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A potential difference across mouse ovarian follicle

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Summary. A small potential difference (antrum positive) has been measured with fine-tipped glass microelectrodes across the epithelial cell layers of the mouse ovarian follicle wall. As ovulation approached the potential in the antrum became more positive compared to the outside. Metabolic inhibitors and locally active hormones also altered the potential difference. The ionic basis and the significance of the potential difference are unknown.
Key words. Mouse; ovarian follicle; potential difference; ovulation.

A potential difference (PD) and a flow of fluid commonly develop across epithelia as a result of active ion transport by epithelial cells. The cell layers of the wall of the mammalian ovarian follicle form a compound epithelium. I have found in mouse, that a very small PD exists across a follicle wall. This PD becomes more positive inside as ovulation approaches, becomes more positive with inhibition of active ion transport processes and shifts in a negative direction after exposure to prostaglandins of the E and F series.

Methods. Naturally cycling and hormonally primed mice of the Porton strain were used. Vaginal smears were taken daily to determine the stage of the natural cycle. Hormonally primed animals were injected i.p. with 5 IU pregnant mare's serum gonadotrophin (PMSG) and 48 h later with 5 IU human chorionic gonadotrophin (HCG), to induce superovulation 11–13 h later[2]. Most experiments were made using an in vitro preparation. (The same results were obtained from experiments in vivo.) Mice were anesthetized with an i.p. injection of urethane. An ovary and adjacent fat was excised and pinned to the Sylgard base of a perspex bath through which pre-warmed (33 °C) physiological salt solution flowed. The composition of the superfusate was, mM/liter: Na Cl, 145; K Cl, 4.7; Mg SO_4, 1.2; K H_2PO_4, 1.2; Ca Cl_2, 2.5; glucose, 10. It was buffered with tris maleate and the pH adjusted to 7.4. Microelectrodes filled with 3 M KCl with tip potentials < 10 mV and resistances of 10–40 MΩ were used for voltage recording. The probing microelectrode mounted on a step-motor was advanced in 1 μm steps (2/sec) through the follicle wall and into the fluid-filled antrum. Potential measurements were amplified and displayed in the convential manner. Results are expressed as mean ± SE of the mean (SEM).

Results. A microelectrode traversing the follicle wall records several negative-going intracellular potentials as it pushes through the cells in its path. A steady potential arises when the fluid-filled antrum is entered and this is maintained as the electrode continues to move through the follicular fluid (fig. 1a). This latter is the trans-follicle wall potential difference (FWPD). To account for variations in follicular anatomy, I define this as any stable potential value recorded with the tip of the electrode more than 60 μm deep to the follicular surface and maintained during continued forward travel of the microelectrode for at least 50 μm.

The FWPD values showed considerable scatter in naturally cycling animals (fig. 1b) and had a mean of $+1.2 \pm 0.3$ mV ($n = 194$). This is significantly different from zero ($p < 0.001$: modified Student's t-test; Bailey, 1981)[3]. No difference was obvious between different stages of the cycle. In hormonally primed animals, 12 h post-HCG, that is around the time of ovulation, FWPD had increased to $+3.8 \pm 0.8$ mV ($n = 19$; fig. 1c). This is significantly different from the mean of the naturally cycling group ($p < 0.01$: modified Student's t-test; Bailey, p. 48, 1981)[2].

Pharmacological experiments. 1) *Sodium pentobarbitone* (10 mM/liter, substituted for equimolar sodium chloride). Six follicles from two animals both hormonally treated and immediately pre-ovulatory were tested. Sodium pentobarbitone in the perfusate caused a small positive going shift in FWPD, ranging from +1 mV to +4 mV, in all follicles (fig. 2a). The mean response was $+2.4 \pm 0.6$ mV. The new potential remained steady until the drug was washed out, as much as 10 min later, and the original value was re-established.

2) *Sodium cyanide* (5 mM/liter, replacing equimolar sodium chloride). Eight follicles were tested from two animals in the estrus stage of the natural cycle. In all cases sodium cyanide caused a small positive shift in FWPD. Responses ranged from 0.5 mV to 1.5 mV with a mean ± SEM of $+1.0$ mV ± 0.1 mV (fig. 2b). The effect was reversible.

3) *Oubain* (10^{-3} M/liter). Eight follicles from four animals were examined. Three of the animals had been primed with hormones and were immediately pre-ovulatory, the other animal was in estrus. Seven of the eight follicles showed positive shifts in FWPD shortly after exposure to oubain. Responses ranged from +1 mV to +7 mV, with a mean value of $+2.0 \pm 0.6$ mV. The potential shift was reversed on wash-out.

4) *Prostaglandin* E_1. A concentration of 1×10^{-5} M/liter was ineffective. 7×10^{-5} *M/liter.* Sixteen follicles from six naturally cycling mice were tested. Three animals were in metestrus, one in diestrus, one in pro-estrus and one in estrus. In fourteen of the sixteen follicles, a small negative shift in FWPD was seen. This ranged from -1 mV to -10 mV, with a

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mean ± SEM of −3.6 ± 0.8 mV (fig. 2c). In some follicles the follicle wall resistance (measured by recording the voltage induced by a current pulse injected into the antrum from a second microelectrode) was abolished while the perfusate contained prostaglandin E_1 (fig. 2c). Both potential and resistance changes were reversed when prostaglandin E_1 was removed.

5) *Prostaglandin $F_{2\alpha}$* (7×10^{-5} M/liter). Five follicles from two diestrus animals were tested. All showed small negative-going shifts in FWPD, with a range from −1 mV to −3 mV. The mean (±SEM) change was −1.8 ± 0.4 mV (fig. 2d). This solution had no effect on follicle wall resistance.

Discussion. The composition of mouse follicular fluid is unknown; that of other species resembles plasma[4]. The FWPD in naturally cycling mice was very small and could simply represent a diffusion potential. However, the metabolic inhibitors used (sodium pentobarbitone, sodium cyanide and ouabain) which would block any active ion transport occurring in the epithelial layers of the follicle wall, all caused an increase in internal positivity of the FWPD. Moreover, as ovulation approached there was an increase in FWPD of a similar size and polarity to that seen with metabolic inhibition. One explanation would be a switching off, or a loss, of some cells normally involved in active ion transport in the follicle wall as this structure thinned down before rupturing at ovulation. The FWPD therefore may involve an active ion transport component.

The concentration of several prostaglandins increases dramatically during the preovulatory period, though the significance

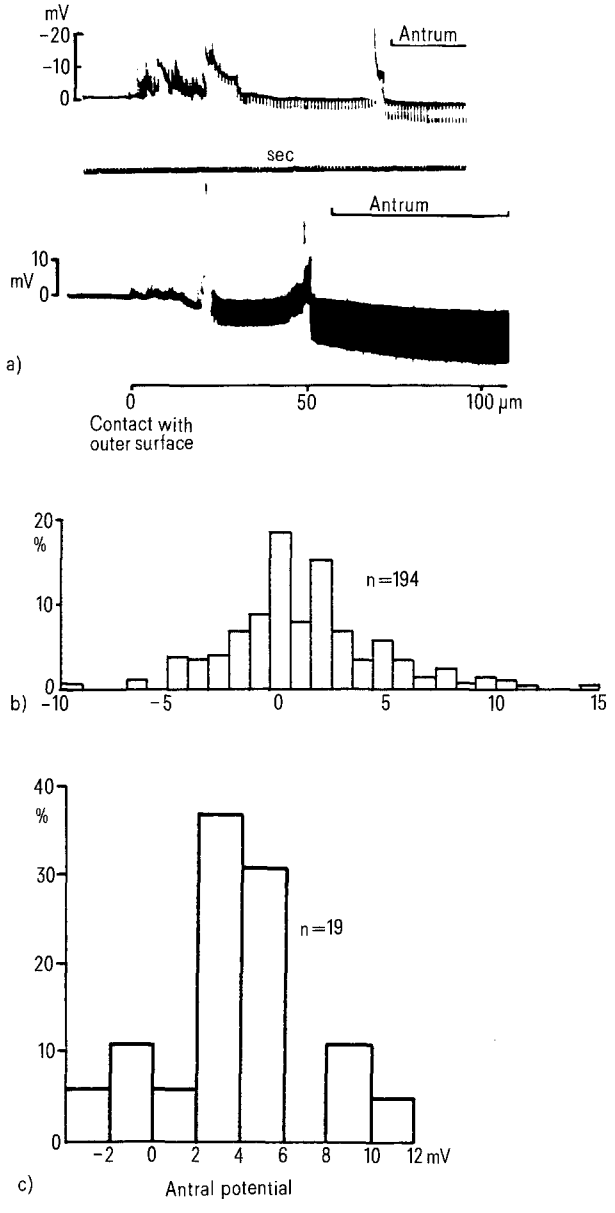

Figure 1. *a* Two examples of recordings made by a microelectrode traversing a follicle wall and entering the fluid-filled antrum. Several intracellular (negative-going, upwards deflections) potentials are seen before a steady, slightly positive potential is attained and held despite continued electrode progress. The electrode is in the antrum. In each case, a second electrode was passing positive current pulses, 1/sec, into the antrum resulting in the downward deflexions. These give a measure of the resistance of the follicle wall. In the lower trace, individual pulses have been blacked in: the upper margin of the trace is FWPD, lower margin, current induced deflection of PD. *b* Distribution of FWPD values from animals at all stages of the natural cycle. *c* Distribution of FWPD values from hormonally treated animals; immediately preovulatory, (48 h post PMSG + 12 h post HCG).

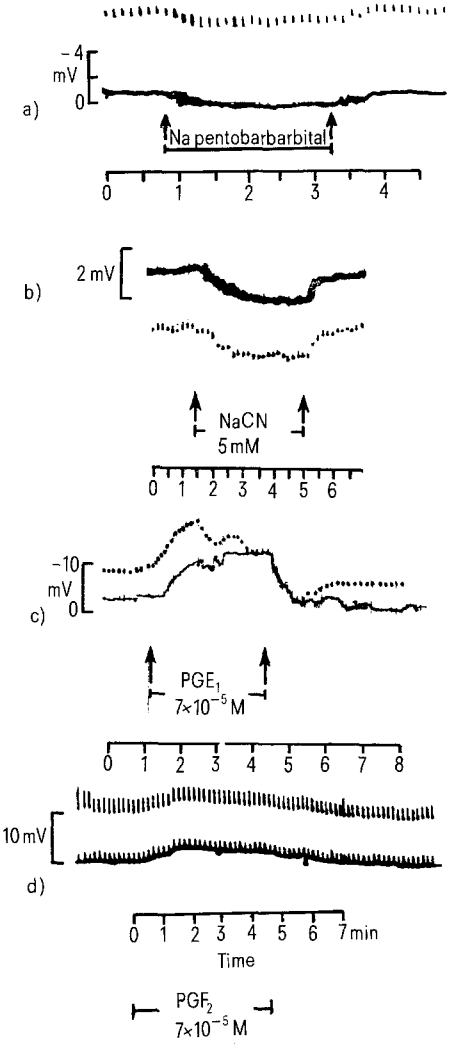

Figure 2. Potential shifts above a baseline are negative-going, below a baseline, positive-going. *a* Effect of sodium pentobarbitone, 10 mM/l, on FWPD. Regular voltage deflexions resulting from current pulses also are present and show that resistance remains unchanged. *b* Effect of sodium cyanide on FWPD. There is no effect on follicle wall resistance. *c* Effect of prostaglandin E_1 on FWPD and follicle wall resistance. Wall resistance (the current induced voltage deflexions) drops almost to zero within 4 min and recovers on washout. *d* Effect of prostaglandin F_2 on FWPD. There is no effect on follicle wall resistance.

of this to the ovulatory process remains unclear[5]. I have found a large negative shift in FWPD and a reversible abolition of wall resistance on exposure to prostaglandin E_1. The ability to alter the FWPD with agents involved in the mechanism of ovulation suggests a possible significance for this PD in these processes. The change in resistance indicates that some resistive barrier to current flow exists in the follicle wall and that the functional characteristics of this structure can be reversibly altered pharmacologically. In other epithelia, specialized intercellular contacts (Zonulae occludentes) form such a resistive barrier and play an important role in the accumulation of fluid brought about by active ion transport[6].

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Negative phototaxis in *Drosophila* associated with a morphological change in the compound eye

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Summary. The ultrastructure of the compound eyes of several photonegative selection lines and their unselected photopositive controls of five species of the *melanogaster* subgroup was analyzed. A qualitative phenotypic change concerning the rhabdomeres in one of the photonegative selection lines of *D. mauritiana* could be detected. It was proved that this structural aberration of the rhabdomeres is caused by a parallel mutation of the mutant *ora (outer rhabdomeres absent)* of *D. melanogaster*.
Key words. Drosophila melanogaster subgroup; phototaxis, negative; eye, compound.

Numerous behavioral traits are accessible to selection but little is known about the structural basis related to the selection response, especially if polygenic systems are involved[1]. E.g., phototactic behavior could be influenced by changing the morphology of the eye or brain, by altering the neural connections or the information processing, by changing the motoric response, and/or by varying the biochemical reactions which participate in these processes[2,3]. In order to investigate altered phototactic behavior it seems obvious to look in a first step for morphological changes in the compound eye. Therefore, the ultrastructure of the eyes of several photopositive and photonegative strains of five *Drosophila* species was analyzed.

Materials and methods. Five species of the *Drosophila melanogaster* species subgroup were involved in the study: *D. melanogaster, D. simulans, D. mauritiana, D. yakuba,* and *D. erecta.* For each of these species there existed a photopositive control and two photonegative selection lines. Phototactic behavior was measured and selected in Hirsch-Hadler mazes (for a detailed description of these mazes see Köhler[4]). The selection had been carried out for 45 generations and had attained mean photoscores, i.e. number of light choices, of less than five in all negative lines, and greater than 11 in the positive lines (e.g. in *D. mauritiana,* fig. 1).

The preparation of the eyes for electron microscopy was as follows: fixation in glutaraldehyde and osmium tetroxide, dehydration in ethanol series, propylene oxide, and embedding in araldite.

Results and discussion. Except for one strain there exist neither remarkable changes in the eye structure in photopositive and negative flies nor distinct differences between species. In one of the negative lines of *D. mauritiana,* N316, the rhabdomeres are strongly degenerated in the distal area of an ommatidium (fig. 2). In the proximal parts of the ommatidium the degeneration of the rhabdomeres 1 to 6 increases until the rhabdomeres disappear completely.

The system of the rhabdomeres 7 and 8 remains intact and the structure is similar to that of the *D. melanogaster* mutants ora and rdgB[5,6]. Compared to the wildtype eye the mutant rhabdomeres 1 to 6 are much smaller and of irregular shape. The degree of degeneration between neighboring ommatidia and neighboring rhabdomeres within an ommatidium varies from slight to nearly complete absence. Degenerated rhabdomeres are found in each of the ommatidia of an eye. Females kept in light and dark adapted females express the trait. No diurnal rhythm was found (preparation at 05.30, 12.00 and 18.00 h) and the trait is expressed during the whole life (preparations from the day of eclosion to an age of 13 days).

Because of the phenotypic similarity of this structural aberration to the mutations *ora* and *rdgB* of *D. melanogaster* crosses were performed between these two mutants and the *D. mauritiana* negative and control lines. Crosses of *D. melanogaster* females and *D. mauritiana* males are possible and give rise to female offspring only. The F_1-hybrids of *D. melanogaster ora* females and males of the negative line N316 had the mutant trait, while the progeny of crosses of *D. melanogaster ora* females and males of control line of N316 looked normal. The mutation in *D. mauritiana* must be a parallel mutation to *ora.* It is highly probable that the mutation occurred spontaneously during selection. This hypothesis is supported twice: Firstly, there was no selection response in the negative direction in the

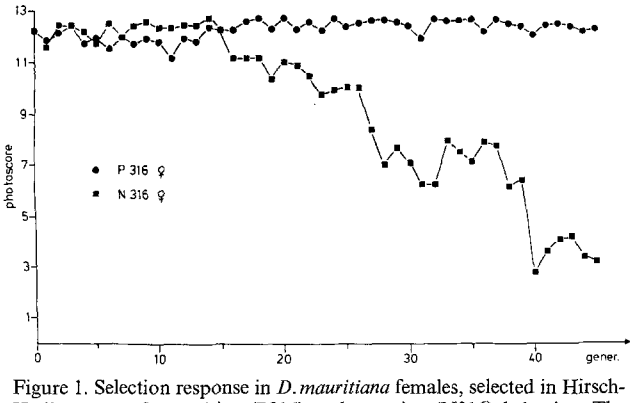

Figure 1. Selection response in *D. mauritiana* females, selected in Hirsch-Hadler mazes for positive (P316) and negative (N316) behavior. The mean number of light choices (photoscores) are plotted against the generations of selection.