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Patterns of Ionic Current through *Drosophila* Follicles and Eggs[1]

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Large steady electrical currents traverse *Drosophila* follicles *in vitro* as well as permeabilized eggs. During the period of main follicle growth (stages 9-11), these currents enter the anterior or nurse cell end of the follicles. This inward current acts like a sodium ion influx with some calcium involvement. During the period of chorion formation (stages 12-14), foci of inward current also appear at the posterior, posterodorsal, and anterodorsal regions of follicles *in vitro*. In stage 14, the posterior in current acts like a chloride ion efflux. In preblastoderm eggs substantial currents continue to enter their anterior end; while weaker and less frequent ones enter their posterior end. We present models in which the currents during follicle growth are driven by the plasma membrane of the oocyte nurse cell syncitium; the external currents during choriogenesis are driven by the follicular epithelium; while the currents through the preblastoderm egg are driven by its plasma membrane. Measurements of pole-to-pole resistances and voltages across preblastoderm eggs indicate that the transcellular currents normally maintain a steady extracellular voltage gradient along the perivitelline space, with the anterior pole kept negative by perhaps 4 or 5 mV. The developmental significance of these currents is discussed.

INTRODUCTION

The development of latent pattern in insect oocytes and eggs in general—and in *Drosophila* in particular—seems particularly dependent upon their anterior and posterior poles (Kalthoff, 1979). On the one hand, there is evidence that these two poles are surprisingly similar: Thus *Drosophila* oocytes homozygous for the single gene, *bicaudal* often develop into embryos with "tails" at both ends and no heads (Nüsslein-Volhard, 1977) and even the introduction of a single *Krüppel* gene by the sperm of *Drosophila* can give phenotypes which are similar to *bicaudal* (Nüsslein-Volhard and Wieschaus, 1980); while in another fly, *Smittia*, similar double abdomen embryos can be produced in high yield by very localized damage to the egg's anterior pole (Ripley and Kalthoff, 1983). Moreover, the still syncitial *Drosophila* egg develops similar pockets of extracellular fluid at its anterior and posterior poles and undergoes similar periodic movements of peripheral cytoplasm to and from these same two poles (Fullilove and Jacobson, 1978). On the other hand, the early events at these two poles also differ in major ways: Thus cytoplasm pours in from the nurse cells only at the *Drosophila* oocyte's anterior pole and so-called "border cells" (which form the micropyle) move only to this pole (King, 1970; Mahowald and Kambysellis, 1980); while germ plasm is laid down only at the *Smittia* posterior pole, even in double abdomen forms (Kalthoff, 1979).

What are the physiological mechanisms which underlie early events at the poles of the insect oocyte and egg? There is now good evidence that a steady inward current of calcuim ions is important in establishing the rhizoidal pole of the fucoid egg (Jaffe, 1979); while an intense ionic current favors the entry of negatively charged (as opposed to positively charged) macromolecules from the nurse cells into the anterior end of *Hyalophora cecropia* oocytes (Woodruff and Telfer, 1980). In this paper we report a first study of ionic currents through wild-type *Drosophila* follicles and eggs, as well as some preliminary observations of currents through *dicephalic* and *tudor* mutants.

MATERIALS AND METHODS

Fly Raising and Mutants

Flies were cultured on an agar-cornmeal-yeast-sugar medium with propionic acid and methyl parasept as fungicides. A 12-hr light:12-hr dark cycle was used. Wild-type flies were an Oregon-R strain of *Drosophila melanogaster*. Mutants with an extreme allele of *tudor*, a maternal effect *grandchildless* type mutation in which pole cells are never formed (Wiechaus, personal communication) were obtained from Dr. Wieschaus. A *dicephalic* stock (Lohs-Schardin, 1982) was obtained from Dr. Lohs-Schardin. Wild-type and *dicephalic*

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stocks were maintained at 25°C; *tudor* stocks were kept at 18°C.

Obtaining and Culturing Follicles

Individual follicles were obtained by an abdomen puncture method (Mahowald, 1980) on 2- to 3-day-old flies kept on fresh media. Follicles were staged according to King (1970) as adapted by Mahowald and Kambysellis (1980). Our chief criterion for judging the critical transition from stage 11 to 12 in living follicles was the appearance of chorionic appendages in stage 12. Only one follicle from each female was selected for measurement. They were stuck onto coverslips coated with poly-L-lysine (Type 1B, MW 85,000, Sigma Chemical Co., St. Louis, Mo., 0.01 mg/ml). In a few cases follicles were placed directly onto polystyrene tissue culture dishes (Falcon Plastics, Los Angeles, Calif.) or 1% agar or gelatin gels. Follicles stuck down in all three ways gave similar results.

Measurements and dissections were carried out in Robb's medium without antibiotics (Robb, 1969). In our hands, Robb's medium supported some *in vitro* development in 93% of stage 11 and 12 follicles ($N = 55$) and 40% stage 10 follicles ($N = 55$). Furthermore, 80% of stage 11 and 12 follicles and 15% of stage 10 follicles develop to stage 14 of oogenesis. Over half of these have abnormally formed chorionic appendages. These survival data roughly agree with those reported by Petri *et al.*, 1979 as well as Gutzeit and Koppa, 1982. The major inorganic ions in Robb's are presented in Table 1. It has a pH of 7.1, a specific resistivity of 100 Ω cm, and an osmolality of 296 mOsm. Osmolality was measured with an Advanced Instruments (Needham Heights, Mass) Hi Precision Osmometer 3R.

Obtaining and Preparing Eggs

General procedure. Eggs were collected from 12- to 19-day-old (or for current measurements 3- to 6-day-old) flies as described previously (van der Meer and Jaffe, 1983); dechorionated in 2.5% sodium hypochlorite; and rinsed twice by pipetting them into 200-ml beakers of distilled water and letting them sink to a layer of agar on the bottom of the beaker. Eggs with two large polar pockets were selected and pipetted onto a coverslip siliconized with Prosil-28 (PCR Research Chemicals Inc., Gainesville, Fla.), or onto the bottoms of polystyrene petri dishes. Well-rinsed eggs stick tightly to such hydrophobic surfaces. Most of the extracellular water was then aspirated off. Small pools left around the eggs were then carefully removed with the aid of filter paper triangles under a dissecting microscope. The subsequent procedure depended upon the kind of measurement as shown in Fig. 1. Eggs were covered with well-hydrated halofluorocarbon oil No. 56 (of 56 cSt viscosity) obtained from Halocarbon Products Corporation, Hackensack, New Jersey or Mediflor, a perfluorinated oil obtained from the medical products division of the Minnesota Mining and Manufacturing Company St. Paul, Minnesota.

Permeabilization. To permeabilize dechorionated eggs, a drop of hydrated *n*-heptane was pipetted onto them and left for 15 sec (compare Limbourg and Zalokar, 1973). Without this permeabilization step, any extracellular currents produced by the egg would be restricted to the perivitelline space by the high resistance of the vitelline membrane and could not be detected by the vibrating probe. Most of the heptane was then suctioned off and any remaining left to evaporate under a stream of moist air for 20 sec before covering with oil or media. Heptane presumably acts by dewaxing the vitelline membrane (cf. Beament, 1946; Davies, 1948). In the course of our experiments we discovered that high-ionic-strength media such as 3 *M* KCl will also permeabilize the intact vitelline membrane and will *further* permeabilize the dewaxed one (see Results).

Medium for current measurements. Permeabilized eggs were covered with polar pocket simulant (PPS) in which the current measurements were made. PPS (54 m*M* NaCl, 12 m*M* Na_2SO_4, 35 m*M* K_2SO_4, 4 m*M* $CaCl_2$, 14 m*M* $MgSO_4 \cdot 7\ H_2O$, 2 m*M* NaH_2PO_4, and 1 m*M* Na_2HPO_4, pH adjusted to 6.8 with NaOH; also see Table 1) was formulated on the determined elemental composition of the perivitelline fluid (van der Meer and Jaffe, 1983). It has a specific resistivity of 64 Ω cm. To test the effect of PPS on their survival, batches of permeabilized eggs were placed in PPS for 0, 10, 15, and 20 min. PPS was then suctioned off, with the remainder left to condense under a stream of moist air for 2 min. Eggs were covered with oil, those with fluid discarded, and the rest left at 25°C for 24 hr before scoring for survival. About 70% of permeabilized eggs survive up to 15 min in PPS but there is a sudden decline in survival by 20 min in PPS (see Table 2). All current measurements on permeabilized eggs were made during their first 15 min in PPS.

TABLE 1
MILLIMOLAR CONCENTRATIONS OF THE MAJOR INORGANIC IONS IN ROBB'S AND POLAR POCKET SIMULANT MEDIA

Medium	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	SO_4^{2-}	PO_4^{2-}
Robb's	61	38	2.4	1.2	76	1.2	2.4
PPS	82	70	14	4	62	61	3
Adult hemolymph	106	25	14.4	7.2	58	—	—

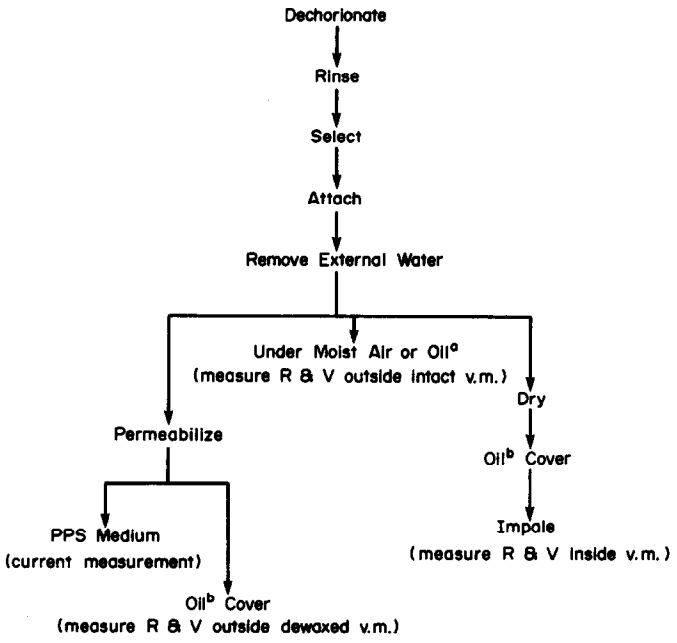

FIG. 1. Protocols for preparing eggs for various measurements. (a) Mediflor; (b) haloflurocarbon oil; v.m.: vitelline membrane; PPS: polar pocket simulant.

Current Measurements

Extracellular currents were measured with a vibrating probe which can reliably and accurately determine the amplitude and direction of the current (Jaffe and Nuccitelli, 1974). Actually the probe functions by sensing voltage drops in the media due to the flow of current. Figure 2a shows the vibrating probe in a measuring position. The pattern of extracellular current around follicles and eggs was mapped by moving the probe to such measuring positions around their surface. Figure 2b shows a representative record of this process. Measurements were made with probes that had platinum black balls with diameters from 16 to 25 μm and a similar range of vibration amplitudes. All measurements were made at 21°C.

Only data from follicles and eggs that had good balances (total in current/total out current less than 2.0 and greater than 0.5) were considered reliable and generally any cases not meeting this criterion were discarded. Among 65 wild-type follicles examined for the pattern of steady current in Robb's medium, the data from 45 met this criterion and are reported in Table 3; while 20 did not and were discarded. Among 63 eggs examined for the pattern of steady current in PPS, the data from 11 met the criterion and are reported in Table 3; while 52 did not and were discarded.

Poor balances were presumably produced by local injury during follicle isolation or egg permeabilization, as well as by patchy permeabilization of some eggs' vitelline membranes. Furthermore, there was an initially unsuspected episode during egg mapping in which a leak from the piezoelectric driver to the probe generated echo currents. About 20 of the egg patterns seem to have been imbalanced in this way before the leak was detected and blocked. In any case, the balance test is an objective and effective culling method. In addition, about 7% of the current patterns were discarded because of visible evidence of follicle injury or the virtual absence of current.

The measured voltage increases linearly with the vibration amplitude (Fig. 3) so that the current is not an artifact due to stimulation by the probe vibration. The decline of current density with increasing distance from a follicle was found to approximate the decline expected from a theoretical point source within the follicle. No current was detected around formaldehyde-fixed follicles, or indeed unpermeabilized eggs. All these artifact checks rule out the possibility that the measured currents are merely reflections from surfaces of current leaking from the probe itself.

Ion substitution and channel blocking experiments. In these experiments, the vibrating probe was left in only one position: either at the anterior pole of stage 10 follicles or the posterior pole of stage 14 follicles that had an inward current at this pole. During measurements, modified media were perfused into the 3-ml chamber using a push-pull two-syringe system: 10 ml of media was drawn through the chamber over approximately 2 min.

In preparing media with ion substitutes or channel blockers, the pH was adjusted to 7.1, osmolality measured and adjusted if necessary to that of normal Robb's, and as far as possible the concentration of only one ion at a time was changed.

100 μM anthracene-9-carboxylic acid (9-Ac) was used as a chloride channel blocker on the basis of reports by Palade and Barchi (1977) as well as Oberleithner *et al.* (1983). The former studied a wide range of aromatic carboxylic acids as inhibitors of anion conductance through the membrane of rat diaphragm muscle cells.

TABLE 2
SURVIVAL OF PERMEABILIZED EGGS IN POLAR POCKET SIMULANT (PPS)

Time in PPS (min)	Number of eggs	% Dead	% Hatched or moving
0	303	20	80
10	109	30	70
15	108	31	69
20	108	54	46

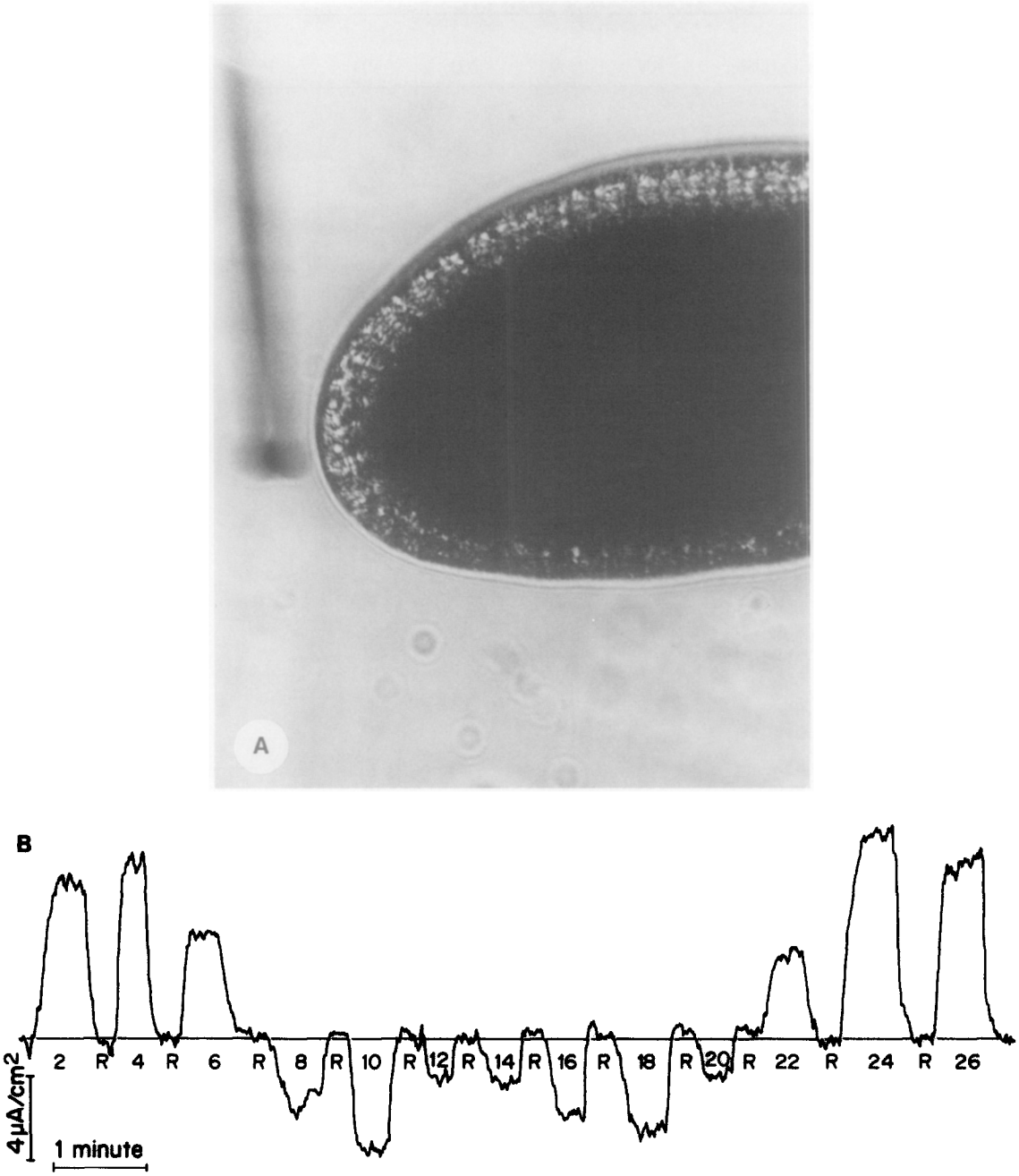

FIG. 2. (A) Vibrating probe in a position to measure current normal to the rear pole of a stage 10 follicle. (B) Record of current densities entering or leaving stage 11 follicle 14. An upward deflection represents inward current. After each measurement, the probe is returned to a reference position (R) 300 μm from the follicle to establish a zero current baseline. Every second measuring position is presented here. A map of the full record is shown in Fig. 4.

9-Ac proved most potent with a K_i of 11 μM and a voltage-independent inhibitory action. The latter studied the inhibition of chloride reabsorption in amphibian kidney and found gross inhibition by 100 μM 9-Ac and no greater effect at 500 μM 9-Ac. Gadolinium ion (50 μM) was used as a calcium channel blocker mainly on the basis of reports by Hambly and dos Remedios (1977) as well as Bourne and Trifaró (1982). The former found that Gd^{3+} is the most potent ion of the lanthanide series in inhibiting skeletal muscle contraction, with 50% inhibition occurring at 6 μM. The latter found that 50 μM Gd^{3+} inhibits $^{45}Ca^{2+}$ uptake induced by depolarization of chromaffin cells by 92%. Gadolinium chloride was obtained from Pfaltz & Bauer Inc. (Stamford, Conn.) and 9-Ac from Aldrich Chemical Co. (Milwaukee, Wisc.).

TABLE 3

FOCI OF FOLLICLE AND EGG CURRENTS

Stage	No.	Orientation	AV	A	AD	PD	P	PV	Balance: in/out	Total out (nA)
7	33	?		4					0.10	
(9	10	?		3					0.43	4.1)
	87	?		2					0.81	0.4
	88	?		1					1.25	0.3
10a	18	Side		21 ——	17				0.58	13.0
	45	Side		13 ——	24				0.66	12.2
	47	Back?		3*					1.90	0.9
	51	Back?		13*					0.91	3.9
	54	Side		30					1.47	9.9
	55	Side		26 ——	62				0.96	14.0
	57	Side		4					1.08	1.3
10b	9	Side		3 ——	7				0.53	6.5
	12	Back		12					0.79	5.6
	44	Side?		18 ——	20				0.70	9.8
	52	Back?		3					1.24	1.2
	56	Side	19	3					0.89	4.1
11	11	Side		2 ——	14				0.57	6.4
	14	Side		14					1.25	3.9
	61	Side		19					0.59	5.3
	65	Side	9 ——	6					1.31	2.5
	77	Side		22					0.97	11.9
	78	Side		35					0.89	10.1
	80	Side		24					0.54	5.9
	81	Side		48					0.65	12.6
12	60	Side		1			7 ——	34	1.68	4.6
	62	Side		7		10			1.00	4.7
	63	Side		3 ——	36				1.52	4.7
	68	Side		4	13		10		1.01	3.1
	70	Side		7	17	6			1.71	4.0
	72	Side		3 ——	16		28		0.80	4.0
12T	*39*	Side		7	15		44		?	?
13	64	Belly		29*					0.57	6.5
	69	Side			5		2		1.08	0.9
	71	Belly		15*					1.11	5.1
	76	Side					30		0.87	4.2
	83	Side		14		16			1.83	3.0
14a	23	Side	16 ——	? ——	11	55 ——	25		0.76	16.8
	25	Side		7	15	11	9		0.92	5.3
	27	Side		5			16		1.33	2.7
	28	Belly		9*					0.70	6.2
	73	Belly		9*			1		0.73	4.3
	85	Side	7		5		4		1.18	2.4
	86	Side			5		18		1.09	2.8
14aT	38	Belly		20					1.4	7.0
	(40	Side		6			6		0.35	8.0)
14b	*75**	Belly		3*			4		1.28	1.2
	(82	Side				417			2.06	21.7)
	84	Side			5		8		0.84	2.5
Eggs: No nuclei seen	16	Side		7					0.57	3.6
	20	Back?		0.5			0.3		0.65	0.2
	21	Side		0.8 ——	0.2				1.11	0.3
	28	Back?		4			0.3		0.77	1.7
	49	Back?		6					0.91	1.6
	57	Back?		3			1		1.17	1.2

TABLE 3 (*Continued*)

Stage	No.	Orientation	AV	A	AD	PD	P	PV	Balance: in/out	Total out (nA)
Eggs: No nuclei	62	Side				0.9 ——	0.7		2.00	0.4
seen	63	Side		8			3		1.88	1.0
Few	23	Back?		4					0.54	0.8
Few	44	Side		6			0.5		1.13	1.2
Many	(25	Back?		20					0.45	3.3)

Note. The currents listed in the main body of the table are peak intensities of inward current (in $\mu A/cm^2$) at foci in anterior (A), posterior (P), anteroventral (AV), anterodorsal (AD), etc. regions. A line between foci indicates marked overlap. For follicles and eggs which lay on their bellies or backs, foci in anterior side locations are listed under anterior (A) with the superscript s. Stage 14b indicates a terminal stage follicle in which the follicular epithelium has peeled off or *was* peeling off (*) the posterior pole. T indicates a *tudor* mutant. Parentheses indicate that the balance criterion was slightly relaxed to provide data not otherwise available. Follicles and eggs were separately numbered. Italicized case numbers are illustrated in Fig. 4.

Measurement of Pole-to-Pole Egg Resistances and Voltages

Across the outside of the vitelline membrane. Measurements of the ultrahigh pole-to-pole resistances across the well-rinsed but otherwise untreated vitelline membrane were made with eggs under moist air or water-saturated Mediflor oil, 30-μm-diameter contact pipets containing 0.008 to 0.8% NaCl plus 0.01% gelatin, Keithley Model 600B or 602 electrometers, and a Model 261 picoammeter source. Erratic meter offset currents and static charges on the leads prevented us from obtaining reliable voltage measurements with this somewhat obsolete equipment.

Measurements across permeabilized eggs were made with 3-μm-diameter micropipets filled with 0.1 *M* KCl or 3 *M* KCl, and a W.P. Instruments, Model 707 microprobe system. Suitable corrections were made for the relativity small resistances and voltages (e.g., about 1 MΩ for the 3 *M* KCl pipets) of the pipets immersed in their own filling solution. For 3 *M* KCl pipets, these corrections might have been underestimated—and thus the resistances across the eggs overestimated—since water may have flowed into the pipet (across the vitelline membrane from the perivitelline space) faster than it evaporated. However, the relatively low variability of these data, as well as their consistency with other estimates of resistance across the egg, argue against this possibility.

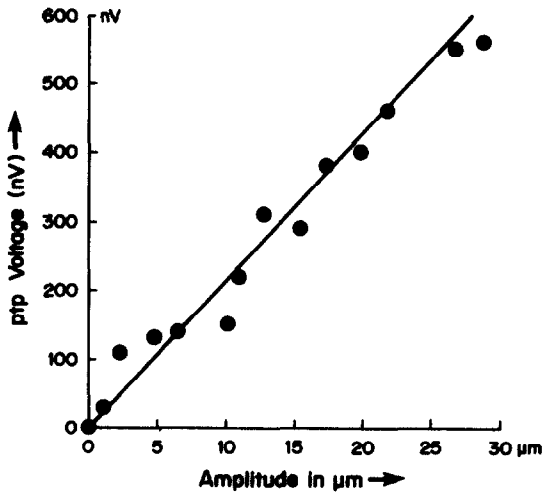

FIG. 3. Peak to peak voltage versus vibration amplitude. Measured with the probe as close as possible to a stage 10A follicle in a lateral position. Its distance from the follicle was smaller than those used in routine current mapping. The data indicate an outward current of 4.2 $\mu A/cm^2$ independent of vibration amplitude.

Inside the vitelline membrane. Eggs were dechorionated, rinsed as usual, selected for polar pockets, and mounted on a double-sided tape holder. At relative humidities above 40%, the turgidity of the eggs was decreased slightly to facilitate their impalement, by drying them over anhydrous $CaCl_2$ for 2–4 min before covering them with oil. Pole-to-pole resistances and voltages within the vitelline membrane were measured by impaling each polar pocket (van der Meer and Jaffe, 1983) with 3 *M* KCl filled glass microelectrodes with tip diameters less than 1 μm. Before and after impalement these same measurements were made in 0.05 *M* KCl. One microelectrode was grounded and the other connected to a microprobe system (W.P. Instruments, Model 707) which can inject constant current and, because of its isolation circuitry, measure the resulting voltage changes. Resistances were calculated from the voltage responses to 1 nA current pulses. Resistances and voltages before impalement generally ran from 15 to 50 MΩ and 0 to 5 mV; afterward, 15–70 mΩ and 0–10 mV. The pole-to-pole resistances and voltages were taken to be the average of the differences between the first impalement value minus the last pretest value *and* the last impalement value minus the first posttest value.

RESULTS AND ANALYSIS

Striking patterns of steady current cross developing *Drosophila* follicles and eggs (Fig. 4 and Table 3). Some features remain fairly constant from early vitellogenic stages through preblastoderm egg stages: First, *inward* currents tend to be more focussed than outward ones. This point is illustrated by the inward currents focussed upon a dorsoanterior region of stage 10A follicle 55; the dorsoanterior *and* posterior ones of stage 12 follicle 72; as well as the anterior pole of egg 49. For this reason we will mainly describe these patterns in terms of their inward rather than their compensating outward currents. Second, throughout the rather long period of development studied, a major focus (or foci) of inward current is almost always—i.e., in 55 out of 58 cases—found in the *anterior* third of the follicle or egg. This point is well illustrated in Fig. 4. Third, while foci of inward current are also frequently found in dorsal and/or posterior regions; they are only rarely found in ventral ones. Only 4 of the 39 cases in which the dorsal and ventral regions could be distinguished showed a clearly ventral focus of inward current.

In order to pursue the analysis beyond these stage-independent features, we would distinguish three main development periods.

1. Main growth period. Almost all of the oocyte's mass accumulates during stages 9–11. In part, this new material enters the oocytes via pinocytosis of hemolymph; in part, it streams in through gross cytoplasmic bridges from the nurse cells.

All of the dozen, relatively reliable, stage 10 cases which were studied showed essentially the same pattern: Current enters all or almost all of the anterior third to half of the follicle and leaves its rear part. The boundary between the anterior cap of inward current and the posterior cap of outward current tends to lie in front of the furrow between the nurse cells and the oocyte, i.e., current tends to leave this furrow. Thus in 17 of 24 sides (12 follicles times 2 sides) the outward current cap extended in front of this furrow; in 6 cases just about to it; while in only 1 of 24 sides did it stop in back of it. These features are illustrated by stage 10A follicle 55 and stage 10B follicle 44. Similar patterns were observed in stage 11 as well as in the small number of stage 9 and 7 cases studied. These are illustrated by cases 11-14 and 9-87 of Fig. 4.

In addition to exploring currents through about two dozen wild-type follicles in vitellogenic stages, we also studied four abnormal ones, from *dicephalic* mutants, which had nurse cells at both poles instead of one. About 1% or less of the follicles from *dicephalic* females express this phenotype. These seem to always form eggs with micropyles at both ends and—in the rare cases where they develop far enough—usually seem to yield double-headed larvae (Lohs-Schardin, 1982). In partial accordance with their bipolar arrangement of nurse cells, two of these four showed substantial inward currents at both poles. Our best guess is that these four corresponded to stage 10 wild-type follicles, and case 10D-92 is shown in Fig. 4. On the average, these *dicephalic* follicles had net (outward) currents of less than a third of those shown by comparable wild-type follicles.

The effects of ion substitutions and of ion channel blockers on the stage 10 in current are summarized in Table 4. Reducing the concentration of Na^+ to a third (actually 61 to 19 m*M*) halves this current; while gross changes in Cl^-, Mg^{2+}, Ca^{2+}, and HCO_3^- have no significant effect. Fig. 5a illustrates the low-sodium effect. These data suggest that Na^+ is a major component of the anterior in current at this stage.

While sharp reduction of external Ca^{2+} (via 1 m*M* EGTA) did *not* affect this current, reasonable concentrations of two calcium ion analogues and channel blockers did: 50 μM Gd^{3+} cut it by about 40% and 1 m*M* La^{3+} by about 25%. This suggests that a Ca^{2+} current may also be involved at the front end of stage 10 follicles.

2a. Choriogenic period: stages 12–14a. At stage 12, the oocyte becomes cut off from the nurse cells and surrounded by a relatively thick and tight epithelium; stops growing in volume; stops accumulating nonyolk protein (Ruddell and Jacobs-Lorena, 1983); and nearly stops streaming (Gutzeit and Koppa, 1982). One thing the follicle does do during this period is to secrete an egg shell or chorion. In accordance with this radical change in structure and function, the patterns of current also change greatly.

Anterior foci of inward current persist; but in the large majority of cases inward currents also appear at the *posterior pole* and/or *posterodorsal* region. Such foci are illustrated by cases 12-72, 13-83, and 14a-86 (as well as the *tudor* mutant 12T-39, discussed below). To be exact, 16 of 21 choriogenic cases showed at least one of these posterior foci, and of the 5 which showed

FIG. 4. Current patterns around representative follicles and eggs. Current enters the blue regions and leaves the orange ones. Each measurement is represented by an arrow drawn to scale. The outlines of the follicles and of the oocytes are taken from photographs but other details are semidiagrammatic. The follicles' and the eggs' front ends point right; where they lie on their sides, their dorsal surfaces point down.

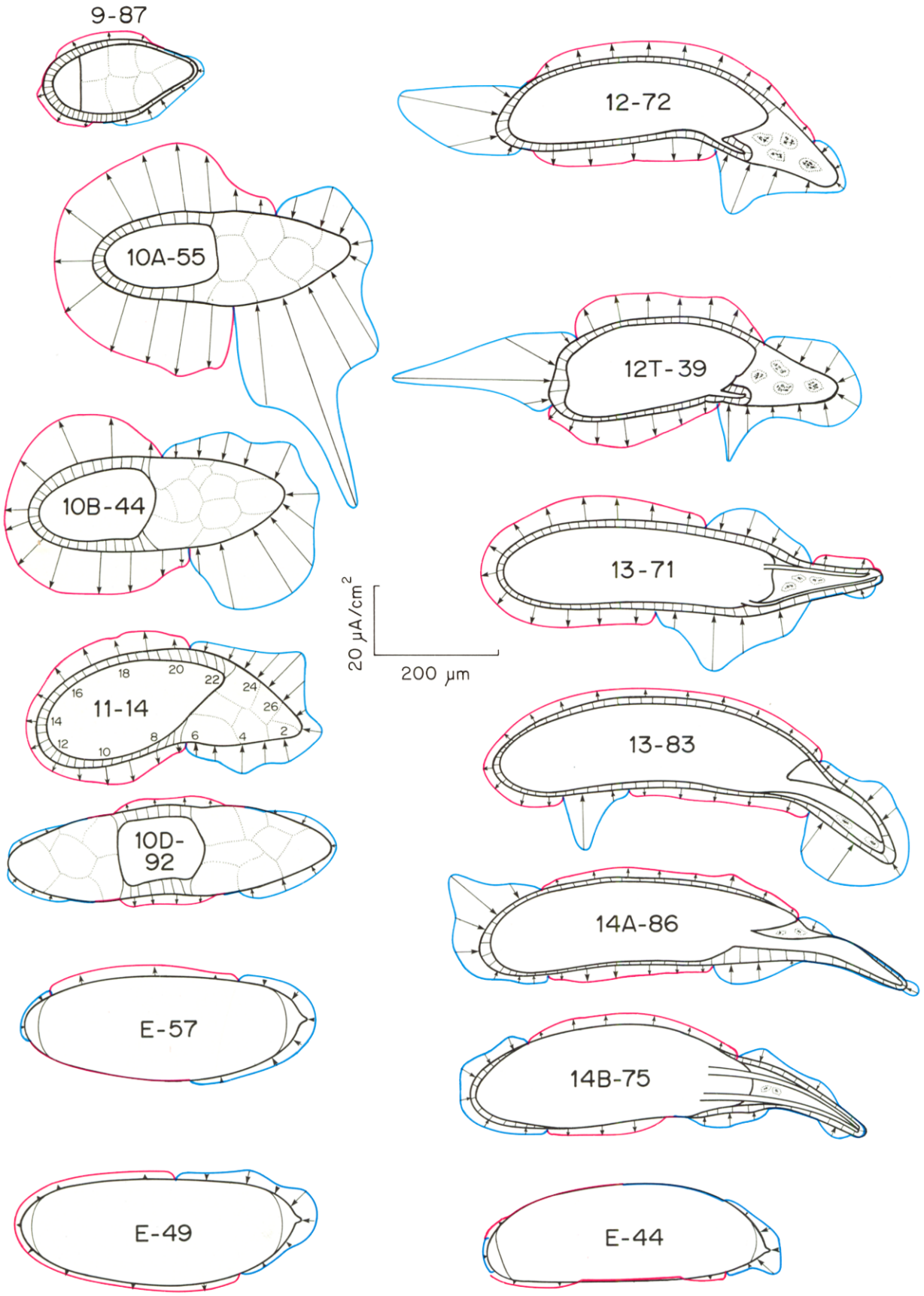
9-87
12-72
10A-55
12T-39
10B-44
13-71
20 μA/cm²
200 μm
11-14
13-83
10D-92
14A-86
E-57
14B-75
E-49
E-44

TABLE 4
EFFECTS OF ION SUBSTITUTIONS AND ION CHANNEL BLOCKERS ON *Drosophila* FOLLICLE POLAR INCURRENTS

Ion changed	Substitute	Percentage of control currents ± SEM[a] Anterior (stage 10)	Posterior (stage 14a)
1/3 Na^+	Tris or choline[b]	49 ± 7 (8)	108 ± 14 (11)
1/5 Cl^-	Isethionate	105 ± 5 (9)	195 ± 9 (10)
Zero Mg^{2+}	Na^+	94 ± 7 (4)	92 ± 8 (9)
Zero Ca^{2+} (1 m*M* EGTA)	Mg^{2+} [c]	110 ± 16 (5)	107 ± 6 (3)
15 × HCO_3^-	*d*	106 ± 4 (6)	118 ± 2 (5)
Ion blocked	Inhibitor		
Cl^-	9-AC[e] (10^{-4} *M*)	102 ± 12 (5)	68 ± 8 (6)
Ca^{2+}	Gd^{+3} (5×10^{-5} *M*)	59 ± 11 (6)	92 ± 11 (6)
Ca^{2+}	La^{+3} (10^{-3} *M*)	76 ± 11 (7)	84 ± 6 (4)

[a] Steady current level reached in test medium was divided by average of currents in control medium as measured before and after test medium. (Parentheses indicate number of cases studied.)
[b] For Tris anterior, 49 ± 11 (5); choline anterior, 49 ± 7 (3); Tris posterior, 130 ± 29 (5); choline posterior 89 ± 10 (6).
[c] 1 m*M* EGTA added.
[d] Mix Robb's with enough isotonic $NaHCO_3$ to reach 3 m*M* HCO_3^-. Gas exchange minimized. No pH change in 15 min.
[e] Anthracene 9-carboxylic acid.

neither, 4 were among those which happened to lie on their bellies rather than their sides. In this orientation, a posterodorsal current would be missed; so only 1 of 21 cases clearly lacked *both* posterior foci.

Furthermore, foci of inward current appeared in the *anterodorsal* regions of most of these choriogenic cases which lay on their sides (where such foci would be accessible). To be exact, 9 of the 14 such cases showed such foci. Examples are shown by numbers 12-72 and 14a-86 of Fig. 4 (as well as *tudor* case 12T-39, see below). They show some resemblance to the dorsal shift of the anterior cap often seen in the main growth period, as in 10A-55 and 10B-44 of Fig. 4. However, they are more isolated and—more important—lie over the anterodorsal part of the oocyte rather than the nurse cell cap.

In addition to exploring currents through 18 wild-type follicles in these choriogenic stages, we also studied a few follicles from *tudor* mutants, an extreme *grandchildless* type mutant which fails to form pole cells. Their patterns of current exhibited no clear differences from those shown by wild-type follicles. In particular, both *tudor* cases which happened to lay on (or nearly on) their sides showed clear foci of inward current at their posterior poles In Fig. 4, *tudor* case 12T-39 is shown and may be compared with wild-type case 12-72.

Finally, Table 4 summarizes the effects of ion substitutions and of ion channel blockers on the stage 14 posterior pole in current. Unlike the (stage 10) anterior in-current, it behaves as though much or all of it consists of Cl^- rather than Na^+: Thus lowering the external concentration of Cl^- to one-fifth (actually 76 to 17 m*M*) doubles this current. (See Fig. 5b for a record of this phenomenon.) Moreover, a reasonable concentration (i.e., 100 μM) of the chloride channel blocker, 9-Ac cuts it by a third. Also, in contrast to the stage 10 anterior in-current, the late posterior in-current shows no evidence of a calcium component: Not only is 1 m*M* EGTA without effect, but so is 50 μM Gd^{3+}.

2b. Terminal follicle: stage 14b. By this we mean stage 14 follicles in which the posterior follicular epithelium has visibly peeled off. Three such cases were studied and two proved similar to choriogenic stages, i.e., they had foci of inward current at both their posterior poles and their anterior dorsal regions. Case 14b-75 is shown in Fig. 4. A third, anomalous case, No. 82 only showed one focus of inward current: an extraordinarily intense one in its posterodorsal region.

3. Preblastoderm eggs. In these stages, the vitelline membrane was dewaxed with heptane, so as to allow the currents to pass through the medium for measurement. Normally, of course, such currents would be restricted to the perivitelline space, i.e., the space between the vitelline and the plasma membranes of the egg.

Eleven such eggs—8 before obvious nuclear multiplication and 3 afterward—yielded relatively reliable data. Of these 11, 10 showed foci of inward current at or near their anterior poles while 5 showed weaker foci at or near their posterior poles. These features are illustrated by cases E-44, E-49, and E-57 shown in Fig. 4. As Table 4 shows, the net currents measured through these eggs were only about 1 to 2 nA as opposed to 5 to 10 nA for follicles.

This raises the question of whether heptane sufficiently permeabilizes the eggs. How much of the egg current in heptane-permeabilized eggs is still confined to the perivitelline space? It also raised another—really quite important—question. Do egg currents, in their normal flow along this extracellular space, generate significant steady voltage gradients along it? In order to answer these questions, we tried to measure the resistances and voltages from egg pole to egg pole across the *outside* of the vitelline membrane (R_o, V_o) as well as from polar pocket to polar pocket, i.e., from

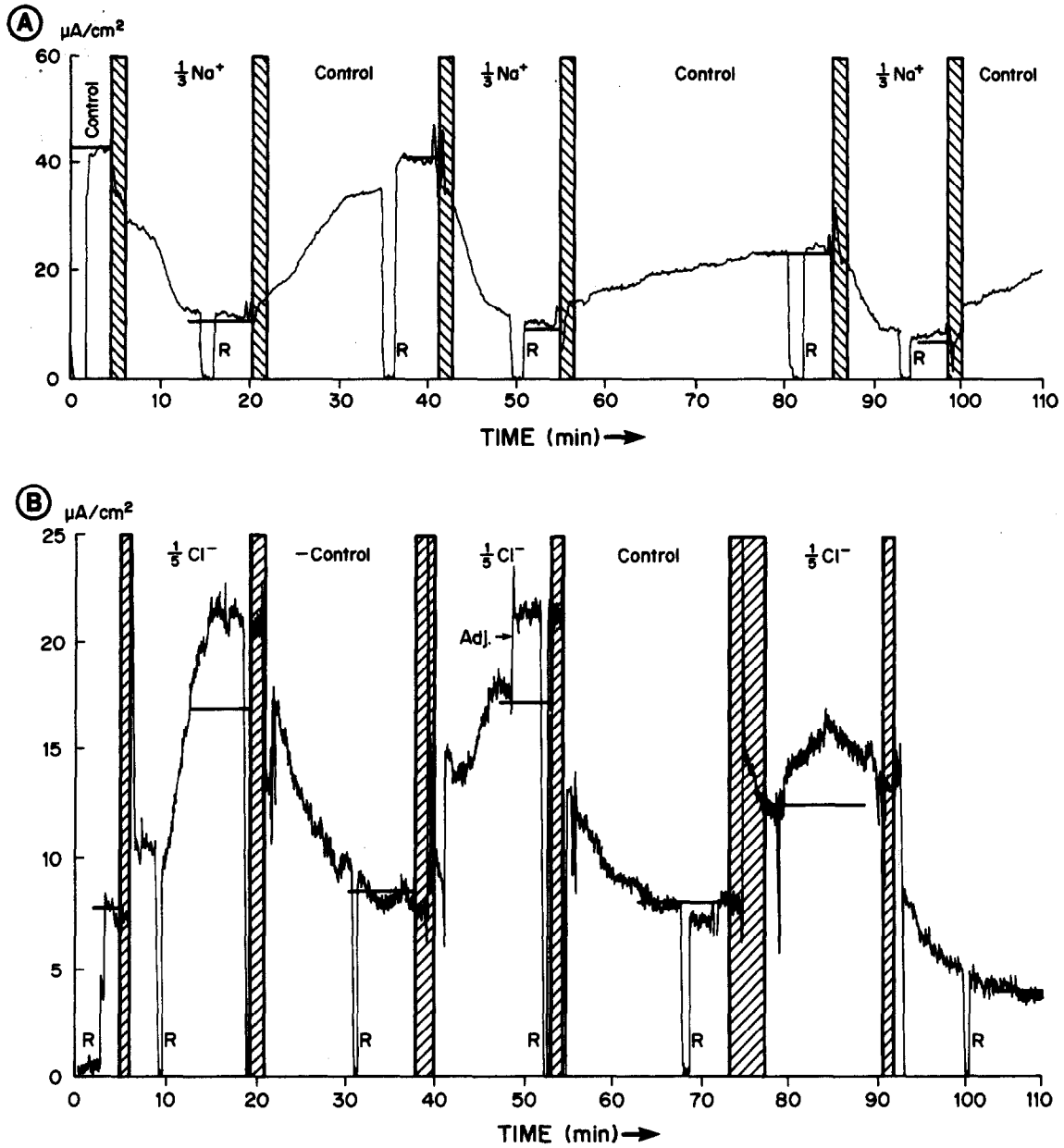

FIG. 5. Records showing the effects of ion substitutions on inward current densities at the poles of follicles. (A) Effect of reducing Na^+ to one-third on the anterior pole current. (Stage 10 follicle with Tris substitution.) (B) Effect of reducing Cl^- to one-fifth on the posterior pole current. (Stage 14 follicle with isethionate substitution.) Cross-hatching shows periods of media change. Horizontal bars represent steady currents after correction for the different conductivities of the substituted media. R indicates reference position. (Adj indicates an adjustment of the probe's position.)

pole to pole *inside* the perivitelline space (R_i, V_i). See Fig. 6. The results are shown in Table 5 and Fig. 7.

We first measured R_o in eggs that were dechorionated, rinsed with deionized water, gently dried with filter paper triangles but otherwise undisturbed. These resistances proved to be remarkably high as long as the egg-laying flies or "mothers" were old enough (i.e., ≥11 days old) and the contact solutions dilute enough (i.e., ≤0.8% or 140 m*M* NaCl). Under these conditions, the large majority of external resistances lay between 10 and 100 GΩ. Since the contact electrodes in these experiments were about 30 μm in diameter, this indicates a specific vitelline membrane resistance of 10^5 to 10^6 Ω cm^2.

However, for younger (i.e., 9- to 10-day old) mothers, most of these same resistances proved to be about a thousand times smaller. Furthermore we found that if such eggs are treated with hypertonic NaCl or KCl solutions, that their polar pockets usually swell—and swell grossly—within minutes. It appeared that this

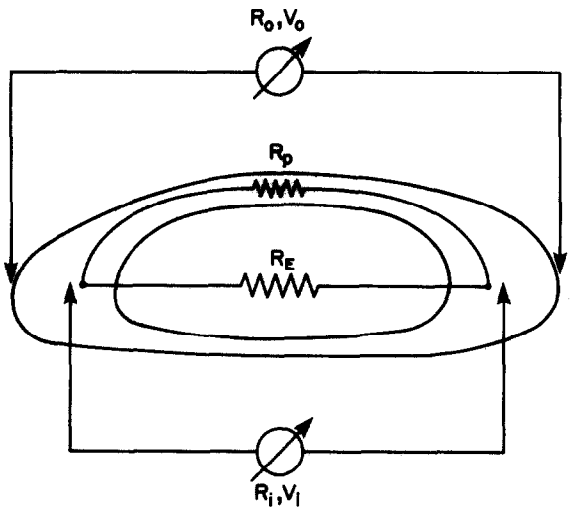

FIG. 6. Symbols for various egg resistances and voltages. See text.

swelling of the polar pockets occurs without any expansion of the vitelline membrane; so it implies a flow of water out of the egg into these pockets. *We infer that when hypertonic, the salt permeabilizes the vitelline membrane to its own entry through this membrane;* hence it enters the polar pockets and then sucks water out of the eggs osmotically. This process tends to start and to go faster in the anterior than the posterior pocket; but it is not simply occurring through the micropyle; for a similar, if somewhat slower shrinkage of both pockets is seen when 1 *M* KCl is applied to only the posterior halves of eggs. This self-permeabilization seems to require a rise in ionic strength rather than (or at least in addition to) a rise in osmotic strength; since even 8 *M* sucrose never induces swelling of the perivitelline space. Nor is such swelling seen in 1 *M* glycerol; indeed eggs develop at normal rates—i.e., to the stage of moving embryos or hatched larvae after a day at 24°C—in 1 *M* glycerol; and only about half of them fail to reach this point even in 2 *M* glycerol. Another curious point is that permeabilization by high-ionic-strength media—as opposed to permeabilization by wax solvents such as heptane—does *not* allow rhodamine B to enter. We did not directly study the change in the vitelline membrane's electrical resistance produced by hypertonic media. However, using the observed swelling rates, we made calculations which suggest that such media reduce its resistivity to the order of 100 Ω cm^2 from the usual 10^5 to 10^6 Ω cm^2.

We were completely unable to reliably measure voltages across ultrahigh resistance eggs, but had some success with eggs that were permeabilized with heptane. To intelligently present these voltage results we must first consider the resistance data in Table 5. There were always some eggs which resisted permeabilization (Limbourg and Zalokar, 1973) and maintained ultrahigh resistances. No attempt was made to accurately measure their resistance or voltage and they will be excluded from this discussion. The external resistances, R_o across heptane-permeabilized eggs contacted with 3-μm pipets bearing 0.1 *M* KCl averaged 260 ± 140 MΩ (SD; N = 8). This indicates a specific resistance of the heptane-treated vitelline membrane of about 10 Ω cm^2, and thus a specific resistance about a million times smaller than that of the untreated eggs. However, the calculated specific resistance of a layer of the same thickness as the vitelline membrane—i.e., 50 nm—and of the same conductivity as the contact fluid would be only about 1 mΩ cm^2 and thus another 10,000 times smaller. *Thus heptane-treated membranes are far from completely permeabilized.* When the same kinds of eggs are contacted with 3-μm micropipets bearing 3 *M* KCl instead of 0.1 *M* KCl, the population of the resultant resistances is distributed as in Fig. 7. This population seems to fall into two nearly discrete groups: A small high-resistance group which averages 19 ± 7 MΩ (SD; N = 9) and a large (and normally distributed) low-resistance group which averages 4.2 ± 1.7 MΩ (SD; N = 53).

Resistance falls from 260 ± 140 MΩ with 0.1 *M* KCl contacts to 19 ± 7 MΩ with 3 *M* KCl contacts in the high-resistance group. This fall could be accounted for simply by assuming that a more conductive solution permeates a vitelline membrane of about the same porosity. However, the reduction to 4.2 ± 1.7 MΩ in the low-resistance group cannot. For 4.2 ± 1.7 MΩ is comparable to the resistance, R_i of 6.5 ± 7.5 MΩ measured *inside* the polar pockets of a similar group[4] of low-resistance eggs. This indicates that the resistance across the two patches of vitelline membrane which were touched by the 3 *M* KCl electrodes was reduced to a level much lower than R_i. Hence it was reduced to a level so low—to ≤1 MΩ from 260 MΩ—as to imply that *3 M KCl not only permeabilizes untreated vitelline membranes (see above paragraph) but also further permeabilizes heptane-treated vitelline membranes.* This inference should not be surprising since heptane presumably acts by dissolving *wax* (cf. Beament, 1946; Davies, 1948) while high-ionic-strength media would be expected to act by weakening salt bridges within or between *protein* molecules. It was also supported by our observation that the polar pockets sometimes swelled after a few minutes of contact by the 3 *M* KCl

[4] The high resistance group of R_i measurements obviously cannot be attributed to imperfect permeabilization of the vitelline membrane. So it is only formally similar to the high resistance group of R_o measurements. Perhaps this group resulted from reversible clogging of the micropipets in some cases. This would fit with the higher V_i for this group, as higher tip potentials are associated with clogging.

TABLE 5
POLE-TO-POLE RESISTANCES AND VOLTAGES ACROSS DECHORIONATED, PREBLASTODERM *Drosophila* EGGS[a]

	R (MΩ)	V (mV)
1. Outside vitelline membrane		
(a) Untreated, with 30 μm φ contacts	10^4 to 10^5	—
(b) Heptane treated, with		
(i) 3 μm φ; 0.1 *M* KCl contacts	260 ± 140 (*N* = 8)	−5.6 ± 8.1 (*N* = 5)
(ii) 3 μm φ; 3 *M* KCl contacts	*4.2* ± 1.7 (*N* = 53) and	−1.4 ± 2.6 (*N* = 53)
	19 ± 7 (*N* = 9)	*−4.5* ± 0.4 (*N* = 9)
2. Inside vitelline membrane		
<1 μm φ; 3 *M* KCl	6.5 ± 7.5 (*N* = 89) and	−0.9 ± 5.5 (*N* = 33)
	48 ± 42 (*N* = 14)	−2.9 ± 14 (*N* = 14)

[a] Negative voltage values indicate that the anterior egg pole has a lower external voltage than the posterior one. Variabilities listed are standard deviations of the individual values. The italicized figures are considered relatively reliable because of their low variability. φ indicates diameter.

electrodes *during* these experiments: Such swelling was noted in about a third of the low-resistance eggs and in only one of the high-resistance ones.

Altogether, we would infer that the resistance from polar pocket to polar pocket is about 5 MΩ. *This value would approximate the perivitelline resistance, R_p, i.e., the resistance from pocket to pocket along the perivitelline space* if it is smaller than the resistance across the egg itself, R_E, i.e., the resistance across the oolemmal caps under the two pockets. Two considerations suggest that this is correct. First, Miyazaki and Hagiwara (1976) reported that the total resistance across the whole oolemma—from cytosol to medium—is 2.3 ± 1.3 MΩ in comparable early stages. It must be borne in mind that this value may overestimate the oolemma's net resistance since the vitelline membranes on their eggs were not permeabilized—only punctured. Nevertheless, a simple calculation based upon their figure and the assumption of uniform resistivity over the oolemma yields a figure of about 30 MΩ for the two oolemmal caps in series, and thus a resistance across the egg itself (R_E) which is six times higher than the pocket-to-pocket resistance (R_i). Second, one can estimate the resistance of the perivitelline space from its dimensions. Scanning electron micrographs of Turner and Mahowald (1976) suggest an effective thickness of the order of 0.1 to 1 μm for this space. Use of these values plus a resistivity of the order of 100 Ω cm yields a perivitelline resistance of the order of 1 to 10 MΩ.

Pole-to-pole *voltages* were measured in all of these more or less permeabilized or invaded eggs and the results are shown in the second column of Table 5. With one striking exception, these voltage data proved to be extremely variable. However, all of the nine eggs which were contacted via 3 *M* KCl but retained a relatively high resistance—*all* of these eggs gave pole-to-pole voltages between −4 and −5 mV and thus an average pole-to-pole voltage of −4.5 ± 0.4 mV (SD, *N* = 9, anterior negative).[5] On the one hand, this low variability cannot be fully attributed to chance; on the other hand, it seems to find a complex of reasonable specific explanations, such as a lack of salt damage in these eggs. So we would consider it to be the most reliable group of our voltage measurements.

Finally we may return to the question of whether heptane sufficiently permeabilized the eggs' vitelline membrane to allow full current escape. This may be answered by comparing the pole-to-pole resistance *along* the *perivitelline* space R_p with the pole-to-pole resistance through the vitelline membrane in the medium used in the current measurements. To the extent that R_i is shunted by the egg itself, R_p will exceed R_i, so the measured 4 MΩ value of R_i may be taken as a lower limit—and thus for these purposes, a conservative estimate— of R_i. The resistance encountered by escaping *current*, R_o can be estimated from the specific resistance of the vitelline membrane when measured in a medium of comparable, relatively low ionic strength, namely 10 Ω cm² through patches touched by 0.1 *M* KCl, *divided by* the area of the vitelline membrane over one pole, i.e., about $(200\ \mu m)^2 = 4 \times 10^{-4}$ cm², *times* 2 for two such polar caps. This gives an estimated average value of 0.5 MΩ for R_c. When compared to the 4 MΩ estimate for R_i, this value suggests that on the *average* the heptane-treated eggs were sufficiently permeabilized but that *some* eggs may have had significant current confined within their vitelline membranes.

DISCUSSION

We find that large currents traverse *Drosophila* follicles and eggs. They are large in terms of peak

[5] One might wonder if this value might be produced or at least affected by a difference in the boundary potentials between the polar pocket fluid and the vitelline membrane at the two poles. Consideration suggests that such a boundary potential difference would have the opposite sign to the observed one and thus tend to mask its true size if it had any effect. We reason that the net surface charges on the vitelline membrane are very likely to be electronegative since—to our knowledge—all known biological surfaces are electronegative at natural pHs (Ambrose, 1965; Mehrishi, 1972). Hence it should be more or less cation selective. The data of van der Meer and Jaffe indicate that the main monovalent cations, Na^+ and K^+, may be about 5% more concentrated in the anterior pocket while the main divalent ones, Mg^{2+} and Ca^{2+} may be about 10% more concentrated there. Given a *perfectly* cation-selective membrane, such differences would generate boundary potentials making the outside of the front pole 1.2 mV *positive* to the rear pole.

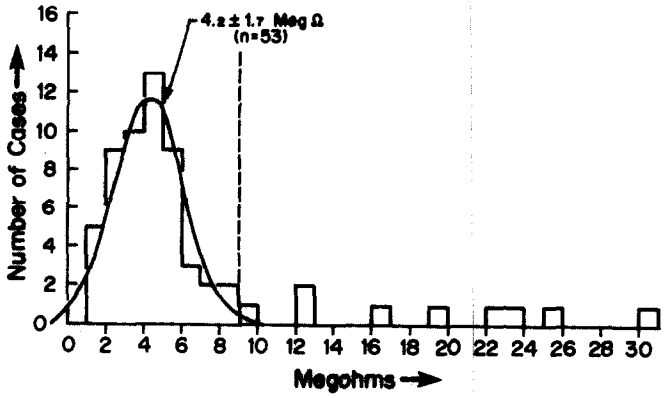

FIG. 7. Histogram of resistances measured across the outside of preblastoderm *Drosophila* eggs. These were the minimum resistances registered with heptane permeabilized eggs and 3 μm, 3 *M* KCl electrodes during successful measurements of pole-to-pole voltages. The population of resistance values seems to fall into two classes: One, a low-resistance class which roughly fits a Gaussian curve with a mean of 4.2 ± 1.7 MΩ; the other, a high-resistance class with a mean of 19 ± 7 MΩ.

inward current densities. Perusal of Table 3 shows that these generally lay between 3 and 30 μA/cm^2. This is large by the standards of other known developmental currents (Jaffe 1979, 1981)—peak current densities through fucoid eggs, for example, are only about 1 μA/cm^2—and even by the standards of adult epithelia. They are also fairly large in terms of ion turnover times. In Table 6 we have converted currents from electrical to chemical units and thus calculated net current per system volume in terms of millimolar (univalent) ion concentration change per 100 sec. From stages 10 to 14 these prove to be about 1 m*M*/100 sec. Except for K^+, concentrations of the major ions (i.e., Na^+, Cl^-, Mg^{2+} and Ca^{2+}) in the cytosol of *Drosophila* follicles and eggs are likely to be of the order of 1 to 10 m*M*. So to the extent that stage 10 to 14 currents consist of these non-potassium ions—and our results indicate major Na^+ and Cl^- components—to that extent some specific ion turnover times will be of the order of only 20 min or less. Beyond these general observations we will consider the three developmental periods separately, and finally the question of follicle cell migrations.

1. Main growth period. Currents with peak intensities of about 3 to 30 μA/cm^2 enter the anterior, nurse cell cap of stages 10 and 11 follicles. Although the data is limited, peak currents of about 1 to 3 μA/cm^2 also seem to enter this region of stage 7-9 follicles *in vitro*. Thus essentially the same pattern of current traverses *Drosophila* follicles throughout the main period of oocyte growth from stages 8 to 11. The pattern and magnitude of these inward currents, as well as the structure of the follicle, resemble those found during comparable stages in cecropia (Jaffe and Woodruff, 1979). These similarities suggest a similar model of internal current flow. In particular, it suggests that *Drosophila*, like *H. cecropia* maintains an intense inner loop of current which returns through the broad bridges between the nurse cells and oocyte. This model is shown in Fig. 8A.

We should note that Woodruff *et al.* have recently suggested another model of the current pattern through vitellogenic cecropia follicles. In this model, current flows *out* of the outer surfaces of the nurse cells (!) and is then held within (and returns to the oocyte along) the space between them and the overlying follicular epithelium. The data behind this new model seem quite weak to us. Moreover, this nurse cap epithelium becomes extremely attenuated during vitellogenesis in *Drosophila* (if not in *H. cecropia*), thinning down to only 0.05 μm in spots (King and Devine, 1958; King and Koch, 1963). So it may not even be grossly continuous, let alone tight enough to hold current in *Drosophila*.

In *H. cecropia*, this inner loop generates a field strong enough to block the movement of electropositive molecules into the oocyte from the nurse cell (Woodruff and Telfer, 1980). A similar field effect in *Drosophila* could help establish a head favoring factor at the front of its oocyte. Furthermore, most of the current entering the *Drosophila* nurse cells in stage 10 seems to consist of sodium ions. Since the nurse cells have about half the follicle's volume at this stage, Na^+ is entering them at more than 1 m*M*/100 sec during this stage (Table 6). Stage 10 is thought to last about 5 hr or 2×10^3 sec at 25°C *in vivo* (King, 1970). If the sodium were not pumped out, it would therefore reach the impossible level of 200 m*M* within the nurse cells. We do not know what happens to all of that sodium; but a specific sodium effect might also help establish a head favoring factor in the oocyte.

2. Choriogenic period. In stages 12-14A, large new foci of inward current appear at the posterior, posterodorsal, and anterodorsal regions of the follicle. What lies beneath this striking change? We think that the source of external current loops changes: Before the transition they are driven by the plasmalemma of the

TABLE 6
AVERAGE TOTAL CURRENTS AND CURRENTS PER SYSTEM VOLUME THROUGH *Drosophila* FOLLICLES AND PREBLASTODERM EGGS

Stage	10a	10b	11	12	13	14	Egg
Total current (nA)	9.2	7.2	9.0	5.8	5.5	6.8	1.8
Current per volume (m*M*/100 sec)	1.2	0.9	1.1	0.8	0.8	0.8	0.2

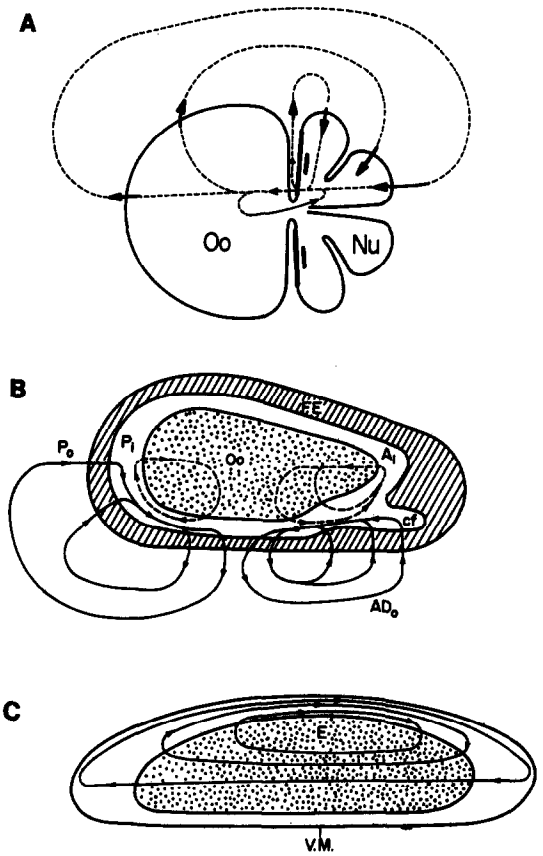

FIG. 8. Models of current patterns through *Drosophila* follicles and eggs. (A) During the period of most follicle growth: stages 9–11. Oo, oocyte; Nu, nurse cells. Battery symbol indicates the hypothetical main ion pump; dark arrows indicate currents that were measured directly with the vibrating probe; light arrow, ones inferred from intracellular measurements of voltages and resistances in cecropia follicles. [Modified from Jaffe and Woodruff, 1979, Fig. 6.] Note that for simplicity, external currents are shown entering or leaving the oocyte nurse cell syncitium directly; actually, some of them may well traverse cells of the follicular epithelium en route to (or from) this syncitium. (B) During chorion formation: stages 12–14. Four loops are shown: The posterior outer loop (P_o) and anterodorsal outer loops (AD_o) are driven by the follicular epithelium, FE. The posterior inner loop (P_i) and anterior inner loop (A_i) are driven by the plasmalemma of the oocyte, Oo. The thickness of the subepithelial space (which contains the growing chorion) is greatly exaggerated. Its projection, cf, represents a growing chorionic filament. (C) In the preblastoderm egg (E), current is driven through the (greatly exaggerated) space beneath the vitelline membrane, V.M. For simplicity, only the anterior in-current is shown.

oocyte-nurse cell syncitium, as shown in Fig. 8A; afterward, by the follicular epithelium as shown in Fig. 8B. We think that current loops driven by the oolemma continue during choriogenesis, but are held within the follicle by its tightened epithelium. So *now* they are hidden, inner loops. This too is shown in Fig. 8B.

Several observations suggest such a change in the source: First, by stage 12, the whole oocyte is surrounded by a relatively thick epithelium (King, 1970). Second, Rubenstein has shown that in comparable stages of cecropia that the follicular epithelium develops an occlusion zone near its inner surface (Rubenstin, 1979). This zone blocks the access of horseradish peroxidase to the oocyte's surface in living follicles and of lanthanum nitrate to this surface in fixed preparations. It seems to be maintained by tight junctions comparable in ultrastructure to those of vertebrate epithelia.[6] Third, the gap junctions which join the oocyte and the epithelium are severed before choriogenesis (Mahowald, 1972). Fourth, our observation that the posterior pole incurrent at stage 14 acts like a chloride outcurrent fits an epithelial source better than a plasmalemmal one; since animal epithelia commonly drive large anion currents (Frizzel *et al.*, 1981; Zadunaisky, 1982) while they seem rare in other animal cells. A good example of an anion current driven by an insect epithelium is the one which accompanies—and, indeed, seems to drive—the resorption of moulting fluid by the integument's epithelium in cecropia (Jungreis, 1979). This epithelium drives an anionic short circuit current of about 10 $\mu A/cm^2$ for hours *in vitro*.

We think that hidden currents flow in through the anterior oolemma throughout choriogenesis because comparable currents can be inferred to flow in there before choriogenesis (Fig. 8A) and are directly observed to flow in there after choriogenesis (Figs. 4 and 8C). We think that hidden currents flow in through the posterior oolemma during the latter part of choriogenesis because currents are seen to flow in there, in terminal stage oocytes, right after the posterior epithelium sloughs off (Table 3; follicle 14b, Fig. 4) as well as in many preblastoderm eggs.

Three of the four main foci of inward current during choriogenesis can be associated with regions of relatively intense chorion secretion. The posterior in-current can be associated with formation of a thickened cap at the posterior pole of the chorion; the anterodorsal and anterior in-currents with the formation of the chorionic filaments and the operculum, respectively (King, 1970, p. 167; Mahowald and Kambysellis, 1980, p. 195; Margaritis *et al.*, 1980). No thickening of the chorion seems to be associated with the fourth, posterodorsal, focus of inward current. Nevertheless, we would suggest that the inward currents during choriogenesis are in, in part, serving choriogenesis.

Specifically, we would venture the suggestion that they serve to resorb water from the newly secreted chorionic materials and thus concentrate them. We

[6] A comparable search for an occlusion zone has not been made in *Drosophila;* but the available ultrastructural evidence seems compatible with one (see Mahowald, 1972).

make this suggestion because epithelial anion currents generally drive net salt currents and hence water currents: cations follow anions driven electrically; then water follows salt driven osmotically. For the same reason, we would suggest that they also serve to dehydrate mature ovarian oocytes (Mahowald *et al.*, 1983).

The dimensions as well as the contents of the subepithelial space during choriogenesis suggest that it has enough lateral resistance to develop significant steady voltage gradients along it. Moreover, our discussion of anion currents suggests that it also develops significant osmotic gradients along it. This combination of forces could provide a powerful mechanism for selectively redistributing mobile macromolecules in this space; that is, proteins, nucleic acids, or polysaccharides which are floating in the oolemma or loosely adsorbed on the matrix within this space. In this way the oocyte and the epithelium might well interact to generate complex developmental patterns: patterns in the oocyte as well as in the epithelium (Margeritis *et al.*, 1980). In particular, the germ plasm—well known at the posterior pole of *Drosophila*—first becomes externally transplantable at stage 13 (Illmensee *et al.*, 1976). Moreover, the symbiotic bacteria associated with a tail-favoring factor in the homopteran, *Euscelis* are seen to "assemble in a cup-shaped depression at the posterior pole of the oocyte . . . and become almost completely engulfed by the . . . cell" at a (comparable?) stage shortly before its shell forms (Sander, 1976, p. 165).

3. Preblastoderm eggs. The current pattern now reverts to that seen during the main growth period, except that the anterior in-current is now complemented by a smaller, posterior one in some eggs (Table 3; egg 57 of Fig. 4). However, under conditions which are known to allow further development of such eggs—i.e., culture inside an intact, impermeable vitelline membrane or under oil—the extracellular current must return along the perivitelline space as shown in Fig. 8C. Obviously this current is driven by the egg's plasma membrane. The work of Miyazaki and Hagiwara (1976) indicates that in a medium containing 84 m*M* K^+ (the concentration found in the perivitelline fluid) that the average membrane potential of the preblastoderm egg is about −20 mV and has both K^+ diffusion components and other (electrogenic?) ones.

A simple calculation suggests that this current should keep the anterior pocket at least a few millivolts negative to the posterior one: for if one multiplies the average measured net current—of about 1 nA (Table 3)—by the measured perivitelline resistance—of about 5 MΩ (Table 5)—one predicts an average standing voltage difference of about −5 mV between the polar pockets. This compares well with our most reliable direct measurement of this difference—namely $-4\frac{1}{2}$ mV (Table 5). Several complications were neglected in this calculation; so there is probably an element of luck in *this* degree of agreement with direct measurement. Nevertheless, we think that the anterior in-current really does keep the front pocket negative by a few millivolts or more during the preblastoderm period.

A wide variety of other developing cells are known to respond with directed growth or movement to applied voltage drops of a few millivolts or less. Table 6 in Jaffe and Nuccitelli (1977; p. 463) documents this for nine different plant cells: In three of these cases, a tenth maximal galvanotropic response occurs at voltage drops of 0.2 to 0.4 mV/cell; in six, 3 to 6 mV/cell. Table 7 in this paper assembles similar data for six different animal cases: at least five of these show significant galvanotropic or galvanotactic responses to voltage drops of 0.2 to 1 mV/cell. These responses are probably mediated by electrophoretic or electroosmotic transport of charged macromolecules along the cell membrane (Jaffe, 1977; McLaughlin and Poo, 1981).

TABLE 7
MINIMUM TRANSCELLULAR VOLTAGES SIGNIFICANTLY DIRECTING GROWTH OR MOVEMENT OF SOME ANIMAL CELLS

Animal	Cell or tissue	Response	Threshold (mV/cell)	Reference
Obelia	Stem section	Grows to +	0.2	Lund, 1924
Xenopus	Embryonic neurite	Grows to −	0.6	Hinkle *et al.*, 1981; Patel and Poo, 1982
Xenopus	Embryonic myoblast	Grows perpendicular	0.6	Hinkle *et al.*, 1981
Xenopus	Embryonic neural crest	Move to −	1	Stump and Robinson, 1983 (Also see Cooper and Keller, 1984)
Quail	Embryonic fibroblasts	Move to −	0.2	Erickson and Nuccitelli, 1984
Fish	Dissociated epidermal cells	Move to −	<4	Cooper and Schliwa, 1984

In any case, it seems clear that a steady extracellular voltage drop of a few millivolts (or more) across preblastoderm *Drosophila* eggs could well act back to advance the process of pattern formation *provided* that latent pattern is not yet well established. There are a number of indicators that, in fact, it is *not* (Schubiger and Wood, 1977; Bownes and Sander, 1976; Okada *et al.*, 1974; Simcox and Sang, 1983; Nüsslein-Volhard and Wieschaus, 1980). So maintenance of an extracellular voltage is one important way that the transcellular current could act back to further patterning in *Drosophila* eggs.

4. Follicle cell migrations. Finally, we propose that these currents help direct follicle cell migrations as well as affecting the oocyte (and later the egg) directly. King (1970) has described five main follicle cell migrations, of which at least the last three or four involve movement through or along a highly restricted extracellular space (see Fig. 9). The latter are: II. The migration of the whole follicular epithelium along the subepithelial space toward the oocyte in stages 8–10. III. The migration of the so-called border cells within the internurse cell space toward the oocyte in stages 9–10. IV. The centripetal migration of some follicle cells into the furrow between the nurse cells and the oocyte in stage 10. V. The anterior migration of some of the latter along the subepithelial space which surrounds the forming filaments in stages 11–14. All of these spaces might well be restricted enough to maintain fields or voltage gradients large enough to direct cell movement.

In particular, there is some reason to speculate that all of these migrations are against the current, i.e., against the direction of positive ion flow and toward the positive pole or anode. Movement II, of the whole follicular epithelium is against the direction of the main external current driven by the oocyte–nurse cell syncitium; movement III, of the border cells, would be against the current if the lateral, as well as the posterior, faces of the nurse cells pump current out; movement IV is against the current leaving the furrow observed in *H. cecropia* and *Drosphila;* movement V, of the filament formers, seems to be against the current as shown in Fig. 8B.

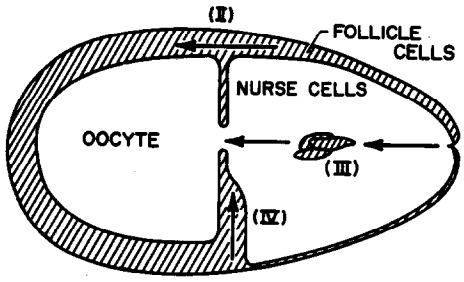

FIG. 9. Diagram of the three main follicle cell migrations which occur in stages 8–10. (Numbered after King, 1970.)

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REFERENCES

AMBROSE, E. J. (1965). "Cell Electrophoresis." Little, Brown, Boston.

BEAMENT, J. W. L. (1946). The waterproofing process in eggs of *Rhodnius prolixus* Stähl. *Proc. R. Soc. London Ser. B* **133**, 407–418.

BOURNE, G. W., and TRIFARÓ, J. M. (1982). The gadolinium ion: A potent blocker of calcium channels and catecholamine release from cultured chromaffin cells. *Neuroscience* **7**, 1615–1622.

BOWNES, M., and SANDER, K. (1976). The development of *Drosophila* embryos after partial U.V. irradiation. *J. Embryol. Exp. Morphol.* **36**, 394–408.

COOPER, M. S., and KELLER, R. E. (1984). Perpendicular orientation and directional migration of amphibian neural crest cells in dc electrical fields. *Proc. Natl. Acad. Sci. USA* **81**, 160–164.

COOPER, M. S., and SCHLIWA, M. (1984). Persistent motility of fish epidermal cells in the presence and absence of DC electric fields. *Biophys. J.* **45**, 98a.

DAVIES, L. (1948). Laboratory studies on the egg of the blowfly *Lucilia sericata* (MG). *J. Exp. Biol.* **25**, 71–85.

ERICKSON, C. A., and NUCCITELLI, R. (1984). Embryonic fibroblast orientation and motility can be influenced by physiological electric fields. *J. Cell Biol.* **98**, 296–307.

FRIZZELL, R. A., WELSH, M. J., and SMITH, P. L. (1981). Electrophysiology of chloride-secreting eipthelia. *In* "Ion Transport by Epithelia" (S. G. Schultz, ed.). Raven Press, New York.

FULLILOVE, S. L., and JACOBSON, A. G. (1978). Embryonic development: Descriptive. *In* "The Genetics and Biology of Drosophila" (M. Ashburner and T. R. F. Wright, eds.). Vol. 26, pp. 105–208. Academic Press. New York.

GUTZEIT, H. O., and KOPPA, R. (1982). Time-lapse film analysis of cytoplasmic streaming during late oogenesis of *Drosophila*. *J. Embryol. Exp. Morphol.* **67**, 101–111.

HAMBLY, B. D., and DOS REMEDIOS, C. G. (1977). Responses of skeletal muscle fibres to lanthanide ions. *Experientia* **33**, 1042–1044.

HINKLE, L., MCCAIG, C. D., and ROBINSON, K. R. (1981). The direction of growth of differentiating neurones and myoblasts from frog embryos in an applied electric field. *J. Physiol.* **314**, 121–135.

ILLMENSEE, K. MAHOWALD, A. P., and LOOMIS, M. R. (1976). The ontogeny of germ plasma during oogenesis in *Drosophila*. *Dev. Biol.* **49**, 40–65.

JAFFE, L. F. (1977). Electrophoresis along cell membranes. *Nature (London)* **265**, 600–602.

JAFFE, L. F. (1979). Control of development by ionic currents. *In* "Membrane Transduction Mechanisms" (R. A. Cone and J. E. Dowling, eds.), pp. 205–211. Raven Press, New York.

JAFFE, L. R., and NUCCITELLI, R. (1974). An ultrasensitive vibrating probe for measuring steady extracellular currents. *J. Cell Biol.* **63**, 614–625.

JAFFE, L. F., and NUCCITELLI, R. (1977). Electrical controls of development. *Annu. Rev. Biophys. Bioeng.* **6**, 445–476.

JAFFE, L. F., and WOODRUFF, R. I. (1979). Large electrical currents traverse developing *Cecropia* follicles. *Proc. Natl. Acad. Sci. USA* **76**, 1328–1332.

JUNGREIS, A. M. (1979). Physiology of moulting in insects. *Adv. Insect Physiol.* **14,** 109-183.

KALTHOFF, K. (1979). Analysis of a morphogenetic determinant in an insect embryo (Smittia Spec., Chironomidae, Diptera). *Symp. Soc. Dev. Biol.* **37,** 97-126.

KING, R. F. (1970). "Ovarian Development in *Drosophila melanogaster.*" Academic Press, New York.

KING, R. C., and DEVINE, R. L. (1958). Oogenesis in adult *Drosophila melanogaster.* VII The submicroscopic morphology of the ovary. *Growth* **22,** 299-326.

KING, R. C., and KOCH, E. A. (1963). Studies on the ovarian follicle cells of *Drosophila. Q. J. Microsc. Sci.* **104,** 297-320.

LIMBOURG, B., and ZALOKAR, M. (1973). Permeabilization of *Drosophila* eggs. *Dev. Biol.* **35,** 382-387.

LOHS-SCHARDIN, M. (1982). *Dicephalic*-A *Drosophila* mutant affecting polarity in follicle organization and embryonic patterning. *Wilhelm Roux's Arch. Dev. Biol.* **191,** 28-36.

LUND, E. J. (1924). Experimental control of organic polarity by the electric current IV. The quantitative relations between current density, orientation, and inhibition of regeneration. *J. Exp. Zool.* **39,** 357-380.

MAHOWALD, A. P. (1972). Ultrastructural observations on oogenesis in *Drosophila. J. Morphol.* **137,** 29-48.

MAHOWALD, A. P. (1980). Improved method for dissecting late ovarian stages. *Dros. Inf. Serv.* **55,** 156.

MAHOWALD, A. P., GORALSKI, T. J., and CAULTON, J. H. (1983). *In Vitro* activation of *Drosophila* eggs. *Dev. Biol.* **98,** 437-445.

MAHOWALD, A. P., and KAMBYSELLIS, M. P. (1980). Oogenesis. *In* "The Genetics and Biology of Drosophila" (M. Ashburner and T. R. F. Wright, eds.), Vol. 2D, pp. 141¼25. Academic Press, New York.

MARGARITIS, L. H., KAFATOS, F. C., and PETRI, W. H. (1980). The egg-shell of *Drosophila melanogaster.* I. Fine structure of the layers and regions of the wild-type egg-shell. *J. Cell Sci.* **43,** 1-35.

MCLAUGHLIN, S., and POO, M.-M. (1981). The role of electro-osmosis in the electric-field-induced movement of charged macromolecules on the surfaces of cells. *Biophys. J.* **34,** 85-93.

MEHRISHI, J. N. (1972). Molecular aspects of the mammalian cell surface. *Prog. Biophys. Mol. Biol.* **25,** 1-70.

MIYAZAKI, S., and HAGIWARA, S. (1976). Electrical properties of the *Drosophila* egg membrane. *Dev. Biol.* **53,** 91-100.

NÜSSLEIN-VOLHARD, C. (1977). Characterization of the maternal-effect mutant *Bicaudal. Wilhelm Roux's Arch. Dev. Biol.* **183,** 249-268.

NÜSSLEIN-VOLHARD, C., and WIESCHAUS, E. (1980). Mutations affecting segment number and polarity in *Drosophila. Nature (London)* **287,** 796-801.

OBERLEITHNER, H., RITTER, M., LANG, F., and GUGGINO, W. (1983). Anthracene-9-carboxylic acid inhibits renal chloride reabsorption. *Pflüegers Arch.* **398,** 172-174.

OKADA, M., KLEINMAN, A. A., and SCHNEIDERMAN, H. A. (1974). Chimeric *Drosophila* adults produced by transplantation of nuclei into specific regions of nuclei into specific regions of fertilized eggs. *Dev. Biol.* **39,** 286-294.

PALADE, P. T., and BARCHI, R. L. (1977). On the inhibition of muscle membrane membrane chloride conductance by aromatic carboxylic acids. *J. Gen. Physiol.* **69,** 879-896.

PATEL, N., and POO, M. -M. (1982). Orientation of neurite growth by extracellular electric fields. *J. Neurosci.* **2,** 483-496.

PETRI, W. H., MINDRINOS, M. N., LOMBARD, M. F., and MARGARITIS, L. H. (1979). *In vitro* development of the *Drosophila* chorion in a chemically defined organ culture medium. *Wilhelm Roux's Arch. Dev. Biol.* **186,** 351-362.

RIPLEY, S., and KALTHOFF, K. (1983). Changes in the apparent localization of anterior determinants during early embryogenesis (Smittia spec., Chironomidae, Diptera). *Wilhelm Roux's Arch. Entwicklungsmech. Org.* **172,** 175-186.

ROBB, J. A. (1969). Maintenance of imaginal wing discs of *Drosophila melanogaster* in chemically defined media. *J. Cell Biol.* **41,** 876-885.

RUBENSTEIN, E. C. (1979). The role of an epithelial occlusion zone in the termination of vitellogenesis in *Hyalophora cecropia* ovarian follicles. *Dev. Biol.* **71,** 115-127.

RUDDELL, A., and JACOBS-LORENA, M. (1983). Abrupt decline in the rate of accumulation of total protein and yolk in postvitellogenic egg chambers of *Drosophila. Wilhelm Roux's Arch. Dev. Biol.* **192,** 189-195.

SANDER, K. (1976). Specification of the basic body pattern in insect embryogenesis. *Adv. Insect Physiol.* **12,** 125-238.

SCHUBIGER, G., and WOOD, W. J. (1977). Determination during early embryogenesis in *Drosophila melanogaster. Amer. Zool.* **17,** 565-576.

SIMCOX, A. A., and SANG, J. H. (1983). When does determination occur in *Drosophila* embryos? *Dev. Biol.* **97,** 212-221.

STUMP, R. F., and ROBINSON, K. R. (1983). *Xenopus* neural crest cell migration in an applied electrical field. *J. Cell Biol.* **97,** 1226-1233.

TURNER, F. R., and MAHOWALD, A. P. (1976). Scanning electron microscopy of *Drosophila* embryogenesis. *Dev. Biol.* **50,** 95-108.

VAN DER MEER, J. M., and JAFFE, L. F. (1983). Elemental composition of the perivitelline fluid in early *Drosophila* embryos. *Dev. Biol.* **95,** 249-252.

WOODRUFF, R., HUEBNER, E., and TELFER, W. The origin of electrical currents in insect ovarioles. *Adv. Invert. Reprod.* **3,** (in press).

WOODRUFF, R. I., and TELFER, W. H. (1980). Electrophoresis of proteins in intercellular bridges. *Nature (London)* **286,** 84-86.

ZADUNAISKY, J. A. (1982). "Chloride Transport in Biological Membranes." Academic Press, New York.