solutions of pH 4.00–4.40), could not be displaced by solutions of high hydrogen ion concentration e.g. pH 2.0 and 1.0. Failure to dislodge this aluminium by H^+ suggest that it must be rigidly bound in a high affinity linkage[9], e.g. covalent linkage, and that groups on the cell surface dissociating in the pH region 4.0–4.4 are the most important factor in the interaction of aluminium with *S. aureus* 893.

Thus 2 types of binding occur: (1) aluminium is bound firmly in a complex with groups on the cell surface; then (2) subsequent binding is as exchangeable cations in the mobile layer of counterions. It has been shown that the binding of aluminium has lethal effects (BRADLEY, FISH and PARKER)[1]. Of the 2 types of binding described complex formation is potentially the more lethal.

The isolated cell walls of *S. aureus* 893 bound a maximum of 82 μ equiv. H^+/g at pH values 4.00–4.40. Figure 2 (compare GALDIERO)[10]; cell walls also bound 130 μmoles Al/g and cell walls with complexed aluminium utilized 94 μ equiv. H^+/g at pH 4.00 with a maximum utilization at pH 3.80 of 138 μ equiv. H^+/g.

From this preliminary data it is evident that aluminium is bound in a high affinity linkage by surface groups of $pK_a \sim 5$ on *S. aureus* 893.

Résumé. Deux types de liaisons ont lieu quand des ions d'aluminium réagissent avec les parois cellulaires isolées de *Straphylococcus aureus* espèce 893. La première interaction a comme résultat la formation d'un complexe stable qui affecte des groupes occupant le mur de la cellule avec un $pK_a \sim 5$. On suppose l'existence d'un composant de surface constitué par de la protéine combinée à de l'acide teichoique. La deuxième liaison consiste en ions interchangeables dans la couche mobile de cations recouvrant la paroi.

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A Preliminary Assessment of the Techniques for Measuring Primary Production in Macrophytic Marine Algae

Attempts to assess the productivity of aquatic plants under natural conditions have produced considerable controversy over the methods employed[1–3]. In this investigation of the rates of primary production of the marine alga, *Caulerpa prolifera* (Forsk.) Lamour, in Canary Waters, oxygen production and ^{14}C incorporation were measured and compared under varying conditions.

Fronds of the alga were collected from a depth of 5 m by divers and immediately on reaching shore they were rinsed in seawater and transferred to incubation bottles. At no time during this transfer were they taken out of water or exposed to direct sunlight. The sealed bottles were attached to wire frames underwater at appropriate depths. The frames were secured to ropes buoyed at the surface, and buffeting of the buoys produced sufficient agitation in the bottles to ensure an even mixing of dissolved gases and nutrients around the tissue. Photosynthetic oxygen production was determined using the light/dark bottle technique[4], oxygen concentration changes in the seawater being measured with a polyethylene-lead-silver electrode system[5]. Estimates of organic production were derived from oxygen figures assuming a photosynthetic quotient of 1.0. ^{14}Carbon incorporation measurements were based on the method of STEEMAN-NIELSEN[6]. A 1 mC ampoule (Radiochemical Centre, Amersham, England) of $Na_2{}^{14}CO_3$ (56 mC/m*M*) was diluted to 500 ml, giving a final activity of 2 μC/ml, and 2.5 ml of this stock solution was added to 197.5 ml of freshly drawn seawater. After incubation (see Tables) the algal fronds were washed quickly, then transferred to 80% ethanol for transport to Britain. Radioactivity was measured in a Packard Tricarb Scintillation Counter at 53% efficiency.

Preliminary experiments with varying tissue/incubation volume ratios indicated no apparent nutrient deficiency effects under the conditions used in the 24-h studies with *Caulerpa*. Similarly, no apparent nutrient deficiency effects were noted in concurrent experiments with algae photosynthesizing at much higher rates, if ratios of 0.3 g dry weight tissue/l seawater or lower were used.

From Table I, it can be seen that with the oxygen method direct 24-h and short-term measurements gave similar estimates for daily production. However, if the

Table I. A comparison of net daily production estimates for *Caulerpa prolifera*, established by oxygen and ^{14}C methods

Method	Daily production mgC/g dry weight
Oxygen method[a]	
Direct 24-h	4.52[c]
Calculated from 3-h[b]	4.56
^{14}Carbon method	
Direct 24-h	4.60
Calculated from 3-h[d]	5.18

[a] Carried out in incubation bottles of 1 l capacity; tissue dry weight approximately 0.2 g. [b] Based on 40 incubations at different 3-h periods throughout the daylight period. [c] This value is remarkably close to an estimate based on a recalculation of the oxygen production figures obtained for *C. prolifera* in the Mediterranean, i.e. 4.19 mgC/g dry weight[7]. [d] This figure is based on 2 sets of incubations under conditions of bright sun between 11.00–14.00. A 12-h daylength period was assumed, i.e. net daily production = $12x - 12y$, where x is ^{14}C incorporation/h in light and y is the respiratory loss of carbon/h calculated from oxygen measurements.

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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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