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.

R. V. TYSON
*Department of Earth Sciences,*
*The Open University,*
*Milton Keynes, Bucks, UK*

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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 ($p$Ca = 5.2). Moreover, Baker and Whitaker derive an apparent stability constant[3] from a Ca-EGTA binding constant[4] ($p$K) of 11. However, in physiological media a lower binding constant is appropriate[5]. We assumed a $p$K 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 $p$H (6.5) and the monovalent cation concentration was kept to 0.13 M. We measured an apparent binding constant, $p$K′ = 5.3, corresponding to a primary Ca-EGTA binding constant of $p$K = 10.6. Since high $p$H[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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ROBERT S. ZUCKER
*Department of Physiology-Anatomy*

RICHARD A. STEINHARDT
*Department of Zoology,*
*University of California,*
*Berkeley, California 94720*

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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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physiological reaction[2] suggest a less trivial explanation. As experiments with *Lytechinus* were performed in the absence of added ATP, the decrease in calcium affinity which results from removal of ATP in *Echinus* may well be the factor responsible for the apparent differences in calcium affinity in the two species.

P. F. BAKER
M. J. WHITAKER
*Department of Physiology,*
*King's College,*
*London WC2, UK*

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## Fusion or lysis of vesicles by $Ca^{2+}$?

GINSBERG showed[1] that sonicated phosphatidylserine (PS) vesicles in the presence of large concentrations of $Ca^{2+}$ or $Mg^{2+}$ did not retain sucrose and he concluded that the final structures had lost the form of closed vesicles. As such, he proposes the cation effect to be one of lysis and the $Ca^{2+}$–PS system to be an inappropriate one for the study of membrane fusion. It is not surprising that the final product of the PS–metal interaction cannot retain solutes such as sucrose, as the vesicles collapse and internal volume is lost[2]. The PS membrane repeat determined by X-ray diffraction is 53 Å in 2 mM $Ca^{2+}$; 67 Å in 10 mM $Mg^{2+}$ (ref. 2) and 71 Å in 1 M $Na^{+}$ (ref. 3). The term 'membrane fusion' refers to the formation of larger membranous structures by contact and mixing of the parent membranes. This is in contrast to the mixing of membrane lipids by diffusion of the components, as proposed for the dimyristoyl lecithin–dipalmitoyl lecithin system[4]. Recent experiments[3] have shown that release of contents is nearly second order in vesicle concentration and is concomitant to aggregation, demonstrating vesicle contact in leakage experiments. That $Ca^{2+}$ induces fusion, that is, mixing of membrane components, is indicated by the formation of large cochleate structures, which on addition of EDTA, become huge vesicles capable of entrapping large molecules[5]. $Mg^{2+}$ alone is less effective[6] but in its presence, only small concentrations of $Ca^{2+}$ are required for obtaining larger structures[2,3]. Similarly, in the dimyristoyl phosphatidylglycerol–dipalmitoyl phosphatidylglycerol vesicles[7] and in phosphatidic acid–phosphatidylcholine vesicles[8], $Ca^{2+}$ induces mixing of the lipids, that is, fusion. Our view is that fusion requires a destabilisation of the membranes in contact until the joint membrane is arranged. Model systems are intended to be approximations to the *in vivo* systems. A definition of membrane fusion as a process without leakage is too restrictive and so far no experiment has ruled out leakage in fusion events. In model systems better approximating the *in vivo* system, such as mixed PA/PC vesicles[8], mixing and retention of contents has been demonstrated. The interactions postulated to occur between the $Ca^{2+}$ and PS in the pure system are still relevant to the mixed systems.

S. NIR
*Roswell Park Memorial Institute*
*Buffalo, New York 14263*

W. PANGBORN
*Medical Foundation of Buffalo,*
*Buffalo, New York 14203*

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GINSBERG AND GINGELL REPLY—The comments of Nir and Pangborn stem from an inadequate definition of membrane fusion. They claim that fusion is equivalent to the mixing of components from two contacting parent membranes. However, physiological membrane fusion apparently involves the transfer of aqueous contents from one membrane-bounded compartment to another without spillage into inappropriate spaces, as seen in phagosome–lysosome interaction and secretion. Any artificial system should fulfil this additional criterion to be biologically relevant[1,2]. Thus, the X-ray data cited by these authors[3] seem to invalidate their PS system as a paradigm for membrane fusion: $Ca^{2+}$ converts PS into a multilayer containing no removable water. Such collapsed multilayers could result from the lysis of closed membranous forms by the mechanism we suggest[1]. The experiments described by Nir and Pangborn where loss of contents is reported to accompany vesicle aggregation may also be explained in terms of lysis: $Ca^{2+}$-induced vesicle rupture with loss of contents may provide the antecedent for immediate aggregation of the resultant membrane fragments.

Although $Ca^{2+}$ is strongly implicated in biological membrane fusion[4], there is no compelling reason to suppose that its action on dispersions of single acidic phospholipids resembles its interaction with mixed lipid membranes nor with the biomembrane systems in which fusion was first studied[5]. This point is underlined by the far greater $Ca^{2+}$ sensitivity of natural vesicle fusion[6]. Gershfeld has recently shown[7] that sonicated vesicles are in a metastable state at temperatures exceeding the lipid phase transition temperature. Thus, addition of divalent cations to PS vesicle suspensions may merely trigger a return to equilibrium by a variety of unknown pathways.

L. GINSBERG
D. GINGELL
*Department of Biology as Applied to Medicine,*
*The Middlesex Hospital Medical School,*
*London, UK*

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## Some real communities are unstable

THE mathematical stability analyses of randomly constructed food webs of Pimm and Lawton[1,2] have emphasised the destabilising influence of omnivory. Their examination of a number of real food webs[3] seems to support their hypothesis that webs with many omnivores should be rare except in insect host–parasitoid systems. However, I analysed the real food webs cited as corroborative by Pimm and Lawton, that is, those of Askew[4], Force[5] and Richards[6], and found that none meet the criterion for Lyapunov stability. The validity of the models and their inherent assumptions appears questionable.

All webs were analysed using the observed signs of interaction and Pimm and Lawton's constraints on the selection of random magnitudes for parasitoid–host and herbivore–plant interactions. Signs for variable interaction, species A and B each serving as prey or predator for the other, were arbitrarily assigned. To investigate whether the occurrence of self-limited species influenced stability, runs were repeated with self-regulation terms removed (all principal diagonal elements equal to zero). Finally, self-limitation was introduced at the lowest trophic level (plant), a criterion used by Pimm and Lawton in constructing their random webs. Analysis of Richard's web was performed twice, once with the parasitoid–host and again with vertebrate predator–prey constraints used for the predatory arthropods. In no case were any of 50 runs for either the original or adjusted webs stable.

I included only primary interactions in abstracting a matrix from Force's web. The excluded terms (dashed lines in Force's paper[5]) represent secondary interactions, such as the rejection of a potential host which has been previously

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