www.sciencemag.org/cgi/content/full/327/5963/338/DC1

Supporting Online Material for

Unidirectional Airflow in the Lungs of Alligators

C. G. Farmer* and Kent Sanders

*To whom correspondence should be addressed. E-mail: cg.frmr@gmail.com

Published 15 January 2010, *Science* **327**, 338 (2010)
DOI: 10.1126/science.1180219

This PDF file includes:

Other Supporting Online Material for this manuscript includes the following: (available at www.sciencemag.org/cgi/content/full/327/5963/338/DC1)

Supporting Online Information

Materials and Methods

We used several methods to measure airflow: a pneumotach (Hans Rudolph, Inc., Shawnee, KS, USA) for airflow into and out of the trachea and dual thermistor flow meters (HEC 132C Thermistor Flow Meter, Hector Eng. Co., Inc., Ellettsville, IN, USA) or single microbead thermistors (Thermometric BB05JA202, Edison, NJ, USA thermistor, 90% of full scale in 6 ms) for intrapulmonary flow. We implanted the dual thermistor meters into the cervical ventral bronchus and a dorsal bronchus of the excised lungs and into the cervical ventral bronchus *in vivo*. We also implanted single thermistor beads into the cervical ventral bronchus *in vivo*. To implant a probe we made an incision in the lung, approximately 1 cm long for the dual thermistor probes and 2 mm long for the single thermistors, inserted the probe and closed the incision using a purse string suture of silk. Experiments were approved by the University of Utah Committee for Animal Care and Use.

We created negative pressure inspiration by placing an excised lung in an airtight container with the trachea open to atmospheric pressure and created a vacuum in the container. For positive pressure inspiration we pushed air into the lung with a syringe. We used resting tidal volumes, approximately 21 ml kg^{-1} (S1).

To visualize flow, saline containing fluorescent microspheres (Invitrogen, Frederick, MD, USA , C14837) was alternately pushed into and withdrawn from the lung with a

syringe. We imaged the movement using a 2X objective, Olympus (Center Valley, PA, USA) IX70 Microscope, Olympus DP70 CCD camera (Center Valley, PA, USA) at 60 frames second^{-1}, and TRITC filter (543/22 Ex, 593/40 Em, 570LP Dichroic, Center Valley, PA, USA).

We used a 128 slice dual energy Siemens (New York, NY, USA) SOMATOM definition computed tomography unit to collect data from an 11 kg alligator during a natural apnea. We used a Siemens Wizard workstation and proprietary software to make coronal and sagittal reconstructions and 3-dimensional volume renderings in various included density based filtration algorithms. We used Amira 5 DICOM image processing software to segment DICOM image files to generate 3-dimensional models.

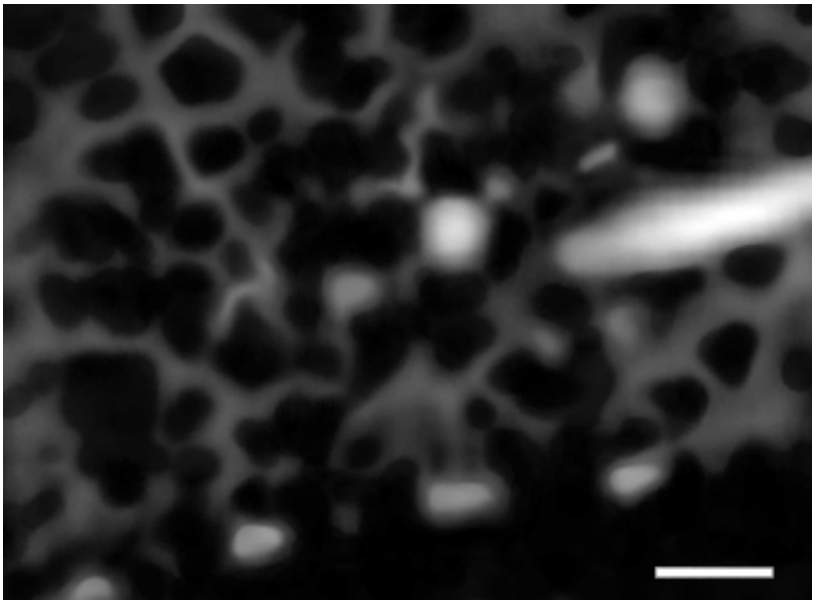

Fig. S1.

Reconstruction of CT data in the dorsal region of the lung showing numerous small passages that anastomose and form a meshwork, the parabronchi. The two white circles in the upper right quadrant of the image are arteries. The four white ovals near the bottom of the image are veins and mark the orifices from a bronchus into 3 parabronchial passages. We used the same magnification in the accompanying videos while we pushed a saline solution containing fluorescent microspheres (Invitrogen, Frederick, MD, USA , C14837) into excised lungs and subsequently withdrew the solution. Both gases and liquids are fluids and will obey the same physical principles under most biological conditions (S2). Like the flow of gases, we observed the microspheres unidirectionally. The saline was injected in volumes equal to, or a little less than, a normal tidal volume (21 ml kg^{-1}). The movement of the microspheres was imaged with a 2X objective, Olympus IX70 microscope, Olympus DP70 CCD camera at 60 frames $second^{-1}$, and TRITC filter (543/22 Ex, 593/40 Em, 570LP Dichroic). Scale bar, 1 mm.

Supplementary Movies

Movies S1 and S2: Microspheres flowing through the cervical ventral bronchus (green bronchus of Fig. 1). In the cervical ventral bronchus most of the microspheres move in a cranial to caudal direction (from the right side of the image toward the left) as fluid is injected into the lung (Movie S1) and when fluid is withdrawn (Movie S2). A few microspheres are no longer suspended in the fluid and are caught in lung tissue. These move at a slower speed and spread apart as the injection takes place and move closer together as fluid leaves the lung. The black line in the lower left visual field is a piece suture on the outside of the lung.

Movie 3: Microspheres flowing through parabronchi. In this movie numerous microspheres have been caught in lung tissue and can be seen moving to the right when fluid is injected and to the left when it is withdrawn and then back to the right as fluid is injected again, which occurs within the same movie. Other microspheres remain suspended in the fluid and move at a greater speed from a dorsocranial (top of the screen) to ventrocaudal direction (bottom of the screen) as saline was injected and as it was withdrawn. These latter, faster moving microspheres do not move in a tidal pattern but flow unidirectionally.

Supplemental references

S1. C. G. Farmer, Respiration Physiology and Neurobiology **154**, 89 (2006).

S2. G. K. Batchelor, *An Introduction to Fluid Dynamics*. (Cambridge University Press, Cambridge, 1990), pp. 615.