never occurs; the cartilage persists through life, and it is never completely eroded nor sealed off as are the epiphyseal plates.

4) Endocrine experiments in rats have revealed differences in the hormonal control of growth activity between the epiphyseal plates and the condylar heads. After thyroidectomy, growth hormone elicited a greater response in the condyles than in long bones, while the latter responded better to thyroxin (*7*).

The condylar cartilage holds a unique position among endochondral growth centers.

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23 January 1961

Blood Trehalose and Flight Metabolism in the Blowfly

Abstract. The concentration of trehalose in the blood of *Phormia regina* was found to determine the rate of energy expenditure during flight as reflected in measurements of the wing-beat frequency. Fat body was found to be the source of blood trehalose; either endogenous or exogenous substrates are used for its synthesis.

The function of the nonreducing disaccharide, trehalose, in insect blood has been the subject of a number of studies (*1*). In the adult blowfly, *Phormia regina* (Meig.), it has been found previously that trehalose is the main carbohydrate in the blood (up to 3 g/lit.), that glucose also is present but at much lesser concentrations, that both sugar concentrations are a function of nutrition, and that trehalose concentration falls during flight (*2*). We have since examined this last point in more detail and have sought the source of blood trehalose.

For flight, males were maintained on 1*M* glucose for 4 to 5 days after eclosion and then mounted by a thin support glued to the dorsum of the abdomen. To promote flight, the tarsi were removed (*3*). About 60 percent of such flies flew regularly for long periods; these flies were selected for study. The wing-beat frequency, shown to be a reliable measure of the rate of energy expenditure (*4*), was measured stroboscopically. Blood carbohydrates were assayed chromatographically and colorimetrically as before (*2*).

The wing-beat frequency fell during prolonged flight, as reported by others (*5*, *6*), and blood trehalose fell with it (Fig. 1); blood glucose did not change significantly. The duration of flight to exhaustion was 2 to 3 hr. These results suggested that substrate availability might directly determine the wing-beat frequency, since it has been shown that, after flight to exhaustion, feeding of suitable carbohydrates can bring about almost immediate resumption of flight for long periods (*7*). Accordingly, the effect of trehalose injections on the wing-beat frequency was measured over the physiological range of the frequency.

Flies were flown to complete exhaustion (*7*), and the wing-beat frequency at which they stopped was designated as the "exhausted wing-beat frequency." Each fly was then injected serially with 52, 105, and 210 μg of trehalose dissolved in saline. In all cases 0.524 μl (standard error, $\pm$0.008) was injected through a fine glass needle on a microinjection apparatus. After each injection, the fly was rested for a 3-min equilibration period and then flown. The wing-beat frequency was recorded every 10 sec for the first minute of flight; then these values were averaged and designated as the wing-beat frequency after injection. The flies were again flown to exhaustion after each injection. After the last injection, each fly was fed 2*M* glucose to repletion, rested for 30 min and flown again. The wing-beat frequency thus obtained was designated as the wing-beat frequency after feeding. This value may be considered as the maximum for a given fly.

Table 1 summarizes the results of these experiments. Clearly, the injections of trehalose did increase the wing-beat frequency, and the amount of the increase over the level at exhaustion was directly related to the amount of trehalose injected. The greatest amount (210 μg) restored the wing-beat frequency to the level found after feeding. These results indicate that the wing-beat frequency under these conditions was determined by the concentration of trehalose available to the flight muscles. Glycogen in flight muscle undoubtedly is also an important energy source, and its concentration has been related to the wing-beat frequency in *Drosophila* (*5*).

Table 1. Wing-beat frequency (WBF) as a function of blood trehalose administered by injection. Each exhausted fly was serially injected with the three quantities of trehalose, in the same volume of saline, and re-exhausted between injections. The "fed" value was obtained from the same flies after the last injection by feeding to satiation and resting for 30 min.

Treatment	Mean WBF (cy/min)	%
Exhausted	7,460	67
Injected		
52 μg	8,800	79
105 μg	9,750	88
210 μg	10,950	99
Fed	11,020	100

Phormia regina has been estimated to expend carbohydrate during flight at a rate of about 15 μg/min (*8*). At this rate the total amount of trehalose in the blood (about 200 μg maximum) could support flight for only a few minutes unless it was continually being replaced. The origin of blood trehalose was therefore a question of considerable interest. Fuel for the flight of Diptera comes in large part from glycogen stored in the fat body (*7*,

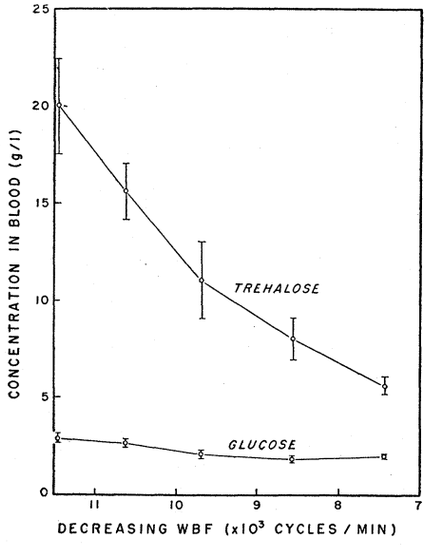

Fig. 1. Relationship between the wing-beat frequency (WBF) and the concentration of blood glucose and trehalose. The data were grouped by averaging observations of wing-beat frequency (at least 10 individual ones per point) made over intervals of 1000 cy/min from 12,000 to 7000 cy/min (which is approximately the exhausted frequency). The standard error of the mean (bars) was calculated for the sugar concentrations.

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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11. This investigation was supported by research grants from the National Science Foundation (G 5927) and the National Institutes of Health (E2358). One of us (J.S.C.) received support from a National Institutes of Health predoctoral fellowship, and from the Woodrow Wilson National Fellowship Foundation.
12. A paper describing the relationships between energy sources in fat body, blood, and muscle is in preparation.

20 February 1961

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-