PHOTOSYNTHESIS WITH RADIOACTIVE CARBON, AND THE DISTRIBUTION OF THE INTERMEDIATE PRODUCTS IN THE PLANT CELL

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Recent experiments with radioactive carbon (C^{11}) have shown that green plants take up CO_2 reversibly in the dark (**2, 3, 5**). The evidence accumulated thus far indicates the presence of high molecular weight compounds which bind the CO_2 in a combination stable in boiling concentrated hydrochloric acid.

In this note experiments are described in which the distribution of the initial photosynthetic products within Nitella cells have been investigated. Radioactive carbon was used as a tracer according to the technique described by RUBEN, KAMEN, and HASSID (**3**).

Experimentation

Nitella plants were used in this work, since these plants release their cell contents very readily on slight crushing. Since no grinding was necessary, it was possible to obtain a maximum of intact chloroplasts by crushing the cells in a 0.5-M glucose solution (**1**). The cytoplasm and vacuolar sap were separated from the chloroplasts and other bodies by centrifuging. Microscopic investigation of the chloroplasts showed that the majority of them were left intact after this treatment.

1. Intact Nitella plants were exposed to $\overset{*}{C}O_2$ for 25 minutes both in the light and in the dark. At the end of this period the plants were removed from the reaction flasks and immediately crushed in 0.5-M glucose. The cell materials were then separated as stated and their activity tested. Each sample was boiled with 12-N hydrochloric acid, in order to expel any $+ 4\ \overset{*}{C}$ present. After several washings of the cell wall material with water and of the chloroplasts with 0.5-M glucose, no activity was found either in the chloroplasts or the cell wall material from the plants which were kept in the dark during the exposure to $\overset{*}{C}O_2$. The aqueous, colorless solution obtained from centrifuging of the crushed plant material contained 90 per cent. of the total activity after the first centrifugation, two further washings of the residue removed almost all of the activity from the water-insoluble material. In contrast, the plants which concurrently were kept in the light during this period contained nearly four times as much activity in their chloroplasts as in the non-chloroplast material. This activity in the chloroplasts could not be removed by further washings with 0.5-M glucose.

2. In another series of experiments, Nitella plants were crushed immediately before exposure to C^*O_2. This material was filtered through cheese cloth, transferred to Warburg vessels and exposed to C^*O_2 for 25 minutes in the dark and in the light. No uptake of C^*O_2 was observed either by the cytoplasm and vacuolar sap or by the chloroplasts in a form resistant to boiling hydrochloric acid.

3. Intact Nitella cells were exposed to C^*O_2 in the dark for 25 minutes and then crushed also in the dark in 0.5-M glucose. Intact cells were filtered out. The suspension was exposed to light for 20 minutes. No decrease in the activity of the aqueous fraction was observed and no activity was found in the chloroplasts.

Thus it appears that intact plant cells are a prerequisite not only for this particular dark reduction of CO_2 but also for the further reduction of the carboxyl group formed in the dark (**4**). Whatever compound reduces CO_2, whether in the light or in the dark, it seems evident that it is non-chloroplastic, or only weakly adsorbed on the chloroplasts and easily removed by physical injury to the living cell.

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