THE CONTROL OF CELL VOLUME: FROG EGGS FACE AN EXTREME CHALLENGE*, **

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INTRODUCTION

When frog eggs are laid they change their environment from an isotonic one to fresh water involving a change in osmotic pressure gradient across the cell wall of about 4 atmospheres. This sudden pressure change presents a mechanical stress to the cell which must have mechanical consequences. Thus, although metabolic and chemical processes may have evolved to help cope with this extraordinary challenge to species survival, the mechanical consequences of placing eggs in fresh water should also provide important information on coping mechanisms.

METHODS

We harvested immature oocytes and mature ova from Xenopus laevis frogs and maintained them in modified Barth's medium (1). Three series of experiments were performed.

1. We immersed both ova and oocytes in fresh water and measured the percentage of cells which burst as a function of time.
2. We measured the relationship between intracellular pressure (Pi) and cell volume (Vc) when both oocytes and ova were placed in hypotonic media. As oocytes are spherical we used the Laplace Law to calculate cell wall tension (Tc) and its relationship to cell surface area (Ac).
3. We placed oocytes and ova in hypotonic media and after one hour measured the change in Vc and Pi. We did this in lieu of a continuous measurement of Pi during swelling in hypotonic medium because in the latter circumstance cytoplasm frequently leaked out around the micropipette during cell swelling and we wished to avoid this result of our intervention.

Pi was measured with a micropipette (~5 μm o.d) which we inserted into the cytoplasm with the aid of a micromanipulator and an inverted microscope. The micropipette was attached to a servo-null pressure

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transducer, an instrument with a rapid dynamic response (90% to a square wave pressure change in <20 msec) and a volume displacement coefficient which is virually zero (2). It is ideally designed to measure dynamic pressure changes in very small spaces. As oocytes are spherical Vc was calculated by measuring their diameter and using the formula $Vc = \pi Dc^3/6$. Ac was calculated using $Ac = \pi Dc^2$ and Tc from $Tc = Pi \cdot Dc/4$.

The digital output from the pressure transducer was recorded with a videocamera and mixed with the videoimage of the cell and the date and time. These were monitored on a television screen and recorded on videotape for later analysis.

The output of the servo-null micropipette system was calibrated *in vitro* against a water manometer and *in situ* by increasing the level of liquid above the cell and plotting the change in extracellular hydrostatic pressure calculated from the height of the liquid column above the cell with the measured change in Pi. These data fell along the line of identity.

The measurement of Vc in ova was less accurate than oocytes because they did not remain spherical in isotonic media but tended to flatten with time. As will be seen the resulting error in calculating Vc was unimportant with regard to the conclusions we make from our experiments.

RESULTS AND DISCUSSION

Figure 1 shows values of Pi obtained in mouse and frog oocytes in isotonic media. As these cells are spherical and return to this shape after deformation it follows that there is circumferential tension within the cell wall leading to the minimum energy configuration (sphericity) with a positive transmural pressure across the cell wall. This we confirmed but our values for Pi in frog oocytes were about 1 order of magnitude greater than estimates of Pi previously made in cells using indirect techniques which require distortion of the cell and subsequent extrapolation to a zero distorted state (3–6). Our measurements in mouse oocytes showed an even higher value of Pi. The reasons for these discrepancies are unclear but could be related to the fact that Xenopus laevis oocytes are invested in a vitelline membrane which might be the source of wall tension. Another possibility is that indirect techniques require knowledge of the distortion produced in those elements of the cell wall which produce Tc. This is not interfacial tension between cell membrane lipids and extracellular liquid. If it were the cell membrane would be smooth whereas in fact it is redundant with many folds or microvilli. The tension may result from the submembranous circumferential network of cross-linked actin polymers. In any event, because of the membrane redundancy with

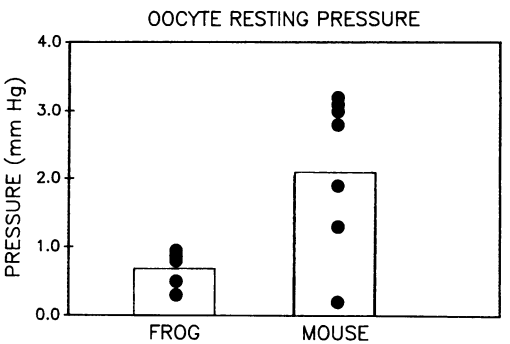

FIG. 1. Mean and individual values of Pi in frog and mouse oocytes in isotonic media.

many microvilli, equating cell membrane distortion with the distortion of the tension-producing elements might lead to a systematic underestimation of Pi.

Knowledge of Pi and Tc in isotonic media does not allow predictions to be made of the pressure-volume relationships during cell swelling or shrinkage in hypo- or hypertonic media. If Tc is independent of Ac as in a soap bubble, Pi must decrease as Vc increases. Such a situation is potentially unstable. A fall in extracellular osmolarity would cause an increase in Dc and Vc but a fall in Pi, leading to a further increase in the net (hydrostatic minus osmotic) pressure difference across the cell wall, increasing Vc and decreasing Pi still further—an "explosive" situation.

On the other hand a positive pressure volume slope in which Pi increases with Vc results is a potentially stable situation. According to the Laplace Law, for Pi to increase with Dc, dTc/dDc must be > 1. This is the condition for mechanical stability. Figure 2 shows that the Pi/Vc diagrams all had positive slopes for oocytes and that Tc increased as Ac increased. The values for Tc of $\simeq 20$ dynes/cm in isotonic media increasing in some instances to several hundred dynes/cm in the swollen state are much too high to be the result of interfacial tension between cell membrane lipids and extracellular liquid. The tension must reside elsewhere.

All cells within the body must be equipped to withstand small changes in osmotic pressure. A change in osmolarity in the order of only one milliosmole causes a change in hydrostatic pressure gradient of about 25 cm H_2O. Cells of the GI, urinary, respiratory tracts and the skin are continuously exposed to changes in osmotic pressure quite independently of any random changes of extracellular osmolarity that may occur. If their pressure volume curves have a positive slope, this at least raises the

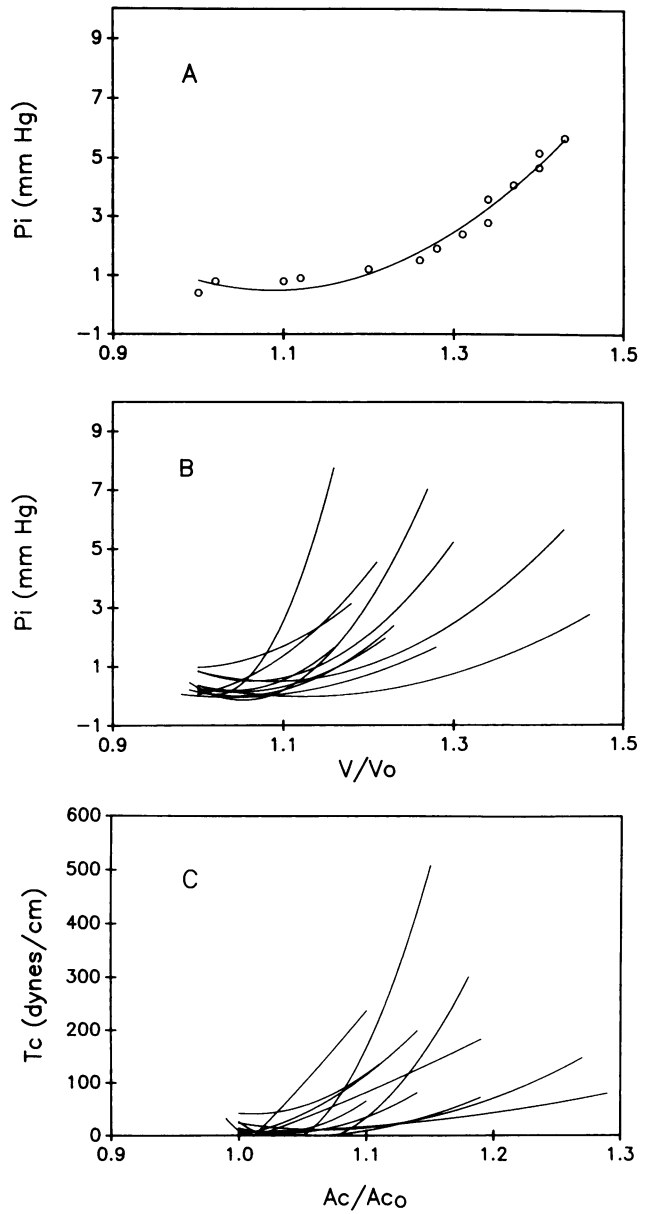

FIG. 2. Upper panel A: Example of a pressure-volume curve of a frog oocyte obtained during swelling when the cell was placed in hypotonic medium. The volume is expressed as a ratio of the volume at any point in time during swelling to the volume of the cell in isotonic medium (V/Vo). The line is the best-fit polynomial. Middle panel B: best-fit polynomial regressions for the pressure volume curves of 11 frog oocytes. Lower panel C: Wall tension (Tc) as a function of cell surface area expressed as a ratio of the surface area at any point in time during swelling (Ac) to the surface area in isotonic medium (Aco) for the 11 oocytes shown in panel B. The curves were calculated using the Laplace Law for a sphere to estimate Tc from Pi and the formula relating Ac to cell volume.

possibility that equilibrium can be achieved by an increase in Pi to offset the decrease in extracellular osmotic pressure causing influx of water into the cell. Thus for small changes in extracellular osmolarity, equilibrium can potentially be achieved by purely mechanical means, not requiring any source of external energy.

Such a mechanism cannot possibly account for the mature ovum's ability to survive in fresh water. We found that after 2 hours in fresh water approx. 60% of oocytes burst whereas none of the mature ova did. We also observed that the mature ova in isotonic media lost their spherical shape and tended to flatten under the influence of gravity even though the difference between their specific gravity and that of the isotonic extracellular liquid was very small. The failure to retain the spherical shape under the influence of very small gravitational forces led us to the prediction that Tc and Pi were close to zero. This we confirmed by direct measurement (Figure 3). In fresh water Pi remained zero in spite of the fact that Dc increased substantially more when mature ova were placed in fresh water compared to isotonic media.

How then does the mature ovum withstand the osmotic pressure challenge? We can conclude: 1) it is not because the cell membrane is

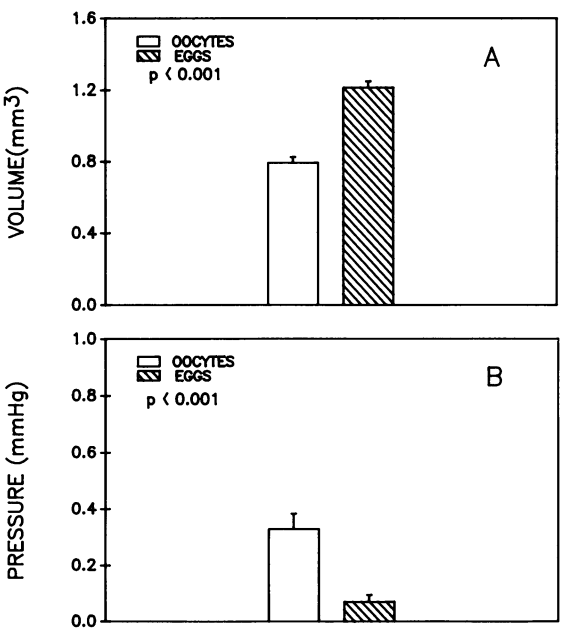

FIG. 3. Histograms showing volume of (upper panel, A) and pressure in (lower panel, B) frog oocytes, and mature eggs. Bars indicate standard errors. Although eggs are larger than oocytes their intracellular pressure is less and is not significantly different from zero.

impermeable to water. The membrane must be permeable to O_2 and CO_2 in order for respiration to take place and these molecules are larger than water molecules. But in addition, even though we could not measure Vc accurately in ova, the rate of increase in cell dimensions of the mature ovum was considerably greater in hypotonic than in isotonic media. To account for this difference the cell wall in mature ova must be water permeable; 2) In the transition from oocyte to ovum there are profound changes in the mechanical properties of the cell; 3) It is reasonable to hypothesize that these changes are an important determinant of cell survival. How could this be?

The fact that mature ova flatten and lose sphericity under the influence of small gravitational forces indicates that whatever structures are responsible for wall tension in oocytes no longer are in ova, resulting in Tc and Pi = 0. The fact that these cells swell, do not burst and do survive indicates that they are permeable to water but manage to reach a quasi-equilibrium state which prevents cells imbibing self-destructive quantities of water in spite of osmotic pressure gradients in the order of 3 atmospheres.

This is compatible with complete gelation of the cell cytoplasm so that the cross-linked polymer network is no longer distributed circumferentially but randomly within the cytoplasm. If so, tension resulting from forces in the polymer strands will be random in direction and should not result in a positive Pi. The Laplace Law does not apply. More importantly the Van't Hoff Law governing liquid flux across a semipermeable membrane so that at equilibrium the hydrostatic pressure difference is exactly balanced by the osmotic pressure difference does not apply either.

The amount of water a gel will imbibe until it reaches a new equilibrium depends upon "gel compliance". A gel is in equilibrium if the elastic forces generated by the polymer strands are fully balanced by the electrostatic attractive or repulsive forces between the strands and the pressures in the liquid phase of the gel in the interstices between the polymer strands (7). Given a substantial number of polymer strands, a high degree of cross-linking, and strands already in a stretched state, gel compliance could be low. If so gel swelling might be minimal in order for the liquid pressure in the interstices to balance the elastic pressure of the polymer strands. We suggest that this may be a basic mechanism by which frog ova meet the challenge of surviving in fresh water.

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DISCUSSION

Nathan (Boston): That was very interesting. You presented at least two options, perhaps linked, to explain this phenomenon. One is the condition of the polymeric proteins and the redundancy of the membrane in the newly formed egg. The two, you think, may be linked, if I understood that correctly. There is a third possibility, Kevin Strange in Ohio has recently shown that cells exposed to hypertonic, at sudden hypertonic shifts, have enormous changes in the production of inositol phosphates. Inositols, of course, have enormous internal osmotic effects. One excellent way for a cell to adapt to these kinds of environments is to make a sudden and rapid shift in inositol production. Although probably your explanations are all correct, it might be wise to look at the osmotic balance in the cell with particular focus on inositol.

Macklem: We haven't looked at inositol, but we've given various drugs to prevent polymerization of actin and tubulin because we thought that polymerization of large molecules might markedly decrease the osmotic pressure gradient across the cell. That did not appear to have any major effect, but we haven't looked at inositol.

Abboud (Iowa City): A few years ago Guharay and Sachs (Guharay, F., Sachs, F. Stretch activated single ion-channel currents in tissue-cultured embryonic chick skeletal muscle. J. Physiol. (London) 1984; 352: 685) demonstrated the opening and activation of specific ion channels that conduct selectively cations during stretching of or suction on various membranes. These have been referred to as specific "stretch-activated ion channels". I was wondering whether such channels might play a role in the polymerization of the gel that you are referring to. What are your thoughts on that?

Macklem: I don't understand the mechanism by which the gel might polymerize and we haven't begun to investigate it. However, I learned a couple of weeks ago that horticulturalists use a material to surround plant roots which when the earth is dry, forms a salt and allows water to enter into the roots. When there are heavy rains, in other words, it is put into a fresh water medium, it forms a gel and prevents or delays the diffusion of water into the roots. This, apparently, is well known to amateur gardeners. I'm not an amateur gardner so I didn't know about it, but the mechanism behind that sol-gel transformation in the presence of fresh water may give us a clue. We have not yet begun to investigate how this transformation might take place. There is no question, however, that the transformation of the oocyte into the mature ovum is dependent upon choriogonadotrophin so it is the action of a hormone on the cell.

Schreiner (Washington): If you take the egg back again to isotonic or hypertonic medium, is the polymerization reversible? I ask the question because with the oceans rising, if we do have a catastrophe of a flood, it is much more likely to be with salt water than with fresh water.

Maklem: No, I think the egg is probably gelated at the time that it is laid. I don't know whether that happens at the point at which it is deposited in fresh water, but it behaves as if it is completely gelated even in isotonic media because it tends to flatten in isotonic

media and the intracellular pressure is zero as opposed to the oocyte. That happens in isotonic media with the mature egg.

Ferris (Minneapolis): I certainly enjoyed your talk. When one thinks in terms of tonicity challenges, the transitional cells of the bladder are being subjected to differences from 50 milliosmoles to 1200 in man and up to 7,000 in the gerbil.

Maklem: Your point is very well taken. The changes in osomolarity to which many cells are exposed is really quite extreme. What I've done here is apply principles of respiratory mechanics because I am a respirologist to individual cells, but a nephrologist is going to have to answer the question. I don't know what the answer is.