SUMMARY

This thesis describes the mechanism for bicarbonate utilization in water plants. Photosynthesis, depending on CO_2 only, in water plants is limited by the slow diffusion of CO_2 in water, which is about 10.000 times as slow as in air. Diffusion is the only way of transport through the unstirred layer around the leaf. Under net photosynthetic conditions the CO_2 concentration in this layer will drop as replenishment from the bulk of the medium is slow. However, a proportion, its size depending on the pH, of the dissolved inorganic carbon is present as bicarbonate and carbonate. A number of aquatic organisms has the ability to make use of this carbon source for photosynthesis.

In the aquatic angiosperms Elodea and Potamogeton the mechanism to utilize bicarbonate depends on the property of the leaves that they can become polar, that is, the ability of these leaves to acidify the medium on the lower side of the leaf, while at the same time on the upper side the medium becomes alkaline. Two models have been proposed for the mechanism by which the acidification stimulates the bicarbonate utilization. The first model is a H^+/HCO_3^- symport system for which the acidification provides the driving force. According to the second model the low pH shifts the HCO_3^-/CO_2 equilibrium to the CO_2 side. The increase of the CO_2 concentration increases the CO_2 diffusion into the leaf cells (Chapter 1).

Prerequisite for the second model is a strong, localized acidification. This is only possible when the acidic and alkaline zones are spatialy separated and when a large unstirred layer exists. For the first model these conditions are not so strict. Protoplasts, which cannot meet the requirements, are not capable of utilizing bicarbonate (Chapter 7 and 8). Furthermore, leaves of plants cultured under high CO_2 conditions lose the ability to become polar and therewith the possibility to use bicarbonate (Chapter 8).

The regulation of the polar reaction is described in the Chapter 2 to 6. In Chapter 2 the effect of inhibitors on the

polarity is presented. The polarity is light dependent and inhibited by DCMU, an inhibitor of the non-cyclic electron transfer chain. Inhibitors of membrane-bound ATPase activity inhibit the polarity. And the polar reaction is sensitive to SH-reagents. Based on these results a model for the regulation was proposed: the polar reaction depends on activation of a proton pumping ATPase in the plasma membrane; the ATPase activity is stimulated by a product of the light reaction (probably reducing equivalents (NADPH)); analogous to activation/inactivation mechanisms in other enzymes, the activation of the ATPase could be effected by reduction of essential SH groups.

For this model to be applicable a number of conditions must be met:

-reductase activity has to take place in the plasma membrane. In Chapter 3 it is demonstrated that trans-plasma membrane electron transport does occur.

-treatments that lead to a decrease in reducing equivalents must inhibit the polarity. Increasing the availability of CO_2, stimulating the CO_2 fixation and thereby the consumption of reducing equivalents, reduces the polarity. This effect can be counteracted by increasing the light intensity, stimulating the production of reducting equivalents (Chapter 4). Another way to create an extra sink for reducing equivalents is the addition of ferricyanide. This treatment also inhibited the polarity; an inhibition also releaved by extra light. It was observed that the reduction of ferricyanide increased with the light intensity and decreased when the carbon was added. As the redcution of ferricyanide, a membrane impermeable ion, has to take place at the plasma membrane, this indicates that an additional requirement, namely that the production and consumption of reducing equivalents in the chloroplast do have an effect on the availability of these equivalents at the plasma membrane, is met (Chapter 5).

-ATPase activity is influenced by SH reagents and the redox poise of the cytoplasm. In experiments with isolated plasma membrane vesicles it is demonstrated that the ATPase activity

can indeed be modulated by changes in the NAD/NADH ratio and is inhibited by SH reagents.
In the proposed model, increase of the ATPase activity leads to the generation of the polar reaction. However, as ATPase activity could be demonstrated on both the upper and the lower side of the leaf, it is not easy to envisage how stimulation of the activity could lead the the dramatic difference between the two sides. In Chapter 8 an attempt is made to give a mathemetical description of the generation of the polarity without the necessity to assume a difference in membrane characteristics on the upper and lower side.