DEVELOPMENT AND ASSESSMENT OF AN ON-LINE AEROBIC MEASUREMENT SYSTEM

U. S. ARMY RESEARCH INSTITUTE OF ENVIRONMENTAL MEDICINE
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Marc G. Cote, Dan M. White, Robert P. Mello,
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Abstract

An on-line aerobic measurement system was developed to meet the needs of an exercise physiology laboratory. It utilized a HP 9820 desk top calculator to compute parameters from signals averaged over a 13 second sample period. An infrared CO₂ analyzer and fuel cell O₂ analyzer were used for gas concentrations and a Fleisch pneumotachograph for air flow. A variable volume mixing chamber was utilized to avoid breath-by-breath variations in gas concentrations. Reliability and validity studies between this on-line system and the traditional Douglas bag collection method showed no significant differences in VO₂, VE or FE₂O₂ for both submaximal and maximal work loads.
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Introduction

Considerable work in exercise physiology requires the accurate and timely measurement of oxygen uptake (\( \dot{V}O_2 \)) during exercise. This determination consists of assessing the expired ventilation (\( V_E \)) and its oxygen (\( FE_{O_2} \)) and carbon dioxide (\( FE_{CO_2} \)) fractions. Traditionally, this determination is executed with the use of a collection system (e.g. Douglas bags, tissot) and chemical (e.g. Scholander analysis (1)) or electrical oxygen and carbon dioxide analysis. A major drawback of these systems is that a pooled sample of ventilation is collected over a period of time such that transient and short term phenomena are masked within the sample. If steady state conditions are desired, the investigator must estimate at what point in time the test subject has achieved a metabolic plateau. Thus, the Douglas bag collection system does not give the investigator a basis to instantaneously determine if he should maintain the subject for a longer or shorter period of time while exercising in order to obtain a metabolic steady state for a particular work load. Furthermore, real time decisions on determining the point at which maximal \( \dot{V}O_2 \) has occurred cannot be made and may require the subject to exercise again if one employs criteria of Taylor, et al. (2).

An on-line aerobic measurement system was developed in answer to these specific needs. The on-line system provides: 1) continuous sampling 2) the computation of \( \dot{V}O_2 \) within seconds of the expired sample 3) the examination of specific physiological responses during the performance of exercise (e.g. anaerobic threshold phenomena (3)) and 4) examination of oxygen uptake kinetics (4).
This paper discusses an on-line aerobic system, its configuration, and its validity based on actual field use. The system was developed at the U.S. Army Research Institute of Environmental Medicine, Natick, MA. Field testing of the system was conducted during studies conducted at Ft. Jackson, South Carolina.

Methods and Procedures

General:

An overall schematic view of the system is outlined in Figure 1 and can be categorized into two major sub-systems: 1) analyzing instruments and 2) the data processing components. The system components consisted of the following:

a) Hewlett-Packard 9820 calculator
b) Hewlett-Packard timing generator
c) Hewlett-Packard A/D convertor
d) Hewlett-Packard memory cassette drive
e) A hard copy terminal printer
f) Hewlett-Packard four channel recorder with carrier amplifier (8805B) and respiratory integrator (8815).
g) Kroehn-Hite Corp. adjustable low pass band electronic filter
h) Validyne Engineering Corp. MP-45 differential pressure transducer (+2.5 cm H₂O).
i) Hans Rudolph Co. pulmonary model thermostatically controlled heated pneumotachograph.
j) Yellow Spring Co. telethermometer with thermistor probe (series 400).
k) Applied Electrochemistry Inc. Model S-3A O₂ analyzer (100 m sec. to 90% of full value with ± 0.01% accuracy).
1) Beckman Co. LB-2 CO$_2$ analyzer (100 m sec. to 90% full value with ± 1% accuracy).

m) An adjustable volume stainless steel mixing chamber with baffle and mixing fan.

n) Koegel Co. 'Y' breathing valve.

The Hewlett-Packard (H/P), four channel recorder with carrier amplifier (8805B) and respiratory integrator (8815) was used in conjunction with the pneumotach and Validyne transducer. The two extra channels allowed for the permanent recording of O$_2$ and CO$_2$ if desired. The Kroehn-Hite low pass filter conditioned the respiratory signal from the carrier prior to integration (Fig. 1). The integrated signal output was in turn interfaced with the A/D convertor. The use of coaxial cables with BNC connectors allowed flexibility in the interfacing and shielding of all the analog signals.

The HP 9820 calculator served as the processor in the data processing system with the four channel A/D convertor interfacing the analog signals to the calculator. The A/D convertor also provided for triggering of the respiratory integrator reset. The memory cassette provided for additional storage and data organization.

Physical Connections:

Plumbing of the system is shown in Figure 2. The Koegel 'Y' breathing valve (5) (Fig. 2 'A', Fig. 3) was utilized due to its low resistance, (< 0.8 cm H$_2$O, @ 300 l/min), deadspace (64 cc.) and low weight (72.5 g). Collins spiral plastic respiratory tubing (1-1/2" I.D., CAT. #22263) was utilized throughout the system. The YSI thermistor (Fig. 2 'B') was placed in the expired flow immediately prior to the heated pneumotach (Fig. 2 'D'). The Validyne transducer (Fig. 2 'E', Fig. 4 'A') was held in a vertical plane so that its diaphragm was
Fig. 3 Koege, breathing valve.
Fig. 4 Flow measuring components.
A: Pneumotach
B: Pressure transducer
C: Mixing chamber
D: Incoming line from subject
E: Calibration flow line
perpendicular to any source of mechanical vibration. The tygon tubing (1/4" I.D.)
between the transducer and pneumotach (Fig. 4) was kept as short as possible to
minimize compliance and mechanical shock. The thermostat setting for the
pneumotach was 37°C to prevent any condensate from forming on the pneumotach
and affecting its performance since it relies on the Poiseuille principle. Since all
measurements were made at sea level, it was not necessary to correct for the
pneumotach measurement problems encountered with light and dense atmospheres
(6).

A stainless steel variable volume mixing chamber, developed at the Cardio-
vascular Pulmonary Research Laboratory, Univ. of Colorado Medical Center, and
further modified by Cymerman and Sacco (7) (Fig. 2 'F', Fig. 5, Fig. 6), allowed for
the adequate mixing of expired air prior to O$_2$ and CO$_2$ sampling. The chamber
volume could be adjusted for low or high ventilations. When the plunger was
extended to a length of 23 centimeters mixing was adequate for both light and
heavy work loads encountered with treadmill running. A two way stainless steel
stopcock (Fig. 2 'G') with Luer fittings allowed for the calibration of the gas
analyzers, sampling of room air or actual chamber sampling. A desiccant trap (Fig.
2 'H') immediately preceded the O$_2$ and CO$_2$ sensors (Fig. 2 'I, 'J'). Although the
trap increased the lag time of the sample it was necessary to insure that no
condensate would enter and damage the 700°F fuel cell sensor. Also the correction
for the partial pressure of water vapor was unnecessary with the use of the
desiccant. Both gas sensors were isolated from mechanical shock and vibration by
suspending them with nylon cord since sensor vibration can result in unstable
readings. The use of a 'Y' connector in the gas analyzer line allowed for the
simultaneous sampling of O$_2$ and CO$_2$. Separate pumps (Fig. 2 'K, 'L') avoided the
instability encountered with high flows on the CO$_2$ sensor. Thus, maximal response
Fig. 6 Mixing chamber interior showing muffin fan (A) and baffle (B).
time was achieved without compromising stability and reliability of the gas analyzers.

When the on-line system was not connected in series to the Douglas bag system for validation studies, a three feet piece of Collins respiratory tubing was left on the effluent port of the mixing chamber to prevent accidental intrusion of room air. A Fisher-Porter rotameter (20-210 cc., Fig. 2 'C', Fig. 7) using a compressed gas flow was used to calibrate the respiratory integrator. Table I summarizes the system specifications and response times.

Programm:ing:

The appropriate analog input ranges within the A/D convertor were selected in order to maximize the digital conversion from the analog signal (e.g. the O₂ analyzer has an output voltage of 0–5 volts over a 0–100% O₂ range; a range of 1.0 volt was chosen in the A/D convertor because the O₂ analyzer would not sample any sample greater than 21% O₂ resulting in an output voltage of 1 volt).

On initiation of program execution, the calculator would trigger the respiratory integrator to reset via the A/D convertor RVS channel. Sampling of Vₑ occurred both at the start and the end of the 13 seconds sample interval. The difference between the start and end of Vₑ interval was used as the actual Vₑ value thereby eliminating any possible baseline drift problems.

Expired O₂ and CO₂ fractions (Fₑ) and expired temperature (Tₑ) were collected three times per sample and sampled at 0, 4, 8, and 13 seconds. The means of the FₑO₂, FₑCO₂ and Tₑ samples within this total 13 second interval were incorporated with the Vₑ from the preceding 13 second Vₑ interval which was temporarily stored for this purpose. This timing compensated for the delay encountered between the measurement of Vₑ and the time it took for the Vₑ
Table 1. Specifications for various components of the on-line system.

<table>
<thead>
<tr>
<th>Component Variable</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of respiratory tubing</td>
<td>1.8 m</td>
</tr>
<tr>
<td>Volume of respiratory tubing</td>
<td>2.1 l</td>
</tr>
<tr>
<td>Ventilatory sample interval</td>
<td>13 sec</td>
</tr>
<tr>
<td>Data printout interval</td>
<td>15 sec</td>
</tr>
<tr>
<td>O₂, CO₂, Texp sample interval</td>
<td>4 sec</td>
</tr>
<tr>
<td>Pneumotach heater temp.</td>
<td>37°C</td>
</tr>
<tr>
<td>Filter (low pass max flat)</td>
<td>80 l/min</td>
</tr>
<tr>
<td>Response time from mouthpiece</td>
<td>5 sec</td>
</tr>
<tr>
<td>Mixing chamber equilibration times</td>
<td>(80 l/min flow)</td>
</tr>
<tr>
<td>(80 l/min flow) and volumes</td>
<td></td>
</tr>
<tr>
<td>with chamber extended:</td>
<td></td>
</tr>
<tr>
<td>1. 13cm</td>
<td>14 sec, 2.4 l</td>
</tr>
<tr>
<td>2. 23cm</td>
<td>20 sec, 4.2 l</td>
</tr>
<tr>
<td>3. 31cm (maximal)</td>
<td>24 sec, 5.6 l</td>
</tr>
<tr>
<td>System resistance</td>
<td>0.86 cm H₂O/80 l/min</td>
</tr>
<tr>
<td>Integrator drift</td>
<td>± 15 mv/ml</td>
</tr>
<tr>
<td>Integrator frequency response</td>
<td>dc to 500 Hz</td>
</tr>
<tr>
<td>Integrator output</td>
<td>1V/10V-sec input</td>
</tr>
</tbody>
</table>

* Three samples are taken of each parameter at 0, 4, 8 and 13 sec and averaged at the end of 13 sec.

** Mixing chamber plunger extended 23 cm.
Fig. 7 Rotameter and connections for flow calibration.
fraction to travel down the Collins tubing to the gas analyzers (see system response specifications in Table 1). A two second interval existed between each 13 second \( \dot{V}_E \) sample for program maintenance. Computed data results were output via the hard copy printer after each \( \dot{V}_E \) sample interval. Data output was formatted so that the following information was printed after each \( \dot{V}_E \) sample: \( \dot{V}O_2 \) (ml/kg.min), \( \dot{V}_E \) (BTPS), \( R(CO_2/O_2) \), \( T_E \) (Exp), \( \dot{V}_E \) (ATPS), \( \dot{V}_E \) (STPD), \( F_ {E O_2} \) and \( F_ {E CO_2} \). Prior to program execution the subject number, subject weight, barometric pressure and inspired \( O_2 \) and \( CO_2 \) concentrations were inputed and printed in order to verify that the appropriate information was in the program and to identify the output data. The complete program in HP 9820 language may be obtained from this Institute upon written request.

**Calibration Procedures:**

The power for all of the components was turned on a minimum of 20 minutes before calibration commenced. (A detailed initial set up guide and trouble shooting procedure is listed in Appendix A. A daily procedure and calibration routine is listed in Appendix B). After calibrating the \( O_2 \) and \( CO_2 \) gas analyzers and the YSI telethermometer, the pneumotach integrator was balanced and calibrated using compressed gas and a rotameter (Fig. 2 'C'). Care was taken while balancing the integrator to insure that no artifacts due to Collins tube movement or convection currents interfered with the integrator balancing. Failure to critically assess the preceding details resulted in an improperly balanced respiratory integrator that would not hold a stable baseline. After calibrating the integrator at a flow of 80 l/min and sampling room air, the system was ready for use. Initially, electrical drift checks were performed every 10 to 20 minutes with flow calibrations every 20 to 30 minutes to establish system stability and reliability. It was found that offset
Table 2. Comparison of $\dot{V}O_2$, $\dot{V}E$ and $FE_{O_2}$ between Douglas bag and on-line systems at different ventilation ranges (mean ± SD).

<table>
<thead>
<tr>
<th>Ventilation Range (l/min BTPS)</th>
<th>n</th>
<th>$\dot{V}O_2$ (ml/kg*min) Douglas Bag</th>
<th>$\dot{V}E$ (l/min BTPS) Douglas Bag</th>
<th>$FE_{O_2}$ (%) Douglas Bag</th>
<th>$FE_{O_2}$ (%) On-Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>40-60</td>
<td>27</td>
<td>35.6 ± 3.2</td>
<td>36.1 ± 3.5</td>
<td>54.7 ± 3.5</td>
<td>54.7 ± 6.3</td>
</tr>
<tr>
<td>60-80</td>
<td>51</td>
<td>38.8 ± 6.3</td>
<td>37.5 ± 5.0</td>
<td>72.0 ± 5.6</td>
<td>70.6 ± 5.7</td>
</tr>
<tr>
<td>80-100</td>
<td>43</td>
<td>41.3 ± 6.4</td>
<td>41.3 ± 6.4</td>
<td>88.4 ± 6.6</td>
<td>88.1 ± 3.6</td>
</tr>
<tr>
<td>100-120</td>
<td>44</td>
<td>46.1 ± 5.9</td>
<td>45.6 ± 6.2</td>
<td>108.6 ± 7.0</td>
<td>109.6 ± 5.9</td>
</tr>
<tr>
<td>&gt;120</td>
<td>39</td>
<td>50.4 ± 5.4</td>
<td>50.5 ± 5.6</td>
<td>137.9 ± 11.2</td>
<td>140.0 ± 11.0</td>
</tr>
</tbody>
</table>

*p < 0.05
balancing was necessary once an hour with flow calibration remaining stable for an average of three to four hours.

**Data Collection:**

In order to validate this system against the standard Douglas bag technique, data was collected over four separate days on different men and women during varying exercise intensities while treadmill running. All work loads involved running at both submaximal and maximal work intensities that resulted in \( V_E \) (BTPS) > 40 l/min. \( V_E \) (BTPS) ranged from 40 to 160 l/min with \( VO_2 \) ranging from 26 ml/kg/min to 60 ml/kg/min. Connecting the on-line and Douglas bag systems in series allowed the calculated values from the same expired samples to be directly compared.

The Douglas bag system consisted of a series of four polyvinyl chloride gas bags (100 l capacity) connected with Collins respiratory tubing and low turbulence three way "Y" valves. A thirty second sample of expired air was collected in each bag during the final two minutes of every work load on the treadmill. The \( O_2 \) and \( CO_2 \) content was determined by withdrawing a 300 ml aliquot of air through the gas analyzers. Expired air volumes were measured with a Collins 120-l chain-compensated gasometer (Tissot).

Comparisons of \( VO_2 \), \( V_E \) and \( F_{O_2} \) between the Douglas bag and on-line systems were made using a paired t-test.

**Results**

Table 2 presents the data for \( VO_2 \), \( V_E \) and \( F_{O_2} \) between the two systems for the following ranges of ventilation: 40-60 l/min; 60-80 l/min; 80-100 l/min; 100-120 l/min; > 120 l/min. Significant differences (p < .05) were found for \( F_{O_2} \).
at a ventilation range of 40-60 l/min and in $V_E$ at ranges of 60-80 l/min and > 120 l/min. These differences, however, are randomly found among the various ventilation ranges such that a consistent discrepancy between systems does not appear evident. Furthermore, the small differences seen in $F_{E_{O2}}$ and $V_E$ were not of sufficient magnitude to significantly effect the values for oxygen uptake between the two systems.

**Discussion**

As seen in Table 1, the total resistance of the on-line system is very minimal (0.86 cm H$_2$O/80 l/min). This low resistance can be attributed to the incorporation of the Koegel 'Y' breathing valve and the design of the mixing chamber. Traditionally, the breathing valve and the mixing chamber are the greatest source of ventilatory resistance in an on-line system. A secondary advantage of the Koegel valve was that naive test subjects were less intimidated by it than a larger valve such as the Triple-3 and adapted quickly to breathing into it. In addition, the subjects were less prone to losing the mouthpiece and becoming fatigued from the weight of the Koegel valve in their mouth. This fact was also noted by Lenox (5).

Extreme care must be taken to make certain that net flow resistance for the gas sensors remains the same from the room air sample port to the chamber sample port. The use of manifolds and solenoids must carefully be assessed if incorporated into a gas sensor system, since flow resistance is a function of the diameter and length of the sample lines.

Calibration of the pneumotach at 80 l/min flow was necessary in order to avoid the linearity at low (< 50 l/min) and high (> 200 l/min) flows. Selection of a 80 l/min calibration flow was within the linear performance of the pneumotach for
ventilations we encountered during treadmill running ($V_e$ BTPS 40-160 l/min) Finucane (8) discusses the pneumotach's limitations as a function of the stream geometry before, after, and within the pneumotachograph. Yet, if calibration of the pneumotach is done with thought to the range of expected performance, one may easily avoid this drawback. This would necessitate separate calibrations for walking treadmill loads ($V_e < 60$) and running loads ($V_e > 60$). Consideration must also be given to the atmospheric conditions where the pneumotach is utilized (6). The most critical factors affecting pneumotach performance/accuracy according to Van Der Harot (9) are the temperature and gas viscosity.

Since the pressure transducer is sensitive to pressure changes as a result of the pressure drop across the two sample ports on the pneumotach, it also is affected by pressure changes within the Collins respiratory tubing. The pressure changes within the Collin's tubing are a direct result of mechanical jarring of the tubing and to flow turbulence at high $V_e$. Filtering with a low pass electronic filter at 0.6 Hz prior to integration eliminated inflated $V_e$ values. The electronic filter eliminates the higher frequency artifacts that were caused by this aforementioned turbulence.

The selection of two gas analyzer pumps maximized as much as possible the sampling of the same fraction of $V_e$ for both $O_2$ and $CO_2$. Concomitantly, the use of only one desiccating trap eliminated the concern over changing lag/sample times that one could encounter if two traps would be used (e.g. trap packing affects flow and resistance).

Although the trap increased lag time slightly, it eliminated the concern over the partial pressure of $H_2O$ vapor in the $V_e$ sample. Even though sampling was slower by using a HP 9820 calculator compared to computer processors used by
other investigators (10), the HP 9820 proves adequate in determining plateaus in $\dot{V}O_2$.

The reliability of the on-line system at various ranges of ventilation was shown in Table 2. The small differences seen in $V_E$ between systems possibly demonstrates a drawback of the use of a pneumotach. If the pneumotach is not precisely calibrated an error may be inadvertently introduced which would effect the $VO_2$ value. In the present situation, however, this error was random and not significant enough to affect $VO_2$ determination.

The mixing chamber capability was assessed by comparing the $FEO_2$ values at various ranges of ventilation for both systems (Table 2). Even though a difference was found at the 40-60 l/min range, the absolute difference between the two means of 0.08 per cent was not sufficient to adversely affect the corresponding values for $VO_2$.

The fact that the filtration at 0.6HZ (low pass) did not over dampen the $VE$ signal prior to integration is suggested by the non-significant differences found for work loads with $VE > 80$ l/min. There was an initial concern that as $VE$ increased the fast rise component of the $VE$ curve would be lost, thus resulting in a falsely low $VE$.

In summary, the use of a calculator in conjunction with the aforementioned mixing chamber allows for a valid, transportable and field oriented on-line aerobic measurement system.
Acknowledgements

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References


7. Cymerman, A. and Sacco, M. Personal communication.


Appendix A

Set Up and Trouble Shooting Procedure

1. All equipment must be connected into the same AC source.
2. Connect the instruments to the A/D convertor using BNC cables. Complete interfacing using coax cables with BNC connectors between the carrier, filter and integrator.

System Test Procedure

1. Power up equipment.
3. Allow for an initial warm up time of one hour or until the O₂ fuel cell temperature reaches equilibrium.
4. Balance the differential pressure transducer, set on proper sensitivity once balanced.
5. With recorder chart drive running, verify that a response can be obtained by blowing through the pneumotach.

If trouble occurs:
   a. Insure that carrier power supply is powered.
   b. Insure that carrier amplifier is in use function (the switch on the sensitivity lever).
   c. Check that system (Collins respiratory tubing) is open for breathing.
   d. Insure that transducer is plugged into the carrier.
   e. Check that the tygon lines between the transducer and pneumotach are connected properly.
   f. If response is in the wrong direction (e.g. not from right to left), reverse the tygon lines on the transducer.
6. Adjust the integrator.

   a. Adjust the baseline and upper limit (level).

   **Settings to adjust baseline:**
   - chart on tidal
   - channel on V
   - reset on manual

   **Settings to adjust upper limit (level):**
   - chart on XI
   - channel on V
   - reset on manual

   The baseline and level adjustments are made using the display output on the calculator.

   Baseline reading is 0.00 ± 0.06, upper level should read 150.00 ± 0.06.

   b. Adjust the offset on the integrator.

   **Switch settings:**
   - chart on tidal
   - channel on V
   - reset on dot position
   - sensitively switch on adjust offset
   - mode on sinusoidal

   Using offset knob, adjust pen drift until it is relatively stable; no flow or disturbance of the Collins tubing can occur during this adjustment.

   If offset is unstable:
   a. rearrange coax cables positioning
   b. insure that signal cables are not crossing over any AC power cords
   c. insure that all components are chassis grounded or shielded
d. ascertain that the filter is powered and set on proper filtration and frequency (0.6 HZ low pass).

7. Integrator calibration.
   a. Switch settings:
      chart on minute
      channel on V
      reset on dot position
      sensitivity on 20
      mode on positive on going curve (→)
   b. With an 80 l/min flow through the flowmeter and pneumotach, initiate calculator V calibration subroutine, adjust integrator gain as necessary in order to obtain 80 l/min calculator read out.


10. Calibrate YSI meter.
    a. Shut off YSI meter; adjust the electrical zero on the A/D convertor
    b. With the thermistor probe disconnected from the meter and with the meter on, adjust meter to red line; concomitantly adjust the electrical gain on the A/D convertor.
    c. Plug probe into meter and leave meter on

**Plumbing Test Procedure**

1. Assuming the gas analyzers are calibrated:
   a. Pass the same calibration gas used in calibrating the analyzers through the on-line system (mixing chamber) agreement may be written ± 0.02%.
   b. Concomitantly collect this same calibration gas in the Douglas bags to insure that gas analyzer plumbing from the bags is correct. Once collection is complete, recalibrate the gas analyzers using the same type of line and fittings that will be used in sampling the bags.
c. Measure the Douglas bags, agreement must be written $\pm 0.02\%$.

2. Adjust gas analyzer plumbing and fittings as needed until reproducibility is within $\pm 0.02\%$. 
Appendix B

ON-LINE PROCEDURE

Start Up

1. Insure that the following components are powered on for 20 minutes.
   A. 9820 calculator
   B. Timing generator
   C. A/D convertor
   D. Terminal printer
   E. H/P recorder
   F. Mixing fan in chamber
   G. Pneumotach heater
   H. Pumps for fuel cell and LB-2
   I. YSI meter
   J. Electronic filter
   K. Memory cassette

2. Change desiccant and check all plumbing connections, especially transducer, pneumotach and gas analyzer lines.

3. A) Zero and span analyzers
   B) Electrically zero and span A/D convertor for O₂, CO₂ and YSI meter.

4. Balance transducer

5. Set lower (0.00 ± .06) and upper limits (150.0 ± 0.06) on the integrator.

6. Adjust offset

7. Set flow in rotometer on 80 l/min, calibrate integrator by adjusting gain.

8. Sample room air, input subject data, RUN PROGRAM when ready.

   Routinely:
   A. Check offset at 30 minute intervals.
   B. Check flow calibration every two hours while operating.
   C. Sample room air as needed.

D. ----- WATCH DESICCANT -----------

Shutting Down For The Evening:

A. Shut power off on following items only:
   1. A/D convertor
   2. Terminal printer
   3. Mixing fan
   4. Pneumotach heater
   5. Pumps
   6. YSI meter
   7. Electronic filter
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