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Elemental Composition of the Perivitelline Fluid in Early *Drosophila* Embryos

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Samples of the perivitelline fluid in the polar pockets of preblastoderm *Drosophila* embryos were analyzed with an electron microprobe, and the results compared with analyses of adult hemolymph. The concentrations of sodium, magnesium, calcium, chlorine, and phosphorus are about the same in these two fluids; but potassium and sulfur are three to four times higher in perivitelline fluid. Moreover, the concentrations of these elements in the anterior and posterior pockets of the same embryos were compared. The former five elements seem to be about 10% more concentrated in the anterior pocket; but the latter two show no significant difference between pockets.

INTRODUCTION

There is reason to believe that the establishment of pattern in early development (or really prepattern—see Stern (1968)) is a centripetal process beginning in or near the plasma membrane (Jaffe, 1969) and involving transcellular ionic currents (Jaffe, 1982). Hence, in order to understand the establishment of pattern, it is important to know the ionic composition of the extracellular milieu during this process. In the case of *Drosophila,* a large part of it seems to occur between oviposition and cellularization a few hours later (Schubiger and Wood, 1977; Illmensee, 1978). During this period, the extracellular medium is confined to the space between the vitelline membrane and the oolemma; a space only a fraction of a micrometer thick except in the two polar pockets (Fig. 1). These pockets form about 15 min after fertilization; disappear shortly after pole cell formation, about 2 hr after fertilization; are 10 to 20 μm deep, and contain about 30 to 140 pl of fluid (Imaizumi, 1958; Turner and Mahowald, 1976; Bownes, 1975). It is the fluid within these curious pockets that we have begun to analyze.

MATERIALS AND METHODS

Obtaining and preparing eggs. An Oregon-R wild-type strain of *Drosophila melanogaster* was kept at 25°C on an agar–cornmeal–yeast–sugar medium with propionic acid and methyl parasept as fungicides. We used a 12-hr light:12-hr dark cycle to secure a maximum number of eggs around the onset of darkness. Eggs were collected on black filter paper which was soaked with apple juice and also bore drops of yeast to encourage oviposition. Eggs collected in the first 2 hr were discarded. After collection eggs were handled at 15°C, except during desiccation (and chemical dechorionation), which were done at room temperature.

To prevent the formation of an exovate upon puncturing the vitelline membrane, the egg's turgor was reduced by desiccation or, in some cases, by puncture. Eggs to be desiccated were manually dechorionated on double-coated tape, because chemically dechorionated eggs took much longer to desiccate. Eggs were oriented with their dorsal or lateral side down on double-coated tape (Van Deusen, 1976) using a dissecting needle with a curved tip. They were then desiccated for 7–8 min over $CaSO_4$ at room temperature and covered with hydrated halofluorocarbon oil No. 56 (Halocarbon Products Corp.). This inert oil was hydrated by shaking with an equal volume of distilled water under which it was stored. Eggs treated in this way developed about as well as did controls.

Eggs to be punctured were dechorionated in 2.5% sodium hypochlorite and pipetted on to a plastic petri dish where most of the water was removed and oil added. They were then punctured halfway between their poles with a sharp glass needle mounted on a Leitz micromanipulator. While this method of turgor reduction has the disadvantage of preventing development, it does allow one to obtain much larger samples than does desiccation.

Obtaining polar pocket samples. Sampling pipets were pulled from thick-walled borosilicate capillaries (o.d., 1 mm; wall thickness, 0.2 mm) which were siliconized with an organosilane solution (Prosil-28, PCR Research Chemicals Inc.). Puller settings were chosen to produce

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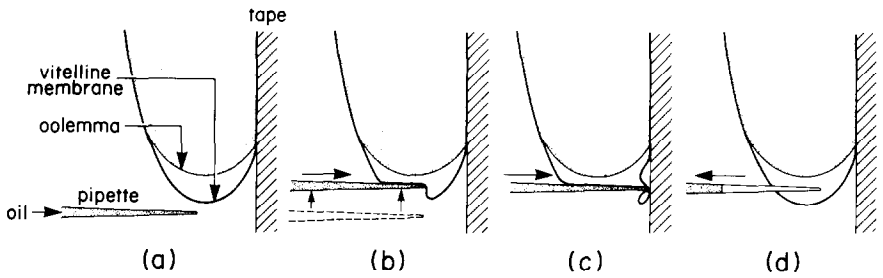

FIG. 1. Procedure for obtaining perivitelline fluid by puncturing the vitelline membrane surrounding a polar pocket. Side view of posterior egg pole. (a) Bring pipet precisely into the median plane of the egg. (b) Indent the vitelline membrane so as to minimize damage to the plasma membrane. (In practice, we were usually unable to avoid touching the latter; but generally avoided puncturing it. Data from punctured eggs are not included in this report.) (c) Puncture. In many cases the tape was a suitable substrate against which to puncture the vitelline membrane. Slight overpressure of oil in the pipet prevented perivitelline fluid from being sucked up too forcefully and the egg from being punctured as a result of its concomitant elongation. (d) Partial withdrawal of pipette and uptake of sample. During uptake the egg elongates and fills the polar pocket, so that the pipet has to be moved towards the tip of the pocket to collect the fluid without puncturing the egg.

an inflexible tip tapering gradually over about 5 mm. The tip was broken to produce a sharp-edged tip with an o.d. of 2-3 μm.

Leitz microinjection equipment, a Leitz micromanipulator, and a Zeiss inverted microscope were installed on an air-isolated table (Micro-g: Technical Manufacturing Co.). The injection system and pipets were filled with paraffin oil (Fisher 0-119) except for the pipet holder and a small part of the tubing. Without this air buffer, removal of the pipet from the holder resulted in air being sucked into the tip of the pipet and loss of the sample.

Reduction of turgor in the egg was such that the vitelline membrane became slightly loose and wrinkled and could be punctured as indicated in Fig. 1. After uptake of the sample, hydrated paraffin oil was taken up to protect the sample and its volume was estimated geometrically. In a typical experiment sample volumes ranged from 30 to 130 pl and averaged about 60 pl.

Each pipet was immediately stored in a holder (W.P.I. Model E2 micropipet storage receptacle for o.d. 1 mm) on dry ice, to prevent concentration of the sample (see Lechene and Warner, 1979). The pipets were later shipped for analysis to the National Biotechnology Resource in Electron Probe Microanalysis, Harvard Medical School, Boston, Massachusetts (Dr. C. P. Lechene, director).

Sampling adult Drosophila hemolymph and human serum albumin. Hemolymph was collected from 17 days (25°C) old adults which were CO_2 anesthetized, rinsed in distilled water, and mounted with their back on double-coated tape. A slit was cut in the ventral abdomen with a sharp sterile tungsten needle, and hemolymph was collected with a 1-μl capillary. Freeze-dried human Ortho normal control serum (Ortho Diagnostics) was made up to a volume in distilled water. A drop of hemolymph or serum was then expelled under hydrated fluorocarbon oil and subsequently collected and treated

TABLE 1
RATIOS OF APPARENT CONCENTRATIONS IN UNDILUTED: 3× DILUTED; AND 3×:6× DILUTED SAMPLES OF PERIVITELLINE FLUID[a]

Ratio of dilutions	K	Na	Mg	Ca	Cl	P	S
1×:3× (n = 5)	1.79 ± 0.10	1.58 ± 0.07	1.71 ± 0.03	1.62 ± 0.11	1.73 ± 0.10	1.90 ± 0.10	1.59 ± 0.15
3×:6× (n = 7)	1.87 ± 0.10	1.68 ± 0.08	1.94 ± 0.13	1.72 ± 0.15	1.76 ± 0.10	2.06 ± 0.12	1.95 ± 0.14

[a] Ratios are averages ± standard error of the mean.

TABLE 2
COMPARISON OF ELECTRON MICROPROBE AND MACROSCOPIC ANALYSES OF HUMAN SERUM ALBUMIN (mM)

	K	Na	Mg	Ca	Cl	P	S
Microprobe[a]	4.2 ± 0.2	136 ± 0.4	0.7 ± 0.2	2.3 ± 0.2	119 ± 3	3.7 ± 0.5	13.3 ± 0.5
Macro[b]	4.3 ± 0.1	139 ± 0.4	1.0 ± 0.03	2.5 ± 0.02	107 ± 1	1.2 ± 0.02	—

[a] Average ± SEM of 10 samples.

[b] From data sheets provided by the commercial supplier. Phosphorus is higher in our analysis than the macroscopic ones because the latter measured only inorganic phosphorus while ours measured total phosphorus.

TABLE 3
ELEMENTAL COMPOSITION OF *Drosophila* EGG PERIVITELLINE FLUID AND OR ADULT *Drosophila*-HEMOLYMPH (m*M*)

	Perivitelline fluid via microprobe[a]	Adult hemolymph via Microprobe[b]	or Macro method[c]
K	84 ± 7	25 ± 2	24 ± 1
Na	98 ± 6	106 ± 7	87 ± 2
Mg	16.5 ± 1.5	14.4 ± 0.8	26 ± 2
Ca	5.0 ± 0.3	7.2 ± 0.8	10.6 ± 0.5
Cl	62 ± 3	58 ± 3	
P	41 ± 2	39 ± 5	
S	30 ± 1	7.3 ± 0.4	

[a] Average of 38 samples from both polar pockets ± SEM.
[b] Average of eight pairs of samples ± SEM.
[c] Atomic absorption (from Larrivee, 1979).

as samples of perivitelline fluid. The hemolymph samples did not contain cells (mag. 320×).

Preparation of samples for electron probe analysis. Before expulsion of samples pipets were transferred from −80 to −20°C and stored overnight. Direct transfer to room temperature occasionally resulted in a breakage of pipets or "spontaneous" expulsion of the sample. For expulsion and subsequent sample preparation: see Lechene (1974).

Protein can interfere with quantitative electron probe analysis of elements by absorbing soft X rays and thereby artificially lowering the intensity of characteristic X-ray emission from protein-containing solutions versus standard solutions that are protein free (Lechene and Warner, 1979). We tested their procedure of two- or threefold dilution, which gave satisfactory results for a solution containing 70 g/liter bovine serum albumin, on our samples of perivitelline fluid, the protein content of which is unknown. The ratio (as averaged over all elements) of apparent elemental concentration of undiluted samples to threefold diluted samples proved to be 1.70 ± 0.04 (n = 35) rather than 3.0 indicating a large error due to absorption by proteins in the undiluted samples; however, when these threefold diluted samples were further diluted by twofold, the average ratio proved to be 1.85 ± 0.05 (n = 49), not far from the expected value of 2.0. A breakdown of the small residual protein error is provided in Table 1. It will be seen that significant errors—of about 15%—remained for Na, Ca, and Cl. However, we elected to proceed with the routine use of threefold dilutions to maintain a large enough total signal.

As a further check on the reliability of our method, we included coded control samples of human serum albumin in our shipments. Our results agreed satisfactorily with those of macroscopic analysis (Table 2).

RESULTS AND DISCUSSION

Table 3 shows our main result: Perivitelline fluid proves to have about the same elemental composition as adult hemolymph except for potassium and sulfur. The latter two elements are three to four times more concentrated in perivitelline fluid than in hemolymph. Table 3 also includes a comparison with Larrivee's (1979) analysis—using atomic absorption—of one microliter samples of adult hemolymph, i.e., samples 10,000 or more larger than the samples we analyzed. The general agreement between Larrivee's and our values may be taken as a further indicator of our methods' reliability. We also obtained very similar results with samples obtained from eggs depressurized via puncture instead of desiccation. Puncture depressurization may provide a way of obtaining larger, yet relatively natural samples of perivitelline fluid despite the fact that it produces such gross damage as to block development.

Included in the 38 samples averaged in Table 3 are 32 from 16 eggs in which it proved possible to obtain and analyze a sample from *both* the anterior and the posterior polar pockets. In Table 4 we present the average ratios of measured compositions between these pockets. Sodium, magnesium, calcium, chlorine, and phosphorus all seem to be about 10% more concentrated in the anterior than in the posterior pocket; while potassium and sulfur do not differ significantly between the two pockets. The probability that the apparent deviations from equality (for sodium, etc.) are due to sheer chance varies from about 1% for chlorine to 16% for phosphorus. If the apparent differences of sodium, etc., are real—and not due to chance or some subtle artifact of measurement—then they are probably maintained by a substantial ionic current through the egg; presumably one similar to the steady currents generally found to traverse developing systems.

TABLE 4
RATIOS OF ANTERIOR:POSTERIOR POLAR POCKET COMPOSITIONS[a]

K	Na	Mg	Ca	Cl	P	S
1.03 ± 0.05	1.08 ± 0.05	1.15 ± 0.07	1.10 ± 0.06	1.10 ± 0.04	1.07 ± 0.05	1.02 ± 0.04

[a] Average ± SEM of 16 pairs of samples.

TABLE 5
ANALYSES OF EXTRACELLULAR *Drosophilia* FLUIDS (m*M*)

	Egg	Larva[a]	Adult
K	84	40-55	25
Na	98	57-63	106
Mg	17	21-33	14
Ca	5	6-8	7
Cl	62	42-?	58

[a] First figure is from Begg and Cruickshank (1963); second from Larrivee (1979).

Finally, in Table 5 we compare our results for perivitelline fluid and adult hemolymph with previous analyses of last instar larval hemolymph. By far the sharpest change during the developmental cycle is the three- to fourfold rise of potassium in going from the maternal hemolymph to the perivitelline fluid.

These data should prove valuable in interpreting electrical measurements on *Drosophila* eggs; particularly measurements of natural voltage gradients along this egg. They may also prove valuable in interpreting electron microprobe studies of frozen hydrated sections of these eggs.

The elemental analysis were carried out at the National Biotechnology Resource in Electron Probe Microanalysis, Harvard Medical School, Boston, Massachusetts. We would like to thank its director, Dr. Claude Lechene, for his patient help in this process. We would also like to thank Ms. Mona Lambert for her skillful technical assistance. This work was supported by NSF grants to L.F.J.

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