The influence of the herbicide, DCMU, on the oxygen-evolving system of photosynthesis

A new herbicide, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), is such a powerful inhibitor of the reduction of 2,6-dichlorophenolindophenol by chloroplasts that, according to WESSELS[1], $2 \cdot 10^{-7} M$ is sufficient to cut the reaction rate in half. This concentration is lower than that of the chlorophyll present in WESSEL's experiments. The affinity of the new inhibitor for some specific reaction component in the chloroplast-Hill reaction must be very high.

At present, poisons of photosynthesis, effective in very low concentrations, can be divided into two classes: Those which mainly inhibit the reduction of carbon dioxide (*e.g.*, cyanide, sulfide, azide) and those which stop the evolution of oxygen (*e.g.*, NH_2OH, napthoquinones, *o*-phenanthroline). Only the latter poisons are strong inhibitors of chloroplast reactions, *i.e.*, Hill reaction. WESSEL's experiments indicated that DCMU had to be classified with this second group of inhibitors. Its chemical structure however, is so unlike that of the substances cyanide, hydroxylamine, or napthoquinone that it seemed useful to consider the existence of a third class of poisons that perhaps would not act on either of the known sensitive parts of the photosynthetic mechanism, but rather function like a narcotic, *e.g.*, phenylurethane, and affect the photochemical apparatus.

Whether a certain effect is restricted to the mechanism of oxygen evolution can be easily tested with algae adapted to do photoreduction with hydrogen. In *Scenedesmus* and some other hydrogenase-containing algae, photochemistry and the reduction of carbon dioxide may continue under conditions where oxygen evolution is virtually eliminated (2, 3, 4, 5). As a result, those poisons which normally inhibit oxygen evolution in photosynthesis have no effect on photoreduction but do prevent the deadaptation of the algae which normally occurs at high light intensities. A poison reacting with the chlorophyll directly should, on the other hand, be an inhibitor of all types of photochemical activity including photolysis.

The effect of DCMU upon photosynthesis and photoreduction of adapted *Scenedesmus* at different light intensities is shown in Figure 1. Photosynthesis is reduced to about one-half by $5 \times 10^{-7} M$ DCMU and becomes completely inhibited in the presence of $3 \times 10^{-6} M$ DCMU. Inhibition occurs at all light intensities, which is typical for poisons of the hydroxylamine type. After adaptation to hydrogen, the effect of DCMU disappears. Photoreduction is not inhibited, but the usual reversion to photosynthesis at higher light intensities is prevented by $3 \times 10^{-6} M$ DCMU. Reversion to photosynthesis does occur, however, in $5 \times 10^{-7} M$ DCMU, but at a much higher light intensity (3000 lux) than the control (1200 lux). Thus, effects caused by this substituted urea are surprisingly similar to those reported by GAFFRON for *o*-phenanthroline, napthoquinone, etc., except that the potency of DCMU in stopping oxygen evolution surpasses that of the other poisons. Apparently DCMU reacts specifically with some intermediate chemical or enzyme concerned with the production of oxygen in photosynthesis.

Recently, KESSLER[5] has shown that the reversible inhibition of photosynthesis, which PIRSON[6] found in manganese-deficient algae, can be traced to the oxygen-producing mechanism.

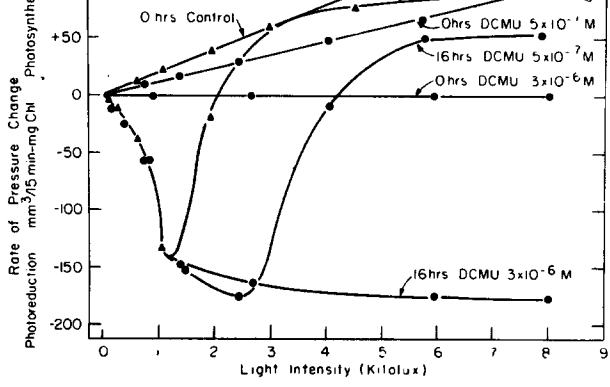

Fig. 1. Rates of photoreduction and photosynthesis at various light intensities for *Scenedesmus* D_3 with and without DCMU. Manometric readings correspond to oxygen production in photosynthesis (corrected for respiration) and to absorption of carbon dioxide and hydrogen for photoreduction. Experimental conditions: phosphate buffer, 0.15 *M*, pH 6.85; gas phase, air + 4 % CO_2 for photosynthesis and 96 % H_2 + 4 % CO_2, purified by a "Deoxo" cartridge, for photoreduction. Dark period for adaptation was 16 h; periods of 15 min for each light intensity. Temperature: 25°.

Ankistrodesmus braunii from manganese-deficient cultures retain a very good photoreducing ability despite the fact that they have little capacity for evolving oxygen. The possibility that the poison, DCMU, might be reacting with either Mn^{++} or Fe^{++} necessary for oxygen production was tested by adding an appreciably large quantity ($2 \times 10^{-3} M$) of these ions to a suspension of algae which had been treated with the inhibitor. In neither case was there a reversal of the inhibition of photosynthesis.

To learn more about the place where DCMU acts, we have raised some manganese-deficient *Ankistrodesmus* and repeated KESSLER's experiment which gave the very high rates of photoreduction. Treatment of these algae with the concentrations of DCMU used above gave the results illustrated in Fig. 2.

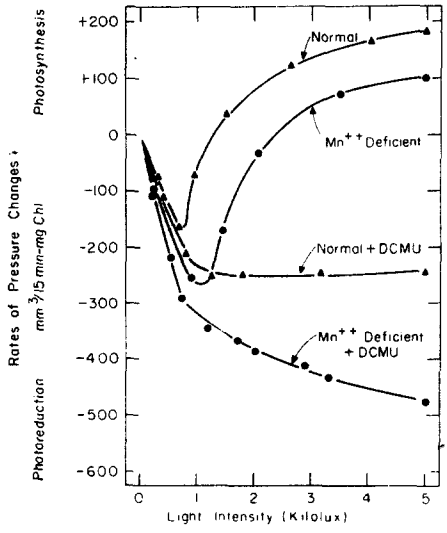

Fig. 2. Rates of photoreduction and photosynthesis for normal and manganese-deficient *Ankistrodesmus braunii* at various light intensities. Experimental conditions were otherwise identical to Fig. 1. DCMU concentration was $3 \cdot 10^{-6} M$.

It should be pointed out how specifically DCMU acts on the oxygen evolving system in green plants. Neither respiration of living cells nor the decomposition of hydrogen peroxide by catalase is affected by concentrations of DCMU that stop photosynthesis. Of course, the negative outcome of the experiments with added metal ions does not preclude the participation of manganese and/or iron in the oxygen production and their possible inhibition by DCMU. The reactivity of the inorganic forms of iron and manganese may easily be quite different from that in their properly bound state, where steric factors may render them highly susceptible to inhibition by this poison.

Not yet fully understood are the circumstances which determine the maximum rates of photoreduction attainable and why these rates vary so conspicuously with different ways of treatment. The highest capacity for photoreduction in our algae reported so far is that observed by KESSLER in his manganese-deficient cultures. The removal of an essential catalytic compound apparently opens the way to photoreduction more completely than the blocking of a catalyst through its inactivation by a poison.

The fact that photoreduction in the deficient algae is not inhibited by DCMU (Fig. 2) indicates that either DCMU interferes with a reaction following the action of Mn^{++} or that the poison pins down a catalyst useful in both pathways, probably in its oxidized form. Under conditions of manganese deficiency, a greater part of a catalyst useful both in the path to oxygen and in that to hydrogenase can be reduced and thus be put into service for photoreduction.

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