methacholine (acetyl-β-methylcholine) the Ca^{2+} influx returned to basal levels within 1–2 minutes (*a* in the figure).

In agreement with ref. 1, we found that the extract-induced $[Ca^{2+}]_i$ signals tend to oscillate irregularly, while methacholine- or thapsigargin-induced signals do not. One explanation for this behaviour is that when the extract is applied the stores are full, and thus there may be mechanisms inactivating the Ca^{2+}-entry signal. But when we examined the response of astrocytoma cells to the extract following depletion of their intracellular stores with thapsigargin, we still saw large $[Ca^{2+}]_i$ oscillations (*c* in the figure on page 481).

In the mouse lacrimal acinar cell, a cell type known to exhibit capacitative calcium entry[6], the extract induced a clear intracellular mobilization of Ca^{2+}, as well as a sustained entry of Ca^{2+} (not shown). The response was entirely blocked by the intracellular application of the inositol trisphosphate receptor antagonist heparin, or by the external application of the muscarinic receptor antagonist atropine. The muscarinic-like activity is always found in the soluble fraction of Jurkat cells, indicating that this is a different principle from the one eliciting the Ca^{2+} entry responses in astrocytoma cells. When this muscarinic agonist activity was blocked, lacrimal cells did not respond to the Ca^{2+}-entry-stimulating principle in the Jurkat cell extract. However, when an extract of lacrimal cells is prepared by the same method as used for Jurkat cells, this extract induces a response in astrocytoma cells similar to the one induced by the Jurkat extract. Thus, although lacrimal cells contain the material, they do not respond to the activity in Jurkat extracts which was designated CIF.

Our findings indicate that the Ca^{2+}-signalling principle (or principles) in acidic extracts of Jurkat cells is unlikely to be the messenger for capacitative Ca^{2+} entry. It is not clear from our data whether the activity described by Randriamampita and Tsien[1] has a novel physiological role in Ca^{2+} signalling in certain cell types, or whether the extraction procedure has created a material which induces Ca^{2+} fluxes by non-physiological means.

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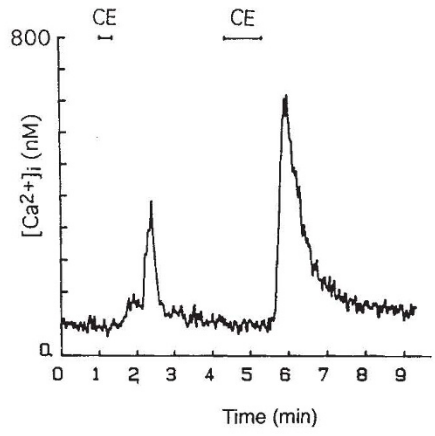
RANDRIAMAMPITA AND TSIEN REPLY — We agree with Bird *et al.* that a crude cell extract is likely to contain several biological activities, not just the desired one. The muscarinic activity towards lacrimal cells found by Bird *et al.* is an example of just such contamination. Lacrimal cells may also be relatively insensitive to externally applied CIF, because cell types do vary in their sensitivity[1]. However, the activity of Jurkat extract on the astrocytoma cells that we use for our assays is not due to this muscarinic component, because our unpublished results show that the $[Ca^{2+}]_i$ elevations are unaffected by atropine doses that completely inhibit muscarinic activation. We take the evidence for multiple activities as an incentive to purify the key messenger rather than to abandon the entire concept. Indeed, we have three independent chromatographic methods for fractionating cell extract and have seen resolution of multiple activities. The most active fraction has at least 20-fold more activity per unit dry mass than crude cell extract. A conclusive demonstration of the CIF concept will require purification to homogeneity and structural elucidation. The activity is not an artefact of acid extraction, because it is also releasable by sonication, but it then undergoes degradation in a few minutes[2], presumably by enzymes. Okadaic acid inhibits such degradation, which may explain why okadaic acid can potentiate Ca^{2+} elevations[2,3].



Cytosolic free calcium ($[Ca^{2+}]_i$) in a single fura-2-loaded 1321N1 astrocytoma cell in a normal medium with 1 mM Ca^{2+}. During the two periods labelled CE, the cell was superfused with Jurkat cell extract with zero added Ca^{2+} and 1 mM EGTA, delivered from a local micropipette.

The figure (above) shows that the action of Jurkat extract can be briefer and less oscillatory than the results shown by Bird *et al.* During the periods labelled CE, single astrocytoma cells were superfused with cell extract containing zero Ca^{2+}. Because CIF action requires extracellular Ca^{2+} (ref. 1), $[Ca^{2+}]_i$ did not increase until the flow was stopped, which re-exposed the cells to normal medium with 1 mM Ca^{2+} but without cell extract. Then a sizeable $[Ca^{2+}]_i$ elevation appeared with negligible spiking or oscillation and quickly decayed. Our interpretation is that some CIF entered the cells during the superfusion, displayed its activation of Ca^{2+} influx once external Ca^{2+} was available, then decayed by leakage or metabolism, its source having been removed. In support of an intracellular site of action, Thomas and Hanley have shown that Jurkat extract works when microinjected into *Xenopus* oocytes[4]. Although many aspects remain mysterious, we think it premature to conclude that cell extract lacks an activity mediating capacitative Ca^{2+} entry.

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Science and religion

SIR — The report "A Line in the Sea", by J. A. Yoder *et al.* (*Nature* **371**, 689; 1994) not only attempts to explain the open ocean front phenomenon, but it also sheds light on a religious issue concerning a revealed natural phenomenon.

The Holy Book of Islam, al-Qur'an, has explained in two verses (XXV: 53 and LV: 19), the natural phenomenon called the "two adjacent seas" (Maraj al-Bahrain). According to the verses in the Qur'an, these two seas exist side by side with a boundary preventing them from mixing. Indeed, it specifies that the composition of water in these seas, especially the salt concentrations, are quite different.

For years, Islamic scholars have had difficulty in interpreting the concept of Maraj al Bahrain. Although diverse interpretations have been offered by different scholars and a variety of natural phenomena have been used to exemplify this revealed concept, none has been satisfactory from a scholarly perspective. It seems that the advancement of knowledge and technology and recent scientific achievements have made it possible for a better understanding of this natural phenomenon brought to the attention of man through the Qur'an.

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