calculation from a short-term study was based on figures from any one 3-h daylight period, large errors in daily production estimates were possible. Short-term $^{14}C$ measurements produced values somewhere between net and gross photosynthesis as determined by the oxygen method (Table II), and this difference can be attributed, in part at least, to re-fixation of respiratory $^{14}$carbon dioxide[6]. This observation provides part of the explanation for the discrepancy between direct 24-h and short-term estimates of daily production (Table I).

*Discussion.* Although the oxygen method is subject to many problems, with an actively photosynthesizing tissue it appears to be as reliable as the currently favoured $^{14}C$ technique, especially with the greater accuracy introduced with the use of the oxygen electrode. However, the determination of an accurate in situ P.Q. must be considered. The main problem in the $^{14}C$ technique is the assessment of the extent of refixation of respiratory $^{14}CO_2$ in short-term studies. Under conditions of low production such as approaching compensation point (Table II), the greater accuracy of the $^{14}C$ method proves invaluable. The 24-h method appears to produce a direct measure of net daily production with both methods, whereas planimetric measurements from daily light energy curves must be used in the conversion of short-term production figures to net daily values. Similarly, the choice of time for the short-term incubation must be considered carefully, since the relation between photosynthesis and radiant energy has been shown to vary over the daylight period[8,9]. In the majority of ecological studies net production estimates are required, and in many cases comparative net values are important, therefore many of the assumptions taken in the oxygen and $^{14}C$ methods are valid for such purposes[10–12].

Table II. A comparison of techniques for assessing the relation of production rate of *C. prolifera* to increasing depth, with conditions[a] approaching the compensation point

| Depth | Temperature | Production[b] mgC/g dry weight | | |
|---|---|---|---|---|
| | | oxygen | | $^{14}$Carbon |
| (m) | °C | gross[c] | net | |
| 5 | 22 | 0.64 | 0.35 | 0.48 |
| 15 | 22 | 0.52 | 0.23 | 0.39 |
| 35 | 21.5 | 0.34 | 0.06 | 0.28 |

[a] Sky heavily overcast with cloud, therefore all figures low. [b] All figures based on 3-h studies between 11.00 and 14.00. [c] Gross photosynthetic oxygen production was taken as the sum of oxygen production in the 'light' bottle and the loss of oxygen (uptake) by respiration in the 'dark' bottle, and assumed that respiration was the same in the light and dark (an assumption which is certainly not valid under all conditions).

*Résumé.* En employant les méthodes de la production d'oxygène et de l'assimilation du $^{14}C$, on a estimé la productivité primaire de l'algue, *Caulerpa prolifera*, in situ à profondeurs variés aux îles Canaries. On a jugé les mérites respectives des 2 techniques et a comparé les résultats avec ceux des autres auteurs.

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## Blushing Effect of Some Carbohydrates on the Green Alga *Dictyococcus cinnabarinus*

The bleaching effect of glucose on some Chlorellae and Euglenae has been studied thoroughly[1,2]. This effect causes the diminution of the formation of chlorophylls and chloroplasts with a following decolouration of the culture. Dentice et al.[3,4] have studied the variations caused by the addition of glucose to cultures of *Dictyococcus cinnabarinus* grown in an extremely poor medium, showing the blushing effect, which was due to the disappearance of the chlorophylls and the appearance of particular keto-carotenoids.

These data have induced the study of the deformation of the structure of the chloroplasts by means of electron microscopy and the biochemical variations caused by the addition of different carbohydrates which can provoke this blushing effect.

*Materials and Methods.* The strain used was *D. cinnabarinus* 280 (Kol-F. Chodat) Vischer, received from the algal collection of the Botanical Institute of the University of Geneva, Switzerland. Normal growth conditions have already been described[4]. For the study of the blushing effect, the green submerged 15-day-old cultures were transferred to fresh medium which contained 2% (w/v) of carbohydrates (glucose, galactose, fructose, mannose, saccharose or lactose). The initial population was 200,000 cells/ml. The second period lasted another 15 days. The determination of fatty acids, the separation, identification and quantitative determinations of the pigments were previously described[4].

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*Results and discussion.* The extra-cellular carbon sources, particularly glucose and mannose, are utilized by *D. cinnabarinus* for the regulation of the syntheses occurring in the chloroplasts. The most evident feature is the formation of keto-carotenoids which account for about 80% of total carotenoids and the diminution of chlorophylls and chloroplasts (see Tables III and IV). The chloroplasts were degenerated little or more up to the disappearance of the lamellar structure and the formation of large oil drops, in case of glucose and mannose uptake. If the development occurred in the presence of a carbohydrate which could not be assimilated quantitatively the photosynthesis did not disappear and the chloroplasts were degenerated proportionally to the uptake of the carbohydrate. While chlorophylls and chloroplasts disappeared, $\beta$-carotene was still synthetized, and neoxanthin was present in trace amounts only. The change of this epoxy-carotenoid in relation to the photosynthetic activity and its absence in cells without chlorophylls confirm the probable correlation between neoxanthin and the photosynthetic process occurring in *D. cinnabarinus*[3]. The resemblance between the chemical structure of carotenoids and the esterified chain of chlorophyll, phytol, suggests a biosynthetic relationship similar to that in higher plants. As the synthesis of the chlorophylls does not occur, it seems to be plausible that the common precursor, which has no more function in the synthesis of the phytol-chain, will be utilized entirely for the synthesis of carotenoids.

If the cells are transferred to the same medium but without carbohydrate, the chlorophylls and chloroplasts are formed again.

The addition of different carbohydrates to the culture medium has a predominant effect on the synthesis of linolenic acid, which seems to be related closely to the photosynthetic mechanism. This acid is not formed in cultures with glucose or mannose, and it is present in small amounts

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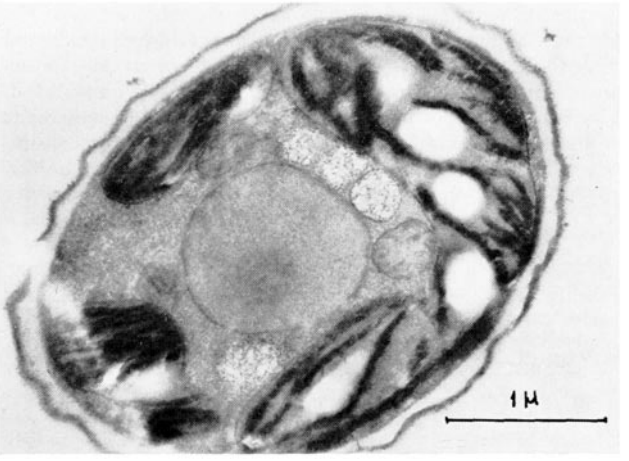


Fig. 1. Without carbohydrates. Chloroplasts along the cell walls with many compact bundles of lamellae, inclusion of starch. Fixation with glutaraldehyde, post-fixed in $KMnO_4$ and inclusion in araldite. All photographs have been taken from samples at the ninth day of growth.

Table I. Pigment content in *D. cinnabarinus* grown in submerged culture in different carbohydrates

| Carbohydrates | Total chlorophyll content mg/g dry weight | | | | Total carotenoid content mg/g dry weight | | | |
|---|---|---|---|---|---|---|---|---|
| | 5° | 9° | 12° | 15° | 5° | 9° | 12° | 15° |
| Glucose | 1.44 | 0.48 | 0.15 | – | 0.39 | 0.52 | 0.81 | 1.25 |
| Galactose | 4.19 | 1.55 | 0.63 | 0.23 | 1.39 | 0.69 | 0.54 | 0.70 |
| Fructose | 1.40 | 0.60 | 0.42 | 0.12 | 0.26 | 0.13 | 0.39 | 0.67 |
| Mannose | 2.03 | 0.89 | 0.42 | – | 0.05 | 0.35 | 0.63 | 0.74 |
| Sucrose | 2.50 | 6.83 | 1.97 | 0.91 | 0.23 | 0.50 | 0.19 | 0.42 |
| Lactose | 4.55 | 6.71 | 3.08 | 1.84 | 0.11 | 0.18 | 0.25 | 0.52 |
| Without carbohydrate | 13.88 | 20.00 | 9.95 | 14.65 | 0.41 | 0.57 | 0.34 | 0.30 |

Table II. Carbohydrate uptake during the growth of *D. cinnabarinus* in submerged culture

| Carbohydrates | Carbohydrate uptake g/100 ml culture medium | | | | |
|---|---|---|---|---|---|
| | 0 | 5° | 9° | 12° | 15° |
| Glucose | 2 | 0.60 | 0.80 | 1.40 | 1.70 |
| Galactose | 2 | 0.60 | 0.80 | 1.00 | 1.00 |
| Fructose | 2 | 0.60 | 0.80 | 0.80 | 0.80 |
| Mannose | 2 | 0.29 | 0.68 | 0.98 | 1.19 |
| Sucrose | 2 | 0.11 | 0.11 | 0.23 | 0.17 |
| Lactose | 2 | – | 0.10 | 0.11 | 0.15 |
| Without carbohydrate | – | – | – | – | – |

Table III. Spectral characteristics of the pigments of *D. cinnabarinus* grown in submerged culture in different carbohydrates

| Compound | Solvent | Absorption maximum (nm) | Identification |
|---|---|---|---|
| Pigment $P_1$ | Hexane | 425, 453, 482 | $\beta$-Carotene |
| Pigment $P_2$ | Hexane | 458 | Echinenone |
| Pigment $P_3$ | Hexane | 462–464 | 3,4-Dioxo-$\beta$ carotene |
| Pigment $P_4$ | Hexane | 465–470 | Canthaxanthin |
| Pigment $P_5$ | Hexane | 415, 437, 466 | Neoxanthin |
| Pigment $P_6$ | Ethyl ether | 430, 660 | Chlorophyll a |
| Pigment $P_7$ | Ethyl ether | 454, 642 | Chlorophyll b |
| Pigment $P_8$ | Carbon disulphide | 500 | Astacin |

Table IV. Relative amounts of keto-carotenoid in *D. cinnabarinus* grown in submerged culture

| Carbohydrates | Relative percentage |
|---|---|
| Glucose | 80 |
| Galactose | 60 |
| Fructose | 35 |
| Mannose | 70 |
| Sucrose | 10 |
| Lactose | 5 |
| Without Carbohydrates | – |

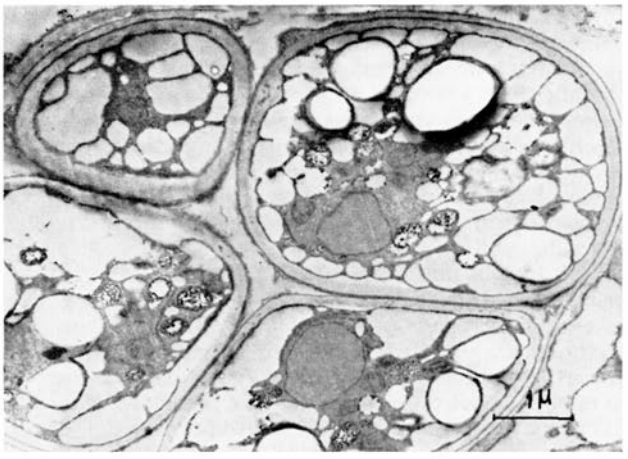


Fig. 2. With glucose. Great lipid drops have replaced the chloroplasts, the cellular mass has been reduced. Fixation as in Figure 1.

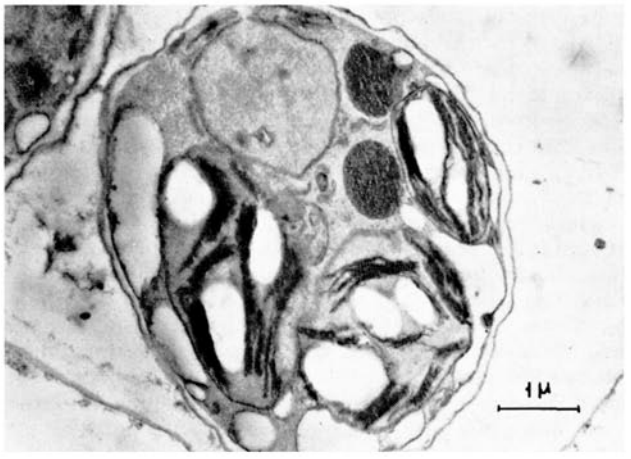


Fig. 3. With galactose. Chloroplasts with many compact bundles of lamellae, lipid and starch. Fixation as in Figure 1.

in those cultures which contain fructose or galactose. It disappeared almost completely in cultures with lactose, while arachic acid was found; in cultures with saccharose, linolenic acid was accompanied by an unidentified fatty acid with 20 carbon-atoms and 4 double bonds.

In all the cultures studied, there is a direct proportionality between carbohydrate uptake and carotenoid synthesis and an inversed relationship between carbohydrate uptake and chlorophyll synthesis (Tables I and II).

The electron microscope photographs confirm the results obtained, demonstrating that the blushing effect is the visible feature of chloroplasts deformation (Figures 1–3)[6].

*Riassunto.* È studiato l'effetto di arrossamento provocato dall'aggiunta di alcuni zuccheri a colture sommerse dell'alga cloroficea *D. cinnabarinus*. Questo effetto è dovuto alla formazione di cheto-carotenoidi, alla diminuzione delle clorofille ed alla degradazione dei cloroplasti. Le osservazioni al microscopio elettronico mettono in evidenza le variazioni della struttura dei cloroplasti.

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## STUDIORUM PROGRESSUS

### Induced Macroconidia Formation in *Neurospora crassa*

Asexual development in *Neurospora* includes the differentiation of laterally growing vegetative hyphae into aerial hyphae and these, in turn, into numerous conidiophores which form vegetative spores called macroconidia. Previous investigations reveal an impressive array of fundamental biochemical differences between vegetative hyphae and the conidia they may eventually yield[1–3] and that the process is under genetic control[4]. However, the first requirement for experimental analyses of macroconidiation is that it be brought under stringent and precise control. A method which improves upon previous attempts to do so[5,6] is given here together with observations which make some contribution to the biology of *N. crassa*.

Both of the earlier methods took advantage of the fact that conidia are never formed on submerged hyphae[7] or in the presence of the wetting agent Tween 80[8]. Strauss[5] reports conditions under which conidiation will begin after about 12 h and continue for a period of 4–5 h. Stine and Clark[6] find that aerial development requires 8 h and the differentiation of conidia continues for another 8 h. Our basic method reduces the durations of both aerial formation and conidiation without decreasing the final conidial yield. In addition, the method can be modified in a number of ways permitting the emergence of new and fruitful experimental situations.

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