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**A PHOTOSYNTHETIC GAS EXCHANGER CAPABLE OF PROVIDING
FOR THE RESPIRATORY REQUIREMENT OF SMALL ANIMALS**

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58-124

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January 1959**

A PHOTOSYNTHETIC GAS EXCHANGER CAPABLE OF PROVIDING FOR THE RESPIRATORY REQUIREMENT OF SMALL ANIMALS

A photosynthetic gas exchanger capable of producing 10 liters of oxygen per day has been designed. The gas exchanger can provide oxygen for, and take up carbon dioxide from, four 30-gram mice on a continuous basis. The production of carbon monoxide by the plant and the successful elimination of the CO produced is described.

The concept of the *sealed cabin* as the passenger-carrying component of the manned space vehicle is well established. In simple terms, such a cabin is defined as a sealed enclosure which carries all the oxygen required by the crew as well as the means of removing all waste gases and capable of withstanding some, as yet undetermined, pressure differential with negligible leaking.

Various methods have been proposed for maintaining the atmosphere in a sealed cabin. Each method is characterized by a certain weight-space penalty based on the requirement that oxygen must be fed into the atmosphere and carbon dioxide must be removed from the atmosphere.

Oxygen tension may be maintained by carrying a supply of liquid oxygen in suitable tanks. Carbon dioxide may be absorbed from the cabin atmosphere with a mixture of calcium and barium hydroxide or lithium hydroxide. There is, however, some practical limit to the length of time that such an arrangement will suffice. Obviously, the oxygen supply, no matter how large, will eventually be depleted and the carbon-dioxide-absorbing system must eventually become saturated. For long-time flights it will be necessary to provide for the regeneration of oxygen from the exhaled carbon dioxide.

With one exception, all methods that have been proposed are characterized by the inescapable necessity of increasing the weight and space in a linear fashion as a function of operational time. This exceptional solution to the problem is to introduce into the sealed cabin a

photosynthetic plant. Such a system has been termed the *photosynthetic gas exchanger*. While apparently characterized by an initial weight-space requirement several times that of competing systems, it has the distinct theoretic advantage that it is regenerative and, therefore, requires the same amount of space and has the same weight over an indefinite period of time.

This unique method makes use of the photosynthetic mechanism of green plants by which, simply stated, carbon dioxide and water are converted to organic carbohydrate and molecular oxygen.

The first problem encountered in the design of a photosynthetic gas exchange system is to decide which plant to use. The general choice was dictated by the following considerations: The unicellular microscopic algae are characterized as a group having the highest photosynthetic rates in the plant kingdom. This type of plant has the further advantage that it can be grown in liquid culture in illuminated tanks through which the cabin air may be pumped. Such a system provides ease of control and manipulation not attainable with other forms of plant life.

Although a large variety of strains is available in the class Chlorophyceae or green algae and the class Cyanophyceae or blue-green algae, the majority of these strains are not endowed with the combination of characteristics required for use in this type of system. The choice of strain to use depends on a number of factors including size of cells, lack of tendency toward clumping, maximum photosynthetic rate,

tolerance to variations in medium composition, ability to utilize urea, broad range of temperature for optimum growth, and minimal excretion of organic matter during growth. A program has been initiated to establish minimum criteria for the above factors, and one or two of the strains with the most favorable combination of characteristics will be selected for future study. In the meantime, interest has been confined principally to two strains: a green alga, *Chlorella pyrenoidosa*, and a blue-green alga, *Anacystis nidulans*. These strains were chosen because of their availability and because they are characterized by reasonable combinations of the above factors.

Following the choice of plants it becomes necessary to establish some rational criteria for the design of the equipment in which the algae will be cultivated. Myers (1) has pointed out the necessity for maintaining a high illuminated area to volume ratio in the design of culture chambers. This requirement results from the fact that the algal suspensions are characterized by very high light absorption. A 1 percent suspension of *Chlorella*, for example, will absorb over 90 percent of incident illumination having an intensity of 400 ft.-c. at a thickness of less than $\frac{1}{4}$ cm. Since gas exchange with the algal cells can occur only within the liquid phase, it is essential to maintain optimal gaseous equilibrium between the gas phase and the liquid phase. Other considerations in design of the equipment should include methods for sterilization, a method for creating a controlled gas-liquid interface under conditions of zero gravity, and an efficient method for harvesting the algal cells.

The recent work of Myers (2) indicates that a photosynthetic gas exchanger can be operated continuously for several weeks in the steady state, provided the algal cells are harvested periodically and fresh liquid medium is added to the culture. Using *Chlorella pyrenoidosa*, Myers found that the photosynthetic gas exchanger produced 100 volumes of oxygen for each 82 to 85 volumes of CO_2 taken up with a thermodynamic efficiency of 18 to 19 percent.

The really important theoretic advantage of the photosynthetic gas exchange system is based on the fact that it can conceivably be operated as one component in a closed cycle, thus making it truly regenerative.

It is entirely reasonable to assume that an adequate medium for growth of the algae can be developed from human waste products. It will be necessary to process these wastes at least to the extent that the fecal organic matter is generally dissimulated to sulfates and phosphates, either by bacterial action or by high temperature oxidation. With the possible exception of boron, all of the required trace elements are excreted in milligram or microgram quantities by man. Since 85 to 90 percent of the nitrogen excreted by man is in the form of urea, this will provide the major source of nitrogen for the plant. The urine may be diluted and filter-sterilized, then mixed with the ash obtained from the fecal incineration. Experiments in Dr. Myers's laboratory (3) indicate that dilute human urine will provide the necessary nitrogen for cultivation of algae while producing little or no inhibition of growth.

As for the disposition of the harvested algal cells, it is likely that they can be used to supplement the diet of the crew. Preliminary calculations indicate that the amount of algae produced during the course of one day in supporting the respiratory requirements of one man is very nearly equal, on a weight basis, to the amount of food required by the man (4). *Chlorella* has an average constitution of approximately 50 percent protein, 16 percent carbohydrate, and 25 percent fat. This would be considered a rather high protein diet for a man. However, these ratios vary somewhat, depending on the conditions under which the algae are grown. If the algae are used as food, probably the greatest difficulty will be encountered in making the algae palatable and in arranging for variety in the diet. Dr. Hiroshi Tamiya in Tokyo has developed a relatively simple procedure for improving both the flavor and color of *Chlorella* (personal communication). Fink and Herold have shown that the biologic value of protein of *Scenedesmus obliquus* is equal to that of skim milk when fed to rats (5).

It is possible that no single microorganism will possess the metabolic capability of completely closing the circle between man's excretions and his material input. Bacterial cultures may be needed to process the waste products to provide a suitable medium for algal growth. Recent experiments by a group at the

University of California headed by Dr. Harold B. Gotaas (6) indicate that unprocessed sewage provides a very favorable medium for growth of algal-bacterial mixed cultures. It is also possible that the entire output of algae from a photosynthetic gas exchanger may not have to be considered the sole or primary food substance. Some fungi have the ability to produce high-quality protein and a large component of the water-soluble vitamins when grown in a simple liquid medium containing a simple sugar as the sole source of organic carbon. In this connection it is interesting to note that we have isolated a mold contaminant from one of our algal cultures which grows quite rapidly in a suspension of algae. The fungus has been tentatively identified as a species of the genus *Fusarium*, a common laboratory contaminant. In this case, as in the case of the bacterial oxidation of sewage, oxygen is consumed by the fungus—a fact which points to the importance of maintaining the algal portion of the system in a condition of favorable oxygen balance if it is finally determined that a portion of the food cycle should include other than algal cultures. In over-all design criteria the *per man* oxygen demand would be calculated on the basis of respiratory oxygen plus a factor for additional oxygen required for waste processing and for accessory food production.

The algal culture chambers designed and used by Myers were of relatively small capacity. Their purpose was to determine the optimum conditions leading to maximum photosynthetic activity in algal cultures.

Since the real value of this method of gas exchange is dependent upon its incorporation into a closed cycle, it was decided to design and construct a gas exchanger of sufficient size to provide for respiratory gas exchange for one or several small animals. This would then be used as a tool in studying the problems associated with the closed cycle.

METHODS AND MATERIALS

The first culture chamber in the series was designed by Gaume (7). Although the essential elements of this design were retained, several modifications were incorporated into the current model, justifying a detailed description.

The major difficulties encountered in the original design resulted from the necessity of

removing 24 bolts from the side of the tank each time it was opened, and from the difficulty in cleaning the tank in which the light wells were integral. These difficulties were eliminated in the design of subsequent tanks.

The model in present use consists essentially of two elements. The main chamber is a rectangular Plexiglas tank having inside dimensions of $3\frac{1}{2}$ in. in depth, $20\frac{1}{2}$ in. in height, and 19 in. in width. The tank is open at the top and is fitted with a reinforcing strip of $\frac{1}{2}$ -in. Plexiglas covered by a thin neoprene gasket. There is one tapered hole 1 in. from the bottom of the tank on each end, designed to take a No. 4 rubber stopper from the inside. The two-element sintered glass bubbler is inserted in one stopper, and a drain tube with a glass stopcock is mounted in the opposite hole.

The illuminator consists of a flat sheet of $\frac{1}{2}$ -in. Plexiglas 4 by $19\frac{1}{2}$ in. in which was drilled 8 evenly spaced round holes 2 in. in diameter. To each of these was cemented a 2-in. Plexiglas tube approximately 20 in. long. These tubes are closed at the opposite end except for a small hole which is connected to a 1-in. Plexiglas manifold which, in turn, rises to the top and is fitted with a $1\frac{1}{2}$ -in. hose connection. In operation, the illuminator is inserted into the tank and held in place by 4 aluminum bars which fasten to the reinforced rim of the tank by winged bolts. A 15-watt fluorescent lamp is inserted into each tube, the lead wires for the bottom lamp contacts being held away from the lamps by means of Bakelite spacers. The manifold is connected to a small squirrel cage blower which forces air up around the lamps. In addition to the 8 lamp ports and the air-cooling port, the top also has 5 other tapered holes, one near the center and two at each end. The center hole is used as the gas outlet port and the inlet for fresh medium. Two holes on one end are fitted with the two pH electrodes. A mercury thermometer is mounted in a No. 4 rubber stopper in one hole in the opposite end and the other port is closed with a blank No. 4 stopper. It is used to gain access to the tank for addition of antifoam and for removal of small samples of the culture. The operating volume of this tank is approximately 15.5 liters of liquid with air space of 1 liter at the top.

Operation of the photosynthetic gas exchanger is dependent upon some accessory equipment

and instrumentation. Accessory equipment includes a water-jacketed reflux condenser mounted above the air outlet and connected to the tank with a 24/40 standard tapered glass joint. This condenser is cooled to approximately 5° C. and serves to dehumidify the effluent air and to prevent reduction of the liquid volume in the tank by evaporation.

Air is forced into the tank through a cotton-wool filter contained in a glass cylinder approximately 10 in. long and 2 in. in diameter. This filter prevents the contamination of the algal culture by bacteria and molds.

Air is forced through this filter from a 4-liter glass ballast bottle by means of a small diaphragm pump (Model 2 Dynapump). A pressure of approximately 80 mm. Hg above ambient is required to overcome the resistance introduced by the bacterial filter, the sintered glass bubblers, and the head of water pressure in the tank. The ballast bottle is fitted with a mercury manometer for visual reference to this pressure and with a Statham pressure transducer for continuously recording the pressure. Other instruments directly associated with the tank are the Beckman Model H2 pH meter and a Weston photocell mounted on one end of the tank. This cell is shielded so that only light from the end fluorescent lamp activates it. A red (640 m μ) Klett filter is positioned between the lamp and the photocell.

Harvest of the algal cells is accomplished by connecting a short length of autoclaved rubber tubing from the outlet port directly to the nozzle of a Sharpless continuous centrifuge. As the suspension enters the centrifuge, fresh sterile liquid medium is introduced at the same rate at the top of the tank. In this way, a continuous record may be obtained of the various parameters associated with the operation of the system during harvest, since the volume is maintained constant. The harvested algal cells are stored at -5° C. and lyophilized when a sufficient batch has been accumulated.

Early experiments indicated that a fairly dense culture of algae in the tank was not light-saturated. For this reason, accessory illumination consisting of two 15-watt lamps underneath the tank plus two 6-lamp units on front and rear were added. The additional heat introduced by the accessory illumination is dissipated by a 16-in. fan located at one side of the tank. No

difficulty has been encountered in maintaining the temperature of the liquid at acceptable levels by this method of air cooling. The nominal output of the 15-watt, T8 lamps is approximately 600 lumens per lamp or approximately 0.9 watts. In calculating the efficiency of this installation it is assumed that the 8 internally positioned lamps transmit 100 percent of this energy to the algal suspension and that the 14 externally positioned lamps transmit 50 percent of their output to the algal suspension. These assumptions appear reasonably valid with algal suspensions having densities of 0.1 to 1 percent. Thus the estimated total input is approximately 13.5 watts or 278.6 kilogram calories per day.

Information from the pH meter, the photocell, the pressure transducer, and from a Beckman oxygen analyzer and a Liston-Becker CO₂ analyzer is fed into a multichannel Brown recorder. It is thus possible to obtain a continuous record of a number of variable parameters associated with the operation of the gas exchanger. Unless otherwise stated, the algal strain used in the experiments reported here was a culture of *Anacystis nidulans* kindly provided by Dr. William Kratz. Characteristics of this species was described by Kratz and Myers (8). The composition of the liquid medium used is shown in table I.

In operation, the algal chamber is routinely coupled to a rectangular Plexiglas animal chamber. This chamber, originally designed for two mice, has inside dimensions of 9 in. in height, 5 in. in width, and 5 in. in depth. The lower one-third of the box is separated from the upper two-thirds by a removable plastic grid constructed of 1/4-in. rod. The top of the chamber is held in place by 12 small machine bolts which thread directly into the sides of the box and is sealed with a neoprene gasket.

Below the grid there is a sliding plastic drawer to collect urine and feces. This drawer is held in place with an aluminum retaining bar fitted with two tightening screws. The drawer is sealed with a neoprene gasket. The top is drilled to accept one No. 10 rubber stopper and one No. 4 rubber stopper. The large hole is fitted with a 100 ml. watering bottle and the small hole with a mercury thermometer. An air inlet port is mounted at the upper rear of the box and an outlet port is fitted below the grid

on one side. The rear side is drilled to accept a No. 10 stopper. This hole is used to insert animals and feed. A semiautomatic feeder was designed to bolt over this opening but operation of the feeder was not entirely satisfactory and will not be described. The other three faces of the box have holes drilled to accept No. 4 rubber stoppers. A mercury manometer is mounted in one of these. The other holes are not ordinarily used and are plugged with blank stoppers. A general view of the experimental setup is shown in figure 1.

RESULTS

Some data will be reported which were obtained in early experiments before the oxygen and carbon dioxide gas analyzers were available and when the illumination consisted only of 6 internally positioned 15-watt daylight fluorescent lamps. Periodic analysis for carbon dioxide and oxygen was made on a Haldane gas

analyzer using 20-ml. samples withdrawn from the system. The total gas volume was so large (approximately 9 liters) that withdrawal of these small samples had no observable effect. Because of the fewer number of lamps, the liquid volume in the tank was approximately 18.5 liters instead of the present 15.5 liters.

On the basis of studies conducted with other plant material, Wilks (personal communication) had predicted that carbon monoxide might be produced in measurable quantities by the large algal cultures used in these studies. Analysis of effluent gas samples when room air was pumped through the algal suspension indicated the presence of little or no CO. However, when effluent gas was recycled in a closed system, the CO content of the gas was routinely found to increase in a more or less linear fashion.

Figure 2 is a record of the percent of oxygen, carbon dioxide and carbon monoxide in the gas phase when a dense suspension (0.5 to 0.7 percent, v/v) of *Anacystis nidulans* was cultured

TABLE I
Nitrate medium

Calcium chloride (CaCl ₂)	1.08 gm.
Magnesium sulfate (MgSO ₄ ·7H ₂ O)	2.70 gm.
Sodium nitrate (NaNO ₃)	9.00 gm.
Potassium nitrate (KNO ₃)	9.00 gm.
Potassium acid phosphate (Monobasic) (KH ₂ PO ₄)	4.50 gm.
Tris buffer	9.00 gm.
Iron sulfate (Fe ₂ (SO ₄) ₃ ·6H ₂ O)	
0.04 gm./500 cc. distilled water (40 mg.)	90 ml.
Microelement solution	1,800 ml.
<hr/>	
Final dilution with distilled water	18,000 ml.
<i>Microelement solution for algae media</i>	
Na ₂ MoO ₄ ·2H ₂ O	1.2 gm.
FeCl ₃ ·6H ₂ O	156 mg.
ZnSO ₄ ·7H ₂ O	26.4 mg.
MnCl ₂ ·4H ₂ O	172 mg.
Co(NO ₃) ₂ ·6H ₂ O	0.600 mg.
CuSO ₄ ·5H ₂ O	0.188 mg.
H ₃ BO ₃	1.36 gm.
Trisodium EDTA	1.2 gm.
Dilute to a final dilution of —	4,000 ml.

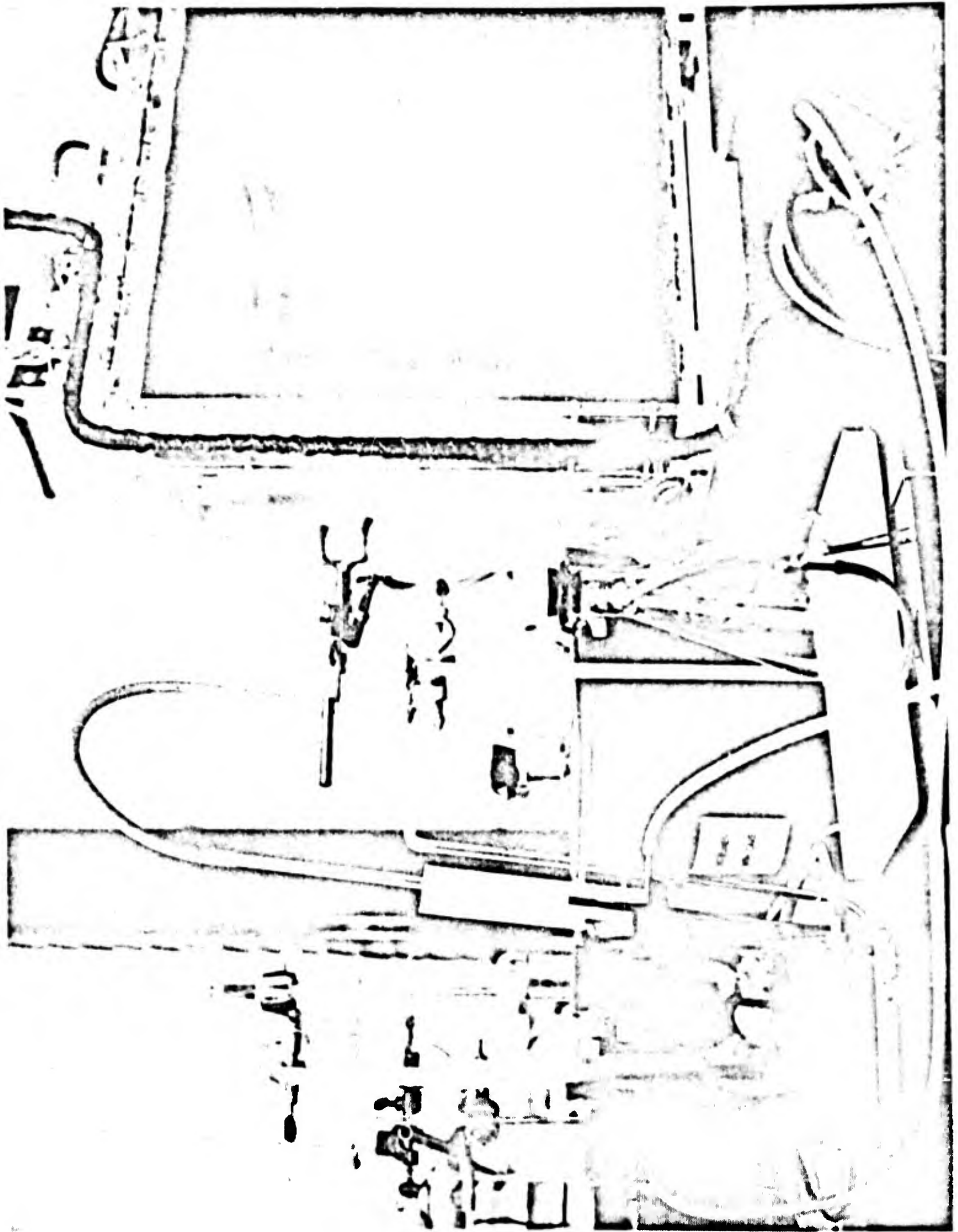


FIGURE 1
General view of photosynthetic gas exchanger and associated equipment.

in a closed system without animals. The CO determinations were made by Dr. Wilks on a Liston-Becker CO analyzer. Details of this method including an assessment of specificity are reported elsewhere (9). The optical density has only relative meaning since it is calculated from the percent transmission taking zero percent as no light and 100 percent as the point where the tank is filled with medium but contained no algal cells. The rate of oxygen evolution, calculated from the total gas volume, was approximately 3 liters per day.

The apparent increase in rate of CO evolution at the end of the experiment is interesting and requires further investigation. It seems likely, from the data presented here, that a culture of *Anacystis* produces CO at a higher rate when it is in the stationary phase in the growth curve.

The decrease in rate of gas exchange was due in part to the fact that the culture was very dense and approaching the stationary phase of the growth curve during the later hours of the experiment, as indicated by the graph of optical density. It was also due in part to the decreased photosynthesis resulting when accessory illumination in the form of daylight and room lights was no longer available after about the sixth hour of the experiment. This effect is more pronounced in the experiment represented by figure 3. This figure is a record of approximately a three-day (71.5 hours) mouse-algal experiment with some data on the algal culture alone just preceding the experiment. Oxygen and carbon dioxide determinations were by the Haldane method. Optical density was measured by the Weston photocell. The algal strain was *Anacystis nidulans*. At zero time the culture was being gassed with a mixture of 4 percent CO₂ in air. Oxygen concentration was 20.7 percent. At time A the system was closed with no mice present. As expected, oxygen concentration increased rapidly and carbon dioxide concentration decreased during the next 5½ hours. At time B, one mouse was inserted into the mouse chamber and the system was again closed.

The effect of accessory illumination provided by daylight and by room lighting during working hours is very evident in the graph of oxygen concentration and apparent but somewhat less evident in the tracings of the relative optical density of the culture and the carbon dioxide

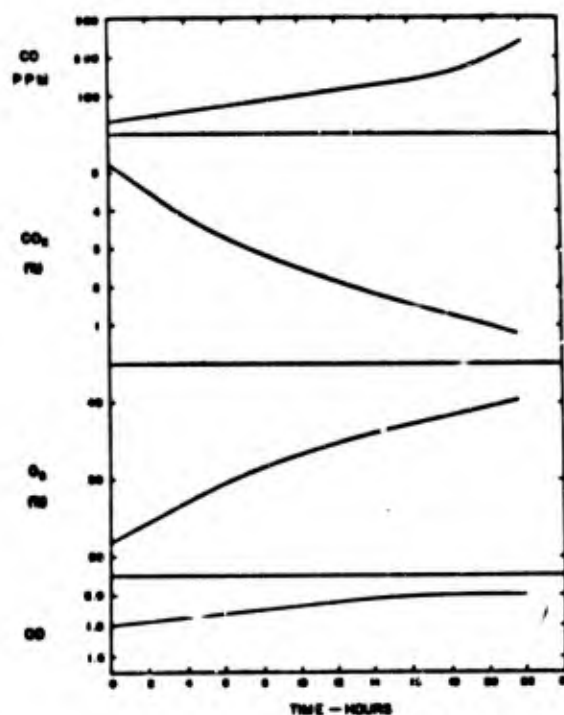


FIGURE 2

Data from an experiment with *Anacystis nidulans* illuminated by six internal 15-watt fluorescent lamps.

concentration in the gas phase. It should be noted that the measure of carbon dioxide in the gas phase is not a direct function of carbon dioxide metabolism since the culture medium is well buffered with Tris buffer. For this reason, the assimilatory quotient

$$AQ = \frac{CO_2 \text{ taken up}}{O_2 \text{ evolved}}$$

cannot be directly estimated from these data. The assimilatory quotient may be calculated if dissolved CO₂ is estimated by use of the Henderson-Hasselbalch equation, but this estimate must be taken only as an approximation.

Since the internal illumination was obviously inadequate, the illuminator was rebuilt to incorporate eight 15-watt fluorescent lamps. Data from an experiment conducted with increased illumination is shown in figure 4. In addition to the 8 internal lamps, six 15-watt lamps were mounted at the rear of the chamber and, at time B, a 100-watt incandescent lamp was placed to illuminate the front face of the chamber.

In this three-day experiment with three mice in the mouse chamber, the carbon dioxide in the gas phase varied between 1 and 2 percent, while the oxygen concentration increased from

approximately 22 to 30 percent. Growth of the algal culture was evidenced by the increase in optical density. At time A, a harvest of algal cells was accomplished. The gradual increase in oxygen, unaccompanied by corresponding decrease in carbon dioxide, results from the fact that the assimilatory quotient of the algal culture did not correspond to the RQ of the mice. Note that there are measurable quantities of carbon monoxide produced, increasing from a value of 300 p.p.m. to 750 p.p.m.

The strain of *Anacystis* employed was unable to assimilate urea nitrogen; however, as previously stated, Myers has found that *Chlorella*, grown at the expense of urea nitrogen, maintains a steady-state assimilatory quotient of 0.82 to 0.85. Furthermore, since energy is required for the algal cell to reduce nitrate nitrogen, it would be expected (and Myers has, in fact, observed) that a measurable difference occurs in thermodynamic efficiency of urea grown cultures over that of nitrate grown cultures.

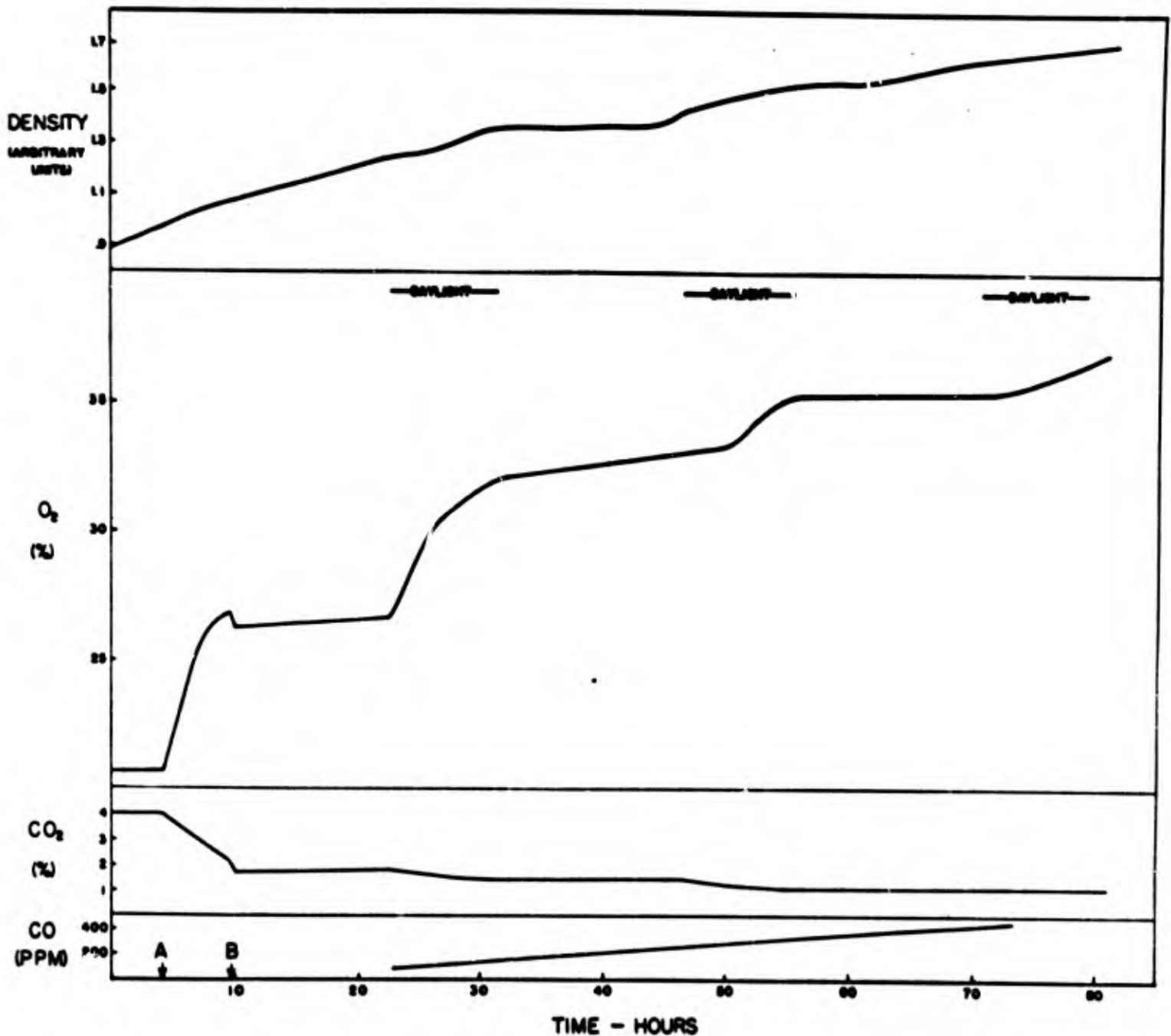


FIGURE 3

Data from an experiment with *Anacystis nidulans* coupled to a 30-gram mouse. Algal culture illuminated by six internal 15-watt fluorescent lamps.

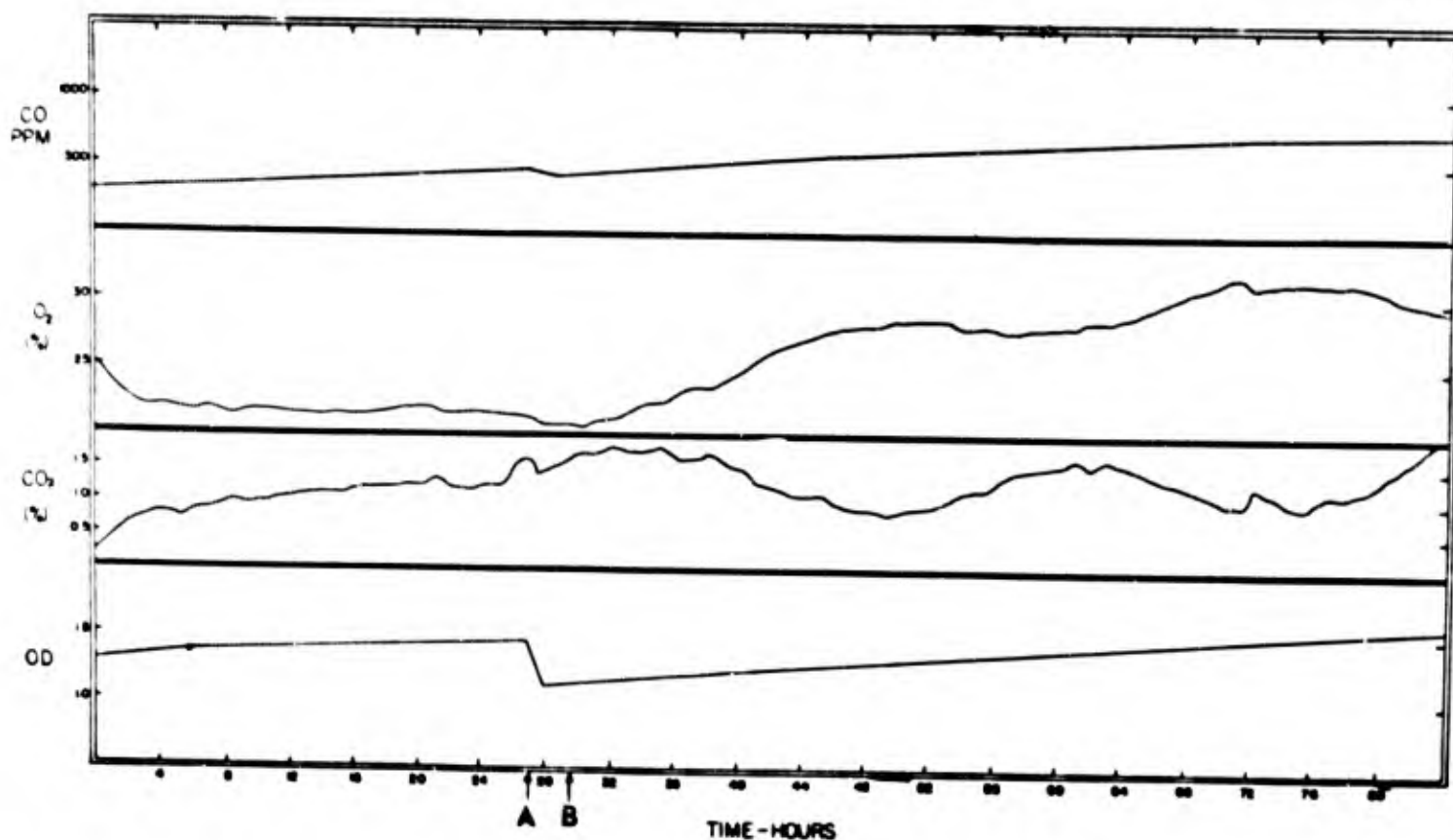


FIGURE 4

Data from an experiment with *Anacystis nidulans* coupled to three 30-gram mice. Algal culture illuminated by eight internal and six external 15-watt fluorescent lamps. At point B a 100-watt incandescent lamp was directed at the front of the tank.

TABLE II
(From Myers)

Experiment No.	Nitrogen source	AQ	Input energy (cal./day)	Energy yields (cal./day)		Efficiencies (%)	
				Cells	Oxygen	Cells	Oxygen
78	Nitrate	0.70	1,690	254.5	296.6	15.06	17.57
80	Nitrate	0.72	1,143	165.7	199.9	14.50	17.49
83	Urea	0.82	1,281	214.4	253.8	16.74	19.81
84	Urea	0.85	1,380	221.8	260.2	16.07	18.85

Representative data from Myers (2) are shown in table II.

Since both the practical efficiency and the relative assimilatory quotient are improved when urea is used as a nitrogen source, it was decided to change over to urea nitrogen. In order to do this it was necessary to obtain a different algal strain. Another blue-green alga, *Synechocystis* spp. was kindly provided by Dr. William Kratz. The composition of the cul-

ture medium used is shown in table III. The cells of this strain are slightly larger than those of *Anacystis* and are nearly round in contrast to the rod-shaped cells of *Anacystis*. The strain employed can be maintained in homogeneous suspension by the turbulence created by air flow in the culture chamber. Urea nitrogen is rapidly assimilated. Data from an algae-only experiment with this strain are shown in table IV. This experiment was conducted with

the full complement of 8 internal and 14 external lamps and, for this reason, rate of photosynthesis is not directly comparable to any *Anacystis* experiment.

Since the absolute pressure, temperature, and relative humidity of all components of the system are known or can be calculated, it is possible to resolve the total gas volume to 760 mm. Hg at 0° C. dry. From this figure the absolute volume of O₂ and CO₂ in the system can be calculated.

The oxygen evolved during the course of one hour was 274 ml. corresponding to a rate of 4.6 ml. per minute. If the assumptions regarding the energy input are approximately correct, this represents an efficiency of approximately

11 percent. The carbon dioxide taken up was calculated to be 254 ml. during the same hour. This figure is somewhat unreliable since the main component (183 ml.) is calculated as dissolved and combined bicarbonate in the liquid phase. Such calculations (Henderson-Hasselbalch equation) depend for their accuracy on the precise determination of pH. The calculated assimilatory quotient is 0.93. Some idea of the error in CO₂ estimation may be obtained if it is assumed that the A:Q is 0.85, as would be expected if the metabolism of urea by *Synechocystis* were comparable to that by *Chlorella*. In this case the expected volume of CO₂ taken up would be 233 ml., indicating an error in estimation of about 8 percent.

TABLE III
Urea medium

Calcium chloride (CaCl ₂)	1.08 gm.
Magnesium sulfate (MgSO ₄ ·7H ₂ O)	2.7 gm.
Sodium chloride (NaCl)	1.8 gm.
Potassium acid phosphate (Monobasic) (KH ₂ PO ₄)	4.5 gm.
Tris buffer	9.0 gm.
Iron sulfate (Fe ₂ (SO ₄) ₃ ·6H ₂ O) (0.04 gm./500 cc. distilled water)	90 ml.
Microelement solution*	1,800 ml.
Urea solution †	10 ml.
Final dilution with distilled water	18,000 ml.

*See table I.

†0.4 gm. per ml., filter sterilized and added separately.

TABLE IV
Gas exchange during 1-hour experiment with Synechocystis sdb.

	Volume O ₂ (ml.)			Volume CO ₂ (ml.)		
	Gas phase	Dissolved	Total	Gas phase	Dissolved	Total
Initial	1491	129	1620	159	1015	1174
Final	1761	133	1894	88	832	920
Difference	270	4	274	71	183	254

$$AQ = \frac{254}{274} = 0.93.$$

$$O_2 \text{ evolved / min.} = \frac{274}{60} = 4.57 \text{ ml.}$$

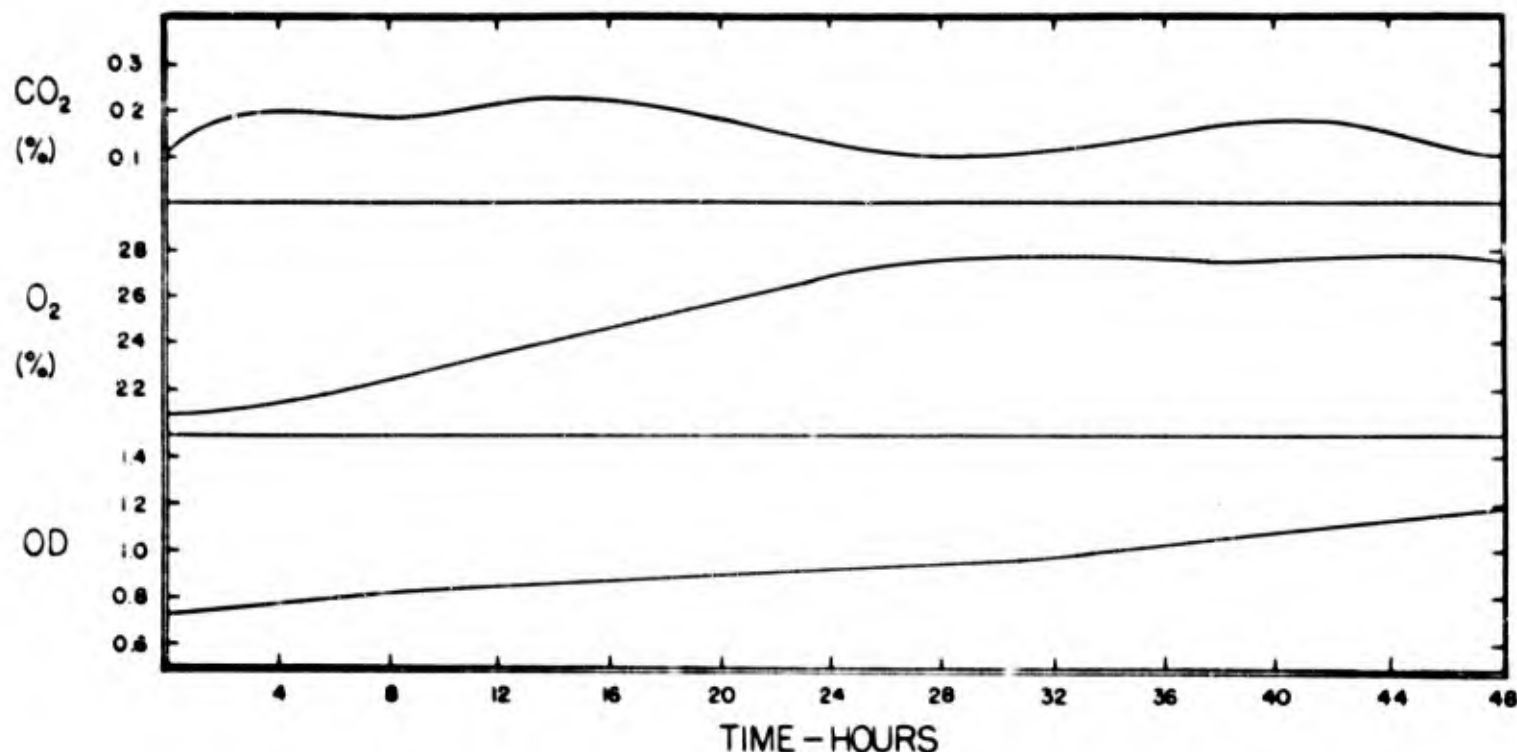


FIGURE 5

Data from an experiment with *Synechocystis* spp. coupled to four mice. Illumination as described in text.

Subsequent experiments with four mice in the animal chamber indicate that the gas exchanger with *Synechocystis* as the photosynthetic organism is capable of producing oxygen at the rate of approximately 7 ml. per minute. Again depending upon the accuracy of the estimate of energy input, this represents a thermodynamic efficiency of approximately 17 percent. Furthermore, in these experiments (typical data are shown in figure 5) oxygen evolution was maintained at a rate equal to or slightly in excess of the rate of consumption by the mice while CO₂ concentration was held relatively constant at less than 0.5 percent. This indicates that the AQ of the algal culture is near to or slightly less than the RQ of the mice.

As previously indicated, a small but steadily increasing concentration of carbon monoxide was observed in the closed algal-mouse system. That the carbon monoxide is the product of the growing plant is indicated by figure 6. This shows the linear increase in carbon monoxide as a function of time in a closed algae system without the mice. The algal strain in this experiment was *Anacystis nidulans*. The rate of CO evolution is roughly estimated at about 1 ml. of CO per gram (wet weight) of algae per

hour. Control experiments indicate that carbon monoxide is not produced by the culture apparatus when operated without the algae.

The maximum concentration of carbon monoxide observed in the algal-mouse system to date is on the order of 800 parts per million. This concentration is high enough to require consideration since it is eight times the allowable concentration for continuous exposure of humans. Levels of carbon monoxide on this order of magnitude could result in saturation of mouse hemoglobin with CO to the extent of 40 to 50 percent, depending on concurrent oxygen concentration. Observation of the mice during these experiments indicated no apparent weakness or loss of appetite. Ears and feet were not unusually red in color. It should be noted that tolerance to high carbon monoxide concentrations by rodents is not unusual (10) and further that these mice were subjected to a very gradual increase in concentration over a relatively long period of time.

In a practical application of the photosynthetic gas exchanger some method of carbon monoxide removal will be necessary. The solution presently employed is the catalytic oxidation of carbon monoxide to carbon dioxide.

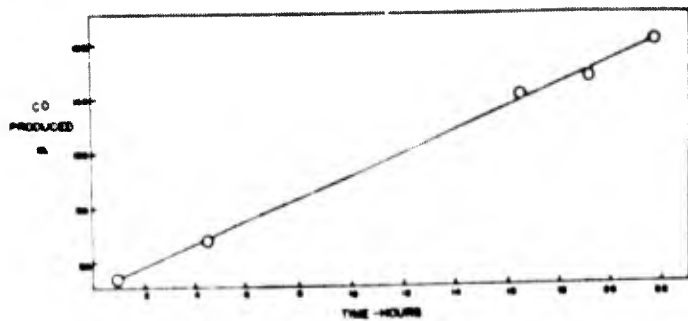


FIGURE 6

The production of carbon monoxide by a culture of *Anacystis nidulans*. During the 22 hours the CO_2 concentration fell from 4.9 to 0 percent and the O_2 concentration increased from 20.7 to 46.2 percent.

Since the observed level of carbon monoxide is quite low on a total volume basis, the oxidation to carbon monoxide has very little effect on the over-all gas exchange balance. A small bed of loosely packed Hopcalite, a commercial catalyst, inserted in the system and maintained at a temperature of 125°C . will completely eliminate all measurable traces of carbon monoxide.

Since this "CO burner" was added to the system, there has been no carbon monoxide problem. The catalyst appears to be quite stable at the temperature used. If the temperature falls below 100°C . the catalyst is "poisoned" by liquid water and is no longer effective. It can be partially regenerated by reheating. The installation of the CO burner having preceded the change-over to *Synechocystis*, no quantitative data on CO evolution by this strain is available. It has been observed, however, that when the CO burner is by-passed, measurable quantities of CO appear in the gas phase in the closed system within 1 to 2 hours.

DISCUSSION

Collection of data in the kind of experiments reported here is a lengthy process. Furthermore, considerable mathematical manipulation of the data is required if precise, quantitative results are to be obtained. The phase of investigation reported here is essentially preliminary to the main purpose of this project—namely, a study of the feasibility of a closed ecologic system.

As presently constituted, the system is capable of maintaining a reasonably well balanced gas exchange between plant and animal components. It now remains to place the harvest

operation in an automatic or semiautomatic status so that the algal culture can be maintained at the optimum density indefinitely. (Such a system has been in operation in Myers's laboratory on a small scale for several years.) Consideration of the preservation and processing of the algal harvest into an acceptable food for the mice can be initiated at once.

The collection of clean excreta from the mice has not been possible in the animal chamber now used. This results from the fact that the mice are fed a standard lab chow which crumbles so that the urine and feces are contaminated with ground food. Modification of the mouse chamber is required so that feeding is restricted to a definite area in the chamber and food waste is collected separately. An alternate solution to this problem would be to feed the mice on a liquid or semisolid diet so that food waste would be negligible.

Aside from the question of material balance, which will be further investigated, probably the two most important unsolved problems associated with the photosynthetic gas exchanger are the weight-space penalty and the question of reliability.

Although neither of these problems has yet been attacked directly, the direction signs are posted. Present thinking indicates that the weight-space oxygen evolved ratio may be materially improved by using very thick algal suspensions grown under extremely high levels of illumination (11). Ultimately systems must be designed which utilize solar radiation since this will be the source of energy in any space operation. It will be necessary to employ algal strains having optimum growth temperatures of 40°C . or higher.

So far as reliability is concerned, the large culture chamber described above has been operated for many weeks at a time by periodically harvesting the algae and replenishing the medium. In one experiment not reported in detail, the algae-mouse system was kept in continuous operation for 15 days. Very long term experiments should be set up in a continuous culture apparatus to determine what, if any, are the really long-range limiting factors associated with the continuous culture of algae.

The authors acknowledge the invaluable assistance of Robert M. Adams in installing and maintaining the electronic recording equipment used in these studies.

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