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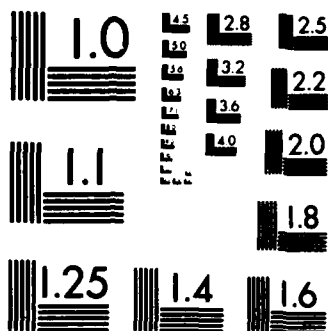
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U S ARMY RESEARCH INSTITUTE
OF
ENVIRONMENTAL MEDICINE
Natick, Massachusetts

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Vincent Forte, Jr.

Stephen Muza

Reed Hoyt

Allen Cymerman



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FOREWARD

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TABLE OF CONTENTS

	PAGE
LIST OF FIGURES	iv
ABSTRACT	v
I. INTRODUCTION	1
II. SYSTEM DEVELOPMENT	1
1. Description	1
2. Layout	3
3. Review of Barometric Method	4
4. Calculation	5
5. Frequency Response Testing	6
6. Data Acquisition and Analysis	7
III. DISCUSSION	8
IV. SUMMARY	12
V. ACKNOWLEDGEMENTS	13
VI. REFERENCES	16
VII. APPENDIX	17

LIST OF FIGURES

<u>FIGURE</u>	<u>LEGEND</u>	<u>PAGE</u>
1	Specific Views and Dimensional Layout Of the Plethysmograph	13
2	Plethysmograph System Schematic	14

ABSTRACT

A plethysmograph was developed to permit non-invasive measurement of pulmonary ventilation in small animals. In this system, precise measurements of temperature, humidity, and barometric pressure are used to calculate tidal volume, while a BASIC computer program directs the collection and display of data on tidal volume, frequency, body temperature, chamber temperature, and relative humidity. The long acoustical time constant of the plethysmograph permits accurate measurement of tidal volume in a wide variety of small unrestrained animals such as mice, rats, and hamsters.

INTRODUCTION

The two-chambered plethysmograph described in this paper permits non-invasive measurement of ventilatory parameters in unrestrained experimental animals such as mice, rats, and other small rodents. Ventilatory rate and tidal volume are derived from pressure changes within the animal chamber. Tidal volume is calculated using the Drorbaugh and Fenn equation (5). This equation corrects for temperature and vapor pressure changes which occur as inspired tidal volume is warmed and humidified and as expired gas is cooled and loses water vapor. The resulting changes in pressure, transmitted throughout the animal chamber, are detected by sensitive transducer and used to calculate tidal volume.

An on-line digital computer collects, displays, and prints out all data during an experiment. The software program, which calculates physiological parameters, is written in BASIC and was developed at the U.S. Army Research Institute of Environmental Medicine. The program which has automated the process of collecting data from large numbers of animals, collects data on respiratory rate, the temperature of the animal, chamber temperature, relative humidity, and uses this information to calculate tidal volume and minute ventilation.

SYSTEM DEVELOPMENT

Description:

The plethysmograph has two identical chambers, each with an internal volume of 11 liters. Each chamber is constructed of acrylic plastic and has removable O-ring gasketed lid (Fig. 1). Each chamber's working volume is adjusted to 10 liters by adding 1 liter of water. The addition of water to the

chamber insures that adequate humidification is maintained. Animals are supported above the water by a plastic grid. Chamber access for set-up and cleaning is provided by a removable lid which is secured by six-evenly spaced 90° latch clamps which provide a gas-tight seal. Temperature and humidity are measured by a dual-element sensor (#LIS-2052-PW, Newport Scientific Co.). A set of NBS traceable calibration curves are supplied with each sensor.

A balanced ventilating system is critical for the operation of this system. Ventilating gases are introduced into the chamber via a pair of matched inlet resistances consisting of small diameter tubing. The resistances are adjusted to provide desired flows and maintain equal pressures in both chambers. The inlet resistances eliminate baseline shifts due to changes in temperature and barometric pressure. The desired gas composition is drawn through each chamber by a regulated vacuum reservoir (approx. 380 mmHg). The outlet resistances (Fig. 2, section #5) are adjusted to maintain a flow of approximately 250 ml/min and equal pressures of approximately -1 cm H₂O with respect to atmospheric pressure in each chamber (P_{box}).

For calibration purposes, 1 ml of ambient air, rapidly injected into the box, should result in a rise in P_{box} of 0.1 cm H₂O. A 3.4 sec acoustical time constant was chosen (C_{gas} equals 10ml/cmH₂O and R_{inlet} of 0.34 cmH₂O/ml/sec), since it is satisfactory for the measurement of respiration in rodents and other small animals with high ventilatory rates and low CO₂ production. (1,2,4,5,13).

Layout:

The plethysmograph is composed of two identical acrylic chambers: a reference and an animal chamber (Fig. 2). The 2.54 cm acrylic wall thickness prevents distortion of the chamber (Fig. 2, sections #1 and #2).

The pressure differential is measured with a transducer (Validyne MP45-1, range ± 2 cm H₂O) (Fig. 2, section #8) and amplified with an amplifier (Validyne CD-12). The gain is adjusted so that a full-scale pen deflection (50 mm on the strip chart) represents a pressure change of 0.125 cm H₂O. Temperature and humidity measurements are displayed by a digital thermohygrometer. Analog D.C. signals for oxygen, carbon dioxide, and volume are displayed on a recorder (Gould/Brush Instruments, Model 200). A mass spectrometer (Perkin Elmer Model 1100A) samples gas at a rate of 60 ml/min for measurement of oxygen and carbon dioxide fractions. The slow leak allows a gas sample to be taken downstream of the system's last resistor (Fig. 2, section #10). The coarse pressure balance in the box is maintained by an adjustable flow meter, (Matheson Model 7640 with Tube #602) (Fig. 2, section #9) and the fine pressure balance is controlled by a micro-adjustable valve (Nupro Model B-4LA) (Fig. 2, section #3). The slow leak of gas into the box is used to stabilize box pressure (Fig. 2, section #10).

Solenoids used for flushing and venting can be activated to rapidly change gas composition in the boxes. Solenoids, (Asco Red-Top™, 1-1/4" NPT), are used as flush valves to change environmental gas mixtures (Fig. 2, section #6) and as gas input valves (Asco Red-Top™ solenoid valves, 3/8" NPT) (Fig. 2, section #7). Solenoid and gas mixture control boxes are used to control the sequence of events and composition of gases in the chambers.

Review of Barometric Method:

Plethysmography requires two chambers, one for the animal and one as a reference connected by a differential pressure transducer (1-5,7,8,11,12). A slow leak mechanism minimize ambient pressure disturbances. If there is a

change in ambient pressure the slow leak mechanism transmits these changes with equal magnitude and phase shifts to each chamber without effecting the differential pressure measurements.

Early systems were unable to maintain stable baselines due the inadequate chamber sealing (4,5). Bartlett and Tenney (2) attempted to alleviate unsteady baseline problems by connecting the experimental chamber to the reference chamber via a high resistance leak. This design change eliminated some baseline drift found in closed systems. However, earlier continuously purged systems had short time constants which resulted in a significant underestimation of the tidal volume. In contrast, Fappenheimer's design (12) has high resistance inlet and outlet ports and requires a pressurized gas to the inlet as well as a vacuum to the outlet to produce a flow through the chamber. This and other similar designs were plagued by poor frequency responses, excessive drift and distorted respiratory patterns due to excessive or rapid leakage from the chamber.

The system described here employs a design which is very similar to that of Jacky (8), except that the plethysmograph's flow rate is greater than that of Jacky's system (8), and its resistance to gas flow is less than 4 cm H₂O with respect to atmosphere. The present system also has individual resistance-inductance networks and provides smaller inlet resistances and a greater flow through the chamber. A regulated vacuum reservoir allows a ventilating flow through the chamber which is adequate for use with 300-600 gram rats who produce a moderate amount of CO₂. This level of flow (250-270ml/min) ensures that the CO₂ concentration does not rise in the box. This system differs from airtight chambers because it behaves as a first-order high-pass filter with a corner frequency of 5.3 cycles/min and has a flat frequency response above 50

cycles/minute. The 3.4 sec time constant is large enough to minimize errors and provide an accurate representation of tidal volume through the range of breathing frequencies exhibited by rodents. The reference and animal chambers are provided with identical inlet and outlet pathways so that ambient pressure disturbances, some which are similar in duration to those of the respiratory cycle, are applied with equal magnitude and phase shifts to both chambers. Therefore, only the peak-to-peak pressure changes which occur when the animal is ventilating are observed.

Calculations:

Drorbaugh and Fenn (5) derived the following formula to calculate tidal volume:

$$V_T = \frac{P_t}{P_k} \cdot V_k \cdot \frac{T_r(P_b - P_c)}{T_r(P_b - P_c) - T_c(P_b - P_r)} \quad \text{where:}$$

V_T = tidal volume (ml);

V_k = 1 cc volume injected into chamber;

P_t = pressure deflection associated with each tidal volume (cm H₂O);

P_k = pressure deflection associated with the injection of calibration volume (cm H₂O);

T_r = body temperature (rectal temperature, °C);

P_c = vapor pressure of water in the chamber at room temperature (mmHg);

P_b = barometric pressure (mmHg);

T_c = air temperature in animal box (°C); and

P_r = vapor pressure of water at body temperature (mmHg).

There are several significant advantages to the type of system (12) described in this paper: 1) a constant flow is established with a minimal pressure drop across the inlet pathway; 2) no high pressure gas source or fine-tuning is needed to produce chamber pressure close to barometric pressure; 3) there is no noise due to turbulent gas flow caused by forcing a moderate flow of gas through inlet orifices under pressure, 4) the acoustical time constant of 3.4 sec is long enough to avoid effecting the respiratory pressure signal in the range of frequencies encountered in rats; and 5) the modified barometric method (8) allows continuous purging of the chambers. This purging reduces CO_2 levels to 0.02% (P_{ICO_2}) under steady state conditions and eliminates errors in ventilatory measurements caused by carbon dioxide build-up in the plethysmograph. This approach avoids the problems with systems which required frequent interruption of experiments to eliminate carbon dioxide build-up in the chamber.

Frequency Response Testing:

The barometric method has been compared by previous investigators (2,5,11) with conventional pneumotachographic techniques and was found to be useful as long as the researcher is aware of known artifacts in this method. Although most investigators did not purge their chambers continuously, this plethysmograph's modifications, which include the gas-flush component, does not affect the pressure-volume relationship of the plethysmograph. Jacky (8) determined the dynamic response of his plethysmograph by injecting a bolus volume of gas, measuring the immediate pressure increase, and calculating a time constant of 1.8 s from the resulting decay. This method was also used to

measure the time constant (frequency response testing)(FRT) in this system. The excessively short acoustical time constant in Jacky's system may result in an underestimation of respiratory volume at elevated breathing frequencies. Thus, the time constant in the our system was lengthened to accommodate awake unrestrained animals whose breathing frequencies are significantly greater than that of a sleeping mammal. Our calculation of the tidal volume using Jacky's method (FRT) assured that our system also had a flat frequency response over the entire ranges of frequencies encountered in rodents under a variety of conditions.

Data Acquisition and Analysis:

Semi-automated data collection and analysis is performed by a MINC 11/23 computer (Digital Equipment Corporation). Software consists of three chained programs which provide calibration, data acquisition, and data analysis functions, respectively (Appendix).

The first program allows calibration of the three A/D channels for volume change, carbon dioxide and oxygen tension. All three channels can be calibrated by providing a baseline voltage (volume reference, zero for PCO_2 , and PO_2) and then injecting a known calibration volume of 1 ml to measure the voltage for the volume channel as well as known gas concentrations to be used as gains for PCO_2 and PO_2 . Respective calibration factors are then determined and stored.

The second program performs the data acquisition and consists of three subroutines. The first prompts the operator for a data file name, the animal's rectal temperature, barometric pressure, box temperature, and relative humidity. The second subroutine provides a CRT strip chart display of the animal's

respiratory pattern (ie. box pressure) and, when signaled by the operator, collects 10 sec of data at a digitizing rate of 50 Hz. The third subroutine stores the data collected through the A/D channels and keyboard entries. The program allows three opportunities for data collection per experimental run.

The third program, which analyzes collected data, has three subroutines. After calibration file and data file identification are made, volume data are graphically displayed. Ten consecutive breaths can be selected for analysis. Tidal volume (V_T (BTPS)) of each of the breaths is determined using the Drorbaugh and Fenn equation (5). Mean tidal volume (V_t), respiratory frequency (f), and minute ventilation ($\dot{V}_E$) are calculated. Mean PCO_2 and PO_2 are also obtained. A hardcopy printout is provided of all identification and respiratory variables measured and calculated.

DISCUSSION

Several investigators have noted limitations in previous plethysmographic systems (6,8). These have included an unsteady baseline, fluctuations in temperature and vapor pressure equilibrium, excessive ventilation due to CO_2 accumulation, and inaccurate measurements of differences between body and ambient temperatures.

The present system overcomes most of these limitations by providing a flow-through design with individual resistance-inductance networks compensating for the baseline drift. Because the respiratory signal is so small it is susceptible to distortion caused by decreases in pressure associated with compression of intra-abdominal gas. This problem fortunately produces an easily recognizable elongation of the expiratory pressure signal.

Continuously purged plethysmographs also avoid problems with temperature and humidity gradients in the chamber which can occur in studies lasting more than 10 minutes. Epstein and Epstein (6) have recently shown that errors in the measurement of temperature in the plethysmograph are responsible for even greater underestimation of tidal volume and developed equations for correcting the tidal volume calculation. Tidal volume calculated by the method of Drorbaugh and Fenn (5) assumes the pressure in the animal chamber increases in proportion to V_T as inspired air is warmed and humidified during inspiration. During expiration, pressure decreases with cooling and water condensation from expired gas. Epstein and Epstein (6) suggest that the pressure changes associated with warming and humidification of inspired air and the subsequent cooling and water condensation are not equal. Errors of 15 to 30% during normal ventilation and 10% during hypercapnic exposures are reported (6).

Our calculations are in agreement with Jacky (9) in that the magnitudes of the errors in tidal volume are less than those of Epstein and Epstein (6) who have used larger animals and infants. The difference in measuring tidal volume by conventional pneumotachographic and plethysmographic methods have been the subject of much research (6,7,9,10) with each group presenting different solutions for correcting the error in the calculation of tidal volume. Plethysmographic measurements of tidal volume have been criticized by Epstein and Epstein as having a greater underestimation of true tidal volume than conventional methods such as pneumotachographic or mechanical methods (2,4,5,11). They believe that the asymmetry of the pressure events (of inspiration and expiration and associated time constants) leads to an overestimation in inspiratory time (T_i) as the total breath duration (T_{tot})

increases. Epstein and Epstein contend that the assumptions of other investigators that expiration mirrors inspiration is incorrect. However, Epstein's findings are not supported by the literature (2,4,5,11) and we believe that the errors which are inherent in other plethysmographs are eliminated by a "leaky" system. When comparing resting data from a recent study with rats with that of stimulated breathing (13) we found no large errors in ventilation. Jacky (9), has reported that the approximate magnitude of potential error in tidal volume measurement is in the order of 15% underestimation during air breathing and decreases to 4% underestimation of the fractional increase in tidal volume during hyperpnea due to hypercarbia, associated with changes in timing of the respiratory cycle and is varied with different breathing patterns (10,13). Our measurements were not corrected for the underestimation in tidal volume because these errors are only significant when absolute measurements of tidal volume are made, but not when relative changes were considered as in our study (13). Typically our acute studies did not require using any one gas for longer than 5-7 minutes and this duration is short enough to minimize temperature distortion of the respiratory signal.

Practical and theoretical considerations must be reviewed when the plethysmograph is used in research studies. The plethysmograph must be well constructed and undergo rigorous testing. The investigator must be familiar with the system because the small respiratory signal is easily distorted by internal and external factors. Once these considerations are addressed, the operation of the system is suitable for measuring ventilation in awake, unrestrained animals. The following are critical characteristics and components of the plethysmographic system: 1) the external reference chamber, 2) a stable and

vibration-free base, 3) a relatively long time constant to damp out slow changes in pressure due to temperature and humidity effects, 4) CO₂ scrubbers or a continuously flushed "leaky" system to avoid CO₂ build up, and 5) an effective system for measuring the partial pressures of carbon dioxide and oxygen. Although more direct methods, such as pneumotachography, have been shown to have smaller errors in measuring "true" tidal volume, the plethysmograph is clearly suitable for monitoring changes in ventilation in small unrestrained animals.

SUMMARY

A plethysmograph system, with an automated gas-control system has been developed for the non-invasive measurement of pulmonary ventilation in small animals. A relatively long time constant preserves signal fidelity and removes drift while a slow-leak system limits the effects of ambient pressure disturbances. The proper selection of the time constant and accurate measurement of temperature and humidity reduces the underestimation in tidal volume in the plethysmograph. The ease with which the gas mixtures can be administered is also conducive to longer and more variable study designs. Early problems with carbon dioxide buildup are easily eliminated by using a continuously flushed system whose ventilation flow rate is set at 250-270 ml/min. Precise measurement of temperature and humidity in the animal chamber permit accurate tidal volume corrections. In conclusion, the plethysmograph design presented in this paper reflects several years of design and research to develop a functional tool to measure pulmonary ventilation in unrestrained small animals.

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APPENDIX

Computer Program:

```

10 REM*****PROGRAM: VFT*****
20 DIM X(2)
30 DISPLAY_CLEAR
40 PRINT 'VFT: A PROGRAM FOR DATA ACQUISITION & ANALYSIS' \ PRINT \ PRINT
50 PRINT 'type: 1) calibrate;'
60 PRINT '         2) collect data;'
70 PRINT '         3) analyze data;'
80 PRINT '         4) stop;'; \ INPUT QX
90 IF QX>=1 THEN IF QX<=4 GO TO 110
100 GO TO 30
110 ON QX GO TO 130,140,150,160
120 REM*****LIST OF CHAINED PROGRAMS*****
130 GO TO 170
140 CHAIN 'VF1T'
150 CHAIN 'VF2T'
160 GO TO 1320
170 REM*****CALIBRATION OF VOLUME*****
180 DISPLAY_CLEAR
190 PRINT 'A/D 0 VOLUME'
200 PRINT 'A/D 1 02'
210 PRINT 'A/D 2 CO2' \ PRINT \ PRINT
220 PRINT 'CALIBRATION OF VOLUME' \ PRINT
230 PRINT 'type 1) to enter calibration factor;'
240 PRINT '         2) to calculate calibration factor;'; \ INPUT QX
250 IF QX>=1 THEN IF QX<=2 GO TO 270
260 GO TO 170
270 IF QX=2 GO TO 300
280 PRINT \ PRINT 'enter calibration factor:'; \ INPUT F3
290 GO TO 650
300 DISPLAY_CLEAR
310 DIM V(100)
320 PRINT 'DETERMINATION OF CALIBRATION FACTOR BY INJECTION OF 1 ML'
330 FOR I=1 TO 5
340 PRINT \ PRINT 'CHECK BASELINE' \ PRINT
350 PRINT 'when ready press RETURN';
360 INPUT X$
370 V=0
380 AIN(V(),.40,.05)
390 FOR I1=0 TO 39
400 V=V+V(I1)
410 NEXT I1
420 B(I)=V/40
430 PRINT 'INJECT 1 ML NOW';CHR$(7)
440 AIN(V(),.100,.05)
450 P=B(I)*1.02
460 FOR I2=0 TO 99
470 PRINT I2,V(I2)
480 IF ABS(V(I2))>=P THEN P=ABS(V(I2))
490 NEXT I2
500 F(I)=P-B(I)
510 PRINT 'BASELINE (volts):';B(I),'CAL #';I;' (volts/ml)';F(I)
520 NEXT I
530 REM*****AVERAGE OF CALIBRATION INJECTIONS*****
540 F3=0
550 PRINT 'do you wish to delete a calibration (Y/N)'; \ INPUT Q$
560 IF Q$='N' GO TO 590
570 PRINT 'hit number of run to delete'; \ INPUT R9
580 I3=0
590 FOR I=1 TO 5
600 IF I=R9 THEN 630
610 F3=F3+F(I)
620 I3=I3+1
630 NEXT I
640 F3=F3/I3
650 PRINT \ PRINT 'CALIBRATION FACTOR (VOLTS/ML) =';F3
660 PRINT \ PRINT 'do you wish to recalibrate (Y/N)'; \ INPUT Q$
670 IF Q$='Y' GO TO 170
680 X(0)=F3
690 DISPLAY_CLEAR

```

```

700 J=J1
710 IF M=10 GO TO 780
720 FOR K=J TO S2X
730 IF B(K)<P THEN P=B(K)
740 IF B(K)>P GO TO 760
750 NEXT K
760 P0=P \ I1=K
770 P=B(I1)
780 NEXT I1
790 FOR I2=1 TO 10
800 V=V+V(I2)
810 NEXT I2
820 V=V/10
830 F=600/((S2X-S1X)*.02)
840 FOR I1=511 TO 609 STEP 2
850 V6=V6+ABS(B(I1))
860 V7=V7+ABS(B(I1+1))
870 NEXT I1
880 V6=ABS((V6/50)/X(1))
890 V7=ABS((V7/50)/X(2))
900 DISPLAY_CLEAR
910 PRINT 'RESPIRATORY RATE = ' ;F
920 PRINT 'TIDAL VOLUME = ' ;V
930 PRINT 'MINUTE VENTILATION = ' ;F*V
940 PRINT 'PO2 = ' ;V6
950 PRINT 'PCO2 = ' ;V7
960 GOSUB 1010
970 PRINT \ PRINT
980 PRINT 'to continue data analysis hit RETURN'; \ INPUT D$
990 NEXT I
1000 GO TO 140
1010 REM*****PRINTER SUBROUTINE*****
1020 OPEN 'LP:' FOR OUTPUT AS FILE #1
1030 PRINT #1 \ PRINT #1
1040 PRINT #1,DAT$,CLK$
1050 PRINT #1,'FILE ID = SY1:'F$QNS
1060 PRINT #1,'RAT NO. = ' ;A$
1070 IF A1$='1' THEN PRINT #1,'NO DRUG - NORMOXIA'
1080 IF A1$='2' THEN PRINT #1,'NO DRUG - HYPOXIA'
1090 IF A1$='3' THEN PRINT #1,'NO DRUG - HYPOXIC, HYPERCAPNIA'
1100 IF A1$='4' THEN PRINT #1,'NO DRUG - HYPERCAPNIA'
1110 IF A1$='5' THEN PRINT #1,'DRUG - HYPEROXIC HYPERCAPNIA'
1120 IF A1$='6' THEN PRINT #1,'DRUG - HYPOXIA'
1130 IF A1$='7' THEN PRINT #1,'DRUG - NORMOXIA'
1140 IF A1$='8' THEN PRINT #1,'DRUG - HYPERCAPNIA'
1150 PRINT #1,'RUN NUMBER = ' ;A2$
1160 IF A3$='0' THEN PRINT #1,'NO DRUG'
1170 IF A3$='1' THEN PRINT #1,'DOSE = 0.005 ML/KG'
1180 IF A3$='2' THEN PRINT #1,'DOSE = 0.050 ML/KG'
1190 IF A3$='3' THEN PRINT #1,'DOSE = 0.500 ML/KG'
1200 IF A3$='4' THEN PRINT #1,'DOSE = 5.000 ML/KG'
1210 PRINT #1,'RECTAL TEMP (C) = ' ;B(1)
1220 PRINT #1,'BOX TEMP(C) = ' ;B(2)
1230 PRINT #1,'BAROMETRIC PRESSURE (TORR) = ' ;B(3)
1240 PRINT #1,'RELATIVE HUMIDITY = ' ;B(4)
1250 PRINT #1,'PH2O (BOX) = ' ;B(5)
1260 PRINT #1,'PH2O (RAT) = ' ;B(6)
1270 PRINT #1,'TIDAL VOLUME = ' ;V
1280 PRINT #1,'RESPIRATORY RATE = ' ;F
1290 PRINT #1,'MINUTE VENTILATION = ' ;F*V
1300 PRINT #1,'PO2 = ' ;V6
1310 PRINT #1,'PCO2 = ' ;V7
1320 PRINT #1 \ PRINT #1
1330 FOR I3=1 TO 10
1340 PRINT #1,V(I3)
1350 NEXT I3
1360 PRINT #1 \ PRINT #1
1370 PRINT #1,X(0),X(1),X(2)
1380 CLOSE #1
1390 RETURN
1400 DISPLAY_CLEAR
1410 CLOSE
1420 END

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10 REM*****PROGRAM NAME "VF1T"*****
20 REM***CHANNEL 0 PRESSURE, CH1 O2, CH2 CO2 *****
30 DIM M(500),G(100),B(610)
40 DISPLAY_CLEAR
50 PRINT 'RAT NO. ='; \ INPUT A$
60 PRINT 'CONDITION' 1=NO DRUG - NORMOXIA'
70 PRINT '          ' 2=NO DRUG - HYPOXIA'
80 PRINT '          ' 3=NO DRUG - HYPOXIC, HYPERCAPNIA'
90 PRINT '          ' 4=NO DRUG - HYPERCAPNIA'
100 PRINT '          ' 5=NO DRUG - HYPEROXIC HYPERCAPNIA'
110 PRINT '          ' 6=DRUG - HYPOXIA'
120 PRINT '          ' 7=DRUG - NORMOXIA'
130 PRINT '          ' 8=DRUG - HYPERCAPNIA'
140 PRINT '          ' 9=OTHER'
150 INPUT A1$
160 PRINT 'RUN # (0-9)'; \ INPUT A2$
170 PRINT 'DOSE' 0= NO DRUG'
180 PRINT '          ' 1=.005 ML/KG'
190 PRINT '          ' 2=.050 ML/KG'
200 PRINT '          ' 3=.500 ML/KG'
210 PRINT '          ' 4=5.00 ML/KG'
220 INPUT A3$
230 A1$='SY1:'&A$&A1$&A2$&A3$
240 PRINT 'DATA SAVED ON FILE:'A1$
250 PRINT 'RECTAL TEMP (C) = ' \ INPUT B(1)
260 PRINT 'BOX TEMP (C) = ' \ INPUT B(2)
270 PRINT 'BAROMETRIC PRESSURE (TORR) = ' \ INPUT B(3)
280 PRINT 'RELATIVE HUMIDITY = ' \ INPUT B(4)
290 REM CALCULATION OF PH2O
300 C=1.23234E+07 \ C1=7.52247 \ C2=-1223.31 \ C3=-222614 \ C4=B(2)+273 \ C5=B(1)+273
310 C6=C1+(C2/C4)+(C3/(C4^2))+(C/(C4^3)) \ B(5)=10^C6
320 C8=C1+(C2/C5)+(C3/(C5^2))+(C/(C5^3)) \ B(6)=10^C8
330 B(7)=0 \ B(8)=0 \ B(9)=0
340 REM
350 DISPLAY_CLEAR
360 PRINT 'type: 1) new file information'
370 PRINT '          2) collect data'
380 PRINT '          3) return to MAIN MENU'; \ INPUT Q%
390 IF Q%>=1 THEN IF Q%<=3 GO TO 410
400 GO TO 350
410 ON Q% GO TO 40,420,780
420 REM*****DATA COLLECTION ROUTINE*****
430 FOR I=1 TO 3
440 IF I=1 THEN N$='1'
450 IF I=2 THEN N$='2'
460 IF I=3 THEN N$='3'
470 OPEN A1$&N$&'.DVF' FOR OUTPUT AS FILE #2
480 AIN('DISPLAY',M(),1,,0)
490 PRINT 'DATA FILE:'A1$&N$
500 PRINT 'press RETURN when ready to sample.'
510 AIN('DISPLAY',M(),1,,0)
520 GET_CHAR(C$)
530 IF LEN(C$)=0 GO TO 510
540 IF ASC(C$)=10 GO TO 560
550 GO TO 510
560 AIN(M(),500,.02) \ AIN(G(),100,.02,1,2) \ REM SAMPLE CHANNELS 0-2
570 PRINT CHR$(7);
580 FOR I1=1 TO 9
590 PRINT #2,B(I1)
600 NEXT I1
610 FOR I1=10 TO 510
620 B(I1)=M(I1-10)
630 PRINT #2,B(I1)
640 NEXT I1
650 FOR I1=511 TO 610
660 B(I1)=G(I1-511)
670 PRINT #2,B(I1)
680 NEXT I1
690 CLOSE
700 NEXT I
710 DISPLAY_CLEAR
720 PRINT 'type: 1) new file information'
730 PRINT '          2) return to MAIN MENU'
740 PRINT '          3) stop'; \ INPUT Q%
750 IF Q%>=1 THEN IF Q%<=3 GO TO 770
760 GO TO 710
770 ON Q% GO TO 40,780,750
780 CHAIN 'VF1T'
790 END

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10 REM*****PROGRAM NAME "VF2T"*****
20 REM***CHANNEL 0 PRESSURE, CH1 O2, CH2 CO2 *****
30 DIM B(610),X(2),V(11)
40 DISPLAY_CLEAR
50 PRINT \ PRINT \ PRINT
60 REM*****OPEN CALIBRATION FILE*****
70 PRINT 'ENTER CALIBRATION FILE NAME': \ INPUT C$
80 OPEN 'SY1:'&C$&'.CAL' FOR INPUT AS FILE #2
90 FOR I=0 TO 2
100 INPUT #2,X(I)
110 NEXT I
120 CLOSE #2
130 PRINT \ PRINT
140 PRINT 'CALIBRATION FILE IS:'&C$
150 REM*****MAIN MENU*****
160 PRINT \ PRINT \ PRINT
170 PRINT 'type: 1) for new calibration file'
180 PRINT '          2) to analyze new data file'
190 PRINT '          3) to collect new data'
200 PRINT '          4) to stop': \ INPUT G$
210 IF G$>=1 THEN IF G$<=4 GO TO 230
220 GO TO 150
230 ON G$ GO TO 50,240,250,1400
240 GO TO 260
250 CHAIN 'VFT'
260 REM*****OPEN DATA FILES*****
270 DISPLAY_CLEAR
280 PRINT \ PRINT
290 PRINT 'ENTER DATA FILE NAME': \ INPUT F$
300 FOR I=1 TO 3
310 IF I=1 THEN N$='1'
320 IF I=2 THEN N$='2'
330 IF I=3 THEN N$='3'
340 OPEN 'SY1:'&F$&N$&'.DVF' FOR INPUT AS FILE #3
350 FOR I1=1 TO 610
360 INPUT #3,B(I1)
370 NEXT I1
380 CLOSE #3
390 A$=SEG$(F$,1,1)
400 A1$=SEG$(F$,2,2)
410 A2$=SEG$(F$,3,3)
420 A3$=SEG$(F$,4,4)
430 REM*****DATA ANALYSIS*****
440 DISPLAY_CLEAR
450 S1%=0 \ S2%=0
460 GRAPH(.500,.B(10))
470 LABEL(.,'RAT: ' &F$&N$)
480 PRINT 'shade graph (Y/N)': \ INPUT Q$
490 IF Q$='N' GO TO 510
500 SHADE
510 PRINT 'select beginning of first breath (':S1%:'): \ INPUT Q$
520 IF LEN(Q$)<>0 THEN S1%=VAL(Q$)
530 PRINT 'select end of tenth breath (':S2%:'): \ INPUT Q$
540 IF LEN(Q$)<>0 THEN S2%=VAL(Q$)
550 POINT('BRAND',S1%,B(S1%))
560 POINT('BRAND',S2%,B(S2%))
570 PRINT 'regraph points (Y/N)': \ INPUT Q$
580 IF Q$='Y' GO TO 460
590 PRINT 'change start or stop point? (Y/N)': \ INPUT Q$
600 IF Q$='Y' GO TO 510
610 REM*****MEASUREMENT OF TIDAL VOLUME*****
620 P0=B(S1%) \ P=B(S2%) \ M=0 \ V=0 \ F=0 \ V6=0 \ V7=0
630 FOR I1=S1% TO S2%
640 IF B(I1)>P GO TO 770
650 M=M+1
660 V(M)=P-P0
670 V(M)=(V(M)/X(0))*((B(1)+273)*(B(3)-B(5)))/((B(1)+273)*(B(3)-B(5))-(B(2)+27
680 IF V(M)>.5 GO TO 700
690 M=M-1

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700 REM*****CALIBRATION OF GASES*****
710 PRINT 'CALIBRATION OF O2 AND CO2' \ PRINT \ PRINT
720 PRINT 'type 1) to enter calibration factor;'
730 PRINT '      2) to calculate calibration factor'; \ INPUT G%
740 IF G%>=1 THEN IF G%<=2 GO TO 760
750 GO TO 690
760 IF G%=2 GO TO 820
770 PRINT \ PRINT 'enter calibration factor for O2 (volts/torr)';
780 INPUT X(1)
790 PRINT 'enter calibration factor for CO2 (volts/torr)';
800 INPUT X(2)
810 GO TO 1040
820 DISPLAY_CLEAR
830 PRINT 'DETERMINATION OF GAS CALIBRATION FACTORS'
840 DIM G(20) \ N=0 \ G=0
850 PRINT \ PRINT 'sample zero O2, when ready press RETURN';
860 INPUT X%
870 GOSUB 1150
880 PRINT 'zero O2=';G1;'volts'
890 PRINT \ PRINT 'sample zero CO2, when ready press RETURN';
900 INPUT X%
910 GOSUB 1150
920 PRINT 'zero CO2=';G2;'volts'
930 PRINT \ PRINT 'sample peak O2, when ready press RETURN';
940 INPUT X%
950 GOSUB 1150
960 PRINT 'peak O2=';G3;'volts'
970 PRINT 'enter peak O2 torr:'; \ INPUT D5
980 PRINT \ PRINT 'sample peak CO2, when ready press RETURN';
990 INPUT X%
1000 GOSUB 1150
1010 PRINT 'peak CO2=';G4;'volts'
1020 PRINT 'enter peak CO2 torr:'; \ INPUT D6
1030 X(1)=ABS((G3-G1)/D5) \ X(2)=ABS((G4-G2)/D6)
1040 PRINT \ PRINT 'O2 CALIBRATION FACTOR (VOLTS/TORR)=';X(1)
1050 PRINT \ PRINT 'CO2 CALIBRATION FACTOR (VOLTS/TORR)=';X(2)
1060 PRINT \ PRINT 'do you wish to recalibrate (Y/N)'; \ INPUT G%
1070 IF G%='Y' GO TO 690
1080 PRINT 'ENTER CAL FILE NAME (day,month,number)'; \ INPUT N%
1090 OPEN 'SY1:'&N%&'.CAL' FOR OUTPUT AS FILE #2
1100 FOR I=0 TO 2
1110 PRINT #2,X(I)
1120 NEXT I
1130 CLOSE #2
1140 GO TO 30
1150 N=N+1
1160 IF N=1 THEN D=1
1170 IF N=2 THEN D=2
1180 IF N=3 THEN D=1
1190 IF N=4 THEN D=2
1200 AIN(G(),20,.1,D)
1210 FOR I=0 TO 19
1220 G=G(I)
1230 NEXT I
1240 IF N=1 THEN G1=G/20
1250 IF N=2 THEN G2=G/20
1260 IF N=3 THEN G3=G/20
1270 IF N=4 THEN G4=G/20
1280 FOR I=0 TO 20
1290 G(I)=0
1300 NEXT I
1310 RETURN
1320 END

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