EVALUATION OF ABNORMAL LUNG FUNCTION

◆6660

A. F. Wilson, R. D. Fairshter
California College of Medicine, Department of Medicine, University of California, Irvine, California 92668

J. R. Gillespie
School of Veterinary Medicine, Department of Physiological Sciences, University of California, Irvine, California 92668

J. Hackney
Rancho Los Amigos Hospital and Department of Medicine, University of Southern California, School of Medicine, Downey, California 90242

INTRODUCTION

Impairment of lung function is often not apparent symptomatically or by clinical examination until substantial and largely irreversible damage has occurred (1, 2). Since many substances that are toxic or potentially toxic to the lung are present in our environment (3–5) and since clinically obvious lung disease may be evident only after prolonged exposure to atmospheric contaminants, it is useful to have sensitive and noninvasive means for detection of pulmonary toxicity from inhaled substances.

Recent investigations have demonstrated that sensitive physiologic tests are capable of detecting abnormal lung function at an early, and, presumably reversible stage of development (1, 2, 6–12). These methods include such tests as maximal expiratory flow rates (6–8), single-breath and multibreath nitrogen washout (9, 10), closing volume (11), frequency dependence of compliance (1), and radioisotopic regional ventilation-perfusion studies (12). Since some of the methods are invasive and/or require expensive equipment, they are not all equally suitable as screening tests for abnormal lung function. Yet, a variety of physiologic tests are necessary since tests, such as maximal expiratory flow rates, detect airway obstruction whereas others, such as single-breath diffusing capacity for carbon monoxide (DLCO SB), are affected primarily by abnormalities of the pulmonary parenchyma or vasculature. Some methods, which are not suitable for screening purposes, are excellent confirmatory tests in specific circumstances.

Part I of this review describes a sequential approach to pulmonary function testing following experimental and natural exposure of humans or animals to inhalants, which includes suggestions as to screening and initial testing as well as later, more detailed investigation procedures. Approaches are suggested for differing circumstances (awake vs anesthetized) and species (large and small animals vs man). Part II, mainly presented in tabular form, offers a critical review of the limitations and applications of individual tests of pulmonary function.

I Sequential Approach to Pulmonary Function Testing

A. Man (Unanesthetized)

Preexposure Testing for Control Values

↓

Exposure

↓

1. Screening
   a. Closing volume (CV)
   b. Single-breath (SB) N_2 washout
   c. Single-breath diffusing capacity of the lung for CO (DLCO SB)
   d. Maximum expiratory flow-volume (MEFV) curves
   e. Spirometry
   f. Airway resistance (R_{AW}) and thoracic gas volume (Vtg)
2. Follow-up testing
   a. Arterial blood gases
   b. Multibreath N_2 washout (7 min)
3. Further analysis, appropriate tests from Table 1

For screening purposes following exposure to inhalants, closing volume (11) and SB N_2 washout (9) are noninvasive, easy to perform, and both tests may be calculated from the same expiratory maneuver. The test is moderately sensitive but is quasistatic since low flow rates are employed. Dynamic methods such as MEFV curves and spirometry, particularly if flow rates are measured at low lung volumes (FEF 25–75, FEF 75–85), may be abnormal when static tests are normal (15). Both procedures are noninvasive, easy to perform, and therefore well suited for screening purposes. All measurements can be made technically from a single maneuver although the tests are usually repeated to insure "best effort." The MEFV curve is probably best utilized with the subject serving as his own control since flow rates are highly variable from person to person (16). The sensitivity of the technique may be increased by comparison of curves obtained after inhaling air and helium-oxygen mixtures (17). Airway resistance is easy to perform but is sensitive to abnormalities, primarily of large central airways (1, 18) and should not be used alone. Changes in maximal expiratory flow (particularly at low lung volumes) with no change in R_{AW} would suggest that the site of the lesion is in the small airways. DLCO SB is simple

and easy to perform; this test and, possibly, SB N_2 washout might be the most sensitive detectors of early (interstitial) pulmonary edema or microatelectasis (19, 20).

Arterial blood gas analysis is a very sensitive parameter of change in respiratory status. The procedure is invasive and is best performed with local anesthesia; it therefore probably should not be considered a screening procedure. Multibreath nitrogen washout is highly sensitive to abnormalities of distribution of ventilation (10); however, not only does this procedure require more time to perform than single-breath tests, but also analysis of results is more time consuming. Multibreath nitrogen washout is best utilized in selected circumstances (i.e. to confirm borderline results, to differentiate degrees of abnormality not apparent from screening tests, and to further investigate suspected abnormalities in the presence of negative screening tests).

A variety of other tests such as radioisotopic regional ventilation and perfusion studies, static or dynamic compliance, pulmonary artery catheterization, multiple inert gas washout are detailed in Table 1. These tests will provide answers to specific questions. They are, in general, more invasive, more expensive and require greater expertise to perform.

B. Large Animals (Unanesthetized)

Preexposure Testing for Control Values

↓

Exposure

↓

1. Screening tests
   a. Spirometry—respiratory rate, tidal and minute volume
   b. Arterial blood gases—PaO_2, $PaCO_2$, pH
   c. Multibreath nitrogen washout—distribution of ventilation, functional residual capacity (FRC)
2. Confirmatory tests
   a. Dynamic compliance
   b. Dynamic resistance (airway)
   c. Total pulmonary resistance (oscillatory)
   d. Steady state diffusing capacity for carbon monoxide (DLCO SS)
   e. Other appropriate tests from Table 1

C. Large Animals (Sedated or Anesthetized)

Preexposure Testing for Control Values

↓

Exposure

↓

1. Screening tests
   a. Spirometry—respiratory rate, tidal and minute volume
   b. Lung volumes—total lung capacity (TLC), vital capacity (VC), functional residual capacity (FRC), residual volume (RV)
   c. Arterial blood gases—PaO_2, $PaCO_2$, pH
   d. Multibreath nitrogen washout—distribution of ventilation, FRC
   e. Lung compliance—static (Cst), dynamic (Cdyn) including measurements at different respiratory rates (frequency dependence of compliance)
   f. Dynamic resistance (airway)
   g. Total pulmonary resistance (oscillatory)
2. Confirmatory tests
   a. Closing volumes
   b. DLCO SS
   c. DLCO SB
   d. Maximal expiratory flow-volume (MEFV) curves
   e. Radioisotopic regional ventilation-perfusion ($\dot{V}/\dot{Q}$) studies
   f. Other appropriate tests from Table 1

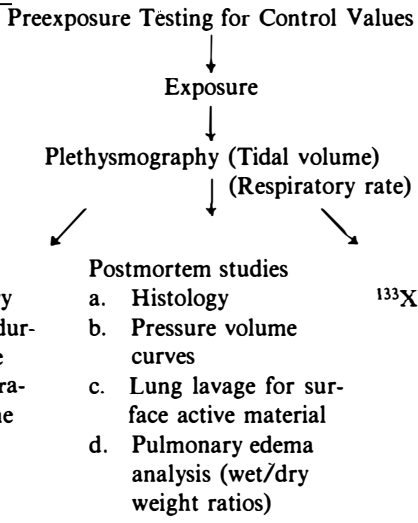
Unanesthetized large animals may be tested using either a face mask (21) or chronic tracheostomy (22). These techniques are used most frequently in relatively cooperative, trainable animals such as dogs. Alternatively, any animal may be tested using general anesthesia, although this method seems less desirable since the effects of anesthesia must also be assessed. Recently, however, Muggenburg & Mauderly have shown that general anesthesia, using triflupromazine HCl, is associated with minimal respiratory side effects (23).

Screening procedures, such as spirometry or multibreath nitrogen washout, are usually easy to perform, noninvasive, and require relatively little equipment or training of animals (see Table 1). Arterial blood gases, although invasive, also require relatively little time and equipment. They probably are best obtained from an indwelling catheter in a femoral or exteriorized carotid artery. Multiple samples can be obtained without causing pain and agitation, which are often associated with reflex changes in respiration.

The confirmatory tests either require intubation and are, therefore, invasive (dynamic compliance, dynamic resistance, radioisotopic regional ventilation studies, MEFV curves), require expensive equipment (DLCO, dynamic compliance, dynamic resistance, radioisotopic regional ventilation-perfusion studies, MEFV curves), or are time consuming (dynamic compliance, dynamic resistance, radioisotopic regional ventilation-perfusion studies) and, therefore, are poorly suited for screening purposes except in anesthetized animals. However, many of these latter methods are also the most sensitive and yield the most information. MEFV curves, for example, have recently been used successfully in monkeys to evaluate the effects

of coal dust upon the small airways (24). Although relatively elaborate equipment was required, individual subjects (monkeys) could be completely tested within 10–12 min following induction of anesthesia (24).

D. Small Animals



The most suitable technique for in vivo screening for response to inhalants is plethysmography. The plethysmograph is relatively easy to construct, and small animals such as rats may be monitored even (with difficulty) without anesthesia. The technique is sensitive and tidal volume and respiratory rate can be measured. Postmortem studies may be sensitive but are both time consuming and tedious and therefore poorly suited for screening purposes in large numbers of small animals. Pulmonary resistance as measured by the technique of Amdur & Mead (25) requires placement of an intrapleural catheter; a plethysmograph and physiologic recorder are also required. The procedure has merit for in vivo testing of small animals but probably should not be considered a screening procedure. ^{133}Xe washout requires expensive equipment as well as use of radioisotopes. As yet, this procedure has not been widely used in small animals. Its utility is not definitely established and therefore is currently under evaluation.

II Specific Types of Tests

The following is a description of pulmonary function methods, their sensitivity and limitations, physiologic interpretation, and the equipment needed for their performance. The material is presented in tabular form as a convenient reference for the inhalation toxicologist (Table 1). This description of methods is divided into the following categories: 1. Ventilatory exchange, measurement of volumes of gases exchanged during (usually quiet) breathing. These tests are ordinarily not very useful. 2. Static lung volumes. These tests are simple and provide useful information

about the strength and elasticity of the respiratory system. They are usually combined with other types of tests since they, by themselves, do not provide definitive information. 3. Tests of respiratory mechanics, analysis of the forces that provide resistance to airflow and inflation or deflation of the lung. Tests of respiratory mechanics vary from the simple to the complex. They are important for the detection and analysis of most types of exposure. 4. Tests of distribution of ventilation, description of the degree of uniformity of alveolar ventilation. Maldistribution of the inspired gas is often associated with early lung disease even though total ventilation is normal or increased. Methods of detecting airway closure are also considered in this section of the table. 5. Tests describing the pulmonary circulation including measurement of vascular pressures, right to left shunting, and distribution of perfusion. Since these measurements are relatively insensitive and require special equipment, they are usually reserved for particular circumstances. 6. Tests describing regional ventilation/perfusion matching ($\dot{V}/\dot{Q}$). This includes direct evaluation of regional ventilation and perfusion as well as several different tests. These measurements are more important to understanding the mechanism of pulmonary function abnormalities than early detection. 7. Tests of diffusion. Although impaired diffusion of oxygen across the alveolar-capillary membrane is not a common cause of hypoxemia (13, 14), diffusing capacity is affected by a wide variety of lung diseases and therefore is a good screening test. 8. Blood gas measurement. These important parameters are affected by abnormalities, single or combined, including altered respiratory mechanics, right to left shunting, altered $\dot{V}/\dot{Q}$ relationships, control of respiration, and acid-base balance. Although not specific for any one type of abnormality, arterial hypoxemia is a sensitive indicator of impaired pulmonary function from a multitude of causes, while mixed venous oxygen tension is more indicative of tissue oxygenation and more often reflects the state of cardiac rather than pulmonary function.

DISCUSSION

Pathologic studies indicate that a surprisingly high percentage of autopsied nonsmoking adults have pulmonary emphysema (135, 136), while a higher incidence of emphysema in both smokers and nonsmokers has been found in areas with high atmospheric concentrations of sulfur oxides, nitrogen oxides, hydrocarbons, and particulates (136). Clinical chronic bronchitis is more common in urban areas (137), and children from urban environments have maximal expiratory flow rates lower than predicted (138). Though much of this data can be explained by high atmospheric levels of common pollutants, viral respiratory infections and cigarette smoking probably have additive deleterious effects upon lung function (136, 138).

The toxicity of all potential atmospheric contaminants, alone or in combination, as well as safe exposure limits, needs to be defined. One approach to this problem is exposure of humans or animals, under strictly controlled experimental conditions, to varying concentrations, durations, and combinations of inhalants. The results of such investigations must be objective and reproducible, and the methods must be sensitive and capable of large-scale utilization. The use of the pulmonary

Table I Characteristics and limitations of pulmonary function tests

	Name of test	Physiological interpretation	Animal species	Experimental conditions	Sensitivity	Limitations	Equipment needed	References
Ventilatory exchange	Respiratory rate.	Frequency of breathing.	Any.	Many.	Low, large animals; moderate, small animals.	Very few.	Spirometer, face mask for large animals or plethysmograph, pressure transducer, recorder for small animals. Pneumograph and transthoracic impedance are noninvasive.	26
	Tidal volume.	Depth (volume of breathing).	Any.	Many	Low, large animals; moderate, small animals.	Very few.	Spirometer, face mask for large animals or plethysmograph, pressure transducer, recorder for small animals. Pneumograph and transthoracic impedance are noninvasive.	26
	Minute ventilation.	Total volume of breathing in one minute (may measure inspiration or expiration) Equals the product of respiratory rate multiplied by tidal volume	Any.	Many	Low, large animals; moderate, small animals.	Very few.	Spirometer, face mask for large animals or plethysmograph, pressure transducer, recorder for small animals. Pneumograph and transthoracic impedance are noninvasive.	26
Static lung volumes[a]	Total lung capacity.	Elasticity of lungs and thorax, muscle strength.	Any (see experimental conditions).	Only man without external forces; animals (30 cm H_2O) distending pressure.	Good.	Requires maximal effort or external distending and/or withdrawing pressures	Spirometer plus helium catharometer, nitrogen meter, or other inert gas measuring device or body plethysmograph.	27–30
	Vital capacity.	Elasticity of lungs and thorax, muscle strength.	Any (see experimental conditions).	Only man without external forces; animals (30 cm H_2O) distending pressure	Good.	Requires maximal effort or external distending and/or withdrawing pressures.	Spirometer.	30, 31

Table 1 *(Continued)*

	Name of test	Physiological interpretation	Animal species	Experimental conditions	Sensitivity	Limitations	Equipment needed	References
Static lung volumes[a] *(continued)*	Residual volume.	Elasticity of lungs and thorax, muscle strength.	Any (see experimental conditions).	Only man without external forces; animals (30 cm H_2O) distending pressure.	Good.	Requires maximal effort or external distending and/or withdrawing pressures.	Spirometer plus inert gas measuring device of body plethysmograph.	30, 31
	Expiratory reserve volume.	Expiratory force, diaphragm position.	Any (see experimental conditions).	Only man without external forces; animals (30 cm H_2O) distending pressure.	Good.	Requires maximal effort or external distending and/or withdrawing pressures.	Spirometer	31
	Functional residual capacity.	Elasticity of lungs and thorax.	Any (see experimental conditions).	Resting.	Good.	Almost none.	Spirometer (body plethysmograph, with cooperation).	30, 32, 33
	Inspiratory capacity.	Inspiratory force, diaphragm position.	Any (see experimental conditions).	Only man without external forces.	Good.	See TLC.	Spirometer (body plethysmograph, with cooperation).	31
Respiratory mechanics Compliance	Static lung and thoracic cage compliance (C total).	Stiffness of respiratory system. $\frac{1}{C_{total}} = \frac{1}{C_{stat\ (\varrho)}} + \frac{1}{C_{stat\ (w)}}$ (liter/cm H_2O)	Any (see experimental conditions).	Results uncertain without anesthesia and paralysis of respiratory muscles.	Moderate or less.	Requires cooperation or relaxing anesthesia plus external forcing.	Pressure and volume transducers, amplifiers and recorders. One method requires head-out body chamber.	34, 35
	Static lung compliance. (Static volume pressure curves) [Cst (ϱ)]	Stiffness of the lungs quasistatic. A measure of distensibility, reciprocal of elastance. (liter/cm H_2O)	Mammals, any (see experimental conditions).	Only in man without relaxing anesthesia, for full curves. Animals: for Cst (ϱ) in tidal volume range in unanesthetized or full range with relaxing anesthesia.	Moderate.	Requires cooperation or relaxing anesthesia plus external forcing (see experimental conditions).	Pleural (animals only) or esophageal balloon, pressure and volume transducers, amplifiers, recorder, or CRT, or X-Y plotter, or tape.	36

Table 1 *(Continued)*

Respiratory mechanics								
Compliance *(continued)*	Static thoracic cage compliance [C_{stat} (w)].	Stiffness of chest wall. (liter/cm H_2O)	See Cst and C total.	See Cst and C total.	See Cst and C total.	See Cst and C total.	See Cst and C total.	37
	"Specific" compliance.	Cst/V_{tg} where V_{tg} is usually at functional residual capacity. (liter/cm H_2O per liter)	Mammals, any (see experimental conditions).	For Cst (ℓ) in tidal volume range. Relaxing anesthesia may not be required.	Moderate or less.	See Cst (ℓ), requires measurement of FRC.	See Cst (ℓ) and FRC.	37
	Dynamic lung compliance [Cdyn (ℓ)].	Stiffness of lung at specified frequency. Frequency dependent if Cdyn is a function of frequency. (liter/cm H_2O)	Mammals, any (see experimental conditions).	Usually only man without relaxing anesthesia for studies at a full range of frequencies.	Moderate or less.	Usually requires cooperation or relaxing anesthesia plus external forcing.	Same as for static volume pressure curves plus a pneumotachograph measure flow.	1, 38, 39
	Static volume pressure curves of saline-filled excised lungs.	Tissue (quasi static) distensibility. (liter/cm H_2O)	Mammals, any (at necropsy).	Saline-filled excised lungs.	Moderate.	Leakage and lack of uniform filling can create problems. Excised lungs.	Pressure and volume transducers, amplifiers, and recording devices.	40
Airflow	Spirometry-forced expired volume vs time.	Overall mechanical function of lungs and thoracic wall including flow rates at various parts of the expiratory curve (e.g. maximum midexpiratory flow in liters/sec) and FEV in liters at various times (e.g., 0.75, 1, 2, 3 sec).	Any (see experimental conditions).	Usually only man without relaxing anesthesia with external forcing.	Good to moderate.	Usually requires cooperation, a relaxing anesthesia plus external forcing.	Low resistance spirometer or pneumotachograph with integrator.	41

Table 1 *(Continued)*

	Name of test	Physiological interpretation	Animal species	Experimental conditions	Sensitivity	Limitations	Equipment needed	References
Respiratory mechanics								
Airflow *(continued)*	Flow-volume maximum expiratory flow-volume curves (MEF).	Overall mechanical function of lung and thoracic wall including Peak Flow Rates (PEFR) and flow rates at various volumes (e.g. MEF_{50} at 50% of vital capacity).	Mammals, any (see experimental conditions).	Usually only man without relaxing anesthesia. Measurements with gases of different physical properties can be done (e.g. He or SF_6).	Good to moderate.	Usually requires cooperation or relaxing anesthesia plus external forcing.	Storage oscilloscope or a photographic X-Y recorder or tape. Low resistance spirometer or pneumotachograph with integrator.	42
	Flow-volume inspiratory maximum inspiratory flow-volume curves (MIF).	Overall mechanical function of lung and thoracic wall including Peak Flow Rates (PIFR) and flow rates at various volumes (e.g. MIF_{50} at 50% of vital capacity).	Mammals, any (see experimental conditions).	Usually only man without relaxing anesthesia. Measurements with gases of different physical properties can be done (e.g. He or SF_6).	Good to moderate.	Usually requires cooperation or relaxing anesthesia plus external forcing.	Storage oscilloscope or a photographic X-Y recorder or tape. Low resistance spirometer or pneumotachograph with integrator.	42
	Lung and thoracic cage flow resistance (total resistance) (Rrs).	Changes in total respiratory system. (cm H_2O/liter per sec)	Mammals, any.	Can use several frequencies (e.g. 3, 6, 12, 24 Hz).	Good to moderate.	Specificity of interpretation limited.	Oscillatory equipment, transducers for flow, pressure, amplifiers, recorders: stripchart, tape.	43, 44
	Total lung flow-resistance (Rℓ).	Flow-resistance of airways and lung tissue $R\ell = Raw + R\ell t$. (cm H_2O/liter per sec)	Mammals, any; (see Cst (ℓ).	See Cst (ℓ).	Moderate.	See Cst (ℓ).	Pleural (animals only) or esophageal balloon, pressure and flow transducers, amplifiers, recorder or CRT or X-Y plotter, or tape.	45, 46

Table 1 *(Continued)*

Respiratory mechanics Airflow *(continued)*	Airway flow-resistance (Raw).	Flow-resistance of airways. $Raw = R_{peripheral} + R_{central}$. (cm H_2O/liter per sec)	Mammals, usually only man.	(Method #1, body plethysmograph) Usually only man.	Moderate or less.	Requires panting.	Body plethysmograph.	47
	Small airways flow-resistance (R_p).	Partition of flow-resistance to airways usually of < 2-3 mm diameter. (cm H_2O/liter per sec).	Difficult with small animals.	Only animals or excised human lungs.	Good to moderate.	Requires invasive procedure.	Retrograde catheter, transducers for flow pressure, amplifiers, recorders (see R_ℓ).	48
	Specific airway resistance [SR (aw)].	1/SG (aw).	See SG (aw).	See SG (aw).	See SG (aw).	See SG (aw).	See SG (aw).	49
	Frictional resistance lung tissue (R_ℓ).	Subtraction of Raw from total lung flow-resistance. $R_\ell t = R_\ell - Raw$. (cm H_2O/liter per sec).	See Raw and total lung flow-resistance.	See Raw and total lung flow-resistance.	See Raw and total lung flow-resistance.	See Raw and total lung flow-resistance.	See Raw and total lung flow resistance.	50
	Airway conductance (Gaw).	$Gaw = {}^1/Raw$. (liter/sec per cm H_2O)	See Raw.	See Raw.	See Raw.	See Raw.	See Raw.	28, 51, 52
	Specific airway conductance [SG (aw)].	$({}^1/Raw)/V_{tg}$ where V_{tg} usually = FRC airway conductance per unit lung volume.	See Raw and V_{tg}.	See Raw; see V_{tg}.	Moderate.	Need measure of V_{tg}.	See Raw and V_{tg}.	28, 51, 52

Table 1 *(Continued)*

	Name of test	Physiological interpretation	Animal species	Experimental conditions	Sensitivity	Limitations	Equipment needed	References
Respiratory mechanics								
Work of breathing	Work of breathing, lungs and thoracic wall.	Work of moving lungs and thoracic wall (kgM/min).	Any (see experimental conditions).	Results uncertain without anesthesia and paralysis of respiratory muscles. Work of inspiration and expiration can be separated.	Moderate or less.	Combined measure.	See C total pressure, flow volume (recorder), body respirator.	35, 53, 54
	Work of breathing, lungs.	Work of moving lungs. (kgM/min)	Any.	See Cst and total lung flow-resistance. Work of inspiration can be separated.	Moderate.	See Cst and total lung flow-resistance.	See Cst and total lung flow-resistance.	35, 53, 54
	Work of breathing, thoracic wall.	Work of moving thoracic wall. (kgM/min)	Any.	Results uncertain without anesthesia and paralysis of respiratory muscles. Work of inspiration and expiration can be separated.	Moderate or less.	See experimental conditions.	See C total pressure, flow volume (recorder), body respirator.	35, 53, 54
Distribution of ventilation[b]	Closing volume ^{133}Xe (bolus distribution).	Closure of dependent airways.	Man, rabbit probably minimal size; larger animals better.	Requires cooperation. Only man without anesthesia. May be done in large animals with anesthesia, positive and negative pressure breathing.	Moderate; most sensitive if measured as closing capacity/TLC (Closing capacity = CV + RV).	See experimental conditions.	Spirometer; flow meter; scintillation counter; digital rate meters, physiologic recorder.	55, 56
	Closing volume (helium bolus distribution).	Closure of dependent airways.	Man, rabbit probably minimal size; larger animals better.	Requires cooperation. Only man without anesthesia. May be done in large animals with anesthesia, positive and negative pressure breathing.	Moderate; most sensitive if measured as closing capacity/TLC (Closing capacity = CV + RV).	See experimental conditions.	Spirometer; flow meter; critical orifice helium analyzer; physiologic recorder.	57

Table 1 *(Continued)*

Distribution of ventilation *(continued)*	Closing volume (argon bolus distribution).	Closure of dependent airways.	Man, rabbit probably minimal size; larger animals better.	Requires cooperation. Only man without anesthesia. May be done in large animals with anesthesia, positive and negative pressure breathing.	Moderate; most sensitive if measured as closing capacity/TLC (Closing capacity = CV + RV).	See experimental conditions.	Spirometer; flow meter; mass spectrometer, physiologic recorder.	11
	Closing volume (nitrogen dilution).	Closure of dependent airways.	Man, rabbit probably minimal size; larger animals better.	Requires cooperation. Only man without anesthesia. May be done in large animals with anesthesia, positive and negative pressure breathing.	Moderate; may be slightly less sensitive than bolus techniques. Measure as CC/TLC.	See experimental conditions.	Spirometer, flow meter, nitrogen analyzer, physiologic recorder.	58
	Nitrogen washout, single breath.	Distribution of ventilation.	Man, rabbit probably minimal size; larger animals better.	Requires cooperation. *Only man without* anesthesia. May be done in large animals with anesthesia, positive and negative pressure breathing.	Moderate; may be *normal when* dynamic measurements are abnormal.	See experimental conditions.	Spirometer, flow meter, nitrogen analyzer, physiologic recorder.	9, 59
	Nitrogen washout, multi-breath.	Distribution of ventilation.	Man, large animal, beagle dog, Shetland pony, monkey, baboon.	Only man and beagle without anesthesia; animals require tight-fitting face mask; restraints.	High; sensitivity increased by washout at high respiratory rates and poor collateral ventilation.	See experimental conditions.	Spirometer or pneumotachograph; nitrogen analyzer; physiologic recorder.	60–62
	Regional pulmonary function, ^{133}Xe technique.	Topographical distribution of ventilation.	Man, baboon, monkey.	Only man without E-T tube; anesthesia, controlled ventilation.	High, man; low, animals (due to limited experience).	See experimental conditions.	Spirometer; multiple scintillation counters or Anger camera; computer or strip charts, physiologic recorder, ventilator, air pump (animals).	12, 63
	^{133}Xe washout multi-breath.	Distribution of ventilation.	Man, baboon.	Only man without E-T tube; anesthesia, controlled ventilation.	High, man; low, animals; not yet well quantitated or subjected to compartmental analysis in animals.	See experimental conditions.	Spirometer; multiple scintillation counters or Anger camera; computer or strip charts, physiologic recorder, ventilator, air pump (animals).	63–65

Table 1 *(Continued)*

	Name of test	Physiological interpretation	Animal species	Experimental conditions	Sensitivity	Limitations	Equipment needed	References
Pulmonary circulation	Cardiovascular pressures.	Intravascular diastolic, systolic, and/or mean pressures. Detects hyper- or hypotension in vascular system. Necessary for calculations of vascular resistances and ventricular work.	Any (see conditions). Difficult in small rodents.	Awake animals following preparation of indwelling catheters. Anesthetized animals usual.	Can be done with good accuracy and reproducibility. Moderate to low sensitivity for pulmonary disease.	Few limitations if one has adequate training and equipment. Frequency-response of equipment and proper application essential. Animals should be studied under similar conditions and levels of activity.	Cardiac catheters, strain gauges, and recorder. Surgical equipment, fluids, drugs, and variety of stopcocks.	65-75
	Cardiovascular volumes, flows, resistance, and work.	Cardiovascular performance.	Any (see conditions). Difficult in small rodents.	All can be done on reasonably tractable awake animal *except* pulmonary capillary blood volume and pulmonary capillary blood flow.	Can be done with good accuracy and reproducibility. Moderate to low sensitivity for pulmonary disease.	Calculated (derived) values. Each value depends upon several variables. Otherwise limitations as listed above.	As above. Dye dilution equipment, body plethysmograph, and appropriate strain gauges. Appropriate drugs and test gases.	65-81
	Distribution of perfusion ^{133}Xe technique.	Regional distribution of pulmonary blood in the lung.	Meaningful only in animals the size of cats *or larger*.	Animals usually anesthetized and positioned with limited movement.	Moderate to low.	Expensive equipment. Useful only on larger animals. Requires use of radioactive material.	Four scintillation detectors and cylindrical collimetors. Magnetic tape recorder. Rate meter.	82-84
	^{135}I-macroaggregated albumin technique.	Regional distribution of pulmonary blood in the lung.	Meaningful only in animals the size of cats *or larger*.	Animals usually anesthetized and positioned with limited movement.	Moderate to low.	Expensive equipment. Useful only on larger animals. Requires use of radioactive material.	Scanner. Radiological equipment.	82-84
	Right to left pulmonary vascular shunt during O_2 breathing.	Percentage of the cardiac output that is bypassing ventilated exchange area in the lung.	More conveniently done in animals at least the size of cats.	Animal must breathe 100% O_2 without rebreathing. Animals usually anesthetized. Must measure O_2 concentration in inspired air, expired air, arterial and mixed venous blood.	Depends upon how rigorously each measurement is made for the shunt calculation. Moderate to good for advanced, chronic lung disease.	Accuracy of measure of O_2 content in mixed venous blood and alveolar O_2.	Blood gas analysis equipment; blood gas pressure analysis, Scholander and perhaps Van Slyke or gas chromatograph with gas extractor.	85, 86

Table 1 *(Continued)*

Pulmonary circulation *(continued)*	Matching of ventilation and perfusion.	Regional distribution of ventilation relative to perfusion in the lungs.	Meaningful only in animals the size of cats or larger.	Animals usually anesthetized and positioned with limited movement.	Moderate to low.	Expensive equipment. Useful only on larger animals. Requires use of radioactive material.	Expensive equipment. Useful only on larger animals. Requires use of radioactive material.	55, 87-93
	Reflexes							
	Pulmonary vascular effects of breathing O_2.	Measurement of effect of high O_2-concentrations upon pulmonary vascular resistance (see Cardiovascular volumes, flows, resistance, and work, above) and and distribution of perfusion (see Distribution of perfusion, above, and Matching of ventilation and perfusion, above).	All experimental animals.	Awake animals following preparation of indwelling catheters. Anesthetized animals usual.	Moderate to low.	Few limitations if one has adequate training and equipment. Frequency-response of equipment and proper application essential. Animals should be studied under similar conditions and levels of activity.	As in Cardiovascular pressures, above, and breathing equipment for giving O_2.	94-102
	Histamine, fibrinopeptide B, bradykinin analysis.	Chemical and/or biological analysis for concentration of pulmonary vasoactive agents.	All experimental animals.	Chemical and/or biological analyses of concentrations in plasma, blood or tissue.	Low.	Availability of techniques.	Cardiac catheters, strain gauges, and recorder. Surgical equipment, fluids, drugs, and variety of stopcocks. Breathing equipment for giving O_2.	103, 104
	Diffusion perfusion ratio studies.	Measure of the distribution of pulmonary diffusion relative to pulmonary perfusion.	Animals at least the size of cats.	Animals anesthetized and usually terminal preparation. Requires special gas handling equipment.	Moderate to unknown.	Requires excellent experimental control and measurement of pulmonary and cardiovascular variables.	Respiratory gas chromatograph. Ability to handle and analyze labeled O_2. Breathing equipment.	105-107
	Edema evaluation.							
	In vivo, 112 indium-transferrin.	Estimate of extravascular fluid accumulation in the lung.	Animals at least the size of cats. Technique confirmed only on sheep.	Animal must be restrained by counters.	Moderate to unknown.	New technique must be confirmed on species other than sheep.	Four scintillation detectors and cylindrical collimetors. Magnetic tape recorder. Rate meter.	108-111

Table 1 *(Continued)*

	Name of test	Physiological interpretation	Animal species	Experimental conditions	Sensitivity	Limitations	Equipment needed	References
Pulmonary circulation *(continued)*	Edema evaluation. (continued)							
	In vitro wet/dry weight ratios.	Measurement of total lung H_2O.	All experimental species.	Study of lung tissue after death.	Moderate to good.	Animals must be sacrificed.	Laboratory balance and desiccating oven.	108-111
	In vivo pulmonary tissue volume.	Estimate of tissue volume exposed to and in equilibrium with gas in airways.	Meaningful only in animals the size of cats *or larger*.	Anethetized controlled airways in animals.	Moderate.	Indirect measurement.	Gas analysis gas-volume measurement.	76
	Postmortem pulmonary arterial and bronchial arterial casts.	Relative distribution of pulmonary and bronchial circulations.	All experimental species and postmortem material from human beings.	Postmortem material.	Moderate to low.	Access to material. Tedious work requiring long man hours.	Vascular canula, latex, or other appropriate injection material.	112
Regional ventilation/perfusion matching ($\dot{V}/\dot{Q}$)	Arterial PO_2.		See Blood gases					
	$AaDO_2$.	$\dot{V}/\dot{Q}$ or shunt.	Any but larger better.		Good.	Requires arterial blood and measurement of alveolar gas (Scholander, gas chromatograph, mass spectrometer).		86, 113
	$AaDN_2$.	$\dot{V}/\dot{Q}$.	← Similar to $AaDO_2$.	Good.		Requires arterial blood and measurement of alveolar gas (blood gas extractor, gas chromotograph, recorder).		114
	Radioisotopes.	Regional $\dot{V}/\dot{Q}$.	Rabbit probably minimal size.	Any.	Fair.	Restraint, cooperation or anesthesia required.	Multiple probes or scintillation camera.	83
	Radioisotopes.	Regional ventilation.	Rabbit probably minimal size.	May be measured during breath holding or during breathing.	Fair.	Deposited aerosols do not measure ventilation; ^{133}Xe most convenient isotope.		115
	Radioisotopes.	Regional perfusion.	Rabbit probably minimal size.	May be measured during breath holding or during breathing.	Fair.	^{113}In, ^{131}I, or ^{99m}Tc, combined with albumin or other 30 μ particles most widely used; ^{133}Xe dissolved in saline useful when studies need to be repeated rapidly.		115

Table 1 *(Continued)*

Regional ventilation/ perfusion matching ($\dot{V}/\dot{Q}$) *(continued)*	Single expiration PCO_2 and R.	$\dot{V}/\dot{Q}$	Any, but larger better.	Slow, complete expiration.	Fair	Cannot quantitate.	CO_2, O_2 meters of mass spectrometer.	5, 116
	V_D/V_t.	$\dot{V}/\dot{Q}$ particularly high ratios.	Any, but larger better.	Need constant breathing pattern.	Fair.	Hard to quantitate.	Analysis of CO_2 in mixed expired gas and arterial blood.	113
	Lobar gas sampling.	Regional $\dot{V}/\dot{Q}$.	Large.	Requires lobar catheters.	Fair.	Invasive, anesthesia.	Catheter, gas analyzers.	117
	Multiple inert gas washout.	Distribution of $\dot{V}/\dot{Q}$.	Any, but larger better.	Collection of expired gas, venous infusion, cardiac output measurement.	Good.	Somewhat complicated.	Gas chromatography mass spectography; dye dilution or Fick cardiac output.	118
Diffusion	D_LCO (SB).	D_M, V_C, Hgb (see below).	Larger better.	Timed breath holding at TLC.	Good.	Cooperation or anesthesia.	Co and He meters or gas chromatograph.	119, 120
	D_LCO (SS).	Above plus $\dot{V}/\dot{Q}$.	Larger better.	Regular breathing.	Good.	Cooperation or anesthesia.	Same plus measurement of V_D (physiol).	121-123
	DM.	Thickness and quantity of membrane.	Larger better.	Timed breath holding at TLC.	Good.	Cooperation or anesthesia.	CO, O_2, and He measurement.	124
	V_C.	Pulmonary capillary blood volume.	Larger better.	Timed breath holding at TLC.	Good.	Cooperation or anesthesia.	CO, O_2, and He measurement.	124
	D_LO_2.	D_LO_2.	Larger better.	Regular breathing.	Good.	Computation of mean capillary PO_2 difficult.	Measurement of VO_2, V_{CO2} at 2 levels of oxygenation.	107, 125
	D_LCO (RB)	Less affected by $\dot{V}/\dot{Q}$.	Larger better.	Breath by breath analysis.	Good.	Complex method and computation.	Rapidly responding analyzers.	126, 127

Table 1 *(Continued)*

	Name of test	Physiological interpretation	Animal species	Experimental conditions	Sensitivity	Limitations	Equipment needed	References
Blood gases	Arterial PCO_2.	Total alveolar ventilation.	Any, but larger better.	Any.	Good.	Requires accessibility of artery.	Anaerobic blood collection, anticoagulant (blood gas analyzer).	128–130
	Mixed venous PCO_2.	Total alveolar ventilation.	Any, but larger better.	Any.	Good.	Requires mixed venous blood.	Anaerobic blood collection, anticoagulant (blood gas analyzer).	131
	Arterial pH.	HCO_3/PCO_2.	Any, but larger better.	Any.	Good.	Arterial blood.	Anaerobic blood collection, anticoagulant (blood gas analyzer).	129, 130 132, 133
	Mixed venous pH.	HCO_3/PCO_2.	Any, but larger better.	Any.	Good.	Mixed venous blood.	Anaerobic blood collection, anticoagulant (blood gas analyzer).	132–134
	Arterial PO_2.	Regional V/Q $R \rightarrow L$ shunt, alveolar ventilation.	Any, but larger better.	Any.	Good.	Arterial blood.	Anaerobic blood collection, anticoagulant (blood gas analyzer).	128, 129
	Mixed venous PO_2.	Above plus cardiac output and $\dot{V}O_2$.	Any, but larger better.	Any.	Good.	Mixed venous blood.	Anaerobic blood collection, anticoagulant (blood gas analyzer).	128, 129

[a] In general, FRC is measured by inert gas dilution, nitrogen washout, or body plethysmography; VC, ERV, and IC by spirometry; TLC and RV are usually calculated.

[b] Distribution of ventilation may be defined as a description of the uniformity of distribution of inspired gas. In a hypothetical lung with perfectly uniform distribution of ventilation, the ratio regional tidal volume per regional lung volume is equal for all alveoli.

function tests described in this manuscript would seem satisfactory for these purposes.

Similar considerations apply to the pulmonary toxicology of a variety of other inhaled (and, in some cases, ingested) substances. If a potentially toxic inhalant is to be investigated, certain tests are more valuable than others. While the factors of reproducibility, sensitivity, and specificity are important, the choice of specific pulmonary function tests also depends upon other factors such as anatomic characteristics of the species to be tested. For example, in dogs, multibreath nitrogen washout might be expected to be a relatively insensitive detector of mild physiologic abnormality since the dog lung has a highly developed collateral ventilation system, a factor known to decrease the sensitivity of tests measuring distribution of ventilation. On the other hand, since the pig lung has poorly developed collateral pathways, multibreath nitrogen washout should be an effective means of detecting maldistribution of ventilation. Multibreath nitrogen washout would be expected to be a reasonably sensitive technique in man, whose lung has collateral pathway development intermediate between that of the dog and the pig. Hence, effective selection of pulmonary function methods will depend not only on the scientific information sought but also the nature of the subject, the test itself, and the experimental conditions. These factors should all be considered in designing inhalation toxicology protocols.

ACKNOWLEDGMENT

Preparation of this review was aided by USAF Contract F33615-76-C-5005 and by California Air Resources Board Contract 4–611 with the Departments of Medicine and of Community and Environmental Medicine, California College of Medicine, University of California, Irvine, California.

Literature Cited

1. Woolcock, A. J., Vincent, N. J., Macklem, P. T. 1969. *J. Clin. Invest.* 48:1097–1105
2. Macklem, P. T. 1972. *Am. J. Med.* 52:721–24
3. Lundgren, D. A. 1970. *J. Pure Air Pollut. Control Assoc.* 20:603–8
4. Gorden, R. J., Bryan, R. J. 1973. *Environ. Sci. Technol.* 7:645–47
5. Bates, D., Hazucha, M. 1973. In *Proc. Conf. Health Eff. Air Pollut., Nat. Acad. Sci.*, 507–40
6. McFadden, E. R. Jr., Lincoln, D. A. 1972. *Am. J. Med.* 52:725–37
7. Morris, J. F., Koski, A., Breese, J. D. 1975. *Am. Rev. Respir. Dis.* III:755–62
8. Gelb, A. F., Zamel, N. 1973. *N. Engl. J. Med.* 268:395–98
9. Buist, A. S., Ross, B. B. 1973. *Am. Rev. Respir. Dis.* 108:1078–87
10. Wanner, A., Zarzeck, S., Atkins, N., Zapata, A., Sackner, M. A. 1974. *J. Clin. Invest.* 54:1200–1213
11. McCarthy, D. S., Spencer, R., Greene, R., Milic-Emili, J. 1972. *Am. J. Med.* 52:747–53
12. Milic-Emili, J., Henderson, J. A. M., Dolovich, M. B., Trop, D., Kaneko, K. 1966. *J. Appl. Physiol.* 21:749–59
13. Bates, D. V., Macklem, P. T., Christie, R. V. 1971. *Respiratory Function in Disease*, p. 71. Philadelphia: Saunders
14. Cole, R. B., Bishop, J. M. 1963. *J. Appl. Physiol.* 18:1043–48
15. McFadden, E. R. Jr., Kiker, R., Holmes, B., De Groot, W. J. 1975. *Am. J. Med.* 57:171–81
16. Black, L. F., Offord, K., Hyatt, R. E. 1975. *Am. Rev. Respir. Dis.* 110:282–92
17. Hutcheon, M., Griffin, P., Lewison, H., Zamel, N. 1974. *Am. Rev. Respir. Dis.* 110:458–65

18. Hogg, J. C., Macklem, P. T., Thurlbeck, W. 1968. *N. Engl. J. Med.* 278:1355–60
19. Arndt, H., King, T. K. C., Briscoe, W. A. 1970. *J. Clin. Invest.* 49:408–22
20. Bouhys, A. 1974. *Breathing*, p. 102. New York: Grune & Stratton
21. Mauderly, J. L. 1972. *Am. J. Vet. Res.* 33:1485–91
22. Thelenius, O. C. 1963. *J. Appl. Physiol.* 18:439–40
23. Muggenburg, B. A., Mauderly, J. L. 1974. *J. Appl. Physiol.* 37:152–57
24. Moorman, W. J., Lewis, T. R., Wagner, A. D. 1975. *J. Appl. Physiol.* 39:444–48
25. Amdur, M. O., Mead, J. 1958. *Am. J. Physiol.* 192:364–68
26. Comroe, J. H. Jr., Forster, R. E. II, Dubois, A. B., Briscoe, W. A., Carlsen, E. 1962. *The Lung: Clinical Physiology and Pulmonary Function Tests*, pp. 27–51. Chicago Yearb. Med.
27. Gilson, J. C., Hugh-Jones, P. 1949. *Clin. Sci.* 7:185–216
28. DuBois, A. B., Botelho, S. Y., Bedell, G. N., Marshall, R., Comroe, J. H. Jr. 1956. *J. Clin. Invest.* 35:322–26
29. Baldwin, E. de F., Cournand, A., Richards, D. W. Jr. 1948. *Medicine* 27: 248–78
30. Robinson, N. E., Gillespie, J. R. 1973. *J. Appl. Physiol.* 35:317–21
31. Kory, R. C., Callahan, R., Boren, H. G., Syner, J. C. 1961. *Am. J. Med.* 30:243–58
32. Tierney, D. F., Nadel, J. A. 1962. *J. Appl. Physiol.* 17:871–73
33. Darling, R. C., Cournand, A., Richards, D. W. Jr. 1940. *J. Clin. Invest.* 19:609–18
34. Rohrer, F. 1916. *Arch. Gesch. Physiol.* 165:419–44
35. Rahn, H. et al 1946. *Am. J. Physiol.* 146:161–78
36. Fry, D. L. 1954. *Am. J. Med.* 16:80–97
37. Comroe, J. H., Forster, R. E. II, Dubois, A. B., Briscoe, W. A., Carlsen, E. 1968. See Ref. 26, pp. 172–73
38. Neergaard, K., Wirz, K. 1927. *Z. Tschr. Klin. Med.* 105:35–51
39. Otis, A. B., McKerrow, C. B., Bartlett, R. A., et al. 1956. *J. Appl. Physiol.* 8:427–443
40. Neergaard, K. 1929. *Z. Gesamte Exp. Med.* 66:373–94
41. Gaensler, E. A. 1961. *Ann. Rev. Med.* 12:385–408
42. Hyatt, R. E., Schilder, D. P., Fry, D. L. 1958. *J. Appl. Physiol.* 13:331–36
43. DuBois, A. B. et al 1956. *J. Appl. Physiol.* 8:587–94
44. Goldman, M. et al 1970. *J. Appl. Physiol.* 28:113–16
45. Mead, J., Whittenberger, J. L. 1953. *J. Appl. Physiol.* 5:779–96
46. Milic-Emili, J. et al 1964. *J. Appl. Physiol.* 19:207–11
47. Mead, J., Whittenberger, J. L. 1954. *J. Appl. Physiol.* 6:408–16
48. Macklem, P. T., Mead, J. 1967. *J. Appl. Physiol.* 22:395–401
49. Bates, D. V., Macklem, P. T., Christie, R. V. 1971. See Ref. 13, pp. 27–28
50. Marshall, R., DuBois, A. B. 1956. *Clin. Sci.* 15:161–70
51. DuBois, A. B., Botelho, S. Y., Comroe, J. H. 1956. *J. Clin. Invest.* 35:327–35
52. DuBois, A. B., van de Woestijne, K. P., eds. 1969. *Int. Symp. Body Plethysmography*, Nijmegen, 1968, *Prog. Respir. Res.*, Vol. 4. Basel & New York: Karger
53. Otis, A. B. 1954. *Physiol. Rev.* 34:449–59
54. Otis, A. B. 1964. *Handb. Physiol.* 1: 463–76
55. Dolfuss, R. E., Milic-Emili, J., Bates, D. V. 1967. *Respir. Physiol.* 2:234–46
56. Leblanc, P., Ruff, F., Milic-Emili, J. 1970. *J. Appl. Physiol.* 28:448–51
57. Green, M., Travis, D. M., Mead, J. 1972. *J. Appl. Physiol.* 33:827 (Abstr.)
58. Anthonisen, N. R., Danson, J., Robertson, P. C., Ross, W. R. D. 1969. *Respir. Physiol.* 8:58–65
59. Fowler, W. S. 1949. *J. Appl. Physiol.* 2:283–99
60. Fowler, W. S., Cornish, E. R., Kety, S. S. 1952. *J. Clin. Invest.* 31:40–50
61. Mauderly, J. L., Pickrell, J. A. 1972. *Proc. Nat. Conf. Lab. Animals Med.*, NIH, Washington DC
62. Dubin, S. E., Westcott, R. J. 1969. *Am. J. Vet. Res.* 30:2027–30
63. Tsai, S. A., Loken, M. K., Ponto, R., Griswa, P. 1969. *Minn. Med.* 52: 1893–97
64. Heidendal, G. K., Fontana, R. S., Tauxe, W. N. 1972. *Cancer* 30:1358–67
65. Deland, F. H., Wagner, H. N. Jr. 1970. *Atlas of Nuclear Medicine*, Vol. 2, *Lung and Heart*. Philadelphia: Saunders
66. Bergofsky, E. H. 1974. *Am. J. Med.* 57:378–94
67. Roos, A., Thomas, L. I., Nagel, E. L. 1961. *J. Appl. Physiol.* 16:77–84
68. Duke, H. N., Lee, G. 1963. *Br. Med. Bull.* 19:71–75
69. Comroe, J. H. Jr. 1966. *Circulation* 33:146–58
70. Caro, C. G. 1963. *Br. Med. Bull.* 19:66–70

71. Eberly, V. E., Gillespie, J. R., Tyler, W. S. 1964. *Am. J. Vet. Res.* 25:1712–15
72. Eberly, V. E., Tyler, W. S., Gillespie, J. R. 1966. *J. Appl. Physiol.* 21:883–89
73. Steffey, E. P., Gillespie, J. R., Berry, J. D., Eger, E. I., Rhode, E. A. 1974. *Am. J. Vet. Res.* 35:1315–19
74. Steffey, E. P., Gillespie, J. R., Berry, J. D., Eger, E. I., Rhode, E. A. 1974. *Am. J. Vet. Res.* 35:1289–93
75. Brody, J. S., Stemmler, E. J., DuBois, A. B. 1968. *J. Clin. Invest.* 47:783–99
76. Cander, L., Forster, R. E. 1959. *J. Appl. Physiol.* 14:541–51
77. Becklake, M. R., Varvis, C. J., Pengelly, L. D., Kenning, S., McGregor, M., Bates, D. V. 1962. *J. Appl. Physiol.* 17:579–86
78. Feisal, K. A., DuBois, A. B. 1962. *J. Clin. Invest.* 41:390–400
79. Lee, G. D. Van de Woestijne, K. P., eds. 1969. See Ref. 52, pp. 140–63
80. Lee, G. D., DuBois, A. B. 1955. *J. Clin. Invest.* 34:1380–90
81. Sackner, M. A., DuBois, A. B. 1965. *7th Conf. Res. Emphysema,* Aspen, 1964; *Med. Thorac.* 22:146–57
82. Anthonisen, N. R., Milic-Emili, J. 1966. *J. Appl. Physiol.* 21:760–66
83. Bell, W. C. Jr., Stewart, P. B., Newsham, L. G. S., Bates, D. V. 1962. *J. Clin. Invest.* 41:519–31
84. Hoppin, F. G. Jr., York, E., Kuhl, D. E., Hyde, R. W. 1967. *J. Appl. Physiol.* 22:469–74
85. Riley, R. L. 1951. *Am. J. Med.* 10: 210–20
86. Riley, R. L., Cournand, A. 1949. *J. Appl. Physiol.* 1:825–47
87. Dollery, C. T., Hugh-Jones, P. 1963. *Br. Med. Bull.* 19:59–65
88. Kaneko, K., Milic-Emili, J., Dolovich, M. B., Dawson, A., Bates, D. V. 1966. *J. Appl. Physiol.* 21:767–77
89. Maloney, J. E. 1967. *Phys. Med. Biol.* 12:161–72
90. Markello, R. A., Olszowka, A., Winter, P., Farhi, L. 1973. *Respir. Physiol.* 19:221–32
91. West, J. B. 1963. *Br. Med. Bull.* 19:53–58
92. Yokoyama, T., Farhi, L. E. 1967. *Respir. Physiol.* 3:166–76
93. West, J. B., Dollery, C. T. 1960. *J. Appl. Physiol.* 15:405–10
94. Dawson, A. 1969. *J. Clin. Invest.* 48:301–10
95. Cotes, J. E., Pisa, Z., Thomas, A. J. 1963. *Clin. Sci.* 25:305–10
96. Arborelius, M. Jr. 1969. *J. Appl. Physiol.* 26:101–4
97. Dugard, A., Naimark, A. 1967. *J. Appl. Physiol.* 23:663–71
98. Thilenius, O. G., Hoffer, P. B., Fitzgerald, R. S., Perkins, J. F. Jr. 1964. *Am. J. Physiol.* 206:867–74
99. Harris, P., Segel, N., Green, I., Housley, E. 1968. *Cardiovasc. Res.* 2:84–92
100. Said, S. I. 1974. *Science* 185:1181–83
101. Vane, J. R. 1969. *Br. J. Pharmacol.* 35:209–42
102. Piper, P., Vane, J. 1971. *Ann. NY Acad. Sci.* 180:363–85
103. Hauge, A. 1968. *Circ. Res.* 22:371–83
104. Hauge, A., Melmon, K. L. 1968. *Circ. Res.* 22:385–92
105. Staub, N. C. 1963. *J. Appl. Physiol.* 18:673–80
106. Piper, J. 1961. *J. Appl. Physiol.* 16:507–16
107. Hyde, R. W., Rynes, R., Power, G. G., Nairn, J. 1967. *J. Clin. Invest.* 46: 463–74
108. Liebow, A. A. 1969. In *The Pulmonary Circulation and Interstitial Space,* ed. A. P. Fishman. Chicago: Univ. Chicago Press
109. Gump, F. E., Mashima, Y., Ferenczy, A., Kinney, J. M. 1971. *J. Trauma* 11:474–82
110. Staub, N. C. 1970. *Hum. Pathol.* 1:419–32
111. Robin, E. D., Cross, C. E., Zelis, R. 1973. *N. Engl. J. Med.* 288:239–45, 292–304
112. Phelan, R. F., Yeh, H. C., Raabe, O. G., Velasquez, D. J. 1973. *Anat. Rec.* 177:255–63
113. Raino, J. M., Bishop, J. M. 1963. *J. Appl. Physiol.* 18:284–89
114. Groom, A. C., Morin, R., Farhi, L. E. 1967. *J. Appl. Physiol.* 23:706–12
115. Wilson, A. F. 1969. *Ann. Int. Med.* 71:155–76
116. West, J. B., Hugh-Jones, P. 1959. *Clin. Sci.* 18:553–59
117. Young, A. C., Martin, C. J., Pace, W. R. 1963. *J. Appl. Physiol.* 18:47–50
118. Wagner, P. D., Laravuso, R. B., Uhl, R. R., West, J. B. 1974. *J. Clin. Invest.* 54:54–68
119. Ogilvie, C. M., Forster, R. E., Blakemore, W. S., Morton, J. W. 1957. *J. Clin. Invest.* 36:1–7
120. Meade, F., Saunders, M. J., Hyett, F., Reynolds, J. A., Pearl, N., Cotes, J. E. 1965. *Lancet* 2:573–75
121. Krogh, A., Krogh, M. 1910. *Skand. Arch. Physiol* 22:236–47
122. Filley, G. F., MacIntosh, D. J., Wright, G. W. 1954. *J. Clin. Invest.* 33:530–39

123. Marshall, R. 1958. *J. Clin. Invest.* 37:394–408
124. Roughton, F. J. W., Forster, R. E. 1957. *J. Appl. Physiol.* 11:290–302
125. Farhi, L., Riley, R. L. 1957. *J. Appl. Physiol.* 10:179–85
126. Lewis, B. M., Lin, T. H., Noe, F. E., Hayford-Welsing, E. J. 1959. *J. Clin. Invest.* 38:2073–86
127. Mittman, C. 1967. *J. Appl. Physiol.* 23:131–38
128. Severinghaus, J. W., Bradley, A. F. 1958. *J. Appl. Physiol.* 13:515–20
129. Severinghaus, J. W., Stupfel, M., Bradley, A. F. 1956. *J. Appl. Physiol.* 9:189–96
130. Astrup, P. 1956. *Scand. J. Clin. Lab. Invest.* 8:33–43
131. Hackney, J. D., Sears, C. H., Collier, C. R. 1958. *J. Appl. Physiol.* 12:425–30
132. Bates, R. C. 1964. *Determination of pH; Theory and Practice,* pp. 379–80. New York: Wiley
133. Wilson, A. F., Simmons, D. H. 1970. *Clin. Sci.* 39:731–45
134. Denison, D., Edwards, R. H. T., Jones, G., Pope, H. 1969. *Respir. Physiol.* 7:326–34
135. Anderson, A. E. Jr., Hernandez, J. A., Holmes, W. L., Foraker, A. G. 1966. *Arch. Environ. Health* 12:569–77
136. Ishikawa, S., Bowden, D. H., Fisher, V., Wyatt, J. P. 1969. *Arch. Environ. Health* 18:660–66
137. Lambert, P. M., Reid, D. D. 1970. *Lancet* I:853–57
138. Zapletal, A., Jech, J., Paul, T., Samanek, M. 1973. *Am. Rev. Respir. Dis.* 107:400–409