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To cite this article: Dvora Ariovich & Richard N. Pienaar (1979) The role of light in the incorporation and utilization of Ca^{++} ions by *Hymenomonas carterae* (Braarud et Fagerl.) Braarud (Prymnesiophyceae), British Phycological Journal, 14:1, 17-24, DOI: [10.1080/00071617900650041](https://doi.org/10.1080/00071617900650041)

To link to this article: <https://doi.org/10.1080/00071617900650041>



Published online: 17 Feb 2007.



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THE ROLE OF LIGHT IN THE INCORPORATION
AND UTILIZATION OF Ca^{++} IONS BY
HYMENOMONAS CARTERAE (BRAARUD ET
FAGERL.) BRAARUD (PRYMNESIOPHYCEAE)

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The coccolithophorid *Hymenomonas carterae* (Braarud et Fagerl.) Braarud produces a complicated external covering of body scales, the outer layer of which is made up of calcified scales called coccoliths. The incorporation of $^{45}\text{Ca}^{++}$ was found to be light dependent whilst calcification itself was found to occur in either the light or the dark depending upon the availability of a storage of intracellular Ca^{++} within the cells.

Hymenomonas carterae (Braarud et Fagerl.) Braarud is a biflagellate marine alga belonging to the Class Prymnesiophyceae (Hibberd, 1976). It is characterized by possessing a complex body covering made up of a number of scale types (Pienaar, 1969a). One of them, the base-plate scale, becomes calcified and these calcified scales are referred to as coccoliths.

Studies by Manton & Peterfi (1969), Pienaar (1971a), and Outka & Williams (1971) have shown that all the scale types are produced intracellularly within the single conspicuous Golgi body. There is evidence to suggest that there is some connection between light and the calcification of the base-plate scale to produce the coccolith (Pienaar, 1976).

According to Paasche & Klaveness (1970), who studied *Emiliana huxleyi* (Lohm.) Hay et Mohler [*Coccolithus huxleyi* (Lohm.) Kampt.], coccolith formation is strongly light dependent and it appears to be associated with the photosynthetic process in a manner that is as yet not fully understood. Wilbur & Watabe (1967) have also suggested a link between calcification and light in coccolithophorids without coming to any definite conclusion.

This report attempts to present some experimental work which illustrates that the actual process of calcification itself is not light dependent and that it can occur in either the light or the dark, but that the incorporation or uptake of Ca^{++} ions is light dependent.

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MATERIALS AND METHODS

Hymenomonas carterae (*Cricosphaera carterae* Plymouth 156) was maintained in full strength Provasoli's E.S. enriched sea water medium (Provasoli, 1968) and exposed to controlled conditions of 16 h of light (2000 lx) alternating with 8 h of darkness at 19°C. The cultures were continuously aerated and grown in conical 500 ml Ehrlenmeyer flasks. Axenic cultures were used in all the experiments and were obtained by exposing cells to the antibiotics Penicillin and Streptomycin (Guillard, 1973) for 24 h.

LIGHT AND ELECTRON MICROSCOPE OBSERVATIONS

Cells were decalcified by bubbling a mixture of 5% CO₂ in air through the culture for 9–10 min (Paasche, 1963). This treatment caused the calcium carbonate associated with the base-plate scale to be dissolved and also caused a sharp drop in the pH of the medium from pH 7.2 to pH 5.2–5.5. Because of this drop in pH the decalcified cells were then concentrated, by gentle centrifugation, and resuspended in fresh sterile medium and placed in either the light or the dark. The cells were observed immediately after decalcification and resuspension in fresh medium and were found to be actively motile. No visual ultrastructural change other than the loss of coccoliths was observed in cells that had been decalcified. The decalcified cells were then monitored to observe the production of newly calcified base-plate scales.

TABLE I. A summary of the experimental procedure adopted in the incorporation of ⁴⁵Ca⁺⁺ by cells of *H. carterae*. The numbers in parentheses represent the sample numbers in Table II

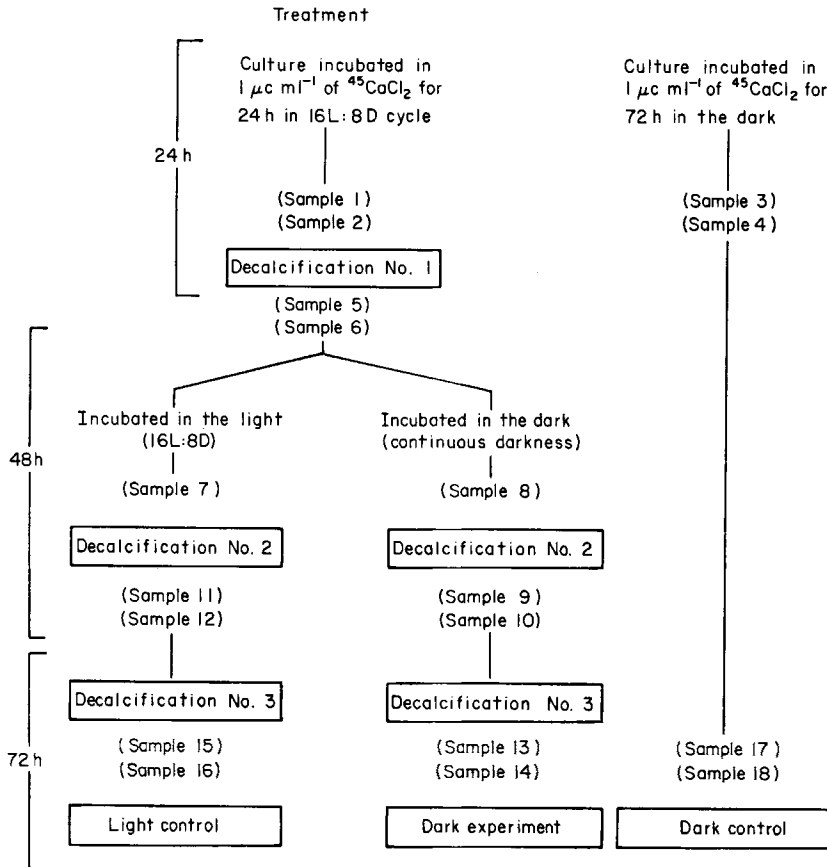


TABLE II. Results of the incorporation and utilization of ⁴⁵Ca⁺⁺, that was fed for 24 h, during the 72 h duration of the experiment

Time in hours	Sample no.	Sample details	Form of ⁴⁵ Ca ⁺⁺	DPM-blank	Radioactive Ca ⁺⁺ ($\mu\text{mol ml}^{-1}$)	Total Ca ⁺⁺ ($\mu\text{mol ml}^{-1}$)	Radioactive Ca ⁺⁺ uptake ($\mu\text{mol } 10^{-6}$ cells)	Total Ca ⁺⁺ uptake ($\mu\text{mol } 10^{-6}$ cells)
Pre-decalcification incubation								
0	—	standard	—	1644912.8	0.75	—	11.09	—
24	1	medium + ⁴⁵ Ca ⁺⁺	—	1644717.9	0.75	—	11.09	—
24	2	cells	whole cell content	126535.5	0.059	0.118	0.87	1.74
Dark experiment								
24	5	supernatant after decalc. No. 1	external	76401.6	0.036	0.532	0.072	1.06
24	6	cells after decalc. No. 1	internal	58094.8	0.027	0.399	0.054	0.79
48	8	cells	whole cell	58386.9	0.027	0.399	0.054	0.79
48	9	supernatant after decalc. No. 2	external	64957.13	0.028	0.414	0.056	0.81
48	10	cells after decalc. No. 2	internal	250.4	0.00009	0.00133	0.00018	0.0027
72	13	supernatant after decalc. No. 3	external	1069.3	0.00068	0.010	0.0013	0.019
72	14	cells after decalc. No. 3	internal	372.6	0	0	0	0
Light control								
48	7	whole cells	whole amount	54815.6	0.023	0.34	0.046	0.68
48	11	supernatant after decalc. No. 2	external	9984.1	0.004	0.05	0.008	0.12
48	12	cells after decalc. No. 2	internal	262.4	0.00009	0.0013	0.00018	0.0027
72	15	cells	internal	100.2	0	0	0	0
72	16	supernatant	external	0	0	0	0	0
Dark control								
24	3	medium + ⁴⁵ Ca ⁺⁺	in medium	1659285.6	0.75	11.09	—	—
24	4	cells	whole amount	11200.3	0.0051	0.075	0.010	0.15
72	17	medium + ⁴⁵ Ca ⁺⁺	in medium	1606279	0.73	10.8	—	—
72	18	cells	whole amount	9574.56	0.0045	0.066	0.009	0.13

Specific activity = 6.76%. Efficiency = 70.45%. Cell numbers = 0.5×10^6 cells ml^{-1} .Note: The calcium concentration in $\mu\text{mol ml}^{-1}$ refers only to the radioactive calcium that was added to the medium.

For electron microscopy cells were prepared according to a method described in an earlier paper (Pienaar, 1971a). Sections were cut on a Reichert OMU3 ultramicrotome using glass and diamond knives and were double stained with uranyl acetate and lead citrate.

ISOTOPE INCORPORATION STUDIES

Isotope incorporation studies were undertaken using $^{45}\text{CaCl}_2$ obtained from the Radiochemical Centre (Amersham).

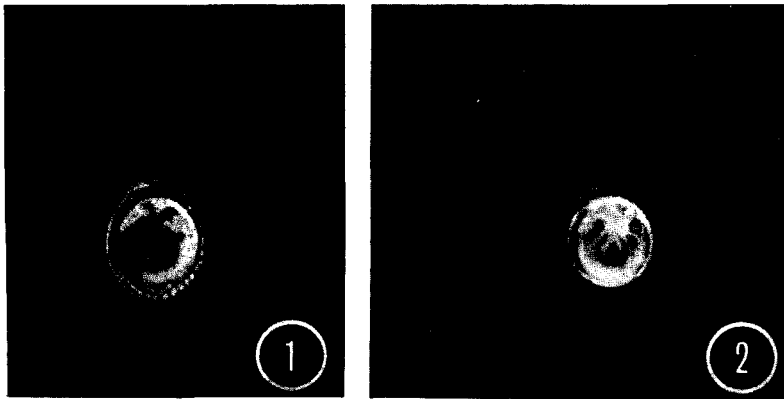
All isotope incorporation studies were done using 10-day-old cultures (towards the end of the exponential phase of growth). Cell counts indicated that there was a negligible increase in cell number during the experimental period.

Cells were incubated for 24 h (16L:8D) in fresh medium to which was added $1\ \mu\text{C ml}^{-1}$ of $^{45}\text{CaCl}_2$. A dark control was set up under continuous darkness. The cultures were then decalcified, and resuspended in fresh non-radioactive medium. This procedure was followed in order to study the utilization of the radioactive calcium that was incorporated during the first 24 h of the experiment. Samples of the naked cells and resulting supernatant were taken to determine their radioactivity. The decalcified culture was then divided into equal halves, one of which was returned to the normal 16 h light: 8 h dark cycle (as a control) and the other was placed under continuous darkness. Every 24 h over a 72 h period samples of cells and media were taken before and after decalcification. All decalcification was done at the beginning of the light period. Triplicates of each sample of cells and supernatant were prepared for counting on a Packard 3320 Spectrometer by suspending 0.75 ml of sample in 15 ml of scintillation fluor (Lips & Beever, 1966). Table I summarizes the procedure that was followed. The figures in brackets represent the sample number as indicated in Table II.

RESULTS

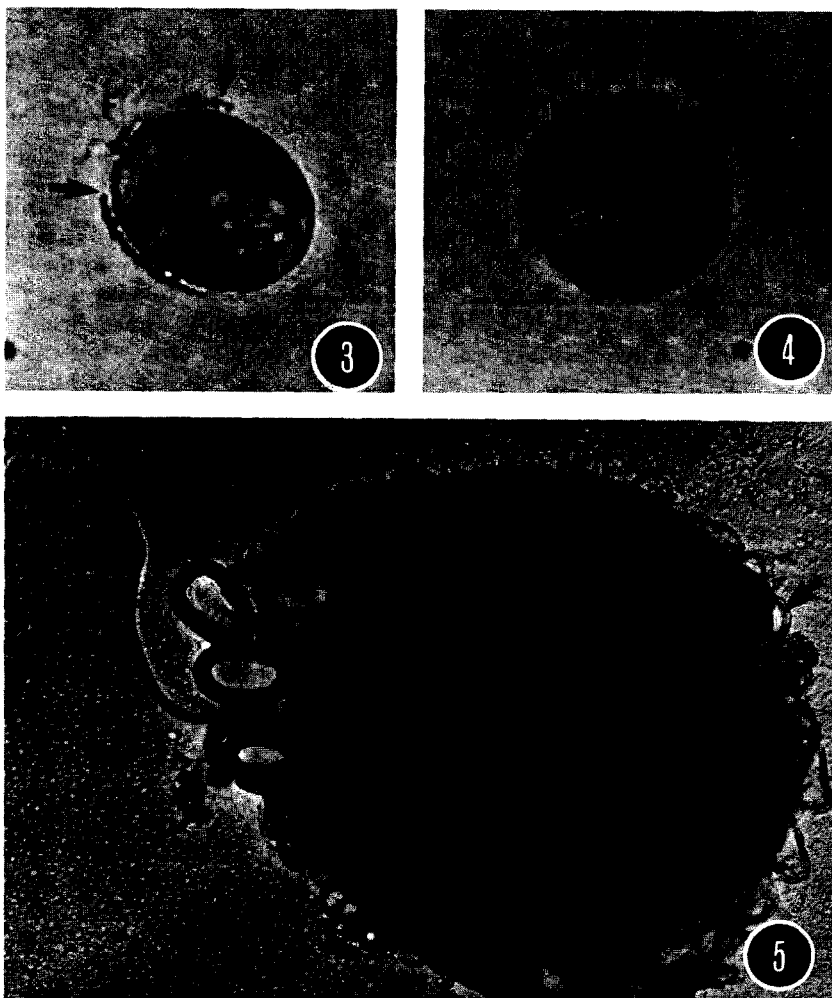
DECALCIFICATION AND RECALCIFICATION

Cells which had been recently decalcified and resuspended in fresh medium did not show any visible damage and were actively motile (Figs 1, 2). The decalcified cells produced new coccoliths intracellularly and these were always found to be released from the flagellar pole (Fig. 3). Decalcification took place at the beginning of the 16 h light period, since it has been shown that cells studied at the ultrastructural level after 8 h darkness were rarely found to possess intracellular coccoliths (Pienaar, 1976). When cultures were decalcified



Figs 1-2. Motile cells of *Hymenomonas carterae*. Fig. 1. Motile coccolith-bearing cell prior to decalcification. $\times 350$. Fig. 2. Cell immediately after decalcification. Note the conspicuous absence of calcified scales. When the coccoliths are decalcified the small bulbous haptoneuma becomes visible (arrow). $\times 350$.

at the beginning of the 16 h light period, and then placed in the dark, only about half of the cell became covered by new coccoliths (Fig. 4). These cells never produced any further coccoliths and remained with an incomplete coccolith covering even after 5-days incubation in the dark.



FIGS 3-5. Decalcified cells of *Hymenomonas carterae*. Fig. 3. Decalcified cell which has been placed into the light for 8 h. Note the coccoliths (arrows) are being released from the flagellar pole (F). $c \times 1,000$. Fig. 4. Cell which had been decalcified at the beginning of the 16L cycle and then placed into continuous darkness. Note only half the cell became covered with coccoliths (Coc.) $c \times 1,000$. Fig. 5. Heavy metal shadowed preparation of a decalcified cell which was incubated in the light. Note the 4 newly produced coccoliths (Coc) situated at the flagellar pole. The decalcified base plate scales can be seen covering the remainder of the cell (arrows). F = Flagellum. $\times 5,300$.

Decalcified cells incubated in the light produced 1–2 new coccoliths within the first hour and within 6 h in the light the cell was half covered with newly produced coccoliths (Fig. 5). In contrast those decalcified cells which were placed in the dark produced new coccoliths, the first of which could be detected at the flagellar pole 6 h after decalcification.

When the cells were decalcified at the end of the 16 h light period and then placed in the dark the entire cell became covered by newly produced coccoliths within 24 h.

These results have led to the proposal of a hypothesis that cells of *H. carterae* incorporate and store Ca^{++} ions during the light period and are able to utilize this store of Ca^{++} during both the light and dark period for the production of coccoliths. It was these results that initiated the study of $^{45}\text{Ca}^{++}$ incorporation in an effort to prove this hypothesis.

The results of the $^{45}\text{Ca}^{++}$ incorporation experiments are presented in Table II. The calcium concentration in $\mu\text{mol ml}^{-1}$ refers only to the radioactive calcium added to the medium.

After decalcification the radioactivity found in the cell fraction represents the internal radioactive $^{45}\text{Ca}^{++}$ which is stored within the cells whilst the $^{45}\text{Ca}^{++}$ found in the supernatant is considered to represent the external $^{45}\text{Ca}^{++}$ which had been deposited in the form of coccoliths, but had subsequently been dissolved during the decalcification process. $^{45}\text{Ca}^{++}$ uptake in the dark is only about 10% of that of the uptake in the light. This data indicates that the $^{45}\text{Ca}^{++}$ incorporation is strongly light dependent. The internal amount of $^{45}\text{Ca}^{++}$ that was stored during the original 24 h incubation period was 46% of the total amount of calcium that was taken up over the 24 h period. The other 54% of the amount of $^{45}\text{Ca}^{++}$ was deposited as CaCO_3 during coccolith production and later dissolved into the medium during the decalcification process.

Approximately half the $^{45}\text{Ca}^{++}$ which was incorporated during the 24 h incubation period was stored within the cell. This fact can explain the light microscope observations which indicated that these cells can produce enough coccoliths in the dark to cover only one half of the cell surface.

These results also suggest that the rate of Ca^{++} uptake is high enough to support the covering of the whole cell from the exposure to the isotope for 24 h (16 L : 8D).

About half of the $^{45}\text{Ca}^{++}$ uptake during this period was sufficient to support the production of the half cover of coccoliths which occurred within 24 h exposure to darkness. No additional Ca^{++} was incorporated and the calcified scales covering the cell after the treatment are thought to have been produced from Ca^{++} that was incorporated during the previous light period. This was assumed since decalcification took place at the beginning of the light period when no internal coccoliths are produced (Pienaar, 1976). It is however still difficult to differentiate between intracellular calcium in the form of coccoliths and other forms of intracellular calcium.

These cells possessing an incomplete covering of calcified scales were again decalcified (Decalcification 2), and again placed in the dark. They failed to produce new calcified scales since they had utilized all the calcium which had been stored intracellularly. About 99% of the $^{45}\text{Ca}^{++}$ that was stored in the cells was used to produce new calcified scales.

An observation worthy of further comment is that the number of coccoliths which can be produced in the dark depends upon the time during the 16L :8D cycle in which these cells were originally decalcified. Cells decalcified at the beginning of the 16 h light period and then placed in the dark had accumulated an appreciably lower store of calcium within the cell as evidenced by the fact that in the dark they could only produce enough calcified scales to cover half the cell. In contrast cells decalcified at the end of the 16 h light period had stored enough intracellular calcium to produce an entire covering of calcified scales when placed in continuous darkness after decalcification.

DISCUSSION

This work provides additional information concerning the role of light in the calcification process in *Hymenomonas carterae*.

The role of light in the uptake and utilization of Ca⁺⁺ indicates to us that calcification in *H. carterae* is not light dependent as was previously suggested (Pienaar, 1976) but that the actual incorporation of Ca⁺⁺ ions by the cell is light dependent. At the present time it is not known where within the cell the Ca⁺⁺ is stored prior to the calcification of the base plate scale to produce a coccolith. Previous workers have suggested that Ca⁺⁺ could be stored within the cells of *H. carterae* in a densely staining body referred to as the intracellular coccolith precursor (Isenberg et al., 1966; Pienaar, 1969b). However subsequent studies have indicated that this organelle is more aligned to an autophagic vacuole (Pienaar, 1971b) and probably does not have anything to do with the storage of intracellular calcium. In another coccolithophorid, *Emiliana huxleyi* (= *Coccolithus huxleyi*), which has been shown to possess two strains, one which is capable of producing coccoliths and the other which seldom produces coccoliths, workers have described an unusual organelle—the Reticular body = Wilbur Watabe body—which was only detected in the calcifying strain (Wilbur & Watabe, 1963; Isenberg et al., 1966). This led to the suggestion that this body may be involved in the production of coccoliths. No such organelle has ever been described in *H. carterae*. The fact that the coccoliths are produced in the cisternae of the single well defined dictyosome does tend to suggest that a possible calcium store might be a dictyosomal cisterna or some dictyosomal derived vacuole. It is hoped that by extending this investigation to include a detailed ultrastructural study of the various cells in samples before and at various time intervals after decalcification that some further information concerning the possible store of calcium within the cells may be forthcoming.

At present studies are being conducted to examine in some detail the changes in the amount of internal Ca⁺⁺ during a 16L :8D cycle and under constant illumination.

ACKNOWLEDGEMENTS

The authors would like to acknowledge financial support from the Council for Scientific and Industrial Research and the Atomic Energy Board.

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(Accepted 21 July 1978)