R. Gross, *Dev. Biol.* **27**, 457 (1972); R. O. Hynes, R. A. Raff, P. R. Gross, *ibid*, p. 150.
6. J. W. Brookbank, *Cell Differ.* **7**, 153 (1978).
7. R. O. Hynes and P. R. Gross, *Dev. Biol.* **21**, 383 (1970).
8. R. D. Kornberg, *Science* **184**, 868 (1974); G. Felsenfeld, *Nature (London)* **271**, 115 (1978).
9. K. E. Van Holde, C. G. Sahsrabuddhe, B. R. Shaw, *Nucleic Acids Res.* **1**, 1579 (1974); A. J. Varshavsky *et al.*, *ibid.* **3**, 447 (1976); J. P. Whitlock, Jr., and R. T. Simpson, *Biochemistry* **15**, 3307 (1976); M. Noll and R. D. Kornberg, *J. Mol. Biol.* **109**, 393 (1977).
10. K. M. Newrock *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **42**, 421 (1977).
11. L. D. Keichline and P. M. Wasserman, *Biochim, Biophys. Acta* **475**, 139 (1977); R. J. Arceci and P. R. Gross (*3*).
12. R. J. Arceci and P. R. Gross (*3*); R. J. Arceci, thesis, University of Rochester (1980).
13. E. H. Davidson, *Gene Activity in Early Development* (Academic Press, New York, 1976).
14. R. T. Hinegardner, in *Methods in Developmental Biology*, F. H. Wilt and N. K. Wessels, Eds. (Crowell-Collier, New York, 1976).
15. A. Tyler and B. S. Tyler, in *Physiology of Echinodermata*, R. A. Boolootian, Ed. (Wiley, New York, 1966).
16. R. Okazaki, *Am. Zool.* **15**, 567 (1975); *Biol. Bull. (Woods Hole, Mass.)* **149**, 439 (1975).
17. R. J. Arceci, D. Senger, P. R. Gross, *Cell* **9**, 171 (1976).
18. R. A. Laskey and A. D. Mills, *Eur. J. Biochem.* **56**, 335 (1975).
19. We thank M. Gorovsky and R. Angerer for helpful advice and comment. This work was supported by research grants from NIH and the Rockefeller Foundation. R.J.A. was supported by the medical scientist training program at the University of Rochester.
* Send correspondence to P.R.G., Marine Biological Laboratory, Woods Hole, Mass. 02543.

31 December 1979; revised 31 March 1980

Effective Pulmonary Ventilation with Small-Volume Oscillations at High Frequency

Abstract. *At high oscillation frequencies (4 to 30 hertz), effective alveolar ventilation can be achieved with tidal volumes much smaller than the anatomic dead space. An explanation of this phenomenon is given in terms of the combined effects of diffusion and convection and in terms of data consistent with the hypothesis. Theory and experimental results both show that the significant variable determining the effectiveness of gas exchange is the amplitude of the oscillatory flow rate independent of the individual values of frequency and stroke volume.*

Gas exchange during normal tidal ventilation is thought to involve two distinct regions of the lung: (i) the dead space volume, comprised of the conducting airways, where gas transport is primarily convective; and (ii) the alveoli, where molecular diffusion is the predominant mechanism of gas transport. In normal ventilation, therefore, effective gas exchange is possible only if the tidal volume exceeds the dead space volume. Recently, however, it was shown that effective alveolar ventilation can occur with tidal volumes considerably less than the dead space volume if the respiratory frequencies are sufficiently high (4 to 20 Hz) (*1*).

To explain the gas exchange effected by high-frequency, low tidal volume ventilation, we present a theoretical model based on the concept of augmented transport, or diffusion coupled to convection (*2*). We also present experimental data that confirm the observations of others concerning the efficacy of this technique and that are in general agreement with the predictions of the theory.

Augmented mass transport in both laminar and turbulent flow through a long straight tube was originally analyzed by Taylor (*2*). Subsequently, Chatwin, using concepts similar to those of Taylor, provided a solution for oscillatory laminar flow (*3*). The augmented mass transport can be represented in a way analogous to molecular diffusion by replacing molecular diffusivity D_{mol} by effective diffusivity D_{eff}. The effective resistance to diffusion R (in units of inverse volume flow) for a tube of length L and cross-sectional area A may then be expressed as

$$R = L/(AD_{eff}) \quad (1)$$

The appropriate formula for D_{eff} depends mainly on two parmeters: (i) the Womersley parameter, $\alpha = d\sqrt{(\pi/2)f/\nu}$, where d is the tube diameter, f the oscillation frequency, and ν the kinematic viscosity; and (ii) the Reynolds number, $Re = ud/\nu$, where u is the root-mean-square velocity.

For this simple analysis we assume that the stroke volume of oscillation is less than the dead space volume and that there exists a critical Reynolds number, Re_c, such that the flow is laminar when $Re < Re_c$ and well mixed when $Re \geq Re_c$ (*4*). Given the theoretical results of Chatwin (*3*) for laminar flow and the theory of Taylor (*2*) and experiments of Scherer *et al.* (*5*) for well-mixed flow, we employ the following expressions for D_{eff}:

$$D_{eff}/D_{mol} = 1 + K_1 (ud/D_{mol}), \quad \text{if } Re \geq Re_c \quad (2)$$

$$D_{eff}/D_{mol} = 1 + (1/192)\,(ud/D_{mol})^2, \quad \text{if } Re < Re_c \text{ and } \alpha < 1 \quad (3)$$

$$D_{eff}/D_{mol} = 1 + (K_2/192)\quad (ud/D_{mol})^2\alpha^{-7}, \quad \text{if } Re < Re_c \text{ and } \alpha \gg 1 \quad (4)$$

where K_1 and K_2 are dimensionless constants of order unity.

Now consider the lung as a network of branching tubes. As in the calculation of resistance in an electrical circuit, the effective resistance to diffusion of the entire bronchial tree, R_T, may be found by summing the individual resistances. Note that in a particular generation, the area in Eq. 1 would be the total cross-sectional area of that generation.

The rate of CO_2 removal from the lung, $\dot{V}_{CO_2}$, is proportional to the difference between the volume fraction of CO_2 present in the alveoli, F_{A,CO_2}, and at the airway opening, F_{AO,CO_2}, and can be expressed as

$$\dot{V}_{CO_2} = (F_{A,CO_2} - F_{AO,CO_2})/R_T \quad (5)$$

To obtain the results shown in Fig. 1A, we used the anatomic data from the dog lung model of Horsfield and Cumming (*6*) and $K_1 = 0.7$ [the mean value for inspiratory and expiratory flow given by Scherer *et al.* (*5*)]. Results are shown for three cases that bracket the anticipated range of Re_c and α_c, where α_c represents the critical value of α; α_c determines whether Eq. 3 (for $\alpha < \alpha_c$) or Eq. 4 (for $\alpha > \alpha_c$) is used (*7*). The value of Re_c is not known, but is thought to be on the order of 10 (*8*). The discontinuities in the curve for $Re_c = 30$ are an artifact of the model caused by the abrupt transition from laminar to mixed flow as the Reynolds number in a given generation changes through its critical value. In reality these transitions are probably not abrupt, but continuous, due to entrance effects, secondary motions, and asymmetries in the bronchial tree.

The model predicts that CO_2 output will increase as the amplitude of the oscillatory flow increases, regardless of the individual values of f and stroke volume, as long as f is low enough for α to be small in regions of laminar flow. In addition, theoretical results not shown in Fig. 1 indicate that the CO_2 output as a function of tracheal flow is relatively independent of animal size or lung volume for a single animal, provided the lung geometries are similar (*9*).

Experiments were performed on four dogs in which we varied f and the stroke volume and observed the effect on CO_2 elimination. Our experimental setup (Fig. 2) consisted of a standard animal piston ventilator with the following added features: (i) a high-frequency oscillator (HFO), which consisted of four 12-inch-diameter speakers sealed in a special chamber, acoustically coupled in series, and driven by an amplifier connected to a wave-form generator used to

0036-8075/80/0801-0609$00.50/0

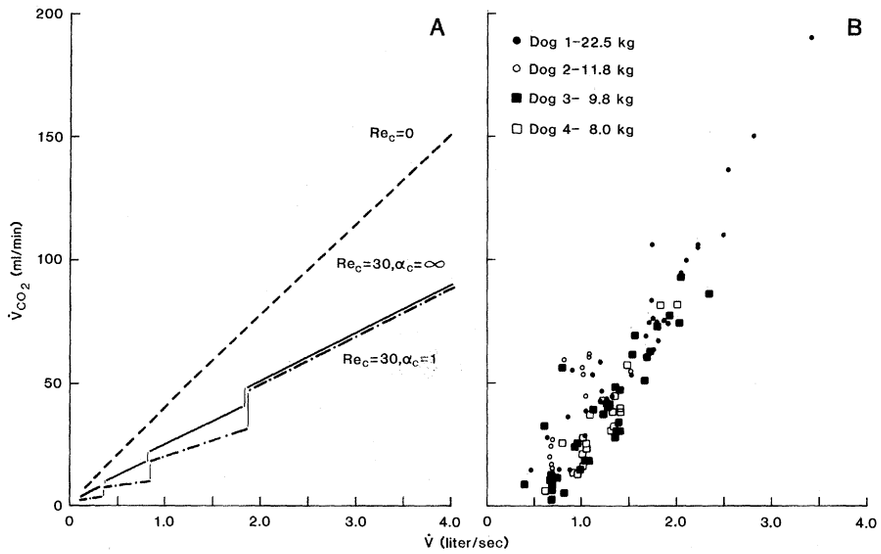

Fig. 1. (A) Predicted values of CO_2 elimination (V_{CO_2}) versus root-mean-square tracheal flow for three cases: (i) $Re_c = 0$; (ii) $Re_c = 30$, $\alpha_c = \infty$; and (iii) $Re_c = 30$, $\alpha_c = 1$. For all cases the oscillation frequency was constant at 30 Hz. (B) Experimental measurements of CO_2 elimination versus tracheal flow for four dogs.

provide a sinusoidal signal of varying frequency and amplitude (*10*); and (ii) a high-impedance bias flow of fresh air adjusted to maintain the mean pressure at the airway opening to within 0.1 cm-H_2O of atmosphere while providing a flow of 700 ml/sec. The oscillatory volume flow rate was measured by a pneumotachograph (screen area, 7 in^2) connected to a pressure transducer (Validyne DP-45, 2 cm-H_2O). This was calibrated prior to all the experiments with a piston pump driven in the range of 3 to 30 Hz with stroke volumes varying from 20 to 100 cm^3. To obtain an index of gas exchange, we measured the rate at which CO_2 was removed by the bias flow (bias flow rate $\times$ CO_2 volume fraction). Since the bias flow rate was constant to within $\pm$ 2 percent and there was no measurable CO_2 in the tubing between the HFO and the pneumotachograph, the bias flow CO_2 removal was equal to the CO_2 output from the lung.

Four dogs were anesthetized with pentobarbital, vagotomized, fitted with tight metal tracheostomy tubes, paralyzed with intravenous succinylcholine chloride, and initially ventilated with the piston ventilator. Multiple measurements of anatomic dead space were then made by a modification of the Fowler technique (*11*), with the CO_2 measured at a tap just proximal to the tracheostomy. The additional dead space from the CO_2 tap to the bias flow was determined by direct measurement. The ventilator settings were then adjusted to maintain the dog's end-tidal CO_2 partial pressure P_{ET,CO_2} at 40 $\pm$ 1.5 mm-Hg, and baseline measurements of CO_2 output were made with the technique described above.

After these measurements, the dog was ventilated by the HFO for 15 to 30 seconds while measurements of CO_2 output were made (*12*). After each measurement, the dog was returned to the piston ventilator and P_{ET,CO_2} was measured. If P_{ET,CO_2} had changed, the ventilatory rate was adjusted transiently to allow it to return to its baseline value. Using this technique, we systematically varied the frequency from 4 to 30 Hz and the stroke volume from about 20 to 85 percent of the dead space volume. Each measurement was obtained only after we had stabilized P_{ET,CO_2} to $\pm$ 1.5 mm-Hg of the control value.

As frequency increased at a fixed stroke volume, and as stroke volume increased at a fixed frequency, the CO_2 output also increased. However, at any fixed product of frequency with stroke volume, the CO_2 output was approximately constant, irrespective of the frequency of oscillation. In Fig. 1B the data from all four dogs are plotted as CO_2 output versus root-mean-square tracheal flow ($\dot{V}_{RMS}$). A single relation is obtained, although the dogs varied in size from 8 to 22.5 kg. These results generally agree with the theoretical predictions (Fig. 1A), except that the experimental data appear to cross the flow axis at positive rather than zero flows.

In conclusion, effective gas exchange can take place at tidal volumes as small as 20 percent of the dead space volume if the oscillatory frequency is sufficiently high to maintain tracheal flow amplitude above some critical level. The coupled effects of molecular diffusion and convection can account for the experimentally observed gas transport.

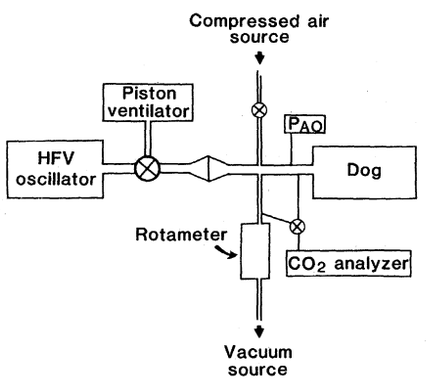

Fig. 2. Experimental setup used in the experiments (P_{AO}, pressure at the airway opening).

ARTHUR S. SLUTSKY
JEFFREY M. DRAZEN
ROLAND H. INGRAM, JR.
Department of Medicine, Peter Bent Brigham Hospital, and Harvard Medical School, Boston, Massachusetts 02115
ROGER D. KAMM
ASCHER H. SHAPIRO
Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge 02139
JEFFREY J. FREDBERG
Cambridge Collaborative, Cambridge, Massachusetts 02142
STEPHEN H. LORING
JOHN LEHR
Department of Physiology, Harvard School of Public Health

References and Notes

1. J. Bunnell, K. Karlson, D. Shannon, *Am. Rev. Respir. Dis.* **117**, 289 (Abstr.) (1978); D. J. Bohn, W. Butler, A. B. Froese, A. C. Bryan, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 951 (Abstr.) (1979).
2. G. I. Taylor, *Proc. R. Soc. London Ser. A* **219**, 186 (1953); *ibid.* **223**, 446 (1954).
3. P. C. Chatwin, *J. Fluid Mech.* **71**, 513 (1975).
4. J. J. Fredberg, *J. Appl. Physiol*, in press; Fredberg's diffusional resistance model is equivalent to assuming an $Re_c = 0$.
5. The experimental justification for using Eq. 2 is based on studies in a five-generation model of the tracheobronchial tree under conditions of steady flow [P. W. Scherer, L. H. Schendalemen, N. H. Greene, A. Bouhuys, *J. Appl. Physiol.* **38**, 719 (1975)]. Although we used an analysis based on effective diffusivities, augmentation of gas mixing in the regions where $Re \geq Re_c$ is likely due to a combination of turbulence, secondary flows, and mixing caused by convection [P. W. Scherer and F. R. Haselton, *AIChE J.* **25**, 542 (1979); F. R. Haselton and P. W. Scherer, *Science* **208**, 69 (1980)].
6. K. Horsfield and G. Cumming, *Respir. Physiol.* **26**, 173 (1975). Since this model provided no dimensions for the respiratory bronchioles, these were determined by extrapolation from Horsfield and Cumming's regression equations. Tracheal dimensions included the endotracheal tube up to the point of the bias flow connection.
7. Although the low α solution (Eq. 3) is expected to be approximately valid up to $\alpha \simeq 2$, the high α solution (Eq. 4) will probably underestimate D_{eff} for values of α less than about 10. Therefore, the curve in Fig. 1A corresponding to $Re_c = 30$ and $\alpha_c = 1$ is likely to exaggerate the influence of the

high-frequency solution. Any theoretical curve calculated by assuming an $\alpha_c > 1$ (and $Re_c = 30$) would lie between the two curves for $Re_c = 30$, $\alpha_c = 1$ and $Re_c = 30$, $\alpha_c = \infty$.

8. T. J. Pedley, R. C. Schroter, M. F. Sudlow, *J. Fluid Mech.* **46**, 365 (1971).
9. For example, if all linear dimensions of the airways increased by a factor of 3, according to this model the CO_2 output would change only by an average of about 15 percent.
10. Our HFO was unable to generate sufficiently large flows at frequencies greater than 30 Hz.
11. J. M. Drazen, S. H. Loring, A. C. Jackson, J. R. Snapper, R. H. Ingram, Jr., *J. Appl. Physiol.* **47**, 657 (1979).
12. Measurements of CO_2 output were made over this short time span to ensure that the alveolar P_{CO_2} would remain relatively constant.
13. Supported in part by a fellowship from the Medical Research Council of Canada (A.S.) and by grants HL-000549 and HL-19170 from the National Heart, Lung, and Blood Institute.

4 February 1980; revised 9 May 1980

Vascular Permeation of Platelet Factor 4 After Endothelial Injury

Abstract. *Antibody to platelet factor 4 was used to demonstrate permeation of this factor into the blood vessel wall after endothelial injury in rabbits. The presence of platelet factor 4 antigen in the vessel wall after removal of the endothelium was shown by immunofluorescence 10 and 30 minutes after injury but not 240 minutes afterward. This study demonstrates that factors carried by platelets can enter the vessel wall and that the movement of these platelet products into the vasculature is a short-lived, self-limiting process.*

After vascular injury, platelets adhere to exposed subendothelium, degranulate, and form aggregate masses (*1*). Following activation, they secrete numerous biochemical mediators including lysosomal enzymes, a potent vasoconstrictor (thromboxane A_2), and a mitogenic protein [platelet-derived growth factor (PDGF)] that stimulates growth of cultured smooth muscle cells. Studies of vascular injury have focused on the morphology of platelet adhesion to the subendothelium, the ensuing aggregation (*2*), and platelet turnover kinetics (*3*). However, until now there was no direct evidence that materials secreted by the platelet enter the vessel wall. To establish that such materials do enter the vessel wall and to follow their course in vivo, we studied a well-known platelet-specific alpha-granule protein, platelet factor 4 (PF4), which is a 7800-dalton polypeptide that can neutralize heparin's anticoagulant effect and may interact with other sulfated glycosaminoglycans (*4*, *5*). In our study, the indirect method of immunofluorescence was used to demonstrate (i) the presence of PF4 antigen in the vessel wall within minutes of platelet attachment to the site of endothelial injury and (ii) the disappearance of the antigen after 4 hours.

We isolated PF4 protein from rabbit platelets (*5*) and then raised in sheep a monospecific antibody to the purified protein (*6*). Rabbit iliac arteries were denuded of endothelium with a low-pressure balloon catheter (*1*). At selected times after injury, the animals were killed by exsanguination and sections of iliac artery were prepared for testing with indirect immunofluorescence techniques adapted for the PF4 antigen (*7*).

The PF4 antigen was identified at the luminal surface and in the tunica media of the artery 10 and 30 minutes after removal of the endothelium (Fig. 1). Immunofluorescent platelets were seen on the vessel surface 240 minutes after injury, although there was a marked diminution in immunofluorescent staining of the vessel wall. These results are consistent with those of morphological studies (*1*) and provide additional information about the reaction between platelets and the vessel wall several hours after injury. They also agree with the results of recent studies of ^{51}Cr-labeled platelet turnover (*3*). In those studies, platelet interaction with the vessel wall was evaluated in rabbits whose endothelium was removed with a balloon catheter. It was shown that only 0.2 percent of the circulating platelets attached to the damaged wall and that turnover was almost undetectable. Similarly, we show here that secretion of antigen by platelets is a short-lived, self-limiting phenomenon, with apparently little secretion occurring 4 hours after injury.

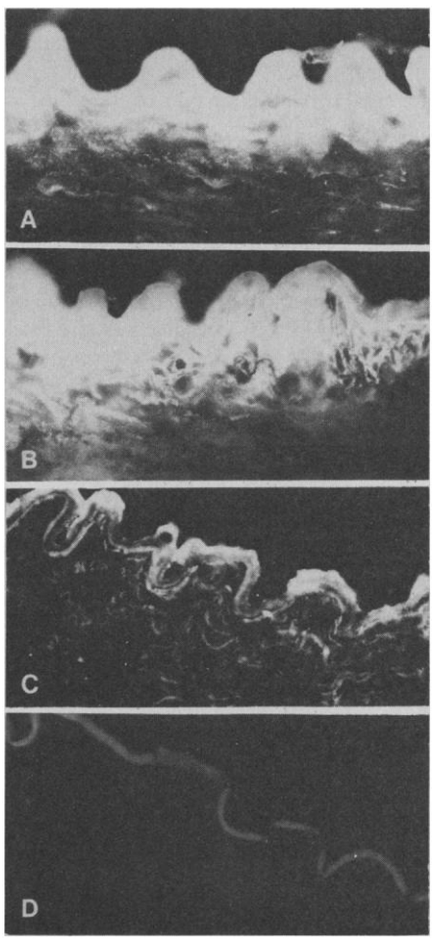

Fig. 1. Localization of PF4 in the vessel wall with sheep antibody to rabbit PF4 at (A) 10, (B) 30, and (C) 240 minutes after endothelial injury (×250). Note permeation of PF4 into the vessel wall after 10 and 30 minutes, and its exclusive presence in the luminal platelets after 240 minutes. (D) Control slide incubated with antigen-absorbed antibody; immunofluorescent staining did not occur (×250). Slides containing hyperimmune sheep serums incubated under the same conditions also did not show immunofluorescence.

It has been well documented that the subendothelium is covered by platelets within minutes of endothelial injury. After 10 minutes, the platelets spread out to completely cover the exposed surface (*1*). Platelets adhering to the subendothelial surface lose 97 percent of their alpha granules after 40 minutes, whereas most of the platelets that aggregate onto the adherent platelets retain their granules (*2*). Our results demonstrate, apparently for the first time, the permeation of a platelet-derived protein into the vessel wall: the platelet-derived antigen appears in the vessel wall within minutes of removal of the endothelium.

Since PF4 and other platelet-secreted proteins like PDGF reside in the same granule population and are secreted in response to the same stimuli (*8*), it seems likely that these proteins also enter the vessel wall after removal of the endothelium. Recent studies suggest that only a transient exposure to PDGF is needed to cause proliferation of cultured cells (*9*, *10*). Previous studies in our laboratory showed that the proliferative response of vascular smooth muscle cells (SMC) is self-limiting, with peak SMC synthesis of DNA occurring after 48 hours, followed by a dramatic decrease (*10*). These studies suggest that the rapid increase in secretion by platelets after injury is fol-

0036-8075/80/0801-0611$00.50/0