If one does believe in stratification one should accept that with the onset of stagnation the O_2–H_2S interface will inevitably rise to the base of the euphotic zone (where it will participate in the plankton dynamics of the basin) unless one of several external influences disrupts the system[7]. Much more convincing evidence is required if Irwin seeks to replace a model which has the weight of Quaternary–Recent modern analogues behind it.

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Calcium activation of the cortical reaction in sea urchin eggs

USING sea urchin (*Echinus esculentus*) eggs broken by shock discharge, or isolated egg cortices, Baker and Whitaker[1] found that about 1 μM Ca was sufficient to activate exocytosis of half the cortical vesicles. They represent this figure as an order of magnitude lower than the figure we reported[2] (between 9 and 18 μM) using *Lytechinus pictus* sea urchin isolated cortices.

However, there is no discrepancy between our results. We measured the level of calcium required for nearly complete (>95%) exocytosis, and we have recently obtained a more precise estimate of 12 μM, determined by breaking the eggs open in the test solutions. From Baker and Whitaker's Fig. 2, they observe 95% exocytosis at a Ca concentration of 6 μM (pCa = 5.2). Moreover, Baker and Whitaker derive an apparent stability constant[3] from a Ca-EGTA binding constant[4] (pK) of 11. However, in physiological media a lower binding constant is appropriate[5]. We assumed a pK of 10.7 in our calculations for our HEPES-buffered solutions, as this value was reported[6] for similar phosphate-buffered solutions. This difference in binding constant accounts for the twofold difference remaining between our calculated Ca threshold for the cortical reaction and that offered by Baker and Whitaker.

The problem of selecting the appropriate binding constant for EGTA is a general one in studies of the role of calcium. We recently attempted to estimate the primary Ca-EGTA binding constant ourselves, using calcium-specific electrodes filled with Ca-DOPP mixed with DOPP-n[7]. We could only obtain accurate measurements of calcium when the apparent binding constant of EGTA for Ca was reduced in PIPES-buffered low pH (6.5) and the monovalent cation concentration was kept to 0.13 M. We measured an apparent binding constant, pK′ = 5.3, corresponding to a primary Ca-EGTA binding constant of pK = 10.6. Since high pH[8] and high ionic strength[9] are both reported to reduce the primary Ca-EGTA binding constant (but see ref. 10), it seems that even our estimate for the Ca threshold of the cortical reaction (12 μM) is too low.

Baker and Whitaker also suggest that ATP is needed to maintain the cortical intracellular Ca store. We found that sperm, the Ca-transporting membrane ionophore A23187, and the parthenogenetic agent urea all release Ca from the same store[11]. Quantitative estimates of the amount of Ca released[2,12] compared to the cortical reaction threshold, suggest that the store is released into a small fraction of the cytoplasm at the cortex. As the store is replenished and can be discharged again about 45 min after release (when the cortical vesicles are gone), it is unlikely to be associated with the cortical vesicles themselves. The calcium store may reside in a subsurface endoplasmic reticulum, but this has not yet been observed in the appropriate cortical region. The calcium may be bound directly to the membrane instead.

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BAKER AND WHITAKER REPLY—We are delighted that Zucker and Steinhardt find no serious disagreement between their results and ours.

It is unfortunate that one cannot be sure of the value of the free calcium concentration in physiological solutions buffered with EGTA to within half an order of magnitude. As Steinhardt *et al.* pointed out[1] the most one can do is to specify total calcium, magnesium and EGTA concentration so that, should reliable affinity constants become available, the free calcium data may be recalculated. Although monovalent cation concentrations were not identical, a rather direct comparison of the results obtained on British and American urchins is possible because the calcium buffers used in our experiments[2] were made up in the same proportions of calcium, magnesium and EGTA as those specified in the study of the cortices of *Lytechinus*.

Much of the apparent discrepancy stems from Zucker and Steinhardt's somewhat peculiar definition of 'threshold' for cortical granule release as the calcium concentration giving release of 95% of the granules. Although the release of individual granules is an all-or-none process, our observations suggest that in a population of granules the rate of discharge is a smooth function of calcium concentration. Physiologically, it is likely that the abrupt onset of the cortical reaction can be attributed to the release of large amounts of calcium from a store[3]. In the presence of 5 mM ATP, a calcium test solution[2] which according to Steinhardt *et al.*[1] contains 1 μM free calcium, initiates a cortical response which spreads as we have described and has discharged all the cortical granules within 2 min. At higher calcium concentrations the rate of discharge of the granules (expressed as a percentage of the total number) increases. A calcium activation curve obtained by scoring the proportion of granules remaining at 30 s has a similar half point to the data (Fig. 2*a* of ref. 2) for eggs subjected to high voltage discharge in solutions of different calcium concentration. Again, using the constant of Steinhardt *et al.*[1] the half activation point is about 3 μM, although its position will depend on the time after addition of calcium at which the reaction is assessed. At its fastest, the discharge of cortical granules over the whole cortical fragment takes about half a minute. This is not dissimilar to the rate *in vivo*[4]. Without information as to the time of observation, the half point of activation in the cortices of *Lytechinus* cannot strictly be estimated, but might at its lowest be 6 μM. At worst, as it is not clear whether concentrations of calcium less than 12 μM cause any granule discharge in *Lytechinus*, there remains an order of magnitude difference between the two species in the lowest calcium concentration which has been demonstrated to produce a cortical response.

Although this difference in the affinity of the cortical reaction for calcium in the eggs of the British and American urchins may well have its explanation in species differences, or the uncertainties in free calcium concentration, our observations as to the critical importance of ATP in maintaining the properties of the

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