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Ptychodiscus brevis toxin (brevetoxin) is associated with "Florida red tide" and causes neurotoxic shellfish poisoning. Saxitoxin is the agent of paralytic shellfish poisoning. Clinical reports of human intoxication suggest that both toxins affect the respiratory system. The toxins were administered by slow intravenous infusion. The effects of the toxins on respiratory function of awake guinea pigs in a pressure plethysmograph were studied. Both toxins caused lactic acidosis of unknown etiology which was compensated by increased minute volume with brevetoxin (PbTx-3) but not with saxitoxin-intoxicated animals. Airways resistance was not increased, nor was dynamic compliance decreased during the course of intoxication, although the data suggest that respiratory system failure was the primary cause of death. The responses seen in these experiments are consistent with the dissimilar molecular actions of these toxins.

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RESPIRATORY EFFECTS OF BREVETOXIN AND SAXITOXIN  
IN AWAKE GUINEA PIGS

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Franz and R.D. LeClaire. Respiratory Effects of Brevetoxin and Saxitoxin in Awake Guinea Pigs. Toxicon xx,xxx-xxx, 19xx.--Ptychodiscus brevis toxin (brevetoxin) is associated with "Florida red tide" and causes neurotoxic shellfish poisoning. Saxitoxin is the agent of paralytic shellfish poisoning. Clinical reports of human intoxication suggest that both toxins affect the respiratory system. The toxins were administered by slow intravenous infusion. The effects of the toxins on respiratory function of awake guinea pigs in a pressure plethysmograph were studied. Both toxins caused lactic acidosis of unknown etiology which was compensated by increased minute volume with brevetoxin (PbTx-3) but not with saxitoxin-intoxicated animals. Airways resistance was not increased, nor was dynamic compliance decreased during the course of intoxication, although the data suggest that respiratory system failure was the primary cause of death. The responses seen in these experiments are consistent with the dissimilar molecular actions of these toxins.

## INTRODUCTION

BREVETOXIN and saxitoxin are marine biotoxins of public health significance; they are produced by dinoflagellates of the genera Ptychodiscus and Protogonyaulax, respectively (HUGHES, 1979). Accidental human exposure to P. brevis-related toxins is typically through ingestion of contaminated clams (neurotoxic shellfish poisoning) (HUGHES, 1979), or inhalation of seaspray containing toxin (PIERCE, 1986). Ingestion typically results in sensory abnormalities, cranial nerve dysfunction, and gastrointestinal symptoms. Inhalation of the toxins may cause cough, rhinorrhea, watery eyes, and sneezing in normal humans and wheezing in asthmatic patients (ASAI et al., 1982). Saxitoxin causes paralytic shellfish poisoning (MEE, 1986). Humans who ingest shellfish containing this toxin may experience progressive numbness of the lips, face, and extremities; muscular incoordination; respiratory distress; and, finally, death (ACRES and GRAY, 1978). Mortality in severe cases is 2.6% to 23.2% and typically results from respiratory paralysis (SAKAMOTO et al., 1987).

BORISON et al. (1985), studying the effects of the brevetoxins in anesthetized cats, concluded that brevetoxins exert major effects on the circulatory and respiratory systems through reflex and central actions, largely sparing neuromuscular transmission; both catecholamines and vagal acetylcholine are released during intoxication. Saxitoxin causes paralysis, hypotension, and respiratory failure in anesthetized experimental mammals, primarily through axonal blockade (KAO, 1972).

In the rat hemidiaphragm (GALLAGHER and SHINNICK-GALLAGHER, 1985), crude brevetoxin increases spontaneous transmitter release (presynaptic action) and, at higher doses, blocks synaptically mediated endplate potentials (postsynaptic action). In canine tracheal smooth muscle preparations (ASAI et al. 1984), low concentrations of brevetoxin lead to contractions through presynaptic effects; the muscle tension returns to baseline as the concentration is increased. In crayfish giant axons, brevetoxin causes concentration-dependent depolarization with initial repetitive discharges followed at higher doses by a block of excitability (HUANG et al., 1984). Finally, in single sodium channels of murine neuroblastoma cells, one of the brevetoxins (PbTx-3) causes activation of channels at membrane potentials approximately 8 mV more negative than normal, while not altering channel inactivation or conductance (Personal

Communication: Robert E. Sheridan and Michael Adler,  
Neruotoxicology Branch, USAMRICD, APG, MD 21010).

In frog sartorius nerve-muscle preparations, saxitoxin blocks peripheral nerve conduction and decreases the excitability of skeletal muscle to direct stimulation (FINGERMAN et al. (1953). In nerve-muscle preparations and isolated axons, end-plate potentials are reduced before miniature end-plate potentials are changed significantly, suggesting failure of conduction in the axon (KAO and NISHIYAMA, 1965). The toxin also interferes with excitation of muscle membranes, postsynaptically. It is now known that saxitoxin produces a reversible use- and voltage-dependant block of the sodium channel (SALGADO et al., 1986)

Therefore, the two toxins affect the sodium channel, but have generally opposite effects: brevetoxin initially increasing the likelihood of the channel opening, promoting depolarization at low doses; and saxitoxin hindering depolarization by blocking early sodium conductance. The purpose of this work was to evaluate and compare the respiratory effects of saxitoxin and the brevetoxin, PbTx-3, both administered by slow i.v. infusion, in the awake guinea pig.

## MATERIALS AND METHODS

All experiments were performed on male, barrier-raised, Hartley albino guinea pigs (CRL:(HA)Br; Charles River Laboratories, Wilmington, MA) weighing 240-260 g. Animals were housed in a bio-safety enclosure (Airoclean Engineering, Inc., Edgemont, PA) and received feed and water ad libitum. Temperature in the animal room was maintained at  $22 \pm 1^{\circ}\text{C}$  with a 12-hr light/dark cycle.

Fed animals were anesthetized with halothane and a polyethylene (P.E. 10) catheter was introduced into an external jugular vein, its tip extending into the anterior vena cava. A saline-filled polyethylene catheter (P.E. 60) was placed percutaneously into the pleural space after the method of AMDUR and MEAD (1958) for the measurement of pleural pressure. The guinea pigs were placed in a pressure plethysmograph for measurement of tidal volume; a tight seal was maintained around the neck by a water-soluble gel and two latex collars. The rate of airflow was determined by electronic differentiation of the plethysmograph pressure signal with time. Ventilatory frequency, tidal volume, pulmonary resistance, and dynamic compliance were calculated electronically (Model-6 Pulmonary Mechanics Analyzer, Buxco Electronics Inc.,

Sharon, CN) and confirmed manually from chart recordings. Mean inspiratory flow was calculated manually from the tidal volume trace.

A second group of animals, used only for blood gas, pH, and lactate studies, was prepared as described above except that cannulae were implanted in both the external jugular vein and left external carotid artery (P.E. 50, stretched to reduce its diameter near the tip); the pleural pressure cannula was omitted.

Purified, lyophilized PbTx-3 (Source: Dr. Dan Baden, Univ. Miami, Miami, FL), dissolved in chloroform, was stored at  $-20^{\circ}$  C. Within 3 hr before use, it was dried and redissolved in phosphate-buffered saline containing 0.5% Emulphor (GAF Corp. NY, NY). PbTx-3 was administered i.v. at the rate of 0.63 ug/kg/min until death of the animal. Saxitoxin (5mg/ml in 0.001N acetic acid) (Source: Dr. Sherwood Hall, FDA, Washington, D.C.), stored at  $-25^{\circ}$  C, was diluted in phosphate-buffered saline within 3 hr before use. Saxitoxin was administered at the rate of 0.315 ug/kg/min until death. A variable-speed syringe pump (Harvard Bioscience, South Natick, MA) was used to administer the toxins via the P.E. 10 venous catheter. Toxin challenge was begun after all measured parameters had stabilized, typically 60-180 min after completion of the surgical

preparation.

Arterial blood samples (approx. 70-100 ul each) were collected before the start of toxin infusion and at 5-min intervals until death; an Instrumentation Laboratories Model 1301 blood gas analyzer (Lexington, MA) was used to measure blood gases and pH. For lactate analysis, 100 ul of arterial blood was collected before the start of toxin infusion and at 10-min intervals until death of the animal. Lactate was measured by a fluorometric method for enzymatic determination (ANTONIS et al., 1966). Multivariate repeated-measures analysis of variance (SYSTAT Inc., Evanston, IL) was used in analyzing data. Values are expressed as means  $\pm$  S.E.; n = 5 for all studies.

#### RESULTS

The mean time to respiratory failure was  $25.0 \pm 4.8$  min for guinea pigs challenged with PbTx-3 and  $25.4 \pm 2.4$  min for those challenged with saxitoxin. The mean dose of toxin infused at the time of respiratory failure was  $15.8 \pm 3.0$  ug/kg for PbTx-3 and  $8.0 \pm 0.8$  ug/kg for saxitoxin. Just before respiratory failure, a PbTx-3-intoxicated animal typically breathed with a frequency of 200/min and tidal volume of 2.0-2.5

ml; apnea occurred suddenly, lasting for 15-45 sec, and was followed by several minutes of slow, variable (gasping) ventilation before death. Failure occurred more gradually with saxitoxin, although the terminal events were similar.

Guinea pigs given PbTx-3 demonstrated a biphasic increase in minute volume (Fig 1); frequency of ventilation was increased only in the second phase. Neither minute volume nor ventilatory frequency was increased during saxitoxin intoxication (Fig 2); animals breathed quietly, at decreasing frequencies, until failure ensued.

Neither toxin caused a significant increase in airways resistance or decrease in dynamic compliance (Figs 1-2). In PbTx-3-intoxicated animals, an early increase in resistance ( $p < 0.086$ ) occurred 3 min after the start of infusion; subsequently, resistance decreased as ventilatory frequency increased before death. In saxitoxin-challenged animals, a distinct period (1-3 min) of increased (approx. 30%) resistance occurred at  $8.8 \pm 0.4$  min after start of infusion. Airways resistance after the brief rise was lower than before ( $p < 0.01$ ).

Mean inspiratory flow ( $V_T/T_I$ ), a general measure of inspiratory drive (AUBIER et al., 1981), typically increased

until death in PbTx-3-intoxicated animals and decreased or was unchanged in saxitoxin-intoxicated guinea pigs (Fig 3a-b).

Both toxins caused metabolic acidosis which began early in the infusion period. Before the start of toxin challenge, lactate levels (mg/dl) were  $9.5 \pm 1.7$  (simultaneous  $PCO_2$ :  $41.8 \pm 1.1$  mmHg) for PbTx-3 and  $8.1 \pm 0.3$  ( $PCO_2$ :  $41.4 \pm 2.3$  mmHg) for saxitoxin. At 2-12 min before failure of ventilation, lactate levels were  $52 \pm 8.2$  ( $PCO_2$ :  $31.3 \pm 0.4$  mmHg) in PbTx-3 animals and  $44.0 \pm 7.3$  ( $PCO_2$ :  $40.4 \pm 2.2$  mmHg) with saxitoxin. In the case of PbTx-3 intoxication, animals tended to counter the metabolic acidosis with respiratory compensation (Fig 4a), while saxitoxin-intoxicated guinea pigs, uncompensated, actually demonstrated trends toward concurrent respiratory acidosis (Fig 4b).

#### DISCUSSION

Both toxins caused progressive metabolic acidosis in our experimental animals. Brevetoxin increased respiratory drive, partially compensating for the metabolic acid load by decreasing

blood  $P_{CO_2}$  levels. A brief excitement stage occurred at the onset of PbTx-3 infusion which was not seen with saxitoxin infusion. The increased minute volume during the first 8 min of PbTx-3 intoxication may have been a result of increased activity, while it is likely that acidosis stimulated the increased minute ventilation observed during the last 8 min of PbTx-3 intoxication. The ventilatory drive appeared strong since both frequency and tidal volume increased during this period, and thus mean inspiratory flow was increased. Although acidosis was as severe in the latter phase of saxitoxin intoxication as in PbTx-3 intoxication, ventilatory compensation did not occur; neither frequency, tidal volume, nor mean inspiratory flow were significantly changed. The progressive increase in mean inspiratory flow in PbTx-3 intoxication, but not in saxitoxin intoxication, suggests a marked difference in the state of respiratory system drive. It appears that saxitoxin impeded effective ventilation during the period before total failure, while PbTx-3 did not. This concept is compatible with neuronal blockade caused by saxitoxin (KAO, 1972).

Using a rat phrenic nerve-stimulated, hemidiaphragm preparation, BADEN et al. (1984) demonstrated that a complete neuromuscular block occurs at  $\mu M$  to  $nM$  concentrations of PbTx-2 or PbTx-3, thought to be caused by persistent depolarization

without depletion of acetylcholine. At the time that the block is in effect, diaphragm muscle still contracts in response to direct stimulation. Although a similar failure of neural transmission is likely to have caused respiratory failure in our study, we did not identify whether the failure occurred at the level of central respiratory drive, afferent or efferent nerve traffic, or muscular pump function.

The cause of the lactic acidosis in either intoxication is not clear. Oxygen debt in skeletal muscle, as a result of increased metabolism, is unlikely, as guinea pigs were not hyperactive and did not convulse, but rather became sedate during saxitoxin intoxication. We know of no reports of ischemia or uncoupling of oxidative phosphorylation by either of the compounds, though hypotension has been noted to occur in animal models (BORISON et al., 1985, KAO, 1972). Peripheral circulatory control may have been sufficiently disrupted to reduce blood flow to muscles of respiration. Regional anaerobiosis might have resulted, especially in the case of PbTx-3 intoxication, which stimulated ventilation. Finally, there is precedent for lactic acidosis with narrow arterial-venous O<sub>2</sub> gradient found in humans with septic shock (RACKOW et al., 1988).

Airways resistance and dynamic compliance measurements suggest

that neither central airways (upper airways, trachea, and second-  
third- generation airways) nor peripheral airways (DRAZEN, 1976;  
DRAZEN and SCHNEIDER, 1978) responded significantly to toxin  
challenge, although both toxins appeared to cause mild, transient  
central airways constriction early in the challenge period.  
BADEN et al. (1982), measuring insufflation pressures in  
anesthetized, artificially-ventilated guinea pigs, demonstrated  
that i.v. PbTx-3 causes bronchoconstriction and spasm of thoracic  
musculature. A bolus dose of 20 ug/kg of PbTx-3 causes the same  
effect as 0.05 ug/kg acetylcholine, a fivefold increase in  
insufflation pressure which can be blocked by atropine. It is  
not surprising that the bronchoconstrictor response was less  
vigorous in our experiments. Because only  $15.8 \pm 3.0$  ug/kg  
had been slowly infused at the time that ventilation failed, the  
concentration of toxin at the axonal or airways, smooth muscle  
effector sites, even immediately before death, was probably lower  
than that in animals that received a bolus dose of 20 ug/kg.

In summary, we have compared the effects of two sodium  
channel-active marine toxins on the ventilatory function of the  
awake guinea pig. Both toxins caused a progressive metabolic  
acidosis. Respiratory compensation occurred during PbTx-3  
infusion, but not during saxitoxin infusion. Airways mechanics  
were not altered significantly by either toxin, yet both appeared

to cause death by respiratory failure. The pathogenesis of intoxication leading to ultimate systems failure resulted in markedly differing ventilatory responses, which is compatible with the reported dissimilar molecular effects of these two toxins.

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The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. In conducting the research described in this report, the authors adhered to the Guide for Laboratory Animal Facilities and Care as promulgated by the Committee on the Guide for Laboratory Animal Resources, NAS/NRC.

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## LEGENDS FOR FIGURES

Fig 1. GRAPHIC REPRESENTATION OF AIRWAYS RESISTANCE, PULMONARY COMPLIANCE, TIDAL VOLUME, MINUTE VOLUME, AND VENTILATORY FREQUENCY IN AWAKE GUINEA PIGS DURING INFUSION OF BREVETOXIN (PbTx-3, 0.63 ug/kg/min). Data were normalized by using only the first and last 8 min of the infusion period. Time 0 is the point at which toxin infusion started. Twenty-five (25) min is the mean time of initial respiratory failure ( $25.0 \pm 4.8$ ) and times 17 through 24 are values prior to initial failure. Values are expressed as means  $\pm$  SEM (n=5, \*=significance at  $p < 0.05$ ).

Fig 2. GRAPHIC REPRESENTATION OF AIRWAYS RESISTANCE, PULMONARY COMPLIANCE, TIDAL VOLUME, MINUTE VOLUME, AND VENTILATORY FREQUENCY IN AWAKE GUINEA PIGS DURING INFUSION OF SAXITOXIN (0.315 ug/kg/min). Data were normalized by using only the first and last 8 min of the infusion period. Time 0 is the point at which toxin infusion started. Twenty-five (25) min is the mean time of initial respiratory failure ( $25.4 \pm 2.4$ ) and times 17 through 24 are values prior to initial failure. Values are

expressed as means  $\pm$  SEM (n=5, \*=significance at  $p < 0.05$ ).

Fig 3. SCATTER PLOTS OF MEAN INSPIRATORY FLOW ( $V_T/T_I$ ) FROM AWAKE GUINEA PIGS DURING INFUSION OF PbTx-3 (Fig 3a.) OR SAXITOXIN (Fig 3b.). Data were normalized by using only the first and last 8 min of the infusion periods. Time 0 is the point at which infusion started. Twenty-five (25) min is the mean time of initial respiratory failure and time points 17 through 24 are values prior to initial failure (n=5).

Fig 4. SCATTER PLOTS OF ARTERIAL  $PCO_2$  vs pH OF AWAKE GUINEA PIGS BEFORE AND DURING I.V. INFUSION OF PbTx-3 (Fig 4a.) AND SAXITOXIN (Fig 4b.). Note partial respiratory compensation of metabolic acidosis with PbTx-3 and lack of compensation with saxitoxin. Point 1 represents control; points 2-3 represent samples collected at 5 and 10 min after the start of challenge; points 4-6 represent samples collected at 15, 10, and 5 min before respiratory failure. Values are expressed as means  $\pm$  SEM (n=5).

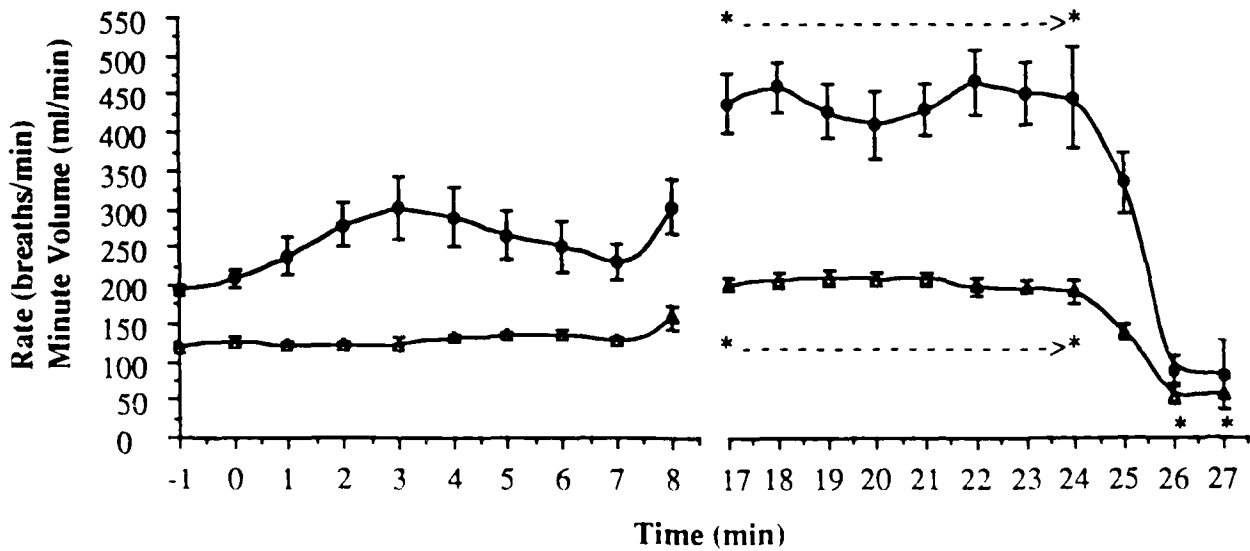
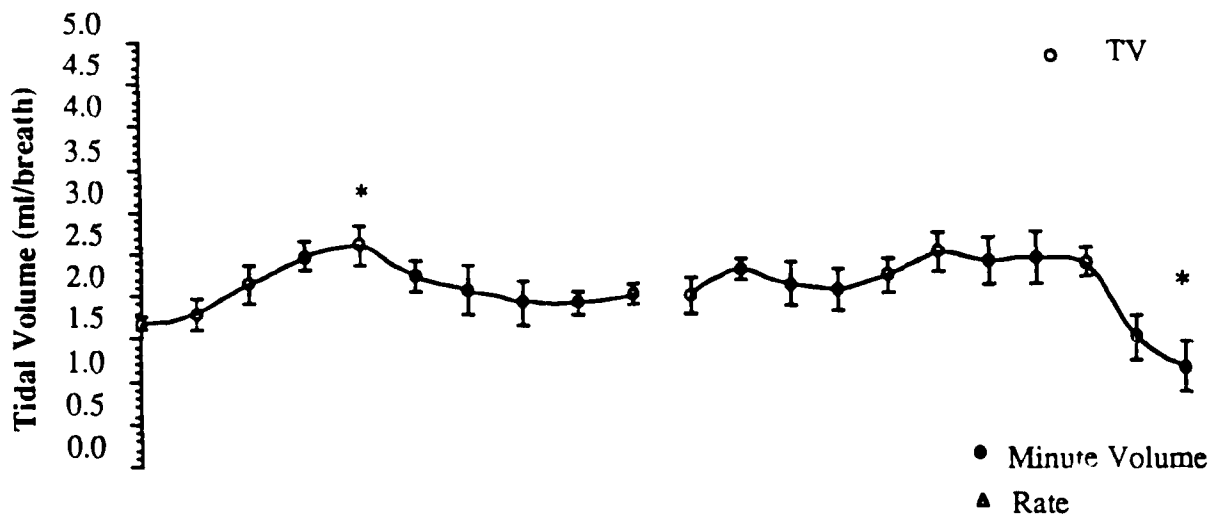
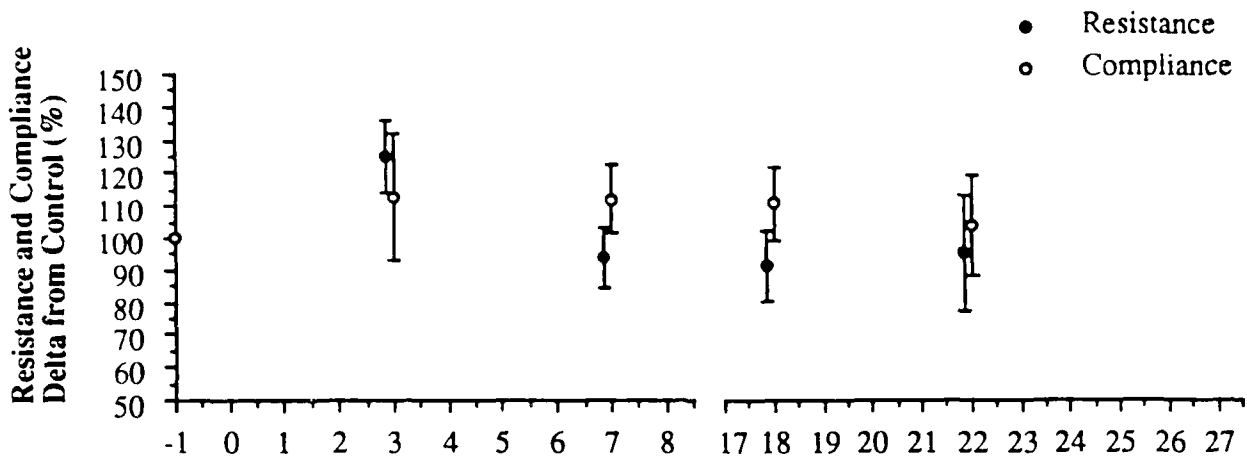
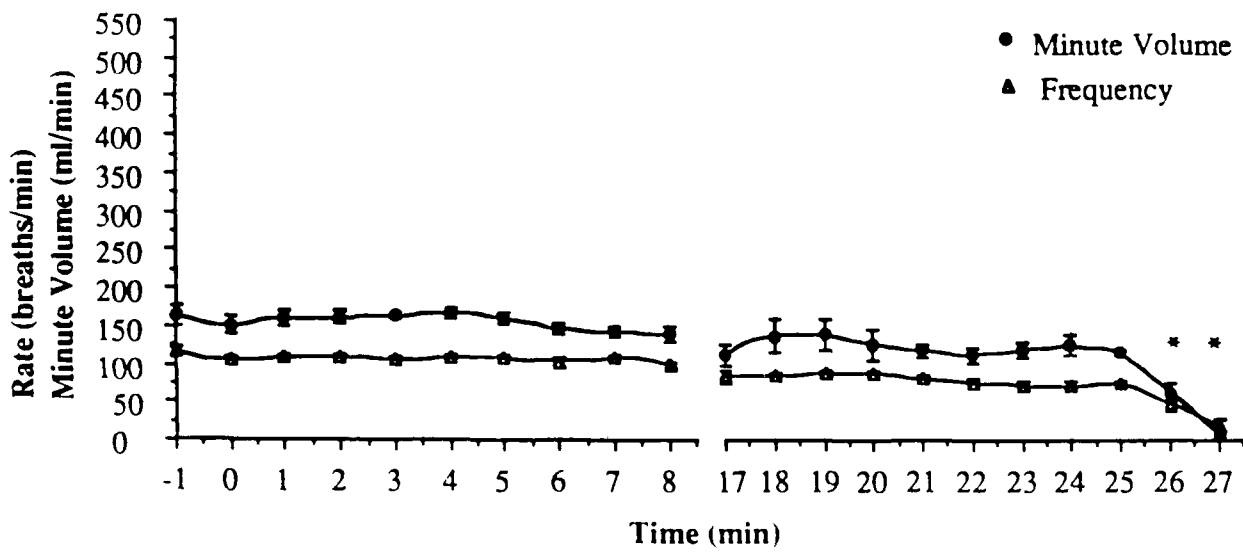
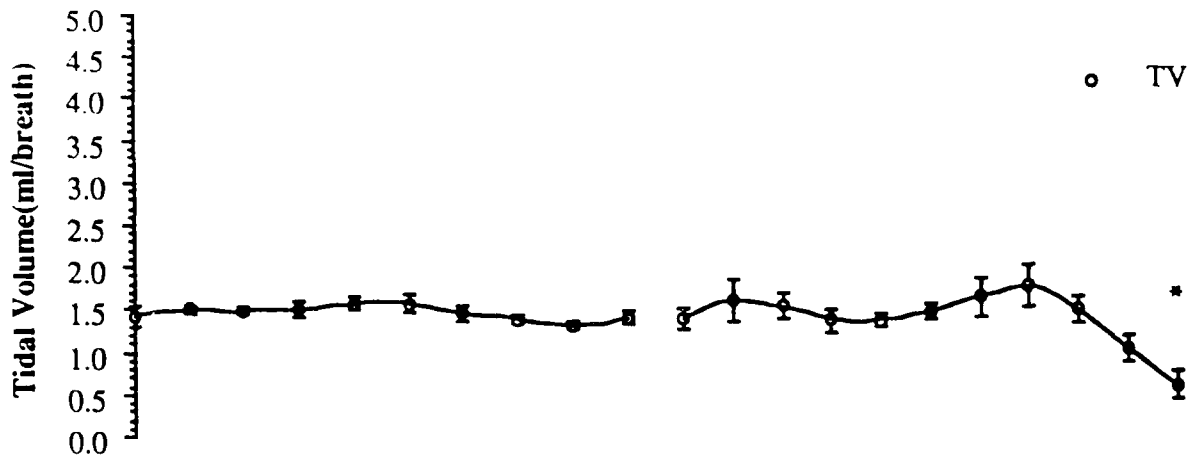
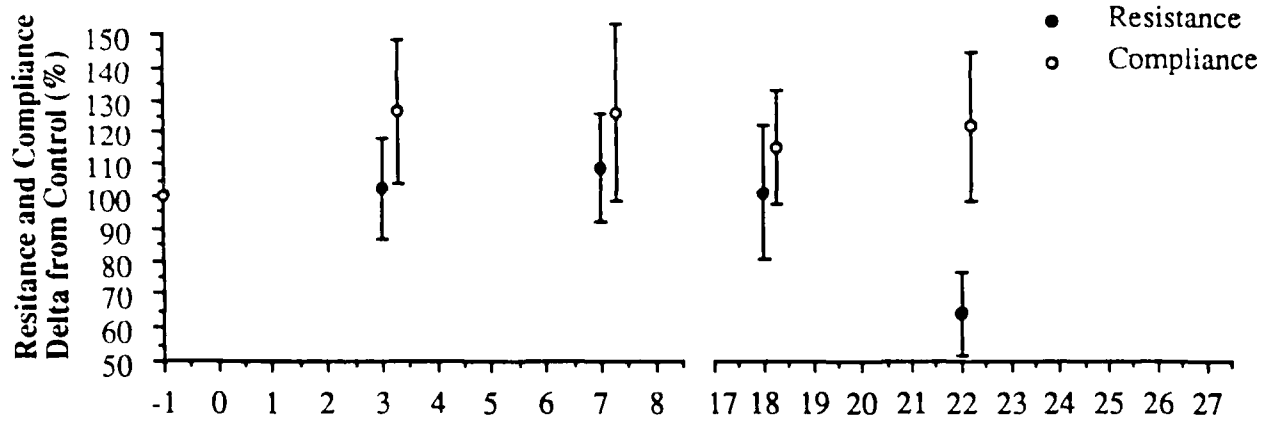
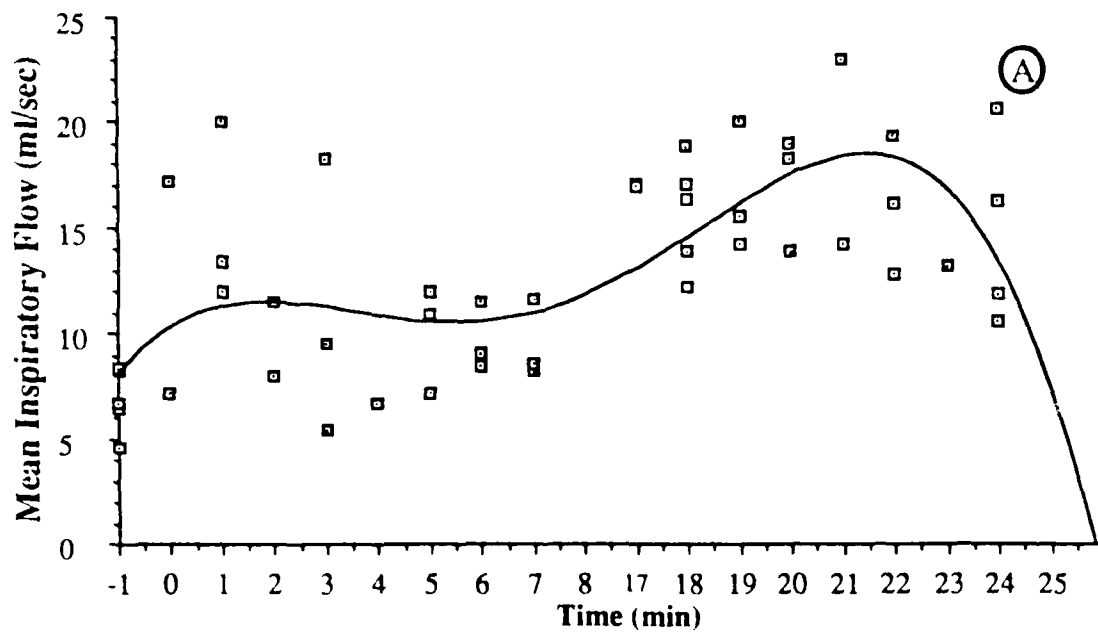


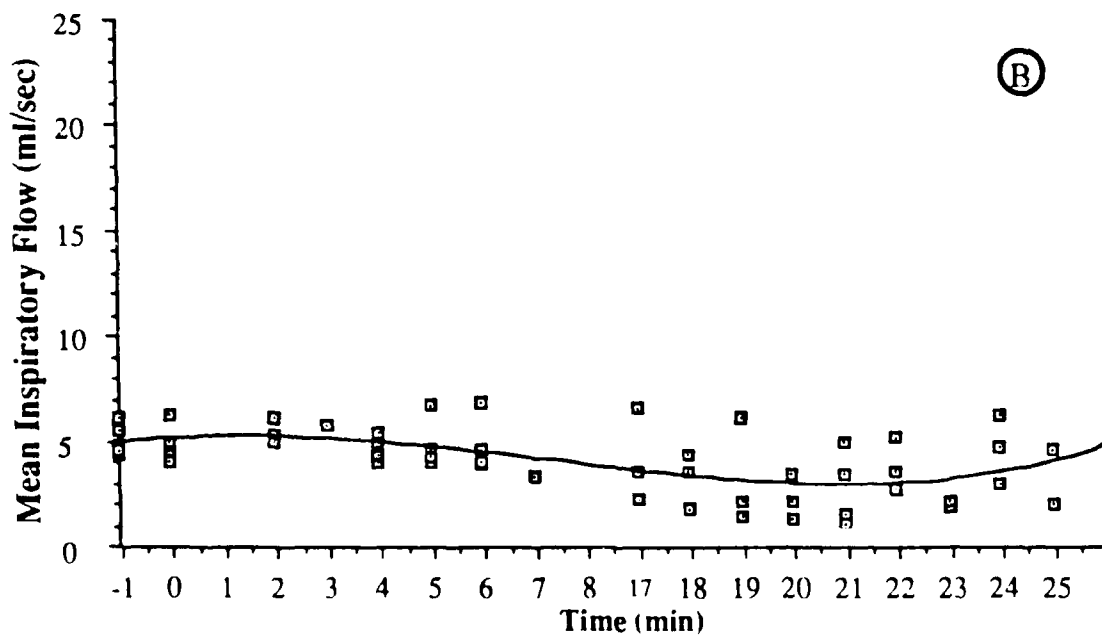
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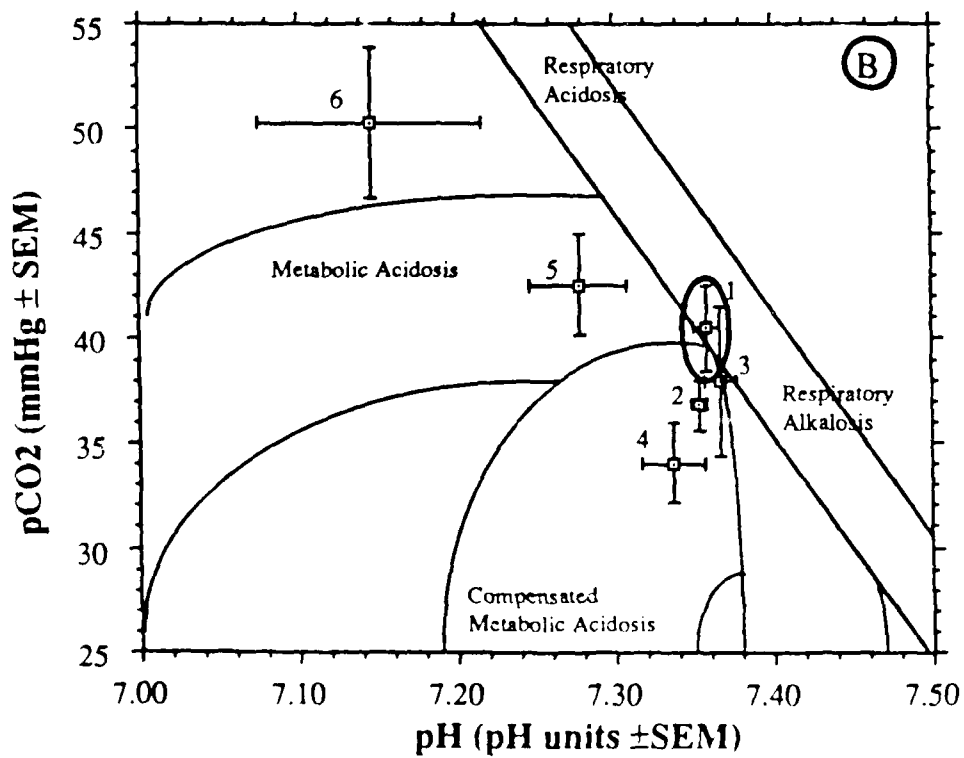
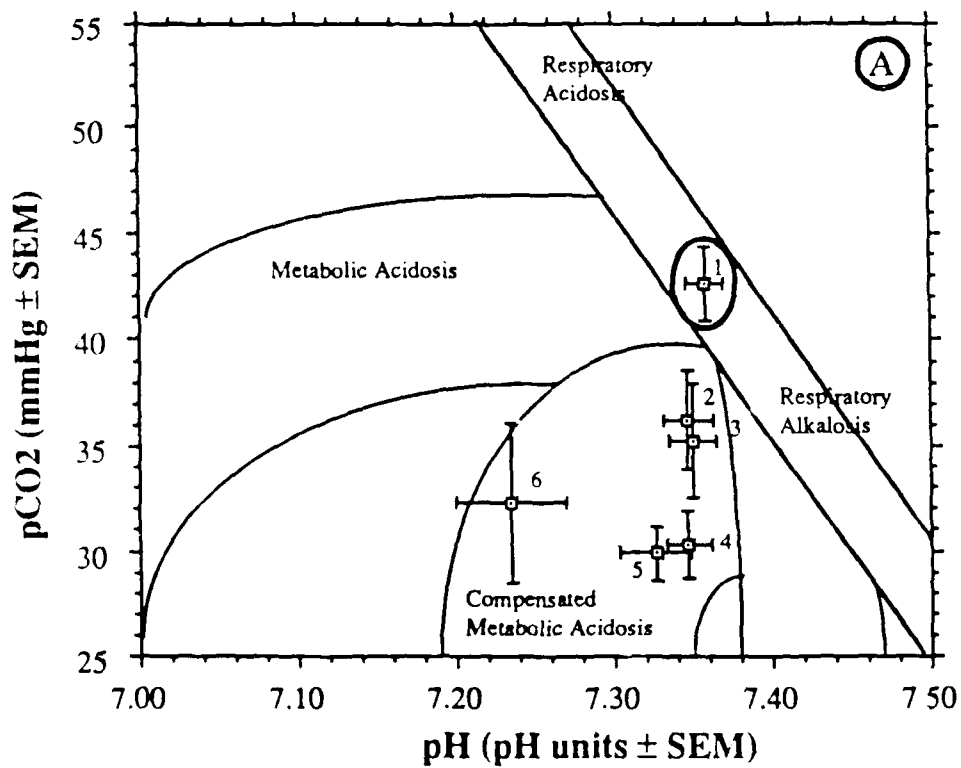
71  
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Pearson's Coefficient of Correlation;  $r = 0.673$  ( $R^2 = 0.453$ ).



Pearson's Coefficient of Correlation;  $r = 0.580$  ( $R^2 = 0.337$ ).



7-1