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Gas chromatographic determination of house fly respiration.

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A Thesis

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INTRODUCTION

Manometric respirometry requires separate oxygen and carbon dioxide measurements (Whitney and Ortman, 1962). This technique allows 1% reproducibility and continuous measurement (Umbreit et al., 1964). However, it precludes simultaneous analysis of oxygen uptake and carbon dioxide production in individual organisms.

Other means for measuring insect respiration have been developed. Measurement of carbon dioxide production from individual insects has been studied by Hamilton (1959) with a recording infra-red spectrophotometer. Infra-red spectrophotometers are capable of .5% reproducibility and continuous measurement (Bausch and Lomb, Inc., 1970). Oxygen uptake in insects has been measured with a Clark oxygen electrode by Hayes et al. (1968). They reported a reproducibility of 4%. This technique also measures continuously. Chromatographic techniques for measuring insect oxygen uptake and carbon dioxide production have been developed by Whitney and Ortman (1962), Carlson (1966) and Dumas et al. (1969). Chromatographic techniques can only sample periodically. Reproducibility is .4%-10%, depending on the specific technique and the skill of the operator (Tranchant, 1969). No technique for simultaneous measurement of insect oxygen uptake, carbon dioxide production and water vapor output is presently available (Wood, 1971; Monro, 1971).

Presented in this thesis is an isothermal gas chromatographic

technique for simultaneous measurement of oxygen uptake, carbon dioxide production and water vapor output. Reproducibility was within 10% for all three calibrations. Complete analysis time was twenty minutes. Two columns of Porapak Q were used for measurement and reference with a thermal conductivity detector. Nitrogen was the carrier gas. Measurements were taken on four day old female house flies. The oxygen uptake and carbon dioxide production data were compared with previous manometric and chromatographic measurements on house flies by Ouye et al. (1961), Edwards (1946), and Whitney and Ortman (1962). The water vapor output measurements were compared with water loss data taken by Bursell (1959 and 1960) on tsetse flies because data was not available for the house fly.

LITERATURE REVIEW

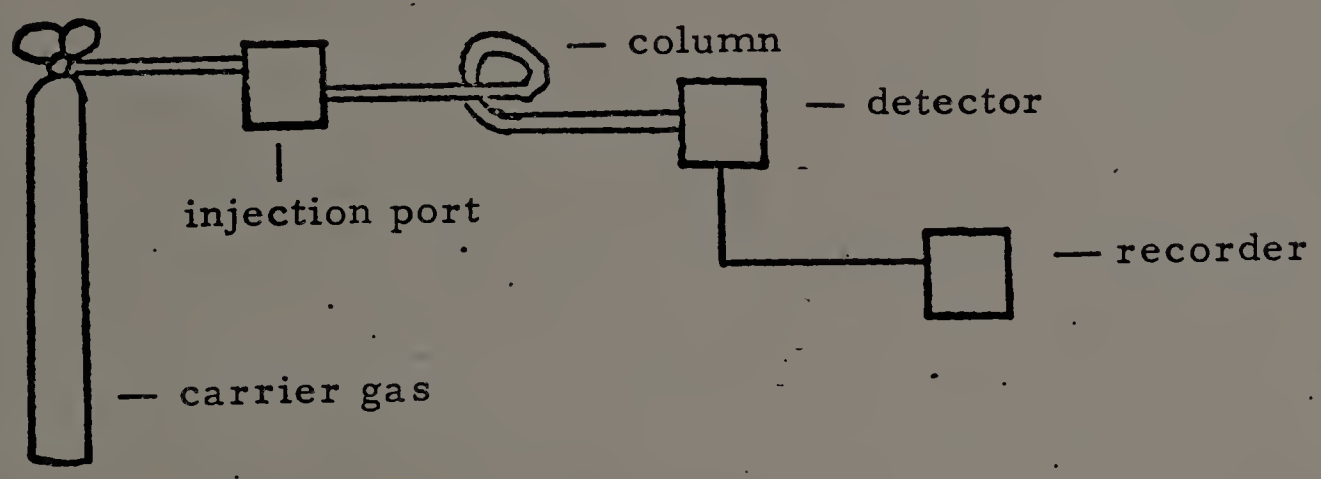
Chromatographic Separation of Respiratory Gases

Origin of Chromatography. Chromatography is an analytical method based on differences in the partition coefficients of substances distributed between a static phase, usually of great surface area, and a moving fluid phase. The earliest reported experiments which can be unequivocally regarded as chromatography are those of Tswett, who separated the components of plant pigments by passing their solutions through columns of solid adsorbents. It was presumably the formation of the colored solute rings that suggested the name chromatography (color writing), but it would be nice to think that Tswett, whose name in Russian means color, took advantage of the opportunity to indulge his sense of humor (Purnell, 1962).

Application of chromatographic techniques to analysis of volatile compounds is generally credited to Martin and Synge (1941) because of their suggestion that the moving fluid phase could be a gas instead of a liquid. The first viable elution gas chromatographic procedure was reported by James and Martin (1952). A basic schematic of a gas chromatograph is shown in Figure 1.

Chromatographic columns for respiratory gas analysis. The column in a gas chromatograph is usually a glass, copper, or stainless steel tube packed with a solid support, (in the form of small particles to increase surface area). A stationary phase consisting of a high boiling point liquid is usually coated on the solid support before it is packed in the column. The gas phase consists of a carrier gas and is often helium or nitrogen. Generally, columns which will re-

Figure 1. Basic diagram of how a gas chromatograph works.



The carrier gas moves the sample, which is injected through a rubber septum at the injection port through the column and detector. The column is usually packed with a large number of small particles to achieve increased surface area. The particles can either be coated with a high boiling point liquid which has different retention times for different species of molecules; or, in some cases, the particle itself has the ability. Once the components in a sample have been separated by the column, they pass through a detector which senses the difference between carrier gas and sample. This difference responded to by the detector causes a deflection or peak on a chart recorder. By relating the size of the peak to known quantities of a sample, quantitative analysis is possible.

solve carbon dioxide will not separate oxygen from nitrogen and argon. Columns which will separate oxygen from nitrogen absorb carbon dioxide and water vapor (Whitney and Ortman, 1962). Various solid supports and stationary phases used in the analysis of respiratory gases by gas chromatography are reported in Table 1.[1]

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[1] In the subsequent papers discussed in this review, helium was used as the carrier gas, and detection was by thermal conductivity, unless otherwise stated.

Table 1. Shows a comparison of column packing materials for use in respiration chromatography.

Reference	Column Packing	Resolutions*
Hamilton and Kory (1960)	Octoil-S[3] (di-ethyl-hexyl sebacate) on diatomaceous earth	$(O_2, N_2, Ar, CO)/CO_2/\underline{H_2O}$
Dressler et al. (1960) Hamilton (1962)	HMPA (hexamethylphosphoramide) on diatomaceous earth	$(O_2, N_2, Ar, CO)/CO_2/\underline{H_2O}$
Ramsey (1959) Hamilton and Kory (1960) Dressler et al. (1960) Jay and Wilson (1960) Luh and Chaudhry (1961) Hamilton (1962)	Molecular sieve	$(O_2, Ar)/(N_2, CO)/\underline{CO_2}, \underline{H_2O}$
Jay and Wilson (1960) Wood et al. (1970)	Activated charcoal	$(O_2, N_2, Ar, CO)/CO_2/\underline{H_2O}$
Luh and Chaudhry (1961)	Silica gel	$(O_2, N_2, Ar, CO)/CO_2/\underline{H_2O}$
Eaton et al. (1968)	Porapak Q	$(O_2, N_2, Ar, CO)/CO_2/H_2O$
Supelco, Inc. (1971)	Carbosieve-B	$(O_2^{**}, Ar)/N_2/CO/CO_2/H_2O$

*Gases enclosed by ( ) are eluted in the same peak.
Gases separated by a / are resolved as distinct peaks.
Gases underlined ____ are absorbed on the column and do not elute.

**Expanded carbon supports are easily oxidized under temperature programmed operation (Supelco, Inc., 1971).

Application of chromatographic measurement to insects was reported by Whitney and Ortman (1962). A column of silica gel resolved carbon dioxide and a column of molecular sieve resolved oxygen. Their data for the house fly is shown in Table 2. Hamilton et al. (1964) applied chromatographic measurement of oxygen and carbon dioxide to human cardiopulmonary physiology. A silica gel column resolved carbon dioxide and a molecular sieve column resolved oxygen. A two loop sampling valve increased the reproducilibity of sample volumes introduced into the chromatograph and resulted in smaller errors both in calibration and in experimental sampling (Hamilton, 1964).

Oxygen and carbon dioxide were resolved with a single column of activated charcoal, nitrogen carrier gas, and a glow discharge detector (Mochizuki and Kawakami, 1965).[2] Normally, nitrogen and oxygen elute together from an activated charcoal column. Since nitrogen rather than helium was the carrier gas, the nitrogen in the sample would not cause a detector response. Although they did not adapt their technique to respiratory work, they did suggest that it would be useful to do so.

Carlson (1966) analyzed respiration in Tribolium confusum with

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[2]This type of detector is discussed in the literature review section under "alternative detectors."

Table 2. Oxygen uptake and carbon dioxide production for individual four day old house flies, converted from µg/mg/min to µl/mg/hr Whitney and Ortman (1962).

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44 µg CO_2 = 22.4 µl @ stp 32 µg O_2 = 22.4 µl

22.4 µl/44 µg = 0.5 µl/µg 22.4 µl/32 µg = 0.7 µl/ug

at 27.5°C Volume correction = $\frac{300.5}{273}$ = 1.1

CO_2 = 0.55 µl/µg O_2 = 0.77 µl/µg

CO_2			O_2		
µg/mg*/min	µl/mg/min	µl/mg/hr	µg/mg/min	µl/mg/min	µl/mg/hr
Fisher					
.135	.074	4.45	.131	.100	6.00
.103	.056	3.36	.062	.047	2.82
.046	.025	1.50	.030	.023	1.38
.086	.047	2.82	.055	.042	2.52
.106	.058	3.48	.074	.057	3.36
R.Q = .98		$\bar{X}$ = 3.12			$\bar{X}$ = 3.21
Beckman					
.080	.044	2.64	.064	.049	2.95
.085	.046	2.80	.093	.072	4.29
.099	.054	3.27	.110	.085	5.08
.091	.050	3.00	.089	.068	4.11
.089	.048	2.93	.058	.044	2.67
R.Q. = .77		$\bar{X}$ = 2.93			$\bar{X}$ = 3.80

* Fresh wet weight

HMPA on diatomaceous earth to resolve oxygen, and molecular sieve to resolve carbon dioxide. The average value for oxygen uptake was 2.68 µl/mg/hr $\pm$ .9, and the average value for carbon dioxide production was 2.80 µl/mg/hr $\pm$ .6.

A possible monitoring system for oxygen, carbon dioxide, water vapor, and trace contaminant levels in manned spacecraft consisted of three columns, three ionization detectors and a 75 minute program of four injections. Two injections on a molecular sieve resolved first oxygen, and second, hydrogen, nitrogen, methane and carbon monoxide. An inert support coated with Amine 220 and Carbowax 4000 resolved carbon dioxide and several trace contaminants. 10% by weight Carbowax 20M on 90% by weight Chromosorb G resolved freon 22, hexane, chloropropane, acetone, methyl ethyl ketone, methylene chloride, benzene, and water (Lem, 1967).

A six minute program using three separate columns of molecular sieve at different temperatures resolved the following: 146°C, nitrous oxide and carbon dioxide; 24°C, nitrogen; -98°C, argon and oxygen (Burford, 1969). However, the 24°C and -98°C columns had to be reconditioned by high temperatures (150°C) after every six injections. A single molecular sieve column was tried but analysis time proved too long (75 minutes).

Both oxygen uptake (resolved by 13X molecular sieve) and carbon

dioxide production (resolved by silica gel) were progressively de-pressed in Tenebroides mauritanicus as atmospheric pressure was reduced below 200 mm (Dumas et al., 1969). Activated charcoal resolved carbon dioxide produced by individual Chlamisus cribripennis and Myzus persicae[3] at 20°C and 5°C, showing a linear relationship between cumulative carbon dioxide output and time during a six and one-half hour period. Carbon dioxide buildup had no significant effect on respiration under the conditions tested (Wood et al., 1970).

In 1971, Supelco Incorporated offered for sale a column packing material called Carbosieve-B. "Carbosieve-B is a highly pure carbon with a surface area of approximately 1000m^2/gm and a pore radius in the range of 10-12 angstroms, making it essentially a carbon molecular sieve. It is not a graphite or charcoal" (Supelco, Inc., 1971). This material elutes water vapor, oxygen and carbon dioxide within 10 minutes. Temperature programming of the column to 175°C is necessary to elute CO_2, but the expanded carbon surface is highly susceptible to oxidation at that temperature (Supelco, Inc., 1971). This packing material was not tested in this thesis. However, if routine oxygen analysis did not oxidize Carbosieve-B, then it would be the fastest, most accurate system for separating oxygen, carbon dioxide and

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[3] The blueberry case beetle and the peach aphid respectively.

water vapor. Use of capillary carbon molecular sieves in the temperature programmed analysis of trace impurities, such as the oxides of nitrogen, hydrogen sulfide, and sulfur dioxide in ethylene, is described by Zlatkis et al. (1970).

Porous polymers. Porous, polyaromatic, polymer beads are synthesized by suspension polymerization of monomers, such as styrene and ethylvinyl benzene, with divinylbenzene as a cross linking agent (Hollis, 1966). The crosslinking of the polymers forms a porous bead. "By controlling the amount of cross-linking agent, and the character of the suspension used in the polymerization process, pore size can be accurately controlled" (Hollis, 1966). Porous polymer beads are stable when subjected to a vacuum, radiation, and temperatures up to 250°C (Waters Assoc., Inc., 1971).

The unusual chromatographic separations achieved with porous polymers can be attributed to basic differences in the way the polymers interact with the fluid phase of the chromatographic system. "Conventional chromatographic partitions are thin film phenomena, involving only the surface of the packing particle. Porous polymer bead partition appears to involve the entire particle" (Hollis, 1966). Porapak (a porous polymer) is compared with other column packing materials in Table 1 and with other porous polymers by Dave (1969).

Porous polymers have been used in the development of analytical

equipment for spacecraft. Nitrogen, oxygen, argon, and carbon monoxide elute as a single peak from Porapak R (a more polar form of Porapak Q) and Porapak Q in a combined gas chromatographic-mass spectrometric analysis of a simulated Martian atmosphere (Wilhite and Hollis, 1968). A six inch capillary column of Porapak T (more polar than Porapak R) resolves the inflight oxygen consumption and carbon dioxide production of the Apollo astronauts (Eaton et al., 1968). Nitrogen as the carrier gas caused a double peak effect (See Discussion Section). A mixture of 10% helium and 90% nitrogen produce normally shaped curves. Eaton's technique was evaluated only for use in atmospheres of 90% oxygen and 10% carbon dioxide at 5 psi.

In gas chromatography, retention time is the length of time a substance is retained on a column. An investigation of the retention times of krypton, carbon dioxide, nitrous oxide and ethane on a column of Porapak Q, using different temperatures (20°C, 52°C, 70°C) and different carrier gases (hydrogen, nitrogen, argon, carbon dioxide, ethane, ethylene), was reported by Rabbani et al. (1968).

Complex gas mixtures can be resolved on Porapak Q. At 90°C, separate peaks for carbon dioxide, hydrogen sulfide, sulfur dioxide, and a composite peak of oxygen, nitrogen, argon, and carbon monoxide are eluted. The components of the composite peaks are resolved on a -70°C column. Carbon dioxide is retained on the -70°C column

until the column is heated to 90°C. Complete analysis time for oxygen, carbon dioxide and water is 40 minutes (Obermiller and Charlier, 1968).

Two columns (Porapak Q and 5-A molecular sieve) and switching valves allow carbon dioxide and water vapor to bypass the molecular sieve, allowing the separation of hydrogen, oxygen, nitrogen, methane, carbon monoxide, carbon dioxide, ethane and water vapor (Forsey, 1968). A third column can be used to keep the baseline from deviating (DiLorenzo, 1970).[4] Still another variation was reported by Marchio (1971), who used columns of Porapak Q and 5-A molecular sieve, with separate carrier gases for each column (nitrogen with Porapak; helium with molecular sieve).

The separation of hydrogen, nitrogen, oxygen, methane, krypton and carbon dioxide at room temperature is possible on a single column of phosphoric acid treated Porapak Q (Carle, 1970). The method was adapted to measuring microbial respiration. Krypton, a metabolically inert gas, served as an internal standard.[5] Water vapor was absorbed

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[4]The baseline had deviated in Forsey's work because of pressure changes when the molecular sieve was by-passed.

[5]Internal standardization is a method in which the concentration of one of the components in a mixture to be separated is known. This allows for quantitation of the other components by relating their chromatographic peak size to the peak size of the internal standard rather than to an external standard such as a calibration graph. It also helps to correct for errors in the sample size injected and changes in atmospheric pressure and temperature.

by the treated Porapak Q (Carle, 1972).

Chromatographic Detectors for Use in Respiratory Gas Analysis

Thermal conductivity detectors. "Thermal conductivity is one of three interrelated transport phenomena; it is the transfer of kinetic energy due to a temperature gradient. The other two phenomena are diffusion, which is the transfer of mass due to a concentration gradient, and viscosity, which is the transfer of momentum due to a velocity gradient" (Lawson and Miller, 1966). "According to the kinetic theory of gases, the coefficient of thermal conductance $\tilde{K}$ of a pure gas is given by $K = 1/2\ p\bar{c}\ \lambda c_v$ where p is the gas density, $\bar{c}$ is the average molecular velocity, λ is the mean free path, and c_v is the specific heat per gram at constant volume" (Bohemen and Purnell, 1958).

In practice, a hot charged wire or thermistor is placed in a stream of gas at a constant temperature, pressure, and flow rate. So long as the composition of the gas remains constant, heat is conducted away from the warm filament or thermistor at a constant rate. When the composition of the gas changes, the rate at which heat is conducted away from the filament or thermistor changes. This has the effect of changing the temperature of the filament or thermistor, which affects the way in which electricity is conducted through the fil-

ament. Usually a pair of filaments or thermistors are connected to a Wheatstone bridge and are set to balance each other in the carrier gas (so that no current flows across the bridge). One filament or thermistor is used as a reference, and the other is used to detect the sample. Thus, when the sample passes over the sampling filament or thermistor, the Wheatstone bridge is unbalanced at the sample side of the detector and activates a recorder (Purnell, 1962). "The first worthwhile device specifically designed to measure thermal conductivity changes in gaseous mixtures was patented by Shakespeare in 1915, and called a 'katharometer' (purity meter)" (Purnell, 1962).

In 1950, Punt described a thermal conductivity device with filaments called a diaferometer. It measured periodic oxygen uptake and carbon dioxide production in insects. This device allowed the continuous minotoring of insect oxygen uptake and carbon dioxide production. Unfortunately, Punt was not able to calibrate the instrument. Therefore, absolute quantities could not be determined, but relative measurements were possible. Another thermal conductivity device employing a thermistor instead of a filament and having a much smaller volume, resolved carbon dioxide bursts from Heliothis zea pupae (Edwards, 1970).

Thermal conductivity detectors behave erratically in carrier gases of low thermal conductivity such as nitrogen (Bohemen and

Purnell, 1958). This is pursued in the discussion section.

Samples with non-linear isotherms[6] should be calibrated over the expected concentration range since theoretical prediction of peak anomalies is not possible (Schmauch and Dinerstein, 1960). General reviews describing the most frequent problems involved in using a thermal conductivity detector are Goedert and Guiochon (1969) and Lawson and Miller (1966).

Alternative detectors. Although thermal conductivity detectors are simple, non-destructive, versatile and cheap, they are not the most sensitive detectors which can be used for analysis of oxygen, carbon dioxide and water vapor. The most sensitive detector for oxygen (2ppb), carbon dioxide (2ppb) and water vapor measurement is the helium ionization detector (Varian Aerograph, Inc., 1970). "The helium ionization detector works on the principle that helium in a high energy state has the ability to ionize all other gases and vapors except neon" (Varian Aerograph, Inc., 1970).

Basically, this detector is a three part system composed of an ionizing radiation source, an anode and a cathode. When an inert gas, such as helium, passes through the detector, free electrons which are liberated from the carrier gas by the ionizing radiation have a low

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[6]Isotherms in this context are graphs which plot detector response as a function of sample concentration at a given temperature.

probability of recombination. These electrons are collected on the anode producing a current. If a vapor which has affinity for electrons passes through the detector, there will be a greater chance for recombination. The subsequent loss of electrons is registered as a drop in the electron current at the anode (Tranchant, 1969).

A technique for analysis of oxygen and nitrogen in air, using a helium ionization detector and commercial grade helium, has been developed by Goldbaum et al. (1968). The only drawback to helium ionization detectors in general is that the linearity of response tends to be narrow (Tranchant, 1969).

Another sensitive detector for oxygen (2ppm), carbon dioxide (3 ppm) and possibly water vapor is the frequency difference radio frequency detector (Williams and Winefordner, 1968). This detector, not commercially available, measures the change in the dielectric constant[7] of a plasma as a means of detecting sample gases in a carrier gas.

Another type of radio-frequency detector measured the change in electron current as a sample passed through the plasma (Mochizuki and Kawakami, 1965). Unfortunately, the authors did not mention the sensitivity or linearity of their detector.

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[7] Dielectric constant of a medium is defined by ε in the equation $F = (Q\,Q^1)/(\varepsilon\, r^2)$ where F is the force of attraction between two charges Q and Q^1 separated by a distance r in a uniform medium (Weast, 1970).

Respiration of House Flies

Intact house flies. The results of measurements with a Scholander volumetric respirometer (Edwards, 1946) and a Warburg manometer (Ouye et al., 1961; Skelton and Hunter, 1970) are shown in Table 3. Results of Whitney and Ortman (1962) are already reported in Table 2.

Table 3. Oxygen uptake values for the house fly.

Edwards (1946) @ 25°C

µl O_2/mg/min	X 60	= µl O_2/mg/hr
.08		
.06		
.05		
.06 = $\bar{X}$		3.6

Ouye, Rai, and Roan (1961) @ 30°C

µl O_2/mg/min	X 60	= µl O_2/mg/hr
.060		3.6

Skelton and Hunter (1970) @ 25°C

µl O_2/gm/hr	X 1/1000	= µl O_2/mg/hr
4239		4.2

There were no water loss measurements available for intact house flies. However, another muscid, the tsetse fly (Glossinia morsitans), weighs an average of 9.8 milligrams (Bursell, 1959) and loses approximately 0.5 milligrams of water per hour (Bursell, 1960).

Flight muscle mitochondria. Flying insects often increase their oxygen uptake fifty to one hundred times over resting values (Chadwick, 1953). Suspensions of house fly flight muscle mitochondria take up oxygen twice as fast when ∝-glycerophosphate is the substrate oxidized as when succinate, glutamate, and ∝-ketoglutarate are the substrates (Sacktor and Cochran, 1958). ∝-glycerophosphate causes an oxygen uptake three to four times faster than pyruvate or Kreb's cycle constituents (Birt, 1961). (See Table 4). This indicates that the main form of energy for flight in house flies might be either glycolysis or lipid catabolism (which produces ∝-glycerophosphate) rather than constituents of the Kreb's cycle, such as succinate, glutamate, or ∝-ketoglutarate (Sacktor and Cochran, 1958).

NADP and NAD were not detectable in house fly mitochondria. This was further evidence that ∝-glycerophosphate was the main source of energy for flight. ∝-glycerophosphate donates hydrogen directly to the flavo-proteins rather than to NAD or NADP as is the case with Kreb's cycle constituents (Chance and Sacktor, 1958). Birt (1961) found small but detectable concentrations of NAD and NADP using a

Table 4. Oxygen uptake in suspensions of house fly flight muscle mitochondria with various substrates (Birt, 1961).

Substrate	Oxygen Uptake ul O_2/mg of protein/hr
∝-glycerophosphate	200-750
succinate	65
pyruvate plus malate	70
malate	3
glutamate	10

spectrophotometric method.

Manometric methods found house fly mitochondrial respiration to be ADP dependent (Gregg et al., 1960; Birt, 1961), contrary to the findings of Sacktor and Cochran (1958). This may have been due to the fact that the former buffered the isolating media while the latter did not. ADP is a mitochondrial respiration controlling mechanism in most other organisms (Sacktor and Cochran, 1958).

Using a vibrating platinum electrode (Oxygraph, Gilson Medical Electronics) Carney (1966) found that the media used in isolating the house fly flight muscle mitochondria significantly influenced the activity of the mitochondria. In a suspension of mitochondria isolated in a medium of potassium chloride, pyruvate was more responsive to the addition of ADP (showed a higher ratio of respiration rate in the presence and absence of ADP) than ∝-glycerophosphate. However, a sus-

pension of mitochondria isolated in sucrose medium showed α-glycerophosphate to be about as responsive to ADP as pyruvate. In mitochondrial suspensions, 2-4-dinitrophenol, 2-4-dinitro-6-secbutylphenol, and benzimidazole (compounds used as insecticides) inhibit respiratory control, as measured with a Clark oxygen electrode (Ilevicky et al., 1967). That is, subsequent addition of ADP to the experimental suspension elicited no response (no oxygen uptake), while control suspensions did react to the addition of ADP.

MATERIALS AND METHODS

General Chromatographic Methods

A Hewlett-Packard 5750 research gas chromatograph was used for all measurements, except for one test which was done with a Varian model 700 autoprep gas chromatograph (Figure 9). Initially, two three-foot copper columns filled with activated coconut charcoal were used with helium, subsequently nitrogen was used as the carrier gas (Figures 5 and 6). The charcoal column could resolve only .1% carbon dioxide, and it absorbed water. These columns were eventually discarded in favor of two twelve-foot columns filled with Porapak Q (Figures 7 and 8). The Porapak columns were found to resolve water vapor and carbon dioxide at ambient concentrations. With the Porapak Q, it was necessary to install a drying tube in the carrier gas line to minimize recording pen noise during measurement of the water vapor peak.

Wilhite and Hollis (1968), using helium as the carrier gas, and a thermal conductivity detector, noted that Porapak Q would not separate oxygen, nitrogen, argon and carbon monoxide. Even though the method presented in this thesis also would not separate these gases, oxygen uptake measurements were possible. It was assumed that the effect of atmospheric carbon monoxide on house flies was negligible*

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*Ambient carbon monoxide concentration is .0001% by volume (Lange and Forker, 1956). This concentration is a thousand times

and argon was constant in the atmosphere and metabolically inert (Hamilton and Kory, 1960). The use of nitrogen as the carrier gas reduced the size of the oxygen-nitrogen-argon-carbon monoxide chromatographic peak by approximately 80%. This was because the detector did not respond to the 80% nitrogen in the sample. Thus the oxygen-nitrogen-argon-carbon monoxide peak on the recorder was almost exclusively a response to oxygen concentration. This made it possible to monitor small changes in oxygen concentration even though the thermal conductivity difference between oxygen and the nitrogen carrier gas was small. This is presented in more detail in the discussion section.

Oxygen, carbon dioxide, and water vapor chromatographic peaks were identified by comparing column retention times of peaks from compressed air samples with those of reference samples of compressed oxygen and carbon dioxide and a sample chromatogram obtained from Waters Associates (Framingham, Massachusetts). The compositions of the reference samples are shown in Table 5.

Reference samples of known volumes were injected into the chromatograph and used to quantitate the oxygen peak height (see Figure 2), and carbon dioxide and water vapor peak areas (see Figure 3).

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smaller than the minimum detectable change in oxygen (.2%).

Table 5. Specifications of compressed gases.*

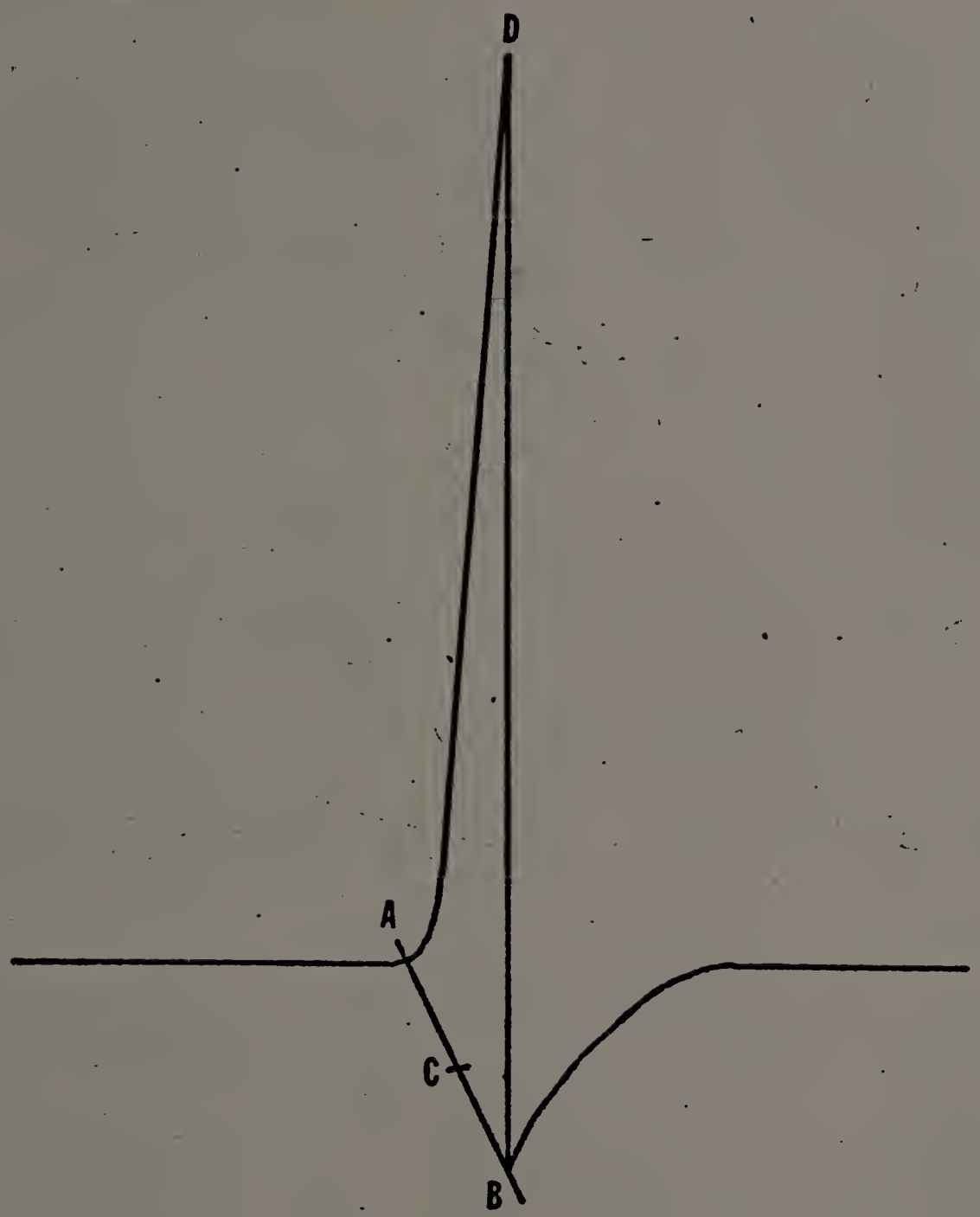
Gas	Grade	Minimum Purity %	Maximum Impurities (PPM)**	Ar	He	H_2	Kr	Ne	N_2	O_2	X_e	N_2O	CO& CO_2	THC	H_2O
Nitrogen	Purified	99.5%	40***	70	—	4	—	20	—	2	—	—	1	1	4
Carbon Dioxide	Purified	99.5%	200	10	8	8	—	—	100	8	—	4	10	20	20
Oxygen	Purified	99.5%	200	100	—	—	24	—	30	—	—	1	2	24	4
Compressed Air	Purified								79%	21%					

* (Airco, Inc., 1968 and Dubuc, 1971)

** Excluding compressed air

*** Excluding argon and neon

Figure 2. Method for the estimation of oxygen peak height.

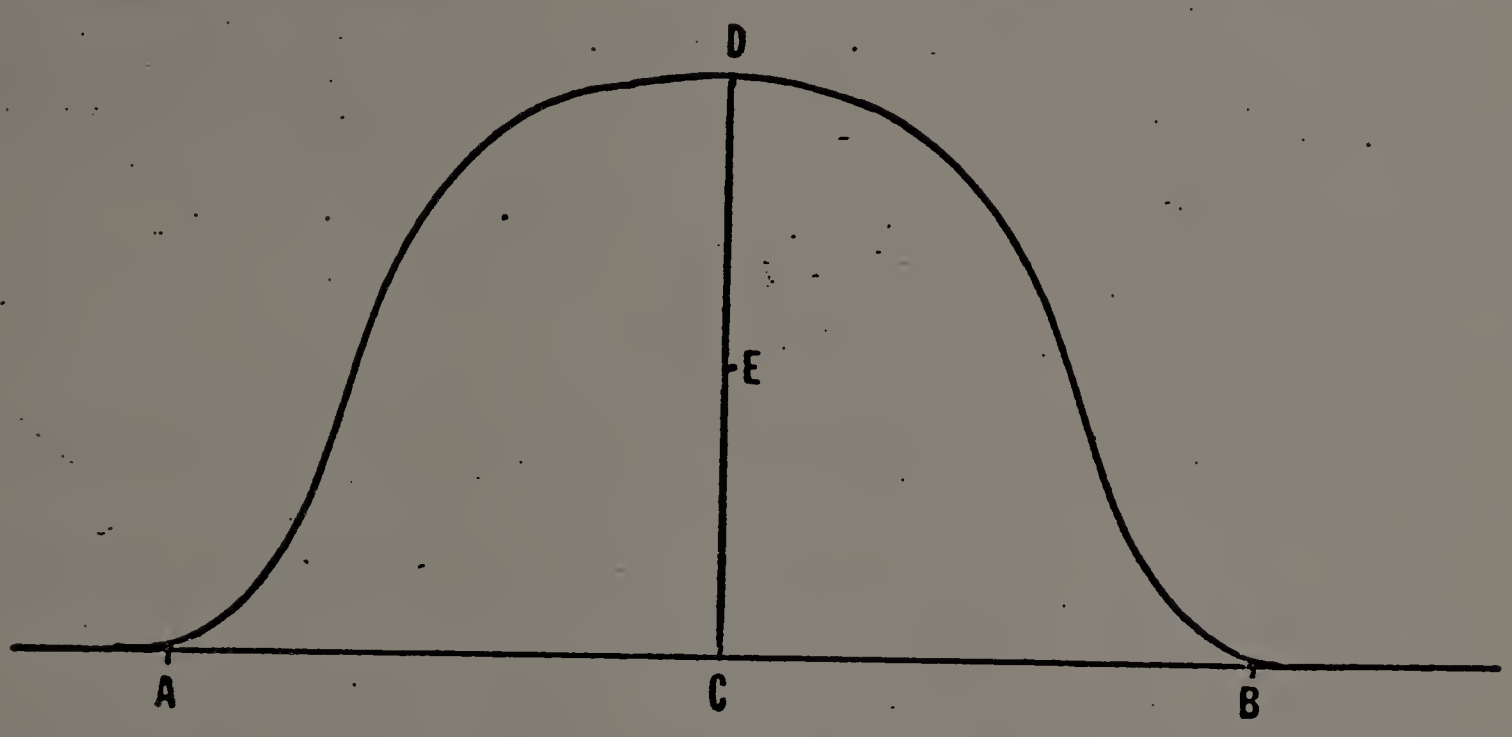


1. The baseline of the chromatographic peak was drawn through inflection points A and B.

2. Half the distance from A to B was measured and marked as point C.

3. Peak height was measured from the top of the line at point C to the top of the peak D.

Figure 3. Method for the estimation of carbon dioxide and water vapor peak areas.



1. The baseline of the chromatographic peak was drawn by laying a straight edge on the baseline to the left of the peak and continuing this baseline across the peak from A to B.

2. Half the distance from A to B was measured and marked as point C.

3. The height was measured from point C to the peak, point D.

4. Half the distance from C to D was measured and marked E.

5. Width at half height was measured parallel to the baseline.

$$Area = \tfrac{1}{2}\ base \times height$$

The measurement was taken from the inside of the left half of the peak, through E, to outside of the right half of the peak.

For a comparison of the accuracies of different methods of gas chromatographic peak size estimation see Table 6. Samples of size 100 microliters or smaller for oxygen and carbon dioxide peak calibrations were made with a 100 microliter gas tight syringe (Hamilton No. 1710). Oxygen and carbon dioxide samples larger than 100 microliters, all water vapor calibration samples, and respiratory measurements were made with a 500 microliter gas tight syringe (Hamilton No.1750).

Oxygen and Carbon Dioxide Calibration

Oxygen and carbon dioxide calibration samples were taken by the following method: The needle on the gas tight syringe was inserted up to its base into the hose connector on the regulator on the compressed gas tank. The syringe was filled to capacity, and then its contents were emptied back into the hose connector and expelled by the gas stream. This was repeated three times in order to reduce contamination in the sample from the dead space in the needle. The fourth time that the syringe was filled, it was transferred to an 8X11 sheet of white paper on the bench near the chromatograph for better visibility. At this point, the syringe was emptied until the desired calibration volume remained in it. The contents of the syringe were then injected into the chromatograph. The water vapor calibration samples were taken by a different method explained in the subsection

Table 6. Comparison of quantitation methods for gas chromatographic peaks. (Dean, 1969).

Integration technique	Standard deviation (%)
Planimetry	4.0
Triangulation	4.0
Height X width (at half height)	2.6
Cut and weigh peaks	1.7
Ball and disk	1.3
Electronic digital	0.44

on water vapor calibration. All calibration samples were analyzed at attenuation four on the chromatograph (one-fourth full signal).

Oxygen calibration graphs were made by plotting peak height against known sample volumes. At each sample volume, four injections were made and the mean peak height was calculated. The mean peak height at each given volume was then plotted. A line was drawn through these points to form the calibration curve (Figure 10). This same procedure was used for carbon dioxide and water vapor calibration graphs, except that peak area was substituted for peak height (Figures 11 and 12).

Water Vapor Calibration

Initially, a 500 milliliter filter flask with a serum cap over the hose connector, a manometer through the stopper, and 200 milliliters of saturated solution of ammonium sulfate inside had been used as a source of water vapor for calibration injections. However, it was found that a humidity gradient had formed inside the flask. Shaking the flask moderately caused the chromatographic water vapor response to be erratic. More vigorous shaking wet the serum cap and caused droplets of salt solution to crystalize on the inside of the needle, stopping up the syringe.

Water vapor calibration samples were finally prepared in the following way. Twenty numbered serum vials of known volume were filled with approximately one milliliter of water and capped. This allowed saturation of the air in the vial with water vapor while retaining an excess of water. The rack which held the serum vials was laid on its side with an approximately one inch tall wooden block under one side. This tilted the serum vials up enough to keep the caps dry, but flat enough to minimize the possibility of a humidity gradient forming in the vial.

The vials were sampled after twenty-four hours. A sample was taken by injecting a volume of air, equal to the size of the sample to be withdrawn, into the serum vial in order to equalize the pressure.

The syringe was then filled and emptied. The third time the syringe was filled to the desired volume of air, it was withdrawn from the serum vial, and injected into the chromatograph.

The weight of water vapor in the sample was calculated from values for the mass of water vapor in saturated air at 27.8°C given in Lange and Forker (1956). Even though the actual temperature of the samples fluctuated between 27°C and 28°C, 27.8°C was the closest value in the table. These calculations are shown in Table 7. Water vapor is present as mass so that the results may be compared with the published values of Bursell (1959 and 1960).

Table 7. Calculations for estimating the mass of water in saturated air.

At 27.8°C Mass of H_2O in saturated air = 26.91 gm/m^3*

$26.91\ gm/m^3 = 26.91 \times 10^{-3}\ mg/ml$

Therefore a 100 µl saturated air sample at 27.8°C should contain

$26.91 \times 10^{-3}\ mg/ml \times .1\ ml = 2.69 \times 10^{-3}\ mg\ H_2O$

*Lange and Forker (1956).

Serum Vial Volume Measurement

Before the volume of the serum vials could be measured, the

volume of a No. 26, 1 1/2 inch Yale needle had to be estimated. In order to do this, a serum vial was filled with water up to the level of where the cap would fit. Then the vial was capped. A No. 26, 1 1/2 inch Yale needle was inserted through the cap. A second needle mounted on a syringe filled with water was then inserted through the cap. The water from the syringe was emptied into the serum vial until the Yale needle was filled to capacity. The volume of water remaining in the syringe was recorded. Next, the Yale needle was pushed further through the cap until the tip could be easily seen in the vial. The syringe was filled from the vial until an air bubble was first seen appearing at the tip of the Yale needle. The volume of water in the syringe at that point was recorded. Finally, the volume of the Yale needle was estimated by subtracting the volume of water remaining in the syringe when the Yale needle was filled from the volume of water in the syringe when the Yale needle was empty.

Now that the volume of the Yale needle was known, the volume of the serum vials could be estimated. The serum vials were numbered with a glass marker. Next, the vials were filled with water from a burette to the level of the cap. This volume was recorded. The vial was capped. The next procedure described was used to estimate the volume of the remaining air bubble. The No. 26, 1 1/2 inch Yale needle was inserted so that the point just protruded through the bottom

of the cap into the vial. A second needle, attached to a syringe filled with water was inserted through the cap. The syringe which was filled with water was emptied until the water filled the Yale needle.

The total volume of the capped serum vial was estimated in the following way. The volume of water transferred to the serum vial by the burette was added to the volume of water injected from the syringe. This figure minus the volume of the Yale needle was the net volume of the capped serum vial.

House Fly Rearing

The house flies used in this thesis were an unknown DDT-susceptible strain reared in the laboratory. House fly eggs were obtained by placing one teaspoon of one week-old CSMA* larval media in a dish and exposing it to the adult flies for three hours. The presence of the CSMA caused gravid females to lay eggs on the media. A half-teaspoon of eggs was then transferred to a half-gallon container of one week-old CSMA. Most larvae completed pupal formation within two weeks from hatching. The mixture of larvae, pupae and CSMA medium was then emptied into a large pan of water. The larva and CSMA

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*The CSMA had been prepared by mixing an equal volume of water with the dry CSMA and allowing this mixture to ferment for one week. The dry CSMA is available from Ralston Purina Specialty Chow Plant, Richmond, Indiana, 47374.

sank to the bottom and the pupae floated. The pupae were collected with a wire sieve and spread to dry. The next day they were placed in adult cages in trays. After some adults had emerged, the tray of pupae was moved to a new cage each day. This allowed grouping of flies of the same age.

Adults were maintained at 23°C to 28°C and 40 to 60% relative humidity. The light regime was controlled automatically and set at 14 hours light and 10 hours dark with the experimental noon synchronized with real noon. Adults had access to water from a plastic container with holes drilled in the top to admit wicks made of absorbal. Adults also had access to dishes of table sugar and powdered milk.

House Fly Handling

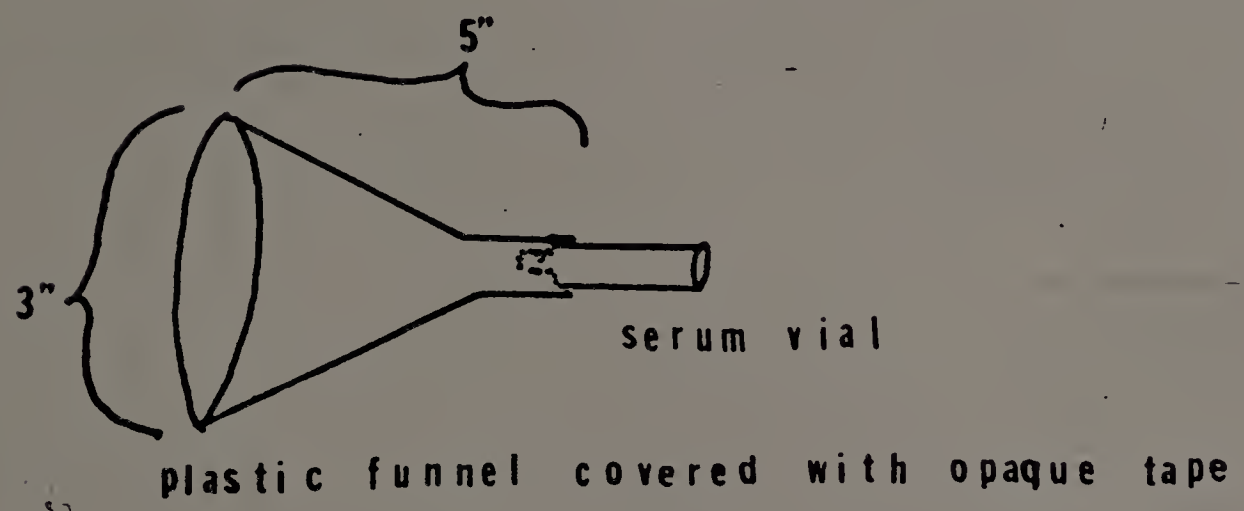
Measurements of oxygen uptake, carbon dioxide production and water vapor output were taken on three successive days. These measurements will be called experiments I, II, III. In experiments I and II, the flies were removed from the cage in which they were raised by a vacuum restraint. This consisted of a vacuum cleaner (model GE AUT 160) with a layer of cheesecloth stretched over the end of the hose and held in place with a rubber band. When the vacuum cleaner was turned on and the hose end inserted into the cage, flies coming within a few centimeters of the hose end were drawn to the surface of

the cheesecloth. When six to eight flies had been caught, the hose was removed from the cage; the vacuum cleaner was turned off, and a small plastic vial was put over the flies. The rubber band holding the cheesecloth on the hose end was rolled down the hose, and the cheesecloth was folded over the top of the plastic vial.

The plastic vial was transferred to a Buchner funnel connected to a tank of compressed carbon dioxide by a length of tygon tubing (Williams, 1946). The flow rate of carbon dioxide was set so that the flies would be anesthetized in 30 to 45 seconds. The flies were sexed, picked up by hand, and placed head down into a serum vial. A small tap on the bottom of the serum vial would knock the flies into the vial. The number of the vial was recorded, and a loose plug of cotton batting was inserted into the mouth of the vial. In experiment III, the flies were caught and sexed individually with the device shown in Figure 4.

The serum vials containing the flies were placed in a test tube rack set approximately 10° from horizontal by a small wedge under one edge of the test tube rack. This kept the vials from falling out of the rack, and helped to prevent the build-up of gradients which would occur if the fly were confined to the bottom of the vial. One-half hour before the air in the vial was to be sampled, it was flushed with approximately 150 ml of compressed air. After the vial was flushed,

Figure 4. A device for catching individual flies. When a fly is trapped under the cone, it will move towards the light and into the serum vial.



the cotton plug was removed. The rubber cap was immediately inserted into the vial and the top of the cap folded over the neck of the vial.

The air pressure in the serum vial had to be equalized. The syringe was filled with 500 microliters of compressed air, using the method described for taking oxygen and carbon dioxide calibration samples. The syringe needle was inserted through the cap on the serum vial. The syringe was emptied and filled four times into the serum vial. On the fourth filling, 500 µl were withdrawn from the vial and injected into the chromatograph.

The vial containing the fly was weighed on a Mettler H-20 balance

to the nearest milligram and the weight recorded. The cap was removed from the vial and the fly released into a general breeding cage. The vial and cap were then weighed again and the weight recorded. The weight of the fly was the difference in these two weights.

Oxygen Uptake, Carbon Dioxide Production and Water Vapor Output Measurements

Experiments I, II, and III were carried out approximately (± five minutes) the same time each day. During each day, the temperature in the laboratory varied from 26° C to 28° C. Barometric pressure was not measured.

Each day's measurements were taken in the following way: At approximately 8:00 A. M., the Wheatstone bridge on the thermal conductivity detector was turned on and the carrier gas-flow rate was turned up to the specifications in Figure 8. The system was then allowed to equilibrate until the first sample was injected at 1:45 P. M.

Injections of samples were made every twenty minutes. In Experiment I, every sixth injection was from a sealed serum vial with no fly in it. In Experiments II and III, every sixth injection was from the tank of compressed air used to flush the serum vial at the beginning of the measurement. The compressed air sample was taken from the tank in the same way described in the section on calibration

injections of oxygen and carbon dioxide. Tests had shown that no detectable change took place in an empty serum vial within a half hour (Table 10). Thus, the injections from the compressed air tank served as a control and as a reference for estimating oxygen uptake.

Basically, gas volume (oxygen uptake, carbon dioxide production, water vapor output) change was estimated by comparing the chromatographic responses of the air sample from the vial with the fly in it, to the mean values of the compressed air samples. These values for gas change were corrected for serum vial volume, fly weight, and length of time in the vial. The method for calculating these corrections is shown in Table 8.

Table 8. Method for calculating the gas change in respiratory measurements.

(height) (width at 1/2 height) = peak area

*(4.04 cm) (.55 cm) = 1.8 cm^2

Experimental peak area - Control peak area = gas uptake in syringe

1.8 cm^2 - .3 cm^2 = 2.1 cm^2

From the calibration graph

2.1 cm^2 = 1.7 µl CO_2/500 µl sample

(1.7 µl CO_2/500 µl sample) (2) = 3.4 µl CO_2/ml sample

Total air volume = serum vial volume + .5 ml of air injected into vial to equalize the pressure in taking a sample.

Total air volume = 6.6 ml + .5 ml = 7.1 ml

(Total air volume) (Vol. Δ in syringe) = total CO_2 change

(7.1 ml air) (3.4 µl CO_2/ml air) = 24.1 µl CO_2

$$\frac{\text{total } CO_2 \Delta}{\text{wt. of fly}} = \frac{\mu l\ CO_2 \text{ production}}{\text{mg of fly wt.}}$$

$$\frac{24.1}{17} = 1.4\ \mu l/mg$$

(µl/mg) (2**) = µl CO_2/mg/hr

(1.4) (2) = 2.8 µl CO_2/mg/hr

* First experimental value of carbon dioxide from first respiration measurement.

**The experimental exposure time was 30 min, X 2 = 1 hr.

RESULTS

Chromatograms

A chromatogram obtained with a charcoal column and helium carrier gas is shown in Figure 5, and with nitrogen carrier gas in Figure 6. Chromatograms obtained with Porapak Q using helium and nitrogen as the carrier gas are shown in Figures 7 and 8 respectively. One test made with a column of Porapak Q and nitrogen carrier gas on a Varian model 700 autoprep is shown in Figure 9.

Calibration Curves

Oxygen. The calibration curve obtained for oxygen is shown in Figure 10. The relationship between peak height and increasing concentration was found to be linear. The mean values and ranges for the oxygen calibration samples are shown in Table 9.

Carbon Dioxide. The calibration curve obtained for carbon dioxide is shown in Figure 11. The relationship between peak area and increasing concentration was found to be linear from 0 to 9 microliters and from 12 to 20 microliters. The experimental measurements were all in the range of 0 to 9 microliters. The mean and range for the carbon dioxide calibration samples are shown in Table 9.

Sealed vial test. Tests showing that the change in gas composition of sealed serum vials was negligible is shown in Table 10. The

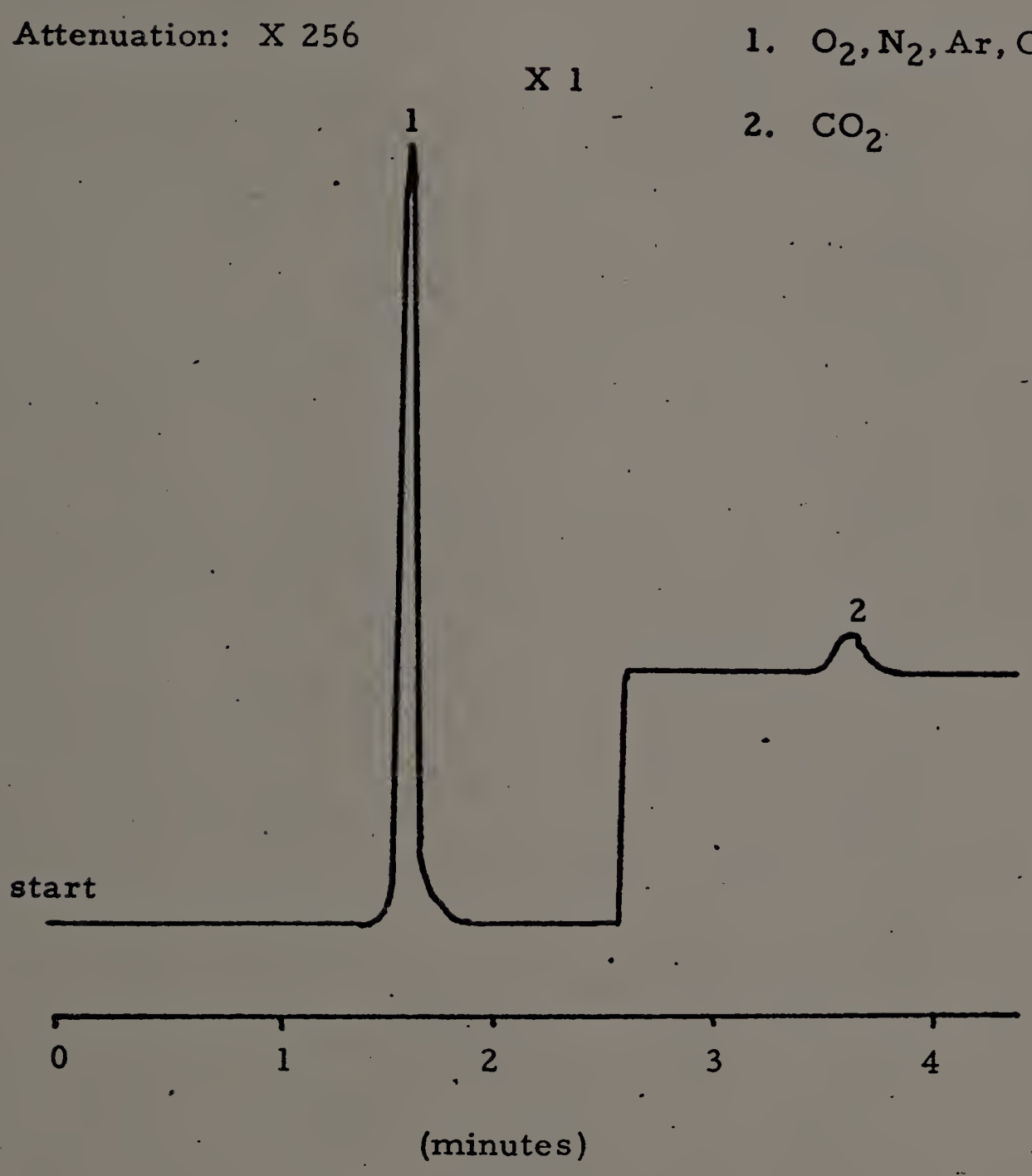
greatest gas change was carbon dioxide. In trial two carbon dioxide appeared to increase approximately five percent. This change, however, was well within the variations introduced by the calibrations (approximately 10%).

Water vapor. The calibration curve obtained for water vapor is shown in Figure 12. The relationship between peak area and increasing concentration appears to be linear. This was due to the necessity of enlarging the points on the calibration curve for photographic reduction. Actually, the bottom point is approximately .2 micrograms above the calibration curve. The mean and range for the water vapor calibration samples are shown in Table 9.

House Fly Oxygen Uptake, Carbon Dioxide Production, and Water Vapor Output Measurements

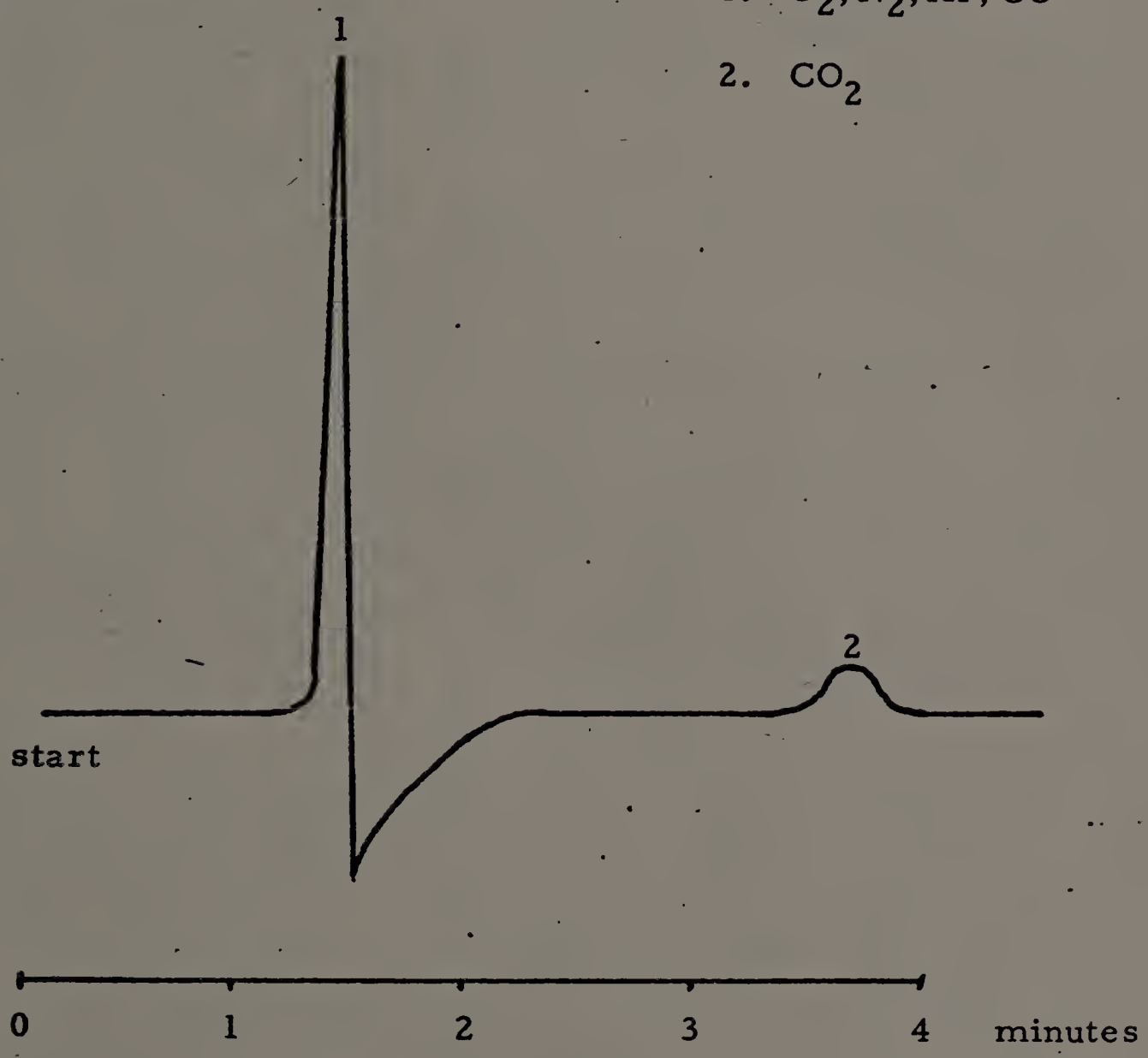
The data for experiments I, II, and III showing each step in the calculations and the control values (marked X) are shown in Tables 11 through 19. The final mean values and respiratory quotients for experiments I, II, III are shown in Table 20. Molar values for oxygen uptake, carbon dioxide production, and water vapor output for the house fly, calculated from the mean values in Table 20, are shown in Table 21.

Figure 5. Chromatogram of .5 ml air sample plus 10% carbon dioxide on columns of charcoal with helium carrier gas.



Columns; 2, 3-feet X 1/4 inch: Support; activated coconut charcoal 50-200 mesh: Carrier; helium, 150 ml/min, 30 psi: Detector; TC, 180 Ma: Temperatures; injection port 200°C, column 160°C, detector 200°C.

Figure 6. Chromatogram of .5 ml air sample plus 10% carbon dioxide on columns of charcoal with nitrogen carrier gas.

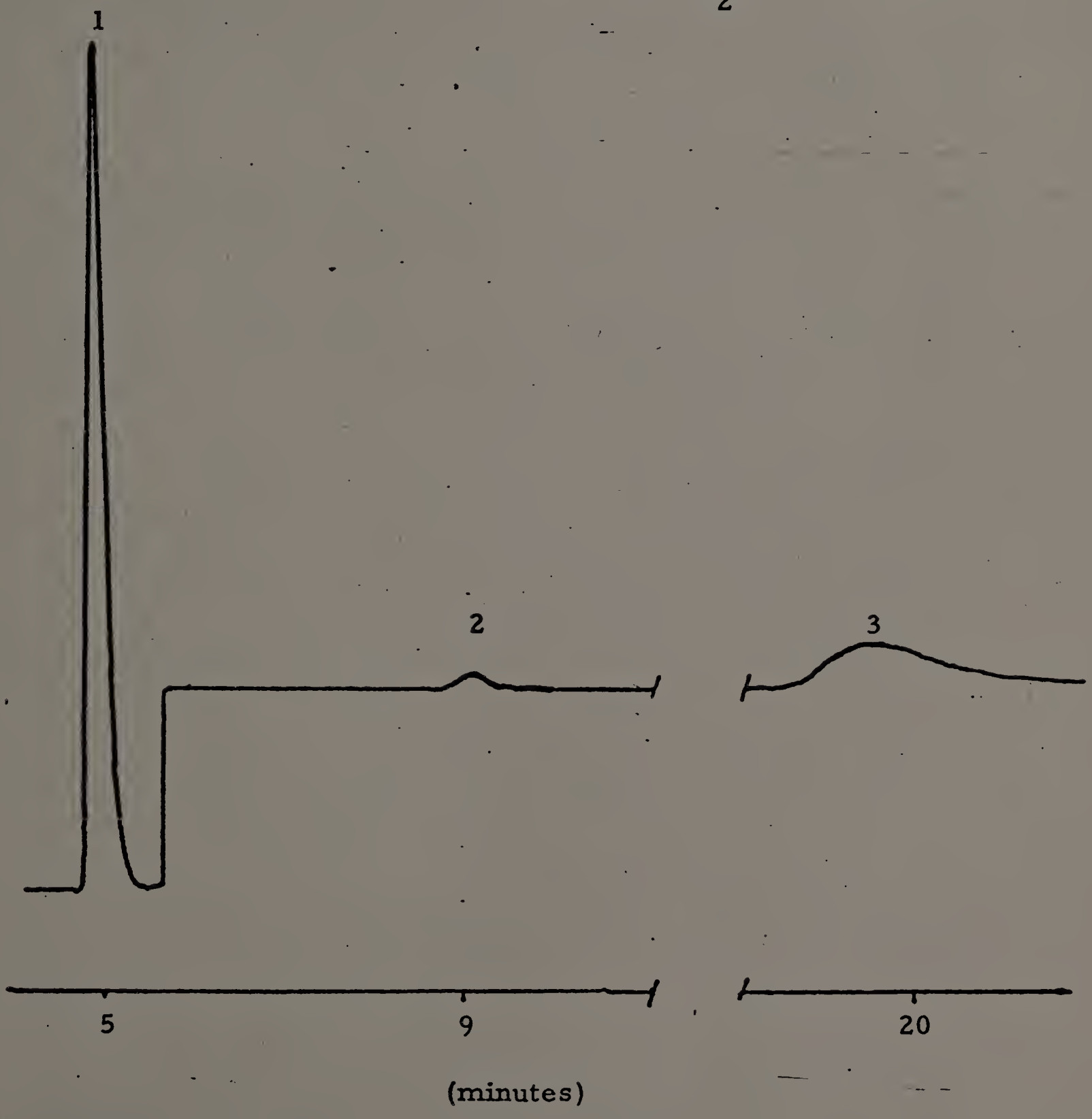


Columns; 2, 3-feet X 1/4 inch: Support; activated coconut charcoal 50-200 mesh: Carrier; nitrogen, 150 ml/min, 30 psi: Detector; TC, 180 Ma: Temperatures; injection port 200°C, column 160°C, detector 200°C.

Figure 7. Chromatogram of .5 ml air sample on columns of Porapak Q with helium carrier gas.

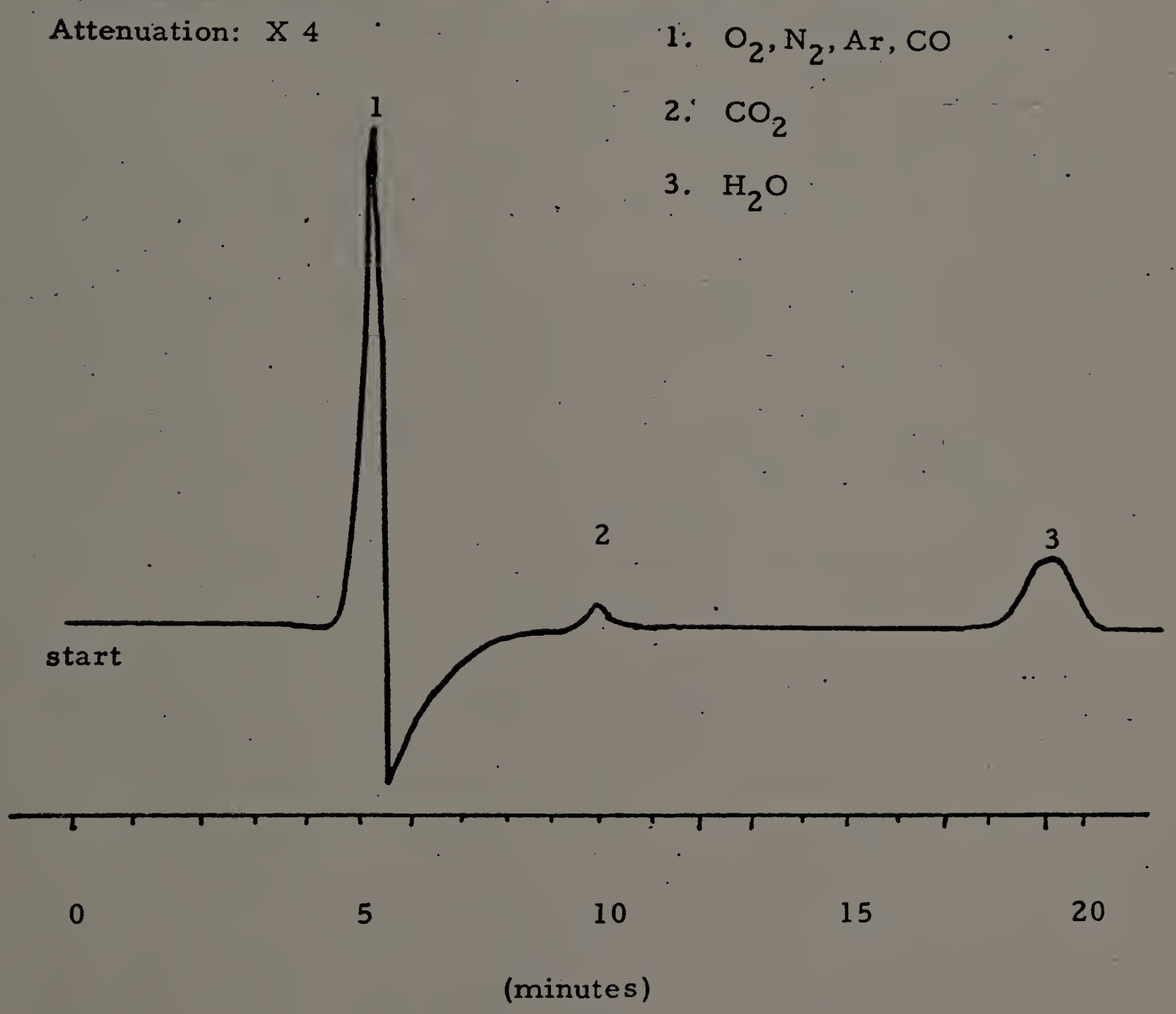
1. O_2, N_2, Ar, CO
2. CO_2
3. H_2O

Attenuation: 64 X 8



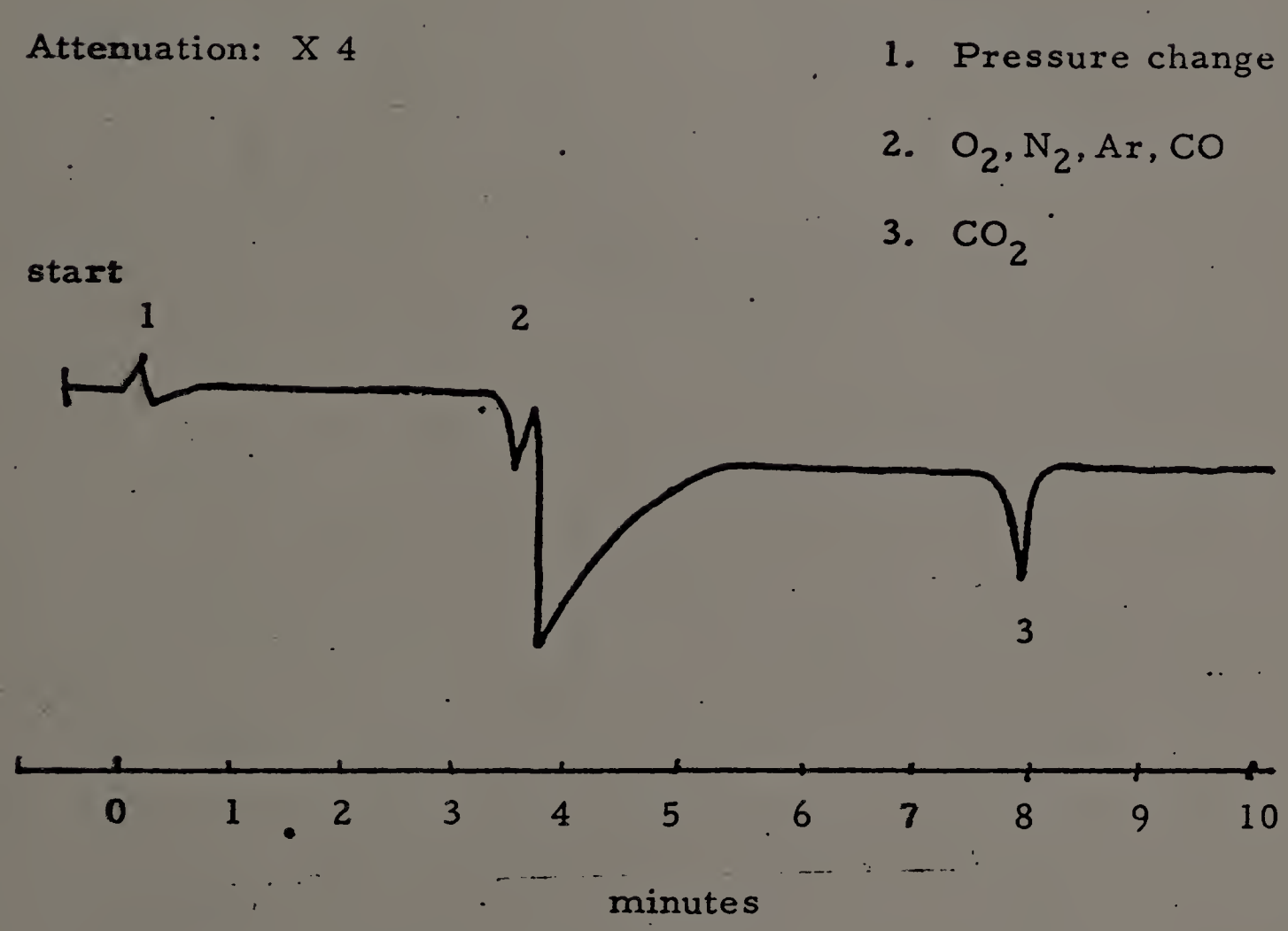
Columns; 2, 12-feet X 1/4 inch: Support; Porapak Q, 80-100 mesh: Carrier; helium, 150 ml/min: Detector; TC, 150 Ma: Temperatures; injection port 150°C, column 60°C, detector 200°C.

Figure 8. Chromatogram of .5 ml air sample with columns of Porapak Q, with nitrogen carrier gas.



Columns; 2, 12-feet X 1/4 inch: Support; Porapak Q 80-100: Carrier; nitrogen, 150 ml/min: Detector; TC, 150 Ma: Temperatures; injection port 150°C, column 60°C, detector 200°C.

Figure 9. Gas chromatogram of .5 ml air sample plus approximately 5% CO_2 on a Varian model 700 autoprep.



Columns; 1, 12-feet X 1/4 inch: Support; Porapak Q, 80-100 mesh: Carrier; nitrogen, 150 ml/min: Detector; TC, 150 Ma: Temperatures; injection port 140°C, column 60°C, detector 200°C.

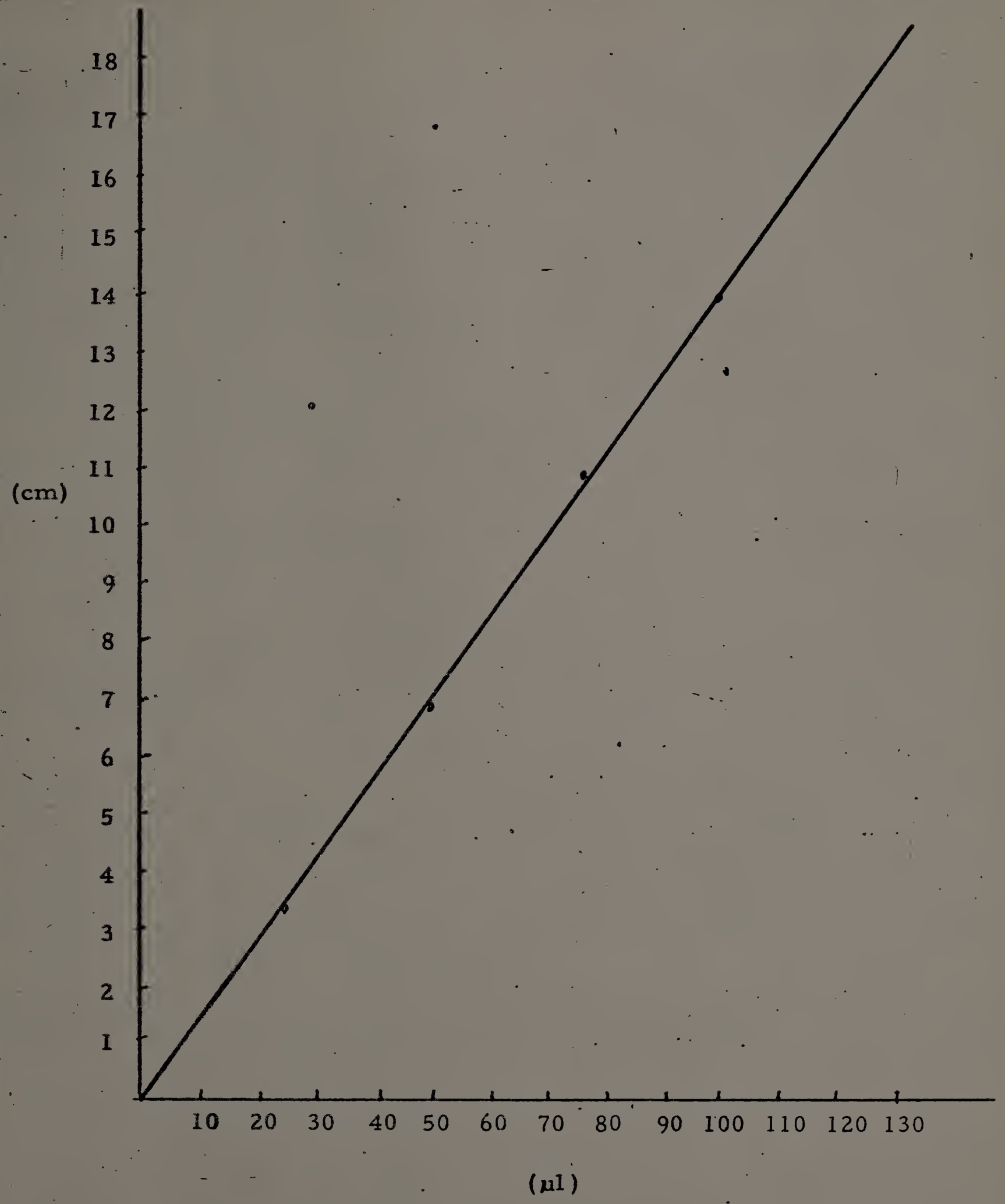


Figure 10. Oxygen calibration graph.

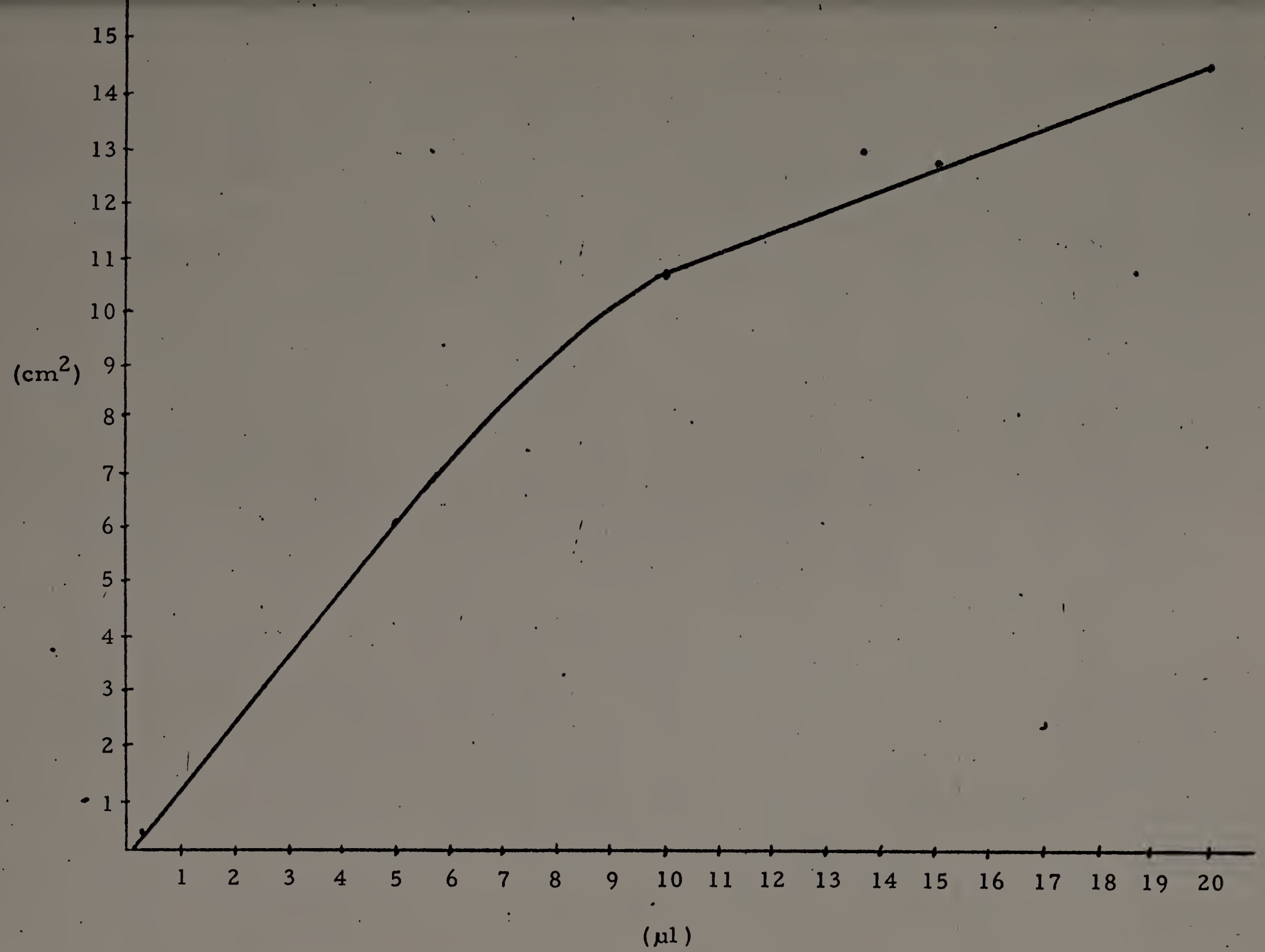


Figure 11. Carbon dioxide calibration graph.

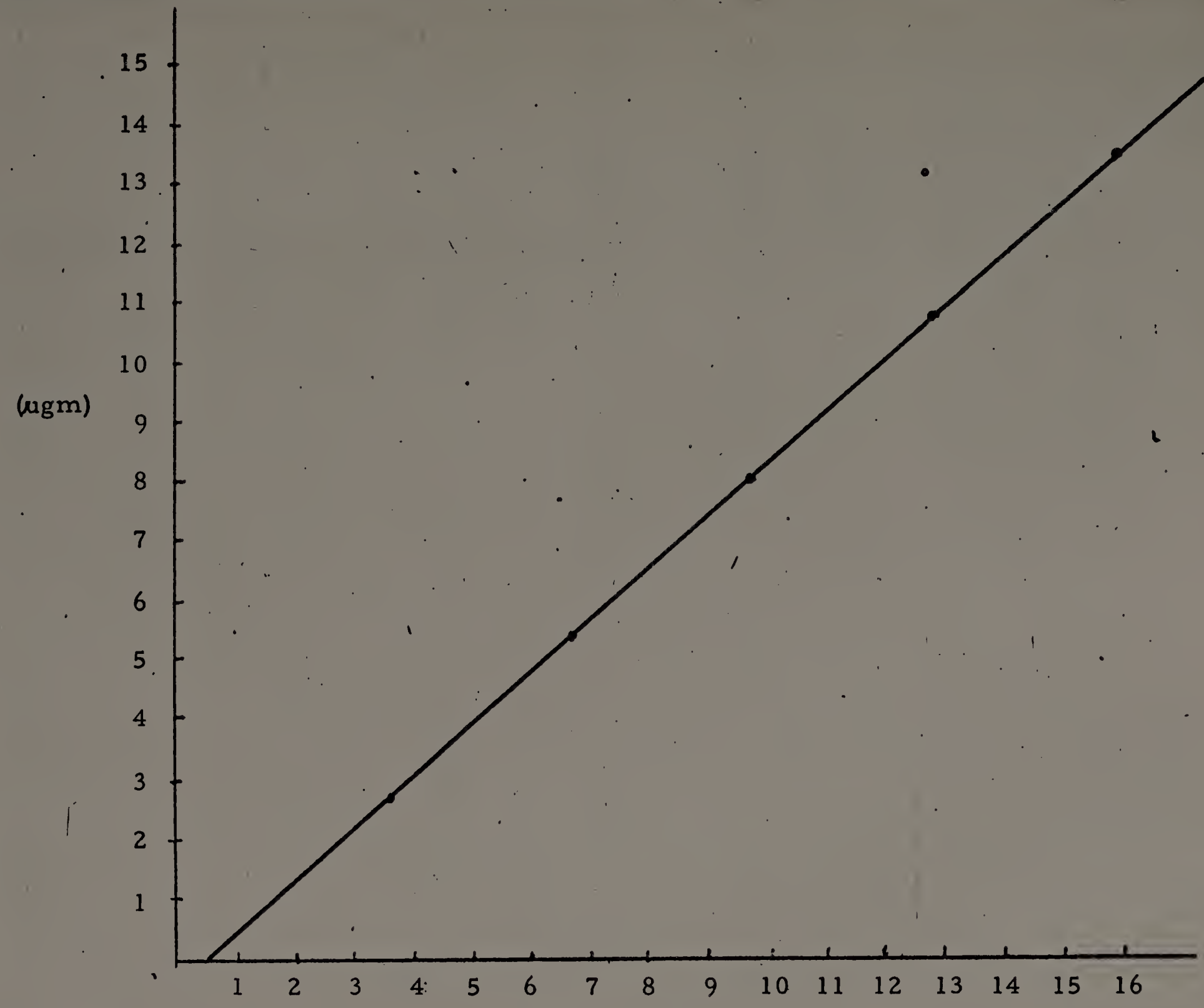


Figure 12. Water vapor calibration graph.

Table 9. Chromatographic peak height (cm) or area (cm^2) of calibration samples.

Oxygen	Volume Injected			
	25 µl	50 µl	75 µl	100 µl
	3.35 cm	6.60 cm	13.00 cm	14.08 cm
	3.35	7.51	10.45	14.09
	3.36	7.39	9.91	14.08
	3.37	5.91	10.50	14.08
$\bar{X}$ =	3.36	6.85	10.96	14.08

Carbon Dioxide	0.3 µl	5 µl	10 µl	15 µl	20 µl
	0.27 cm^2	5.91 cm^2	10.60 cm^2	12.64 cm^2	14.74 cm^2
	0.27	6.13	11.48	12.64	14.76
	0.25	6.05	10.56	12.64	14.56
	0.32	5.84	10.82	12.74	14.00
$\bar{X}$ =	0.28	5.98	10.69	12.66	14.52

Water Vapor					
µl of saturated air —	100 µl	200 µl	300 µl	400 µl	500 µl
mg H_2O x 10^{-3} —	2.69	5.38	8.07	10.76	13.48
	3.60 cm^2	6.74 cm^2	9.98 cm^2	12.74 cm^2	15.98 cm^2
	3.58	7.33	9.90	12.44	15.84
	3.62	6.82	9.20	12.97	15.66
	3.63	6.68	9.88	13.02	15.66
$\bar{X}$ =	3.61	6.89	9.74	12.79	15.78

Table 10. Changes in peak size for air sealed in a 6.5 ml serum vial.

Trial	(Peak Area Cm^2) Laboratory Air			Laboratory Air in vial 30 minutes		
	O_2	CO_2	H_2O	O_2	CO_2	H_2O
I	3.05	0.31	3.61	3.03	0.32	3.81
II	3.07	4.18	3.02	3.04	4.38	2.90

Table 11. Raw data for oxygen uptake in Experiment I.

height (cm)	net height	µl/500 µl	µl/ml	vial vol (ml)	vial + .5 ml	total vol (µl)	fly wgt (mg)	µl/mg	µl/mg/hr
13.84 X									
13.73	.17	1.7	3.4	6.6	7.1	24.1	17	1.4	2.8
13.71	.19	1.8	3.6	6.0	6.5	23.4	21	1.1	2.2
13.69	.21	2.0	4.0	5.4	5.9	23.6	16	1.5	3.0
13.67	.23	2.1	4.2	7.3	7.8	32.8	19	1.7	3.4
13.62	.28	2.5	5.0	6.4	6.9	34.5	19	1.8	3.6
13.67	.23	2.1	4.4	6.6	7.1	31.2	16	1.9	3.8
14.38 X									
13.59	.31	2.7	5.4	6.4	6.9	37.3	24	1.6	3.2
13.61	.29	2.6	5.2	6.4	6.9	35.8	24	1.5	3.0
13.59	.31	2.7	5.4	6.4	6.9	37.3	22	1.7	3.4
13.59	.31	2.7	5.4	6.4	6.9	37.5	25	1.5	3.0
13.88 X									

$\bar{X}$ = 13.9 average control height

Table 12. Raw data for carbon dioxide production in Experiment I.

height	width @.5h	CM^2 area	net area	µl/ 500 µl	µl/ ml	vial vol.	vial +.5 ml	total volume	fly wgt	µl/mg	µl/mg/hr
0.62	0.57	0.33	X								
4.04	0.55	2.22	1.9	1.7	3.4	6.6	7.1	24.1	17	1.4	2.8
4.13	0.58	2.40	2.1	1.9	3.8	6.0	6.5	24.7	21	1.2	2.4
4.77	0.58	2.77	2.4	2.2	4.4	5.4	5.9	25.9	16	1.6	3.2
5.60	0.52	2.91	2.6	2.3	4.6	7.3	7.8	35.8	19	1.9	3.8
5.39	0.59	3.18	2.8	2.5	5.0	6.4	6.9	34.5	19	1.8	3.6
4.55	0.53	2.41	2.1	1.9	3.8	6.6	7.1	27.0	16	1.7	3.4
0.61	0.55	0.34	X								
7.04	0.51	3.59	3.3	2.8	5.6	6.4	6.9	38.6	24	1.6	3.2
5.59	0.54	3.02	2.7	2.4	4.8	6.4	6.9	33.1	24	1.4	2.8
5.83	0.58	3.38	3.1	2.7	5.4	6.4	6.9	37.3	22	1.7	3.4
6.24	0.58	3.62	3.3	2.9	5.8	6.4	6.9	40.0	25	1.6	3.0
0.66	0.51	0.34	X								

$\overline{X}$ = 0.3 average control area

Table 13. Raw data for water vapor output in Experiment I.

height	width @.5h	(CM^2) area	net area	mg/ 500 µl	mg/ ml	vial vol	vial +.5 ml	total vol	fly wgt	mg/mg	mg/ mg/hr
.081	1.70	1.38	X								
6.09	1.68	10.23	8.85	7.30	14.6	6.6	7.1	104	17	6.1	.012
6.72	1.67	11.23	9.85	8.20	16.4	6.0	6.5	107	21	5.1	.010
6.61	1.72	11.38	10.0	8.40	16.8	5.4	5.9	99.2	16	6.2	.012
6.44	1.68	10.83	9.45	7.80	15.6	7.3	7.8	122	19	6.4	.012
0.76	1.48	11.25	9.85	8.20	16.4	6.4	6.9	114	19	6.0	.012
5.54	1.66	9.37	8.00	6.60	13.2	6.6	7.1	94.4	16	5.9	.011
0.82	1.72	1.41	X								
7.43	1.76	13.08	11.70	9.90	19.8	6.4	6.9	137	24	5.7	.011
7.35	1.75	12.86	11.50	9.70	19.4	6.4	6.9	134	24	5.6	.011
7.30	1.75	12.77	11.40	9.60	19.2	6.4	6.9	132	22	6.0	.012
8.28	1.77	14.66	13.20	11.20	22.4	6.4	6.9	155	25	6.2	.012
0.81	1.68	1.36	X								

$\bar{X}$ = 1.4 average control area

Table 14. Raw data for oxygen uptake in Experiment II.

height	net height	µl/500 ul	µl/ml	vial vol	vial +.5 ml	total vol	fly wgt	µl/mg	µl/mg/hr
14.01	.27	2.4	4.8	6.5	7.0	33.6	20	1.7	3.4
14.07	.21	2.0	4.0	6.3	6.8	27.2	18	1.5	3.0
14.11	.17	1.7	3.4	6.5	7.0	23.8	15	1.6	3.2
14.05	.23	2.1	4.2	6.6	7.1	29.8	20	1.5	3.0
13.96	.32	2.8	5.6	6.5	7.0	39.2	22	1.8	3.6
13.93	.35	3.0	6.0	6.5	7.0	42.0	25	1.7	3.4
14.25 X									
14.12	.16	1.6	3.2	6.5	7.0	22.4	15	1.5	3.0
14.07	.21	2.0	4.0	6.5	7.0	28.0	20	1.4	2.8
14.12	.16	1.6	3.2	6.3	6.8	21.8	17	1.3	2.6
13.88	.40	3.4	6.8	6.6	7.1	48.3	24	2.0	4.0
14.32 X									

$\bar{X}$ = 14.3 average control height

Table 15. Raw data for carbon dioxide production in Experiment II.

height	width @.5h	area	net area	µl/ 500 µl	µl/ ml	vial vol	vial +.5 ml	total vol	fly wgt	µl/mg	µl/mg/hr
5.93	0.54	3.20	2.9	2.6	5.2	6.5	7.0	36.4	20	1.8	3.6
4.62	0.55	2.54	2.2	2.0	4.0	6.3	6.8	27.2	18	1.5	3.0
4.34	0.51	2.21	1.9	1.8	3.6	6.5	7.0	25.2	15	1.7	3.4
5.72	0.58	3.32	3.0	2.7	5.4	6.6	7.1	38.3	20	1.9	3.8
4.65	0.59	2.74	2.4	2.2	4.4	6.5	7.0	30.8	22	1.4	2.8
6.75	0.55	3.71	3.4	3.0	6.0	6.5	7.0	42.0	25	1.7	3.4
0.57	0.53	.30	X								
3.57	0.54	1.92	1.6	1.6	3.2	6.5	7.0	22.4	15	1.5	3.0
4.98	0.51	2.54	2.2	2.0	4.0	6.5	7.0	28.0	20	1.4	2.8
3.69	0.51	1.88	1.6	1.6	3.2	6.3	6.8	22.8	17	1.3	2.6
8.08	0.52	4.20	3.9	3.4	6.8	6.6	7.1	48.3	24	2.0	4.0
0.58	0.53	.31	X								

$\overline{X}$ = .3 average control area

Table 16. Raw data for water vapor output in Experiment II.

height	width @.5h	area	net area	mg/ 500 µl	mg/ ml	vial vol	vial +.5 ml	total vol	fly wgt	mg/mg	mg/ mg/hr
6.88	1.72	11.83	10.30	8.70	17.4	6.5	7.0	122	20	6.1	.012
6.31	1.74	10.98	9.45	7.80	15.6	6.3	6.8	106	18	5.9	.011
5.18	1.74	9.03	7.50	6.10	12.2	6.5	7.0	85.5	15	5.7	.011
6.52	1.76	11.48	9.95	8.30	16.6	6.6	7.1	118	20	5.9	.011
6.84	1.70	11.63	10.10	8.50	17.0	6.5	7.0	119	22	5.4	.010
8.94	1.77	15.83	14.0	11.4	22.8	6.5	7.0	160	25	6.4	.012
.84	1.72	1.45	X								
5.41	1.69	9.14	7.61	6.20	12.4	6.5	7.0	87	15	5.8	.011
6.75	1.67	11.28	9.75	8.10	16.2	6.5	7.0	114	20	5.7	.011
5.62	1.69	9.49	7.96	6.50	13.0	6.3	6.8	88.4	17	5.2	.010
9.03	1.76	15.88	14.35	12.2	24.4	6.6	7.1	173	24	7.2	.014
0.95	1.70	1.62	X								

$\bar{X}$ = 1.5 average control area

Table 17. Raw data for oxygen uptake in Experiment III.

height	net height	µl/500 µl	µl/ml	vial vol	vial +.5 ml	total vol	fly wgt	µl/mg	µl/mg/hr
14.12	X								
13.67	.23	2.1	4.2	6.6	7.1	29.8	13	2.3	4.6
13.55	.35	3.0	6.0	6.4	6.9	41.4	22	1.9	3.8
13.59	.31	2.7	5.4	7.3	7.8	42.1	20	2.1	4.2
13.71	.19	1.8	3.6	6.4	6.9	24.8	19	1.3	2.6
13.71	.19	1.8	3.6	6.4	6.9	24.8	19	1.3	2.6
13.64	.26	2.3	4.6	6.4	6.9	31.7	24	1.3	2.6
13.50	X								
13.63	.27	2.4	4.8	6.4	6.9	33.1	24	1.4	2.8
13.64	.26	2.3	4.6	6.0	6.5	26.0	17	1.5	3.0
13.63	.27	2.4	4.8	6.4	6.9	33.1	24	1.4	2.8
13.63	.27	2.4	4.8	5.4	5.9	28.3	19	1.5	3.0
14.08	X								

$\bar{X}$ = 13.9 average control height

Table 18. Raw data for carbon dioxide production in Experiment III.

height	width @.5h	Cm^2 area	net area	µl/ 500 ul	µl/ ml	vial vol	vial +.5 ml	total vol	fly wgt	µl/mg	µl/mg/hr
0.64	0.52	.33	X								
4.77	0.56	2.67	2.3	2.1	4.2	6.6	7.1	29.8	13	2.3	4.6
7.91	0.55	4.35	4.0	3.5	7.0	6.4	6.9	48.3	22	2.2	4.4
6.05	0.55	3.33	3.0	2.7	5.4	7.3	7.8	42.1	20	2.1	4.2
4.57	0.58	2.65	2.3	2.1	4.2	6.4	6.9	29.0	19	1.5	3.0
5.06	0.57	2.88	2.5	2.3	4.6	6.4	6.9	31.7	19	1.7	3.4
6.59	0.54	3.56	3.2	2.8	5.6	6.4	6.9	38.6	24	1.6	3.2
0.62	0.53	.33	X								
6.57	0.54	3.55	3.2	2.8	5.6	6.4	6.9	38.6	24	1.6	3.2
5.66	0.56	3.17	2.8	2.5	5.0	6.0	6.5	32.5	17	1.9	3.8
4.84	0.55	2.66	2.3	2.1	4.2	6.4	6.9	29.0	24	1.2	2.4
4.15	0.55	2.28	1.9	1.8	3.6	5.4	5.9	24.2	19	1.1	2.2
0.67	0.52	.35	X								

$\bar{X}$ = .3 average control area

Table 19. Raw data for water vapor output in Experiment III.

height	width @.5h	Cm^2 area	net area	mg 500 µl	mg/ ml	vial vol	vial +.5 ml	total vol	fly wgt	mg/mg	mg/mg/hr
0.87	1.61	1.40	X								
5.14	1.68	8.64	7.32	5.90	11.9	6.6	7.1	84.5	13	6.5	.013
8.09	1.66	13.41	12.1	10.20	20.4	6.4	6.9	140	22	6.4	.012
6.63	1.71	11.32	10.0	8.40	16.6	7.3	7.8	130	20	6.5	.013
6.30	1.67	10.52	9.21	7.60	15.2	6.4	6.9	106	19	5.6	.011
6.46	1.68	10.85	9.54	7.95	15.8	6.4	6.9	110	19	5.8	.011
7.91	1.65	13.05	11.7	9.90	19.8	6.4	6.9	137	24	5.7	.011
0.75	1.60	1.20	X								
7.77	1.65	12.82	11.5	9.70	19.4	6.4	6.9	134	24	5.6	.011
6.66	1.70	11.32	10.0	8.40	16.8	6.0	6.5	109	17	6.4	.012
8.06	1.69	13.62	12.3	10.4	20.8	6.4	6.9	144	24	6.0	.012
7.70	1.65	12.70	11.4	9.6	19.2	5.4	5.9	112	19	5.9	.011
0.84	1.60	1.34	X								

$\bar{X}$ = 1.3 average control area

Table 20. Mean values for oxygen uptake, carbon dioxide and water vapor production in four day old female house flies at 27°C.*

Experiment	µl O_2/mg**/hr	µl CO_2/mg/hr	mg H_2O/mg/hr	R.Q.***
I	3.1	3.2	.012	1.0
II	3.2	3.2	.011	1.0
III	3.2	3.4	.012	1.1

* Raw data in Tables 11-19
** Live wet weight
*** R.Q. = (µl CO_2)/(µl O_2)

Table 21. Molar values for oxygen uptake, carbon dioxide, and water vapor production in four day old female house flies.

Exp.	Oxygen µ moles/mg/hr	Carbon dioxide µ moles/mg/hr	Water vapor µ moles/mg/hr
I	.13	.13	.35
II	.13	.13	.32
III	.13	.13	.35
$\bar{X}$ =	.13	.13	.34

@ 27.5°C 1 micromole = 24.6 µl = 34 µgm H_2O

DISCUSSION

Chromatographic Method

Accuracy and reproducibility. In a 500 microliter sample the practical lower limit for oxygen uptake, carbon dioxide production, and water vapor output in the house fly was 1.0, 0.3, and 2.2 microliters respectively. Although the reproducibility of the calibrations varied widely over the ranges calibrated, the mean values for the oxygen and water vapor calibration samples formed linear curves (Tables 9 and 11) and oxygen measurements taken on house flies agreed with values taken by other investigators (see discussion section on house fly measurements). The carbon dioxide calibration was not linear, however all of the values obtained with the house flies fell within the linear part of the curve. Also the house fly carbon dioxide production values agreed well with the oxygen uptake measurements taken in this thesis, and produced respiratory quotient values very close to one (see discussion section on house fly measurements).

Oxygen peak distortion. A consequence of small thermal conductivity difference between oxygen and nitrogen is the distortion of the oxygen peak (Figures 3 and 5). When helium was used as the carrier gas, no peak distortion was noticed (Figures 2 and 4). Peak anomalies in nitrogen carrier gas have been studied by a number of people (Bohemen and Purnell, 1958; Schmauch and Dinerstein, 1960; Lawson and Miller, 1966). However, the theory which explains peak anomalies

(in general) is not yet fully developed and fails in many respects; even the experimental data of different investigators vary due to the wide variety of types of thermal conductivity cells (Lawson and Miller, 1966). Distortion of the oxygen peak with nitrogen carrier gas has been noted by Eaton et al. (1968) in analyzing oxygen and carbon dioxide in spacecraft. They reported an -M- shaped oxygen peak which disappeared when a five percent helium, ninety-five percent nitrogen mixture was used. Peak anomalies were not observed by Mochizuki and Kawakami (1965) when they analyzed oxygen in a nitrogen carrier gas, probably because they used a glow discharge detector rather than a thermal conductivity detector.

Bohemen and Purnell (1958) suggested three possible reasons for peak anomalies when a carrier gas of low thermal conductivity, such as nitrogen, was used. The first possible reason for peak anomaly, corrosion of the detector filament, may come about because of sample decomposition and interaction with the surface of the filament. When thermal conductivity contrast is high, such as with helium carrier gas and an oxygen sample, the corrosion of the filament surface would be unimportant. However, when thermal conductivity contrast is low, such as with nitrogen and oxygen, corrosion might significantly interfere with the detector response and lead to peak distortion. In the present thesis, the possibility of corrosion was minimal. This

was assumed because switching the reference side of the detector with the sampling side after three months showed no change in the oxygen peak shape.

The second possible reason for peak anomaly is maxima or minima in the thermal conductivity isotherm of oxygen in nitrogen carrier gas. Ordinarily, the relationship between detector response and sample concentration would be positive and linear. However, when the thermal-conductivity coefficients of carrier gas and sample are close, then the relationship can become non-linear and hence exhibit maxima or minima on a graph (Lawson and Miller, 1966). This could cause distortion in a peak in the following way: the concentration of the sample reaching the detector is low at the beginning and increases as the center of the sample is reached. Yet, as the concentration around the filament increases, the detector response may fall. Then, as the less concentrated tail of the sample reaches the detector, the detector response may rise and then fall again. However, maxima or minima in the thermal conductivity isotherm of oxygen in nitrogen carrier gas did not occur in this thesis, as can be seen in Figure 9. The relationship between detector response (peak height) and increasing oxygen concentration was linear.

A third possible reason for peak anomaly is based on the fact that heat is lost from the detector filament "by mechanisms other than

thermal conductivity. Probably the most important competing mechanism is heat capacity (Bohemen and Purnell, 1958)." Heat capacity or specific heat[1] in the present context is the amount of heat needed to change the temperature of 1 gm of gas by 1°C, whereas, thermal conductivity is the rate at which heat is transferred in a gradient. It may be possible that when thermal conductivity "contrast" is low and carrier gas flow rate high, heat capacity becomes an important part of the detector response (Miller and Lawson, 1966). As can be seen from Table 22, the thermal-conductivity coefficient of oxygen is higher than that of nitrogen, while that of carbon dioxide is lower than that of nitrogen. This means that oxygen conducts heat away from the filament faster than the nitrogen carrier gas while carbon dioxide is slower than the carrier gas. Therefore, oxygen and carbon dioxide thermal-conductivity peaks should appear on opposite sides of the baseline on the recorder. However, as can be seen from Table 23, the specific heat of both oxygen and carbon dioxide is higher than that of nitrogen. This means that oxygen and carbon dioxide take up more heat before they change temperature than nitrogen does. Thus, one would expect peaks based on heat capacity differences for oxygen and carbon dioxide to be on the same side of the baseline. A sample which has a specific heat higher than the carrier gas has the same effect on

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[1]Heat capacity is measured as specific heat.

Table 22. Thermal conductivity values for gases important in respiratory physiology.*

Gas	Thermal Conductivity Values $cal/cm^3/sec/^\circ C \times 10^{-5}$
Helium	39.85
Air	7.20
Oxygen	7.43
Nitrogen	7.18
Argon	5.09
Carbon dioxide	5.06
Water vapor	5.51

* Values from Lange and Forker (1956).

Table 23. Specific heats of gases important in respiratory physiology.*

Gas	$gm\text{-}cal/gm^\circ C$**
Oxygen	.350
Carbon dioxide	.124
Water vapor	.036
Nitrogen	.028

* Specific heat is defined as the quantity of heat needed to raise 1 gram of a substance $1^\circ C$.

** Values from Lange and Forker (1956).

the peak direction as a sample with a coefficient of thermal conductivity lower than the carrier gas. Thus, one would expect heat capacity

peaks for oxygen and carbon dioxide in nitrogen to be in the same direction as the thermal conductivity peak for carbon dioxide. This means that, for oxygen, a thermal conductivity peak would be on the opposite side of this baseline from a specific heat peak.

On the basis of this information, it is suggested that the peak distortion observed for oxygen in nitrogen in this thesis could be a result of competing thermal conductivity and specific heat responses from the detector. In other words, if the carbon dioxide thermal conductivity peak is above the baseline, then the part of the oxygen peak above the baseline is mainly a detector response to specific heat and the part of the oxygen peak below the baseline is mainly a thermal conductivity response.

It is also suggested that the specific shape of the oxygen peak in nitrogen is related to the geometry of the detector cell (Lawson and Miller, 1966). This could account for the difference in oxygen peak shape between the Hewlett-Packard chromatogram (Figure 5) and the Varian chromatogram (Figure 6). For this reason, it is suggested that a research quality gas chromatograph be used with this method to minimize oxygen peak distortion. It is important to note that, although distorted peaks are difficult to measure, the distortion of the oxygen peaks in this thesis apparently did not affect the accuracy of the method, because the relationship between oxygen concentration

and peak height was linear and the mean value for oxygen uptake of the house fly taken with the method presented in this thesis agreed within one µl/mg/hr with measurements taken by Whitney and Ortman (1962).

House Fly Measurements

Comparisons with measurements taken by other investigators. It can be seen, in Tables 2 and 3, that the mean values for oxygen uptake by Edwards (1946), Ouye et al. (1961), Whitney and Ortman (1962), Skelton and Hunter (1970), and this thesis (Table 20) agreed within one µl/mg/hr. The mean value of carbon dioxide measurements taken in this thesis agreed within 0.5 µl/mg/hr with the mean value of the data taken by Whitney and Ortman (1962), (Table 2). Comparison of water vapor output measurements is somewhat less reliable. This is because there were no other measurements available for house flies. However, measurements taken by Bursell (1959 and 1960), showed the mean water loss for another Muscid, the tsetse fly, to be .05 mg of water/mg of fly/hr. This value was approximately five times higher than the mean values measured in this thesis (.012 mg of water/mg of fly/hr).

The fivefold difference in mean water output could arise from three major sources. Firstly, there are taxonomic differences in

the two species. Musca domestica (house fly) and Glossina morsitans (tsetse fly) are in the same family but in different genera. This is equivalent to comparing chimpanzees and gorillas—differences should be expected. Secondly, the flies have adapted to different habitats. House flies have adapted to human environments which tend to be drier than the environment of tsetse flies. The house flies used in this thesis were bred in laboratory colonies, whereas the tsetse flies used by Bursell were captured in the field. Both of these factors, humidity of native habitat and familiarity with confinement, would be expected to influence water output in insects. Thirdly, there was a difference in the techniques used to take the measurements. Bursell used a gravimetric method to detect water loss; a chromatographic method was used for the measurements in this thesis. The gravimetric method of weighing the fly before and after the experimental test period measured all liquid water lost from the mouth and anus, as well as water vapor lost by transpiration. While the chromatographic method measured only vaporized losses from these same sources.

Comparison among oxygen uptake, carbon dioxide production and water vapor output measurements in this thesis. Consider the respiring fly as a system for oxidizing carbohydrates. When a carbohydrate is burned ($6\ O_2 + C_6H_{12}O_6 \longrightarrow 6\ H_2O + 6\ CO_2$), one mole of oxygen is consumed for every mole of carbon dioxide produced. Thus, if the

volume or molar value of carbon dioxide produced by the fly is divided by the volume or molar value of oxygen taken up, the quotient (called respiratory quotient) should be 1.0 when a carbohydrate is burned (Chadwick, 1953).[2] In experiments I, II and III in this thesis, the mean respiratory quotients (R.Q.) were 1.0, 1.0 and 1.1, respectively (Table 20). Respiratory quotient measurements, however, are not usually exact because of variance in individual physiology and variation in the population.

Unfortunately, water vapor output measurements cannot be compared with oxygen uptake in the way that carbon dioxide can because much more water vapor is lost than is made from the breakdown of a carbohydrate. From the data obtained in experiments I, II and III, it appears that the flies lose about three times as much water (.012 mg/mg/hr) as they make (.004 mg/mg/hr). One would expect water loss to exceed metabolic water production since caged adult flies will die within three days at 30°C without a source of liquid water. Comparison on a molar basis of oxygen uptake, carbon dioxide production and water vapor output is shown in Table 21.

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[2] 0.8 for a protein; 0.7 for a fat.

SUMMARY

An isothermal gas chromatographic method has been developed for use in measuring insect oxygen uptake, carbon dioxide production and water vapor output and is presented herein. The method used two columns of Porapak Q, nitrogen carrier gas, and a thermal conductivity detector. Oxygen uptake, carbon dioxide production, and water vapor measurements were taken on four-day old female house flies (mean values were 3.2 µl O_2/mg/hr; 3.3 µl CO_2/mg/hr; and .012 mg H_2O/mg/hr). These measurements were compared with oxygen and carbon dioxide measurements on house flies taken with another chromatographic method by Whitney and Ortman (1962), (mean values 3.50 µl O_2/mg/hr; 3.0 µl CO_2/mg/hr). The water vapor output measurements were compared with water loss measurements taken on tsetse flies with a gravimetric method by Bursell (1959 and 1960), (.05 mg H_2O/mg/hr). Finally, the measurements from Experiments I, II and III were compared among themselves. A respiratory quotient of approximately one was computed. This meant that carbohydrates were being degraded for metabolic energy. It was also calculated that the house flies being studied lost approximately three times as much water through evaporation as they produced metabolically.

CONCLUSIONS

1. Single sample, isothermal, gas chromatographic analysis of oxygen, carbon dioxide and water vapor can be accomplished by using two columns of Porapak Q, nitrogen as the carrier gas, and a thermal conductivity detector.

2. This method can be adapted to study insect oxygen uptake, carbon dioxide production, and water vapor output.

3. Oxygen uptake, carbon dioxide production and water vapor output were measured for four-day old female house flies. Average oxygen uptake was 3.2 μl/mg/hr. Average carbon dioxide production was 3.3 μl/mg/hr. Average water vapor output was .012 mg/mg/hr.

4. If it is assumed that the flies were burning carbohydrates for energy (R.Q. = 1 for experiments I, II, III), and that overall degradation of the carbohydrates conformed to the equation

$$6\ O_2 + C_6H_{12}O_6 \rightarrow 6\ CO_2 + 6\ H_2O,$$

then the flies lost more than three times as much water as they made metabolically.

REFERENCES

Airco, Inc. 1968. Rare and speciality gases catalog. New York, New York. 47 p.

Bausch and Lomb, Inc. 1970. Shimadzu infra-red spectrophotometer. Rochester, New York. 14 p.

Birt, L.M. 1961. Flight-muscle mitochondria of Lucilia cuprina and Musca domestica Estimation of the pyridine nucleotide content and of the response of respiration to adenosine diphosphate. Biochem. J. 80: 623-31.

Bohemen, J., and J.H. Purnell. 1958. The behaviour of katharometers for gas chromatography in carrier gases of low thermal conductivity. J. Appl. Chem. 8: 433-40.

Burford, J.R. 1969. Single sample analysis of N_2-N_2O-CO_2-A-O_2 mixtures by gas chromatography. J. Chromat. Sci. 7: 760-2.

Bursell, E. 1959. The water balance of tsetse flies. Roy. Entomol. Soc. Lond. Trans. 111: 205-35.

_____. 1960. Loss of water by excretion and defecation in the tsetse fly. J. Exp. Biol. 37: 689-97.

Carle, G.C. 1970. Gas chromatographic determination of hydrogen, nitrogen, oxygen, methane, krypton, and carbon dioxide at room temperature. J. Chromat. Sci. 8: 550-1.

_____. 1972. Personal communication.

Carlson, S.D. 1966. Respiration measurement of Tribolium confusum by gas chromatography. J. Econ. Entomol. 59: 335-8.

Carney, G.C. 1966. The effects of different isolation media on the respiration and morphology of house fly sarcosomes. J. Insect Physiol. 12: 1093-103.

Chadwick, C.E. 1953. Aerodynamics and flight metabolism. In Roeder, K.D., editor, Insect Physiology. J. Wiley and Sons, Inc., New York. 615-36.

Chance, B., and B. Sacktor. 1958. Respiratory metabolism of insect flight muscle. II. Kinetic respiratory enzymes in flight muscle sarcosomes. Arch. Biochem and Biophys. 76: 509-31.

Dave, S.B. 1969. A comparison of the chromatographic properties of porous polymers. J. Chromat. Sci. 7: 389-99.

Dean, J.A. 1969. Chemical Separation Methods. Van Nostrand Reinhold Co., New York. 398 p.

DiLorenzo, A. 1970. Trace analysis of oxygen, carbon monoxide, methane, carbon dioxide, ethylene, ethane in nitrogen mixtures using a column selector. J. Chromat. Sci. 8: 224-6.

Dressler, D.P., G.J. Mastio, and F.F. Allbritten. 1960. The clinical application of gas chromatography to the analysis of respiratory gases. J. Lab. Clin. Med. 55: 144-8.

Dubuc, A. 1972. Personal communication.

Dumas, T., C.T. Buckland, and H.A.U. Monro. 1969. The respiration of insects at reduced pressures II. The uptake of oxygen by Tenebroides mauritanicus. Entomol. Exp. and Appl. 12: 389-402.

Eaton, H.G., V.R. Huebner, and L. Mittleman, Jr. 1968. A metabolic rate analyser for the Apollo spacecraft. J. Gas Chromat. 6: 441-5.

Edwards, G.A. 1946. The influence of temperature upon the oxygen consumption of several arthropods. J. Cell. Comp. Physiol. 27: 53-64.

Edwards, L.J. 1970. A thermal conductivity device for detecting the discontinuous release of carbon dioxide by insects. Ann. Entomol. Soc. 63: 627-9.

Forsey, R.R. 1968. A dual column gas chromatographic method for the analysis of light gases. J. Gas Chromat. 6: 555-6.

Goedert, M., and G. Guiochon. 1969. A study of the sources of error in quantitative gas chromatography: reproducibility of the response of a catharometer. J. Chromat. Sci. 7: 323-39.

Goldbaum, L.R., T.J. Domanski, and E.L. Schloegel. 1968. The use of a helium ionization detector for the determination of atmospheric gases. J. Gas Chromat. 6: 394-6.

Gregg, C.T., C.R. Heisler, and L.F. Remmert. 1960. Oxidative phosphorylation and respiratory control in housefly mitochondria. Biochim. et Biophys. ACTA 45: 561-70.

Hamilton, A.G. 1959. The infra-red gas analyser as a means of measuring the carbon dioxide output of individual insects. Nature 184: 367-9.

Hamilton, L.H. 1962. Gas chromatography for respiratory and blood gas analysis. Ann. New York Acad. Sci. 102: 15-28.

Hamilton, L.H. 1964. Two-loop sampling valve for use in gas chromatography. J. Gas Chromat. 2: 302-3.

Hamilton, L.H., and R.C. Kory. 1960. Application of gas chromatography to respiratory gas analysis. J. Appl. Physiol. 15: 829-37.

Hamilton, L.H., J.R. Smith, and R.C. Kory. 1964. The use of gas chromatography in the cardiopulmonary laboratory. Amer. J. Med. Electronics 3: 3-15.

Hayes, D.K., M.S. Schechter, E. Mensing, and J. Horton. 1968. Oxygen uptake of single insects determined with a polarographic oxygen electrode. Analytical Biochem. 26: 51-60.

Hollis, O.L. 1966. Separation of gaseous mixtures using porous polyaromatic polymer beads. Analytical Chem. 38: 309-16.

Ilevicky, J., W. Chefurka, and J.E. Casida. 1967. Oxidative phosphorylation and sensitivity to uncouplers of house fly mitochondria: influence of isolation medium. J. Econ. Entomol. 60: 1404-7.

James, A.T., and A.J.P. Martin. 1952. Gas-liquid partition chromatography. A technique for the analysis of volatile materials. Analyst. 77: 915-32.

Jay, B.E., and R.H. Wilson. 1960. Adaptation of the gas adsorption chromatographic technique for use in respiratory physiology. J.

Appl. Physiol. 15: 298-302.

Lange, N.A., and G.M. Forker, ed. 1956. Handbook of Chemistry. Handbook Publishers, Inc., Ohio. 1969 p.

Lawson, A.E., Jr., and J.M. Miller. 1966. Thermal conductivity detectors in gas chromatography. J. Gas Chromat. 4: 273-84.

Lem, J.D., Jr. 1967. Application of gas chromatography in manned space flight. Aersp. Med. 38: 1110-17.

Luh, B.S., and M.S. Chaudhry. 1961. Gas chromatography of CO_2, H_2, O_2, and N_2 in processed foods. Food Technol. 15: 52-4.

Marchio, J.L. 1971. A dual-channel gc technique for mixtures of permanent gases and C_1-C_2 hydrocarbons. J. Chromat. Sci. 9: 432-5.

Martin, A.J.P., and R.L.M. Synge. 1941. A new form of chromatogram employing two liquid phases 1. A theory of chromatography 2. Application to the micro-determination of the higher monoamino-acids in proteins. Biochem. J. 35: 1358-68.

Mochizuki, M., and Y. Kawakami. 1965. A new detector for a gas chromatograph using electron current following glow discharge. Jap. J. Physiol. 15: 553-6.

Monro, H.A.U. 1971. Personal communication.

Obermiller, E.L., and G.O. Charlier. 1968. Gas chromatographic separation of nitrogen, oxygen, argon, carbon monoxide, carbon dioxide, hydrogen sulfide, and sulfur dioxide. J. Gas Chromat. 6: 446-7.

Ouye, M.T., L. Rai, and C.C. Roan. 1961. Effects of malathion and piperonyl butoxide combinations on the rate of oxygen consumption of house flies. J. Kan. Entomol. Soc. 34: 64-73.

Punt, A. 1950. The respiration of insects. Physiol. Compar. 2: 59-74.

Purnell, H. 1962. Gas Chromatography. J. Wiley and Sons, Inc., New York. 441 p.

Rabbani, G.S.M., M. Rusek, and J. Janák. 1968. Gas chromatographic investigation of some ethylvinylbenzene polymers using different carrier gases. J. Gas Chromat. 6: 399-400.

Ramsey, L.H. 1959. Analysis of gas in biological fluids by gas chromatography. Science 129: 900-1.

Sacktor, B., and D.G. Cochran. 1958. The respiratory metabolism of insect flight muscle. I. Manometric studies of oxidation and concomitant phosphorylation with sarcosomes. Arch. Biochem. and Biophys. 74: 266-76.

Schmauch, L.J., and R.A. Dinerstein. 1960. Response of thermal conductivity cells in gas chromatography. Analytical Chem. 32: 343-52.

Skelton, T.E., and P.E. Hunter. 1970. Flight and respiration responses in house flies following topical application of sterilization levels of tepa. Ann. Entomol. Soc. Amer. 63: 770-3.

Supelco, Inc. 1971. Carbosieve-B product information. Bellefonte, Penn. 7 p.

Tranchant, J., ed. 1969. Practical Manual of Gas Chromatography. Elsevier Publishing Co., New York. 387 p.

Umbreit, W.W., R.H. Burris, and J.F. Stauffer. 1964. Manometric Techniques. 4th ed. Burgess Publishing Co., Minn. 305 p.

Varian Aerograph, Inc. 1970. Instruments and accessories. Walnut Creek, California. 44 p.

Waters Associates, Inc. 1971. Porapak gas chromatography column packing material. Framingham, Mass. 6 p.

Weast, R.C., ed. 1970. Handbook of Chemistry and Physics. 51st ed. Chemical Rubber Co., Ohio. 2364 p.

Whitney, W.K., and E.E. Ortman. 1962. Gas chromatography for measuring insect respiration. J. Kan. Entomol. Soc. 35: 323-32.

Wilhite, W.F., and O.L. Hollis. 1968. The use of porous-polymer

beads for analysis of the Martian atmosphere. J. Gas Chromat. 6: 84-8.

Williams, C.M. 1946. Continuous anesthesia for insects. Science 103: 57.

Williams, H.P., and J.D. Winefordner. 1968. Several high sensitivity radio-frequency detectors for permanent gas analysis. J. Gas Chromat. 6: 11-17.

Wood, G.W. 1971. Personal communication.

~~Wood,~~ G.W., F.A. Wood, and R.A. Dickinson. 1970. Carbon dioxide output of individual small insects measured with a gas chromatograph. Can. J. Zool. 48: 902-3.

Zlatkis, A., H.R. Kaufman, and D.E. Durbin. 1970. Carbon molecular sieve columns for trace analysis in gas chromatography. J. Chromat. Sci. 8: 416-7.