8, 9), and blood trehalose obviously might be the transport form of this glycogen. Fat-body extracts of locust do synthesize trehalose from glucose (*10*). When the fat body of *Phormia* was examined, it was found that intact cells in vitro "secreted" trehalose into the medium at rates up to 300 μg/mg (dry wt.) per hour. In view of the observations listed above, and since four analyses of fat body showed trehalose concentrations of less than 26 μg/mg (dry wt.), glycogen is probably the main source of this trehalose. That the fat body can also synthesize trehalose from exogenous glucose and release it into the medium was demonstrated by incubating intact fat body with glucose-U-C^{14} having a specific activity of 2620 count/min per micromole. Trehalose isolated from the medium after 30 min of incubation had a specific activity of 822 counts/min per micromole, and this accounted for 93 percent of the total counts, other than glucose, found in the medium. Preparations of midgut, flight muscle, and blood were unable to produce trehalose when assayed in this way.

The present results (*11*) and other observations (*12*) lead to the conclusion that the fat body produces trehalose during flight and that the concentration of this sugar in the blood determines, at least in part, the rate of energy expenditure by the flight muscles. This finding complements Sacktor's views of the regulation of flight-muscle metabolism (*1*).

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12. A paper describing the relationships between energy sources in fat body, blood, and muscle is in preparation.

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Long-Term Nontoxic Support of Animal Life with Algae

Abstract. One 40-g male albino mouse was kept in good health for 66 days in a chamber containing an algal photosynthetic gas exchanger. Carbon dioxide was well controlled, oxygen was slowly increased, and nitrogen was decreased in the chamber. Photosynthesis can support life in a hostile environment for extended periods of time.

One method of reducing the weight of the contents of an inhabited space capsule or vessel for long voyages in a hostile environment is to recycle the constituents of the initial load, utilizing solar energy to reverse the entropy-increasing reactions required for living. Such recycling is done on a much larger scale on the earth's surface, where chlorophyll-containing plants use waste CO_2 from animals, decay, and combustion to create food and fuels, and to restore O_2.

Photosynthetic gas exchangers are of use in life-support systems. Unicellular plants or algae have been used with animals because they are efficient in the use of light, they can be cultured in relatively simple liquid media, and they produce little fiber or nonfood material. The algae take up CO_2 and give off O_2; the animal in the system uses O_2 and produces CO_2 which regulates the growth of the algae. In theory, when the respiratory quotient of the animal is the same as the assimilation quotient of the plant, a stable state is reached.

Myers maintained mice for various periods of time by gas regeneration with algae (*1*). The U.S. Air Force School of Aerospace Medicine has supported various animals, including monkeys (*2*), on algae for short periods of time. At the Chance Vought Research Center mice have been maintained for as long as 28 days (*3*). A 66-day run was completed successfully in August 1960.

The apparatus used was described previously (*3*). The algal chamber contained 4 liters of 1 percent packed cell volume of Sorokin thermophilic strain of *Chlorella pyrenoidosa* (*4*). The mouse chamber was a Scheibler desiccator, 250 mm in diameter, containing vermiculite bedding, a supply of Purina food pellets, and a supply of water. Air was circulated from the mouse chamber through the algal culture at 39°C; it was dehumidified by a condenser cooled to 5° to 15°C and returned to the mouse chamber. The algal culture received 300 ft-ca of fluorescent light on its outer surface and about 400 ft-ca on its inner surface; the culture thickness was ¾ in. The algal medium was that recommended by Myers (*3*). It was made from Knops-urea, microelements, iron sulfate, and ethylenediamine tetraacetate as chelating agent and adjusted to *p*H 6.8 with 2*N* potassium hydroxide. One drop of Silicone Antifoam A was added to new medium occasionally to prevent foaming of the culture. The medium was harvested and replenished by adding fresh medium from a separatory funnel at the top of the algal chamber at the same time that the culture was withdrawn from the bottom with the pump turned off. A water manometer was watched so that pressure in the system was maintained with minimal change during, and with no change after, harvesting.

A male mouse, weighing 38.9 g, about 1 year old, was sealed up in the desiccator at noon on 7 June 1960. Leaks in the system were sought with a soap solution, and none were found; later checks were also negative. Harvesting and replacement of the medium was done each Monday, Wednesday, and Friday. The packed cell volume of the algae was kept between 0.6 and 1.7 percent and usually varied from 0.7 percent after harvest to 1.3 percent before. A total of 53 liters of algae were removed during the experiment; this contained approximately 131 g of air-dried algae. After the system was opened on 12 August at 3 P.M., there was about 200 ml more of algal culture than there had been at the start. Since 400 ml of gas were removed for analysis, and since the food and water consumed occupied some space, these decreases in the total gas volume in the system must be balanced by the extra culture fluid and also by added gases in the medium admitted at 28°C and by removed gases in the culture at 39°C. The total gas volume was estimated at 5 liters. Room temperature was stabilized by air-conditioning at 28° ± 1°C.

Gas was removed from the latex rubber tubing to the mouse chamber with a hypodermic needle and syringe. On 19 occasions, three times weekly at first and once weekly later, samples of 20 to 22 ml of system gas were analyzed in duplicate, within 2 hr, on a Perkin-Elmer gas chromatograph standardized with known gas mixtures and controlled each time with room-air analyses. A silica gel column and 1-ml samples were used for CO_2; a molec-

ular sieve column and 0.25-ml samples were used for O_2. At the start of the experiment the chamber air contained 0.4 percent CO_2 and 21 percent O_2. Before the chamber was opened on the last day, it contained 0.13 percent CO_2 and 63 percent O_2. Oxygen had slowly accumulated to 21.5 percent after 1 day, 23.5 percent after 3 days, 26 percent after 8 days, 34 percent after 13 days, 40 percent after 17 days, 43 percent after 30 days, 53 percent after 40 days, 55 percent after 48 days, and 58 percent after 61 days. Carbon dioxide was well controlled, usually being analyzed at 0.2 percent or less. Only one analysis exceeded 0.8 percent (31st day). A value of 5 percent was obtained on the 28th day, after a weekend when algal growth had attained 1.7 percent packed cell volume. The mouse did not show respiratory signs or lack of activity consistent with this high value. Analysis had shown 0.2 percent CO_2 on the 24th day, so the analysis was considered to be in error for unknown reasons.

At no time were gas chromatograph peaks for methane or ethane seen. The chamber contents were checked for CO with the Mine Safety Appliances analyzer at the end of the experiment, and no accumulation of CO was found. Both room air and chamber gas were alike, containing less than 0.001 percent CO. The CO reached toxic levels in blue-green algal gas exchangers (*5*).

The mouse was normally active, though confined, throughout the run. It weighed 43.5 g when removed, a weight gain of 4.6 g. It remained healthy for more than 9 months after the experiment. The increased O_2 in the atmosphere (30 percent rising to 60 percent over a period of 50 days) and restoration to 21 percent had no adverse effect.

Odor in the mouse chamber containing the accumulated excreta of 66 days was considerably less than that of an uncleaned cage after 1 week. This is explained by the fact that relatively dry air entered the chamber and by the deodorant action of the algal culture. No activated charcoal or other absorbent was used. Ammonia, volatile fatty acids, hydrogen sulfide, and mercaptans would be metabolized and removed by the algae. Chlorophyll has also been claimed to be a deodorant.

No precautions were taken to begin with or to maintain a pure culture of *Chlorella*. Bacteria were present, but created no problem. Fecal bacteria in a previous experiment (*3*) had demonstrated that their aerobic metabolism can disrupt the functioning of the system. Close to 400 ml of tap water were used by the mouse, except for a slight loss by wastage and evaporation. Exhaustion of this supply was the reason for terminating the experiment.

The variation in chamber N_2 is unexplained. With the rise in O_2 and the maintenance of CO_2, the N_2 decreased progressively. Nitrogen storage in the mouse's weight gain could explain a very small part of the decrease. It is estimated that about 2 liters of N_2 disappeared from the chamber air. Removed culture should have contained less than the added medium because the temperature change reduced the nitrogen's solubility. Undetected leaks should also have the effect of increasing low values of N_2. *Chlorella* does not fix N_2. Bacteria could, but this seems to be a large amount of nitrogen fixation, and in other experiments no evidence for nitrogen fixation has been found. The oxygen buildup can be explained by imbalance in the respiration quotient of the mouse and the assimilation quotient of the algae. This could be prevented by use of ammonium salts as the nitrogen supply in the medium, and such an experiment might supply an answer to the N_2 picture.

One minor accident occurred on the 22nd day. While the medium was being admitted, the separatory funnel was inadvertently emptied, and room air estimated to be 100 ml or less entered the chamber. This would be 2 percent or less of room air added to chamber air of 0.2 percent CO_2, 40 percent O_2, and 59.8 percent N_2.

An average man's weight is three orders of magnitude greater than that of this rather large mouse. The respiratory exchange is two orders of magnitude higher. The present experiment indicates 100 gallons of 1 percent packed cell volume of algae could support life for one man. The lighting was designed for the standard strain of *Chlorella*. Optimum lighting for the thermophilic strain is much higher (*6*). Improved performance can be developed even in the absence of gravity where the separation of gas from liquid is a problem. Relatively long-term safety and reliability have been shown for this system.

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Diageotropism in Vanilla Roots

Abstract. Diageotropic growth in the dark and geotropic growth in the light occurred in the roots of cuttings of three *Vanilla* species. The diageotropic response also occurred in far-red, red, orange, and green light, while positive geotropism resulted only if blue light was present.

Diageotropism, or the orientation of plant parts at right angles to the direction of gravity, was mentioned by Darwin (*1*) as occurring in certain rhizomes and probably in some secondary roots. Bennet-Clark and Ball (*2*) reported that the diageotropic response of the rhizome of *Aegopodium* occurred only in the absence of light and that positive geotropism occurred in the presence of light of any color. They suggested that growth curvatures in response to light and darkness served as a depth-regulating mechanism. A search of the available literature did not reveal reports of a similar light-dependent mechanism in roots, or of a plant with a diageotropic terrestrial roots system.

The pendent roots of *Vanilla* vines may grow downward as far as 30 ft before reaching the ground. After they reach the ground, the roots turn at right angles and ramify through the accumulated organic matter and seldom penetrate the mineral soil. Although the physical resistance of the soil may be a factor in controlling root growth, recent work at this station indicates that light may play a major role in determining the direction of growth of *Vanilla* roots.

To determine the effect of light on root growth, single-node cuttings of *Vanilla planifolia* Andrews with at-