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Electrical currents through full-grown and maturing *Xenopus* oocytes

(cell polarity/progesterone/calcium)

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ABSTRACT **An extracellular vibrating electrode was used to map the current pattern around *Xenopus laevis* oocytes. Current was found to enter the animal hemisphere and leave the vegetal hemisphere; in fully grown oocytes from which the follicle cells had been removed, the maximal current density was about 1 $\mu A/cm^2$. This current decreased to nearly zero in response to progesterone and several other maturation-producing agents. In the case of progesterone, the decline began within a few minutes of the addition of the hormone and proceeded with a half-time of about 20 min.**

An analysis of the effects on the current of the removal or addition of various ions and drugs led to the inference that the major current-carrying ion was chloride and that the chloride permeability was controlled by calcium.

An ubiquitous feature of nonmammalian animal eggs is the presence of an animal–vegetal axis. The nucleus of the egg is generally displaced toward the animal pole and this pole is the site of polar body formation. In many amphibian eggs, the axis is readily distinguished by the pigment accumulation at the animal pole and the yolk mass at the vegetal pole. Superimposed on this visible polarity is a developmental polarity: the animal pole gives rise to the ectodermal parts of the embryo and the vegetal pole, to the endodermal. (For a recent review, see refs. 1 and 2.) When the full-grown anuran oocyte is exposed to progesterone, a sequence of events is triggered, leading to the reinitiation of meiosis and formation of a fertilizable egg. This process is also a polar one. The basal portion of the germinal vesicle is the first to break down as the entire germinal vesicle moves toward the animal pole (3).

Our knowledge of the physical forces that might produce such asymmetries and direct polar movements remains limited. The oocytes are randomly oriented in the ovary; thus gravity is not the causal agent. Strong evidence has now accumulated in the case of fucoid algae eggs that electrical currents and ion gradients are involved in transducing environmental asymmetries into cellular polarity (4–6). Electrical currents associated with growth and localization processes have also been shown to exist in insect oocytes (7), pollen tubes (8), and regenerating *Acetabularia* (9). There are also a few older surface potential measurements from which the existence of polar currents in animal eggs can be inferred (10). It is against this background that this investigation of animal–vegetal currents in *Xenopus* oocytes and eggs was begun.

METHODS AND MATERIALS

Sexually mature *Xenopus laevis* females were obtained from the South African Snake Farm, Fish Hoek, South Africa. To obtain oocytes, females were anesthetized by chilling in an ice bath, portions of their ovaries were removed, and the oocytes were then manually dissected from their follicles. In some cases, the remaining follicle cells were removed by a 5-min treatment with Pronase (50 μg per ml in Steinberg's solution).

A vibrating platinum electrode was used to measure extracellular currents. The design, calibration, and operation of this instrument have been reported extensively elsewhere (11, 12). Briefly, a 30-μm-diameter platinum black ball is vibrated between two points in the medium surrounding a cell. If there is a current flowing between those two points, a sinusoidal voltage will be sensed by the probe; this signal is fed into a phase-lock amplifier and the root mean square value of the voltage is displayed on a chart recorder. By moving the probe around the cell, the current pattern produced by it can be mapped. In the present case, current densities as small as 0.1 $\mu A/cm^2$ could be detected.

The oocytes were placed in the measuring chamber and oriented with the aid of a stereomicroscope so that their animal–vegetal axes were parallel to the vibrational plane of the probe. In some cases, the oocytes were held in place by fine gold wires that were cemented to the bottom of the chamber, but usually they were held only by their own weight.

Three different media were used: Ringer's solution which contained 112 mM NaCl, 1.9 mM KCl, 1.0 mM $CaCl_2$, and 0.8 mM $NaHCO_3$ (pH 7.8); 0R2 solution (13) was 82.5 mM NaCl, 2.5 mM KCl, 1.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.0 mM Na_2HPO_4, and 5 mM Hepes (pH 7.8); and Steinberg's solution was 58 mM NaCl, 0.67 mM KCl, 0.3 mM $Ca(NO_3)_2$, 0.8 mM $MgSO_4$, and 5 mM Tris·HCl (pH 7.6). Similar results were obtained in all three media; therefore most measurements were done in Steinberg's solution, because, due to its higher resistivity, a larger signal was produced for a given current density. When the medium in the chamber was to be changed, a double syringe system was used so that the level of the fluid did not change.

Progesterone was prepared as a stock solution (5 mg/ml) in ethanol and was added to oocytes at a final concentration of 1 μg/ml. The appearance of a white spot in the animal hemisphere [indicative of germinal vesicle breakdown (GVBD)] was the criterion for maturation.

Eggs matured *in vivo* were obtained by injecting 800 units of human chorionic gonadatropin (Sigma) into the dorsal lymph sac of females. The eggs were then stripped from the animal 8–10 hr later and were dejellied by treating them with 1% papain and 0.2% cysteine in Steinberg's solution at pH 7.8. All experiments, unless otherwise noted, were carried out in a controlled-temperature room at 20°C.

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Abbreviation: GVBD, germinal vesicle breakdown.

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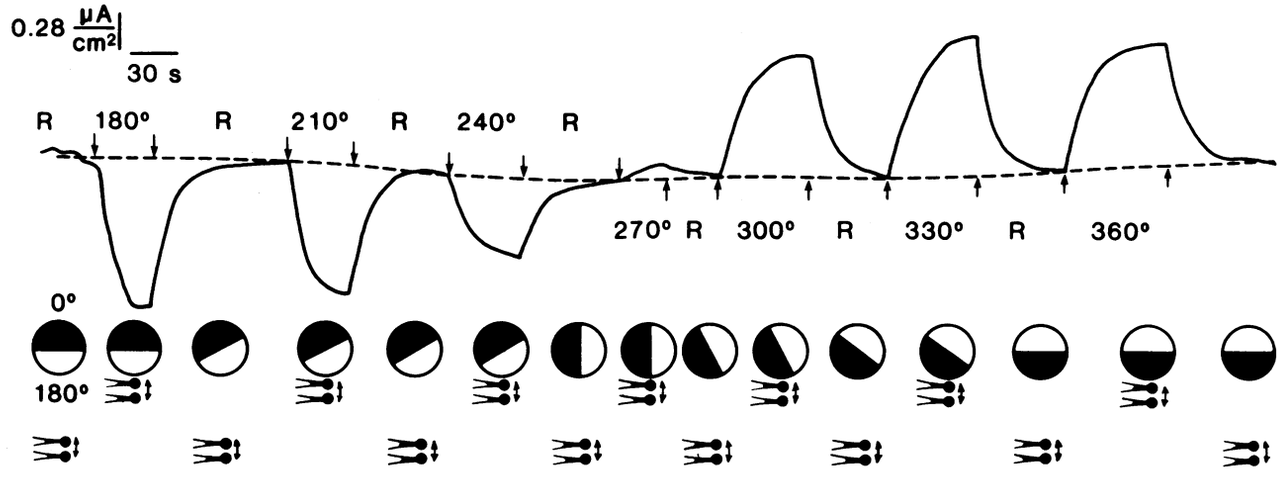

FIG. 1. Mapping of current around a fully grown oocyte. The graph is a tracing of a portion of a chart recording. The position of the vibrating probe is shown schematically. A downward deflection of the trace from the reference level represents current out of the oocyte; an upward deflection is current into the oocyte. The animal pole is taken as 0°; "R" indicates times at which the probe was moved to a reference position far from the oocyte.

RESULTS

Oocyte Animal–Vegetal Currents. When the perimeters of mechanically defolliculated, fully grown oocytes were explored with the probe, it was found that there were substantial currents. This current (taken as the movement of positive charge) entered the animal hemisphere and left the vegetal hemisphere, and had a maximum density of about 1 $\mu A/cm^2$ when mapped at a distance of 40 μm from the surface. Fig. 1 shows a tracing of a portion of a chart recording made during the mapping of one such oocyte. Measurements were taken at 300 intervals around the oocyte, and the complete result for this oocyte is shown in Fig. 2. The measurements shown in Figs. 1 and 2 were made with the center of vibration of the probe 40 μm from the surface. The measured current density was not very sensitive to changes in this distance; as it varied from 30 to 90 μm, the current density fell only by 17%. Therefore, the current density at 40 μm may be taken as a close approximation to the current density at the surface of the oocyte.

The averages of all measurements on fully grown, mechanically defolliculated oocytes that were mapped at three or more positions are given in Table 1. It will be noted that the pattern was not completely symmetrical about the animal–vegetal axis and that the total current entering the oocytes was somewhat larger than the exit current. However, the mappings were done around only one circumference of each oocyte, and each mapping tended to be asymmetrical. Presumably, therefore, the asymmetries in the aggregate are due to the small number of measurements. One cause of the asymmetrical current pattern was the presence of patches of intact sheets of follicle cells. When such patches were encountered, the currents were always greatly reduced near the centers of the patches and increased near the edges. In other cases, however, areas of large currents were found at positions relatively far from the poles that were not associated with follicle cell patches.

Some measurements were done on oocytes that had been treated with Pronase to remove the remaining follicle cells. These oocytes had similar current patterns to untreated oocytes, but the magnitude of the current was only 10–20% of the current of untreated oocytes from the same ovary.

When oocytes in their intact follicles were examined, currents could be detected, but they were less than 10% of the currents measured around defolliculated oocytes. Even here the same pattern was seen: current entered the animal pole and left the vegetal pole.

Effect of Progesterone and Other Maturation Inducers on the Current. When progesterone in ethanol was added to the medium to a concentration of 1 $\mu g/ml$, the oocyte's current always began to decline within a few minutes, as shown in Fig. 3. The addition of ethanol alone to the medium produced no effect on the currents. The current typically fell with a half-time of about 20 min and within 2–3 hr became nearly undetectable. This decline preceded GVBD by 3–6 hr; no currents could be detected during the remainder of the maturation process. By injecting females with gonadatropin, *in vivo* matured eggs were obtained. No currents could be measured around these eggs,

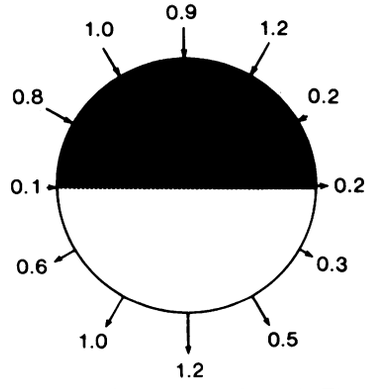

FIG. 2. Summary of the mapping shown in Fig. 1. The lengths of the arrows are proportional to the magnitude of the current ($\mu A/cm^2$).

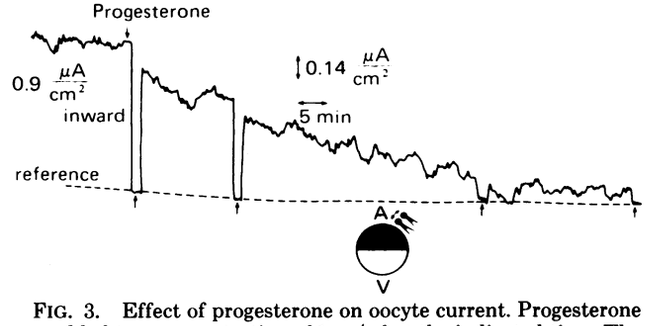

FIG. 3. Effect of progesterone on oocyte current. Progesterone was added to a concentration of 1 $\mu g/ml$ at the indicated time. The current decreased to one-half in about 20 min; this was typical in such experiments. The arrows indicate the points at which reference readings were taken. A, animal pole; V, vegetal pole.

Table 1. Summary of current measurements around fully grown oocytes

	Position						
	AP	30°	60°	90°	120°	150°	VP
Current, $\mu A/cm^2$	0.92 ± 0.12 (13)	0.73 ± 0.12 (9)	0.53 ± 0.19 (8)	0.19 ± 0.20 (9)	−0.34 ± 0.09 (9)	−0.63 ± 0.11 (8)	−0.72 ± 0.15 (7)

The figures shown are the average currents from all mappings in which the current was measured in three or more positions. Positive current is into the oocyte, negative outward. The animal pole (AP) is taken as 0°; the vegetal pole (VP), as 180°. The numbers in parentheses are the number of oocytes included in the averages. ± indicates SEM.

either in Ringer's solution or 5% deBoer's solution (a dilute medium that will support fertilization and development).

Lanthanum is known to be a potent inducer of maturation (14). The addition of 1 mM La^{3+} to the medium caused an immediate, transient increase in current lasting 10–20 sec, followed by a fall in current (Fig. 4). The La^{3+}-induced decline was much more rapid than that caused by progesterone; the times required for the current to decrease by one-half were 1 and 20 min, respectively. One rapid effect of La^{3+} on these cells is to block Ca^{2+} entry; 1 mM La^{3+} causes $^{45}Ca^{2+}$ influx to fall to 20–30% of its original value (C. M. O'Connor and K. R. Robinson, unpublished observation). In view of this, it might be expected that Mn^{2+} would also interfere with the current (15). Table 2 shows that 2 mM Mn^{2+} reduced the current by 50%; 10 mM Mn^{2+} reduced the current to 20% of its original value. It was noticed that oocytes to which Mn^{2+} had been added subsequently underwent GVBD. As shown in Table 3, 10 mM Mn^{2+} is an effective inducer of GVBD although it is slower than progesterone [as is La^{3+} (14)].

The drug Verapamil (D-200), which blocks calcium entry in cardiac muscle (16), also induces maturation in *Xenopus* oocytes (17). The addition of 1 mM Verapamil caused the current to fall to 35% of its initial value (Table 2).

Ionic Components of the Current. These data strongly suggested that calcium was involved in the animal–vegetal currents of *Xenopus* oocytes. However, tracer measurements indicated that Ca^{2+} could not be the main ionic component of the current. Previous measurements of calcium fluxes in these oocytes showed that the total calcium influx was 3 pmol/hr (18). If all of the calcium influx were in the animal hemisphere and the efflux in the vegetal hemisphere, the total current would be about 0.1 nA. However, the total measured current was on the order of 10 nA (calculated by assuming the oocyte to be a sphere of radius 0.06 cm and an average current density of 0.5 $\mu A/cm^2$). Thus it was clear that some other ion(s) was carrying the current, and a systematic effort was made to identify it (them).

When the normal Steinberg's solution in which the oocytes were bathed was replaced by a Na^+-free Steinberg's solution in which choline was substituted for sodium, the current increased by 50–100% (Table 2). This increased current was stable for at least 5 min. The diuretic drug, amiloride, which blocks passive sodium fluxes in amphibian epithelia (19), had no effect on the current at a concentration of 1 mM. It was concluded, then, that Na^+ was not the current-carrying ion, but that Na^+ did indirectly affect the current. The removal of Mg^{2+} had a similar but smaller effect on the current when compared to Na^+-removal: the current increased by 20%.

The effect of K^+-free Steinberg's solution on the current was variable: at one extreme there was a 10% reduction in the current; at the other, the current decreased by 50%. The average effect on three oocytes was a 25% reduction in the current. When the potassium concentration was increased 10-fold, a small (0–20%) increase in current resulted. Ouabain (0.1 mM), which blocks sodium–potassium exchange in a wide variety of cells [including these oocytes (20)] caused the current to decline by 20%.

The removal of Cl^- from the medium (by replacement with methanesulfonate) resulted in an average 50% increase in the current. An example of such an experiment is shown in Fig. 5. The current always returned to its original level when normal Cl^- medium was reintroduced. As will be discussed later, the membrane potential and chloride concentration of these oocytes is such that the net passive movement is outward. Thus, if Cl^- were the major current-carrying ion, the expected effect of reducing external Cl^- would be to increase the current.

In view of the effect of Cl^--removal, the effect of increasing HCO_3^- was explored, both because HCO_3^- is the only other anion present in all three of the media in which currents were measured and also because of the well-known interaction of Cl^- and HCO_3^- in erythrocytes (21). When the HCO_3^- concentration was increased to 2.2 mM (this is approximately a 10-fold increase in HCO_3^- concentration over the concentration of Steinberg's solution at pH 7.8 and equilibrium with atmospheric

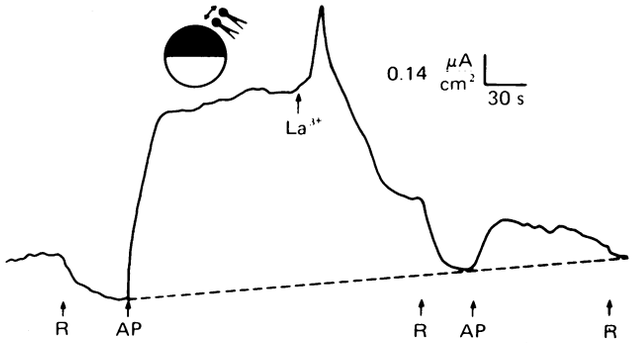

FIG. 4. Effect of La^{3+} on oocyte current. La^{3+} (1 mM) caused a transient (10–20 sec) increase followed by a rapid decline in current. Lanthanum, like progesterone, is a potent inducer of maturation. The arrows marked "R" indicate times at which the probe was moved to a reference position far from the oocyte; the arrows marked "AP" indicate the times at which the probe was brought near the animal pole of the oocyte.

Table 2. Effects of certain ions and drugs on *Xenopus* oocyte currents

Ion or drug	Change to	Effect on current, % of initial value
Na^+	0	200
Mg^{2+}	0	120
La^{3+}*	1 mM	10
Mn^{2+}*	2 mM	50
Verapamil*	1 mM	35
(Ca^{2+})	1 μM	Oscillation
Amiloride	1 mM	No change
K^+	0	75
K^+	10X	100–120
Cl^-	0	150
HCO_3^-*	2.2 mM	50
Ouabain	0.1 mM	80

* Agents that induce maturation.

Table 3. Maturation effect of HCO_3^- and Mn^{2+}

Time, hr	% GVBD by Progesterone	Mn^{2+} (1 mM)	Mn^{2+} (10 mM)	HCO_3^- (2 mM)	HCO_3^- (20 mM)
5	50	0	0	0	0
6		0	0	0	0
8	60	0	10	0	0
9.5	90	20	70	0	0
22		50		10	90

The oocytes were all taken from the same female. Each sample had 25–50 oocytes. The criterion for maturation was the appearance of a white spot in the animal hemisphere. This experiment was done at 22–24°C.

CO_2) the current briefly increased, but by 5 min decreased to about one-half of the original value. Increasing HCO_3^- to 4.4 mM caused another transient increase and a subsequent decrease to 15% of the original current. It was observed that some of the oocytes that were left in the high bicarbonate media underwent GVBD (Table 3).

The effects of HCO_3^- mentioned above were not mediated by changes in extracellular pH. The addition of $NaHCO_3$ to the well-buffered Steinberg's solution increased the extracellular pH by less than 0.1 pH unit. Increasing the pH alone by 0.1 pH unit had no detectable effect on the current; increasing the pH by 0.4 pH units to 8.0 caused a 25% increase in the current.

Relation of the Current to Ovulatory State and Oocyte Size. It has recently been reported that full grown oocytes taken from females that had been recently induced to ovulate with human chorionic gonadotropin matured more rapidly in response to progesterone than oocytes from females that had not been stimulated (22). The time required for 50% GVBD was about 3 hr for oocytes from females stimulated 7–8 days previously compared with 8 hr for females that had not been stimulated previously. When 1.2-mm oocytes from a female that had been injected with human chorionic gonadotropin 8 days previously were examined with the vibrating probe, the maximal current was less than 0.1 $\mu A/cm^2$. However, three oocytes from this same female that ranged in size from 0.75 to 0.85 mm all had maximal currents of 1.0 $\mu A/cm^2$ or larger. Furthermore, the current pattern around these smaller oocytes was the same as that shown in Fig. 2: current entered the animal hemisphere and left the vegetal hemisphere.

One experiment was done on small oocytes from a female that had not recently been stimulated. These oocytes were approximately 500 μm in diameter, and the pigment accumulation in the animal hemisphere could just barely be detected. The currents of three such oocytes were about one-third the magnitude of the currents of 1.2-mm oocytes from the same ovary and had a similar spatial distribution.

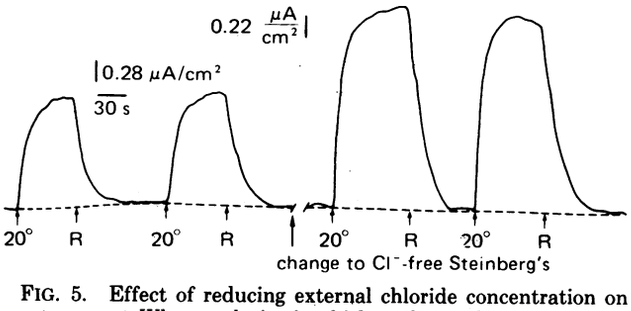

FIG. 5. Effect of reducing external chloride concentration on oocyte current. When a solution in which methanesulfonate had been substituted for Cl^- was introduced, the current increased 50%. Note that the scale is different in the two cases due to the lower conductivity of the methanesulfonate medium. The gap during the change to Cl^--free medium was about 3 min.

DISCUSSION

The data presented here clearly establish the existence of an animal–vegetal current in growing and fully grown *Xenopus* oocytes. The maximal current density 40 μm from the surface of mechanically defolliculated oocytes is about 1 $\mu A/cm^2$. Currents of the same orientation but much smaller were measured around oocytes in intact follicles. It is presumed that most of the current was confined within the epithelium, thus resulting in an attenuated signal outside the follicle. Currents could be measured around oocytes as small as 500 μm, which is as early as the pigment accumulation in the animal hemisphere can be detected. An important matter for additional investigation will be to determine how early the current can be detected; it may be that these current measurements will provide a method for establishing the polarity of small, featureless oocytes.

In response to progesterone, the animal–vegetal current decreases to less than 10% of its original value; the fall-off has a characteristic half-time of about 20 min. *In vivo* matured eggs also had no detectable currents. All other maturation-inducing agents that were tested—La^{3+}, D-200, Mn^{2+}, and HCO_3^-—also reduced the current. Two of these (Mn^{2+} and HCO_3^-) were not known to cause maturation. Several treatments that are known to speed GVBD in response to progesterone also produced reduced currents. These treatments were prior injection of the female with human chlorionic gonadotropin (22), ouabain (20), and Pronase (C. M. O'Connor and K. R. Robinson, unpublished observation).

One can infer that the current is a calcium-controlled chloride one. The evidence supporting this conclusion is as follows. (*i*) Agents that would be expected to reduce Ca^{2+} entry into cells (La^{3+}, D-200, and Mn^{2+}) reduce the current. (*ii*) External sodium is known to compete with calcium for entry into various cells, both plant (23) and animal (24); thus, reducing external sodium increases calcium entry. In the present case, reduced sodium increased the current. Likewise, the removal of external magnesium caused the current to increase. (*iii*) Reducing external chloride increased the current. If chloride were the major current-carrying ion, then the net chloride flux through passive channels would be increased (assuming that the cytoplasmic chloride concentration is greater than the equilibrium value as will be discussed below). (*iv*) The changes in the current in response to changes in external potassium (Table 2) are consistent with the idea of a chloride current. Wallace and Steinhardt (25) have shown that lowering potassium depolarizes and raising potassium hyperpolarizes the membrane by way of an electrogenic pump. Thus, the outward driving force on chloride (and hence the current) would be lowered by reducing potassium and raised by increasing potassium.

The membrane potential of *Xenopus* oocytes that have been dissected from their follicles is known to be between −30 and −70 mV (25, 26). Kusano *et al.* (26) have recently shown that acetylcholine produces a depolarization in the *Xenopus* membrane potential, and they concluded that this was due to the opening of chloride channels. The reversal potential for this effect was −25 mV which would put the internal chloride concentration at about 40 mM. This 40-mM figure agrees well with Morrill's measurement of 43 mM for *Rana pipiens* oocytes (27). Under these circumstances (a membrane potential of −50 mV and a chloride equilibrium potential of −25 mV), the passive efflux of chloride would be 2.5 times as great as the passive influx, and, if the chloride permeability in the animal hemisphere were larger than at the vegetal end, a current in the observed direction would be generated.

The existence of a calcium-controlled chloride permeability is known in other cellular systems. Berridge *et al.* (28) conclude that increased intracellular calcium increased chloride permeability in the blowfly salivary gland. Chloride action potentials in the giant algal cell, *Nitella*, seem to be controlled by calcium (29). However, the most striking parallel is found in the developing embryo of the marine alga, *Pelvetia*. Episodes of current are found to enter the growing rhizoid and chloride is the major current-carrying ion (30). Furthermore, this current is dependent on extracellular ions in a remarkably similar way to the *Xenopus* oocyte current. It was concluded that the *Pelvetia* chloride permeability was calcium dependent.

The effects of HCO_3^- on the current are difficult to interpret. One possibility is that HCO_3^- simply substitutes for chloride and thus reduces the net efflux. However, if that were the case, the initial transient increase in current that was seen remains inexplicable. A second possibility is that the added bicarbonate exerts its effect by increasing the dissolved CO_2 content of the medium, and this CO_2 enters the oocyte and lowers the cytoplasmic pH. It has been recently reported that the acidification of the interior of the squid axon causes a decrease in cytoplasmic free calcium (31). Because calcium seems to be involved in the control of the oocyte current, it may be that raising the external bicarbonate acts via a similar mechanism.

The role that these currents might play in oogenesis and maturation is not clear, but certain inferences may be drawn from these experiments to guide future work. The simplest model is to assume that the current is produced by a larger calcium permeability in the animal hemisphere than in the vegetal hemisphere. This would lead to a calcium gradient within the oocyte, with more calcium at the animal end. This gradient would then cause the chloride permeability to be greater at the animal pole, producing the detectable current. In response to progesterone, the calcium gradient would, by this model, break down, perhaps due to a release of calcium from internal stores. There is some evidence for this; O'Connor *et al.* (18) observed a rapid increase in calcium efflux from *Xenopus* oocytes after progesterone treatment. This model also may explain why agents that block calcium entry (D-200 and La^{3+}) and increase calcium entry (A23187) both can induce maturation. The hypothesized calcium gradient would be disrupted in either case.

Several features of this model are experimentally testable. For example, it should be possible to put 50–100 oocytes in a screen with holes just smaller than the oocytes such that the animal hemispheres are all on one side of the screen. The influx and efflux of various ions could then be measured from the two poles; the model predicts that calcium entry and chloride efflux would be larger at the animal than the vegetal end. This method was successfully used with fucoid eggs (5).

It will be interesting to determine whether these polar currents are a common feature of oogenesis. Preliminary experiments on the oocytes of a fish, the medaka, indicate that they have currents in the same sense and of a similar magnitude as *Xenopus* oocytes. Also like *Xenopus*, the mature medaka egg seems to produce no current (32).

In conclusion, it has been shown that both growing and fully-grown *Xenopus* oocytes produce an animal–vegetal current. This current, which seems to be a calcium-controlled chloride one, fell to nearly zero in response to all maturation-inducing agents that were tested. The role of the current in oogenesis is not yet understood; however, it certainly can provide a reference axis within the growing oocyte for charged entities. How large this intracellular field might be depends on several unknowns, but Jaffe *et al.* (33) estimate that in the *Pelvetia* egg there may be fields as large as 0.1 V/cm. Thus, the current may provide a physical force for segregating components along the animal–vegetal axis, both in the cytoplasm and in the plasma membrane.

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