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NH_4Cl and other weak bases in the activation of sea urchin eggs

TWO recent reports[1,2] have demonstrated the importance of intracellular alkalinisation in initiating development of sea urchin eggs. However, in accounting for the ability of certain weak bases to initiate development by raising intracellular pH (pH_i), the authors used *ad hoc* assumptions, where a straightforward physiological explanation would have sufficed.

Loeb[3] first showed that ammonia can trigger development of unfertilised sea urchin eggs. In 1976, Johnson *et al.*[1] observed that pH_i rises rapidly in the first minutes after fertilisation, and showed that this rise is associated with both an efflux of acid (fall in pH_o) and the influx of an equivalent amount of Na^+. Egg development and the changes in pH_i and pH_o could be prevented both by amiloride and by incubation in Na^+-free media. The authors concluded that fertilisation activates an amiloride-sensitive $Na^+:H^+$ exchange mechanism which raises pH_i, thereby causing the metabolic derepression that initiates development. More recently Lopo and Vacquier[2] showed that the rapid initial pH_i rise is followed by a slower fall which is largely prevented by 2,4-dinitrophenol (DNP).

In addition, Johnson *et al.*[1] found that certain weak bases (ammonia, nicotine, procaine), when added to fertilised eggs in Na^+-free sea water, even in the presence of amiloride, caused the characteristic changes in pH_i and pH_o and initiated development. The authors made the *ad hoc* assumption that these pH changes were caused by the amines either inducing only one component (that is, H^+ efflux) of the $Na^+:H^+$ exchange, or substituting directly for Na^+. Lopo and Vacquier[2] showed that procaine, known to reversibly stimulate DNA synthesis in unfertilised eggs[4], also causes a reversible increase in pH_i. They resorted to the explanation that procaine raises pH_i by increasing membrane permeability to H^+. We propose to replace these *ad hoc* assumptions by a simpler explanation, namely, that the weak base enters the cell as the uncharged molecule (B), which then combines with protons to yield the charged form (BH^+). The authors discarded this mechanism, apparently because they considered it incompatible with the observed fall in pH_o. However, such a fall is precisely what would be expected. The equilibrium $BH^+ \rightleftharpoons B + H^+$ in the medium shifts to the right as B leaves the medium for the cells, thereby lowering pH_o. The entering B simultaneously raises pH_i, as has been demonstrated in several cells with pH-sensitive microelectrodes[5–7]. These changes in pH_o and pH_i can thus occur in the absence of the transmembrane movement of H^+ *per se*, and clearly should be neither Na^+-dependent nor amiloride-sensitive. When the weak base is removed from the bathing medium, B leaves the cell, the above reactions reverse, and both pH_o and pH_i return towards their initial values. Indeed, Lopo and Vacquier observed such a fall in pH_i. It follows that this fall in pH_i is DNP-insensitive as these authors found. We propose that weak bases circumvent the normal mode of raising pH_i in sea urchin eggs by directly penetrating the cell membrane and consuming protons.

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EPEL ET AL. REPLY—Fertilisation initiates a defined sequence or programme of events and one of these, the massive extrusion of protons referred to as the fertilisation acid, was described almost 50 years ago[1]. Ammonia (and other amines) acts like a parthenogenetic agent in turning on certain 'late' events of this sequence, such as K^+ transport[2], protein[3] and DNA synthesis[4,5], and in fact its mode of action was postulated as a pH effect[2,3]. The subsequent finding that ammonia mimicked fertilisation in causing a rapid acidification of the seawater when added to eggs[6] led us to assume that ammonia and the various amine compounds were inducing yet another step of the fertilisation sequence, that is, the fertilisation acid. This discovery, of course, led to the subsequent descriptions of the Na^+–H^+ exchange and the resultant alkalinisation of the cytoplasm which seems essential for biosynthesis[7]. The explanation for the external acidification that is proposed by Boron *et al.* above, and which has since been confirmed experimentally by Winkler and Grainger[8], provides a simpler explanation and we concur with them.

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Nitrogen-fixing root nodules in Ulmaceae

A SURVEY of symbioses of *Rhizobium* spp. with members of the Ulmaceae and Urticaceae in Java and Bali (Indonesia)[1] has revealed nitrogen-fixing nodules only in *Parasponia parviflora* Miq. (Ulmaceae). None were found in specimens of Trema, which are morphologically closely related to *Parasponia* spp. The survey has shown that reports that nodulated specimens of the Ulmaceae from Papua New Guinea belonged to *Trema aspera*[2] or *T. cannabina* Lour. variety scabra (Bl.) de Wit[3] were incorrect. The specimens have now been shown to belong to *P. rugosa* Bl. We believe that *Trema* and *Parasponia* spp. have been confused frequently in the past. Symbioses of *Rhizobium* spp. with members of the Ulmaceae probably occur only in the Asiatic genus Parasponia.

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