response[3,5] and includes reactions which are independent of one another and of macromolecular synthesis. Therefore, it was necessary to invoke an intermediary agent between external signal and intracellular response with the capacity to modulate many different metabolic pathways at the same time. The only specific enzymatic steps then known to be accelerated by the stimulatory treatments were the transphosphorylation reactions of glycolysis[7-10], all recognised as control points in this pathway and all requiring Mg^{2+} (refs 11–13). It has also become evident that some key regulatory enzymes vary in activity with their degree of phosphorylation[14,15] and that the activities of the protein kinases or phosphatases which determine this degree are sharply dependent on $[Mg^{2+}]$ in the physiological range. In a slightly different cellular domain, we have shown that the rates of uptake of hexoses and uridine into chicken embryo cells, which are governed coordinately by external effectors, can be controlled by Mg^{2+}, but not by Ca^{2+}, in a manner that simulates in detail the kinetics of the physiological response[13].

It is generally agreed that over 90% of the Mg^{2+} in cells is bound, largely to membranes and macromolecules[17-20]. Changes in configuration of the binding structures or in their microenvironment would alter the availability of Mg^{2+} for its metabolic tasks. Increasing the permeability of the cell membrane to Mg^{2+} would also tend to drive up the intracellular concentration of Mg^{2+} (ref. 21). Because the estimated concentration of free intracellular Mg^{2+} is less than that required for maximal activity of many key regulatory enzymes[11-14] any small change would have far reaching effects[22].

By contrast, the concentration of free Ca^{2+} in cells is far too low[23] to affect the regulatory enzymes of the pathways involved in the coordinate response[3,5]. Perhaps some mechanical responses which involve proteins with a very high affinity for Ca^{2+} are under its control. But, the remarkable sequestering power of the cell for Ca^{2+} (ref. 24) would ensure that such a response would be short-lived, unlike the coordinate response which is maintained for many hours, requires continuing stimulation, and culminates in accelerated cell division.

There would seem to be some merit in Durham's suggestion that the inhibition of cell metabolism which accompanies infection by cytocidal viruses is caused by a gross increase in intracellular Ca^{2+} following damage to the cell membrane. This follows from the observation that an internal Ca^{2+} level, $[Ca^{2+}_i] \geqslant 10^{-4}$ M interferes with key Mg^{2+}-dependent reactions in the cell[25,26]. Beyond such pathological effects, however, the role of Ca^{2+} seems likely to be restricted to short-term structural and mechanical responses in keeping with the pulse-like, localised nature of its fluctuations, and to the limited number of proteins which can respond to $[Ca^{2+}]$ within the physiological range of $<10^{-7}$–10^{-5} M. Long-term inhibition of metabolism by Ca^{2+}_o deprivation may be the indirect result of lowering free Mg^{2+} within the cell[4].

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DURHAM REPLIES—The best current estimates of ion concentrations in resting eukaryotic cytoplasm are around 10^{-3} M for Mg^{2+} and 10^{-7} M for Ca^{2+}. This 10^4-fold difference explains why Ca^{2+} and not Mg^{2+} is used as a short-term intracellular signal in movement, hormone action, synaptic transmission, protozoan chemotaxis, vision and bioluminescence. Powerful homeostatic mechanisms tend always to maintain very low cytoplasmic Ca^{2+} concentrations. Prolonged overwhelming of these mechanisms would produce a spectrum of changes strikingly like those produced by many lytic or transforming viruses.

Rubin and his colleagues have shown clearly that changes in extracellular Mg^{2+} concentrations can greatly affect cells. Others have shown analogous responses to extracellular K^+ or H^+ levels, and to agents that affect polyamine metabolism. Responses to extracellular Ca^{2+} are notoriously variable, however, even for processes that undoubtedly involve intracellular Ca^{2+}. One reason is that eukaryotic cells conduct most Ca^{2+} fluxes across internal membranes, to and from substantial calcium reservoirs for which there are probably no magnesium equivalents. In the long term, feedback relationships between different ions tend to obscure the primary ion fluxes, so that one is probably wise not to make categorical statements about any one ion.

Rubin's statement that the cytoplasmic Ca^{2+} concentration is too low to affect the "coordinate response" is wrong. Micromolar Ca acts on adenyl and guanyl cyclases and phosphodiesterases, with consequent effects on cyclic nucleotide levels and kinase activities. It also acts on K^+ and other ion fluxes, and on DNA precursor synthesis. Rubin's implication that intracellular Ca^{2+} ions act via Mg^{2+}-dependent reactions can be only partly true.

Rubin and I agree that cell biologists frequently postulate, and then expensively seek, macromolecules to fill roles that ions can fill much more simply.

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Cholinergic link in yawning

HOLMGREN *et al.*[1] recently focused attention on the basic mechanism behind the act of yawning. They reported that physostigmine and pilocarpine induce yawning in young male rats and hypothesised that a central cholinergic link may be involved in the reflex. Our results support such a proposal.

Yawning is a characteristic sign of withdrawal from morphine in man[2] and monkeys[3]. When naloxone (0.5 mg per kg body weight), but not physiological saline, was injected subcutaneously (s.c.) into three 'ex-addict' baboons (two male and one female, 4.4–5.2 kg), 98 d after abrupt withdrawal of morphine, a low incidence of yawning (2–4 episodes) occurred within 15 min; on this occasion, other signs of long-term withdrawal were absent. Seven days later, the same baboons were again challenged with naloxone, 20 min after physostigmine (0.05 mg per kg s.c.). Although this dose of physostigmine *per se* did not elicit yawning, with each animal there was a threefold increase in the incidence of yawning.

Although a cholinergic link may indeed be involved in yawning, it should be recognised that other factors are also important. Thus, dimethyltryptamine causes

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