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# Comparison of Defolliculated Oocytes and Intact Follicles of the Cockroach Using the Vibrating Probe to Record Steady Currents

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**Follicle cells were removed by dissection from early vitellogenic oocytes of the cockroach *Blattella germanica*. The vibrating probe was used to record steady currents from 19 defolliculated oocytes and 19 intact follicles of the same developmental stage. Defolliculated oocytes generated currents that were stable and distinguishable (by intensity or selective direction) from background reference values. Distributions of the intensities of reference values and experimental values were, in general, similar in both intact and defolliculated preparations. The patterns of currents generated by preparations recorded in the mid-sagittal plane were analyzed for both defolliculated oocytes ($n = 8$) and intact follicles ($n = 10$). The larger, generally more mature preparations in both groups generated patterns of current similar to the pattern seen in mid-vitellogenic follicles (focused inward near the germinal vesicle (GV), the presumptive ventral side, and broadly outward on the apo-GV side, the presumptive dorsal side). Smaller sized preparations in both groups showed inward or outward current on the apo-GV aspect and, typically, inward current at the GV. Only two defolliculated oocytes, and no intact follicles, appeared to generate outward current at the GV, and we believe this observation resulted from recording slightly outside the mid-sagittal plane. We conclude that preparations during early-vitellogenesis initially generate currents without an asymmetric pattern and that the inward flux at the GV is the first step in developing patterns of currents. The results suggest that the oocyte (and not the follicle cell epithelium) is responsible for generating the various patterns of currents observed in early-vitellogenic stages. At the end of early-vitellogenesis, the follicle cell epithelium begins to adhere tightly to the oocyte. The possibility is considered that the follicle cells may influence the currents generated during mid-vitellogenesis.** © 1994 Academic Press, Inc.

## INTRODUCTION

A fundamental question in modern biology is how polarities are established in developing organisms. One physiological feature correlated with polarity is the generation of patterned extracellular currents by oocytes or follicles, which can be recorded using the vibrating probe. Successful vibrating probe studies have used defolliculated oocytes (but not intact follicles) of *Xenopus* (Robinson, 1979) and intact follicles (but not defolliculated oocytes) of several insects (*Drosophila*, Overall and Jaffe, 1985; Bohrmann *et al.*, 1986; Sun and Wyman, 1989; *Hyalophora*, Woodruff *et al.*, 1986; *Rhodnius*, Diehl-Jones and Huebner, 1989, 1992; *Sarcophaga*, Verachtert and DeLoof, 1989; and *Blattella*, Kunkel, 1986; Kunkel and Bowdan, 1989; Bowdan and Kunkel, 1990). In addition, Bohrmann *et al.*, (1986), using *Drosophila*, Woodruff *et al.*, (1986), using *Hyalophora*, and Diehl-Jones and Huebner (1992), using *Rhodnius*, have reported results of vibrating probe studies of partially defolliculated oocytes.

Except for the cockroach *Blattella*, all of the insect follicles studied have been meroistic—i.e., had nurse cells. Thus, the follicle included the oocyte, an epithelium of follicle cells, and either a cluster of nurse cells (polytrophic) or a connection to a remote tropharium (telotrophic). The panoistic follicle of the cockroach is somewhat simpler, because it has no nurse cells. Still, the oocyte is surrounded by a layer of follicle cells that could potentially generate currents detected by the vibrating probe.

We have found that, in early vitellogenic stages (0.4–1.0 mm in length), the panoistic follicles of the cockroach *Blattella germanica* may be completely defolliculated with watchmaker's forceps, leaving a preparation consisting of a single, viable germ cell (Anderson and Kunkel, 1990). With this capability, we used the vibrating probe to record and compare extracellular currents generated during early vitellogenesis by both intact follicles and defolliculated oocytes. To our knowledge, the *Blatella* follicle is the only insect preparation completely defolliculated and recorded from with the vibrating probe.

Because the follicle cells begin to adhere tightly to the oocyte once it reaches a length of about 1.0 mm, it was

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not possible to defolliculate oocytes in later stages of vitellogenesis. Previous vibrating probe studies of intact, more mature mid-vitellogenic follicles of *B. germanica* (Kunkel, 1986; Bowdan and Kunkel, 1990) revealed currents with an asymmetric pattern—inward at the concave, presumptive ventral aspect of the animal and outward at the convex, presumptive dorsal aspect. Preliminary observations of intact, early vitellogenic follicles (Bowdan and Kunkel, 1990) indicated that extracellular currents are generated initially without a clear asymmetric pattern and that they increase in intensity over the course of vitellogenesis. The data of the present study confirm and extend those results to defolliculated oocytes.

## METHODS AND MATERIALS

*Animals.* Cultures of *B. germanica* were raised synchronously from hatching to adult at 30°C (Kunkel, 1966). Adult females were held in the previtellogenic state by keeping them at 18°C without food. At 3 to 4 days before an experiment, a first or second parturition female was transferred to 30°C and provided food. At this temperature, all of the terminal oocytes of the approximately 20 ovarioles in each ovary undergo vitellogenesis. With the accumulation of yolk, the oocytes increase in size. They typically reach a length of about 2 mm at the time of ovulation (Roth and Stay, 1962), which occurs after 6–7 days at 30°C (Kunkel, 1973). In this study, we used early- to mid-vitellogenic oocytes ranging in length from 0.4–1.0 mm. In all preparations used, the germinal vesicle, which at still earlier developmental stages is located at the center of the oocyte, had migrated to the cortex at the equator between the anterior and posterior poles of the follicle.

Ovaries were removed and cleaned of adhering tissues in a dish containing physiological saline solution. Individual ovarioles were transferred to the recording chamber (a plexiglass oval 8 × 22 mm, 5 mm deep, with openings at the ends of the oval for perfusion tubing) which contained saline solution. Defolliculated oocytes were prepared in the recording chamber by removing the follicle cell epithelium with sharpened forceps. For recording from intact ovarioles, the glass coverslip bottom of the chamber (replaced with each use) was treated with 0.01% poly-L-lysine (Sigma P 1274) to hold the ovarioles in place, and a saline solution containing 4 m*M*/liter Ca was used. For recording from defolliculated oocytes, poly-L-lysine was not used because the oocytes adhered directly to the glass substrate, and the saline solution contained either 1 or 4 m*M*/liter Ca. During a recording session, the chamber contained from 1 to 4 oocytes or ovarioles. The recording medium was maintained at 28–30°C with a Peltier regulated heating coil.

*Solutions.* The control physiological saline solution had the following composition (m*M*/liter): NaCl, 145.4; KCl, 14; $CaCl_2 \cdot 2H_2O$, 4; $MgSO_4 \cdot 7H_2O$, 5; $H_3PO_3$, 11; sucrose, 110. Penicillin (125 mg/liter) and streptomycin (50 mg/liter) were added as preservatives. The osmotic concentration was 290 mOsm/liter (Bowdan and Kunkel, 1990). In those experiments using a medium containing 1, instead of 4, m*M*/liter $CaCl_2 \cdot 2H_2O$, the minor change in osmotic concentration was either left uncorrected or corrected by adding sucrose. Both solutions were adjusted to a pH of 6.8. Solutions were refreshed or changed by perfusing through the chamber.

*Recording.* A 2-D vibrating probe built by Applicable Electronics was used at the University of Massachusetts in Amherst. The system is controlled by an 80286 MS-DOS computer. An A/D board (Metrabyte, DAS-8) provides four 12-bit input lines for vibrating probe data and one line of temperature input from a thermocouple in the recording chamber. An 8-bit 640 × 480 (square pixel) frame grabber (Matrox PIP-640) records an image via a TV camera (DAGE MTI CCD 72A) mounted on an Olympus trinocular stereomicroscope. Microsoft C software for this instrumentation was written by Applicable Electronics and J. G. Kunkel.

The tip diameter of the probe was approximately 30 μm. The vertical position of the probe tip was set at about half the height of the oocyte, and vertical adjustments were made to achieve the maximum signal possible. For most preparations, all scans around the perimeter of the preparation were done with the probe at the same vertical position. The probe tip was brought to about one tip diameter's distance from the surface of the oocyte or ovariole. Reference recordings were taken frequently between measurements at a distance of > 0.5 mm from the preparation. Each vector was determined by averaging 3000 individual measurements over a period of 5 sec. Several preparations were recorded from for periods up to 3 hr. All data included in this study were stable over at least 20 min.

*Experimental design.* Two groups of oocytes—intact within the follicle and defolliculated—of approximately the same developmental stage were studied using the vibrating probe. In some cases, we were able to record from both an intact and a defolliculated oocyte in the same chamber. However, we did not consistently strive to record from such pairs. While intact follicles usually required a poly-L-lysine substrate to adhere to the base of the chamber, defolliculated oocytes adhered without treatment of the glass. We believed it preferable not to use poly-L-lysine when recording from defolliculated oocytes, both because the poly-L-lysine would tend to flatten the oocyte, and thereby make it more difficult to approach with the vibrating probe, and because it might affect the function of membrane proteins involved in generating currents. In addition, in the course of these

TABLE 1
ORIENTATIONS AND LENGTHS (IN mm) OF DEFOLLICULATED AND INTACT TERMINAL OOCYTES

| Orientation | Defolliculated | Intact |
|---|---|---|
| GV on one edge (mid-sagittal recording) | 9 (.43–1.0) | 10 (.54–.93) |
| GV facing upward (frontal recording) | 5 (.44–.93) | 7 (.45–.69) |
| Position of GV unknown | 5 (.8–1.05) | 2 (.69–.83) |
| Total | 19 | 19 |

experiments, we found that a bathing medium containing less calcium tended to maintain the defolliculated oocytes in better condition than the control medium. We concluded that it was preferable to record from intact ovarioles and defolliculated oocytes separately in different media. We therefore present here a comparison of extracellular currents recorded from 19 successfully defolliculated oocytes with those from 19 intact ovarioles.

Effort was made to orient the oocyte or ovariole so that it was attached to the substrate either on its lateral aspect with the germinal vesicle (GV) visible on one edge (yielding a midsagittal record) or on its dorsal aspect with the germinal vesicle facing upward (yielding a frontal record). Table I shows the orientations of all the preparations included in this study. The ranges of length, in millimeters, of each group of defolliculated oocytes or terminal oocytes within an ovariole are shown in parentheses.

## RESULTS

In its development, the terminal oocyte of *Blattella* grows and changes from oval to banana-shaped as it accumulates yolk; thus the size and shape of the oocyte correlate in a general way with its physiological development (Roth and Stay, 1962; Kunkel, 1973). All of the results presented in this paper were obtained from early-vitellogenic preparations, which were either oval in shape or slightly concave near the germinal vesicle.

Figure 1 shows recordings from two preparations, one intact (A) (No. 9) and the other defolliculated (B) (No. 7), taken from the same ovary and placed in the recording chamber at the same time. The terminal oocyte of the intact follicle is enclosed within the follicle cell layer, which is continuous with the posterior oviduct (pointing left) and the anterior chain of immature follicles (pointing right). Both preparations rested on the lateral aspect, with the germinal vesicle visible at the middle of the top (presumptive ventral) surface. Current vectors were recorded at several different positions in a single, mid-sagittal scan around each preparation. The probe can be seen in the lower right of each panel. Both of these preparations show an asymmetry of currents, with net inward current near the GV and net outward current on the opposite (apo-GV) side of the oocyte. However, such an asymmetric pattern, although typical of intact mid-vitellogenic ovarioles, was rarely recorded from early vitellogenic preparations (see below).

*Y currents.* In these experiments, the chamber with the oocyte was rotated to make the long axis of the oocyte parallel with the $X$ axis. The two-dimensional vibrating probe was vibrated in the $XY$ plane. It reported currents both perpendicular to (Y currents) and parallel with (X currents) the surface of the membrane and also reported the sums of X and Y vectors as total currents. For this analysis, we reasoned that Y currents would be the most faithful representation of current flowing through the membrane in the vicinity of the probe. (Currents tangential to the membrane, and therefore having a large component of X current, could have origi-

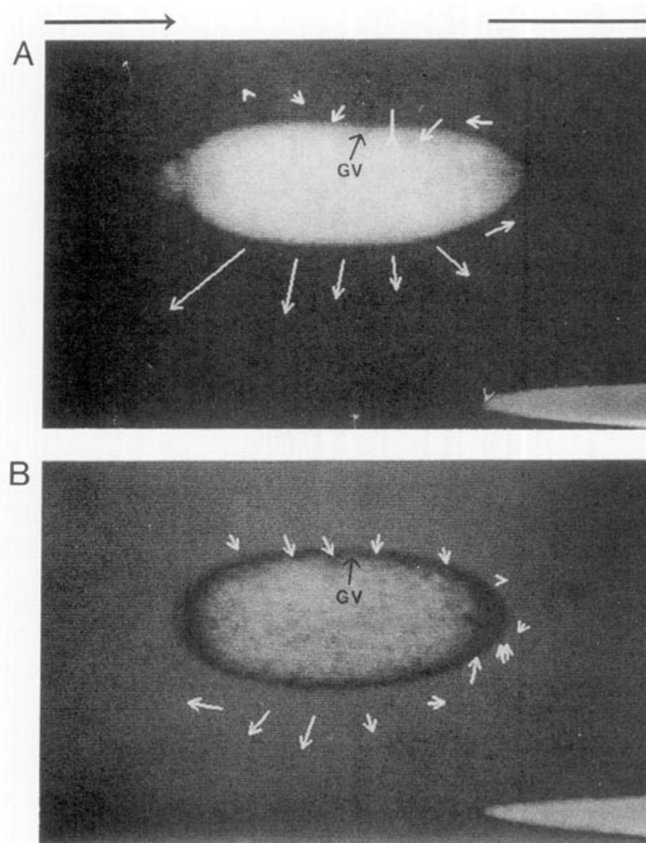


FIG. 1. Vectors of two preparations, an intact follicle (A) and a defolliculated oocyte (B), taken from the same ovary and placed in the recording chamber at the same time. Each set of vectors was recorded from a single scan of the probe around the mid-sagittal plane of the preparation. Black arrows point to the position of the GV. The substrate was not treated with poly-L-lysine. The control medium contained 4 m$M$/liter Ca. Calibrations, 10 $\mu A/cm^2$ (left arrow); 0.5 mm (right bar).

nated at unknown sites remote from the region of membrane closest to the probe and would give misleading information about the local source). In addition, total currents (the sums of X and Y vectors) would not only be complicated by the X component, but, because the error of X and the error of Y are independent and thus additive, they would also increase the total degree of error. To check that X and Y errors were independent of each other, we plotted the X and Y components of all reference readings (the indicators of error in recording currents) taken at points distant from the oocytes and found correlation coefficients for both intact and defolliculated preparations to be insignificant. All means of X and Y components were well within 2 standard errors of zero. On the basis of this analysis, we concluded that the error components of X and Y were indeed independent. Therefore, all analyses reported here were carried out using Y currents, which carry the major information of interest.

*Comparing reference and experimental vectors.* Figure 2 shows plots of Y current (*Y* axis) versus probe position (*X* axis) for two mid-sagittal scans taken around each of the preparations shown in Fig. 1. Icons indicate the orientation of the oocyte and arrows the main directions of the plotted Y vectors. By convention, net inward currents are shown as negative (downward) values. Reference values (open symbols) are clustered around the left vertical line and experimental values (closed symbols) around the right vertical line. Experimental values (squares) on the right of the vertical line were taken on the apo-GV side of the preparation, while values (diamonds) to the left of the vertical line were taken on the GV side. To avoid vectors that may have been influenced by tangential currents near the ends of the defolliculated oocytes, or by injury currents generated by the cut oviducts in the intact follicles, experimental values at the extreme anterior and posterior ends of the preparations were not plotted. (All reference values were plotted).

In both Figs. 2A and 2B, the intensities of the experimental values are distinct from those of reference values. In Fig. 2A (intact follicle No. 9), the negative values indicate inward currents near the GV and the positive values indicate outward currents on the side away from the GV. In Fig. 2B (defolliculated oocyte No. 7), the reference values are in the same size range as those of Fig. 2A; the experimental readings are somewhat smaller than the experimental readings of Fig. 2A, but the pattern of inward currents near the GV and outward at the apo-GV surface is the same.

*Stability of currents in defolliculated oocytes.* Figure 3 shows that, although currents generated by defolliculated oocytes were small, they were stable and distinct from reference values. Figure 3A (oocyte No. 1) shows all vectors recorded at five different positions along the

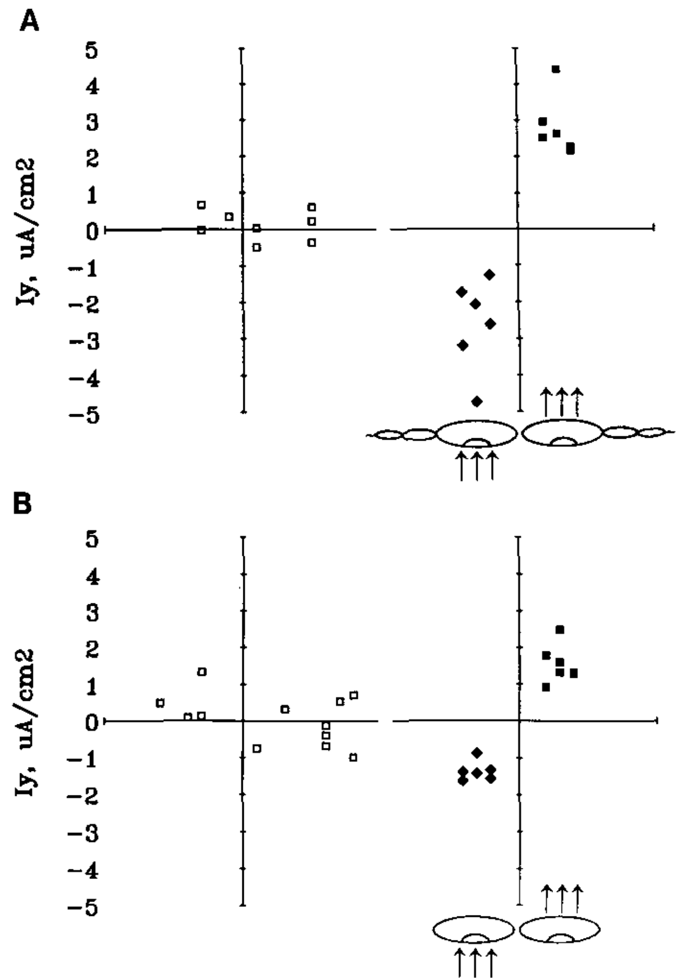


FIG. 2. Reference (open symbols, left) and experimental (closed symbols, right) Y currents for the intact follicle (A) and defolliculated oocyte (B) shown in Fig. 1. Vibrating probe recordings taken on the GV side of each preparation are shown to the left of the vertical lines and recordings taken on the apo-GV aspect to the right of the vertical lines. Inward currents are shown below the horizontal axis and outward currents above the horizontal axis. The probe was placed near the surface of the preparation for experimental readings and moved at least 0.5 mm away for reference readings. While Figs. 1A and 1B show a single scan each, these data were obtained from two scans around each preparation. Both generated inward currents at the GV and outward currents at the apo-GV aspect.

apo-GV aspect of a defolliculated oocyte over a period of 10 min. The experimental currents were consistently inward, had intensities within an envelope of about 2 to 7 $\mu A/cm^2$, and were by and large distinct from the reference values. Vectors recorded from the GV aspect of this oocyte (not shown) were indistinguishable from reference values. Figure 3B (oocyte No. 8) shows a series of successive currents, recorded over a period of 16.5 min at a single position on the apo-GV aspect of a different defolliculated oocyte. At this single position, the experimental vectors were consistently outward, varied in intensity over a few $\mu A/cm^2$ (but were consistent within a

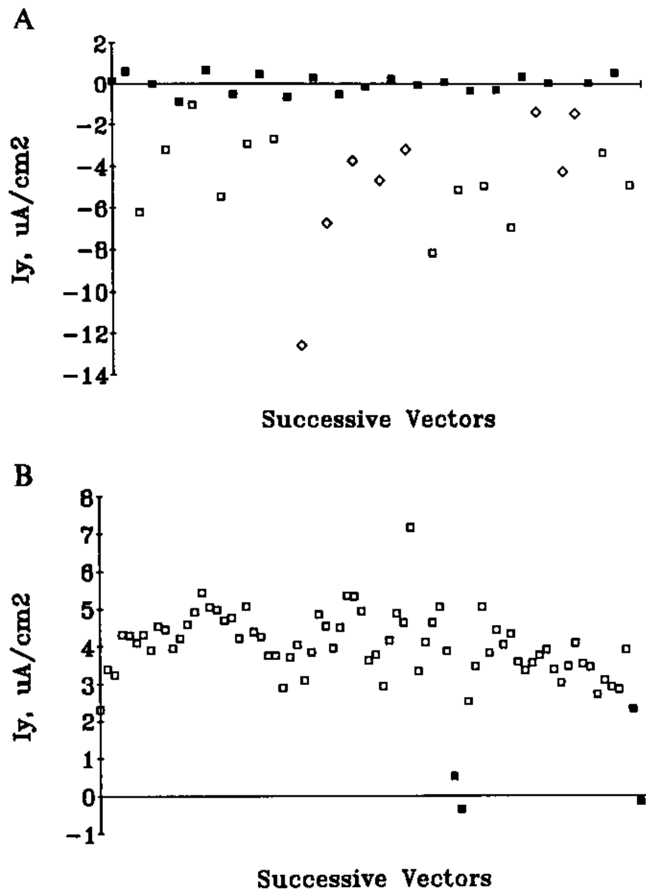


FIG. 3. Stability of experimental (open symbols) and reference (solid symbols) currents in defolliculated preparations. In A, experimental readings were alternated with reference readings approximately every 15 sec over a period of 10 min. Inward currents were recorded at five different, equidistant positions (alternating squares and diamonds) sequentially along the length of the apo-GV surface of a defolliculated oocyte. In B, successive outward vectors were recorded approximately every 15 sec over a period of 16.5 min from a single position on the apo-GV surface of a different oocyte. The probe was moved away four times to record reference values and then returned to the same experimental recording site. All experimental and reference vectors were determined relative to zero. After each reference measurement, the reference value was reset to zero.

stable envelope of values), and, for the most part, distinct from reference values. Vectors recorded on the GV aspect of this oocyte (not shown) were inward and about 3 $\mu A/cm^2$ in intensity. Thus, current vectors in defolliculated oocytes were consistent in direction and intensity over periods of many minutes' duration and were distinguishable from reference values.

*Relative intensities of reference and experimental vectors.* As a first test of the functional capacity of all the early vitellogenic intact and defolliculated preparations in this study, we compared the intensities of reference and experimental vectors recorded from each preparation. We then compared the vectors of both groups by preparing the ordered histograms shown in Fig. 4. To avoid outliers, the upper 95% confidence limits of the measured current intensities for each oocyte (95% cl) were determined and used for both histograms. Figures 4A and 4B show that the upper 95% cl intensities of experimental vectors were distinct from those of reference values in nearly all preparations. The two exceptions were defolliculated oocytes No. 9 and No. 18, which did not generate vectors greater in intensity than reference values. This result might suggest that they were not viable. However, one of these oocytes (No. 9) was subsequently bumped by the vibrating probe, and sizable inward injury currents (which would not be expected were the preparation dead) were observed. Closer examination of the vectors in the other oocyte (No. 18) showed that, while the vectors on one surface varied in direction (as did the reference values), those on the opposite surface were consistently outward. This observation strongly suggests that, although not exceeding the intensities of reference values, the directional vectors were indicative of controlled current flux on one surface of the oocyte. Both of these oocytes, therefore, were very

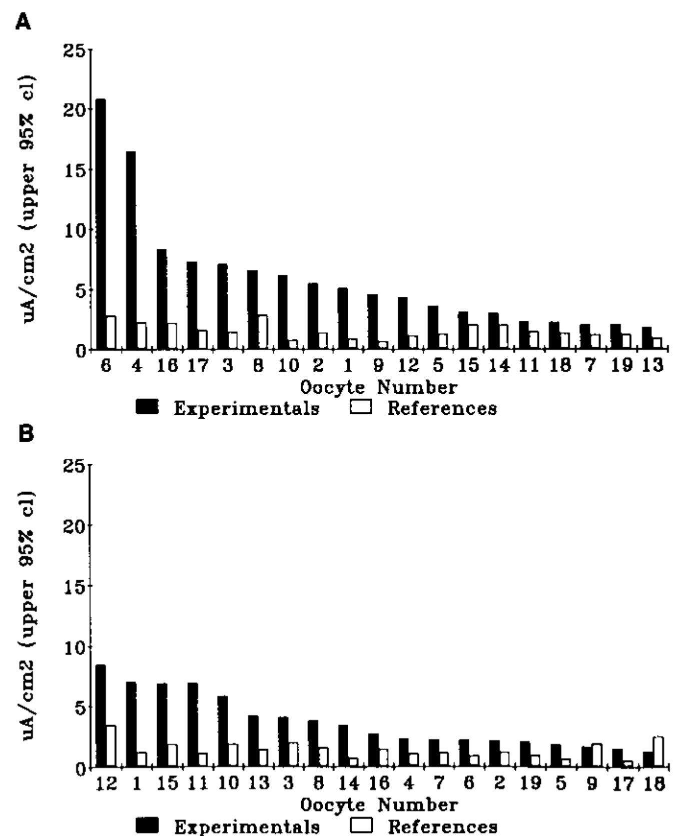


FIG. 4. Intensities of Y currents in 19 intact (A) and 19 defolliculated (B) preparations. Upper 95% confidence limits (cl) of the intensities of reference values (open bars) and experimental values (solid bars) are shown for each preparation included in this study. To permit comparison of intact and defolliculated preparations, the experimental currents were sorted in descending order of their magnitudes. Identification numbers shown here are used in further figures and text.

likely viable, but not generating currents of greater intensities than reference values.

Except for those of two intact follicles (No. 4 and No. 6), the intensities of experimental vectors in the 38 preparations studied had a similar size distribution in both intact (Fig. 4A, $n$ = 19) and defolliculated (Fig. 4B, $n$ = 19) preparations. A Fisher exact test (for 0:19 and 2:17) yielded approximately a 1/4 probability ($P$ = 0.2432) that the two extreme observations could have happened by chance to fall in the intact category. The two large values found in the intact group could have occurred in the defolliculated group with a similar probability. Therefore, with this sample, we have no strong evidence that the intact and defolliculated groups are different in the distribution of extreme values of intensities.

It is important to note that, in both defolliculated and intact groups, the intensities of the current vectors were not correlated with the lengths of the oocytes. Indeed, the lengths of the defolliculated oocytes that generated experimental vectors smaller than the reference vectors were both 0.8 mm, and the lengths of the two intact follicles that generated unusually large currents were 0.74 (No. 4) and 0.8 (No. 6) mm. These lengths are in the middle range of sizes of preparations used in this study. Therefore, while the capacity to generate steady currents is a general indicator of both viability and development to vitellogenesis, absolute intensities of current vectors recorded from early vitellogenic preparations are not indicators of exact stage of development. Thus, Fig. 4 shows that early vitellogenic intact follicles and defolliculated oocytes generate steady currents over a similar spectrum of intensities that are usually distinguishable from reference values.

*Transition from early vitellogenic current patterns to mid-vitellogenic current patterns.* To look for evidence of the development of asymmetric patterns of current, we examined the data from intact and defolliculated preparations recorded mid-sagitally [10 intact follicles and 8 defolliculated oocytes; the experimental currents of the 9th defolliculated oocyte did not exceed background values (preparation No. 9)]. From plots such as those in Figs. 5 and 6, we determined the net directions of Y currents (inward or outward) on the GV and apo-GV surfaces. Figure 7 plots the number of preparations that generated each type of current behavior. The greatest number of preparations exhibited either net inward current near the GV or net outward current on the apo-GV aspect. Thus, they had one of the features of the more mature mid-vitellogenic pattern. Two defolliculated and 4 intact preparations had the complete mid-vitellogenic pattern. A few preparations (3 defolliculated and 3 intact) exhibited inward current on the apo-GV aspect. All but 1 of these also showed inward currents near the GV. (To complete current loops, preparations with all inward currents in the mid-sagittal plane would have generated outward currents in regions of surface membrane remote from the probe). The exception was a defolliculated oocyte (No. 1) that showed no distinguishable currents near the GV, and it was the smallest oocyte used in this study (0.43 mm). Outward currents near the GV were observed in two defolliculated oocytes (No. 3 and No. 6). Although these oocytes may indeed have generated outward currents at the GV, an alternative explanation is that they may have been tilted slightly, so that any focused inward current at the GV was just out of range of the probe. Such an orientation would yield a band of outward current, which was observed. It is of interest to note that both of these examples were defolliculated oocytes. These are the preparations that adhere to the substrate without poly-L-lysine.

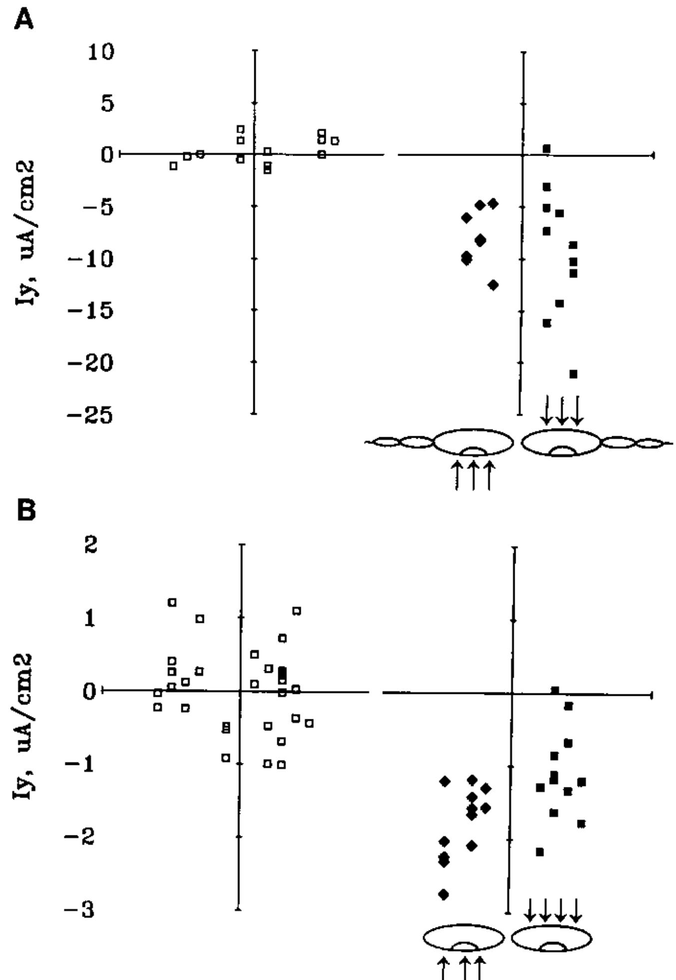


FIG. 5. Plots (like those described in Fig. 3) of Y currents from an intact (A) and a defolliculated (B) preparation showing net patterns of currents generated in the mid-sagittal plane. (A) (No. 4 intact, 0.74 mm) and (B) (No. 4 defolliculated, 0.72 mm) show inward currents at both GV and apo-GV aspects. The intensities of the currents recorded around the intact follicle were significantly greater than those around the defolliculated oocyte. This follicle is one of the preparations showing unusually large currents in Fig. 4A.

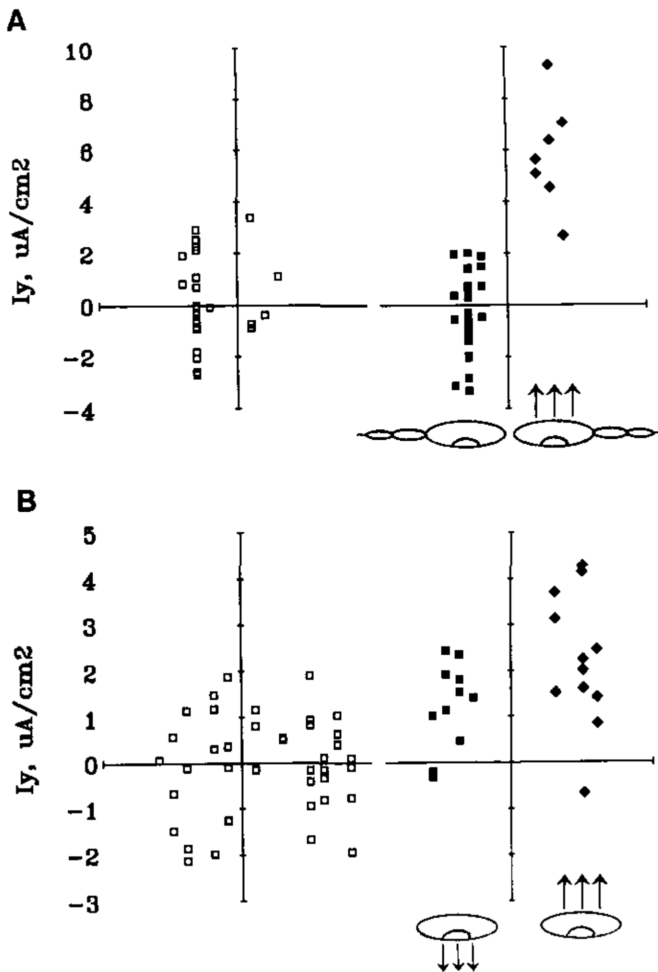


FIG. 6. Plots of Y currents from an intact (A) and a defolliculated (B) preparation showing net patterns of currents generated in the mid-sagittal plane. (A) (No. 8 intact, 0.86 mm) and (B) (No. 3 defolliculated, 0.72 mm) show outward vectors on the apo-GV aspect, but no vectors above background reference values at the GV. While the experimental vectors recorded at the GV in (A) are approximately evenly distributed between inward and outward directions, those at the GV in (B) are consistently in the outward direction. The consistency in direction of these vectors, even though their intensities do not exceed background levels, suggests a functionally significant generation of outward currents. The vectors recorded around the intact follicle were somewhat greater in magnitude than those around the defolliculated oocyte.

Unlike the intact ovarioles, the positions of which can (to a degree) be "adjusted" to assure correct placement for mid-sagittal recording, the defolliculated oocytes, once touching the substrate, cannot easily be repositioned for optimal mid-sagittal recording. This fact may provide a technical explanation for the appearance of outward current generated near the GV.

Figures 8A and 8B illustrate with three-dimensional plots the relation between the size of the oocyte and the total patterns of current generated. Currents at the GV aspect are shown on the left (outward in the top quadrant and inward in the bottom quadrant) and currents on the apo-GV aspect on the right. Preparations are identified by number, and oocyte lengths are plotted in mm, from 0.4 mm at the center of the plot to 1 mm at the periphery. Figure 8B shows that the two defolliculated oocytes with the mature mid-vitellogenic pattern (No. 7 and No. 8: inward GV, lower left quadrant, and outward apo-GV, upper right quadrant) were the largest of the mid-sagittally recorded preparations. Figure 8A shows that 3 of the 4 intact follicles with a mature pattern (No. 7, No. 9, and No. 10) were also in the upper range of lengths.

None of the defolliculated oocytes with inward currents on the apo-GV surface (lower right quadrant) had lengths in the upper range of the preparations studied. The intact follicles with inward currents on the apo-GV surface were in the low-to-mid range of lengths. Further, two of the intact preparations with inward currents on the apo-GV surface were the preparations (No. 4 and No. 6) that generated significantly larger currents than all the other preparations (Fig. 4). Thus, large intensities of currents are not necessarily correlated with occurrence of the mature mid-vitellogenic pattern.

The two defolliculated oocytes with outward current on both GV and apo-GV sides were 0.72 (No. 3) and 0.8 (No. 6) mm in length. No intact preparations showed this current behavior, and it seems likely that the recordings made from these defolliculated oocytes may have resulted from a tipped orientation of the oocyte.

Two of the defolliculated oocytes and 3 intact follicles generated currents on only one aspect of the preparation (open symbols). Recording only outward current on the apo-GV aspect may be explained if an inward

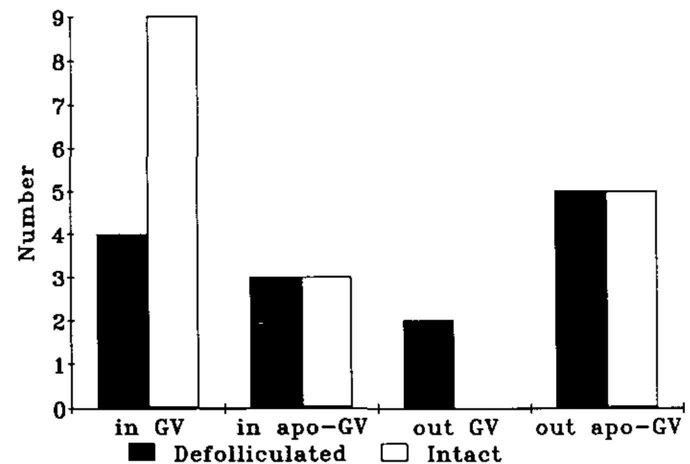


FIG. 7. Proportions of mid-sagittally recorded preparations generating net inward or outward currents on the GV or apo-GV aspects. Ten intact follicles and 8 defolliculated oocytes are represented. In Fig. 8, these patterns of currents are related to the lengths of the preparations from which they were recorded.

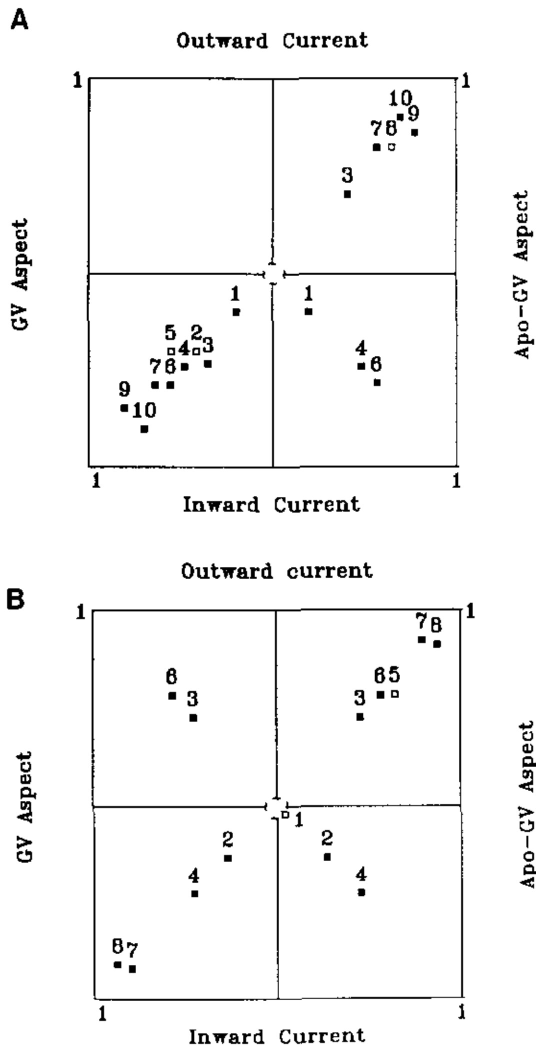


FIG. 8. Total patterns of current plotted with respect to length of oocyte (a rough indication of degree of development) for 10 intact (A) and 8 defolliculated (B) preparations (numbered as in Fig. 4) recorded in the mid-sagittal plane. Lengths are plotted from 0.4 mm (center) to 1.0 mm (periphery). Preparations that generated inward currents are indicated below the $X$ axis, outward currents above. Preparations with currents at the GV are shown to the left of the $Y$ axis and those with currents on the apo-GV aspect to the right. Preparations that generated above-background currents on both GV and apo-GV aspects are indicated by closed symbols; those that generated above-background currents on only one aspect are indicated by open symbols. In (A) 4 intact oocytes (No. 7, No. 9, No. 10, and No. 3) exhibited inward current at the GV and outward opposite the GV; 3 (No. 1, No. 4, and No. 6) inward on both aspects; 2 (No. 2 and No. 5) generated inward current at the GV only, while 1 (No. 8) generated outward current on the apo-GV aspect only. In (B) 2 defolliculated oocytes (No. 8 and No. 7) exhibited the mature mid-vitellogenic pattern of inward current at the GV and outward opposite the GV; 2 (No. 4 and No. 2) inward on both aspects; 2 (No. 3 and No. 6) outward on both aspects; and 2 generated either inward (No. 1) or outward (No. 5) currents on only the apo-GV aspect.

current flux at the GV is highly focused, and therefore missed by the probe. Intact No. 8 and defolliculated No. 5 fall into this category. However, recording inward current at the GV, without encountering the more easily detected broad outward current on the apo-GV side, suggests lack of mature mid-vitellogenic current behavior. Intact follicles No. 2 and No. 5 fall into this category. It is of interest that these preparations were in the lower range of lengths of the preparations included in this study. The smallest preparation in this study (No. 1 defolliculated) showed inward current on the apo-GV aspect. These results, therefore, suggest that preparations during early vitellogenesis initially generate currents without an asymmetric pattern and that the inward flux at the GV is the first step in developing patterns of currents.

The fact that the defolliculated preparations yielded results similar to those of the intact follicles strongly suggests that the oocyte (and not the follicle cell epithelium) is both responsible for generating the various patterns of currents observed in early vitellogenic stages and also capable of giving rise to the asymmetric pattern of currents seen consistently in intact mid-vitellogenic follicles. The results do not provide information on whether the follicle cell layer may influence the currents during mid-vitellogenesis. The data are consistent with the idea that pumps and/or channels tend to congregate in specific regions during the course of vitellogenesis. As vitellogenesis proceeds, the populations of these specific membrane components would function to produce currents with net directions detectable by the vibrating probe. The data indicate that outward currents are very unlikely to occur near the GV, and they suggest a trend from an initial inward flux at the GV toward the development of a broad efflux of current opposite the GV.

## DISCUSSION

The results reported here indicate that defolliculated early vitellogenic oocytes of *B. germanica* generate extracellular currents similar to those recorded from intact follicles of the same developmental stage. For several reasons, we are confident that the defolliculated oocytes were viable. First, we knew from intracellular recordings in the presence and absence of azide (Anderson and Kunkel, 1990) that defolliculated oocytes maintain resting membrane potentials that depend on active metabolic cellular processes. Second, in these experiments, injuring a defolliculated oocyte by touching it with the vibrating probe always resulted in large inward currents, which would not be generated in a dead oocyte. Third, currents recorded in successive scans over periods of (in some cases) up to 2 hr did not show deterioration, even in oocytes that had been placed in the recording chamber over an hour prior to recording. Finally, the similarity of the patterns of current seen in both groups of preparations supports the conclusion

that the defolliculated oocytes were both viable and functional.

The results suggest that the pattern of currents typically observed during mid-vitellogenesis is acquired with development and that the oocyte itself is capable of generating an asymmetric pattern of currents. However, in the course of these experiments, we found that a bathing medium containing a $[Ca]_o$ of 1 m*M*, instead of the control $[Ca]_o$ of 4 m*M*, maintained the defolliculated oocytes for longer recording periods, although it had no effect on the intensities of the currents recorded. Further, previous studies of the cockroach *Nauphoeta* (Koenig *et al.*, 1988) showed that a low [Ca] is required for vitellogenin binding to receptors on the oocyte membrane. Both of these observations suggest that a low-calcium microenvironment distinct from the hemolymph exists between the oocyte and the follicle cells and that it may influence the currents. It is of interest in this context that Bohrmann (1991) showed several functions of intact *Drosophila* follicles, including potassium uptake, to vary in different media, and he emphasized the importance of incubating tissues in appropriate media in order to analyze their normal functions. In addition, Treherne and Schofield (1979) discussed the idea of glial cells providing a stable fluid environment, distinct from the hemolymph in its ionic composition, around the nervous system of insects. We believe that, in the *Blattella* follicle, the epithelium may play a similar role by either actively or passively influencing the concentrations of ions, and perhaps other substances, within the space enclosing the oolemma.

Indeed, although a rudimentary asymmetric pattern was seen in some defolliculated oocytes, it is not possible to reject a role of the follicle cells in influencing the more clearly defined patterns and larger intensities of currents recorded from more mature oocytes. The follicle cell epithelium could, for example, present a barrier to current flow with regional differences in resistance; in fact, Zhang and Kunkel (1992) found a greater patency in the ventral follicular epithelium than in the dorsal epithelium. Or, the epithelium could generate currents itself, or contribute metabolically to the microenvironment between the oocyte and the epithelium and actually enhance the oocyte's capability to generate currents, or it could engage in some combination of these functions.

Investigators of meroistic insect follicles have used partial defolliculation as a way to determine the individual contributions of germ cell, nurse cells, and epithelium to extracellular currents recorded with the vibrating probe. From their studies on polytrophic meroistic follicles, Bohrmann *et al.* (1986) and Woodruff *et al.* (1986) suggested that the follicle cell epithelium may influence extracellular currents. Woodruff *et al.* (1986), using *Hyalophora*, found that the extracellular currents generated by intact follicles are typically inward at the trophic cap containing the nurse cells and outward at the oocyte. However, when a part of the epithelium overlying the nurse cells was removed, outward current was recorded in the region of the nurse cells. Anatomical studies indicated that the epithelium of the trophic cap could provide a high resistance barrier to current flow. On the basis of their observations, these investigators proposed that, in intact follicles, outward currents generated by the nurse cells are prevented by the epithelium from flowing out of the anterior end of the follicle and instead are directed posteriorly toward the oocyte through lower resistance extracellular pathways. They also suggested that the inward currents recorded from intact follicles may be produced by the epithelial cells.

Recently, Diehl-Jones and Huebner (1992) removed the epithelium from different regions of the telotrophic meroistic follicles of *Rhodnius*. They found a slight reduction in intensity, but not in direction, of extracellular currents recorded over defolliculated regions of the terminal oocyte. Currents recorded over the trophic cord were similar in both intact and partially defolliculated preparations. Currents over the apical tropharium were inward in both intact and defolliculated conditions. However, currents at the base of the tropharium were outward over a broader region in the absence of the epithelium, so that outward currents emanated from regions typically characterized by inward currents in the intact condition. Diehl-Jones and Huebner (1992) suggested that the basal lamina overlying the epithelium in this region of the follicle may present a resistance to outward currents which are then shunted toward more basal regions of the tropharium. They also pointed out that it is simply not known if the *Rhodnius* follicle cell epithelium presents sufficient resistance to current flow to direct extracellular currents.

Using *Drosophila*, Bohrmann *et al.* (1986) observed that, when a patch of epithelium was removed from over the nurse cells, the extracellular currents recorded in the defolliculated region remained inward. However, in the one experiment in which a patch of epithelium was removed from the surface of the oocyte, no current was recorded over the exposed oolemma, although outward currents were recorded from regions covered by epithelium. They interpreted this result to mean that the epithelium itself generated the outward extracellular currents. In other experiments, Bohrmann *et al.* (1992) used mutants of *Drosophila* that produce follicles lacking an oocyte. In early stages of development morphological features indicated that the follicular epithelium retains anterior–posterior polarity. However, at later stages, this organization is lost. Current vectors recorded from later stages had current densities similar to wild-type follicles, but only two of the six follicles tested had wild-type patterns of vectors. These results suggest

that the oocyte is required to maintain the organization of the entire follicle.

In *Blattella,* the range of current intensities was similar in both defolliculated and intact preparations, except for two intact follicles with unusually large current intensities (Fig. 4). It is possible that the follicle cells may have played some functional role in the generation of these large currents. In addition, there were several intact preparations with slightly greater intensities (1–2 $\mu A/cm^2$) than the defolliculated preparations (Fig. 4). Although a contribution by the follicle cell epithelium to these slightly greater intensities can not be ruled out, an alternative explanation is that there was a tendency to keep the probe at a greater distance from the unprotected surfaces of the defolliculated oocytes. Models show that a difference in probe distance of some tens of micrometers from the source can yield a sharp drop in the intensity of currents (Kunkel and Bowdan, 1989).

It is also of interest that the follicle cell epithelium begins to adhere tightly to the ventral aspect of the oocyte close to the time that the asymmetric pattern of extracellular currents becomes apparent—the beginning of mid-vitellogenesis. The tight adherence of the follicle cells to the presumptive ventral aspect of the *Blattella* oocyte may be the beginnings of coupling to the oocyte by means of gap junctions. Woodruff and his coworkers have reported that dye coupling between the oocyte and follicle cells appears near the onset of vitellogenesis in *Oncopeltus* (Woodruff and Anderson, 1984) and *Hyalophora* (Woodruff and Telfer, 1990). Verachtert and DeLoof (1989) reported dye coupling in *Sarcophaga,* but not in *Manduca* and *Drosophila.* However, in their ultrastructural study of *Drosophila,* Giorgi and Postlethwait (1985) reported the presence of gap junctions on microvilli between oocyte and follicle cells correlated with the onset of vitellogenesis. Thus, in several insects, there is evidence of the development of gap junctional contacts between oocyte and follicle cells near the beginning of vitellogenesis.

Gap junctions between oocyte and follicle cells have also been reported in several mammals (Anderson and Albertini, 1976) and in the amphibian *Xenopus* (Browne *et al.,* 1979; Dumont and Brummett, 1978; Browne and Werner, 1984). In *Xenopus,* very interesting functional interactions between the oocyte and follicle cells have been investigated. Supplisson *et al.* (1991) have provided data that are consistent with a Na/Ca exchanger located in the follicle cells of *Xenopus.* They propose that Ca ions brought into the follicle cells move into the oocyte through gap junctions. Thus, the intraoocyte [Ca] is regulated by the exchanger located in the follicle cells. Such an integrated association regulating ionic fluxes between two compartments might also be found in developmental stages of *Blattella* follicles later than those from which we were able to remove the epithelium. Further, in a series of voltage-clamp experiments, Miledi and Woodward (1989a,b) showed that outward potassium currents elicited from intact *Xenopus* follicles were consistently abolished by defolliculation, although oscillatory chloride currents were maintained in defolliculated oocytes. The K currents were elicited by bath applications of a variety of neurotransmitters and hormones, including catecholamines, gonadotrophins, and vasoactive intestinal peptide (Miledi and Woodward 1989a) as well as prostaglandins, oxytocin, and atrial natriuretic factor (Miledi and Woodward, 1989b). Miledi and Woodward concluded that the K currents generated in the follicle cells could be recorded in the oocyte due to electrical coupling between these cells. The chloride currents, generated by the oocyte itself, were elicited by muscarinic agonists and several divalent cations (Miledi and Woodward, 1989a) as well as angiotensin II (Woodward and Miledi, 1991). These results indicate a clear dichotomy of placement of receptor molecules and channels involved in the generation of electrical responses by *Xenopus* follicles.

Although not yet investigated directly in any insect, the possibility of the follicle cell epithelium generating currents, possibly in close association with the oocyte, is not unreasonable. For example, at the beginning of vitellogenesis the follicle cells of *Rhodnius* respond to juvenile hormone by actively pumping out fluid in order to reduce their volume and create intercellular spaces (patency) through which vitellogenin can move to reach the oocyte (Abu-Hakima and Davey, 1977). Juvenile hormone stimulates a ouabain-sensitive Na-K-ATPase (Ilenchuk and Davey, 1982, 1987a,b) by activating protein kinase C (Sevala and Davey, 1989, 1990) to trigger the change in cell volume. One could speculate that an electrogenic Na-K-ATPase would generate net outward currents from the follicle cells and that a preparation in which the follicle cells of one region were more active than those of another region would generate asymmetric extracellular currents that may be detectable by the vibrating probe. In *Rhodnius,* the follicle cells are connected to the oocyte and to each other by gap junctions (thereby establishing ionic continuity); moreover, the anterior follicle cells, which are not altered in shape during vitellogenesis, differ in morphology from the lateral follicle cells (Huebner, 1984), further supporting the possibility of different functional capacities.

In *Blattella,* a constellation of features leads up to mid-vitellogenesis. These include initially the germinal vesicle coming to rest at the presumptive ventral surface (Wheeler, 1889). Extracellular currents are at first small in magnitude, with an apparent sink at the GV and variously directed on the apo-GV aspect. As the follicle reaches about 1 mm in length, the follicle cells begin to adhere tightly to the oolemma. Further, the follicle cell epithelium, at first morphologically homoge-

neous, becomes polarized in structure, with the dorsal cells columnar in shape and closely apposed and the ventral cells flattened in shape, with arm-like extensions connecting adjacent cells across large intercellular spaces (Zhang and Kunkel, 1992). As the follicle increases in length, proceeding toward mid-vitellogenesis, the presumptive ventral surface becomes concave and a strong, broad efflux of currents from the apo-GV region flows toward a sink at the GV. These phenomena, taken in conjunction with the suggestion that a unique environment exists between the follicle cell epithelium and the oolemma, lead us to speculate that there is very likely a dynamic, and perhaps morphological, interaction between the oocyte and the follicle cells. Woodruff and Telfer (1990) have also commented on a variety of changes that occur together in *Hyalophora* follicles at the onset of vitellogenesis—a milestone in development of this organism.

Insect oocytes are useful models for studying polarity, because dorsoventral and anteroposterior axes are established prior to fertilization (Kunkel, 1991; Melton, 1991). It would be very helpful to approach the problem of polarity from two directions: to identify, clone, and sequence genes involved in developmental processes and to characterize the functions of gene products, which could include channels or pumps involved in generating steady extracellular currents. Great strides have been made using *Drosophila* mutants to study the molecular genetic basis of polarity (Slack, 1991, and Anderson, 1987, for overviews; Nusslein-Volhard *et al.*, 1987; Schupbach, 1987). Ideally, vibrating probe studies of extracellular currents on this preparation would complement the genetic work. However, the results of such studies are currently unclear.

Overall and Jaffe (1985) recorded significant extracellular currents generated by *Drosophila* egg chambers, with an inward direction at the nurse cells and outward at the oocyte. These directional currents were of interest in view of other studies arguing for (Woodruff *et al.*, 1988) and against (Bohrmann and Gutzeit, 1987) electrophoretic transport of materials from the nurse cells to the oocyte. Bohrmann *et al.* (1986) also used the vibrating probe to record extracellular currents from *Drosophila* follicles; while they saw a pattern of extracellular currents similar to that described by Overall and Jaffe (1985) in some preparations, they saw considerable variation from preparation to preparation, and they found much smaller intensities of currents. Sun and Wyman (1989) reported current vectors of very small intensity and no evidence of a consistent pattern of current flow. These contradictory observations may be the result of varying placement of the probe near the very small *Drosophila* egg chamber. *Drosophila* oocytes are about 150 $\mu$m in diameter—one-third the size of the smallest preparations described in this study. Or they could, as Bohrmann (1991) suggests, result from the use of different culture media. Interpretation is further complicated by the fact that three types of cells may all contribute to the generation of extracellular currents, and partial defolliculation experiments (Bohrmann *et al.*, 1986) have not fully solved that problem.

These complex results highlight both the value of the vibrating probe technique in detecting extracellular currents underlying integrated functions and the problem of interpreting currents recorded from structures composed of various types of cells. For example, in the case of the experiments on *B. germanica* described in this paper, we were able to record currents generated independently by the oocyte. We observed various patterns of currents, including the asymmetric pattern which is consistently, and more robustly, generated by intact mid-vitellogenic oocytes. However, we found a bathing medium containing a lower $[Ca]_o$ (1 m*M*) appeared to maintain the defolliculated oocytes for longer periods of time than did a medium containing a higher $[Ca]_o$ (4 m*M*). This observation suggests that the extracellular fluid in which the oocyte is normally bathed does not have the same composition as that of the hemolymph, and raises the important consideration of finding the appropriate medium in which to bathe any tissues that are found enveloped, or in laminar arrangements, *in situ*. Further, once the oocyte reached about 1 mm in length, we found it impossible to remove the follicle cell layer without injuring the oocyte, and therefore impossible to measure the independent functions of these intimately associated cells. Thus, to exploit the unique power of the vibrating probe, it will be important to continue to develop techniques (including partial as well as complete separations of different types of cells) to permit measuring the independent and interactive functions of closely associated cells.

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