THE CARBON DIOXIDE MACHINE, OR PLANTS ON THE MAKE

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Plants cover the world. They may be edible and intensely cultivated, or natural ground cover which is less useful but may be valuable as a natural resource or for cooking, heating, and industrial use, or just beautiful to look at; and sometimes they are serious pests. Usually plant growth is quite lush. However, there are many problem areas where plants have to face real problems in order to survive, such as salt pans in Australia or the rugged and inhospitable terrain north of the Great Wall in China. In fact, it is suprising that plants could grow in these unpleasant places at all. Once, in early geological times, plant growth was more lush, much more so than now, although we depend as much on today's plants for food as on yesterday's for coal and oil.

Why is plant growth not so lush as formerly? What are the problems that plants face growing in difficult or inhospitable situations? How are these problems solved? Plants are carbon dioxide machines. Carbon dioxide is the major nutrient for plants, and it is in short supply. Once there was a great deal of carbon dioxide in the atmosphere, but the plants of early days made profligate use of their raw materials, and now its concentration in the air is only 0.03%. For plants, the expression 'thin air' has special meaning! Plants also face a second problem: oxygen is a by-product of plant growth, and it is poisonous to plants. Finally (we will not consider minerals, although fertilizers represent an increasingly important problem in the world food supply) there is the problem of water that faces many plants. Land that is considered fit for agricultural use in Australia would be classified as a national disaster in North America! Plants nevertheless manage to grow in these areas in Australia. Let us look at some of the tricks plants have for dealing with this situation.

Water and carbon dioxide present a compound problem. Carbon dioxide, because of its low concentration, diffuses into the leaves down a very shallow gradient, while water diffuses out of the leaves down a very steep gradient (Fig I). This problem can be somewhat alleviated 38 by the deposition of an impervious cuticle on the epidermis of the plants. Unfortunately, although it is impervious to water it is also impervious to CO_2. This problem can be solved by having small holes, called stomata, in the surface of the epidermis. CO_2 readily 'squeezes up' as it passes through these holes, so that the cuticle and epidermis offer very little barrier to the passage of CO_2 into the leaf. Water vapor, being saturated inside the leaf, cannot squeeze up very much, and consequently the epidermis strongly hinders its diffusion outwards. This arrangement works satisfactorily for many plants. However, when a drought occurs, the plants must close their stomata in order to avoid water loss. Then, even though the sun is shining (and the sun is the source of all energy for plant growth) no photosynthesis can take place.

There are thus three basic problems: water, carbon dioxide concentration, and oxygen. I shall examine different solutions that have evolved in the plant kingdom, and a recent suggestion (from work done in collaboration with staff of the Atlantic Regional Laboratory while I was a guest there last year) on how seaweeds cope with these problems.

First I shall present the basic mechanisms of photosynthesis in broad outline. CO_2 enters a leaf through the stomata, and diffuses through the rather open mesophyll cells to the main photosynthetic tissue, which is the palisade layer near the top of the leaf. Sugars there formed must then diffuse to the leaf veins for translocation to other parts of the plant. The characteristic large air spaces require CO_2 to diffuse over long distances inside the leaf. Once the CO_2 has diffused to the photosynthetic cells, it moves (perhaps as bicarbonate - HCO^{3-}) into the chloroplast, where it encounters the photosynthetic carboxylase. In the majority of plants this is ribulose bisphosphate carboxylase (RuBPc'ase) which works as shown in Figure 2. The product of this carboxylase, two molecules of phosphoglyceric acid (PGA), are reduced, and used to make sugars, starch, and to regenerate RuBP, the CO_2-acceptor molecule. An overall diagram of the process is shown in Figure 3., illustrating the stomata, the problem of water loss, and the sources of energy that make the photosynthetic cycle run. This cycle is known as the C_3 cycle, because the first product of carboxylation is a C_3 compound. It is also known as the Calvin cycle, after its discoverer.

Certain plants have developed a special technique to enable them to live in extremely dry environments. Cacti can survive under conditions where ordinary plants would die. They take advantage of the fact that, although it may be very hot and dry in the daytime, it is often cool and quite moist at night. Their photosynthetic metabolism uses a different

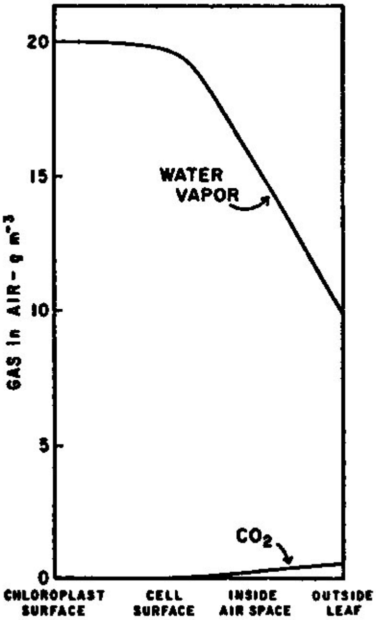


Fig 1. Diffusion gradients of water and carbon dioxide.

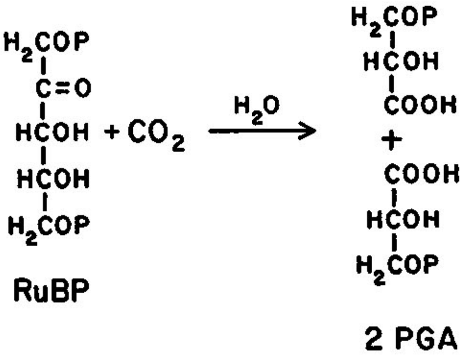


Fig 2. Ribulose bisphosphate carboxylase (RuBPcase).

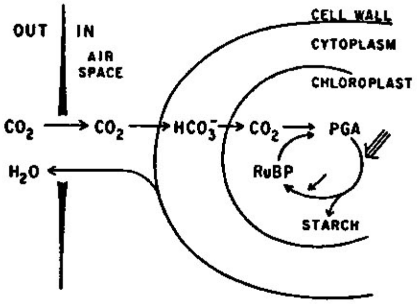


Fig 3. Outline of C_3 photosynthesis; arrows indicate input of light energy.

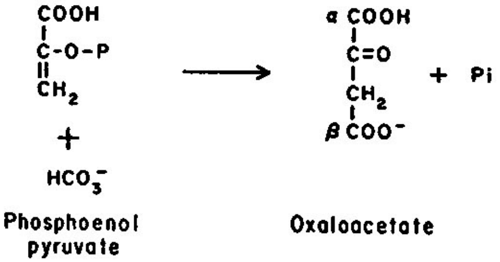


Fig 4. Phosphonenol pyruvate carboxylase (PEPcase).

primary carboxylase, called phosphenol pyruvate carboxylase (PEPcase), whose function is shown in Figure 4. This carboxylase uses bicarbonate (which is hydrated CO_2) instead of CO_2 as a substrate. The operation of this kind of photosynthesis, called Crassulacean Acid Metabolism (CAM) because it is characteristic in crassulacean plants, is shown in Figure 5. At night the stomata are open, the plants absorb CO_2 which is fixed by PEPcase with the manufacture of organic acids which build up in the plant. The substrate, PEP, is formed from the breakdown of starch that was made in the previous day's photosynthesis.

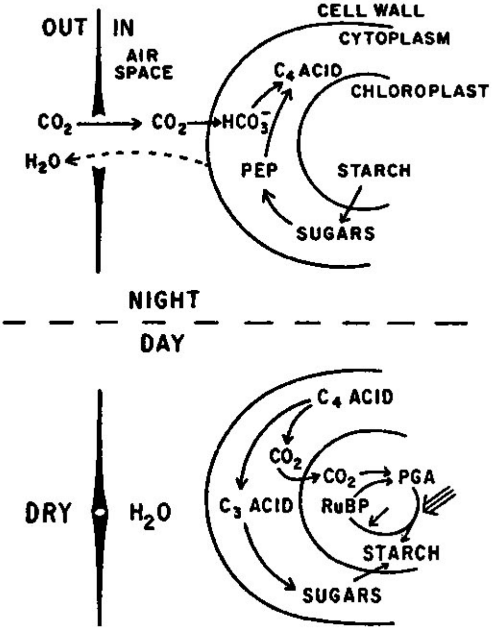


Fig 5. Outline of Crassulacean Acid Metabolism (CAM).

During the day, the stomata close to prevent water loss. Then all the acids that were formed the previous night are again decarboxylated, and the CO_2 so produced is fixed photosynthetically by RuBPc'ase in the usual way. The C_3 compound formed from the decarboxylation of the C_4 acids can also be made into sugars or starch, and is not wasted. CAM metabolism is not very fast, but it does enable the plant to survive under extreme conditions of drought. It may be so dry that the stomata can never open, day or night, for a period of months. This would kill ordinary plants because they would lose weight by respiration and eventually die. CAM plants, however, are able to refix any respiratory CO_2 that they lose, and so can survive even if they cannot grow.

A different problem is oxygen. I mentioned earlier that oxygen is poisonous. It turns out that RuBPcase is a 'short sighted' enzyme and is unable to distinguish easily between oxygen and CO_2. It evolved early in geological times when there was a lot of CO_2 and very little oxygen in the atmosphere, so that this characteristic of the enzyme was not important. However, during earlier eras plants behaved in a profligate manner and used up all their raw materials and poisoned the environment with their by-products (oxygen). In this respect they resemble the behavior of some more recent biological systems! Thus, the carboxylase also functions as an oxygenase. and when it fixes oxygen it makes one molecule of PGA and one molecule of a C_2 compound called glycolic acid, instead of two molecules of PGA (Fig 6). Glycolate is not of much use to the plant, but a C_2 cycle has evolved which enables the plant to trap much of the wasted glycolate and feed it back into the system (Fig 7). In this way, three-quarters of the carbon passing into glycolate (which may amount to a large proportion to total photosynthate) is salvaged and fed back in as PGA to the Calvin cycle. This helps, but the process, called photorespiration (because oxygen is consumed and CO_2 is produced in light), is very wasteful. Now PEPcase, which I described in connection with CAM metabolism, absorbs bicarbonate (HCO_3^-) instead of CO_2 and consequently has no problem with oxygen. An enzyme that recognizes bicarbonate has no difficulty in distinguishing between bicarbonate and oxygen. Certain plants have evolved a mechanism that uses this enzyme, PEPcase, to pump CO_2 into the inside of the leaf. This has two effects: oxygen ceases to be a problem to the initial carboxylase, which can thus absorb CO_2 readily, and CO_2 can be concentrated inside the leaf where it can then be fixed much more effectively by RuBPcase. In a sense, this is rather like throwing a football: if you

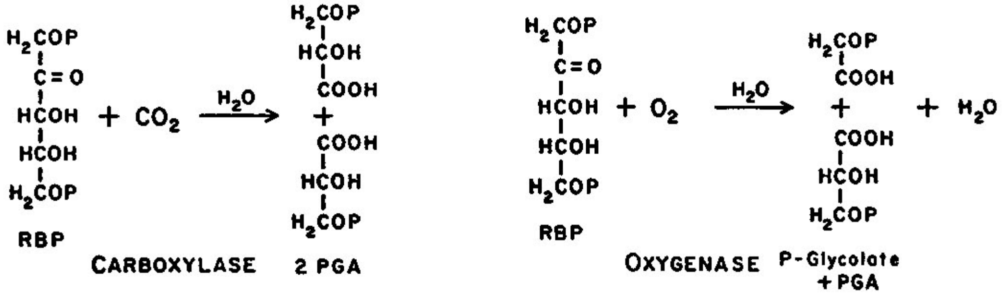


Fig 6. Ribulose bisphosphate oxygenase (RuBPcase).

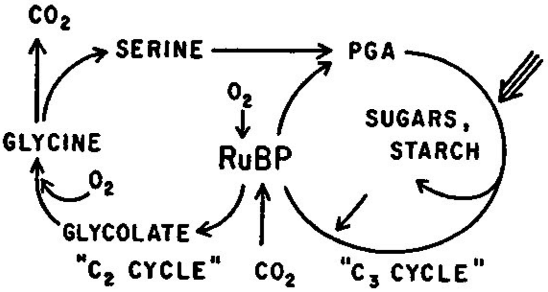


Fig 7. Outline of the C_2 cycle of photorespiration.

want to throw a handful of air from one place to another, the easiest way to do so is to wrap it up in a leather skin and throw that. In the same way, CO_2 is 'wrapped up' by being fastened onto a C_3 compound, PEP, to make a C_4 acid which can be 'thrown much more readily to the inside of the plant. Since first product of this kind of photosynthesis is a C_4 acid, it is called C_4 photosynthesis.

C_4 photosynthesis is shown in Figure 8. Carbon is fixed by PEP carboxylase in mesophyll cells (see Fig 14) and is then transferred, in the form of C_4 acids, to the bundle-sheath cells (cells that surround the vascular tissue) where it is released by decarboxylation of the C_4 acid and fixed by RuBPcase. The system thus requires cooperation between two cell types. In a conventional leaf there are considerable open spaces, and a long diffusion path that CO_2 must travel before it is picked up by the photosynthetic cells. In a C_4 leaf the air spaces are much smaller, the mesophyll more dense, and there is a pronounced ring of cells around each vascular bundle where the final carbon fixation takes place. This anatomical structure (called Kranz anatomy) is essential for effective operation of the C-$_4$ cycle. C-$_4$ plants usually have higher productivity (such as corn, sorghum, sugar cane, and a number of extremely persistent weeds such as fox grass, bermuda grass and crab

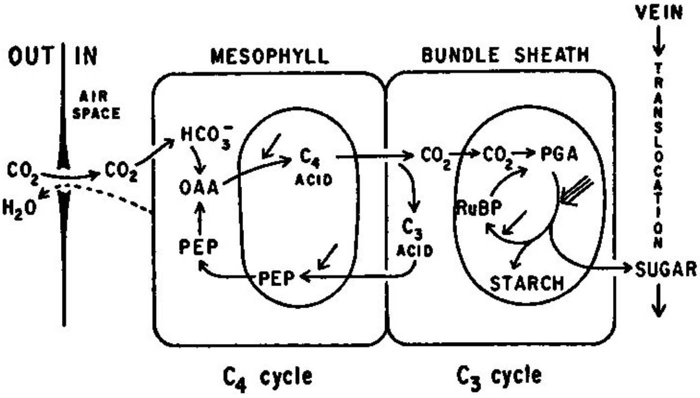


Fig 8. Outline of C_4 photosynthesis - integration of the C_4 (Hatch and Slack) cycle and the C_3 (Calvin) cycle; arrows indicate input of light energy.

grass) because they can make better use of carbon in bright light and under conditions when there is a shortage of water. They require some additional energy input to drive the C-4 cycle, but this is more than offset by the fact that PEPc'ase is a much more effective CO_2 trap, that the CO_2 has a much shorter diffusion pathway and so enters the leaf more quickly, and (because the C_4 cycle works well at low CO_2 concentration) the stomata need not open so widely and less water is lost. These plants, therefore, are able to function effectively under drought conditions that would stop a C_3 plant. Ultimately, of course, the productivity of the plant depends on the RuBPcase. In optimum conditions, C_3 plants may photosynthesize as rapidly as C-4 plants, and be just as productive. C_4 plants, however, are usually more effective because they can get ahead in hot dry weather when C_3 plants would have to shut down.

I shall now briefly consider seaweeds. They are important for food and for the chemical industry. In Nova Scotia, following the development of an experimental technology at ARL under Dr Neish and Dr Simpson, a number of industrial research stations have been developed for growing Chondrus and other seaweeds. The problem facing seaweeds is, of course, not water, but low CO_2, and its slow diffusion into and through seawater.

I worked at ARL last year on the gas exchange of seaweeds, which was measured with an infrared-gas analysis apparatus. It was necessary to measure CO_2 exchange in these plants in air because the sea contains large amounts of bicarbonate, which tends to equilibrate more or less rapidly with CO_2. It is virtually impossible to measure photosynthesis effectively, without very special precautions, when the plants are under water. With this apparatus, it was possible for the first time to make effective measurements of gas exchange in underwater plants. We noted at first that there is no effect of oxygen on the photosynthesis of marine plants, and they do not exhibit photorespiration. These are characteristics of C_4 plants. However we quickly found that although the plants were capable of some C_4 carboxylation, they did not contain a C_4 cycle. Kinetic analyses showed that the C_4 compounds formed in the light were not reused, and their carbon did not enter the C_4 cycle. Other data from Australia suggest that marine plants have the same carboxylase - a normal RuBPcase. Since the carboxylase is susceptible to oxygen poisoning, it follows that these plants must have some alternative method for concentrating CO_2. The only way that the oxygen effect can be prevented in a plant having normal RuBPcase is to present the carboxylase with a high enough concentration of CO_2 that the oxygen can no longer compete. We considered the possibility that such a CO_2-concentrating mechanism might be based on the bicarbonate in the sea. The sea contains a great deal of bicarbonate: its concentration is 2 mM, as compared to an equivalent concentration of 10μM for CO_2. There is, in other words, 200 times more bicarbonate around than CO_2.

CO_2 uptake by seaweeds appears normal (Fig 9) but, if the CO_2 concentration is increased to unusually high levels, an anomalous curve appears (Fig 10). Normally, photosynthesis increases at a more or less steady rate with increasing CO_2 concentration until a CO_2 concentration is reached at which the plant can handle no more. Then the curve flattens out. The curve in Figure 10 is quite different from this. We then, by careful manipulation, measured the rate of photosynthesis as

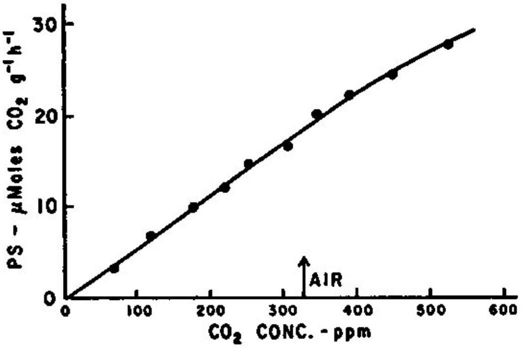


Fig 9. Photosynthesis of Fucus vesiculosus at low and normal concentrations of CO_2 in air.

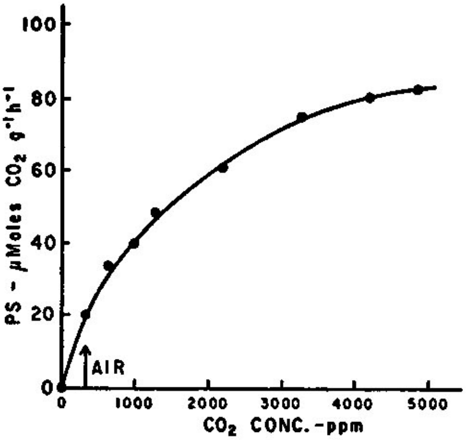
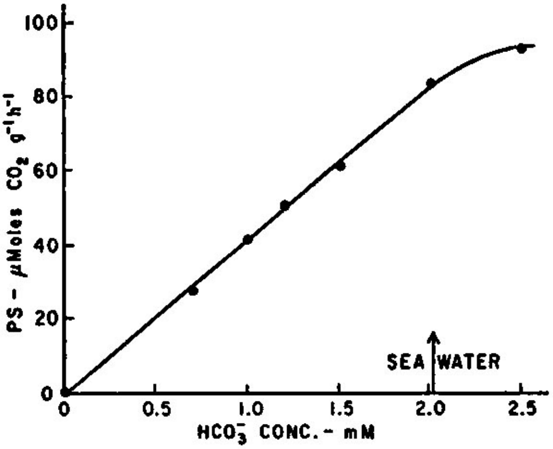


Fig 10. Photosynthesis of Fucus vesiculosus at elevated concentrations of CO_2 in air.

affected by the concentration of bicarbonate, and found that we got an absolutely normal curve (Fig 11) that levels off at a bicarbonate concentration slightly above that in seawater. In another experiment, bicarbonate was varied by varying the pH (Fig 12). You can see quite clearly that photosynthesis of this seaweed varied with the bicarbonate content and not the CO_2 content. From this and much other data that I am not

Fig 11. Photosynthesis of Fucus vesiculosus at low to normal concentrations of bicarbonate in seawater.



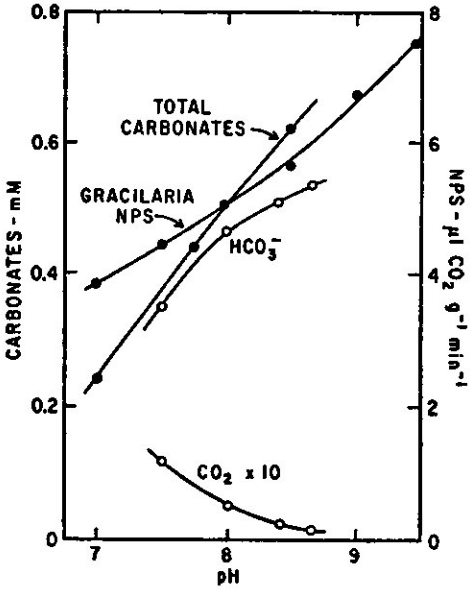


Fig 12. Photosynthesis of Gracilaria tikvahiae compared with bicarbonate $[HCO_3^-]$ and CO_2 concentrations varied by pH.

presenting here, we conclude that these marine plants absorb bicarbonate rather than CO_2. This finally settles a long standing controversy, which could not have been solved without the use of the technology that we have developed for studying CO_2 exchange in underwater plants.

The next question to be answered is how do the plants use bicarbonate. Bicarbonate is a charged ion, which can be absorbed and pumped (it has a handle, its ionic charge, and is like the football analogy that I made earlier - it can be picked up and thrown to another place, which CO_2 cannot). Work in Canberra, Australia and in Toronto supports the fact that there is a mechanism in underwater plants that enables them to pump bicarbonate into the cell. Bicarbonate can diffuse through the cytoplasm to the chloroplast. Since the cytoplasm has about the same pH as seawater, there will be little tendency for the bicarbonate to convert to CO_2 until it reaches the chloroplast. There it encounters an enzyme, carbonic anhydrase, which greatly speeds the interconversion of bicarbonate and CO_2. In addition, the chloroplast has a lower pH than the cytoplasm. Both these facts favor the conversion of bicarbonate into CO_2. This will permit the buildup of a substantial concentration of CO_2 in or at the chloroplast - the same trick as performed by the C_4 cycle in C_4 plants, with the same result. Calculations of the bicarbonate and CO_2 concentrations in various plants are shown in Table I.

Table I Estimated bicarbonate and CO_2 concentrations inside leaves, fronds and cells of terrestrial and marine plants

		CO_2 + HCO_3 conc	
		HCO_3^- mM	CO_2 ppm
Air		-	330
Inside leaf	C_3	-	50
air space	C_4	-	5-50
In chloroplast	C_3	-	2-5*
at RuBPcase	C_4	-	300-600*
Seawater		2	330 (10µM)
Algal cytoplasm		1.5	-
Algal chloroplast		-	150-300*

*Estimated value.

It can be seen that the calculated CO_2 concentration inside or at the chloroplast of algae is in the same range as that in C_4 plants, and much higher than that in C_3 plants. This, then, explains how algae can have a high rate of photosynthesis (they are among the most productive plants in the world), no photorespiration, and no poisonous oxygen effect.

Thus seaweeds have also learned a trick or two. In fact, it is possible that land plants also use a bicarbonate-transfer mechanism to move carbon through the mesophyll cells to photosynthetic sites. This would explain the almost universal presence of carbonic anhydrase in photosynthetic cells. Land plants would be foolish if they did not use such a mechanism! It is much easier, if you want to kick air from one place to another, to wrap it up!

Seaweeds may not be so beautiful as some land plants, but beauty may be more than skin deep. The photosynthetic mechanisms under the epidermis of CAM and C_4 plants, and also of the seaweeds, are really rather beautiful. That, after all, is how plants make plants.