ual virus was removed by washing the cultures three times with phosphate-buffered saline or Eagle medium. Medium containing from 5 to 40 μM methisazone was added to respective groups of cultures, and a control group was set up in medium without methisazone. Cultures were incubated for 42 hours and were then subjected to three cycles of freezing and thawing. The amount of virus in the supernatant fluids was determined by titrating the hemagglutinin with patas monkey red cells in perspex plates. The titer of hemagglutinin from the cultures without methisazone was between 512 and 1024. In the presence of 5 μM methisazone this was reduced to 64; a further reduction occurred at 10 and 20 μM, and in the presence of 30 to 40 μM methisazone the formation of hemagglutinin was completely inhibited.

The dose-response curve derived from this experiment is shown in Fig. 1a, which shows that complete inhibition of hemagglutinin production is obtained with 30 μM methisazone. A similar experiment was carried out with 5-iodo-2′-deoxyuridine (idoxuridine) (Fig. 1c), which has been found to inhibit multiplication of adenovirus 5 (*7*). This compound also suppressed formation of hemagglutinin but was much less active than methisazone, since complete inhibition was not obtained even with a 200 μM concentration.

The main hemagglutinin in most types of adenovirus is attached to the virus particle (*8*), and therefore these results imply that methisazone also inhibits the production of infective virus. In order to establish this beyond doubt, the content of infective virus in the cultures was determined by serial titration in HeLa cells, the end point being read after 4 days. The dose-response line for infectivity thus obtained lay parallel to the dose-response line for hemagglutination but two to three $\log_2$ units higher (Fig. 1b). The infectivity titer of control cultures was 4096, and the production of infective virus was completely inhibited in cultures treated with 30 and 40 μM methisazone. Inhibition of hemagglutinin production was observed in similar experiments with adenovirus types 3 and 9, and also types 7, 14, 16, 17, 21, and 28. The hemagglutinin of types 9 and 17 was titrated with rat red blood cells. In types 7 and 14 the hemagglutinin is produced as the result of infection but is separate from the infective particle (*8*). In a similar experiment in primary patas monkey kidney cells the hemagglutinin of SV15, a simian adenovirus, was inhibited to a similar extent.

The effect of methisazone on the growth curve under single-cycle conditions was investigated. Parallel series of HeLa cell cultures were infected with adenovirus 11. After residual virus had been washed off, the cultures were incubated with normal medium and with medium containing 30 μM methisazone, respectively. At intervals up to 72 hours, two tubes were taken from each series and pooled, and the content of hemagglutinin was titrated in twofold dilutions with patas monkey red cells; results are shown in Fig. 2. In the absence of methisazone hemagglutinin appeared after 10 hours; the titer then rose logarithmically and attained a maximum after 48 hours. In the presence of methisazone no formation of hemagglutinin occurred over a period of 72 hours except for a small amount found in the tubes sampled after 28 hours. Formation of hemagglutinin was thus inhibited over a period extending beyond the normal growth cycle. In a similar experiment tubes were sampled at intervals and the content of infective virus was determined by titration in HeLa cells. Results obtained were similar to those of Fig. 2 and showed that methisazone also inhibited the formation of infective virus.

An attempt was made to determine the time during the growth cycle of the virus at which methisazone acts. Replicate cultures of HeLa cells were infected with adenovirus 11 under single-cycle conditions, and at increasing intervals after infection the medium was removed from two cultures and replaced with medium containing 30 μM methisazone. Additions of the compound were made at hourly intervals up to 24 hours. Cultures were incubated further for a period of 18 hours, and the hemagglutinin content of all cultures was determined. Production of hemagglutinin was completely inhibited when the addition of methisazone was delayed until 13 hours after infection. Increasing amounts of hemagglutinin appeared when addition of the compound was delayed beyond this time. It therefore appears that methisazone acts at a late stage in the cycle, when infective virus is just about to appear.

A preliminary study of the structure-activity relations of antiviral activity of methisazone against adenoviruses showed that this compound followed the same pattern that it does with pox viruses. Thus, activity was abolished by replacing sulfur with oxygen in the side-chain (isatin 3-semicarbazone) and by substituting two alkyl groups in the 4′-position [isatin 3-(4′,4′-dibutyl) thiosemicarbazone]; it was reduced to one-half by removal of the 1-methyl group (isatin 3-thiosemicarbazone) and reduced still further by substitution in the 5-position (5-methylisatin 3-thiosemicarbazone).

Successful use of methisazone in pox virus infections of man makes it reasonable to expect that this compound may find some application in the prevention and treatment of adenovirus infections of man.

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Airflow Control by Auditory Feedback: Respiratory Mechanics and Wind Instruments

Abstract. *The auditory signal provided by a soprano recorder in a breathing circuit can help human subjects to regulate inspiratory and expiratory airflow rates at constant preset levels. This method of airflow control is useful in studies of the static and dynamic mechanical properties of the lungs and may have additional applications in human respiration physiology.*

Production of a tone of constant pitch and loudness on a soprano or descant recorder requires a low and constant driving pressure (1 to 7 cm H_2O for tones of increasing pitch) and a low and constant airflow rate (0.05 to 0.11 liter/sec for tones of increasing pitch). The auditory signal provided by

the recorder can be used to control airflow rates at selected and constant levels during respiratory experiments with human subjects. The use of this method of airflow control in measurements of mechanical properties of the human lungs (that is, the elastic and flow-resistive characteristics of the lungs and airways) is discussed. The method may also be useful for other respiratory measurements (for example, determination of anatomical dead space and gas distribution in the lungs) in which constancy of flow rates is desirable.

The mechanical properties of the lungs can be measured in terms of the transpulmonary pressure required to change lung volume, and to overcome viscous resistive forces in the airways and lungs. During spontaneous breathing, all three variables—transpulmonary pressure, lung volume, and airflow rate —vary cyclically with time. If airflow rate is kept constant, the mechanical properties of the lungs can be analyzed from the relations between the other two variables, that is, from the pressure-volume diagram of the lungs. The properties of recorders and whistles make these wind instruments especially suitable for achieving constant airflow rates. They have a fixed slit and edge for tone production and do not require embouchure (precisely controlled contact between the player's lips and the mouthpiece of the instrument); they can therefore be played through tubing and flowmeters. Only minimal practice is required to play a tone of constant loudness and pitch on recorders. As pressures and flow rates are small and vary within a narrow range for all tones, precise control of pitch is immaterial for the present purposes. Whistles and recorders differ from other wind instruments in which both driving pressures and flow rates vary widely with pitch and loudness (*1*). Pressures that are too high are easily recognized because they result in over-blowing the instrument.

Pleural pressures were measured with the esophageal balloon technique (*2*), changes in lung volume were measured with a volume-displacement body plethysmograph, and airflow rates were measured with a Fleisch pneumotachograph (*3*) (Fig. 1). The subject (wearing a noseclip) sits in the air-conditioned plethysmograph, and breathes through a mouthpiece. A spirometer connected to the plethysmograph records the changes of lung volume during breathing (ΔV). A differential transducer connected to the esophageal balloon and to the tube close to the mouthpiece, respectively, records pleural pressure *(Ppl)* relative to pressure at the airway opening *(Pao)*. At any instant, *Ppl-Pao* equals the algebraic sum of the pressure difference across the elastic walls of the lungs [static recoil pressure, *Pst* (L)], and any pressure difference between the alveoli *(Palv)* and the airway opening. Thus $Ppl\text{-}Pao = -Pst(l) + Palv\text{-}Pao$, or: $Ppl = -Pst(l) + Palv$ . . . Eq. 1. If the breath is held with the glotis open, $Palv = 0$, and $Ppl = -Pst(l)$. By performing this maneuver at different lung volumes, the static recoil curve of the lungs, that is, their static pressure-volume relationship, can be determined (Fig. 2, broken line). The breath-holding method, however, presents difficulties in untrained subjects and in patients. In such subjects, a curve very similar to the static recoil curve can be obtained as follows.

After a maximal inspiration, the three-way stopcock (Fig. 1) is turned to its lower position, and the subject is asked to play (during a full expiration) a soft tone on the recorder which is connected to that stopcock. Before entering the plethysmograph, the subject has been given some previous training in producing soft and constant tones

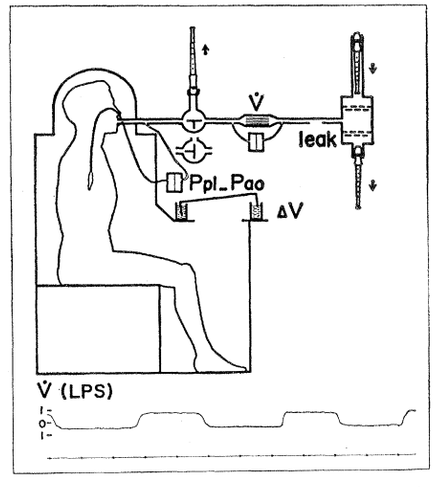

Fig. 1. Experimental arrangement. Both positions of the three-way stopcock near the mouth are shown. Arrows indicate direction of airflow. Airflow rate ($\dot{V}$) is sensed by a differential transducer connected to a Fleisch pneumotachograph. Bottom: recording of airflow rates as controlled by the inspiratory and expiratory recorders. Deflection above zero is inspiration. Time base in seconds.

on the instrument. During the production of such constant tones, pleural pressures are at all volumes slightly higher than $-Pst(l)$ (Fig. 2). The difference between *Ppl* and $-Pst(l)$ at any volume equals *Palv* (Eq. 1). In this case, *Palv* equals the pressure difference required for expiratory flow through the airways. As flow rates are small and constant, *Palv* is also small and constant. In Fig. 2 both the static recoil curve and the curve during tone production were obtained after the subject performed three vital capacity maneuvers to standardize lung volume history *(2)*. The curve during tone production has the same shape as the static recoil curve and is only slightly displaced to its right by the amount of *Palv*. Calculation of lung compliance ($\Delta V/\Delta P$) in the approximately linear midportions gives similar results with both curves. Noise introduced by pleural pressure variation with the heart beat is inherent to both curves but has been eliminated by averaging only in the static recoil curve of Fig. 1. This method of obtaining a nearly static pressure-volume curve of the lungs has proven helpful in subjects who cannot perform the breathholding technique, and may be particularly useful in persons with dyspnea. Auditory feedback is here used to maintain *Palv* at a small and constant value throughout expiration.

During breathing, the alveolar pressure component of pleural pressure indicates the pressure required to overcome viscous resistance to airflow. Since *Palv* depends on the rate of airflow ($\dot{V}$), it is customary to express these viscous properties of the lungs in terms of resistance ($Palv/\dot{V}$). During spontaneous breathing both *Palv* and $\dot{V}$ change cyclically, and a loop is inscribed on the pressure-volume diagram during each breathing cycle. The horizontal distance between any point on the loop and the static recoil curve indicates *Palv*, but since simultaneous values of $\dot{V}$ are not represented in the diagram, one cannot relate instantaneous values of *Palv* and $\dot{V}$ to one another. This problem can be eliminated by using recorders to keep airflow rates constant over a considerable portion of both inspiration and expiration. This simplifies the interpretation of the pressure-volume diagram and enables one to calculate viscous flow resistance in a simple way.

In healthy lungs viscous resistance to

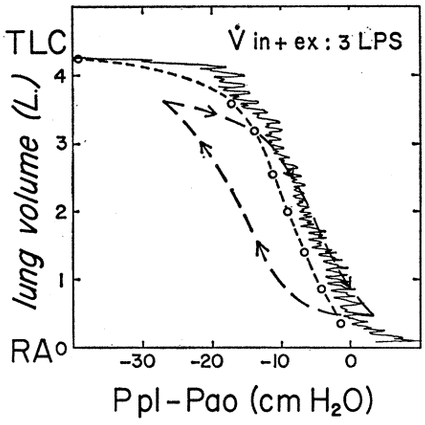

Fig. 2. Pressure-volume diagram of the lungs. Broken line (--O--), static recoil curve of the lung, obtained from pleural pressures during breathholding. Circles are experimental points averaged over pressure excursion with the heart beat at each volume. Continuous line shows pressure swings caused by heart beat: pleural pressures were recorded during one slow and maximal expiration, while producing a soft and constant tone on a recorder at maximum inspiration. *TCL* = total lung capacity. *RA* = residual volume. Dashed loop (— — —), average pressure-volume loop during inspiration (left limb) and expiration (right limb) at controlled flow rates (see $\dot{V}$ recording in Fig. 1).

airflow is small, and to measure viscous resistive pressures adequately requires relatively high flow rates (much higher than those needed for tone production on the recorder). Devices to achieve such higher constant flow rates have been used by Mead and by Allander *et al. (4)*. Their flow regulators keep airflow rate constant, regardless of subject effort. The present method, on the other hand, provides the subject with an audible signal which helps him to regulate his own effort in order to maintain a constant flow rate. The higher flow rates needed for resistive pressure measurements can be obtained by placing a leak parallel with an inspiratory and expiratory recorder (Fig. 1) (*5*). A Fleisch flow-meter records total flow rate. The total flow is divided into flow through the leak and flow through one of the two recorders connected to both sides of a low-resistance breathing valve. The inspiratory recorder (above the valve in Fig. 1) is inverted; it is placed in a tube with only the mouthpiece open to atmosphere. This recorder produces a tone when the subject inspires at a sufficiently high flow rate. The other recorder is connected to the expiratory side of the breathing valve and sounds during expiration. The size of the leak determines the total airflow rate at which both recorders will sound. The choice of total flow rate is a matter of compromise: it should be large enough to require measurable resistive pressures and small enough to be maintained over most of the lung volume. In practice we used total inspiratory plus expiratory flow rates of 2 to 3 liters/sec for healthy subjects. The $\dot{V}$ record in the lower part of Fig. 1 illustrates the constancy of flow rates. Pressure-volume loops recorded during such constant flow breaths are shown in Fig. 2. The loops are displaced to the left of the static recoil curve because inspiratory flow rate is larger than expiratory flow rate (see flow curve in Fig. 1). This difference is caused by a slight difference between the resistances of the inspiratory and expiratory parts of the breathing circuit. The resistive pressure required for the sum of inspiratory and expiratory flow rate can be read from the diagram as the horizontal distance between the inspiratory (left) and the expiratory (right) limbs of the loop. Pulmonary viscous resistance can then be calculated as the ratio of resistive pressure to flow rate. At the flow rate used for the dynamic *P-V* loops, the slope of both limbs of the loop differs from the slope of the static lung recoil curve. Apparently, dynamic compliance is lower than static compliance in this subject. Such changes in the slope of *P-V* loops with increasing flow rates, if they occur, demonstrate that compliance is dependent upon breathing frequency (*6*). Preliminary experiments have shown that these constant-flow *P-V* loops demonstrate the bronchoconstrictive effect of an inhaled histamine aerosol on the airways by an increase of resistive pressure, at equal flow rates, and by an increased frequency-dependence of lung compliance.

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Reversal in Tactile and Visual Learning

Our conclusion [*Science* **153**, 205 (1966)] that tactile and visual learning take place in independent functional systems was based on results of experiments in which monkeys were required to make tactile and visual object discriminations. Biederman refers to work [*Science* **154**, 677 (1966)] with rats and spatial discriminations. We are not certain how closely one can compare results obtained with these two different species and discriminations. We know of no work that shows an increase of the reversal-learning score by the monkey with overtraining of original learning for tactile object discriminations.

Furthermore, Biederman's argument applies only to our control ratio of 75:1, relating to original and reversal learning by touch. However, our conclusion was based upon two separate lines of evidence: first, the small reversal ratio across modalities when compared with the large reversal ratio within one modality; and, secondly, the good performance of our animals during 80 test trials in the light and dark. Biederman's point cannot bear upon either the small reversal ratio across modalities of 1.6:1 or upon the good performance during the 80 test trials. Finally, we cannot agree with him that original learning light and reversal in the light would have provided either helpful or necessary evidence: because our experimental animals were originally trained in the dark, it was useful only to compare dark-to-light reversal (experimental) with dark-to-dark reversal (control)—which we did.

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