Direct Measurement of Intracellular pH Changes in *Xenopus* Eggs at Fertilization and Cleavage

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ABSTRACT We have used Thomas-type recessed-tip pH-sensitive microelectrodes to measure the intracellular pH (pH_i) in *Xenopus* eggs during both fertilization and ionophore activation. The average pH_i in unfertilized eggs is 7.33 ± 0.11 (SD; n = 21) with a resting membrane potential of −10.1 ± 3.5 (SD; n = 38) mV. Within 2 min after the onset of the fertilization potential, there is a slight, transient pH_i decrease of 0.03 ± 0.02 (SD, n = 8), followed by a distinct, permanent pH_i increase of 0.31 ± 0.11 (SD; n = 7) beginning ~10 min after the start of the fertilization potential and becoming complete ~1 h later. The pH_i remains near this level of 7.67 ± 0.13 (SD, n = 10) through at least 10 cleavage cycles, but it is possible to discern pH_i oscillations with a mean amplitude of 0.03 ± 0.02 (SD, n = 38). Eggs perfused for at least 2 h in Na^+-free solution with 1 mM amiloride exhibited all of these pH_i changes, so these changes do not require extracellular Na^+.

Similar cytoplasmic alkalinizations that accompany the activation of metabolism and the cell cycle in a wide variety of cell types are discussed.

The activation of development at fertilization results in a dynamic change in cellular activity including an increase in the metabolic rate and the initiation of cell cleavage. Recent investigations have revealed that among the earliest events triggered by sperm-egg fusion are changes in intracellular free Ca^{2+} and H^+ concentrations. A large, transient increase in the intracellular free Ca^{2+} concentration occurs within a minute after fertilization in both invertebrate (43) and vertebrate (15, 34) eggs, and a permanent increase in intracellular pH (pH_i) of ~0.4 occurs after fertilization in marine invertebrates (23, 24, 38). This pH_i rise requires a small amount of extracellular Na^+ (24, 39), and has been implicated in the dramatic increase in the rate of protein synthesis that occurs at fertilization in sea urchin eggs (18, 48–50). We now report the first direct measurement of the intracellular pH during fertilization in the egg of the frog *Xenopus laevis*, a freshwater vertebrate. Here we also find a significant pH_i rise accompanying the activation of development. In addition, we have detected an initial, small transient pH_i fall preceding the larger permanent pH_i rise, and a small, cyclical pH_i fall associated with cleavage. None of these pH_i changes appears to require extracellular Na^+.

MATERIALS AND METHODS

Mature eggs were obtained from *Xenopus laevis* females injected with 800 IU of human chorionic gonadotropin (Sigma Chemical Co., St. Louis, Mo.). The unfertilized eggs with their jelly coat intact (as required for fertilization) were placed in the dry perfusion chamber to which they adhered. The chamber was then flooded with modified F_1 solution (21) and perfused either continuously or intermittently. Modified F_1 solution (referred to as F_1 throughout) contained (in mM): NaCl, 31.25; KCl, 1.75; $CaCl_2$, 0.25; $MgCl_2$, 0.06; Na_2HPO_4, 0.5; NaOH, 1.9; $NaHCO_3$, 2.0; Tricine, 10.0; pH 7.8.

Intracellular pH (pH_i) was measured with Thomas-type recessed-tip pH-sensitive microelectrodes (44) and 3 M KCl-filled voltage microelectrodes. pH electrodes were made using a one-stage pull and typically had tip diameters of <0.5 μm with full response times of 6–30 s for a unit change in pH. Their typical response was 55–59 mV per pH unit although a few responding as low as 50 mV were also used. The pH electrode was connected to a high input impedance operational amplifier (311J; Analog Devices, Inc., Norwood, Mass.) and the output was recorded on a four-channel chart recorder (Watanabe Instruments, Tokyo).

The pH electrode was usually inserted first by sharply tapping the electrode holder with a pair of forceps. The voltage electrode was then introduced by increasing the negative capacitance to oscillate the electrode tip. 5–10 mM of the anaesthetic, chlorobutanol (1,1,1-trichloro-2 methyl-2 propanol; Sigma Chemical Co.), was usually included in the F_1 solution to prevent prick activation during electrode insertion. This substance causes a slight but reversible pH_i decrease. After successful impalement, the chlorobutanol was washed off and appeared to have no adverse effect on normal development. The potential recorded by the voltage electrode was subtracted from that of the pH electrode at the differential input of the chart recorder to give pH_i as shown in the upper trace of all the figures. This method of recording pH_i allows changes in the tip potential of the voltage electrode to be registered as mirror-image deflections on the two traces. If the electrode tip potential changed while in an egg, the experiment was terminated. All experiments were done at 22°–24°C.

RESULTS

A typical recording from an unfertilized egg after impalement is shown in Fig. 1*A*. Penetration of the egg jelly, vitelline envelope, and plasma membrane by the voltage electrode is accompanied by potential changes similar to those first reported by Palmer and Slack (33) in eggs that were already

THE JOURNAL OF CELL BIOLOGY · VOLUME 91 NOVEMBER 1981 562-567

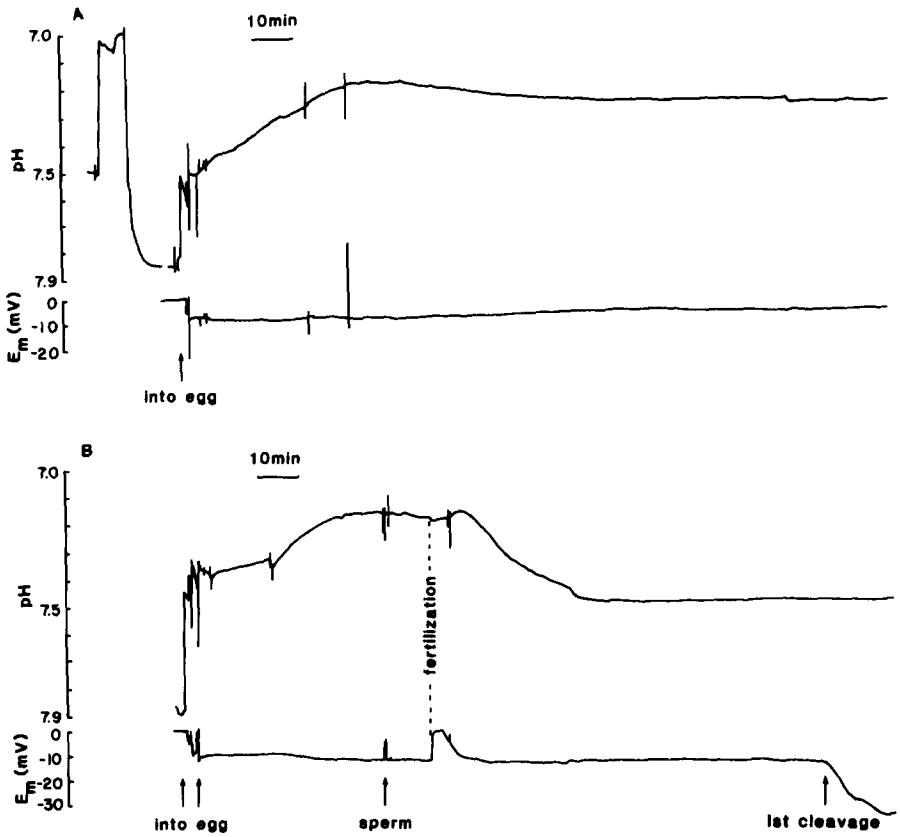

FIGURE 1 Intracellular pH and membrane potential (E_m) recorded in *Xenopus* eggs. (*A*) A typical recording from an unfertilized egg preceded by calibrating solutions. (*B*) An unfertilized egg is impaled and sperm are added resulting in the typical fertilization response and cleavage. Note that the pH voltage scale is twice as large as that for the E_m in Figs. 1 and 2 but in Fig. 3, they are the same size.

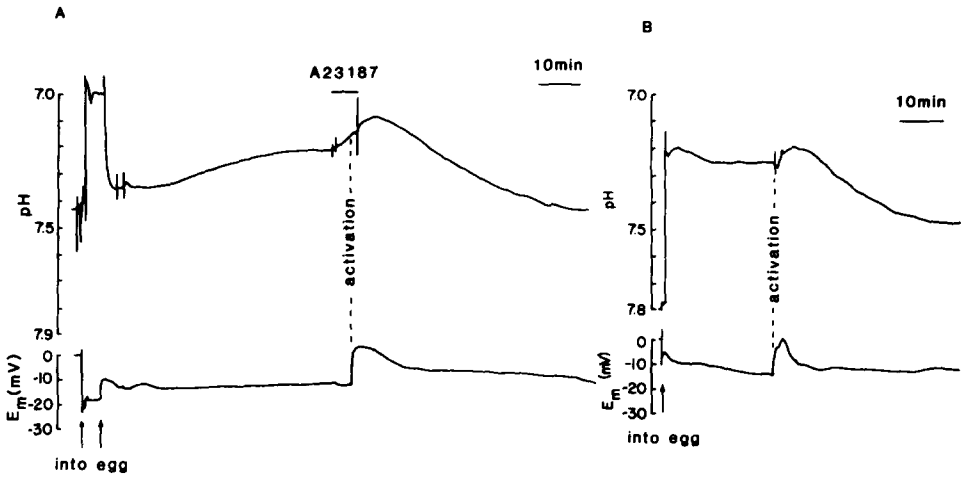

FIGURE 2 Intracellular pH and membrane potential (E_m) recorded in *Xenopus* eggs during artificial activation. (*A*) Ca^{2+}-H^+ ionophore, A23187, activation. The voltage electrode was inserted first, followed by the pH electrode. In this example the chlorobutanol-containing F_1 solution was at pH 7.4 but after successful impalement was replaced with F_1 at pH 7.8. 2×10^{-6} M A23187 (Calbiochem-Behring Corp., San Diego, Calif.) in F_1 solution was added for the time indicated by the bar. 0.1% ethanol was also present but had no apparent effect when added alone. (*B*) Spontaneous activation in Na^+-free F_1 solution. The egg was perfused with Na^+-free solution (choline chloride substitute) for ~10 min before impalement in the absence of chlorobutanol. Spontaneous activation followed some minutes later.

fertilized. The mean resting potential in the unfertilized egg was −10.1 ± 3.5 mV (SD, n = 38) just before the activation potential, the same value as found ~1 h after fertilization or activation, −10.1 ± 3.9 mV (SD, n = 42). This value for the fertilized egg agrees with that reported by other investigators (7). Although no previous studies have been reported for the unfertilized *Xenopus* egg, a similar resting potential (−12 mV) was found in the toad *Bufo vulgaris* (27), and the frogs *Rana rugosa* (−13 mV) (22) and *Rana pipiens* (−28 mV) (6). In common with other freshwater species, the membrane of *Xenopus* eggs seems to possess a very high resistance (7). Insertion of the electrodes may cause some depolarization, because eggs impaled with a single voltage electrode initially had higher membrane potentials of −20 ± 2 mV (SD, n = 16). However, even these would gradually depolarize to a lower value of −13 ± 6 mV (SD, n = 8) in 30–60 min. Upon insertion of the pH electrode there was usually a gradual fall in pH_i over 30 min. This was sometimes followed by a slow rise before stabilizing at the unfertilized pH_i. Such stabilization could take 2 h or more after the initial impalement but would remain steady for

many hours thereafter. Occasionally, recovery from penetration occurred more rapidly, as in Fig. 2*B*. Because fertilization becomes increasingly difficult the longer *Xenopus* eggs remain in F_1 solution and the likelihood of spontaneous activation is greater, sperm were occasionally added before complete stabilization of pH_i had occurred, as in Fig. 3. Penetration of already fertilized eggs resulted in a steady pH_i rise to a stable level over the next 30 min.

Fertilization results in changes in the membrane potential and pH_i, as shown in Fig. 1*B*. The fertilization potential, a depolarization of ~10 mV, is followed ~2 min after its onset by a slight, transient pH_i decrease, 0.03 ± 0.02 (SD, n = 8). This is followed by a distinct, permanent pH_i increase of 0.31 ± 0.11 (SD, n = 7) beginning ~10 min after the start of the fertilization potential and becoming complete ~1 h later. We have summarized our pH_i measurements in Table I. Egg rotation after activation might be expected to cause minor damage and thus affect the results presented here, but the pH_i changes were similar whether recorded in the animal or vegetal hemisphere.

A similar sequence of pH_i changes followed prick activation, application of the Ca^{2+}-H^+ ionophore, A23187, and spontaneous activation. An example of the changes in membrane potential and pH_i elicited by application of A23187 is shown in Fig. 2*A*. The main difference between this response and that after fertilization is in the initial transient acidification. The ionophore-triggered acidification often begins before the onset of the activation potential and can have a larger amplitude than occurs with fertilization, as indicated in Table I. This suggests that A23187 can cause a pH_i fall before the activation response.

Shen and Steinhardt (38) reported a pH_i rise in the sea urchin egg after fertilization that began sooner, changed faster, and had a greater amplitude than that found in *Xenopus*. No initial transient acidification was reported, and a few millimolar extracellular Na^+ was required for the pH_i rise (39). Fig. 2*B* shows an example of an unfertilized egg impaled in Na^+-free solution in the absence of chlorobutanol, yet avoiding prick activation. Subsequent spontaneous activation resulted in pH_i changes similar to those triggered by fertilization. In

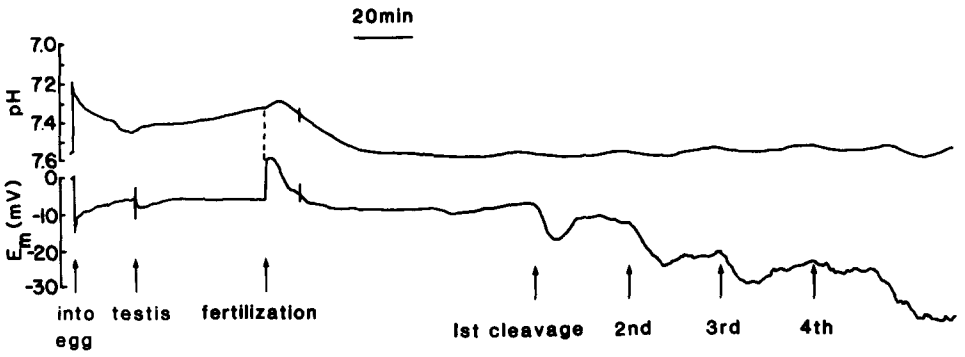

FIGURE 3 Intracellular pH and membrane potential (E_m) recorded in a *Xenopus* egg during fertilization and early cleavage. This egg was impaled before the chamber was flooded with F_1 solution in the absence of chlorobutanol. A fresh piece of testis was added to the chamber and fertilization ensued some 50 min later. The electrodes remained in the embryo up to the 5th cleavage division, and after electrode removal this embryo developed to the swimming tadpole stage.

TABLE I

Changes in the Membrane Potential and pH_i at Fertilization and Activation

	Before activation		After activation*	
Resting potential (mV) mean ± SD (n)	−10.1 ± 3.5 (38)		−10.1 ± 3.9 (42)	
	Fertilized	Spontaneous activation	A23187 activation	
Transient pH_i fall				
Amplitude	0.03 ± 0.02 (8)	0.06 ± 0.05 (7)	0.08 ± 0.04 (6)	
Onset time,‡ *min*	2.6 ± 1.1 (8)	0.1 ± 1.3 (7)	−1.3 ± 2.0 (6)	
Duration, *min*	10.7 ± 4.6 (7)	9.6 ± 2.6 (7)	17.6 ± 7.9 (6)	
	Total Feb.-Dec. 1980	May-Dec. 1980§	ΔpH_i	Time
				min
Permanent pH_i rise				
Unactivated	7.33 ± 0.11 (21)‖	7.39 ± 0.11 (11)	—	—
Fertilized	7.67 ± 0.13 (10)‖	7.69 ± 0.05 (6)	0.31 ± 0.11 (7)	67 ± 15 (6)
Activated¶	7.64 ± 0.12 (28)	7.70 ± 0.10 (14)	0.30 ± 0.09 (15)	64 ± 14 (15)
	ΔpH_i amplitude	Periodicity	Onset time before cleavage	
		min	*min*	
Cyclical pH_i changes at cleavage	−0.03 ± 0.02 (38)	33 ± 6 (38)	24 ± 5 (9)	

* Includes fertilized and artifically activated.
‡ Measured from the beginning of the fertilization potential.
§ These results were obtained with improved technique and during the same period that our ^{31}P-NMR experiments were carried out (31).
‖ Range for unfertilized pH_i, 7.13–7.57; fertilized or activated, 7.44–7.95.
¶ Includes all forms of artificial activation.

three cases in which eggs were perfused for at least 2 h in Na^+-free solution with 1 mM amiloride present in two cases, subsequent activation produced the same pH_i changes. Therefore, the pH_i rise in activated *Xenopus* eggs does not appear to require extracellular Na^+.

Because fertilized *Xenopus* eggs undergo normal cleavage while impaled, it was possible to monitor pH_i during early development. Fig. 3 shows an example of such a record during the first few cleavage divisions. This particular egg was impaled in the absence of chlorobutanol without prick activating. The usual pH_i changes can be seen after fertilization, but additionally it is possible to discern a series of small rhythmical pH_i oscillations. These oscillations precede each cleavage division, as evidenced by the rhythmical membrane hyperpolarizations. Eggs impaled before or after fertilization all exhibited these cleavage-associated pH_i oscillations with a mean amplitude for 38 oscillations from 11 eggs of 0.03 ± 0.02 (SD). In one particular embryo the oscillations were followed for 10 cycles. Preliminary evidence indicates that ionophore- and prick-activated eggs also undergo rhythmical pH_i oscillations beginning at similar intervals after the activation potential and having a similar periodicity even though such eggs do not cleave. The cyclical pH_i changes also seem to be unaffected by removal of Na^+ or Cl^- or by lowering of the ionic strength of the extracellular solution.

Even though the pH and voltage electrodes were introduced into the egg in close proximity (~200 μm), at later divisions they may have ended up in different blastomeres. Fortunately, the blastomeres remain ionically coupled (47) and cleave synchronously for the first dozen or so divisions in the animal hemisphere (20).

DISCUSSION

These first direct measurements of pH_i during fertilization in a vertebrate egg indicate that the activation of development is accompanied by a permanent pH_i increase of 0.3. They also reveal a slight, transient acidification preceding this increase and small pH_i oscillations associated with the cell cycle. We will discuss each of these three pH_i changes separately.

Initial Transient Acidification

Beginning ~2.6 min after fertilization, we detect a slight acidification with a duration of ~11 min. In ionophore-activated eggs, this acidification often begins before the activation potential and has nearly three times the amplitude. This suggests that it may be linked to an increase in the intracellular free Ca^{2+} concentration (Ca_i^{2+}). Such a Ca_i^{2+} increase accompanies the cortical reaction in both the freshwater vertebrate *Oryzias* (15, 34) and the marine invertebrate *Lytechinus* (43), and most likely also accompanies the *Xenopus* cortical reaction, which lasts for ~3.5 min in this 1.3-mm-diameter egg (19). Because the cortical reaction probably does not begin until ~30 s after fertilization (15, 43), one would expect a wave of elevated free Ca^{2+} to cross the egg between 0.5 and 4 min after fertilization. This narrow region of elevated Ca_i^{2+} would be expected to reach the pH_i electrode halfway through this interval on average, i.e., ~2.3 min after fertilization. The average onset time of 2.6 min is very close to this, and the standard deviation of 1.1 min indicates a wide range of onset times, as would be expected for such a mechanism.

How might a Ca_i^{2+} increase cause a local acidification? Meech and Thomas (29) have shown that in snail neurons, Ca^{2+} injection causes an immediate decrease in pH_i that is directly proportional to the amount of Ca^{2+} injected. They conclude that most of this Ca^{2+} is probably removed from the cytoplasm by mitochondria in exchange for protons. Moreover, the recovery of pH_i in snail neurons after a 15-s injection of $CaCl_2$ is on the order of 10 min, which is very similar to the duration of the transient acidification after *Xenopus* egg fertilization. Thus, a wave of increased free Ca_i^{2+} passing through the *Xenopus* egg might well generate a transient acidification through a similar H^+ exchange mechanism.

Permanent pH_i Increase

The unique feature of this study was the measurement of pH_i in mature, jelly-coated eggs before, during, and after fertilization. Prick activation often results from electrode impalement, making these fertilization experiments difficult, but the application of 5 mM chlorobutanol was found to solve that problem. Other investigators have measured a pH_i of 7.7 in full-grown oocytes removed from the ovary (26) and have found a similar value of 7.65 in fertilized eggs (27). Therefore, our measurement of the lower pH_i of 7.33 in the mature, jelly-coated, unfertilized egg squeezed from the uterus was surprising. The possibility that chlorobutanol is itself causing the lowered pH_i was ruled out by several successful impalements in its absence, as shown in Figs. 2 *B* and 3. The possibility that the unfertilized egg is more sensitive to electrode impalement-induced damage than the fertilized egg was ruled out by obtaining very similar pH_i values of 7.42 and 7.66 for unfertilized and fertilized eggs, respectively, with the noninvasive ^{31}P nuclear magnetic resonance (^{31}P-NMR) technique (31). That technique averages the pH_i of ~1,000 eggs with a time resolution of 40 min. We therefore feel quite confident that this lower pH_i in mature, jelly-coated, unfertilized eggs is real and conclude that, after ovulation, as the eggs move down the oviduct into the uterus, a fall in pH_i occurs. It was hypothesized by Bataillon (2) 50 yr ago that eggs were "anaesthetized" by CO_2 as they pass through the genital tract and Brachet (4) has presented some evidence that ovulated oocytes are exposed to high CO_2 tensions after entering the oviductal-uterine environment. Smith and Ecker (42) also reported uterine suppression of biochemical and morphogenetic events in *Rana pipiens* oocytes. However, although it is true that a higher CO_2 tension could cause a cytoplasmic acidification (45), it is unlikely that this is the only mechanism involved in maintaining the low pH_i, because unfertilized eggs remain in the low pH_i state for many hours after leaving the uterus. Unless it is fixed in a nondiffusible state, CO_2 will leave the egg and cause the pH_i to rise again after the egg has left the high CO_2 environment. For example, a 30-min exposure to CO_2 renders *Rana* unresponsive to activation stimuli for 2 h (17), so after that period most of the CO_2 effect has worn off. Yet the unfertilized *Xenopus* egg pH_i remains low for at least twice that period.

Previous pH_i Measurements in Amphibian Eggs

In a much earlier study of pH_i in amphibian eggs using a pH-sensitive antimony electrode with a large tip diameter (50 μm), the pH_i recorded after fertilization was >1 pH unit higher than in the oocyte (5). However, these results are unreliable, because, besides using such a large electrode, the investigators failed to use a reference voltage electrode so that membrane potential changes would have been reported as pH_i changes. More reliable measurements were made a decade later by

Dorfman in 1938 (8) using a platinum wire to impale the amphibian egg in an H_2 atmosphere. He reported an unfertilized pH_i of 7.06 that increased to 7.19 in early cleavage embryos. Here we have used much finer electrodes and believe that our data more accurately indicate the true pH_i, particularly with our corroborating ^{31}P-NMR results.

EXTERNAL Na^+ IS NOT NEEDED FOR THE pH_i CHANGES: Approximately 13 min after fertilization the pH_i begins to rise steadily, reaching a final level of pH 7.7 ~50 min later. This pH_i change, as well as the transient acidification and small oscillation at cleavage, is independent of external Na^+. This is not surprising when one realizes that *Xenopus* develops in a freshwater environment in which the Na^+ activity is normally much lower than the 20 mM intracellular Na^+ activity of the egg (7, 41). Na^+-H^+ exchange would, therefore, require movement of Na^+ against its activity gradient. Amphibians have apparently dispensed with a dependence upon extracellular ions for the generation of the activation potential by relying instead upon an efflux of Cl^- (22, 28). It is possible that the pH_i rise after fertilization is also independent of extracellular ions although we have not ruled out exchange with ions other than Na^+. This membrane has an exceptionally low water and ion permeability (7, 41), and alterations in extracellular pH over a range of 5–9 for at least 40 min have very little effect on pH_i in either the unfertilized or fertilized egg. It is worth noting that, at the extracellular pH of 7.8 used here, H^+ is very nearly in equilibrium across the plasma membrane for both the unfertilized and fertilized eggs if their membrane potentials are −20 mV and −10 mV, respectively. However, we have not observed any pH_i dependence on the membrane potential.

WHAT CAUSES THE PERMANENT pH_i INCREASE? The unreliable extracellular ionic environment of pond water and exceptionally low membrane permeability make ion exchange across the plasma membrane a less likely candidate for the pH_i change than in marine systems. However, even in the sea urchin egg much of the fertilization-triggered pH_i rise is apparently attributable to CO_2 release from the egg (14). This would generate a change in the extracellular pH that our preliminary measurements have failed to detect in *Xenopus*. Furthermore, the slow, 50-min time-course suggests that some other rate-limiting step might be involved. Other possibilities that we are investigating include the synthesis of an organic base and H^+ exchange across an intracellular membrane system such as the mitochondrion or endoplasmic reticulum.

Small pH_i Oscillations Preceding Cleavage

Superimposed on the permanently increased pH_i level of 7.7 are small acidifications that average 0.03, but are occasionally three times larger than this average. They have the same period as the cleavage cycle but are out of phase with it, beginning ~24 min before the hyperpolarization associated with each cleavage. Lee and Steinhardt (27) have also detected similar pH_i oscillations. The pH_i oscillations reported here coincide very closely with the surface contraction waves that also begin ~20 min before the onset of first cleavage and have a periodicity of 30–35 min (20). As in the case of the initial transient acidification after fertilization, these small cytoplasmic acidifications may indirectly reflect free Ca_i^{2+} oscillations occurring during the cortical contraction waves. Measurements with Ca^{2+}-sensitive microelectrodes have failed to detect Ca_i^{2+} increases at these times (35), but injection of Ca^{2+} beneath the plasma membrane or ionophore-induced release from intracellular stores will induce cortical contractions in frog eggs (16, 36). Therefore, it remains an appealing possibility that free Ca_i^{2+} increases, which are perhaps beyond the spatial resolution of the recent electrode measurements, may accompany the surface contraction waves and lead to a pH_i decrease through exchange for H^+ across the mitochondrial membrane. An oscillating Na_i^+ activity has also been reported during the cleavage cycle in dejellied embryos, rising transiently by 2–3 mM at about the same time that the surface contraction wave begins (41). This is in the wrong direction to support a Na^+-H^+ exchange mechanism at the plasma membrane, but it would be consistent with a Na^+-Ca^+ exchange mechanism moving Ca^{2+} out in exchange for Na^+ entry to bring cytoplasmic Ca_i^{2+} back down to normal levels after its increase.

Another good candidate for the generation of these small pH_i oscillations is CO_2 production. The mitotic cycle of some dividing embryos, including these from the frog *Rana platyrrhina*, is associated with a rhythm in the rates of oxygen uptake and CO_2 output. In the frog embryo the cycling of CO_2 output was observed from first cleavage onwards (52), whereas in the embryos of two marine invertebrates *Urechis* and *Dendraster*, this rhythm began after the first five or six divisions (10). This observation suggested to the investigators a gradual fall in pH_i during mitosis and a gradual rise during interphase, which would agree with our findings in *Xenopus*.

Somewhat larger oscillations in pH_i during cell division have been reported in *Physarum* (12), *Tetrahymena* (13), and lymphocytes (11) by other pH_i-measuring techniques. However, these larger changes are in the opposite direction so they appear to be unrelated to the pH_i oscillations reported here. A recent study of sea urchin embryos did not detect significant changes in pH_i through the first two cleavage divisions (23).

Similar Findings in Other Systems

A pH_i increase of 0.3–0.4 follows sea urchin egg activation in both *Lytechinus pictus* (23, 38) and *Strongylocentrotus purpuratus* (23). The rate of protein synthesis is influenced by pH_i in the sea urchin, and it has been postulated that the increases in pH_i and Ca_i^{2+} play a regulatory role in the control of protein synthesis (18, 48–50). In the frog *Rana pipiens*, the rate of protein synthesis increases by 50% at fertilization (40), and in *Xenopus* both the polysome content and the percentage of ribosomes present as polysomes double between 10 and 45 min after activation (51), which would suggest a 100% increase in protein synthesis. This correlates well with the time-course of the permanent pH_i increase. Furthermore, the next significant increase in polysome content, which occurs 4 h after fertilization, correlates well with the beginning of an alkalinization of 0.18 pH unit observed by Lee and Steinhardt (27). Although these two correlations suggest a possible link between pH_i and rates of protein synthesis, measurements of pH_i during in vitro maturation of *Xenopus* oocytes do not support any direct relationship with the twofold increase in protein synthesis occurring at that time (32). Therefore, direct measurements of protein synthesis rates during imposed changes in pH_i should be made to clarify this relationship.

Imposed pH_i changes can also be used to determine the importance of the permanent pH_i rise to activation. Does blocking the permanent pH_i rise block activation? This question, with respect to cleavage, was asked by Lee and Steinhardt (27) in another paper in this issue. They lowered pH_i with a weak acid beginning after the permanent pH_i increase had

occurred and observed normal cleavage with pH_i as low as 7.32. This implies that a sustained elevated pH_i is not required for cleavage, and we will attempt to prevent the permanent pH_i rise entirely in order to assess its importance for activation.

Cytoplasmic alkalinizations have been measured in several other systems during the activation of the cell cycle or increased metabolic rates. These pH_i increases fall into two broad classes. One class exhibits a large pH_i increase of a full pH unit, typically rising from pH 6.5 to 7.5. This class is composed mainly of cells being activated or released from a dormant state and includes bacterial and yeast spores (1, 37; the activated pH_i is an unpublished result from Drs. J. A. den Hollander, T. R. Brown, K. Ugurbil, and R. G. Shulman) and hydrating brine shrimp cysts (W. B. Busa and J. H. Crowe, Zoology Department, University of California, Davis, personal communication). The second class exhibits pH_i increases of 0.2–0.5 pH unit and includes the "start" signal in the cell cycle of slime molds (12), ciliates (13), and lymphocytes (11); the increase in the glycolysis rate upon insulin (9, 30) or glucose (46) addition; the regulation of actin polymerization (3); and the initiation of sea urchin sperm motility (25). Therefore, the cytoplasmic alkalinization that follows fertilization in sea urchin and frog eggs may be an example of a very general cellular regulation mechanism that can influence metabolic rates, the cell cycle, and contractile protein organization.

We thank Dr. Gary Meeker for his help during the early stages of this project, and Drs. Bill Moody, Roger Thomas, Sheldon Shen, and Rick Steinhardt for suggestions on recessed-tip pH electrode manufacture.

This investigation was financially supported by National Science Foundation grant PCM 78-26022.

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Received for publication 11 May 1981, and in revised form 20 July 1981.

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