

during the precipitation, indicating formation of a solid phase with an average Ca/P ratio greater than 1.39. Readjustment of the reagent solution concentrations to a Ca/P ratio of 1.45 in a subsequent experiment (experiment 1777) resulted in crystal growth in which all solution parameters remained constant to within the experimental error in the analytical determinations, ± 0.3 percent. In addition, it was found that the rate of reaction was directly proportional to the inoculating seed concentration, confirming that growth of the crystals occurred without interference from secondary nucleation. To have obtained a kinetic precipitation stoichiometry to this precision by techniques previously used would have required concentration analyses to at least ± 0.03 percent. It is significant that in previous crystal growth experiments at physiological pH, using both synthetic and natural enamel and dentin seed, the molar Ca/P ratio actually precipitating on the seed material was normally in the range of 1.45 ± 0.05 (16). Although it has been assumed (11) that this ratio is close to that of β -TCP, and thus TCP has been invoked as the precursor, it is now clear that the ratio is significantly lower than the 1.50 required for TCP. Hydrolysis would be expected only to increase the molar Ca/P ratio, which further rules out TCP as the precursor phase.

In preliminary experiments it was observed that as the rate of precipitation exceeded a critical value of about 3×10^{-3} g of precipitate per liter per minute, the apparent Ca/P ratio of the solid phase became less than 1.45. To examine this phenomenon further, in experiment 2577 the initial supersaturation was increased, but to a value still below that for DCPD. The results shown in Table 1 clearly indicate a Ca/P ratio corresponding to OCP in the early stages of the reaction, and this phase was confirmed by x-ray analysis. At longer times (15 to 20 minutes) hydrolysis to a more basic phase took place with a Ca/P ratio of approximately 1.45, the value observed in so many previous calcium phosphate precipitation studies. It is highly significant that, using the constant composition method, more than twice the original seed material could be grown as OCP in the early stages of the reaction. The results support a model for calcium phosphate precipitation in which OCP, formed as a precursor phase, hydrolyzes either partially or completely to HAP, depending on the relative rates of the hydrolysis and precipitation reactions. The observed Ca/P values that are normally greater than 1.33 can be accounted for by

assuming that one in every three molecules of OCP transforms into HAP, leading to a Ca/P ratio of 1.44, within 0.01 of the observed value in experiment 1777. The hydrolysis probably takes place one layer at a time, as Brown *et al.* (13) proposed on the basis of a unit-cell x-ray analysis of the phases.

To our knowledge, the results presented here provide the first direct experimental evidence for OCP as the kinetically favored precursor in calcium phosphate precipitation at physiological pH from solutions supersaturated with respect to OCP, TCP, and HAP but not supersaturated with respect to DCPD or DCPA. The new method will enable studies to be made at very low supersaturations, and the influence of factors such as temperature, ionic strength, the nature and concentration of the seed, trace inhibitors, and fluid dynamics can be investigated unambiguously since these parameters do not change the supersaturation significantly. In our laboratory the method is being successfully used to study not only calcium phosphate mineralization (17) but also carbonates (18), sulfates (19), and oxalates (20) of calcium, as well as other important reactants such as magnesium hydroxide (21).

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Land Floras: The Major Late Phanerozoic Atmospheric Carbon Dioxide/Oxygen Control

Abstract. *Since at least the late Mesozoic, the abundance of terrestrial vegetation has been the major factor in atmospheric carbon dioxide/oxygen fluctuations. Of modern ecosystem types occupying more than 1 percent of the earth's surface, productivity/area ratios of terrestrial ecosystems (excepting tundra and alpine meadow, desert scrub, and rock, ice, and sand) exceed those of marine ecosystems and probably have done so for much of late Phanerozoic time. Reduction of terrestrial ecosystems during marine transgression would decrease the world primary productivity, thus increasing the atmospheric carbon dioxide concentration and decreasing the oxygen concentration. Regression would produce opposite effects.*

It is now widely accepted that the varying abundance of photosynthesizing phytoplankton in the oceans has had major impact on controlling atmospheric CO₂/O₂ ratios through geologic time. Elaborate models have been constructed that relate the terminal Paleozoic and Mesozoic worldwide faunal extinctions to phytoplankton failure (1-3). Of the factors that control phytoplankton abundance, for example, continental physiography, sea-level changes, upwelling, climatic fluctuation, and nutrient abundance, all relate to or are affected by relative land/sea ratios. Because it was

long believed that the productivity of marine phytoplankton exceeded that of terrestrial plants, it was believed that flooding of continental areas by epeiric seas would increase the total world primary productivity and that regression of epeiric seas to the ocean basins would reduce it. Thus, in the geographical areas that have been alternately flooded and drained by transgressions and regressions of epeiric seas, little attention has been focused upon the net primary productivity of terrestrial ecosystems that would have replaced marine ecosystems during a regression. Evaluation of mod-

ern vegetation in terms of biomass production, and of land/sea and productivity/area ratios (P/A), strongly suggests that the importance of terrestrial plants in the overall world productivity in the geologic past has not been recognized. Today, and for much of the Mesozoic and possibly late Paleozoic, the role of terrestrial vegetation in the total world productivity, and its impact on atmospheric CO_2/O_2 ratios, has likely been more important than that of marine phytoplankton.

In the past century ecologists have made great strides in assessing world primary productivity. Before about 1960, various ecologists believed that the bulk of the primary productivity occurred in the oceans; Riley (4), Muller (5), Nodack (6), and Fogg (7) estimated that the oceans produced 74, 71, 65, and 57 percent of the total world productivity, respectively. Mid-1960 estimates of marine and terrestrial productivity were roughly equivalent; Lehninger (8) and Vallentyne (9) estimated that the oceans produced about 50 percent of the total world productivity. By the late 1960's and early-middle 1970's, with increasing knowledge of world vegetation, estimates of the world primary productivity showed terrestrial productivity exceeding marine. Whittaker and Likens (10) indicated that the oceans account for only about 32 percent of the world's primary productivity; Leith (11) and Golley (12) recorded similar estimates of 35 and 38 percent, respectively. Some estimates of marine productivity are even lower; Bowen (13) and Bazilevich *et al.* (14) estimated that the oceans produce only 21 and 26 percent of the total, respectively.

Difficulties in estimating marine productivity arose for two reasons. (i) The oceans cover about two-thirds of the earth's surface, and productivity data for the highly productive nearshore ecosystems were extrapolated over the entire marine realm. (ii) Earlier estimates were based on potential rather than actual productivity (15). As is now known, productivity in much of the sea is limited by a poverty of nutrients; the oceans are relative deserts rimmed by productive waters in areas of upwelling and on continental shelves where nutrients are more readily available (15). Because recent estimates of marine productivity average about 30 percent of the total world productivity, far below the 50 to 90 percent estimates for modern marine phytoplankton often cited (1, 2, 16), marine phytoplankton is not as important in controlling atmospheric CO_2/O_2 ratios, at least today, as has been thought.

In the geologic past, before the middle

Table 1. Estimates of Late Cretaceous and modern plant diversity. From Hughes (17).

Period	Gymnosperms	Pteridophytes	Angiosperms
Recent	640	10,000	286,000
Late Cretaceous	500	2,000	20,000

Paleozoic colonization of the lands by terrestrial vascular plants, marine phytoplankton certainly produced the bulk of the world's organic matter. However, as vascular plants began to effectively utilize the terrestrial realm, marine/terrestrial productivity ratios would have progressively declined through time to their present value. By the latest Cretaceous, long after angiosperms had become widespread, terrestrial floras were relatively diverse and abundant; estimates which compare Late Cretaceous and modern plant diversity are given in Table 1 (17).

In spite of relatively low Late Cretaceous terrestrial plant diversity, there is no reason to believe that Late Cretaceous terrestrial ecosystems were less effectively utilized than today. As Hughes (17, p. 48) noted, "if the [Cretaceous] equatorial belt was at least 80° of latitude wide, as suggested by the distribution of *Classopollis*, there would be more appearance of uniformity. This relatively low diversity does not imply any shortage of numbers of individual plants, if the general frequency of fossil remains in the sediments may be taken as a guide." A comparison of modern productivity with that of Late Cretaceous,

with the objective of assessing the impact of a phytoplankton failure on atmospheric CO_2/O_2 ratios, is illuminating.

Of modern ecosystem types (Table 2) which occupy 1 percent or more of the earth's surface, and evaluated in terms of 10^9 metric tons of carbon production per year per 10^6 km^2 , the terrestrial ecosystems (excepting tundra and alpine meadow, desert scrub, and rock, ice, and sand) are the most productive on earth. Of the marine ecosystems which occupy more than 1 percent of the earth's surface, the continental shelf lags behind all terrestrial ecosystems except for tundra and alpine meadow, desert scrub, and rock, ice, and sand; the productivity of the open ocean approximates that of tundra and alpine meadow.

If the present can be used as a guide to help interpret past geologic history, transgression of epeiric seas onto continental areas would replace relatively productive terrestrial ecosystems by less productive marine ecosystems. Conversely, during a regression of epeiric seas back to the ocean basins, almost any terrestrial ecosystem that would develop on the previously flooded terrain would be more productive than the marine ecosystem it replaced. The only question is how far back into the geologic past this principle would apply. Because the latest Cretaceous vegetation cover was probably not markedly dissimilar from modern, it certainly would seem applicable to latest Cretaceous time.

During the latest Cretaceous, epeiric seas, which had flooded continental areas earlier in the period, were under-

Table 2. Relationships between ecosystem types, showing the area ($\times 10^6 \text{ km}^2$) and percentage of the earth's surface occupied by each, their primary productivity ($\times 10^9$ metric tons of carbon per year), and their productivity/area ratios. Area and productivity data are from Whittaker and Likens (10).

Ecosystem type	Area (A) and percent of earth's surface	Total net primary productivity (P)	P/A
<i>Terrestrial ecosystems</i>			
Tropical rain forest	17.0 (3.4%)	15.3	0.90
Tropical seasonal forest	7.5 (1.5%)	5.1	0.68
Temperate evergreen forest	5.0 (1.0%)	2.9	0.58
Temperate deciduous forest	7.0 (1.4%)	3.8	0.54
Boreal forest	12.0 (2.4%)	4.3	0.36
Savanna	15.0 (3.0%)	4.7	0.31
Woodland and shrub land	8.0 (1.6%)	2.2	0.28
Temperate grassland	9.0 (1.8%)	2.0	0.22
Swamp and marsh	2.0 (0.4%)	2.2	1.10
Tundra and alpine meadow	8.0 (1.6%)	0.5	0.06
Desert scrub	18.0 (3.62%)	0.6	0.03
Rock, ice, and sand	24.0 (4.84%)	0.04	0.002
<i>Marine ecosystems</i>			
Lake and stream	2.5 (0.50%)	0.6	0.24
Continental shelf	26.6 (5.4%)	4.3	0.16
Open ocean	332.0 (66.9%)	18.9	0.06
Upwelling zones	0.4 (0.08%)	0.1	0.25
Algal bed and reef	0.6 (0.12%)	0.5	0.83
Estuaries	1.4 (0.28%)	1.1	0.79

going their final major regression back to the ocean basins (18). Productivity in the epeiric seas can only be guessed at, but a reasonable assumption is that it approximated the present day *P/A* value for the continental shelf of 0.16. At the time of the terminal Cretaceous regression, angiosperm vegetation covered wide areas and the tropical regions were more latitudinally widespread than they are today. Thus, during the regression, replacement of the epeiric seas (*P/A* value, 0.16) by tropical and temperate vegetation (average *P/A* value, 0.68) should have resulted in an increase in the total world primary productivity; any reduction of phytoplankton productivity would have been more than offset by the expansion of terrestrial plants. Because of the expanded Late Cretaceous tropical regions, the total productivity then could easily have exceeded the modern value.

During an interval of relatively sudden late Mesozoic or Cenozoic phytoplankton failure, as, for example, the catastrophic late Maestrichtian reduction in diversity and abundance of the coccolithophorids, a minor, geologically short-term interval of reduced productivity could have occurred. However, because of the great productivity and wide distribution of Cretaceous terrestrial vegetation and because other phytoplankton species would have quickly occupied the vacated niche, such a failure would probably have been reflected as a minor inflection on the world productivity-time scale. The failure, and the slight increase in atmospheric CO₂ and decline in O₂, unless they disrupted the atmosphere/ocean CO₂ balance, would in themselves probably have been insufficient to generate worldwide terrestrial and marine faunal extinctions.

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Scanning Electron Microscope Study of Early Lichen Synthesis

Abstract. *In the early stages of cellular interaction between the symbionts of Lecidea albocaerulescens, the phycobiont produced an extracellular sheath which bound to it hyphae of the mycobiont. Such a sheath may be a means by which the symbionts recognize each other. Hyphae of the mycobiont formed flattened appressoria as they grew over the algal cells and in this way secured the autotrophic population necessary for the development of a lichen thallus.*

There have been many attempts to reconstitute lichens from their separate fungal and algal symbionts. Some of the attempts have been successful (1), but, in general, they have not contributed much to our understanding of the cellular events that occur during resynthesis. Our study, the first at the ultrastructural level of the early stages of lichen synthesis, provides new clues on the mechanism of interaction between the symbionts of lichens.

The symbionts of *Lecidea albocaerulescens* (Wulf.) Ach. (2) were isolated and maintained in separate cultures for about 1 year before they were recombined. A culture of the fungal component (mycobiont) was obtained from several spores that were discharged from a fruiting body onto an agar surface. The fungus was cultivated in malt extract-yeast extract medium. A culture of the algal partner (phycobiont) was obtained from a single cell that was isolated by the micropipette method (3). The alga was cultured in a tris-buffered inorganic medium (4) and identified as *Trebouxia glomerata* (Warén) Ahm. Colonies of the fungus that had grown in liquid medium were washed in sterile distilled water for 1 hour and then placed onto a purified agar substrate (Difco 0560-02) in plastic petri dishes (60 by 20 mm) together with cells of the alga taken from an agar slant. The mixed cultures were incubated at 18°C under 2140 lm/m² of illumination and with a daily cycle of 12 hours of light and 12 hours of darkness. Observations with a scanning electron microscope (SEM) were made about 4 months after incubation of the cultures.

Colonies of the resynthesized lichen were cut out of the agar and fixed for 30 minutes at room temperature in 2 percent glutaraldehyde in 0.1M phosphate

buffer, pH 7.2. The colonies were rinsed briefly in the phosphate buffer and then fixed for 30 minutes at room temperature in 1 percent OsO₄ in 0.1M phosphate buffer. The specimens were rapidly dehydrated through an ascending alcohol series, placed in a critical-point drying apparatus (model DCP-1, Denton Vacuum, Inc., Cherry Hill, N.J.) and dried by the method of Anderson (5). Specimens were cemented to specimen stubs, coated with carbon and then with 150 Å of gold-palladium, and examined at 10 kv in an SEM (JEOL model JSM 35U).

Lichenized interactions between mycobiont and phycobiont were observed with a light microscope 25 days after the beginning of the joint culture. Early contacts between the symbionts as well as a thallus-like structure were seen with an SEM in some areas of our mixed cultures (Fig. 1). The algal cells associated with fungal hyphae were dark green whereas those algae free from the fungus were yellow and filled with large droplets. Thus, the influence of the fungus on the phycobiont appears in the earliest stages of synthesis. Hill and Ahmadjian (6) reported that the fungus influenced the metabolism of the phycobiont even in a mixed culture where there was no visible physical contact between the symbionts.

One of the most evident features of lichens at the ultrastructural level is an extracellular, fibrillar material that surrounds the symbionts. Such a substance, presumably a polysaccharide (7, 8), binds hyphae of the cortex and medulla and provides a structural integrity to the lichen thallus. It has been assumed that this material is mostly of fungal origin since it is evident mainly in the cortex and medulla which are thallus layers that consist of fungal hyphae.