and have relatively high half-saturation concentrations for inhibition (K_i for penicillin ~ 50 μM; K_i for LTC_4 >> 1 μM (Table 1) (*6, 10*). Certain prostaglandins are also transported from CSF into blood by a probenecid-sensitive system in choroid plexus (*6*). The relation of the leukotriene transport system in choroid plexus (Table 1) to the transport systems for penicillin, prostaglandins, and other weak acids remains to be determined. Fourth, the fact that leukotrienes, when injected into the blood, achieve extremely low levels in brain and presumably CSF (*2*), is probably due to their poor penetration into CSF and active transport back into blood. Penicillin is handled in this fashion by the choroid plexus in vivo (*6, 10*).

The transport system for leukotrienes in choroid plexus can probably transport leukotrienes effectively out of the extracellular space of brain, since there is no anatomical barrier to the diffusion of substances from the extracellular space of brain into CSF (*7*). It is also possible that cerebral capillaries have a comparable system for direct transfer of leukotrienes from extracellular space of brain into blood.

Finally, we speculate that the transport system for leukotrienes in choroid plexus would protect the central nervous system from the vasopermeability-inducing and vasoconstrictor activities of leukotrienes synthesized in brain after cerebral ischemia (*3*) or when blood enters the CSF as in a subarachnoid hemorrhage (*4*). Blood in CSF may also interfere with choroid plexus function. Answering these questions will require in vivo studies to define the function and specificity of this system and the effect of disease states on leukotriene transport.

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Mechanism of Aeration in Rice

Abstract. *Mass flow of air to the submerged parts of the plant constitutes the major mechanism of aeration in partially submerged rice. It is proposed that the flow of air results from reduction of pressure in the air-conducting system of the plant caused by consumption of oxygen and solubilization of respiratory carbon dioxide in the surrounding water.*

Most of the world's rice is grown on land that is flooded for at least part of the season. These conditions greatly limit the availability of O_2 to the submerged parts of the plant. Aeration problems are especially severe for deep-water or floating rices, which grow in water as deep as 6 m and reach a height of up to 7 m (*1*). It has been postulated that the aeration requirements of the submerged organs of rice and other plants tolerant of partial flooding are met by O_2 entering the above-water parts of the leaves and moving by diffusion through internal air spaces to the submerged organs (*2, 3*). In rice these air spaces are particularly well developed in the culms (*4*) and roots (*5*). Using excised leaves, we have demonstrated that continuous air layers trapped between the hydrophobic surface of rice leaves and the surrounding water form a low-resistance pathway for gas movement (*6*). We now report that air is moved to the submerged parts of the plant through the external air layers and internal air spaces primarily by mass flow rather than diffusion and provide evidence that this mass flow is driven by solubilization of respiratory CO_2 in water.

To demonstrate mass flow of air to the submerged parts of a rice plant, leaf tips protruding from the water were placed in a small leaf chamber that was inverted into a submergence tank and connected to a volumeter. Movement of air into the plant was monitored by recording the disappearance of air from the headspace of the leaf chamber with an angular transducer (Fig. 1). The rate of air intake was 4.9 ml/hour during the first period of light (Fig. 2). When the lights were turned off after 3 hours the rate of air intake abruptly increased fourfold but gradually declined over a period of 3

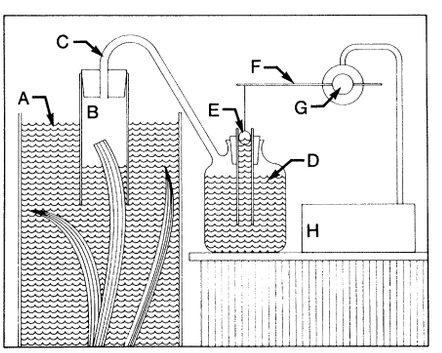

Fig. 1. Experimental setup to measure mass flow of gases. A rice plant was placed in a submergence tank (A) filled with water. One to six leaves were introduced into a leaf chamber (B) with an initial headspace volume of 50 ml. Before the start of the experiment the tips of these leaves were cut at the same height so that only a 5- to 6-cm portion of each blade was above the water level in the leaf chamber. All other parts of the plant were kept submerged. The leaf chamber was connected with Tygon tubing (C) to a volumeter (D) such that the height of the water column in the glass tube with the Styrofoam float (E) was always equal to the difference between the water level of the submergence tank and that of the leaf chamber. When air was withdrawn from the headspace by the plant the water level in the leaf chamber rose and the height of the water column under the float dropped. The float was linked by a rigid steel rod (F) to the lever of an angular transducer (G) to amplify the movement of the float. The transducer was connected, via a power source and an amplifier, to a chart recorder (H). Displacement of the recorder pen was directly proportional to the changes in the headspace volume of the leaf chamber and was calibrated in milliliters of gas withdrawn from the headspace or expelled into it. Calibration was performed by injecting into or withdrawing from the headspace known volumes of air with a gas-tight syringe inserted into the leaf chamber through the rubber stopper. Every 1 or 2 hours the leaf chamber was lifted from the submergence tank for 1 minute to replace the gases in the headspace with air and to readjust the height of the water column in the volumeter. We verified that the rate of gas movement into the rice plant was not affected by the small increase in pressure in the leaf chamber. The results were essentially the same with atmospheric or a slightly negative pressure inside the leaf chamber.

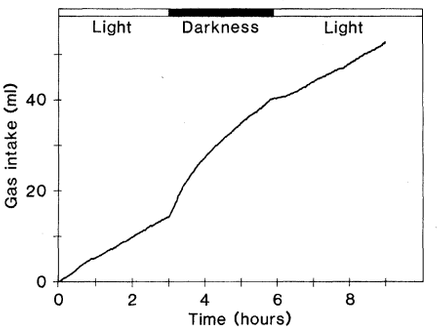

Fig. 2. Mass flow of air into the underwater parts of a partially submerged rice plant. A rice plant (*Oryza sativa* L., cv. Habiganj Aman II), raised as described previously (*12*), was lowered into a 120-cm-high, 300-liter plastic tank filled with deionized water. Photosynthetic photon flux density at water level was 350 μmol/sec-m^2, air temperature 24°C, and water temperature 28°C. All experiments were performed with 33- to 55-day-old plants. While Habiganj Aman II is a deep-water rice, similar results were obtained with plants belonging to other varietal groups. To monitor the mass flow of air into the rice plant the top six leaves were enclosed in the leaf chamber. After 3 hours the lights were turned off and air uptake in darkness was monitored for 3 hours. After this period the lights were turned on again. The graph shows a direct tracing of the chart recorder. This experiment was repeated five times with similar results. When the dark period was extended to 12 hours the plant continued to take up air until the end of the experiment, at which time the rate of uptake was about one-fifth that of the initial rate.

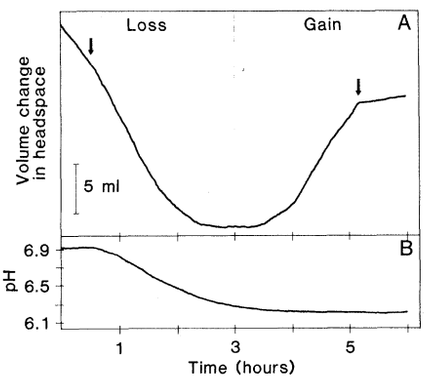

Fig. 3. (A) Effect of the concentration of CO_2 in the solution around the plant on the rate and direction of gas flow. A rice plant was placed in a 71-cm-tall plastic tank filled with 14 liters of 90 m*M* phosphate buffer (*p*H 6.91). Mass flow of gases was monitored as described in the legend to Fig. 2, except that four leaves were used for the measurements and the experiment was performed at a photosynthetic photon flux density of 100 μmol/sec-m^2 at an air and water temperature of 24°C. The recorder tracing on the left side of the graph represents loss of gas from the headspace of the leaf chamber (flow of air into the plant); the tracing on the right side represents gain of headspace volume (release of gas into the headspace). After the initial rate of air intake was determined for 30 minutes, CO_2 was bubbled through the solution with an air stone placed at the bottom of the submergence tank (left-hand arrow). To verify that the gain in headspace volume was not due to direct release of CO_2 from the solution, the leaves were removed from the leaf chamber after 5 hours, 10 minutes (right-hand arrow) and changes in headspace volume were monitored for another 50 minutes. Gas expelled by the plant between 4 and 5 hours after the start of the experiment contained 78.6 percent CO_2 and 2 percent O_2. Changes in the direction of gas flow were completely reversible. When a plant expelling gases from a CO_2-saturated solution was switched to a solution that had not been saturated with CO_2, air intake resumed at a rate comparable to that of a plant not previously exposed to high external CO_2 concentrations. This experiment was repeated seven times with similar results. (B) Change in *p*H in the submergence tank, as monitored with a *p*H meter equipped with a combination electrode and connected to a chart recorder. We verified that a change in *p*H from 6.9 to 6.2 did not in itself alter the mass flow of gases into the plant.

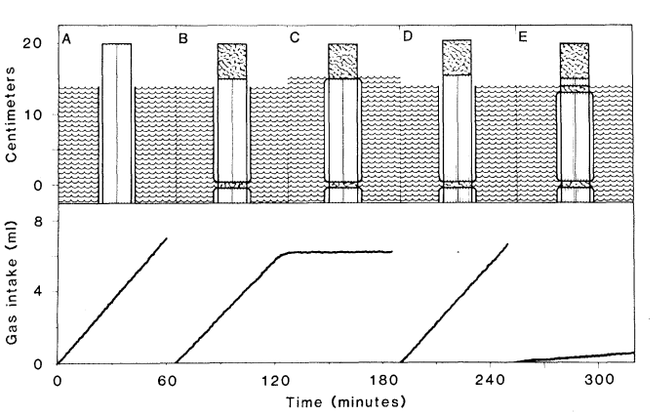

Fig. 4. (A to E) Role of air layers in the conductance of gases. The experimental procedure was the same as that outlined in the legend to Fig. 2, except that only one leaf was kept above water for the air intake measurements. Before the experiment the top 6-cm portion of the leaf tip was cut off and the cut surface was sealed with transparent nail polish. Of the remaining leaf, 6 cm protruded from the water into the leaf chamber. Stippled areas represent regions of the leaf blade that were covered with nail polish. This experiment was repeated three times with similar results.

hours to about 6 ml/hour. In the second light period the rate of air intake returned to that observed during the first light period (*7*).

The rate and direction of gas flow depended on the concentration of CO_2 in the solution surrounding the plant. When CO_2 was bubbled through the submergence tank filled with 90 m*M* phosphate buffer at *p*H 6.9, air uptake by the plant slowed progressively (Fig. 3A). The direction of mass flow was eventually reversed, with gas being expelled from the plant into the headspace of the leaf chamber. The degree of saturation of the phosphate buffer with CO_2 was monitored as the decrease in *p*H of the solution (Fig. 3B).

We suggest that uptake of air into the submerged organs of the rice plant is driven by the difference in the solubility of O_2 and CO_2 in water. At 25°C and *p*H 7, CO_2 (as CO_2, H_2CO_3, and HCO_3^-) is 140 times more soluble in water than is O_2. Consumption of O_2 in the submerged organs and solubilization of respiratory CO_2 reduce the pressure in the extensive network of internal air spaces and in the external air layers of the plant. The resulting pressure gradient causes a mass flow of air from the atmosphere into the internal air spaces through the stomata of the above-water foliage and the stomata of the submerged parts of the leaves, which are surrounded by air layers. Intake of air from the atmosphere continues as long as the CO_2 activity gradient favors net movement of CO_2 from the internal air spaces into the solution around the plant. The direction of gas flow is reversed when CO_2 activity in the solution becomes greater than that in the internal air passages and air layers. This causes net movement of CO_2 from the solution into the plant and expulsion of gas into the atmosphere.

The rate of air intake is lower in light than in darkness because photosynthesis reduces the concentration of CO_2 and increases the concentration of O_2 inside the air spaces of the shoot. In light the main "pull" for mass flow is created by the roots, whose well-developed internal air passages are separated from the soil water by a thin layer of cells (*5*). When the root of an illuminated rice plant, partially submerged in 120 cm of water, was severed from the shoot under water, the rate of air intake abruptly dropped tenfold (from 4.6 to less than 0.5 ml/hour) (*8*). The continuous decline in the rate of air intake in darkness (Fig. 2) has also been observed with isolated leaves (*6*). We ascribe this decrease to the replacement of O_2 consumed during respiration by an equal volume of air, that is,

by only one-fifth of the amount of O_2 used. Thus the concentration of O_2 in the submerged organs decreases continuously in darkness while that of N_2 increases, leading to a depression of the respiratory rate and a steady decline in the rate of air intake. As the concentration of O_2 inside the plant drops with increasing length of the dark period, the diffusional component of O_2 movement into the plant must increase (*9*).

Both surfaces of the submerged rice leaf are separated from the water by continuous air layers, the volume of which reaches 44 percent of that of the leaf (*6*). The role of air layers in the transport of gases from the atmosphere into the internal air passages is illustrated in Fig. 4. A single leaf protruding 6 cm from the water into the leaf chamber drew air from the atmosphere at the rate of 7.2 ml/hour (Fig. 4A). After 60 minutes both sides of this leaf were covered from the tip to a point 1 cm above the water with transparent nail polish to block the stomatal pores. At the same time, a 5-mm-wide ring of nail polish was painted on the leaf blade 14 cm below the water surface to interrupt the continuity of the air layers and to demonstrate that nail polish did not interfere with air conductance inside the leaf. The rate of air flow into the plant was not affected by these treatments (Fig. 4B). Withdrawal of air by the leaf caused the water level in the leaf chamber to rise. When the water level reached the area of the leaf blade that was covered with nail polish, the entrance to the air layers closed. This resulted in immediate and complete cessation of air intake (Fig. 4C). After the water level in the chamber was lowered to open the entrance to the air layers, the original rate of air intake was restored (Fig. 4D). The importance of the air layers was shown by the experiment represented in Fig. 4E. When another 5-mm-wide ring of nail polish was painted on the leaf just below the water surface so that the entrance to the air layers was blocked and only 1 cm of uncovered leaf remained above the water, the rate of air intake was reduced 12-fold (*10*). These results indicate that air from the atmosphere is drawn into the air layers and then passes into the internal air passages via the stomata, provided that a small portion of the leaf protrudes from the water and the entrance to the air layers is open. Air layers greatly decrease the leaf area that must be kept above the water to replace O_2 consumed in respiration and are essential under conditions of high flooding.

We know of only one other report of aeration in an aquatic plant, the water lily, proceeding by mass flow (*11*). In that case it was suggested that the movement of air is caused by thermal transpiration and hygrometric pressurization. Such a system of aeration could not operate in darkness, when the availability of O_2 inside the plant is particularly restricted. The mechanism of aeration that we propose for partially submerged rice plants is driven by solubilization of respiratory CO_2 in water. It functions in light as well as darkness and may also operate in other semiaquatic plants.

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7. Mass flow of air into rice plants was also demonstrated by the following experiment. A rice plant was partially submerged and all tillers except two were cut off below the water surface. The remaining two culms with leaves attached were trimmed to 7 cm above the water surface. They were enclosed in an inverted 30-ml syringe that was lowered into the water such that a 25-ml headspace of air remained above the two culms. The syringe was sealed with a serum-vial cap through which gas samples could be taken for O_2 analysis by gas chromatography. A rising water level inside the syringe indicated uptake of air by the plant. In light the plant took up 20 ml of air in 2 hours. The concentration of O_2 in the remaining 5 ml of headspace was unchanged (21 percent).
8. From four experiments in which we measured air uptake by illuminated plants with and without roots, we estimated that gas uptake by roots, 100 to 120 cm under water, was on the order of 4 ml/hour. Diffusion of O_2 over this distance could have contributed but a small fraction of the gas taken up by the roots. This was calculated from the equation for the diffusion of gases through a tubular structure (*2*), according to which the rate of diffusion is equal to $[DA\ (C_2 - C_1)]/L$, where D is the diffusion coefficient of O_2 in air, A is the cross-sectional area of the tube, C_2 is the concentration of O_2 at the source, C_1 is the concentration of O_2 at the sink, and L is the length of the diffusion path. The combined cross-sectional area of the air layers and of the internal air spaces of six leaves protruding into the leaf chamber of the volumetric setup is 0.08 cm^2 (*6, 13*). On the basis of the diffusion equation, the rate of diffusion of O_2 from a source containing 21 percent O_2 to a sink containing 0 percent O_2 through a 100-cm-long tube with a cross-sectional area of 0.08 cm^2 would be 0.12 ml/hour. This value is an overestimate for the diffusion of O_2 from the atmosphere to the roots of a rice plant 100 cm below the surface because the diffusion equation does not take into account the diffusional resistances in the plant.
9. The volume of air taken up by a partly submerged rice plant is of the same magnitude as the volume of O_2 consumed during respiration. The average dry weight of the shoot of a rice plant used in our experiments was 6.4 g and that of the root 1.0 g. Using these dry weight determinations and published respiration rates of rice roots and leaves (*14*), we have calculated an average respiratory rate of 3.8 ml of O_2 per hour for the root and 14.7 ml/hour for the shoot. In the experiment represented in Fig. 2, the initial rate of air intake in darkness was 17.7 ml/hour, or very close to the estimated respiratory rate of a whole plant.
10. If less than 15 cm^2 of a leaf with blocked air layers remained above the water, the air layers decreased in size until, after several hours, water was drawn into the plant infiltrating the air spaces. This demonstrates that a significant negative pressure will develop inside the air-conducting spaces of the plant when its access to air is restricted.
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Construction and Recovery of Viable Retroviral Genomes Carrying a Bacterial Suppressor Transfer RNA Gene

Abstract. *The integration of retroviral genomes into cellular DNA can induce mutations by altering the expression of nearby cellular genes and can serve to identify the gene affected. The construction of a retrovirus that stably carries a suppressor transfer RNA gene from* Escherichia coli *has allowed facile recovery of the viral genome in vectors marked with amber mutations. This virus can be used for rapid isolation of cellular sequences at the site of proviral insertion.*

Retroviruses are unique among viruses of vertebrates in that a DNA copy of the viral genome integrates into the host genome as an obligate step in the life cycle. This integration event often affects the expression of host genes near the site of the insertion (*1–4*). The insertions can be useful in identifying and cloning the affected host genes because the new provirus can be used as a hybridization probe for this region of the host genome.

One difficulty in identifying new provirus insertions in mouse cells is the presence of endogenous sequences with homology to the exogenous viral DNA (*5*). These sequences obscure the presence of the newly inserted viral DNA in hybridization experiments, even though probes with less homology to the endog-