DEVELOPMENTAL BIOLOGY 35, 349-361 (1973)

Ion Movements in a Developing Fucoid Egg[1]

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Accepted July 26, 1973

Radioisotopes were used to measure the fluxes of K, Cl, Na, Ca (and to some extent, Mg) across the membranes of the eggs of the brown algae *Fucus* and *Pelvetia* at different stages of development. The membranes of unfertilized eggs have a relatively nonselective permeability to K, Na, and Cl but develop a high degree of specificity for K within a few hours after fertilization. At this time, the outward leakage of Cl falls to a very small fraction of the inward flux. This allows the eggs to accumulate KCl, which provides the osmotic driving force for growth. The internal [K]:[Na] ratio increases by about 6-fold by the time of first division; this results (at least partially) from a 60% fall in the sodium permeability. Finally, membrane conductances were calculated from these tracer data. Multiplication of these conductance values by the transcellular voltages previously found sufficient to polarize such eggs gives effective transcellular current values comparable to those driven by the egg through itself. This suggests that the endogenous current acts back to further polarize this egg.

INTRODUCTION

This is the last of three reports that aim to set forth some basic facts about the membrane properties and internal ionic environment of developing fucoid eggs. The first of these dealt with the membrane potential and conductivity (Weisenseel and Jaffe, 1972) and the second with the ionic concentrations and the osmotic pressure of the interior (Allen *et al.*, 1972). In this paper we present the results of flux measurements of the important ions of sea water and relate these to the data of the first two papers. While substantial areas of agreement were found, one serious discrepancy appeared: the electrically measured membrane conductances reported by Weisenseel and Jaffe (1972) are 10–50 times higher than those calculated from the flux data reported here. However, new electrical measurements now leads us to believe that it is the flux data which yield approximately correct conductances. This conclusion, it turns out, has a consequence of considerable developmental interest which we will discuss below.

In calculating the permeability of the membrane to the various ions we have made use of the passive flux equations developed by Hodgkin and Katz (1949). These equations may be summarized as follows:

$$J^j = J^j_{in} + J^j_{out}$$

$$= P_j \frac{Z_j FV}{RT}\left[\frac{C_{in}\exp(Z\,FV/RT) - C^j_{out}}{1 - \exp(Z_j\,FV/RT)}\right]$$

where J^j is the net flux of the jth ion, Z_j is the number of elementary charges of the ion, P_j is the permeability of the membrane to that ion and C^j_{out} and C^j_{in} are respectively the external and internal activities of the jth ion. V, the membrane potential, is $V_{in} - V_{out}$ and the one-way fluxes, J^j_{in} and J^j_{out}, are taken as positive for fluxes into the cell and negative for fluxes out of the cell. If one assumes that the influx and efflux of a particular ion are independent, the above equation may be separated into two parts:

$$J^j_{in} = -P_j \frac{Z_j FV}{RT}\left[\frac{C^j_{out}}{1 - exp\,(Z_jFV/RT)}\right]$$

and

$$J^j_{out} = P_j \frac{Z_jFV}{RT}\left[\frac{C^j_{in}\exp(Z_jFV/RT)}{1 - \exp(Z_jFV/RT)}\right]$$

(See, for example, Shanes, 1958, p. 109 or

[1] From K. R. Robinson's Ph.D. thesis.

Katz, 1966, p. 60.) These two equations can easily be rearranged to give expressions for P_j which are functions of V, C^j_{in} or C^j_{out} and either J^j_{in} or J^j_{out}. Thus, if the direction of passive flux can be established, the permeabilities of the various ions can be calculated by measuring the flux in that direction.

In the various calculations involving membrane potentials and ionic concentrations, we have used the results given in the earlier mentioned first two papers in this series. We have, of course, always used the values appropriate for the particular stage of development in question.

The interior of these eggs is packed with various relatively dense inclusions that occupy perhaps 70–80% of the total volume (Jaffe, 1968). These inclusions are too small (1–2 μm) to be penetrated by the microelectrodes used by Weisenseel and Jaffe (Quatrano, 1972). There is no single, large vacuole as found in *Nitella* and other adult algal cells.

METHODS AND MATERIALS

Most data on fertilized eggs were obtained from zygotes of the monoecious alga *Pelvetia fastigiata* which was collected at Pacific Grove, California, by Mrs. A. Phillips. This material was used because ripe fronds are available all year. Measurements on the unfertilized egg were done on eggs from *Fucus vesiculosus* which was collected on the Massachusetts coast north of Boston in the winter months. It was necessary to change to this dioecious plant because no satisfactory method was found for getting large numbers of undamaged, unfertilized *Pelvetia* eggs.

About 5 hr after fertilization these eggs become very sticky and will hold tightly to almost any substratum. It was therefore necessary to have separate methods for dealing with the stuck and unstuck eggs. Following the application of tracer during uptake experiments, the unstuck eggs were washed by gentle hand centrifugation. Three washes were found to be sufficient to remove the extracellular tracer. The eggs were then distributed on ringed planchets for counting in a gas-flow counter. In order to do washout experiments on unstuck eggs, they were loaded with tracer and placed on a Nitex (Tobler, Ernst, and Traber, New York) nylon net. The net was then placed in a series of 2-ml washes for a measured time, and each wash was dried and counted. The stuck eggs were grown directly on Pyrex planchets for both uptake and washout experiments. Again, during washout studies the planchets were moved through a series of 2-ml washes and the washes dried and counted.

The specific activity of the bathing medium was determined by adding measured amounts of radioactive sea water to preparations of eggs that were identical to the samples. Distilled water was then added to both standards and samples in order to burst the eggs. This was to minimize differences in absorption of radioactivity by eggs and salts between standards and samples. Care was taken to see that the eggs were evenly distributed on the planchets. When washes from efflux experiments were to be counted, standards were prepared by adding radioactive sea water to a volume of sea water identical to the washes.

All experiments were performed in a Tris-buffered artificial sea water at pH = 8.0. The ionic composition of this sea water was: [Cl] = 563 mM, [Na] = 483 mM, [Mg] = 55 mM, [Ca] = 10 mM, [K] = 10 mM, [SO_4] = 28 mM, [HCO_3] = 2.5 mM, [Tris] = 10 mM.

All values of the membrane potential used for various calculations in this report are taken from Weisenseel and Jaffe (1972). The determination of the amount of water per cell was done by Allen *et al.* (1972) for *Pelvetia*. It has been assumed here that *Fucus* eggs have a similar percentage of water. Direct measurement of the size of *Fucus* eggs typically yielded a volume of 170 picoliters (pl) and a surface area of 1.2×10^{-4} cm^2; the water volume was about 75 pl. *Pelvetia* eggs were usually

400 pl in volume and 2.5×10^{-4} cm^2 in area before germination, so the water volume was 180 pl. After germination, corrections were made for the increase in size. All concentrations reported here are in terms of millimoles of ion per liter of cell water.

During all experiments, the eggs were well illuminated; that is, with about 100 foot-candles of "cool white" light.

The number of eggs in each sample was determined either by photographing the sample before putting the eggs in the planchets or by photographing identical aliquots. In either case, the eggs in the photograph were counted.

RESULTS

Potassium

The movement of potassium (as followed by ^{42}K) into unfertilized eggs of *Fucus vesiculosus* shows simple one-compartment kinetics in both uptake and washout studies. A typical uptake result is shown in Fig. 1. The 178 m*M* saturation value agrees well with the 180 m*M* measured by Allen *et al.* (1972) for the just-fertilized *Pelvetia* egg. If the uptake is replotted in appropriate semilogarithmic form (Fig. 2), a straight line results indicating a single internal compartment. The influx calculated from this curve is $J_{in}^{K} = 10.8$ pmoles/cm^2-sec. The results of a washout experiment on a different batch of eggs are shown in Fig. 3a and from this the efflux was calculated to be 9.4 pmoles/cm^2-sec.

The membrane potential, $V = V_{in} - V_{out}$, of the unfertilized *Fucus* egg was measured at -22 mV by Weisenseel and Jaffe (1972). At this potential, the equilibrium concentration of potassium in the egg would be 24 m*M*, so we conclude that potassium is being actively moved into this egg. Assuming that the efflux is entirely passive, the permeability coefficient for K can be calculated from the flux equation given in the Introduction:

$$P_K = \frac{RT}{FV}\left[\frac{1 - exp(VF/RT)}{[K]_i \exp(VF/RT)}\right] J_{out}^{K}$$

This results in $P_K = 8.5 \times 10^{-8}$ cm/sec.

If ^{42}K-sea water is added to just-fertil-

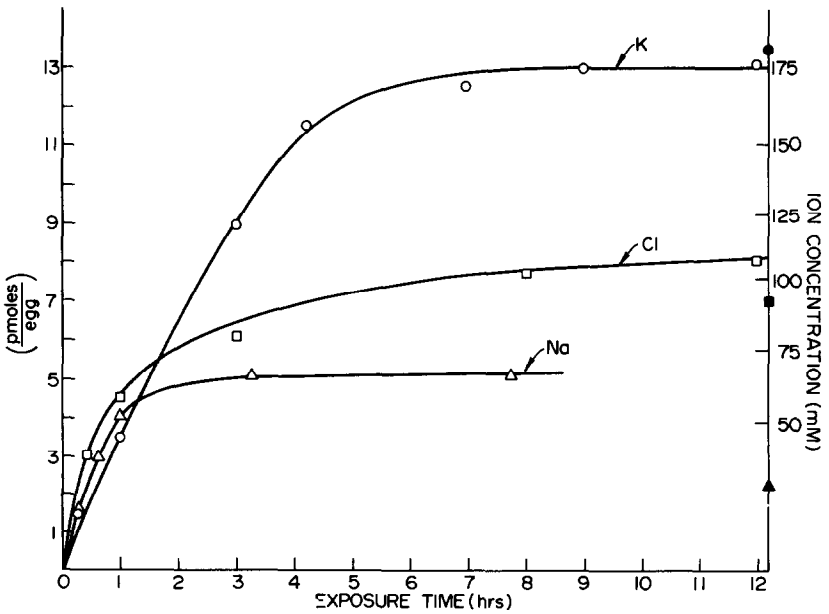

FIG. 1. The uptake of tracer ions into unfertilized eggs of *Fucus vesiculosus*. The right-hand ordinate indicates the measured internal radioactivities per liter of cell water divided by the external specific activities. The chemically determined ion concentrations for just fertilized *Pelvetia* eggs are shown for reference (▲ = [Na], ■ = [Cl], ● = [K]) (Allen *et al.*, 1972).

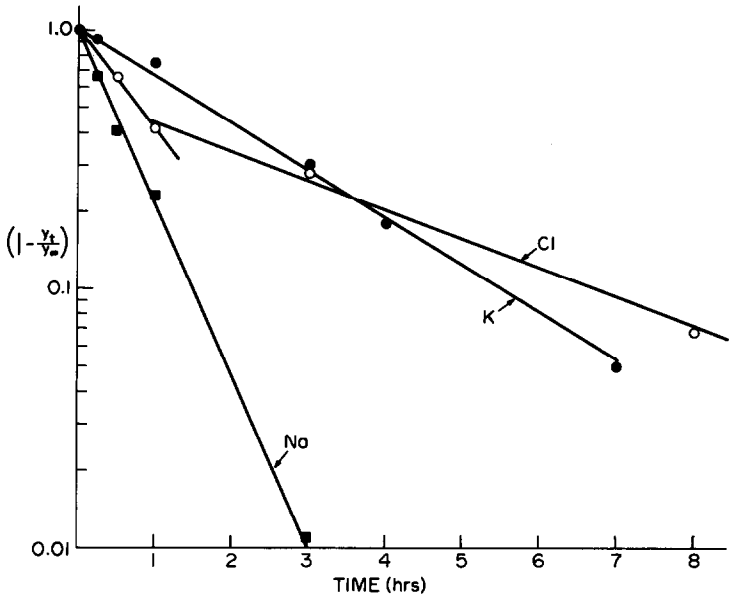

FIG. 2. Uptake of ions into unfertilized *Fucus vesiculosus* eggs replotted from Fig. 1. Y_t is the amount of ion as measured by tracer taken up by the cells at time t and Y_∞ is the saturation value for that ions. For a single internal compartment, $Y_t = Y_\infty (1 - \exp(-JA/Y_\infty)t)$, so the flux, J, can be calculated from the slope of these curves by the equation

$$J = \frac{-Y_\infty}{A} \quad \frac{\Delta \ln [1 - (Y_t/Y_\infty)]}{\Delta t}$$

where A is the area of the eggs.

ized *Pelvetia* eggs, the internal specific activity rises to that of the sea water in about 3 hr, as can be seen in Fig. 4. Since the total amount of potassium changes very little during the first few hours after fertilization, the influx can be taken as a measure of efflux, avoiding the difficulty of having fully labeled *Pelvetia* eggs. (The data from Allen *et al.*, as presented in our Fig. 4, indicate that there may be some fluctuation in the internal potassium concentration between 0.5 hr and 2 hr after fertilization. At worst, these fluctuations would mean a difference between influx and efflux of about 20%, so the permeabilities calculated by this method may be uncertain to that extent.) Short pulses of ^{42}K were given, as shown in Fig. 5, and the influx determined from these. The value of the membrane potential at the middle of each exposure period was used to calculate the permeability, which was found to increase 5-fold during the first 3 hr to reach about 4×10^{-7} cm/sec.

The question of correspondence between the two different genera, *Fucus* and *Pelvetia*, arises. Were the differences that were seen between unfertilized *Fucus* and fertilized *Pelvetia* eggs due to fertilization or did they reflect the genus difference? Two tests of this were done. First, unfertilized *Fucus* eggs were left in ^{42}K-sea water long enough to allow their internal specific activity to become equal to that of the bathing medium (about 10 hr, see Fig. 1) and the sperm, which were also in ^{42}K-sea water of the same specific activity, were added to the eggs. The subsequent increase in ^{42}K (and thus, in internal potassium concentration) was then measured. These results are shown in Fig. 4 and it is clear that the potassium concentrations of *Fucus* and *Pelvetia* eggs are virtually the same.

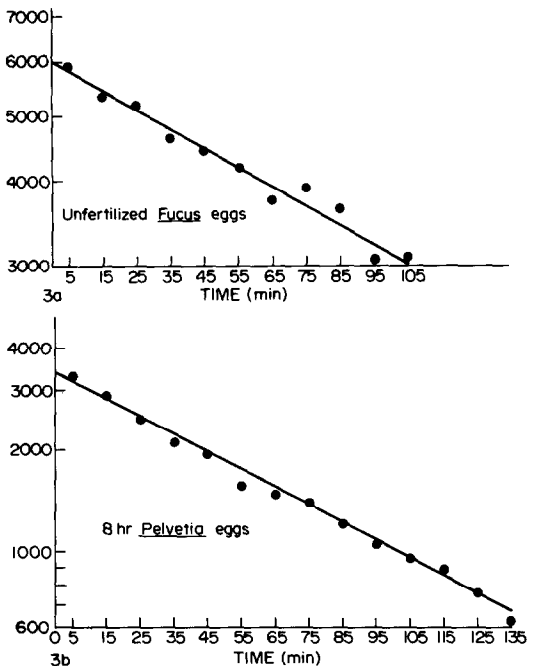

FIG. 3. The efflux of ^{42}K from (a) unfertilized *Fucus vesiculosus* eggs and (b) 8-hr *Pelvetia* zygotes. The eggs were preloaded in ^{42}K-sea water until the internal specific activity was equal to that of the sea water; that is, 9 hr for *Fucus* and 8 hr for *Pelvetia*. The fluxes were determined by extrapolating to $t = 0$ and converting cpm/10 min to pmoles/cm^2-sec using the known specific activity of the sea water, the area per egg, and the number of eggs in the samples. For unfertilized *Fucus*,, $J_{out}^{K} = 9.4$ pmoles/cm^2-sec, and for 8-hr *Pelvetia*, $J_{out}^{K} = 30$ pmoles/cm^2-sec.

A second test was carried out by measuring the influx of ^{42}K in the recently fertilized *Fucus* egg. At 1 hr after fertilization, the influx of potassium was found to 17.2 pmoles/cm^2-sec which is quite similar to the influx in *Pelvetia* at the same time (see Fig. 5). These two tests suggest that the potassium data for unfertilized *Fucus* eggs and fertilized *Pelvetia* can be taken together without serious error.

After 3 hr, it is possible to do efflux measurements directly. The results of a typical efflux measurement on 8 hr *Pelvetia* zygotes are shown in Fig. 3b. During the course of this experiment about 80% of the total radioactivity was washed out, and as can be seen, only one compartment existed. The flux was about 30 pmoles/cm^2-sec. It was noticed in several determinations of influx and efflux during the time of rapid potassium concentration change (i.e., 3–10 hr after fertilization) that the influx exceeded efflux by 1–5 pmoles/cm^2-sec, which would roughly account for the observed accumulation of potassium. However, none of these determinations were done at the same time on the same batch of eggs; so one experiment was done in which influx and efflux were measured at 8 hr after fertilization on one batch of eggs. The influx proved to be 33.0 pmoles/cm^2-sec while the efflux was 29.0 pmoles/cm^2-sec which should result in a potassium concentration change of 20 m*M*/hr. This compares well with the observed change at this stage of 15 m*M*/hr (Allen *et al.*, 1972). The results of a number of determinations of P_K are shown in Fig. 6. The peak shown at 7 hr was seen in every series of experiments that spanned this time.

Sodium

Figure 1 shows the uptake of ^{22}Na into unfertilized *Fucus* eggs. The saturation value was 65 m*M*. These data are replotted in semilog form in Fig. 2 and single com-

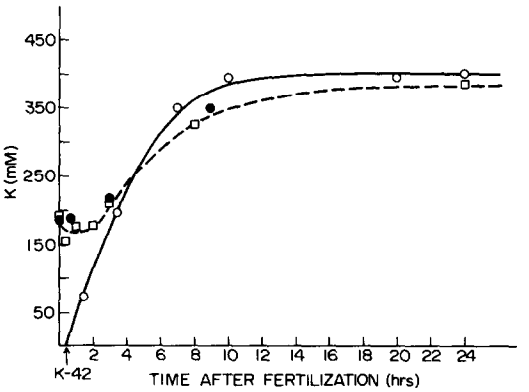

FIG. 4. ^{42}K uptake into developing *Pelvetia* eggs (O——O). The result of an atomic absorption spectroscopic determination of the potassium content of *Pelvetia* (□- - -□) is shown for reference (Allen *et al.*, 1972). The filled circles show the concentration of potassium in *Fucus vesiculosus* eggs as determined with ^{42}K. See text for an explanation of method.

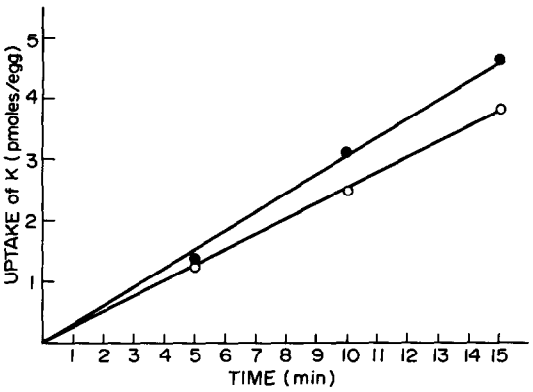

FIG. 5. Uptake of ^{42}K by recently fertilized *Pelvetia* eggs. ^{42}K was applied at 1 hr (○——○) and 2 hr (●——●). The influx (and hence the efflux) at 1 hr and 2 hr was 15.6 pmoles/cm²-sec and 19.2 pmoles/cm²-sec, and potassium permeability, P_K calculated from these values was 2.1×10^{-7} cm/sec and 3.6×10^{-7} cm/sec, respectively, using 40 mV and 55 mV for the membrane potential.

partment kinetics are obeyed. The flux calculated from this curve is 16.1 pmoles/cm²-sec. Since this inward movement is presumably passive, the permeability, P_{Na}, can be calculated from the following equation:

$$P_{Na} = -\frac{RT}{FV}\left[\frac{1-\exp(VF/RT)}{[Na]_0}\right] J_{in}^{Na}$$

This yields a value of 2.3×10^{-8} cm/sec.

The 65 m*M* equilibrium concentration was considerably larger than the 30 m*M* concentration of sodium measured by Allen *et al.* (1973) in just-fertilized *Pelvetia* eggs. To see whether this was a change caused by fertilization or simply a genus difference we added sperm to preloaded *Fucus* eggs, as described for potassium, and followed the change in sodium concentration. It rapidly fell, reaching about 40 m*M* in the first hour after fertilization a figure similar to that for *Pelvetia* at the same stage. The influx in *Fucus* also decreased; 45 min after fertilization it was measured at 6.1 pmoles/cm²-sec. If the membrane potential is taken as 35 mV, P_{Na} is calculated to be 7.5×10^{-9} cm/sec. At 1 hr after fertilization, the sodium influx in *Pelvetia* was found to be 6.8 pmoles/cm²-sec so $P_{Na} = 7.1 \times 10^{-9}$ cm/sec if a membrane potential of 40 mV is used. Sodium permeability remains low for the remainder of the one-cell stage at $5\text{–}6 \times 10^{-9}$ cm/sec. As Fig. 7 shows, the sodium continues to be in only one compartment.

Chloride

Figure 1 shows the uptake of ^{36}Cl into unfertilized *Fucus* eggs. If these data are replotted in semilog form (Fig. 2), it can be seen that there seem to be two compartments. This may be an illusion; it can be shown that a very slow passive accumulation of chloride—a process that might well accompany the slow deterioration of unfertilized eggs after shedding—could also produce such a relationship. However, even if there are indeed two compartments, our general knowledge of chloride compartmentation in algal cells (MacRobbie, 1970) indicates that the cytoplasmic chloride is always much *less* than the average cell chloride. Hence, the cytoplasmic chloride is almost certainly no larger than the average of 110 m*M* reached after 12 hr exposure to ^{36}Cl. The −22 mV membrane potential (Weisenseel and Jaffe, 1972) indi-

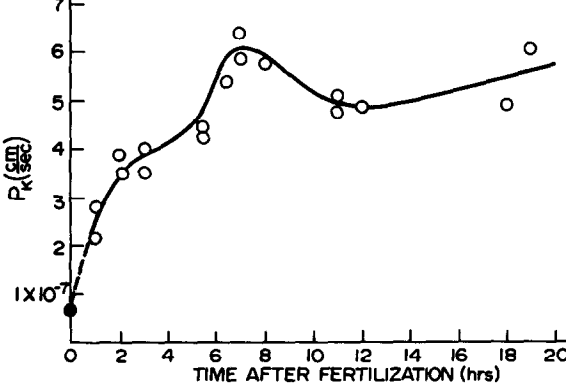

FIG. 6. The potassium permeability of developing *Pelvetia* eggs (○——○) and of unfertilized *Fucus* eggs (●). Each point represents either one experiment or the average of two experiments done at the same time. Several different batches of eggs collected at various times during a year were used.

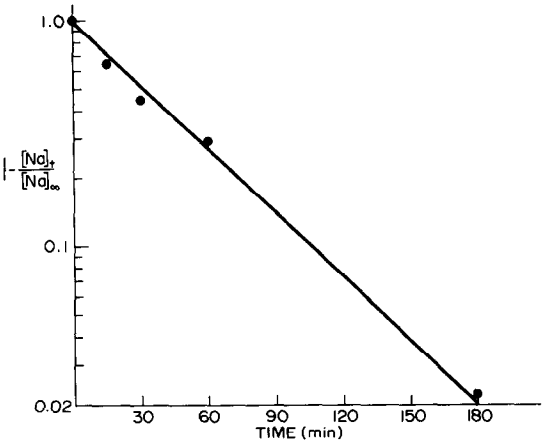

FIG. 7. The uptake of ^{22}Na into 12-hr *Pelvetia* eggs. These data are plotted as in Fig. 2. The value of $[Na]_\infty$ was 35 m*M* and was determined by adding ^{22}Na to eggs just after shedding and measuring its accumulation 12 hr later. Comparison with the data of Allen *et al.* (1972) makes it clear that the internal sodium was completely exchanged in 12 hr.

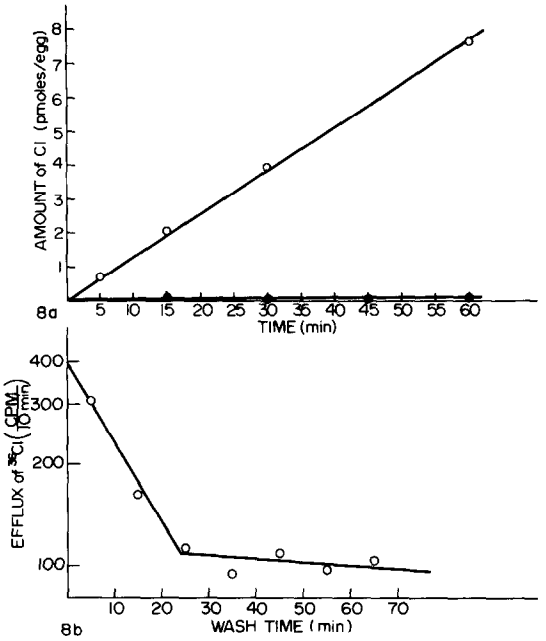

FIG. 8. (a) The uptake (O——O) and efflux (●——●) of ^{36}Cl in 6.5-hr *Pelvetia* eggs. The efflux points show the accumulations of ^{36}Cl in the wash solution. J_{in}^{Cl} = 10 pmoles/cm²-sec. (b) The efflux of ^{36}Cl from 23-hr-old *Pelvetia* eggs. Two phases are clearly seen as discussed in the text. J_{out}^{Cl} = 9.0 pmoles/cm²-sec.

cates an equilibrium cytoplasmic chloride of 230 m*M*, a value *larger* than the whole-cell average. Therefore chloride influx into the unfertilized egg can rather safely be inferred to be in the same direction as the electrochemical driving force and thus passive. Finally, then,

$$P_{Cl} = -\frac{RT}{FV}\left[\frac{1-\exp(FV/RT)}{[Cl]_0\exp(FV/RT)}\right] J_{in}^{Cl}$$
$$= 4.0 \times 10^{-8}\ \text{cm/sec}$$

using an influx value of 14 pmoles/cm²-sec calculated from the initial linear portion of the chloride curve in Fig. 1.

Our attempts to measure the efflux of chloride from preloaded 6-hr zygotes gave the surprising result that the efflux is less than our methods can measure, which means less than 1 pmole/cm²-sec. The results of an influx and efflux experiment are shown in Fig. 8a.

By 18 hr, the efflux kinetics clearly show two Cl compartments. The rapidly exchanging compartment is small, representing less than 5% of the total Cl. Figure 8b shows the efflux from a 23-hr embryo. The flux is about 9 pmoles/cm²-sec, but it is difficult to calculate a permeability coefficient since the Cl concentration in the fast compartment is not known.

One chloride uptake experiment was done in darkness. The influxes at 6 hr and 18 hr were not found to differ from those measured in light. This contrasts with several other algal systems which are known to have much higher Cl influxes in light than in dark (MacRobbie, 1970).

Calcium

Calcium flux measurements were complicated by the fact that the divalent cations tend to be bound to cell wall and membrane components. As shown in Fig. 9a, the efflux of ^{45}Ca from 6-hr eggs is clearly a two-phase process. We have some evidence that the fast phase represents cell wall calcium. Cell wall preparations (supplied by Dr. M. Forman) showed calcium efflux kinetics similar to the fast phase of the intact cells. It seems reasonable to assume that the slow phase represents

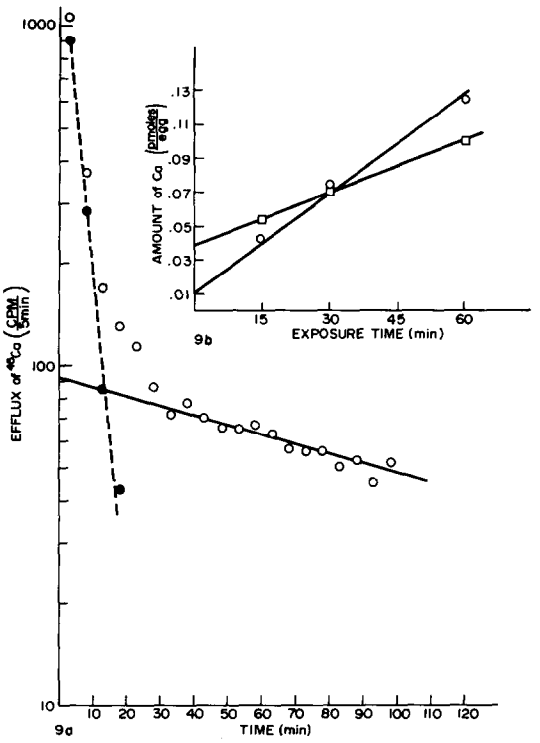

FIG. 9. (a) The efflux of ^{45}Ca from 6-hr-old *Pelvetia* eggs. The observed fluxes (O) are analyzed into a *slow* efflux from the cytoplasm (of 0.13 pmole/cm^2-sec) and a *fast* component (●- - -●) from the cell wall. Each filled circle represents a measured total value minus the extrapolated slow component at that time. (b) The influx of ^{45}Ca at two developmental stages: (O——O), influx at 6 hr; (□——□), influx at 18 hr. Eggs were washed for 10 min following application of tracer.

movement of Ca across the plasma membrane from the cytoplasm. If the slow component is extrapolated back to the beginning of the washing period, a flux of 0.13 pmoles/cm^2-sec is calculated. An estimate of the amount of Ca in the cell wall can also be made from Fig. 9a by subtracting the extrapolated slow component from the measured total value. This calculation shows that there are about 0.15 pmole of calcium in the wall at this stage which is about 20% of the amount of calcium in the cytoplasm.

The results of ^{45}Ca uptake experiments at 5.5 and 18 hr are shown in Fig. 9b. Following the application of ^{45}Ca, the eggs were washed for 10 min before drying and counting. The nonzero intercept of these curves indicates the remainder of the tracer bound to the cell wall. Correcting for this, the fluxes were 0.12 pmole/cm^2-sec at 5.5 hr and 0.074 pmole/cm^2-sec at 18 hr. The permeability can be calculated from:

$$P_{Ca} = \frac{-RT}{2FV}\left[\frac{1 - \exp(2VF/RT)}{[Ca]_o}\right] J_{in}^{Ca}$$

This results in 2.1×10^{-9} cm/sec and 1.3×10^{-9} cm/sec, respectively.

When tracer Ca is allowed to equilibrate with the interior of the eggs, the concentration is found to be 4–5 m*M* after suitable correction for cell wall calcium. This is in good agreement with the value reported by Allen *et al.* (1972).

Magnesium

Owing to the cost of ^{28}Mg, only two experiments with magnesium were done. In one of these experiments, the influx was 0.4 pmole/cm^2-sec at 8 hr and in the other, 1.1 pmoles/cm^2-sec at the same stage. Considering that sea water contains six times more magnesium than calcium, these results indicate that magnesium permeability is of the same order of magnitude as calcium permeability.

DISCUSSION

The results summarized in Table 1 indicate that the unfertilized *Fucus* egg membrane is rather nonselectively permeable to K, Na, and Cl, in agreement with the electrical findings of Weisenseel and Jaffe (1972). We find no evidence for compartmentalization of K in unfertilized *Fucus* eggs such as has been reported in unfertilized sea urchin eggs (Tyler and Monroy, 1959) and frog eggs (Ecker and Smith, 1971).

Just after fertilization, dramatic changes take place in the membrane's ionic properties. The potassium permeability increases rapidly and the sodium permeability decreases. As mentioned earlier, this decrease in P_{Na} causes a decrease in sodium influx of about 6 pmoles/cm^2-sec in *Fucus*. This

TABLE 1

PERMEABILITIES AND CONDUCTANCE OF FUCOID EGGS AT VARIOUS STAGES

Stage →	*Fucus* Unfertilized	Pelvetia		
		1–3 Hr	5–12 Hr	12–24 Hr
P_K ($\times 10^{-7}$ cm/sec)	0.85[a]	3.0[b]	5.0[a]	5.5[a]
P_{Cl}	0.40[b]	0.61[b]	—	—
P_{Na}	0.23[b]	0.07[b]	0.06[b]	—
P_{Ca}	—	—	0.02[b]	0.01[b]
Conductance (mmho/cm²)				
Flux	0.1	0.1	0.2	0.3
Electrical[c]	1.2	1.0	4.0	6.0

[a] From efflux data.
[b] From influx data.
[c] From Weisenseel and Jaffe (1972).

would produce a decrease in internal Na concentration of about 35 m*M* in 1 hr if the efflux remained constant. The observed decrease is 25 m*M*, suggesting that the active transport mechanism continues to expel Na at about the same rate during the first hour after fertilization as it did in the unfertilized egg.

The data suggest that there is little difference in membrane permeability and ionic concentrations between fertilized *Fucus* eggs and *Pelvetia* eggs; this is certainly true for potassium and sodium. Therefore, for purposes of comparison, we have grouped the data on unfertilized *Fucus* eggs with that for developing *Pelvetia* zygotes and have assumed that the pattern of changes is a consequence of fertilization, not of genus difference.

The chloride flux results are not easily interpreted. The membrane potential measurements of Weisenseel and Jaffe indicate that $P_{Cl}/P_K \geq 0.04$ from 5 hr on, so P_{Cl} is at least 2×10^{-8} cm/sec. If all of the internal chloride is in a single compartment bound only by the plasma membrane, the constant-field flux equation

$$J_{out}^{Cl} = P_{Cl} \frac{FV}{RT} \left[\frac{[Cl]_i}{1 - \exp(VF/RT)} \right]$$

predicts an efflux of at least 10 pmoles/cm²-sec at 8 hr. As shown in Fig. 8a, no such efflux was measured. It would be possible to explain this if it were assumed that chloride exists in two compartments, as is clearly the case in the later stages (see Fig. 8b). If the concentration in the compartment that is limited by the plasma membrane is less than the whole-cell value, then the efflux would be proportionally smaller. For example, if the chloride in the fast compartment is in electrochemical equilibrium with the external medium, its concentration would be 35 m*M* rather than 190 m*M* and the efflux could be as little as 1–2 pmoles/cm²-sec, which would be quite difficult to detect by our methods. This suggestion is made more plausible by our knowledge of characean internodal cells; as we have observed earlier, the chloride concentration in their flowing cytoplasm is much lower than that in both their vacuoles and their chloroplasts and hence much lower than the average inside the whole cell (MacRobbie, 1970). By 1 day after fertilization both the cytoplasmic concentration of Cl and P_{Cl} must increase if the results shown in Fig. 8b are to be understood in these terms.

One important point emerges from the Cl data. The dramatic change in Cl efflux provides the mechanism for allowing the accumulation of KCl and increasing the internal osmotic pressure. The development of this osmotic gradient is a requirement for growth; Whitaker and Clancy (1937) and Torrey and Galun (1970) have shown that rhizoid growth can be reversibly blocked in *Fucus* embryos by increasing the osmolarity of the external medium. It is interesting to note that the switch to active anion uptake coincides with the development of a cell wall by the egg. As Dainty (1962) has pointed out, animal cells do not normally have anion pumps, but they are common in plant cells. The unfertilized fucoid egg has no cell wall and in that respect is "animal-like."

The most serious discrepancy between the flux measurements and the electrical

measurements on these eggs seemed to be in the value of the membrane conductance. A crude estimate of the membrane conductance, which may be more physically obvious to the reader than the full calculation (in turn based on the flux equation which is given in the Introduction) can be made by considering the potassium influx. Since the membrane at the later stages behaves approximately like a potassium electrode (Weisenseel and Jaffe, 1972) and the influx is primarily due to the electrical field,

$$g_K \approx \frac{I_K}{V} = \frac{F J_{in}^K}{V}$$

At 18 hr, the influx is about 40 pmoles/cm²-sec and the membrane potential is 75 mV so $g_K \approx 0.05$ mmho/cm², 100 times less than Weisenseel and Jaffe's value of 5 mmho/cm².

A more exact value for the membrane conductance, consistent with the assumptions made in calculating the permeability coefficients, can be obtained from Eq. 6.0 of Hodgkin and Katz (1949):

$$G = \frac{F^3}{(RT)^2} V_m P_K \frac{WY}{Y - W}$$

where

$$W = [K]_0 + \frac{P_{Na}}{P_K}[Na]_0 + \frac{P_{Cl}}{P_K}[Cl]_i$$

$$\text{and } Y = [K]_i + \frac{P_{Na}}{P_K}[Na]_i + \frac{P_{Cl}}{P_K}[Cl]_0$$

This gives a value of 0.05 mmho/cm² for the unfertilized egg, which rises, depending on what assumptions are made about Cl permeability and concentration, to between 0.1 and 0.3 mmho/cm² at the later stages. At the maximum, at about 12 hr, Weisenseel and Jaffe measured a membrane conductivity of 9.0 mmho/cm², nearly 50 times our flux-estimated value. Even at the time of best agreement there was a 10-fold difference. A summary of these results is shown in Table 1. The possibility of a leak during the electrical measurements has been extensively discussed by Weisenseel and Jaffe, and it seemed most unlikely that leakage around the electrode is the root of this disparity. We then considered the possibility that the membrane is extremely sensitive to a small voltage changes across it so that the conductance increased greatly during its electrical measurement. To test this idea, we looked at ^{42}K uptake while a small voltage was applied across the membrane. This was done by placing a nylon net holding eggs in a chamber containing ^{42}K-sea water between two flat platinum black electrodes. An ac signal sufficient to result in an rms voltage of several millivolts across the poles of the membrane was applied to the electrodes and K-uptake measured. When the rms voltage across the membrane was as high as 10 mV, no increase in g_K was observed; the slight increase in K-uptake that was seen was due to the increased electrical driving force. A second, alternate explanation that was considered was that there might be a very small but rapidly exchanging potassium compartment limited by the plasma membrane and that our measured potassium fluxes actually represent the secondary movement of K into a large, noncytoplasmic compartment. However, when the uptake of ^{42}K was examined at 30-sec intervals, it was found to be completely linear with time for 15 min. If there had been a small, rapidly exchanging compartment in contact with the plasma membrane we would have expected to see a much higher uptake rate initially; therefore we concluded that there was no fast second compartment.

Recent electrical measurements shed new light on this discrepancy. The first conductance measurements of Weisenseel and Jaffe (1972) were made within a few minutes after puncturing the egg. New measurements, made upon 1-day-old *Fucus* embryos by Weisenseel, show that *the conductance falls greatly as the electrodes remain in the egg*. It falls quickly at first,

then more and more slowly. This fall in conductance is not due to electrode plugging as a two-electrode method was used and any plugging of the current electrode would cause an apparent increase in membrane conductance. By about 40 min after puncture, it has fallen to one-tenth of its initial value and is now only twice the flux value. At this time, the measurements were discontinued because of the appearance of large fluctuations in the resting potential. (Before this time the membrane potential does *not* change.)

We believe that the lower conductance approached long after puncture is the natural value, while the high initial value is a result of puncture. A similar conclusion has been reached by Spanswick (1970) working on *Nitella*. [Incidentally, the high initial conductance is not due to nonselective leaks around the electrodes, for in this state the membrane is found to be *more* selective for potassium than it is in the low conductance state. Rather, we think, the disturbance of the membrane causes potassium channels to open, perhaps as a result of the entry of calcium during impalement. Small increases in internal calcium seem to markedly increase potassium permeability in several other systems (Baker, 1972)].

Much or most of the conductance discrepancy, then can be easily attributed to puncture injury. On the other hand, there is no apparent basis for explaining much of this discrepancy via uncertainties in the flux-inferred conductances. Such an error would have to lie in the inferred potassium conductance since this ion plainly provides most of the membrane's conductance after 5 hr. To our knowledge, exchange diffusion under physiological circumstances is unknown for potassium ions and in any case would only act to widen the discrepancy. A single-file effect, (which, in theory, is the other source of uncertainty) would reduce the discrepancy; but there is no evidence that such effects can provide more than 2-fold errors (Hodgkin and Keynes, 1955). *We conclude, then, that the tracer-determined conductance value is the more reliable one.*

This is of considerable developmental significance. Jaffe (1966) has determined that developing *Fucus* zygotes produce an electrical current that enters the growing rhizoid tip and leaves the thallus pole, completing the circuit through the surrounding sea water. He has suggested that this current may play an important role in the amplification process by which the egg becomes polarized. If one assumes a simple dipole model for the current distribution (i.e., assumes that the current density entering the egg is proportional to the cosine of the angle which goes from 0° at the rhizoid pole to 180° at the thallus pole), then Jaffe's 1966 data imply a polar current density of 3 $\mu A/cm^2$ at the initial growth stage; the imposition of a current of similar magnitude through the egg should polarize it if indeed the current is essential to the polarization process. We have information from two sources on the imposed *voltage* required to effect 50% polarization of *Fucus* eggs: The data of Lund (1923) imply that 18 mV/membrane is required; while those of Bentrup *et al.* (1967) using potassium gradients imply that 7 mV/membrane is needed. The *current* driven through the eggs by these voltages depends on their membranes' conductivity. With the lower value obtained from our tracer measurements and the extended electrical measurement of Weisenseel, one calculates that the imposed current required to polarize *Fucus* eggs is 1–10 $\mu A/cm^2$, clearly of the same magnitude as the 3 $\mu A/cm^2$ endogenous current.

There are relatively few other developing systems in which both flux and electrical measurements have been made. Tupper and Powers (1972) have done such experiments on the eggs of the starfish *Asterias* and they conclude that the hyperpolarization observed after fertilization is a result of the increase of P_K/P_{Na}. They report that

P_{Na} stays constant at 0.2×10^{-7} cm/sec but that P_K increases 4-fold from 2.8 to 11.3×10^{-7} cm/sec: this pattern is in general agreement with the one we have seen in the fucoid egg.

One striking change in the internal ionic environment of the fucoid egg is the 6-fold increase in [K]/[Na] that occurs between fertilization and cell division. Allen *et al.* (1972) have reviewed the information on the ionic contents of other developing systems. From that paper it can be seen that [K]:[Na] increases by a factor of 2 as the unfertilized frog egg develops to the blastula stage. The amount of exchangeable potassium in the sea urchin egg increases 4-fold in the same developmental time, suggesting that [K]:[Na] may also increase. It may be that this ratio is an important control in protein synthesis. Lubin and Ennis (1964) have shown that while K stimulated protein synthesis in cell-free bacterial systems, Na was antagonistic to this effect.

We had hoped to find some clue to the identity of the ion responsible for carrying the current that the developing *Fucus* zygote drives through itself. To do this we looked for a marked increase in influx of the various ions after the current was known to begin. No such increase was seen. Of course, this method would fail if an increase in influx at one end of the egg was accompanied by a similar decrease at the other end. Table 2 shows the fluxes of the ions expressed as a current for comparison with the measured current. K, Na, Cl, and possibly Mg can be seen to be candidates for the current-carrying ion in the extreme case that all of the measured influxes were entering one end or the other of the egg. A major objective of our future work will be to identify the ion or ions involved in the current.

TABLE 2

MAXIMUM POSSIBLE CURRENT CARRIED BY EACH ION[a]

Actual current	Ion				
	K	Na	Cl	Mg	Ca
100	1000	250	250	50	5

[a] Values given here were calculated by assuming the entire measured influx of a given ion entered the egg at one end. Current is given in picoamperes.

This work was supported by the National Science Foundation and the National Institutes of Health.

REFERENCES

ALLEN, R. D., JACOBSEN, L., JOAQUIN, J., and JAFFE, L. F. (1972) Ionic concentrations in developing *Pelvetia* eggs. *Develop. Biol.* **27**, 538-545.

BAKER, P. F. (1972). Transport and metabolism of calcium ions in nerve. *Progr. Biophys. Mol. Biol.* **24**, 177-223.

BENTRUP, F., SANDAN, T., and JAFFE, L. (1967). Induction of polarity in *Fucus* eggs by potassium ion gradients. *Protoplasma* **64**, 254-266.

DAINTY, J. (1962). Ion transport and electrical potentials in plant cells. *Annu. Rev. Plant Physiol.* **13**, 379-402.

ECKER, R. E., and SMITH, L. D. (1971). Influence of exogenous ions on the events of maturation in *Rana pipiens* oocytes. *J. Cell. Physiol.* **77**, 61-70.

HODGKIN, A. L., and KATZ, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (London)* **108**, 37-77.

HODGKIN, A. L., and KEYNES, R. D. (1955). The potassium permeability of a giant nerve fiber. *J. Physiol. (London)* **128**, 61-88.

JAFFE, L. F. (1966). Electrical currents through the developing *Fucus* egg. *Proc. Nat. Acad. Sci. U.S.* **56**, 1102-1109.

JAFFE, L. F. (1968). Localization in the developing *Fucus* egg and the general role of localizing currents. *Advan. Morphog.* **7**, 295-328.

KATZ, B. (1966). "Nerve, Muscle, and Synapse." McGraw-Hill, New York.

LUBIN, M., and ENNIS, H. L. (1964). On the role of intracellular potassium in protein synthesis. *Biochim. Biophys. Acta* **80**, 614-631.

LUND, E. J. (1923). Electric control of organic polarity in the eggs of *Fucus*. *Bot. Gaz.* **76**, 288-301.

MACROBBIE, E. A. C. (1970). The active transport of ions in plant cells. *Quart. Rev. Biophys.* **3**, 251-294.

QUATRANO, R. S. (1972). An ultrastructural study of the determined site of rhizoid formation in *Fucus* zygotes. *Exp. Cell Res.* **70**, 1-12.

SHANES, A. M. (1958). Electrochemical aspects of physiological and pharmacological action in excitable cells. *Pharm. Rev.* **10**, 59-272.

SPANSWICK, R. M. (1970). Electrophysiological techniques and the magnitude of the membrane potentials and resistances of *Nitella translucens*. *J. Exp. Bot.* **21**, 617-627.

TORREY, J. G., and GALUN, E. (1970). Apolar embryos

of *Fucus* resulting from osmotic and chemical treatments. *Amer. J. Bot.* **57,** 111–119.

TUPPER, J. T., and POWERS, D. R. (1972). Determination of P_{Na} and P_K in an early embryonic system. *Biophys. Soc. Abstr.* **12,** 68a.

TYLER, A., and MONROY, A. (1959). Changes in the rate of transfer of potassium across the membrane upon fertilization of the eggs of *Arbacia punctulata*. *J. Exp. Zool.* **142,** 675–690.

WEISENSEEL, M. H. (1972). Personal communication.

WEISENSEEL, M. H., and JAFFE, L. F. (1972). Membrane potential and impedance of developing fucoid eggs. *Develop. Biol.* **27,** 555–574.

WHITAKER, D. M., and CLANCY, C. W. (1937). The effect of salinity upon the growth of eggs of *Fucus furcatus*. *Biol. Bull.* **73,** 552–556.

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Published monthly by Academic Press, Inc., 111 Fifth Avenue, New York, N. Y. 10003.
Editor: Elizabeth Hay, Harvard Medical School, Boston, Mass. 02115.
Owned by Academic Press, Inc., 111 Fifth Avenue, New York, N. Y. 10003
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