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# Ionic Concentrations in Developing *Pelvetia* Eggs

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Intracellular ion concentrations were measured in the developing *Pelvetia* egg during the first 24 hr after fertilization. Intracellular $K^+$ and $Cl^-$ show large concentration increases during this period. Intracellular $K^+$ increases from 170 mmoles/liter at fertilization to 320 mmoles/liter at 8 hr and reaches 400 mmoles/liter by 24 hr. Intracellular $Cl^-$ increases from 87 mmoles/liter at fertilization to 194 mmoles/liter at 8 hr, and 320 mmoles/liter by 24 hr. Intracellular $Na^+$ shows smaller but significant concentration changes, increasing from 27 mmoles/liter at fertilization to 40 mmoles/liter at 8 hr, and then decreasing to 20 mmoles/liter at 24 hr. Neither $Mg^{2+}$ nor $Ca^{2+}$ vary significantly from 25 mmoles/liter and 4 mmoles/liter, respectively. The changes in $Na^+$ plus $K^+$ are approximately equal to the $Cl^-$ increase over the first 24 hr after fertilization but do not balance at every time interval. In agreement with the ion accumulation of the developing eggs is an increase of the intracellular osmotic pressure preceding germination. The correlation of these data with electrical data on the same system (Weisenseel and Jaffe) and possible physiological consequences of the ion concentration changes are discussed.

## INTRODUCTION

In the fucoid egg, the participation of the ionic environment in the developmental process is indicated by a number of observations. The developing egg can be polarized by external $H^+$ and $K^+$ gradients (Bentrup *et al.*, 1967), electric current begins to flow through the egg about 6 hr after fertilization (Jaffe, 1968), and the membrane potential and conductance increase greatly during the first 8 hr (Weisenseel and Jaffee, 1972).

A thorough analysis of ion concentrations in unfertilized eggs has been done for frog (Morrill, 1965) and sea urchin (Rothschild and Barnes, 1953). But very little information is available on intracellular ion concentrations at later stages of development.

In the investigation reported here we have determined intracellular concentrations of $K^+$, $Na^+$, $Cl^-$, $Mg^{2+}$, and $Ca^{2+}$ at various stages during the first 24 hours after fertilization of the fucoid egg. Coupled with recent investigations of the membrane potential and impedance (Weisenseel and Jaffe, 1972), these findings present a striking picture of physiological changes during development.

## MATERIALS AND METHODS

Fronds of *Pelvetia fastigiata* were obtained from the California coast and stored at 5°C until needed. Zero time for development was taken as 0.5 hour after shedding began. Egg samples for analysis at later developmental periods were grown in artificial sea water (ASW) (Weisenseel and Jaffe, 1972) or natural sea water (NSW) at 15 ± 1°C under continuous white fluorescent light.

Thorough rinsing of each sample in the appropriate washing solution was necessary to remove the ion being measured from the external solution. Samples for $K^+$ analysis were rinsed in isosmotic choline chloride solutions. For $Na^+$ analysis, NaCl and KCl in ASW were replaced with isosmotic mannitol, and for $Cl^-$ analysis, $Cl^-$ was replaced by propionate. For $Ca^{2+}$ and $Mg^{2+}$ analysis $Ca^{2+}$ and $Mg^{2+}$ were simply left out of the ASW (the small change in osmotic pressure was insignificant).

Up to 5 hr after fertilization, the developing egg cells remained unstuck and could be rinsed by quickly centrifuging the cells, pouring off excess solution, and replacing with 10 to 15 ml of fresh wash-

ing solution. This process was repeated 5–7 times. By 8 hr the cells had become firmly stuck to the bottom of their container and were rinsed with 5–7 changes of washing solution. Preliminary checks with radioactive tracers indicated 5 washings sufficient to remove external ions. Usually the washing procedure was completed within 3 min, but for $Ca^{2+}$ and $Mg^{2+}$ it was extended to 10–15 min to remove all ions from the cell wall.

Two methods were used to prepare and analyze samples for ion contents. Samples for $K^+$, $Ca^{2+}$, and $Mg^{2+}$ were analyzed on an atomic absorption spectrometer (Perkin-Elmer Model 303) while $Cl^-$ and $Na^+$ were analyzed using ion-specific electrodes (Orion models 94-11 and 94-17A). For the atomic absorption samples struck eggs were grown in quartz crucibles. After rinsing and drying overnight at 60°, the $K^+$ samples were digested in 0.5 ml 50/50 sulfuric-nitric acid for 15 min and the $Ca^{2+}$ and $Mg^{2+}$ samples were digested in nitric acid and evaporated to dryness two or three times. Chemical and ionization interferences with the atomic absorption measurements of $K^+$ and $Ca^{2+}$ were suppressed as follows: to the $K^+$ samples Cs (1000 ppm) and HCl (0.6 *N*) were added and to the $Ca^{2+}$ samples La (1%), $K^+$ (1000 ppm), and HCl (5%) were added.

For the ion-specific electrode samples, stuck eggs were grown on 1 cm diameter Teflon disks. Immediately after rinsing, 1–2 ml of distilled water was placed on each sample and dried at 75°C. The dried samples were soaked at least 12 hr in distilled water and the resulting solution diluted to 2 ml. The sample ionic strength was adjusted with 10 m*M* $KNO_3$ in $Cl^-$ samples and 5 m*M* $CaCl_2$ in $Na^+$ samples. Sodium samples were buffered with 0.5 m*M* CAPS (Sigma Chemical Co.) at pH 10, and $Cl^-$ samples buffered with 0.5 m*M* Tris-$NO_3$ at pH 8.

The sample size was measured by photographing each sample or a sample aliquot in each experiment and determining the egg number from the photograph. Cell volumes were calculated from measurements of cell diameter. From 3000 to 30,000 eggs were required for each sample, depending on the ion being measured.

The ion content of the cells could be expressed as millimoles per liter of cell water from the ion analysis, cell volume measurements, and percent cell water at the various stages of development. Percent water was determined from wet and dry weights of samples grown on Teflon disks. The wet weights were corrected for extracellular water by rinsing for 30 sec with $^{36}Cl$ sea water and counting after taking dry weights. The 30-sec $^{36}Cl$ rinse allowed exchange with at most 1% intracellular $Cl^-$ and would thus result in less than 1% error in the percent water determinations.

Intracellular osmotic pressure was taken as the osmotic pressure of an external solution causing plasmolysis (microscopically detectable separation of the protoplast from the cell wall) of 50% of the cells in a 100 egg sample. The external solutions were composed of artificial sea water with added mannitol used to increase the osmotic pressure by an amount calculated assuming ideal solutions.

## RESULTS

### *Cell Water Content*

Changes in the egg cell water content during the first 24 hr after fertilization are shown in Fig. 1. The measured water content did not vary significantly from 45% during the first 14 hr, but a large increase to 57% was apparent by 24 hr. However, the cell volume begins to increase (and most likely the cell water also) at the onset of germination. Considering both measured water content and cell volume, the dotted line in Fig. 1 represents the best estimate of cell water and was accordingly used in all later calculations. These values actually represent percent cell water by weight but were la-

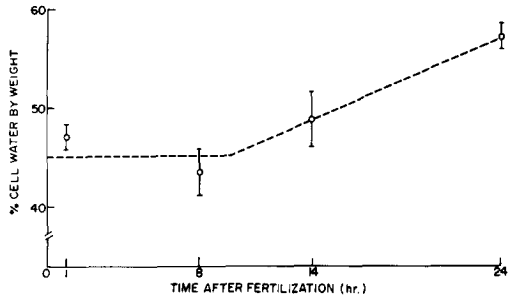


FIG. 1. Change in cell water content (percent by weight) of developing *Pelvetia* eggs. Each point represents the average of all measurements ± SEM. Values for the calculation of ion concentrations were read from the dotted line.

ter used to calculate cell water from the measured volumes. This approximation leads to no significant error; a preliminary comparison of ion concentrations measured from weighed samples and photographed samples yielded measurements which agree within ±3%. Small deviations in percent water will result in larger errors in calculated ion content. For example, ±5% cell water will lead to ±10% in the calculated ion content. However, since the standard error of the mean of percent water measurements at each experimental point was less than ±5%, this variation was not a serious source of error.

*Intracellular Ion Concentrations*

The intracellular ion contents (in mmoles/l cell water) during the 24 hr following fertilization are shown in Fig. 2; the significance of ion concentration changes was determined using the Student *t* test. The most obvious concentration change is the large increase in both $K^+$ and $Cl^-$. Intracellular $K^+$ concentration ($[K^+]$) remains at 170 mmoles/l up to 2 hr after fertilization then rapidly increases to about 320 mmoles/l at the onset of germination (8 hr). As germination proceeds $[K^+]$ continues to increase, but more slowly, reaching 400 mmoles/l at 24 hr.

Intracellular $Cl^-$ concentration ($[Cl^-]$) is initially 87 mmoles/l, somewhat lower than $[K^+]$. Between 0 hr and 1 hr $[Cl^-]$ may decrease slightly, although this change is not statistically significant. By 8 hr $[Cl^-]$ has increased to 194 mmoles/l and by 14 hr to 310 mmoles/l, with no further significant change at 24 hr.

Intracellular $Na^+$ concentrations ($[Na^+]$) show smaller but significant changes. At fertilization $[Na^+]$ is 27 mmoles/l, increasing to 40 mmoles/l by 8 hr, and then decreasing to 20 mmoles/l at 24 hr.

Over the 24-hr experimental period, the net $K^+$ and $Na^+$ entering the cell is approximately equal to $Cl^-$ uptake (Table 1), but the amounts are not equal when considered at each time interval. Between 0 and 3 hr, $K^+$ uptake may exceed that for $Cl^-$. A direct comparison in this time interval is difficult since measurements were not made at corresponding time periods for $Cl^-$ and $K^+$; $[Cl^-]$ may decrease between 1 and 2 hr and thus show a much larger increase between 2 and 3 hr similar to $[K^+]$. Between 8 and 24 hr the $Cl^-$ uptake is substantially higher than the net cation uptake so that by 24 hr the net cation and anion changes after fertilization are nearly equal.

In contrast to $[K^+]$, $[Cl^-]$, and $[Na^+]$ neither $[Mg^{2+}]$ nor $[Ca^{2+}]$ change significantly during the first 24 hr of development. $[Mg^{2+}]$ remains constant at 25 mmoles/l and $[Ca^{2+}]$ remains constant at 4 mmoles/.

*Osmotic Pressure*

In agreement with the $[K^+]$ and $[Cl^-]$ increase there is an osmotic pressure increase of the cell contents (Fig. 3). Up until about 3 hr after fertilization, the eggs are in the process of forming a cell wall, and thus are necessarily at or near osmotic equilibrium with sea water. After cell wall formation is complete at 3–4 hr, the internal osmotic pressure increases rapidly. By the start of germination the osmotic pressure has reached a steady value even though $[K^+]$ and $[Cl^-]$ continue to increase at least until 14 hr.

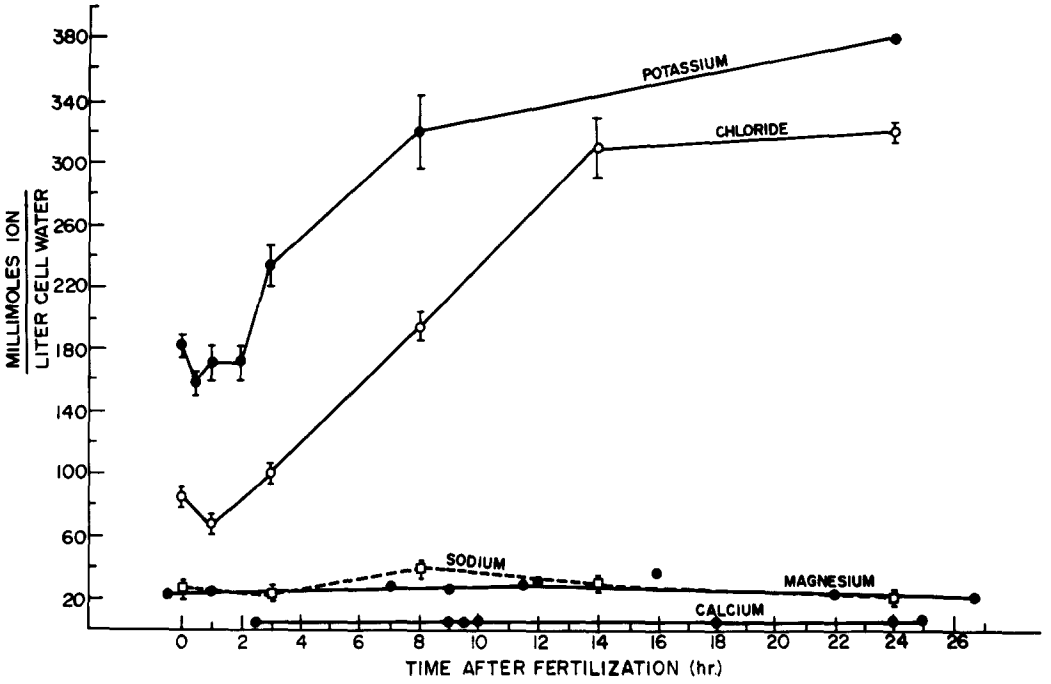


FIG. 2. Intracellular ion concentrations in developing *Pelvetia* eggs. Each point for $K^+$, $Na^+$, $Cl^-$ represents the mean ± SEM of from 6 to 20 measurements. Each point for $Ca^{2+}$ and $Mg^{2+}$ represents the mean of from 2 to 7 measurements. The values reported for zero time may vary slightly from the actual values at fertilization due to the choice of zero time as 30 min after the start of shedding. However, this consideration is probably only important for $Na^+$ (see text).

TABLE 1

ION CONCENTRATION INCREASES DURING THE 24 HR FOLLOWING FERTILIZATION[a]

| Time interval | Ions | | | Cations | Anions |
|---|---|---|---|---|---|
| | $K^+$ | $Na^+$ | $Cl^-$ | | |
| 0–3 Hr | 63 ± 17 | −3 ± 3 | 22 ± 8[b] | 60 | 22 |
| 3–8 Hr | 86 ± 27 | 15 ± 6 | 94 ± 11 | 101 | 94 |
| 8–24 Hr | 80 ± 24 | −20 ± 4 | 126 ± 11 | 60 | 126 |

[a] Increases are expressed as the concentration change (in mmoles/liter cell water) during the stated time interval ±SEM.

[b] Measured between 1 and 3 hr.

## DISCUSSION

During the first 24 hr after fertilization intracellular $K^+$ and $Cl^-$ concentrations increase substantially. Practically all the concentration increase occurs between cell wall formation (at 2–3 hr) and the time at which all cells have germinated (at about 14 hr). Somewhat smaller but significant changes are evident for $[Na^+]$, but $[Mg^{2+}]$ and $[Ca^{2+}]$ show no significant changes during the 24-hr experimental period.

The intracellular concentrations reported here for $Na^+$, $K^+$, $Cl^-$, and $Ca^{2+}$ are in good agreement with radioactive tracer equilibrium determinations (Robinson and Jaffe); unfortunately there were inadequate data for comparison with $Mg^{2+}$. Furthermore, there were no indications that variations in washing

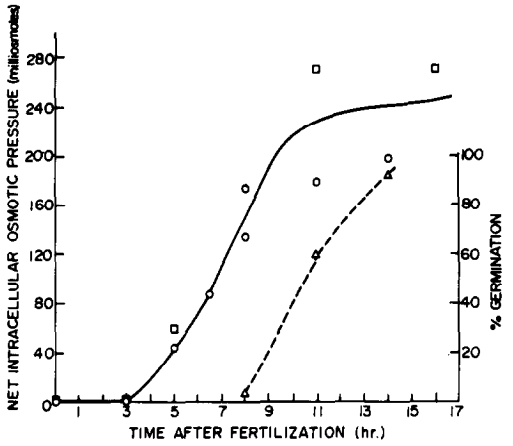


FIG. 3. Increase in the intracellular osmotic pressure in two experiments (O, □) of developing *Pelvetia* eggs. The intracellular osmotic pressure in excess of the osmotic pressure of the seawater medium is shown together with the % germination (Δ). Until after formation of the cell wall at 3 hr, osmotic pressure changes were difficult to measure since the cells tended to "dimple" rather than plasmolyze. The change was less than 20 milliosmoles and probably did not vary significantly from zero.

solutions or digestion and analysis procedures affected our results. For $K^+$ samples washed with a more nearly physiological solution (ASW with $K^+$ and $SO_4^{2-}$ substituted by NaCl) rather than choline chloride, there was no appreciable variation in the results. And for $Na^+$ a comparison of the atomic absorption and ion electrode analysis procedures yielded results agreeing within ±4%.

Owing to the limited information on ion content changes during development no detailed comparison with other systems is possible, but the available information is assembled in Table 2. Not included in the table are specific references to rapid ion content changes within 15 min after fertilization. Rapid changes have been reported for $[Na^+]$ and $[K^+]$ (Monroy-Oddo and Esposito, 1951; Tyler and Monroy, 1959) as well as free $Ca^{2+}$ (Mazia, 1937; Monroy-Oddo, 1946) in sea urchin. But whether these changes are only transient or lead to lasting changes over a longer period of development is not known.

The most obvious similarity among the results listed in Table 2 is the initially low $[K^+]$, increasing as development proceeds. In the case of frog, $[K^+]$ in the unfertilized egg is lower than at any other stage of development, even in fully mature and differentiated cells. Total $K^+$ in sea urchin does not change, but exchangable $K^+$ (probably indicating cytoplasmic free $K^+$) increases by 4-fold. A relatively low level of $[K^+]$ in the unfertilized egg cell is also indicated by the lower $[K^+]$ in the ovulated or unfertilized frog egg relative to the oocyte $[K^+]$.

For the other ions reported there are either insufficient data for an adequate comparison (e.g., $Cl^-$) or the concentration changes are at least apparently different from our system. In the case of frog $[Na^+]$ decreases at later stages of development, and for $[Ca^{2+}]$ and $[Mg^{2+}]$ in sea urchin concentration decreases are also reported. Although it should be mentioned that radioactive $Na^+$ uptake in unfertilized eggs of the dioecious species *Fucus vesiculosus* indicated $[Na^+]$ to be 70 m*M* (Robinson and Jaffe), much higher than reported for our zero time eggs. Perhaps $[Na^+]$ does decrease rapidly in the first 30 min after shedding and is thus not detected by our methods. Determinations of $[Na^+]$ at later times in *Fucus vesiculosus* should help clarify this point.

It should be pointed out that intracellular ion binding or compartmentalization may alter the apparent ion content changes with development. In the sea urchin, tracer studies indicate that 80–90% of the potassium in the unfertilized egg is inexchangeable (Chambers and Chambers, 1949; Tyler and Monroy, 1959), and a recent investigation with $K^+$-specific microelectrodes measures an internal potassium ion activity which does not differ much from its gross concentration (Steinhardt *et al.*, 1971). Taken together, these two investigations suggest intracellular $K^+$ compartments which

TABLE 2
INTRACELLULAR ION CONCENTRATIONS OF EGGS (mmoles/liter cell water)

| Material | Developmental stage | $K^+$ | $Na^+$ | $Cl^-$ | $Ca^{2+}$ | $Mg^{2+}$ | Reference[c] |
|---|---|---|---|---|---|---|---|
| Frog | Oocyte | 108–115 | 69–93 | 43 | 3.0 (2.7)[a] | | 1–4 |
| | Unfertilized egg | 69 | 70 | 75 | 7.7 (1.7)[a] | | 1 |
| | Blastula | 85 | 39 | | | | 5 |
| | Striated muscle | 139 | 15 | | | | 6 |
| | Nerve | 159 | 47 | | | | 7 |
| | Smooth muscle | 129 | 66 | 14 | | | 8 |
| | Erythrocyte | ~120 | | | | | 9 |
| Sea urchin | Unfertilized egg | (50)[b] | | | | | |
| | | 180–240 | 52 | 80 | 2.5–4 | 11–22 | 10–15 |
| | Blastula | 200[b] | | | | | |
| *Pelvetia* | Unfertilized egg | 170 | 27? | 87 | 4 | 25 | This |
| | Polarized egg | 320 | 40 | 194 | 4 | 25 | pa- |
| | Two-cell stage | 400 | 20 | 322 | 4 | 25 | per |

[a] Water-extractable fraction.
[b] Exchangeable fraction.
[c] Key to references:
1. Morrill (1965)
2. Naora, *et al.* (1962)
3. Abelson and Duryee (1949)
4. Morrill *et al.* (1964)
5. Kostellow and Morrill (1968)
6. Hodgkin and Horowitz (1959)
7. Hurlbut (1958)
8. Bozler *et al.* (1958)
9. Mullins *et al.* (1941)
10. Tyler and Monroy (1959)
11. Rothschild and Barnes (1953)
12. Shapiro and Davson (1941)
13. Mazia (1937)
14. Azarnia and Chambers (1970)
15. Steinhardt *et al.* (1971)

are of equal $K^+$ activity. Compartmentalization in frog eggs is suggested for $Na^+$ by the separate analysis of nuclei and cytoplasm (Century, *et al.*, 1970), for $K^+$ by tracer studies (Ecker and Smith, 1971) and for $Ca^{2+}$ by water-extractable fractions (Morrill, 1965). Unsuspected similarities in ion changes during development may become apparent with more detailed investigations of intracellular ion distributions using tracer exchange and ion specific microelectrodes.

Two important questions must now be considered: first, what is the *cause* of the $[K^+]$ and $[Cl^-]$ changes, and second, what is the *physiological significance* of these changes? Regarding the cause of the changes, the large $[K^+]$ increase might be accounted for by the hyperpolarization of the membrane resting potential immediately after fertilization reported by Weisenseel and Jaffe (1972). However, a comparison of external and internal $K^+$ with the membrane potential (see Weisenseel and Jaffe, 1972, Table 6) indicate an inward $K^+$ pump at all times; although $[K^+]$ is close to electrochemical equilibrium at later times. On the other hand, $Cl^-$ is pumped out initially, but after 2–3 hr, $Cl^-$ begins to increase and the apparent pump direction is reversed. Of course, the possibility of cytoplasmic compartmentalization, as discussed above, might alter these conclusions about the existence and direction of pumps. For the other cations ($Na^+$, $Ca^{2+}$, $Mg^{2+}$) where the external concentrations are relatively much higher, an outwardly directed pump will always be necessary to maintain the low internal concentration.

Turning now to the second question just raised, the possible physiological significance of the $[K^+]$ and $[Cl^-]$ increase, two immediate possibilities can be suggested; increased $[K^+]$ and $[Cl^-]$ may activate enzymes necessary for development and/or increase the internal osmotic

pressure.

1. The general effects of ion concentrations on enzyme activity have been extensively discussed (Evans and Sorger, 1966; Suelter, 1970) specifically in the sea urchin (Epel *et al.*, 1969). In cell-free preparations of sea urchin eggs, amino acid incorporation is greatly stimulated by $K^+$ (Molinaro and Hultin, 1965). Moreover, the optimum $[K^+]$ of 300 m*M* is similar to $[K^+]$ attained by *Pelvetia* after fertilization. Molinaro and Hultin (1965) also report that the high $[K^+]$ requirement is confined to the ribosomal functions.

In a species related to *Pelvetia*, *Fucus vesiculosus* some interesting features of protein synthesis are reported which might be related to ion changes. The rate of protein synthesis increases rapidly after fertilization, then decreases after 3.5 hr, but again increases after germination (Peterson and Torrey, 1968). At least initially the size of the heavy polysome fraction increases and decreases with the rate of protein synthesis (Linskens, 1969), but proteins necessary for rhizoid initiation are not synthesized until at least 8 hr after fertilization (Quatrano, 1968). Upon considering these observations on similar species, the high $K^+$ optimum of ribosomal functions and timing of essential protein synthesis, together with the presently reported $[K^+]$ changes, it seems possible that $[K^+]$ may help activate and control protein synthesis after fertilization.

2. The importance of solute accumulation to supply osmotic pressure compatible with cell enlargement has recently been stressed by Steward and Mott (1969). In this respect it is important to note the osmotic pressure increase reported here *precedes* germination and expansion.

In general, the relations between developmental stage, intracellular ion concentration, and osmotic pressure fall into several well defined phases:

1. Before and just after fertilization the eggs are in osmotic equilibrium with sea water (1027 milliosmolar). The total ion content for unfertilized or zero time eggs would only account for about 300 milliosmoles of the initial internal osmotic pressure. A large part of the remaining 700 milliosmoles is possibly accounted for by mannitol and other low molecular weight carbohydrates (Bidwell *et al.*, 1958; Lindberg and Page, 1954). Up to 2 hr there is no net ion uptake and the ion concentrations may even decrease (e.g., $Cl^-$). During this phase the primary physiological event is the deposition of the cell wall.

2. From 2 to 8 hr the internal osmotic pressure increases rapidly by 180–300 milliosmoles. This increase could be totally accounted for by the simultaneous $[K^+]$ and $[Cl^-]$ increase which would predict an osmotic pressure increase of 250 to 300 milliosmolar.

3. Between 8 and 14 hr the cells are in the process of germination. There is no further increase in osmotic pressure, but $[K^+]$ and $[Cl^-]$ continue to increase. The onset of growth and synthesis of new cellular material during germination possibly decreases soluble organic substances in the cell interior, at least initially. At the same time the osmotic pressure could be held relatively constant by the $[K^+]$ and $[Cl^-]$ increases.

4. After 14 hr $[K^+]$ and $[Cl^-]$ have reached steady levels. By this time all the cells have germinated and are in the process of actively growing and expanding.

The changes in intracellular ion concentrations reported here emphasize the intimate relation of ion movements and concentrations with the developmental process. Of even more crucial importance may be the localized ion concentration within the developing cell. These local concentrations may vary due to binding or compartmentalization and regulate specific enzymes or even cell polarity (Jaffe, 1969).

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