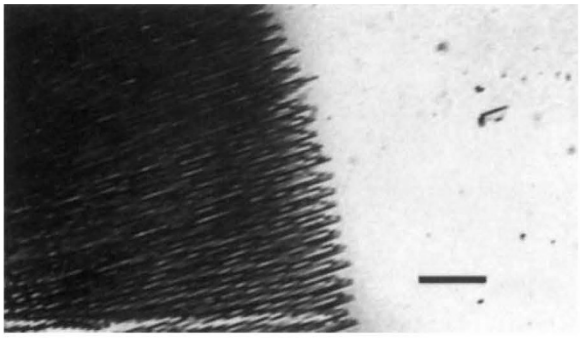
Antifreeze protein in snake venom?

SIR — Rubinsky *et al.*[1] claim that the C-type lectin found in rattlesnake venom can inhibit ice growth in certain crystal planes and that the mechanism is probably similar to that of the antifreeze proteins in the body fluids of polar fishes. But the evidence is inconclusive. The authors were incorrect in their belief that modification of the growth habit of ice crystals by binding to the crystal surface is unique to fish antifreeze proteins. Non-colligative antifreeze proteins have been identified in several invertebrates[2] and antifreeze proteins have also been found in the leaves of cold-acclimated winter rye (*Secale cereale* L)[3].



Ice crystals growing in a 3% solution of xanthan gum at −10 °C. Scale bar, 100 μm.

In the natural world, antifreeze proteins have two functions: to depress the freezing point of fluids to below the surrounding temperature to prevent ice formation; and to suppress ice recrystallization once ice has formed[4]. The two methods generally used to test for the presence of antifreeze proteins are based on these two properties. Thus antifreeze proteins are either identified by measuring their non-colligative freezing point depression[5] or by measuring rates of recrystallization[6]. Rubinsky *et al.*[1] used the first method, the determination of freezing point using a Clifton nanolitre osmometer, but did not detect any non-colligative freezing point depression. They did not use the second method.

To support the view that C-type lectin acts like an antifreeze protein, Rubinsky *et al.* showed that the ice crystals grown in lectin solutions are spicular and resemble those grown in a sea raven antifreeze protein solution. But ice crystals of this shape are quite common in many solutions grown in various circumstances.

Muhr and Blanshard[7] have shown photographs of clouds of ice crystals in the form of straight dendrites (very fine parallel needles) which were grown in a solution of sucrose, alginate and calcium hydrogen phosphate. Tiller[8] grew ice crystals in a solution of potassium chromate and produced fine parallel filaments. Rapatz and Luyet[9] show parallel ice 'spears' growing in partially dehydrated muscle fibre at −16 °C. The figure shows ice crystals grown at −10 °C in my laboratory. The solution is 3% xanthan gum, a commonly used food thickener. The crystal shapes are almost identical to those shown by Rubinsky *et al.* Thus the shape of ice crystals depends on various physical and chemical factors and is not a reliable indication of non-colligative freezing behaviour.

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Phytoplankton growth and CO_2

SIR — By making a case for CO_2 limitation of phytoplankton growth in the sea, Riebesell *et al.*[1] focus long overdue attention on the role of CO_2 in marine phytoplankton ecology. Their case, however, is equivocal. Their theoretical analysis assumes that diatoms use only CO_2 and that the production of CO_2 from HCO_3^- is uncatalysed. These assumptions may be valid for some species but require rigorous testing in the light of work showing that *Phaeodactylum tricornutum* produces extracellular carbonic anhydrase[2] and, together with some freshwater diatoms, has the capacity to utilise HCO_3^- (refs 3–5). Although CO_2 may be a growth-rate-limiting resource, the experimental design of Riebesell *et al.* altered CO_2 concentrations by varying pH. Therefore, it is unclear whether the observed changes in growth rates are caused directly by changing pH or indirectly by CO_2.

A more general point relevant to future attempts to interpret stable carbon isotope ratios in phytoplankton is the need to distinguish between CO_2-limited photosynthesis and CO_2-limited growth. CO_2-limited photosynthesis does not necessarily imply CO_2-limited growth provided that: P_{max}/μ is greater than or equal to the reciprocal of the fractional limitation of photosynthesis by CO_2 availability, where P_{max} is the CO_2-saturated rate of carbon-specific net photosynthetic carbon fixation; and μ is the carbon-specific growth rate.

In cyanobacteria and green algae, HCO_3^- and/or CO_2 transport are induced in response to low levels of dissolved inorganic carbon. Full induction may occur without any appreciable decline in steady-state growth rate[6,7] even though such cells exhibit steady-state CO_2-limited photosynthesis. A decrease in discrimination against $^{13}CO_2$ corresponding to the induction of the CO_2-concentrating mechanism has been observed[8]. Consequently, CO_2-limited photosynthesis can explain the striking inverse relationship between stable carbon isotope composition of marine phytoplankton and ambient CO_2 concentrations[9,10]. But, in isolation, this does not imply that CO_2 limits phytoplankton growth rates in the sea.

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RIEBESELL *ET AL.* REPLY — Turpin makes two important points pertinent to the question of CO_2 limitation of phytoplankton growth. With respect to his first comment, we agree that inorganic carbon use by the common marine diatom species needs to be rigorously tested. With the exception of *Phaeodactylum tricornutum*, a brackish water species which in many aspects is an atypical diatom, information on carbon acquisition of marine diatoms is not available[11]. We have now analysed sixteen species of marine diatoms and two species of Prymnesiophytes for their ability to use bicarbonate (manuscript in preparation). We found no evidence of HCO_3^- use for any of the planktonic diatoms or the Prymnesiophytes. Their rates of carbon uptake were consistently lower than the maximum potential supply of CO_2 over a wide range of CO_2 concentrations. Both HCO_3^- use and the presence of extracellular carbonic anhydrase would result in uptake rates higher than the rate of uncatalysed CO_2 supply at low ambient CO_2 concentrations. We did find evidence of HCO_3^- use for one benthic diatom species. These results further substantiate the assumption of uncatalysed CO_2 use of marine planktonic diatoms.

Based on the information presently available, the question of whether the observed change in growth rate of the