8592

ESTIMATION OF AIRWAY RESPONSES TO PHYSIOLOGICAL STIMULI AND DRUGS[1,2]

MICHIYOSHI HARASAWA[3] AND SIMON RODBARD

University of Buffalo Chronic Disease Research Institute, Buffalo,[4] New York

Received for publication January 27, 1961

A number of methods have been devised to appraise changes in bronchomotor activity. Direct methods have included the contraction of tracheal chains (Castillo, 1948; McDougal and West, 1953), or bronchial strips (Macht and Gin-Ching Ting, 1921), changes in intrabronchial pressure (Petrouskaia, 1939; Rall and Gilbert, 1945), and bronchoscopic (Moersch, 1948) and radiographic (Nicholson, 1939) observations. Bronchomotion has also been evaluated on the basis of changes of tidal air volume in open-chest animals (Alcock *et al.*, 1936–37; deBurgh Daly and Mount, 1951). Other studies have analyzed changes in gross respiratory function by spirometric or pneumotachographic estimations of respiratory rate and tidal volume, vital capacity, functional residual volume, duration of the expiratory phase of respiration, and by calculation of airway resistance (Comroe *et al.*, 1954; Dubois *et al.*, 1956). Data have been obtained by a plethysmograph surrounding part or all of the lungs, or of the entire body of animals (deBurgh Daly and Mount, 1951) and man (Dubois *et al.*, 1956). The respiratory oscillations of the blood pressure have also served as a means of clinical appraisal (Rodbard *et al.*, 1956).

In some of these studies, pressure changes in the airway have been measured during insufflation with a fixed air volume (Loomis, 1956). Others have measured the changes of tidal volume during insufflation with a constant air pressure (Alcock *et al.*, 1936–37; deBurgh Daly and Mount, 1951; Konzett and Rössler, 1940; Konzett, 1956). However, many of these techniques are rather complex, requiring the use of specially designed spirometers and other equipment.

We have found that the pneumatic balance resuscitator (Burns valve) (Otis *et al.*, 1950) can provide a direct measurement of variations in the exchangeable air volume. This valve is in common use for administration of intermittent positive pressure respiration, in resuscitation, and for artificial respiration in animals (Greene *et al.*, 1952). The positive insufflation pressure is determined by the manual setting of a reducing valve. Insufflation from this constant pressure source continues until the pressure in the airway-respirator system rises to the preselected pressure setting of the reducing valve. The valve then cycles and the flow of air from the source is stopped; an exhaust valve opens simultaneously to permit escape of the air from the lungs to the atmosphere. When the tracheal pressure falls to nearly ambient levels, the insufflation phase begins again.

The durations of the insufflation and the expiratory phases of the machine are proportional to the volume of air available for exchange under circumstances of the experiment. Changes in the available air volume may thus be recorded as increases or decreases in duration of the respiratory cycle. The available air volume is affected by the size of the lung, the resistance to flow through the airways, the elasticity of the parenchyma, and the action of surface forces which may close off some of the alveolar spaces. Under the conditions of our studies, it appears that the primary factor affecting available air space is the resistance to air flow through the airways.

This system has been utilized to study the effects of physiological stimuli and drugs on the

[1] This study was aided by Grant H2271(C3) from the National Heart Institute of the U. S. Public Health Service.

[2] Presented at the Fall 1959 meeting of the American Physiological Society (Physiologist 2: 53, 1959).

[3] Dr. Harasawa is Assistant Professor of Medicine, Gumma University, and Assistant in Medicine, Dr. Okinaka's Clinic, University of Tokyo School of Medicine, Japan.

[4] The University of Buffalo Chronic Disease Research Institute is supported in part by the New York State Department of Health.

respiratory cycle. The results indicate that the apparatus is useful in the quantitative assay of factors which modify the available airway volume.

METHODS. I. *Animal experiments*. Deep anesthesia was induced with intravenous pentobarbital sodium (30 mg/kg) and supplements were given to maintain narcosis as indicated by depression of eye reflexes, in 10 dogs weighing 7 to 16 kg. After tracheotomy, positive pressure ventilation was established with the Burns valve, using a mixture of 95% O_2 and 5% CO_2 (fig. 1). Continuous recordings of intratracheal air pressure on a Sanborn electromanometer permitted measurement of the duration of each respiratory cycle. The thorax was opened and the fourth rib removed. An 18-gauge vinyl catheter was inserted in a branch of the pulmonary artery for continuous recordings of pressure and heart rate. Succinylcholine chloride (10 mg) was injected as needed into the anesthetized animals to avoid the effects of movement of the chest.

II. *Isolated lungs*. Isolated dog lungs were connected to a specially designed Burns valve which delivered the exhaled gas to a Monaghan ventilometer for measurement of the tidal volume.

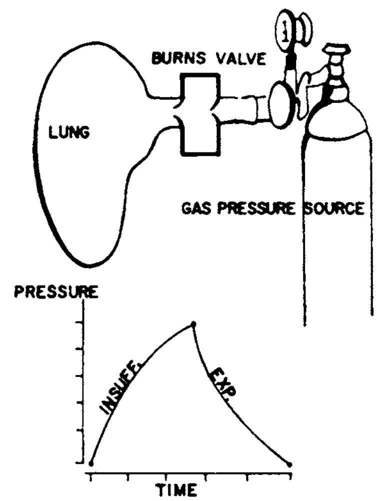

FIG. 1. Method.
The respiratory gas mixture was delivered from the source through a reducing valve which lowered the pressure to 30 pounds/square inch. A second, adjustable reducing valve lowered the insufflation pressure (insuff) to selected values. The gas passed through the Burns valve into the lung. As the gas pressure in the lung increased to the pressure setting of the valve (lower part of figure), flow stopped. An exhaust valve then opened which permitted the elastic properties of the distended lungs to expel (exp) the gas to the atmosphere. As intrapulmonary air pressure fell to a value a few millimeters above ambient, the outlet valve closed and the insufflation phase began again. The duration of the cycle measured in the present study includes both the insufflation and the expiration phases.

RESULTS. I. INTACT ANIMAL. 1. *Stability*. The Burns valve-pulmonary system was remarkably stable for relatively long periods of time. Prior to each test, the system was allowed to cycle for a control period of 10 minutes or longer during which no measurable changes in the duration of the respiratory cycle were noted.

2. *Factors shortening the respiratory cycle*. (a) Serotonin: Serotonin creatinine sulfate (Upjohn), 5 to 40 μg/kg, was injected into the pulmonary artery in 100 trials in 10 dogs (table 1). Doses of less than 5 μg/kg produced no effects. Ten μg/kg or more consistently produced significant changes in the rate of the respirator valve; these effects were more notable with the larger doses. The respiratory cycle began to shorten about 10 seconds after injection and became shortest in 15 to 45 seconds. The respiratory cycle then lengthened gradually to control values over the course of about 5 minutes. A typical tracing is given in figure 2. Cardiac arrest began about 7 seconds after the injection of the serotonin into the pulmonary artery and a bradycardia continued for 4 seconds or more, depending on the dose used, as noted by many investigators.

The effects on the respiratory cycle were reduced but not entirely abolished by vagotomy (fig. 3), even though changes in heart rate were eliminated. The pulmonary arterial pressure increased almost at once after the injection, the response being maximal 30 $\pm$ 15 S.D. seconds after injection; the pressure returned to control levels within 5 minutes.

(b) Histamine: Histamine acid sulfate (5 to 20 μg/kg) was injected into the pulmonary artery in 30 trials in 10 dogs (table 1). Ten seconds after injection, the respiratory cycle became shorter, the greatest effect occurring in 1 to 3 minutes. The degree of change and the duration of the responses were related directly to the amounts given. The duration of the respiratory cycle then lengthened slowly to control values over the next 5 minutes.

The pulmonary arterial pressure increased slightly at first, but fell after 30 seconds. The heart rate slowed somewhat. These responses were not influenced by vagotomy in 10 trials, which are included in the 30 trials of table 1.

TABLE 1

Effect of drugs and procedures on respiratory cycle duration

Experiments	Dose (μg/kg)	No. of Trials	Cycle Duration, sec		% Change	Max. Change at sec	Returned to Control Value (min)
			Control	Response			
Serotonin	0.5	5	0.55	0.55	0		
	1	7	0.63	0.62	−2		
	5	11	0.54	0.50	−8	30	3
	10	11	0.53	0.45	−16	30	4
	20	11	0.53	0.39	−32	30	4
	40	11	0.59	0.37	−35	30	5
After vagotomy	5	11	0.66	0.65	−1	30	1
	10	11	0.66	0.63	−5	30	1
	20	11	0.60	0.57	−5	30	2
	40	11	0.60	0.53	−12	30	2
Histamine	5	9	0.54	0.43	−20	45	4
	10	12	0.48	0.33	−33	45	5
	20	9	0.48	0.26	−45	45	5
Acetylcholine	0.1	3	0.51	0.49	−4	45	1
	10	13	0.39	0.33	−12	45	3
	20	5	0.43	0.33	−25	45	3
	30	5	0.47	0.36	−25	45	3
	50	4	0.47	0.33	−30	45	3
Vagal electrical stimulation		12	0.74	0.70	−5	immed.	
	Dose mg/kg						
Aminophylline	18	3	0.55	0.57	+7	60	11
	35	2	0.57	0.62	+9	60	6
TEAC	3	2	0.56	0.59	+5	60	5
	5	2	0.56	0.62	+10	60	5
	7	1	0.36	0.41	+15	60	5
Occlusion: branch of main pulmonary artery		10	0.53	0.53	0	—	—
Occlusion: 2 branches of pulmonary veins:		5	0.40	0.40	0	—	—
Blood removal		5	0.40	0.40	0	—	—
Blood infusion		5	0.40	0.40	0	—	—
Hypoxia		5	0.59	0.59	0	—	—
Hypercapnia		5	0.45	0.45	0	—	—

(c) Acetylcholine: About 30 seconds after injection of acetylcholine chloride (0.1 to 50 μg/kg) in 30 trials in 10 dogs, the duration of the respiratory cycle decreased markedly, the effect becoming maximal within a minute (table 1). The pulmonary arterial pressure increased slightly. These changes were not influenced by vagotomy.

(d) Vagal stimulation: The duration of the respiratory cycle was shortened during electrical stimulation (3 volts, 50 milliseconds' duration, 5 pulses per second) of the peripheral vagal

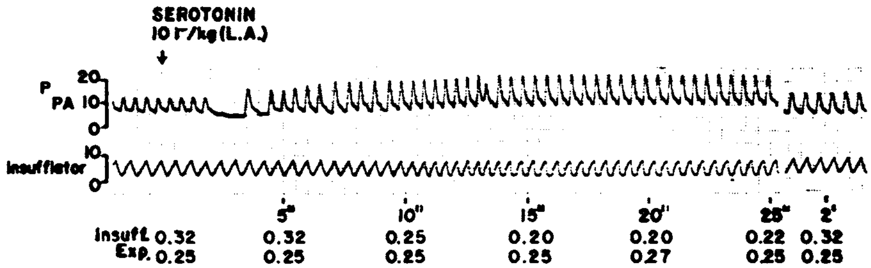

FIG. 2. Serotonin.

Ten μg/kg of serotonin were injected into the left atrium (LA). The pulmonary arterial pressure (P_{PA}) is shown. The insufflation air pressure variations in the trachea are given below. The space between two vertical lines equals 0.5 second. Two seconds after injection, the heart slows transiently. The pulmonary arterial pressure rises for 1 to 3 minutes. The durations of the rises in intratracheal air pressures are given in seconds as the insufflation phase (insuff) and the expiratory (exp) phase. Ten seconds after injection, the insufflation phase has shortened from 0.32 to 0.25 second; at 20 seconds after injection it is 0.20 second. The exhaust phase is relatively unaffected in this test and may be slightly prolonged at 20 seconds. Return to control values is complete in about 2 minutes.

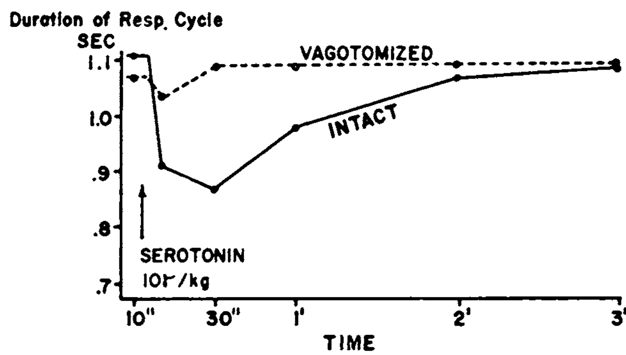

FIG. 3. Effect of vagotomy on the response of the respiratory cycle duration to serotonin.

Duration of the respiratory cycle in seconds is given as the vertical axis; the time during the experiment is given as the horizontal axis. The cycle shortens significantly after serotonin is administered to an animal with intact vagi, and returns to control values in 2 to 3 minutes. The effect is still present, but markedly diminished in the bilaterally vagotomized animal. See table 1.

stump (table 1). Although these changes were very slight, they were observed consistently in 12 trials in 5 dogs.

3. *Factors prolonging the respiratory cycle.* (a) Vagotomy: The duration of the respiratory cycle increased immediately after vagotomy, the new values being maintained for at least several minutes.

(b) Aminophylline: Theophylline with ethylenediamine (Aminophylline—Searle), 18 to 35 mg/kg, injected into the pulmonary artery in 5 trials in 2 dogs produced slight but relatively persistent increases in the pulmonary arterial pressure and the heart rate. The duration of the respiratory cycle was slightly prolonged in every case (table 1).

(c) Tetraethylammonium chloride (TEAC): After injection of TEAC (Etamon—Parke, Davis & Co.), 3 to 7 mg/kg in 5 trials in 3 dogs, the pulmonary arterial pressure and heart rate showed decreases as reported previously (Harasawa and Rodbard, 1961). The duration of the respiratory cycle was consistently and significantly, but only slightly prolonged.

4. *Tests on the vascular system.* Changes in the total volume and pressure of the blood in the pulmonary circuit are known to change the elastic properties of the lung. The possibility that such changes in the blood flow through the lungs and associated changes in compliance might affect the respiratory cycle was tested by occlusion of branches of the pulmonary arteries and veins, and by acute hemorrhage or infusion of blood in quantities sufficient to affect the cardiac output.

(a) Occlusion of the pulmonary arteries: Obstruction of the right or left main branch of the pulmonary artery for periods up to 5 minutes in 10 trials produced a slight increase in the pulmonary arterial pressure, while the heart rate tended to slow. These values returned to normal levels on release of the occlusion. The respiratory cycle showed no changes during these maneuvers.

(b) Occlusion of the pulmonary veins: Obstruction of two of the pulmonary veins for periods up to 5 minutes resulted in no measurable changes in the pulmonary artery pressure, heart rate, or duration of the respiratory cycle.

(c) Blood removal or infusion: Removal of 50 ml of blood from the pulmonary artery within 5 seconds (5 tests in 4 dogs) sharply and transiently decreased the pulmonary arterial pressure but had no significant effects on the heart rate or on the duration of the respiratory cycle.

Rapid infusion of 50 ml of blood into the left main pulmonary artery (5 tests in 3 dogs) produced a transitory increase in the pulmonary arterial pressure but there were no changes in heart rate or in the respiratory cycle.

(d) Hypoxia: After a control period of several minutes during which conditions were observed to be steady, the 95% oxygen–5% carbon dioxide gas mixture ventilating the lungs was changed to 100% nitrogen. As the color of the blood became darker, indicating progressive desaturation, the pulmonary arterial pressure and the heart rate increased. Other studies from this laboratory have shown that the increase in cardiac output accompanying hypoxia accounts for the changes in the pulmonary arterial pressure (Rodbard and Harasawa, 1959). The duration of the respiratory cycle remained unaffected by the change to nitrogen or the return to the control respiratory gas mixture in all 5 experiments in 3 dogs.

(e) Hypercapnia: Animals were ventilated in 5 tests with a gas mixture of 10% carbon dioxide and 90% oxygen. The pulmonary arterial pressure and heart rate showed a slight decrease during the carbon dioxide breathing, but no significant or consistent changes were observed in the duration of the respiratory cycle. Return to 5% CO_2 and 95% O_2 after 10 minutes was also unattended by changes in the cycle.

The foregoing experiments demonstrated that certain maneuvers which affected the pulmonary circulation, such as increases in pulmonary arterial blood flow and pressure, obstruction of a main pulmonary artery or two pulmonary veins, as well as hypoxia or hypercapnia, did not affect the duration of the Burns valve cycle. These factors undoubtedly affect the elastic properties of the lung. By contrast, marked changes were observed after the administration of serotonin, histamine, acetylcholine, TEAC, Aminophylline, and vagal stimulation or section. The possibility that the responses observed were due to effects on the airway was tested in isolated lungs.

II. Isolated lungs. 1. *Respiratory cycle length and tidal volume.* The tidal volume of an isolated nonperfused dog lung was modified by occluding one or more bronchi with a hemostat; this shortened the duration of the respiratory cycle in all trials; release of the occlusion was followed by a rapid return to normal durations. The relation between the tidal volume and the duration of the respiratory cycle in 16 series of tests on 4 isolated lungs is shown in figure 4.

For a given air pressure setting of the valve, the duration of the respiratory cycle was related directly to the tidal volume. Thus, larger lungs had longer respiratory cycles.

The data in figure 4 indicate that the tidal volume is proportional to the product of the insufflation pressure setting times the duration of the respiratory cycle. Each valve must be tested for its flow characteristics if quantitative data are to be established.

Discussion. In a given experiment, two major factors determine the duration of the inspiratory cycle of the Burns valve: insufflation pressure desired and available lung volume. At a constant insufflation pressure, the respiratory cycle will be determined by the available lung volume. When the exchange or tidal air space was decreased by occlusion of one or more bronchi, the tracheal pressure increased more rapidly during insufflation and resulted in a shorter cycle. Enlargement of the pulmonary air space by removing the clamps on the bronchi slowed the rate of pressure rise in the trachea and prolonged the time required to reach the triggering level of the valve. Changes in the duration of the cycle would thus appear to reflect proportional changes in tidal volume and to permit an appraisal of the magnitude of the change in the available air space if all other factors are constant.

Striking reductions were obtained with agents that are known to produce bronchoconstriction: serotonin, histamine, and acetylcholine. The degree of reduction was related to the dose of the agents. Vagal stimulation, known to increase

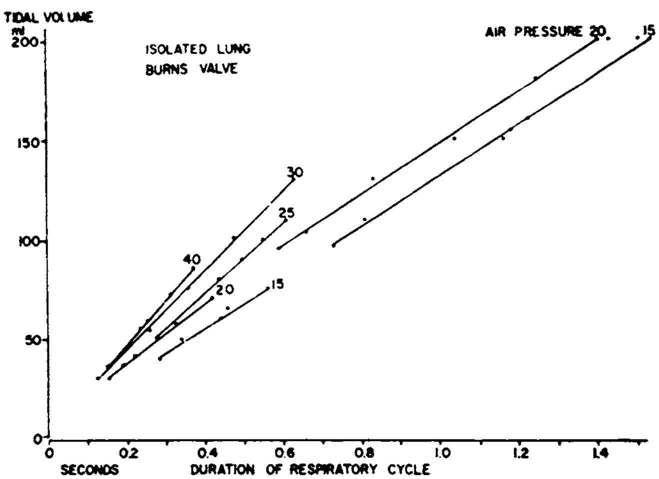

FIG. 4. Relationship between duration of the respiratory cycle and tidal volume in isolated dog lungs. Tidal volume (vertical scale) is given in milliliters, duration of cycle (horizontal scale) is given in seconds. Each line represents a series of measurements on a given lung with insufflation air pressure setting in cm water given at top of each line. The individual measurements in tidal volume and in respiratory cycle duration following clamping of a segment of the airway are shown as dots. A linear relation between tidal volume and duration of the cycle is evident. Discussed in text.

bronchomotor activity, shortened the cycle. On the other hand, bronchodilatation produced by Aminophylline, tetraethylammonium chloride, or by vagal section, diminished bronchomotor activity as indicated by a prolongation of the cycle.

There is little to suggest that pulmonary compliance was changed by the administration of the drugs noted or by vagal stimulation. Admittedly this was not measured directly. Alteration of the pulmonary blood volume and pressure did not change the cycle length. Even if these factors did change, it is unlikely that the changes would be sufficient to account for the observed findings.

In view of the above, it seems that the changes in the duration of the cycle represent variations in bronchomotor tone. If the tone of the bronchial and bronchiolar muscles increased, the freedom of movement of air into and out of the distal chambers of the lungs would be impeded. As a result, the available air volume would be reduced and the duration of the cycle would be shortened. A relaxation of these muscles would increase the ability of the air to enter the distal bronchioles and alveoli and thus prolong the cycle.

The quantitative degree of bronchoconstriction is difficult to estimate. Thus, a reduction in available air volume may represent an actual occlusion of an equivalent space, or it may represent an increased resistance to inflow and escape of air from the air sacs. The relative contributions of obstruction and partial constriction are now under analysis.

The method described above provides a new, simple, relatively quantitative means for the estimation of changes in the available air volume of the lung. These changes are believed to be related to changes in bronchomotor tone.

SUMMARY

Intermittent positive pressure insufflation of the lungs of anesthetized, thoracotomized, curarized dogs was maintained with the Burns valve. During each respiratory cycle, the duration of the insufflation phase with this instrument is determined by the rate of rise of intratracheal air pressure to the selected value at which the valve will close; passive expiration is then triggered. Narrowing or occlusion of a bronchus shortens the cycle; an increase in available lung volume prolongs it. The respiratory cycle was found to be shortened after administration of serotonin, histamine, or acetylcholine; the cycle was prolonged by Aminophylline, TEAC, or vagotomy. The responses increased with the amount given. These results suggest that the drugs used modify the pulmonary air space available for insufflation, presumably by an action on the bronchiolar musculature. Thus, the data suggest that bron-

choconstriction results from serotonin, histamine, or acetylcholine, while bronchodilatation follows TEAC, Aminophylline, or vagotomy. Hypoxia, hypercapnia, tachycardia, occlusions of branches of the pulmonary arteries or veins, and rapid infusion or removal of fluid from the circulation, all had no significant effect on respiratory cycle length. These studies provide a new approach for testing agents or physiological conditions which modify ventilatory lung volume.

ACKNOWLEDGMENTS. The excellent technical assistance of Mr. Roy Fiorella is acknowledged. We are grateful to Dr. David G. Greene, Professor of Clinical Cardiology of the University of Buffalo School of Medicine, for the use of the Burns valve.

REFERENCES

ALCOCK, P., BERRY, J. L., DEBURGH DALY, I. AND NARAYAMA, B.: Quart. J. exp. Physiol. **26:** 13, 1936–37.
CASTILLO, J. C.: Fed. Proc. **7:** 210, 1948.
COMROE, J. H., JR., NISELL, O. I. AND NIMS, R. G.: J. appl. Physiol. **7:** 225, 1954.
DEBURGH DALY, M. AND MOUNT, L. E.: J. Physiol. **113:** 43, 1951.
DUBOIS, A. B., BOTELHO, S. Y. AND COMROE, J. H., JR.: J. clin. Invest. **35:** 327, 1956.
GREENE, D. G., DAMERON, J. T. AND BUSH, E. T.: Surgical Forum of the American College of Surgeons, p. 389, 1952.
HARASAWA, M. AND RODBARD, S.: Amer. J. Physiol., **200:** 287, 1961.
KONZETT, H.: Brit. J. Pharmacol. **11:** 289, 1956.
KONZETT, H. AND RÖSSLER, R.: Arch. exp. Path. Pharmak. **195:** 71, 1940.
LOOMIS, T. A.: Proc. Soc. exp. Biol., N. Y. **92:** 337, 1956.
MCDOUGAL, M. D. AND WEST, G. B.: Brit. J. Pharmacol. **8:** 26, 1953.
MACHT, D. E. AND TING, G.: THIS JOURNAL **18:** 373, 1921.
MOERSCH, H. J.: The Chest and Heart, Charles C Thomas, Springfield, Ill., 1948.
NICHOLSON, W. F.: J. Thorac. Surg. **9:** 194, 1939.
OTIS, A. B., FENN, W. O. AND RAHN, H.: J. appl. Physiol. **2:** 592, 1950.
PETROUSKAIA, B.: Quart. J. exp. Physiol. **29:** 121, 1939.
RALL, J. E. AND GILBERT, N. C.: J. Lab. clin. Med. **30:** 953, 1945.
RODBARD, S. AND HARASAWA, M.: Amer. Heart J. **57:** 232, 1959.
RODBARD, S., KARIV, I. AND HEIMAN, D. F.: Amer. Heart J. **52:** 182, 1956.