

# Recent Aspects in the Development of a Closed Ecologic System

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**T**HE CONCEPT of a closed ecologic system in its entirety has existed for a number of years in the mind of one of our foremost scientists in the field of Space Medicine. Dr. Hubertus Strughold had envisioned man on extended flights into space, his respiratory requirements to be met by an apparatus termed a photosynthetic gas exchanger.

This brilliant idea began to assume its place in reality when Doctor Strughold as Chief of the Department of Space Medicine, School of Aviation Medicine, contacted Dr. Jack Myers and a young graduate student, J. Neil Phillips, at the University of Texas regarding the project on May 14, 1952.

On May 17, 1952, J. Neil Phillips, who recently joined the School of Aviation Medicine staff, was encouraged by Doctor Strughold's enthusiastic discussion and submitted a research proposal entitled "Development of Balanced Respiratory Systems between Plants and Animals for Closed Systems," a proposal which is now recognized as the first known written document concerning the use of a photosynthetic gas exchanger.

In 1960, the same requirements for a closed system exist as in 1952. The original justification for the problem written in May 1952, is still applicable

even though extensive advances have been made in this field at the School of Aviation Medicine and elsewhere. This justification was as follows:

In view of projected long-range objectives of basic research related to high altitude and/or extraterrestrial flight, the need for achievement of balanced systems for maintenance of physiologically tolerable conditions for long periods of time in closed systems involving mammals becomes critical and self-evident.

Today with manned space-flight almost a reality, the need for a closed system is obvious, especially in view of logistics. In a sealed space vehicle with no cycling of wastes involved, the weight of conventional supplies to support an astronaut would be 29.6 pounds per day (13.418 kg.).<sup>13</sup> Obviously, a system that is functional in the recycling of waste materials would be more desirable from the standpoint of weight conservation.

Figure 1 shows a comparison of a 140-pound (64 kg.)\* algal system with conventional systems based on only one phase of respiration, oxygen production. It can be observed from the graph that the algal system becomes more economic than contemporary methods of O<sub>2</sub> supply in approximately forty-five days of space flight.<sup>4</sup>

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\*Dr. W. A. Kratz, an algal physiologist and former expert on photosynthetic gas exchangers, calculated that 140 pounds (64 kg.) would be the final weight of an algal system needed to support the requirement of an astronaut.

# CLOSED ECOLOGIC SYSTEM—BATES

The ability of a gas exchanger to absorb or utilize carbon dioxide is as important as its oxygen-producing capability, which is one critical logistic

less advantageous than conventional oxygen systems.<sup>4</sup>

A photosynthetic gas exchanger can be shown to be of even greater logistic

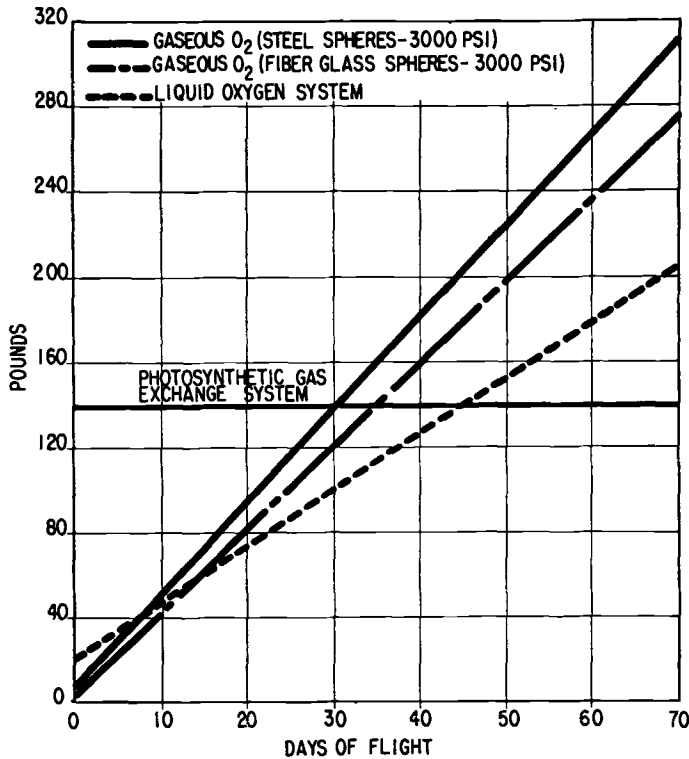


Fig. 1. Comparison of oxygen supply systems for human space flight.

advantage of a photosynthetic gas exchanger. Figure 2 indicates the logistical advantage of a 140-pound (64 kg.) gas exchanger when considering the weights of substances needed to maintain both phases of the respiratory cycle: production of oxygen, and disposal of carbon dioxide. In view of both factors, the 140 pound (64 kg.) algal system becomes economically more feasible than conventional oxygen systems after nearly fifteen man days of space exploration.<sup>4,12</sup> Presently, a chemical system would be even

tic advantage if the following aspects are considered: (1) Excess algae harvested from the algal system would be a source of food for the astronaut. (2) Components of human metabolic wastes would be used for algal nutrition.

Unfortunately, the 140 pound (64 kg.) system is not our present level of attainment. If we extrapolate experimental data from investigations on primate maintenance and relate these values to the respiratory requirements of an astronaut (875 gm. O<sub>2</sub> required and 1085 gm. CO<sub>2</sub> to be absorbed per

day), the theoretic weight of an adequate laboratory gas exchanger would be in the range of 1197 (543 kg.) pounds.

Texas, on contract to the School, published a comprehensive report concerning studies on algal suspensions with a small model photosynthetic gas exchanger.<sup>17</sup>

The first truly successful effort to sup-

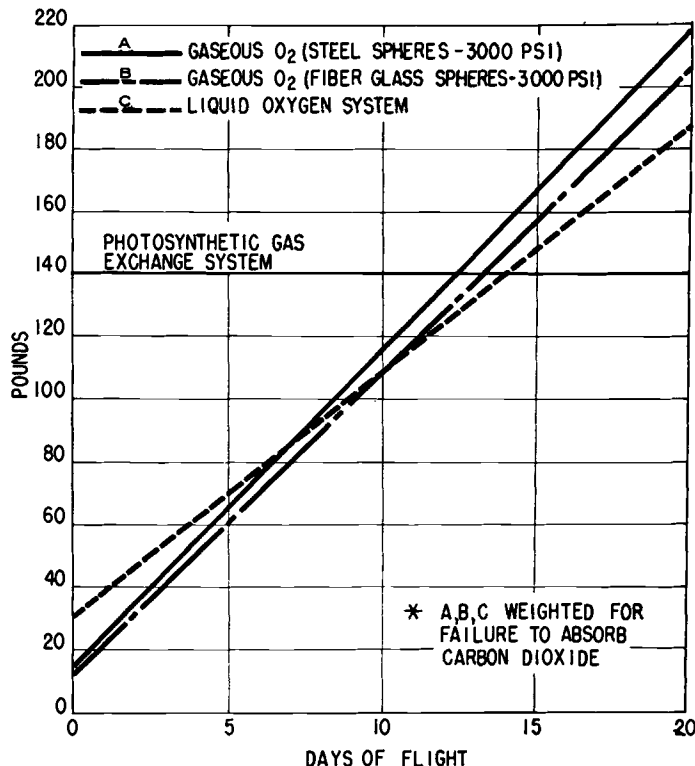


Fig. 2. Comparison of oxygen supply systems.

To date, the majority of the attention in the development of a closed ecologic system has been focused on the algal photosynthetic gas exchanger.

At the USAF School of Aviation Medicine, the photosynthetic gas exchanger has evolved from a "model" to one reliably supporting a primate.

Some of the milestones in this evolution are as follows:

The "Design of an Algal Culture Chamber Adaptable to Space Ship Cabin" was reported by Gaume at the School of Aviation Medicine, USAF, in May, 1958.<sup>11</sup> In the same year, Dr. Jack Myers, University of

port an animal with the use of a photosynthetic gas exchanger at the School was a mouse-algal experiment published in January, 1959.<sup>9</sup> This was a culture chamber retaining essential elements of the Gaume Chamber and utilizing the algae, *Anacystis* in 14 liters liquid medium (Fig. 3). Two hundred seventy-four ml. of oxygen production per hour was reported to be evolved by the system.

This system was replaced by a 16-liter algal suspension gas exchanger with an internal illuminating source consisting of a 107-watt power-groove lamp directly in contact with the algal suspension. This gas exchanger exhibited approximately the same oxygen-producing potential as the previous

model. The system demonstrated the feasibility of maintaining a specific temperature without heavy cooling or heating coils, thus producing a weight-volume advantage. An alga was then selected whose temperature

Aviation Medicine was designed by Dr. J. N. Phillips and H. R. Hair, II (Fig. 5). It presents a radical design in algal culture chambers. Basically composed of two parts, the algae are collected in a hemispheric con-

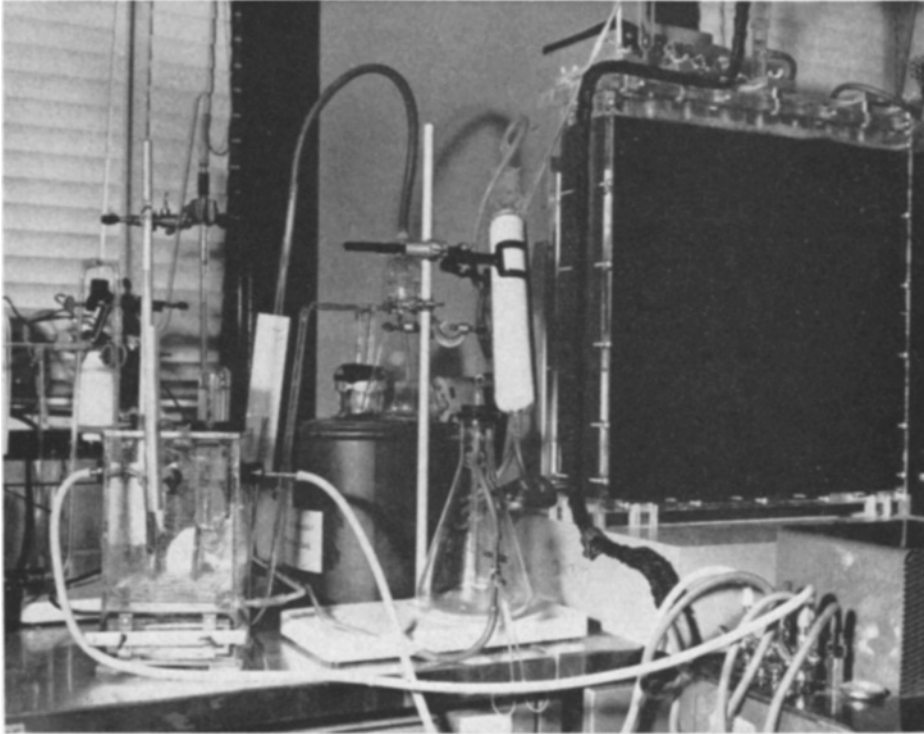


Fig. 3. Culture chamber to support an animal with use of photosynthetic gas exchanger utilizing the algae *Anacystis* in 14 liters liquid medium.

optimum was the same as the operating temperature of the liquid system.

The next photosynthetic gas exchanger was designed to provide for the respiratory requirements of primates (Fig. 4). The design eliminated major areas of deficiencies of the earlier models. It contains 56 liters of working algal suspension and is illuminated internally by nineteen 42" G. E. fluorescent lamps and externally by eighteen 42" G. E. fluorescent lamps. (Each lamp is rated at 25 watts). Temperature can be controlled from 15° C. to 64° C. and light intensity varied. The weight of the system is 171 pounds, excluding the ballasts for the fluorescent lights.

Another type of algal photosynthetic gas exchanger in use at the USAF School of

tainer and continuously recycled over a conical illuminated surface. In this manner, advantage is taken of intermittent thin film exposure of the algal suspension to illumination, and through liquid turbulence in the film, introduces flashing light effects.<sup>16</sup> The present experimental model operates with 15 to 18 liters of an algal suspension. Oxygen-producing potential of the system is undetermined.

#### CURRENT INVESTIGATIONS (INVOLVING THE USE OF THE PHOTOSYNTHETIC GAS EXCHANGERS)

Figures 6, 7, and 8 are examples of results obtained in our laboratory from

a photosynthetic gas exchanger: The information is compiled from a forty-nine-hour experiment utilizing the gas exchanger shown in Figure 4 to main-

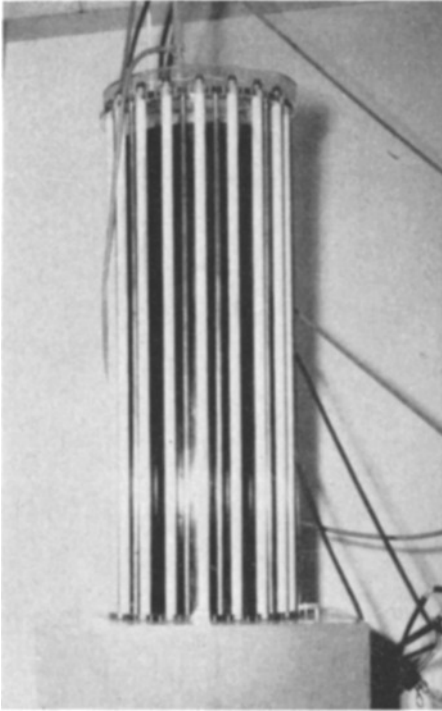


Fig. 4. Photosynthetic gas exchanger designed for respiratory requirements of primates. It contains 56 liters of algal suspension.

tain a Rhesus monkey. Results such as these have been obtained only after prolonged experiments in other gas exchangers maintaining smaller animals, and the elimination of numerous problem areas.

#### PROBLEM AREAS ENCOUNTERED IN INVESTIGATION WITH PHOTOSYNTHETIC GAS EXCHANGERS

One of the most important factors in establishing the validity of results is the ability to reproduce experiments.

To achieve reproducibility of results with photosynthetic gas exchangers, a strict adherence to experimental dis-

cipline must be maintained. Algal inoculum for the photosynthetic gas exchangers is pre-grown in flasks aerated with 3 per cent carbon dioxide. After a twenty-four-hour growth period, the cells are separated by centrifugation at 3,000 r.p.m. for fifteen minutes. The algal cells are resuspended in fresh medium and again aerated for twenty-four hours. The centrifugation process is repeated and the algal cells are used for the basic inoculum of the gas exchanger.

The density of the suspension in the gas exchanger is brought to 35\* (dry weight .27 mg. per ml.). Three per cent carbon dioxide in air is passed through the gas exchanger until the oxygen and carbon dioxide analyses of affluent and effluent gases indicate equivalent respiratory maintenance of the desired animal. After a test for leaks, the system is then sealed with the animal in the closed system. Determination of oxygen is made with an A. B. Beckman Model F-3 analyzer; carbon dioxide, with a Model LB-1 Liston Becker analyzer, and carbon monoxide, with a Liston Becker Model 15-A infrared analyzer. All determinations are made either continually or at one-hour intervals of the algal chamber affluent and effluent gases. Other parameters measured during an experiment are shown in Figures 7 and 8.

Another problem confronting us in the manipulation of algal photosynthetic gas exchangers is bacterial contamination within the exchangers.

This problem of algal growth assumes even greater significance when considering the possibility of utilizing algae as part of a closed ecologic system for manned space or interplanetary operations.

\*Klett-Summerson Photoelectric Colorimeter.

On many occasions, the gas exchange rate of algae in our gas exchange units decreased significantly in thirty-six to 108 hours after original inoculation.

antibiotics have been published.<sup>14,19</sup> As the algal cultures age, these specific antibiotics disappear and contaminating bacterial flora increase.<sup>10</sup>



Fig. 5. Algal photosynthetic gas exchanger basically composed of two parts. The algae are collected in a hemispheric container and continuously recycled over a conical illuminated surface.

This was characterized by a sharp increase in carbon dioxide, a depletion of oxygen in the system, loss of optical density, and development of a distinct yellow-brown color and a foul odor. Invariably large quantities of *Pseudomonas* were cultured from the medium. Initial attempts to control this contamination by sterilization of the physical system with ethylene oxide to eliminate subsequent contamination and to allow maintenance of sterile algal cultures met with little success.

After the autoinhibitor, chlorellin, was found in filtrates from *Chlorella*, many reports on algae excretion of

Polymyxin-B Sulfate\*\* exhibits potential useful antimicrobial activity against a wide variety of Gram-negative microorganisms, and it reportedly functions more efficiently than most other antibiotics and chemotherapeutic agents in the control of infection caused by *Pseudomonas sp.*<sup>2</sup> Although it had been reported that most commercial antibiotics consistently have shown a toxic or inhibitive effect in the test tube, a recent publication cites the feasibility of the practical use of Polymyxin-B Sulfate, to protect open

\*\*Source: Burroughs Wellcome & Co., Tuckahoe, New York.

cultures of *Scenedesmus obliquus* and *Chlorella vulgaris*.<sup>10</sup>

A series of experiments was designed to test Polymyxin-B-Sulfate on algal growth and photosynthesis in a

hibition occurring upon the introduction of 20 units and a range of dilutions up to 1,000 units of the antibiotic per ml. of algal suspension. The tolerance varied with algal species.

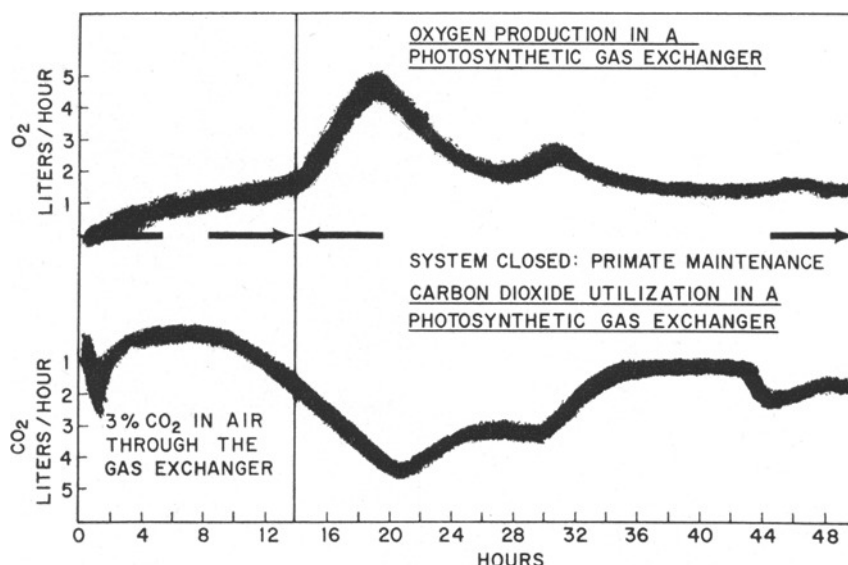


Fig. 6. Forty-nine-hour experiment utilizing gas exchanger shown in Figure 4 to maintain Rhesus monkey.

rapidly metabolizing algal species. Results indicated that proper concentrations of this antibiotic can be used effectively to control bacterial contamination in algal cultures without depressing algal growth. In a series of five experiments, one unit of Polymyxin-B-Sulfate per ml. algal suspension did not inhibit growth in *Synechocystis sp.*, *Oocystis sp.*, *Chlamydomonas sp.* and *Chlorella ellipsoidea* T 37-2. Furthermore, one unit of Polymyxin-B-Sulfate can be added to an algal culture on a 24-hour basis without inhibition of algal growth.

In one algal form tested, *Synechocystis sp.*, the introduction of two units of Polymyxin-B-Sulfate per ml. algal suspension showed slight inhibition of growth, with complete irreversible in-

hibition occurring upon the introduction of one unit of antibiotic in every twenty-four-hour period controlled but did not completely eliminate the contaminating agent, *Pseudomonas sp.*

Another area of primary concern in the development of an algal photosynthetic gas exchanger is the accumulation of carbon monoxide in the closed system and subsequent exposure of the occupant to the gas. Carbon monoxide has been reported to result from growth of green plants in a closed illuminated system.<sup>23</sup> Gafford and Craft previously reported as much as 800 ppm. of CO in a mouse-algal photosynthetic gas exchange system containing the alga *Anacystis nidulans*.<sup>9</sup> As shown in Figure 8, our recent experiments with a primate-algal photosyn-

thetic gas exchanger have shown 34 ppm. carbon monoxide accumulating in approximately four hours, with no further increases throughout the experiment.

of Dow-Corning Silicon Antifoam. In several tests, it has been found that 30 ml. of the antifoam per 14 liters of cell suspension would not inhibit oxygen production in a gas exchanger.

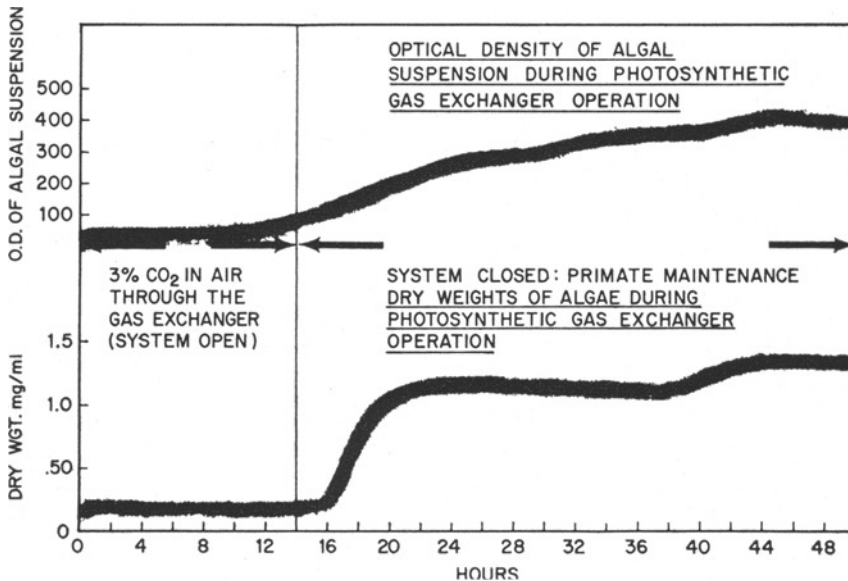


Fig. 7. Forty-nine-hour experiment utilizing gas exchanger shown in Figure 4 to maintain Rhesus monkey.

In a closed ecologic system, accumulation of carbon monoxide in quantities of 50 ppm. would pose a physiologically significant level. Fortunately, it has been found that small amounts of hopcalite† in a closed system, when heated to 100° C., will substantially reduce this deadly gas.

Two problems of mechanical malfunction within our gas exchange systems have been alleviated as follows: (1) Interference in the conduction of air from the gas exchanger and also loss of liquid volume occurs from extensive foam formation. The formation of large quantities of foam from the algal suspension within the gas exchangers has been reduced by the use

(2) It was found that the use of glass air dispersal devices within an algal system proved to be inadequate due to algal growth within the orifices and subsequent failure of the "aerating" system. Tygon and Nalgon tubing with approximately fifty perforations made with a 23-gauge hypodermic needle per cm. of tubing length serves as an excellent aerator, and can be used for thirty days with no malfunctions.

Although the major interest of our research to date has been directed toward the development and use of algal gas exchangers as a mechanism of maintaining the respiratory requirements of animals in a sealed cabin, recent interest has expanded to include other areas in the development of a closed ecologic system: algae as a

†Source: Mine Safety Appliance Co., Pittsburgh, Pennsylvania.



source of food, the recycling of metabolic waste products as nutrients for the algae, and the subjecting of algal cells to conditions simulating those encountered on space "flights."

gas exchanger supplying the respiratory requirements of one man. In view of the nutritional qualities, most of these algae can be used as a source of food for the occupant of a closed sys-

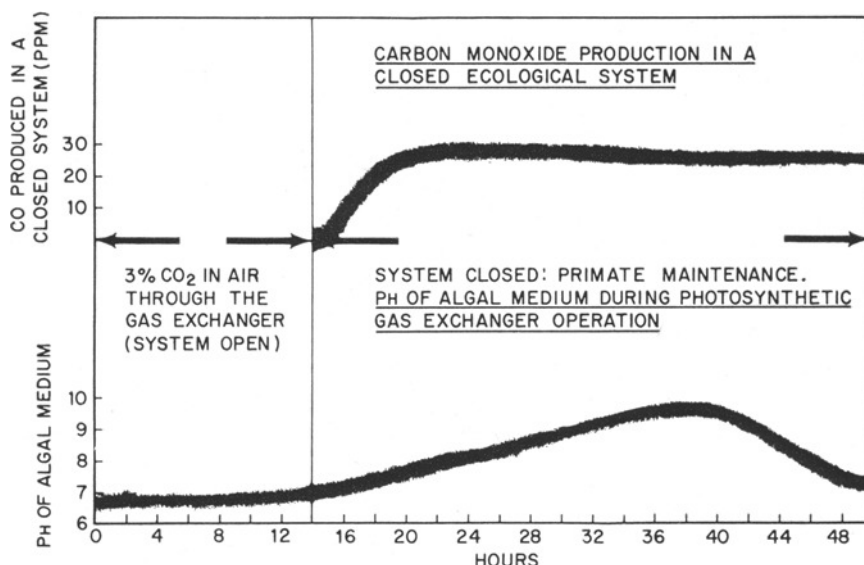


Fig. 8. Forty-nine-hour experiment utilizing gas exchanger shown in Figure 4 to maintain Rhesus monkey.

*Algal Toxicity Studies.*—Algae harvested from a photosynthetic gas exchanger could serve as one source of food for the astronaut; since the use of algae as a source of food for human consumption is now widely known. Several marine algal species have been collected and processed for food in Japan, England, and in the Northeastern United States. The Work of Milner,<sup>18</sup> Fink,<sup>6</sup> and Fisher and Burlew,<sup>7</sup> have confirmed that generally algae contain the essential amino acids, and a high content of vitamins, and have proved in animal feeding tests that at least some algal forms are nutritionally equal to the best animal protein.

It has been reported<sup>5</sup> that 552 gm. of algae containing 50 per cent protein could be harvested per day from a

tem. It would be essential to either cook the algae, or use some other method to make the bulk of the nutrient material available for human enzyme digestion. Studies of this nature will begin shortly at the School of Aviation Medicine. To date, only a few studies to examine toxicity of selected strains of algae have been accomplished.

Blue-green algae have been reported to produce a toxic material that sometimes causes the death of cattle drinking from pond water in which algae have accumulated.<sup>21</sup> In many cases, algal cultures are said to produce a "slow-acting" neurotoxin which results in neurologic symptoms resulting in death of mice in less than three hours.<sup>22</sup> It has been concluded that

the toxic material, not present in the active form in fresh algae, develops or is released by partial putrefaction of the algal material. This work has led to two theories of toxin production in blue-green algal cultures: one theory indicates that the neurotoxin is produced by the bacteria associated with the algal growth and not the algal cells themselves.<sup>22</sup> The second theory called the "infection theory" assumes that algal cells are actually infected with bacteria.

appeared normal with no paralysis or other abnormal activity noted throughout the fourteen days of observation.

*Algal Nutrition in a Closed Ecologic System.*—In a manned closed ecologic system, metabolic wastes will accumulate unless there is adequate disposal or appropriate recycling. One human waste material of potential interest is urine. Concern is focused on its potential capability for furnishing a fixed nitrogen source in the form of urea

TABLE I. TOXICITY TEST FOR SYNECHOCYSTIS SP.

Group*	Procedure	Toxicity
I	<i>Synechocystis</i> sp. was harvested by a Sharples centrifuge from an operating gas exchanger, dried by incandescent lighting, resuspended in saline and injected I.P., 480 mg. dried algae/kg. body weight. Total volume of injection: 2 ml.	All negative
II	Same as Group I except algae resuspended in fresh Medium C <sup>18</sup> .	All negative
III	Same as Group I except algae resuspended in its own supernatant.	One death in six days; four negative
IV	<i>Synechocystis</i> sp. was harvested from gas exchanger by centrifugation. Algae was washed with physiologic saline and wet algae resuspended in saline and injected I.P., 480 mg. wet algae/kg. body weight. Total volume of injection: 2 ml.	All negative
V	Same as Group IV except algae resuspended in its own supernatant.	All negative
VI	Supernatant separated and injected I.P. Volume of injection: 2 ml.	All negative
VII	Controls. Physiologic saline injected: 2 ml.	All negative

\*Each group consisted of five Sprague-Dawley rats of the same age group.

A blue-green algae, *Microcystis aeruginosa*, was reported to produce 100 per cent mortality in mice at doses of 22 mg. per kg. body weight after intraperitoneal injection of resuspended freeze-dried algae when the material had been incubated.<sup>22</sup>

To test the toxicity of *Synechocystis* sp., preliminary experiments were devised following a modification of the method of Thompson.<sup>22</sup> The procedure and results are presented in Table I.

Those animals reported in Table I indicated decreased activity for approximately fifteen minutes after injection. After this period of time, the animals (with the exception of one)

for growth of algae. It is estimated that 432 gm. of fixed nitrogen and 28.8 gm. of nutrient salts per twenty-four hours, with recycling of water, will be required to support necessary algal nutrition in a one-man support photosynthetic gas exchanger.<sup>5</sup>

In order to consider recycling of metabolic waste as nutrients for the algae it was necessary to evaluate the toxicity effects of urine on algae.

To test the toxicity of human urine, the alga *Synechocystis* sp. was grown in Medium C and a range of concentrations of pooled raw human urine. Four series of growth experiments were performed of from one to three

days' duration. Results consistently indicate that one part urine (or less) to nine parts of algal suspension in Medium C would support algal growth for the one- to three-day period. Higher concentrations of urine would occasionally support algal growth, but growth patterns were erratic.

Attempts were then made to determine the effect on growth and nitrogen utilization of algae with the nitrogen in human raw urine replacing the nitrogen in the Medium C.<sup>15</sup> Use of the alga *Synechocystis sp.*, poses a special problem area for there is conclusive evidence of fixation of molecular nitrogen in cultures of blue-green algae.<sup>1</sup>

The problem could be stated as follows: (1) If the atmosphere in a space vehicle contains no inert gas as nitrogen, it would be advantageous to have nitrogen supplied from metabolic waste products. (2) If the sealed cabin atmosphere contains nitrogen, removal of this nitrogen by algal fixation would make it unavailable to the astronaut.

Either of these statements indicates the advantage of utilizing the nitrogen from metabolic wastes for algal nutrition in a photosynthetic gas exchanger.

As *Synechocystis sp.* has not been fully examined with respect to utilization of nitrates from metabolic wastes, experiments were devised to determine the ability of this alga to utilize nitrogen from urine for growth.

Three sets of tubes were prepared: the first set contained algae in stock Medium C,<sup>18</sup> the second contained algae in Medium C minus the nitrate constituents, and the third contained Medium C minus the nitrate constituents ordinarily present but containing a quantity of urine sufficient to replace the exact amount of absent nitrates in terms of nitrogen.

All tubes were aerated in a water bath with the same amounts of carbon dioxide. Optical density readings were made every four hours for twenty-four-hour periods.

Results from sixty tubes in two separate experiments showed that the *Synechocystis sp.* in stock Medium C had a 68.1 per cent increase in optical density after twenty-four hours, whereas the algal cells in Medium C minus nitrate constituents had 55.9 per cent increase in optical density. The cells utilizing the nitrate constituents of raw human urine had an average increase in optical density of 63.5 per cent.

From these experiments, it was concluded that *Synechocystis sp.* will grow with the nitrogen supplied from human urine; however, the growth was somewhat inferior to that in synthetic Medium C.

The use of a photosynthetic gas exchanger in a sealed cabin to provide for the sustenance requirements of the astronaut demands that the living algal cells as well as other plant and animal members of a closed ecologic system be subjected to the same altered physiologic environments as those confronting the astronaut. These conditions include exposure to an artificial atmosphere and the threat of decompression with the ensuing reduced pressure. Several experiments were carried out in which algae were subjected to such conditions.

*Algae in a Space Cabin Environment.*—Alga, *Synechocystis sp.*, in liquid cultures were exposed to a potential space cabin atmosphere of approximately 191 mm. Hg total pressure with no inert gas present. Of the 191 mm. Hg total pressure, 164.8 mm. Hg was oxygen, 5.2 mm. Hg carbon

dioxide and  $P_{H_2O}$  was 21 mm. Hg. After a 120-hour exposure to this atmosphere, the experimental tubes exhibited a 3 per cent increase in optical density as compared to an identical 3 per cent increase in optical density in the control tubes.

A series of experiments was performed in which liquid cultures of algae and potted plants were subjected to reduced pressures of 380 mm. Hg for seven-day periods. Illumination to both control and experimental plants was constant.

Results indicated that *Chlorella ellipsoidea* T 37-2, *Synechocystis sp.*, and an unknown alga collected at Yellowstone National Park (SAM 135), showed no significant decrease in growth when exposed to this pressure for seven days. The broad-leaf plant *Begonia lucerna* showed no objective symptoms of decreased viability when exposed to the same conditions.

To determine if algal cells in a photosynthetic gas exchanger would become ineffective as a life-sustaining mechanism for an astronaut if the algal system should be decompressed in any way, algal cells were rapidly decompressed from ambient to reduced pressures.

The algae used in the test were *Synechocystis sp.* and *Chlorella ellipsoidea* T 37-2.

In the first series of experiments, all culture tubes of these species were rapidly decompressed from sea level to 380 mm. Hg pressure. One group of these tubes was returned to sea-level pressure immediately at a rate of 3000 feet per minute, a second group was recompressed after five minutes at 380 mm. Hg at the same rate, and a third group was recompressed after thirty minutes at 380 mm. Hg—also at the same rate.

This procedure of varying the period of time at altitude after decompression would indicate potential effects other than those due to decompression, that is, altitude.

The tubes containing the decompressed algae and control tubes of algae were immediately placed in a constant temperature water bath and the growth in a twenty-four-hour period was determined.

The series of experiments was repeated in the same manner with the peak altitude achieved on decompression being 100 mm. Hg.

The algal forms, *Chlorella ellipsoidea* T 37-2 and *Synechocystis sp.*, exhibited no adverse growth characteristics.

#### DISCUSSION

Our results to date support the feasibility of an algal life-support system for the maintenance of respiratory requirements in a space cabin. In addition to our preliminary experiments supporting a primate with an algal photosynthetic gas exchanger, it has been shown that there are other advantages achievable through the use of algal cells as the photosynthetic mechanism: (1) Harvested excess algae may provide one source of food for the astronaut, (2) results have indicated that some of the metabolic wastes could be used to an advantage as nutrient material for the algae, and (3) the cells apparently have a high tolerance to those conditions possibly found in the space capsule.

Research with gas exchangers is continuing, with refinement, on the premise that this type of experimental tool offers possibilities of solving known problems and revealing, as yet, unknown areas of research.

## SUMMARY

This résumé presents a review of actual experimentation in progress at the USAF School of Aviation Medicine. The following information has been emphasized: a brief history of a closed ecologic system, the development of our present photosynthetic gas exchangers, results and problem areas associated with the use of these exchangers, and fundamental research on algal cells.

The use of an algal photosynthetic gas exchanger in the closed ecologic system is a feasible approach to the problem of life support in space travel.

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