† MEASUREMENT OF METABOLIC GAS EXCHANGE WITH A RECORDING MASS SPECTROMETER †

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(WITH FIVE FIGURES)

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Introduction

This paper describes a new method for measuring the gaseous metabolism of cells, tissues, and whole organisms. The method incorporates certain features which offer unique advantages for physiological studies. In principle, measurements consist of successive analyses, at frequent intervals, of the partial pressures of several constituents of the gas phase over the biological material under observation. A mass spectrometer is employed as the measuring instrument.

A mass spectrometer is essentially a special kind of vacuum tube through which the gas or vapor being analyzed is flushed continuously at a low pressure. A hot tungsten filament generates an electron beam at one end of the tube (the source), and positive ions of the gas are created there by electron bombardment. These ions are accelerated in an electric field and focused into a beam which projects down the tube. The ion beam is resolved into its mass components by the analyzer, a magnetic field, located part way down the tube. As a charged particle enters the magnetic field, it is deflected by an amount which depends upon the strength of the field and on the velocity mass and charge of the particle. Lighter particles enter the field with greater velocity and hence suffer greater deflection. Thus an ion beam, originating from a mixture of gases having different molecular weights, is resolved into a series of discrete beams according to the respective masses of the constituent ions. Proper adjustment of the ion accelerating voltage and of the field strength of the magnetic analyzer will cause any one of the separate ion beams to come to focus on the collector at the far end of the mass spectrometer tube. The ion current, measured with a vacuum tube amplifier, is proportional to the rate at which ions of the particular mass are formed at the source—proportional, therefore, to the partial pressure of the corresponding component of the gas mixture being flushed through the tube. For general information on the principles of operation of a mass spectrometer and on further technical details, the reader may refer to any of several reviews (**3, 6, 8, 9, 12, 13**).

In our application of the mass spectrometer for analysis of metabolic gases, we operate the instrument at a fixed magnetic analyzer field strength

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and tune in on the desired ion beams by regulating the accelerating voltage. Since we want to effect essentially continuous monitoring of the gas mixture, the accelerating voltage is automatically varied in cyclic fashion tuning to each of several masses in succession. The amplifier output is continuously recorded. Ordinarily no adjustments are necessary once the instrument is set up for the particular analytical sequence desired; the data for the entire experiment are thus collected and recorded completely automatically.

This method has considerable versatility and an exceptionally high degree of analytical specificity because the gaseous constituents are distinguished on the basis of molecular weight which avoids ambiguity in all but a few instances. Since a mass spectrometer distinguishes not only between chemically different gases but also between different isotopes of the same gas, the method is especially applicable to various kinds of tracer experiments.

The use of a mass spectrometer for continuous analysis of a process gas was first described by NIER *et al.* (**10**). Recently ROBINSON *et al.* (**11**) described a similar commercial instrument designed for monitoring continuous industrial processes. KYDD and HITCHCOCK (**4**) apparently were the first to apply these principles to continuous gas analyses in metabolic studies with an instrument described by HUNTER *et al.* (**2**). MILLER *et al.* (**5**) described a portable mass spectrometer designed for respiratory studies on mammals and for use in operating rooms as an aid to the anesthetist.

Apparatus and methods

The apparatus consists principally of a mass spectrometer similar to that described by NIER (**7**). The first instrument used for the kind of studies described herein was constructed in the Physics Department and was modified to facilitate our particular experiments. Provision was made for connecting the gas inlet of the mass spectrometer to one of several types of experimental chambers containing the living material under investigation. Gas from the experimental chamber can diffuse into the mass spectrometer tube at a very slow rate (a few ml. per day) depending upon the size of leak employed. It is this gas which is flushed through the tube at the desired pressure (ca. 10^{-6} mm. Hg). With such a low rate of diffusion through the leak, the gas in the experimental chamber is not seriously depleted during the time of an experiment.

We have used one basic leak design which is of the viscous flow type. The performance of viscous flow leaks was studied by HALSTED and NIER (**1**). The leak was made from copper tubing drawn down to a capillary with an internal diameter of 0.007 inch and pinched by screwing a small steel bar against a backing plate so as to clamp down on the copper capillary soldered to the plate. The leak was cautiously pinched to the desired size which was determined empirically.

The accelerating voltage of the mass spectrometer was controlled by a voltage divider. As part of the divider, a motor-driven potentiometer was arranged to scan a voltage range automatically and to cycle repeatedly over

the preset range thus scanning through the tuned settings for several predetermined masses. A series of intermittent analyses of each of a number of gases was obtained. The voltage output of the mass spectrometer is fed into a recording potentiometer whereupon the data are recorded on a strip chart for subsequent study.

The only changes necessary to adapt a mass spectrometer, designed for routine isotopic analyses of gas samples, to our type of measurements are: (a) provision of the additional leak communicating with the experimental chamber, (b) provision of the voltage divider and automatic tuning, and (c) provision for recording the amplifier output with automatic switching of amplifier sensitivity so that each analysis is recorded at an appropriate sensitivity.

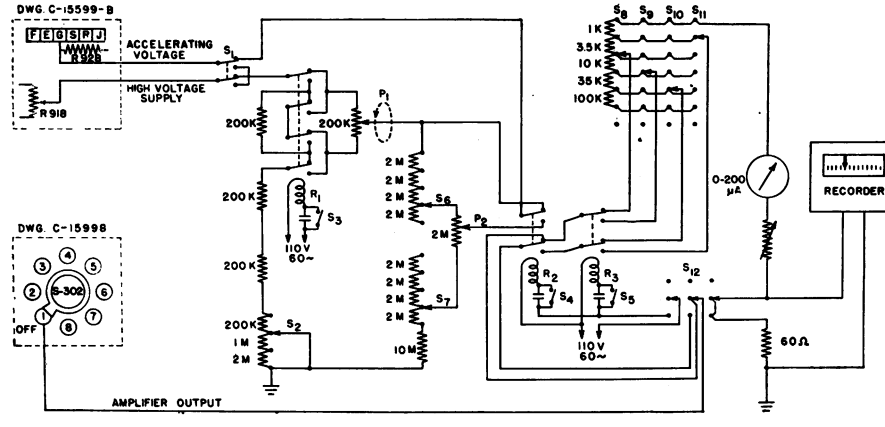

FIG. 1. Accessory circuits for controlling mass spectrometer accelerating voltage and recorder sensitivity. Schematic diagram shows connections to the spectrometer high voltage supply, accelerating voltage control, and amplifier output. Also reproduced within dashed lines are portions of the circuit drawings of the Consolidated Engineering Corp. Mass Spectrometer, Model 21–201 indicating where connections were made in this instrument. See text for description of circuit operation.

These relatively simple changes were incorporated into two different instruments. They do not prevent the use of the spectrometer for the usual isotopic analysis of gas samples according to conventional practice. Pertinent circuit modifications are shown in figure 1. The added circuit components are entirely external to the mass spectrometer chassis with only three leads connecting to the instrument as indicated in the figure. One of the mass spectrometers we have so adapted was a commercial instrument (Consolidated Engineering Corporation Isotope Ratio Mass Spectrometer, Model 21–201). This model instrument is available at a number of institutions where biological problems are under investigation. Figure 1 also indicates the specific points in the circuit drawings of the commercial spectrometer at which connections were made to the accessory circuits. The operation of these circuits may be considered briefly.

Accessory electrical circuits

Referring to figure 1, switch, S_1, can disconnect the voltage divider thus restoring the accelerating voltage control of the mass spectrometer to the usual manual adjustment on the instrument panel. Similarly, setting the panel voltmeter sensitivity control at other than the off position eliminates the added recording circuit. The instrument circuits thus are easily restored to their usual operating condition for isotopic analyses of gas samples introduced into the mass spectrometer manifold. In the opposite position, switch, S_1, introduces a bank of resistors (800K to 4M, depending on the setting of switch S_2) between the spectrometer high voltage and ground. The accelerating voltage of the instrument is picked off from the center tap of the 200K General Radio potentiometer, P_1, which constitutes the top member of the series of resistors. This potentiometer (with end stop removed) is connected through a gear box (with easily changeable gears) to a 2 r.p.m. type K Bodine motor. As the potentiometer is turned by the motor through one revolution, the mass spectrometer accelerating voltage sweeps through a definite fraction of its maximal range. With the sizes of resistors shown, this is 5%, 10%, 20%, or 25% of the range according to the setting of S_2. By changing gears the potentiometer can be turned at several speeds from one fifth to 1 r.p.m. which is about as fast as it is practical to repeat analyses in our experiments.

Switch S_3 controls relay, R_1, which can interchange the scanning potentiometer with the next resistor element thus providing voltage scanning at a lower maximal voltage but over the same voltage range as before. Microswitches S_4 and S_5 are operated by cams attached to the extended shaft of the scanning potentiometer. At a given position in the scanning cycle S_4 operates the relay R_2. As R_2 closes, the mass spectrometer accelerating voltage jumps to a new value determined by the settings of switches S_6 and S_7 and of potentiometer P_2 across their center taps. The other half of the relay R_2 and both sides of relay R_3 are so connected that the amplifier output from the mass spectrometer is read on meter, M, through one of four possible resistances according to the positions of the relays and the setting of switches S_8, S_9, S_{10} and S_{11} connected to R_3. This meter circuit is essentially a replica of the one contained within the mass spectrometer proper, and the sensitivity values obtainable with it are the same as those provided by switch S–302 in the case of the Consolidated instrument.

The voltage output is recorded by a Model 153X17V–X–30 Brown Recording Potentiometer connected across a 60 ohm resistor in the meter circuit. Included in the meter circuit is a variable resistor which can be adjusted so that a full scale reading on the meter corresponds to the full scale of the recorder. With switches S_8, S_9, S_{10} and S_{11} properly set and the cams which activate microswitches S_4 and S_5 adjusted appropriately on the shaft of the motor driven potentiometer P_1, the sensitivity scale on which the different mass peaks are recorded changes during the scanning cycle so that both large and small peaks can be recorded on the strip chart at sensitivities which are respectively appropriate.

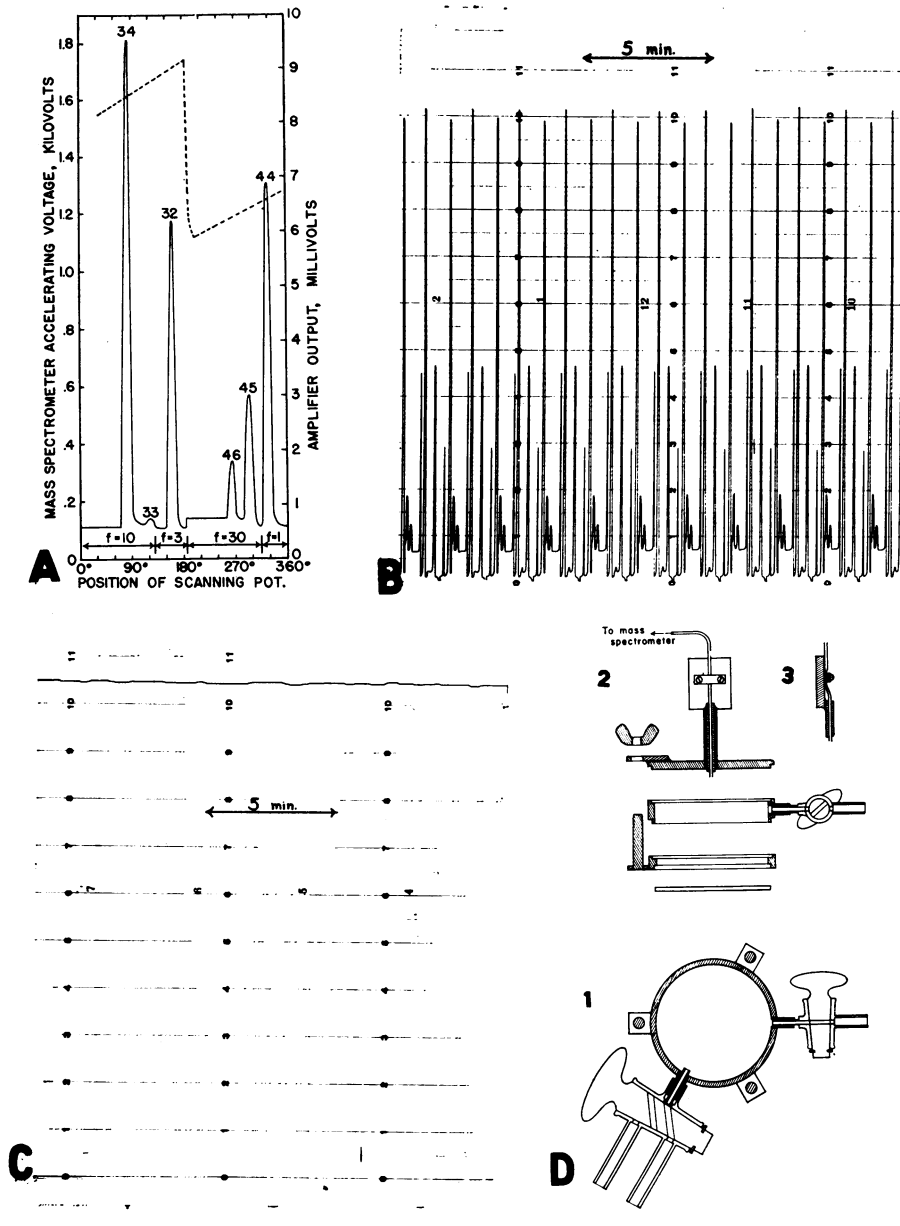

FIG. 2. A. Relation between accelerating voltage (broken line), amplifier output (solid line), and scanning potentiometer position (abscissa) for one complete scanning cycle. Each amplifier output peak is numbered according to the mass represented. Amplification factors (f) show how other peaks are magnified relative to that of mass 44. B. Photograph of sample strip chart data. Same peaks represented as in A. Not to be confused with mass peaks are sharp spikes such as the one, immediately to the left of each mass 32 (highest) peak, which was caused by automatic switching of amplifier sensitivity and other smaller spike, immediately to left of each mass 44 (next to highest)

Operation of mass spectrometer

The dashed line in figure 2 A shows how the mass spectrometer accelerating voltage can be made to vary over two nonadjacent ranges during a scanning cycle. On the same abscissa is reproduced a tracing of part of the strip chart record obtained when the gas being analyzed consisted of a mixture of nitrogen, carbon dioxide, and oxygen which had been enriched in mass 34 ($O^{16}O^{18}$). The apparatus was so adjusted that nitrogen peaks were not recorded. Three isotopic peaks for carbon dioxide and three for oxygen appear in the record. The tops of the peaks are labeled with their respective masses. At the bottom of the graph the amplifier sensitivity factors are indicated. Shifts in sensitivity occur automatically between the 33 and 32 peaks, between the 45 and 44 peaks, and also at the point where the accelerating voltage changes abruptly to a different part of its range. The factors (f) indicate the relative amplification of the spectrometer output voltage for the various mass peaks.

As the cycle repeats, a series of such peak patterns appears on the strip chart constituting a continual succession of analyses as shown in figure 2 B. This figure is a photograph of a section of strip chart upon which is recorded the continual analysis of a gas mixture very similar to the one whose analysis is shown in figure 2 A. Some indication of the reproducibility of our data can be obtained by comparing respective analyses in different cycles over the 20 minutes represented by these data. For certain applications it is not necessary to change amplifier sensitivity or to interrupt the scanning range. To eliminate the operation of relays R_2 and R_3, ganged switches, S_{12}, can be set in the middle position.

In addition to the electrical circuit modifications depicted by figure 1, it was necessary to devise a new mass spectrometer leak since the one incorporated in the commercial instrument is not suitable for our purposes. Otherwise the commercial mass spectrometer was operated in normal fashion and proved to be a dependable instrument for measuring metabolic gas exchange.

The manner in which the data were taken, of course, depended upon the purpose and conditions of the particular experiment. In some instances it was advantageous to record continuously the partial pressure of one particular gas in the experimental chamber in order to observe short term fluctuations. For this purpose the instrument was tuned to the desired mass peak

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peak, which represents the back trace at the end of each scanning cycle. No gas was being evolved or consumed. Cycling period, 1.8 min. C. Photograph of the sample strip chart record of mass 32. Instrument is tuned to oxygen peak and no change in oxygen partial pressure occurs within the apparatus. The fluctuations of 1% indicate the degree of instrument instability. D. Experimental chamber constructed of brass in three sections with circular glass window on bottom. 1. Top view showing insertion of stopcocks into middle chamber section. 2. Exploded view showing all three sections above glass bottom-plate. Mass spectrometer leak assembly attached to top section. Copper capillary leak tubing extends into chamber. 3. Side view of leak assembly only showing copper tubing pinched by clamp to form leak.

and a continuous record was obtained. Figure 2 C is a photograph of such a record. The mass spectrometer was tuned to mass 32 and the gas being analyzed was undergoing no change in oxygen partial pressure. Extreme fluctuations in such records typically are about 1% or less. In most instances, however, our investigations have required analyses of two or more gases or of more than one isotopic species of the same gas. Then, automatic scanning was employed as indicated above to provide a mass peak pattern that was repeated at intervals whose length depended upon the cycling rate.

Experimental vessels

The requirements of the high pressure side of the apparatus, *i.e.*, the experimental chamber, vary with the experiment so that a variety of designs may be needed. Three different designs are illustrated and suffice as examples of what has been found in practice to be useful. Figure 2 D shows a glass-bottom chamber of 38 ml. gas space with inlet and outlet tubes attached. The chamber consists of three sections which fit together on rubber gaskets and are clamped by three bolts and wing nuts. External to the experimental chamber but soldered to the top section is a loop of brass tubing (not shown in the figure) which connects to inlet and outlet pieces of one quarter inch copper tubing. Water may be circulated through this to provide for temperature control since the chamber when in use was not placed in a bath but was surrounded by air.

Into the center of the top section of the chamber a metal capillary tube is inserted through a one quarter inch brass stud. The leak into the mass spectrometer is located at a constriction in this capillary as near as possible to the chamber. The top section thus is attached to the mass spectrometer by this capillary tube which leads directly to the spectrometer gas inlet. The top section is fixed in position by a clamp attached to the frame of the mass spectrometer which holds the stud vertical and supports the whole chamber when closed and in use. A Pyrex plate, three sixteenths of an inch thick, cemented to the bottom section of the chamber provides a circular window through which the contents may be illuminated. For such purposes we have used a horizontal optical bench with light source, lenses, and filters as desired and the light was projected upward into the chamber with a right-angle prism. The middle section of the chamber is provided with two openings to which short pieces of one quarter inch brass tubing are soldered. Capillary bore glass tubing is sealed with wax inside of these brass tubes and glass stopcocks provide for closure of these openings through which the chamber can be evacuated or flushed with gas mixtures as desired. The chamber, of course, must be vacuum tight. In use it is assembled with the biological material (*e.g.*, a plant leaf) resting on the glass bottom-plate; the appropriate gas mixture is introduced; the chamber is closed and observations are begun.

The chamber was suitable for experiments with leaves, leaf sections, or other plant parts. However it could not be used for studying algal or

chloroplast suspensions. When working with suspensions it is of course imperative that the liquid be agitated so that soluble constituents of the gas phase shall remain practically in equilibrium with the liquid. To meet this requirement one must either shake the experimental chamber or bubble gas through the suspension. Both methods have been used.

Figure 3 shows apparatus designed to circulate gas in a closed system in which the experimental vessel is one compartment. This vessel is a flat Pyrex glass cell 35 mm. in diameter and 10 mm. thick. The whole unit was constructed on an apparatus rack and the cell protrudes from the plane of

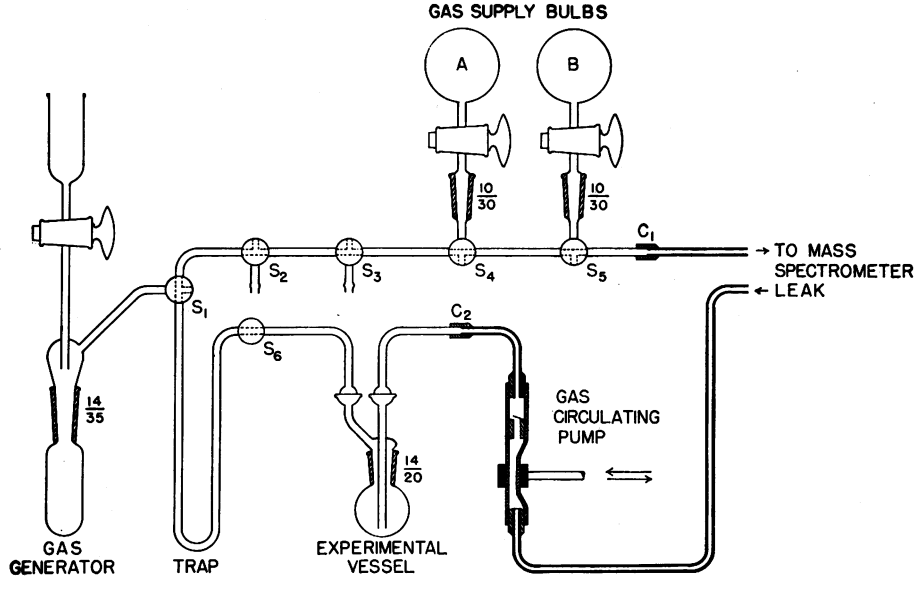

FIG. 3. Gas mixing and circulating system. Stopcocks S_2 and S_3 control flushing of system and addition of background gas. Stopcocks S_4 and S_5 control supplies of special gases from bulbs A and B. Stopcocks S_1 and S_6 control addition of gas from generator. The trap, when immersed in a liquid nitrogen bath, facilitates addition of CO_2 from generator as described in text. Connections C_1 and C_2 are turned brass sleeves waxed to inserted glass tubing and soldered to copper tubes leading to mass spectrometer leak and to gas circulating pump. Experimental vessel is a flat cylinder 1 cm. thick and with 10 ml. useful volume. Contents of vessel are stirred by a bubbling gas stream.

the apparatus so that it may be surrounded by a small water bath whenever required. It is so oriented that the cell contents are easily illuminated horizontally.

PREPARING GAS MIXTURES

Gas may be introduced into the system shown in figure 3 in three ways. Nitrogen or helium, for example, may be flushed through the system in quantity; stopcocks S_2 and S_3 serve as inlet and outlet ports. More precious gases (isotopically enriched), contained in supply bulbs A or B in figure 3, can be introduced by diffusion into a previous evacuated section of the system, for example, between S_3 and S_5. For introducing gases produced from

solid or liquid precursors (radioactive carbon dioxide) the gas generator is used. To produce carbon dioxide from solid carbonate, the generator is evacuated through S_1 and S_2. At the same time the trap also is evacuated to S_6. The trap is surrounded by a liquid nitrogen bath and sulphuric acid is run into the generator from above. The carbon dioxide evolved is frozen out in the trap. The generator is closed off at S_1. The trap is warmed as the pressure is brought to one atmosphere with background gas.

Gas circulating pump

During an experimental run the gas in the system must be circulated to insure representative sampling as well as equilibrium between gas and liquid phases. The pump which accomplishes this must be tight to the outside atmosphere and must pump against a water head of a few centimeters at most. Both rubber tubing and all-metal pumps have been tried. There seems to be no material advantage in employing anything more complicated than the simple type shown schematically in figure 3. This pump was constructed out of short lengths of brass and rubber tubing and was fitted with circular flap valves cut from a sheet of dental dam rubber. The valves were cemented at one point with apiezon wax to the square turned ends of the brass tubing as suggested by the illustration. The internal volume of such a pump can easily be kept small which has been important in some experiments with our apparatus. The intermittent compression of the rubber tubing section between the valves was accomplished by a shaft connected eccentrically to a motor driven flywheel. The motor speed was controlled by a variac and the pump was run at several hundred strokes per minute. Thus operated, the capacities of several pumps of this type were between 100 and 200 ml. per minute. At this rate of bubbling of gas through the experimental vessel, very thorough agitation of the suspension was achieved.

Adaptation to Warburg-type vessel

For studying materials with annoying tendencies to foam (*viz.*, certain algal and chloroplast suspensions) it was found necessary to avoid bubbling, and the experimental vessel illustrated in figure 3 was replaced by a rectangular Warburg-type vessel shown in figure 4 A attached to a joint but not to the conventional Warburg manometer. This joint with gas inlet and outlet tubes was attached to a manometer holder in the same position as is usual for a Warburg vessel with manometer. Thus the experimental vessel can be shaken by a mechanical shaker in a constant temperature Warburg bath of commercial design (J. H. Emerson Co., Cambridge, Massachusetts). A mass spectrometer experiment thus can be run in one vessel with several ordinary Warburg vessels being run simultaneously beside it in the same bath. The gas inlet and outlet tubes of the vessel shown in figure 4 A were connected to the rest of the gas circulating system by metal capillaries and short lengths of rubber tubing so arranged as to insure sufficient flexibility for shaking the experimental vessel.

Mass spectrometer leak

When the apparatus was operated in connection with the modified commercial mass spectrometer it was found convenient to locate the gas leak close to the spectrometer tube; and a new leak assembly (fig. 4 B) was bolted to the aluminum plate upon which the glass manifold of the instrument is mounted. From valve V_2 of this assembly, copper tubing communi-

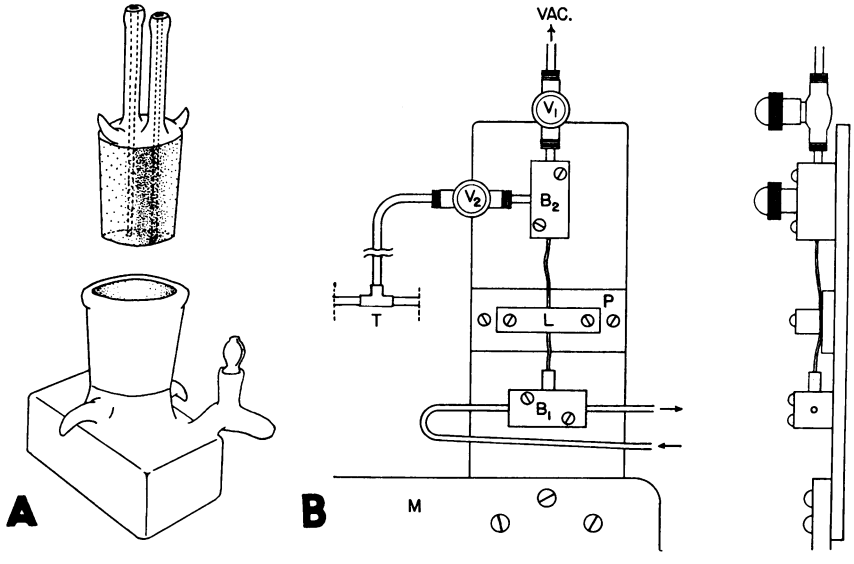

Fig. 4. A. Warburg-type vessel. The vessel is supported by a 24/25 standard taper joint into the male part of which are incorporated gas inlet and outlet tubes. These tubes are connected by short pieces of rubber pressure hose to metal tubing leading to the gas circulating system. It is connected in place of the experimental vessel shown in figure 3. The vessel is rocked in a thermostatically controlled water bath by a mechanical shaker. B. Mass spectrometer leak assembly. Front and side views of the all-metal assembly are depicted. Copper capillary tubing is pinched at L by bar clamped against backing plate P. Gas from circulating system (fig. 3) flows through tubing soldered into block B_1. The open end of the capillary leak tubing is inserted into this gas stream. Diffusion through the leak transfers the gas for analysis into the cavity of block B_2 through needle valve V_2 to the connection T in the spectrometer gas inlet tube. Valve V_1 connected to a mechanical pump permits evacuation of the region between the leak and valve V_2. Entire assembly mounted on one quarter inch brass plate bolted to mass spectrometer manifold supporting frame.

cates via a T-connection with the gas inlet tube of the mass spectrometer. This eliminates the need for the leak normally connecting the instrument manifold with the spectrometer tube. Therefore the high pressure side of the manifold leak is kept evacuated when the instrument is employed as described in this paper so that the mass spectrometer measures only the gas diffusing in through the new leak from the gas stream being circulated through the experimental vessel.

Experimental procedure

When carrying out an experiment with this apparatus the biological material is placed in whatever type of vessel is to be employed. The vessel and the accessory gas circulating system is flushed either with a prepared gas mixture or with a background gas and, after flushing ceases, small amounts of other gases are added as previously indicated. The gas mixture thus is prepared in the apparatus and can be analyzed *in situ* so that, by trial and error, the desired gas mixture may be approximated. In most cases this requires about 10 to 15 minutes. Equilibration is rapid. Metabolic data usually can be considered meaningful about 15 to 30 minutes after the experimental vessel is placed on the apparatus.

Interpretation of data

After the experiment the strip chart must be analyzed to convert a recurring peak pattern, such as figure 2 B, into a more conventional type of graph. Data are taken off the chart by measuring the various peak heights (correcting for base lines) and making the appropriate corrections for amplifier sensitivities. The resulting data are relative and can best be converted to absolute values by direct calibration with known gas mixtures. It may also be noted that a given partial pressure of one gas will not necessarily produce the same mass spectrometer voltage output as the same partial pressure of a different gas. This is because the ionization efficiencies of different gases may differ significantly, hence the need for individual calibrations when absolute values are wanted. Another point to be remembered is that it is infeasible to employ the usual corrections for spectrometer residual peaks (the peak heights obtained when the mass spectrometer inlet valve is closed so that no gas can enter the tube). Apparently it is the presence of water vapor in the metabolic gases which invalidates the usual type of blank correction. Since there is no provision for drying the gas being analyzed, the apparatus is far from clean in the sense this term is used in mass spectrometry. Water vapor has not constituted a source of difficulty in our experiments. If it should prove objectionable in future applications, a drying agent or cold trap may be introduced in the gas circulating system in front of the T-connection to the mass spectrometer leak.

Time resolution

Figure 5 A shows a continuous record of the mass 32 peak when the apparatus was filled initially with helium. Time runs from right to left on the chart. As described previously, a section of the gas circulating system isolated between two stopcocks was filled with 100% oxygen. The stopcocks were turned so as to allow gas circulation, the pump was started, and the slug of oxygen was swept around the system again and again until complete mixing had been accomplished. The instrument detected this oxygen each time it passed the connection to the spectrometer leak tubing giving rise to the oscillating record shown in figure 5 A. The time of one cycle on

the chart corresponds to the circulation time of the gas in the apparatus and is 24 seconds under the particular conditions of this measurement. The time for complete mixing is about two minutes but this obviously depends on the size of the experimental vessel. With a larger vessel the mixing time would be less. Figure 5 A gives some indication of the time resolution achieved by the method.

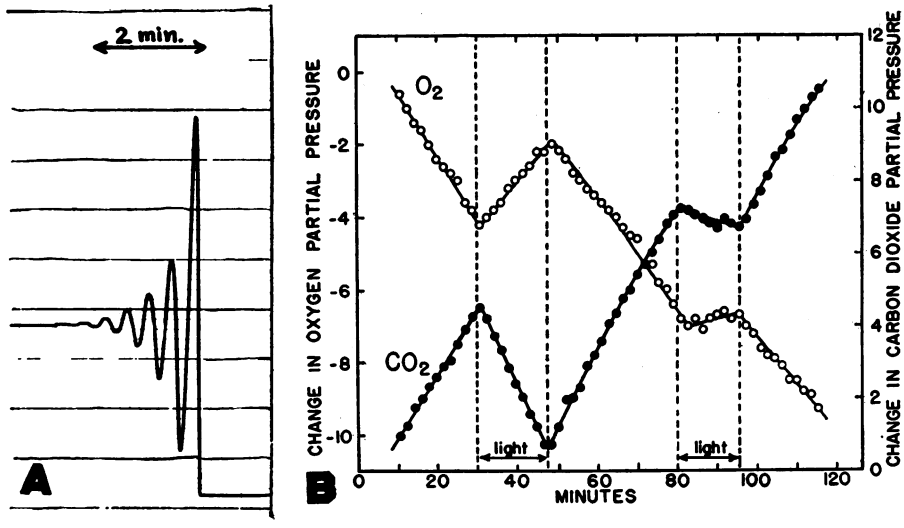

FIG. 5. A. Photograph of chart data showing gas circulating time. Part of the system (fig. 3) was filled with oxygen and the mass spectrometer was tuned to mass 32. The gas circulating pump was started and the gas was completely mixed after six circulations past the spectrometer leak. From this record, circulation time was 24 seconds. The measured system volume was 51 ml. The calculated pumping rate was 128 ml. per min. B. Sample data illustrating mass spectrometric measurement of respiratory and photosynthetic gas exchange of *Chlamydomonas Moewsii*. One hundred microliters of algae were suspended in 2 ml. phosphate buffer, pH 5.4. The gas phase was 3.5% oxygen in helium. Three dark and two light periods are represented. First light period gave photosynthesis 2 × compensation; second light period was near compensation. Points on each curve represent intervals of 108 seconds. The units on the ordinate correspond approximately to 10 microliters of either gas.

Data

After appropriate analysis of the data from the strip chart one may draw a graph of the gas exchange of the cells or tissue observed in the usual manner. Figure 5 B is an example which is chosen merely as an illustration. The biological material was 2.0 ml. of a 5% algal suspension in a rectangular vessel of 10 ml. volume (similar to figure 4 A) shaken in a water bath. The gas phase consisted initially of helium containing about 3% oxygen. The data show two intervals of photosynthetic activity at different light intensities between dark intervals during which only respiratory gas exchange is recorded. The ordinate values have no absolute significance; they have the dimensions of voltage output. By calibration with gas mix-

tures they can be converted to partial pressure increments of the respective gases. Such values can in turn be converted to absolute quantities of gases produced or consumed only if the total gas volume is known. In the experiment illustrated each unit on the ordinate corresponds roughly to 10 microliters of either gas.

Extreme fluctuations in the record typically are about 1% or less. Obviously the precision with which increments in partial pressure of a given metabolic gas can be measured thus depends upon the prevailing partial pressure. This is in contrast with a method such as Warburg manometry for which the precision neither is improved by working at low concentration of the gas which is being metabolized nor is there any sacrifice in precision if one works at high concentration. Practical considerations including quantity of biological material studied, its metabolic rate, the gas volume of the experimental system, and the size of the mass spectrometer leak actually determine the effective upper limit to the partial pressure of the gas being measured. We have taken data on rates of oxygen metabolism by plant tissues in as high as 10% oxygen with a statistical error in rate determination of less than 5% (standard error of regression coefficient of partial pressure on time for data such as those of fig. 5 B).

Various applications of this method will be dealt with in subsequent papers.

Summary

A method is described for measuring gaseous metabolism of cells, tissues, or whole organisms. In principle it consists of an experimental chamber of suitable size either with or without provision for gas circulation according to the requirements of the experiment. The gas phase is continuously sampled by a recording mass spectrometer. This instrument is provided with automatic tuning in such a manner that it records in sequence the partial pressures of several gases in the chamber at frequent intervals. The device accomplishes practically continuous monitoring of a predetermined number of gases. The analyses are of course based on the molecular weights of the respective gases and therefore the method has excellent analytical specificity except for a few ambiguities (like nitrogen and carbon monoxide). The method has good time resolution. Sample data are presented illustrating application of the method in a biological experiment.

Many times during the development of this method a number of individuals have contributed useful advice, helpful suggestions, or manual assistance in ways too numerous to mention in detail. Among those especially to be thanked are Charles Bollenbacher, Jaan Jurrisson, and J. M. Daly. We are particularly grateful to Dr. R. E. Halsted who was responsible for the basic electrical circuit design, essentially similar to that shown in figure 1, and who has advised us on many technical aspects of mass spectrometry. Kent Chapman and Wilma Monserud prepared most of the figures.

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