fluorescent test described by P. Y. Ertel, I. J. Ertel, N. L. Somerson, J. D. Pollack, *Proc. Soc. Exp. Biol. Med.* **134**, 44 (1970). Mycoplasmas were isolated from the lungs of one rat who did not show antibody. The animal had been killed 8 days after inoculation, probably before this antibody was produced.
12. The lack of microscopic pulmonary lesions contrasts with the findings of A. B. Organick and I. I. Lutsky [*J. Bacteriol.* **92**, 1154 (1966); *ibid.*, p. 1166], who studied *M. pulmonis* infection in both conventional and gnotobiotic mice. They reported consolidation with a polymorphonuclear cellular response in the acute stage of infection. In some cases they showed distension of alveolar spaces and disruption of mitochondria. Possibly the rats we used were more resistant than mice to *M. pulmonis*. However, D. F. Kohn and B. E. Kirk [in *Lab. Anim. Care* **19**, 321 (1969)] reported bronchiectasis in rats given *M. pulmonis*. Perhaps our strain was not as virulent as the one they used.
13. We recognize that, theoretically, bronchoconstriction could mimic an increase in surface tension [see E. P. Radford, Jr., and N. M. Lefcoe, *Amer. J. Physiol.* **180**, 479 (1955)]. However, there is no evidence that such a phenomenon occurs in mycoplasma-infected rats.
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Pinocytosis and Membrane Dilation in Uranyl-Treated Plant Roots

Abstract. *Electron-dense crystals formed in plant roots exposed to uranyl acetate have been used to identify binding sites and to follow the pinocytotic uptake of uranyl in the oat rootcap. Before uranyl enters the protoplast, the plasmalemma is greatly dilated. After uranyl is sequestered in vacuoles, the tonoplast is similarly dilated.*

When oat roots were placed in solutions of uranyl acetate, then fixed in phosphate-buffered glutaraldehyde, fixed in osmium tetroxide, and stained with lead citrate (*1*), very dense, needle-shaped crystals, easily visible in electron micrographs of thin sections, were formed. The crystals also formed in material fixed only in phosphate-buffered glutaraldehyde without additional fixation or staining. In view of this and their very high electron density, we conclude that they represent some type of uranium complex. They are probably not simply uranyl phosphate crystals since they were not found when aqueous or phosphate-buffered potassium permanganate was used as a fixative or when uranyl acetate was mixed in a variety of concentrations with phosphate buffer and deposited an collodion-coated grids. We have used these crystals to follow the binding and uptake of uranyl in cells of the oat rootcap (*2*).

The number and distribution of uranyl crystals in outer secretory cells of the oat rootcap varied with the concentration of uranyl solutions used and the length of time the roots were exposed. In one test, roots were exposed to 1 m*M* uranyl acetate for 5, 10, 20, and 30 minutes, then removed and rinsed in three changes of distilled water for a total desorption time of 30 minutes. Although the number of crystals increased as exposure to uranyl increased, uranyl crystals were, in all cases, sharply localized in the cell walls, intercellular spaces, and in secretory products in direct contact with cell

Fig. 1. Localization of uranyl crystals in cells of the oat rootcap. (A–E) Portions of cells in outer secretory layers of the rootcap from roots exposed for 10 minutes (A) or 1 hour (B–D) to 1 m*M* uranyl acetate. Secretory vesicles (*sv*) are common and in (A) secretory products have accumulated between the plasmalemma (*pl*) and the cell wall. Uranyl crystals are abundant in cell walls (*w*), intercellular spaces (*is*), and secretory products (*sp*). (E) Outer secretory cell exposed to 0.1 m*M* uranyl acetate for 20 hours. Note the normal appearance of the nucleus (*n*) and mitochondria (*m*). (F–I) Portions of normally nonsecretory cells in the cap interior of oat roots exposed to 1 m*M* uranyl acetate for 1 (F and G), 2 (H), or 6 (I) hours. Note invaginations of the plasmalemma (*pl*) and presence of pinocytotic vesicles (*pv*) in (F) and transitions in membrane structure (arrows) in (G). In (H), the vacuole (*v*) contains a dense inclusion, many secretory vesicles (*sv*) are present, and Golgi dictyosomes (*g*) are active. (I) Massive deposits of uranyl crystals in walls of cells just beneath the outer secretory layers. (J–L) Portions of interior cap cells from oat roots exposed to 0.1 m*M* uranyl acetate for 10 (J and K) or 20 (L) hours. (J and K) Swollen cell wall (J) and part of vacuole with a dense deposit (K) in interior root cap cells. Arrows (K and enlarged area of J) indicate points of apparent membrane overlap in tonoplast (*t*) and plasmalemma (*pl*). (L) Cell below with uranyl crystals localized in secretory products (*sp*) is in the outer secretory region of the rootcap. Contrast with the normally nonsecretory cell above with crystals in vacuoles (*v*). All roots were desorbed after exposure to uranyl for 30 minutes in distilled water, except for that in (D) which was desorbed 2 hours. Unless otherwise indicated, scale equals 1 μm.