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Analytical and experimental studies on the relationship between Na^+, K^+, and water uptake during volume increases associated with *Fundulus* oocyte maturation in vitro

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Summary. Oocytes of marine and estuarine teleosts often undergo pronounced volume increases during the maturation phase of development that precedes ovulation and fertilization. To examine the physiological correlates of these volume increases, prematuration follicles of the saltmarsh teleost, *Fundulus heteroclitus,* were cultured in vitro with a maturation-inducing steroid (17α-hydroxy-20β-dihydroprogesterone). Mean follicle volume rose significantly (75%) during a 40-h incubation period. Similar to the situation previously found in vivo, uptake of water by the maturing follicle was responsible for this volume increase in vitro, with the water content increasing from 62% to 78% of the total follicle mass. The follicle contents of two probable osmotic effectors – Na^+ and K^+ – also rose, the increase in K^+ being twice that of Na^+. The influx of K^+ even exceeded water uptake, resulting in a net increase in the concentration of this cation. It thus appears that the influx of these cations, in particular K^+, is a major cause of the uptake of osmotically obligated water and subsequent volume increase experienced by maturing *F. heteroclitus* follicles. In a search for operant mechanisms, it was found that follicle hydration, but not maturation, was strictly dependent on external K^+ in a concentration-dependent manner. The mechanism by which K^+ accumulates in the follicle was insensitive to ouabain, so that a typical Na^+,K^+-ATPase mechanism does not appear to be involved. The ability of external K^+ to promote follicle hydration was gradually lost during the maturation process as the oocyte dissociated from the surrounding granulosa cells in preparation for ovulation. Removal of all associated somatic cells prior to maturation prevented subsequent steroid-initiated hydration but not maturation. The results suggest that K^+ may be translocated from surrounding granulosa cells to the oocyte via gap junctions during maturation.

Key words: Cell volume regulation – K^+ transport – Meiotic maturation – Oocyte hydration – Killifish *Fundulus heteroclitus*

Abbreviations: GVBD, germinal vesicle breakdown

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Introduction

Teleost oocytes frequently undergo a significant increase in volume immediately prior to ovulation and fertilization (Wallace and Selman 1981). The proximal cause for this volume change appears to be a rapid uptake of water during the maturation phase of oocyte development (Fulton 1898; Milroy 1898; Watanabe and Kuo 1986; Craik and Harvey 1987; Greeley et al. 1991) when the oocyte overcomes a meiotic arrest at prophase I (which has lasted throughout the previous primary and vitellogenic growth periods) and proceeds to metaphase II to await fertilization (Masui 1985).

Water uptake by the maturing oocyte is most pronounced in marine teleosts in which it apparently functions to increase the buoyancy of spawned eggs, enabling them to become pelagic [i.e., float in full-strength seawater (Fulton 1898; Craik and Harvey 1987)]. Oocytes of estuarine teleosts with demersal or non-floating eggs often undergo a similar, but less pronounced, hydration that appears insufficient to achieve buoyancy (Greeley et al. 1986b). In the case of the saltmarsh killifish, *Fundulus heteroclitus,* it has been suggested that water uptake during oocyte maturation ensures that the fertilized egg or embryo – which is deposited during high spring tides in protected substrates remaining above the waterline during the intervening neap tides – will have sufficient water reserves for embryonic development in such an uncharacteristically arid environment (Greeley et al. 1991).

The water uptake and subsequent volume increase experienced by the maturing teleost oocyte are reminis-

cent of the regulatory volume increase that occurs in many somatic cells following shrinkage in hyperosmotic media [see review by Gilles (1983)]. These processes obviously differ in that the volume increase of the maturing oocyte is developmental in nature and hormonally regulated, while regulatory volume increase occurs in response to cell shrinkage brought about by hyperosmotic stress. However, the physiological mechanisms by which water uptake and volume increases occur may be similar in each situation. In somatic cells, regulatory volume increase is often associated with an intracellular rise in either free amino acids or inorganic cations such as Na^+ and K^+ followed by osmotically obligated water (e.g., King and Goldstein 1983; Vislie 1983; Deaton 1987; Green et al. 1988). Similarly, the generation of free amino acids or the influx of inorganic solutes may be the driving force behind maturation-associated water uptake by teleost oocytes, since volume increases during maturation have been associated with an intracellular elevation of either free amino acids (Craik and Harvey 1987) or inorganic cations (Hirose and Ishida 1974; Hirose 1976; Babiker and Ibrahim 1979; Craik and Harvey 1984, 1987; Watanabe and Kuo 1986; Greeley et al. 1991; LaFleur and Thomas 1991).

In *F. heteroclitus* follicles, volume increases of over two-fold are common during maturation in vivo (Wallace and Selman 1978), with most of this gain being attributable to water uptake (Greeley et al. 1991). An influx of K^+ and Na^+ observed to occur during oocyte maturation in vivo may provide the primary osmotic stimulus for water influx, while the role of free amino acids is minimal in this species (Greeley et al. 1991). In the present study, this hypothesis is tested by examining changes in follicle volume, water, Na^+, and K^+ during the maturation of *F. heteroclitus* follicles in vitro. It is possible to reproduce most of the water and volume increases typical of oocyte maturation in vivo, noting a correlation between a rise in follicle cations (especially K^+) and both volume and water gains by maturing follicles. Possible mechanisms of follicle hydration are investigated, including the role of external K^+ and associated somatic cells. Preliminary notes on some results have already been published (Greeley et al. 1987b; McPherson et al. 1987, 1989).

Materials and methods

Female *Fundulus heteroclitus* were trapped from salt marshes in the vicinity of the Whitney Laboratory near St. Augustine in Northeast Florida. Fish were maintained in indoor tanks supplied with a continuous flow of seawater, and fed a liberal diet of flake food (Nutrafin) and chopped shrimp (Lin et al. 1989).

Follicles containing prematuration oocytes (1.3–1.5 mm in diameter) were dissected free of adhering ovarian tissue and placed in a 75% solution of L–15 culture medium containing glutamine (Sigma) according to Greeley et al. (1986a). After equilibration in culture medium for 1 h, follicles were transferred to 35 × 10 mm petri dishes containing 3 ml fresh medium. A steroid (17α-hydroxy-20β-dihydroprogesterone), previously shown to cause the resumption of meiosis by *F. heteroclitus* oocytes in vitro (Greeley et al. 1986a), was added in an ethanol vehicle (10 μl) to treatment groups at a final concentration of 0.1 $\mu g \cdot ml^{-1}$. Ethanol vehicle alone (10 μl) was added to control groups. In experiments assessing the effect of ouabain, follicles were also pretreated for 1 h with this drug. Unless specified otherwise, incubations were carried out for 40 h at 20 °C by the methods of Greeley et al. (1986a). The incidence of oocyte maturation was indicated by scoring germinal vesicle breakdown (GVBD), a common indicator of meiotic maturation (Wallace and Selman 1978).

As an alternative incubation medium for experiments on the effects of external cations, a simple saline medium [solution "FO–R1" = NaCl 113 $mmol \cdot l^{-1}$, KCl 5 $mmol \cdot l^{-1}$, $CaCl_2$ 2 $mmol \cdot l^{-1}$, HEPES 10 $mmol \cdot l^{-1}$ (pH 7.5), glucose 5 $mmol \cdot l^{-1}$, 50 μg gentamicin $\cdot ml^{-1}$, based on solution "FO" (Wallace and Selman 1978)] was used.

Volumes were calculated from the average diameters of follicles measured with an eyepiece micrometer mounted on a dissecting microscope. Water contents and dry weights of follicles were determined by the methods of Greeley et al. (1991). Na^+ and K^+ were measured in a parallel set of follicles with a Perkin-Elmer 2380 (Norwalk, Connecticut) atomic absorption spectrophotometer. Samples were prepared by homogenizing 10–20 follicles in distilled water, deproteinizing and acidifying the homogenate by the addition of perchloric acid (70% solution at 1/16 sample volume), and centrifuging at $1000 \times g$ to obtain a protein-free supernatant.

Intact follicles consist of an oocyte surrounded in turn by a thin layer of granulosa cells, a theca containing the capillary bed, and a surface epithelium. In one experiment, these cellular layers were successively removed by manual dissection in Ca^{2+}-free FO–R1 as previously described (Greeley et al. 1987a) prior to culture in 75% L–15 medium.

Results

Changes in follicle volume and intracellular water, Na^+, and K^+ content associated with maturation in vitro

Figure 1A shows that prematuration follicles treated with 17α-hydroxy-20β-dihydroprogesterone resumed meiosis and matured in vitro, with nuclear dissolution (GVBD) at approximately 22 h into the incubation. Steroid-treated follicles enlarged and completed maturation by 40 h, reaching a preovulatory stage of development characterized by absence of a nucleus, clearing of the yolk so that the oocyte was relatively translucent, marked increase in volume, and convergence of oil droplets at the upper periphery of the oocyte (Wallace and Selman 1978). Mature oocytes rarely ovulate from their follicles in vitro, so this preovulatory stage (normally followed by the postovulatory or ovulated egg stage in vivo) was typically the final stage of follicle maturation in vitro. Control follicles cultured in the absence of a maturation-inducing steroid neither matured nor enlarged appreciably during the 40-h incubations (Fig. 1A). Steroid-treated follicles enlarged in volume by an average of 75% in vitro, which was only about two-thirds of the volume increase previously noted for follicles in vivo (Fig. 2).

Water contents of steroid-treated follicles increased markedly during culture, coincident with both meiotic maturation and the enlarging volume of the follicle; however, dry weights remained relatively unchanged (Fig. 1B). As a result, the water content of the follicle increased from 62% to 78% during maturation. A nearly 1:1 relationship between volume and water content of maturing follicles was noted (Fig. 3A; slope = 0.97),

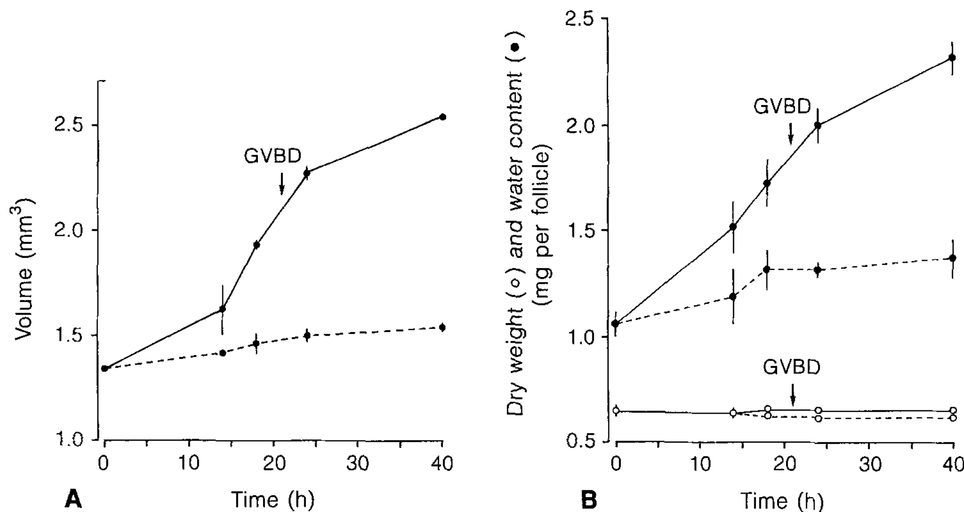


Fig. 1 A, B. **A** Volume and **B** weight changes in *F. heteroclitus* follicles during culture in the presence of either 0.1 μg 17α-hydroxy-20β-dihydroprogesterone (17α-OH-20β-diProg) · ml^{-1} or ethanol vehicle (EtOH). Steroid-treated oocytes matured within the 40-h incubation period (time to 50% GVBD indicated); no ethanol controls underwent maturation. Presented as the means ± SEM of three trials (10–20 follicles per treatment group per time period), ——— *17α-OH-20β-diProg*, - - - *EtOH*

while the dry weight remained essentially constant throughout the volume change (Fig. 3B; slope = 0.01). Hence, water uptake appears to be the fundamental cause of the volume increase in *F. heteroclitus* follicles during maturation in vitro.

A previous study on possible osmotic effectors in *F. heteroclitus* follicles in vivo revealed that inorganic ions play a predominant role (Greeley et al. 1991). Therefore, the changes in intracellular Na^+ and K^+ that occurred during maturation in vitro were monitored. As shown in Fig. 4A, follicle Na^+, and particularly K^+, content increased significantly in steroid-treated follicles during maturation in vitro. The influx of K^+ actually exceeded the accompanying uptake of water, resulting in a net increase in K^+ concentration during maturation (Fig. 4B). In contrast, there was little net change in either the content or concentration of Na^+ or K^+ in non-maturing control follicles (Fig. 4A, B).

Additional evidence of a possible role for cation flux in the water uptake and volume increases of maturing follicles was provided by direct comparisons of intracellular Na^+ and K^+ values with follicle volume (Fig. 5). In both content (Fig. 5A) and concentration (Fig. 5B), each cation maintained a positive linear relationship with follicle volume (and hence water content) during maturation. In both cases, the slopes of the lines calculated for the K^+ data were 2–3 times that calculated for the Na^+ data, indicating that K^+ is the primary osmotic effector.

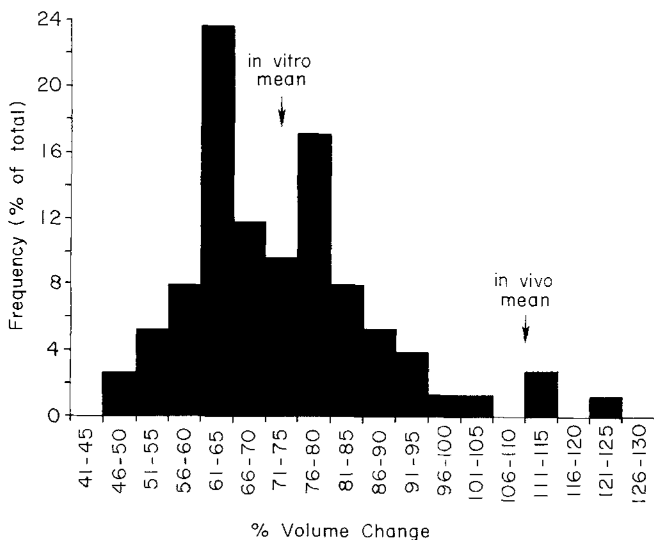


Fig. 2. Variation of percent volume changes of follicles during steroid-induced volume changes (total number of observations = 76). Means in vitro and (for comparison) in vivo are indicated by *arrows* [latter data from Greeley et al. (1991)]

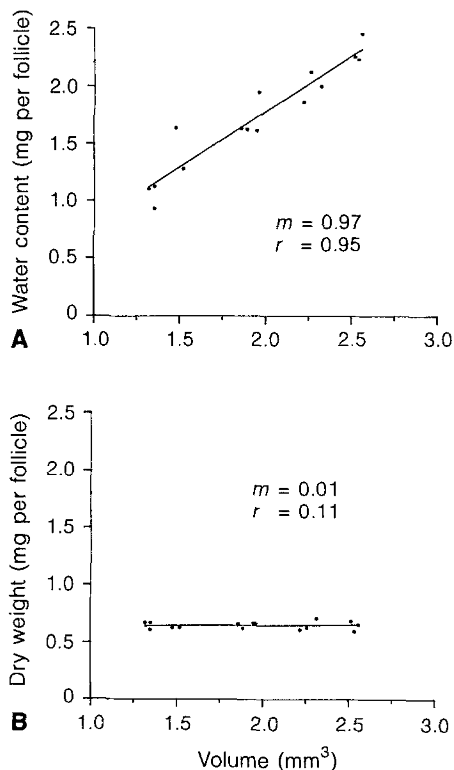


Fig. 3A, B. Relationship of volume to **A** water content and **B** dry weight in steroid-treated follicles undergoing maturation in vitro. Lines fitted by least-squares linear regression, with the slopes and correlation coefficients indicated

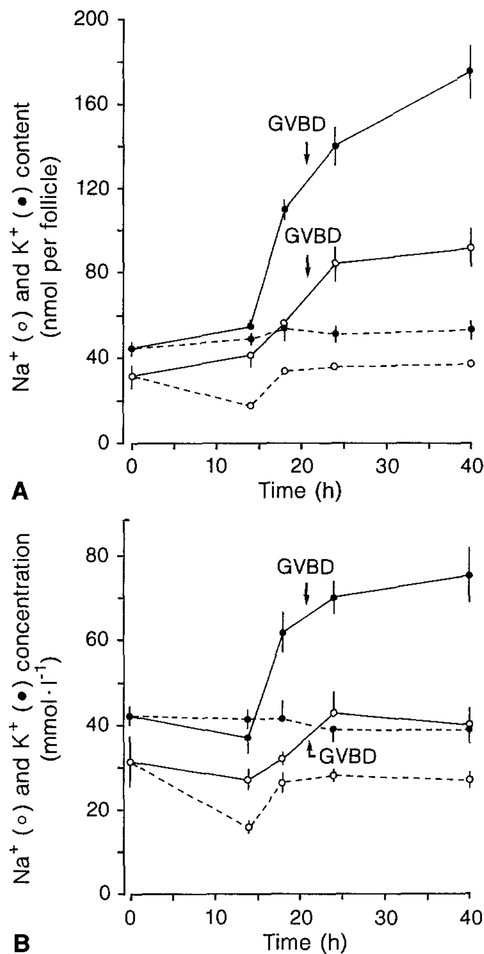


Fig. 4A, B. Changes in **A** follicle content and **B** concentration of Na^+ and K^+ relative to water vs time. The water contents were derived from the data indicated in Fig. 1B. Follicles were treated with either 17α-hydroxy-20β-dihydroprogesterone (———; *17α-OH-20β-diProg*) or vehicle (- - -; *EtOH*). Steroid-treated follicles underwent maturation within the 40-h incubation period (time of 50% GVBD indicated by *arrows*); no ethanol controls underwent maturation. Means ± SEM of three trials (10–20 follicles per treatment group per time period)

Exploration for operant mechanisms

Since the intracellular levels of both Na^+ and K^+ increased during the maturation of *F. heteroclitus* follicles, an experiment was designed to test the relative importance of these two cations to volume regulation more directly. Accordingly, a simple saline medium (solution FO–R1) was devised that was able to support both steroid-initiated maturation, as indicated by scoring GVBD (Fig. 6A), and maturation-associated volume increase (Fig. 6B). Although the response in both cases was less vigorous than achieved with L–15 medium (Fig. 6A, B) as previously observed (Greeley et al. 1986a), the simplified medium contained a physiological level of K^+ (Umminger 1972) and allowed easier investigation of the requirements for either Na^+ or K^+.

Maturation appeared to be uninhibited by the removal of Na^+ and/or K^+ from the saline medium; if anything, the response in K^+-free saline, occurred somewhat faster than in the control group (Fig. 6A), as has been previously observed for amphibian oocytes (Vitto and Wallace 1976). However, follicle hydration was found to be strictly K^+-dependent (Fig. 6B). The extent of follicle hydration in response to the presence of K^+ was also found to be concentration-dependent (Fig. 7).

Steroid-treated follicles were also placed in K^+-free saline and transferred to K^+-containing saline at various times during maturation. The results (Fig. 8) indicated that follicles were still able to hydrate fully when placed in normal saline after 12 h in K^+-free medium. However, when K^+-deprived follicles were transferred to normal saline at the time of 50% GVBD (36 h) only partial hydration occurred, and by 48 h even this partial response was gone.

Attempts were made to disrupt cation and water uptake by treating follicles with ouabain before and during culture in 75% L–15 medium. As seen in Fig. 9, ouabain at a concentration (10^{-5} mol · l^{-1}) that effectively blocks the Na^+,K^+-pump of amphibian oocytes (Vitto and Wallace 1976) had no apparent effect on either the cation

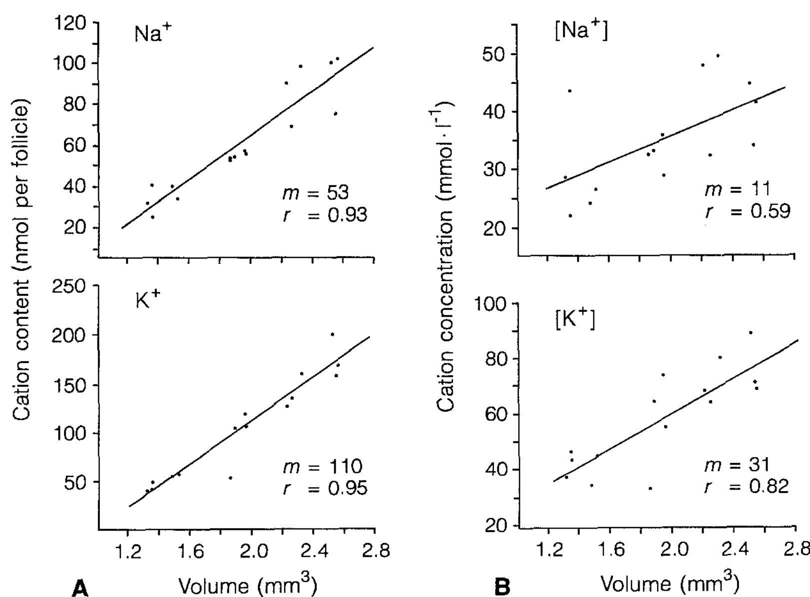
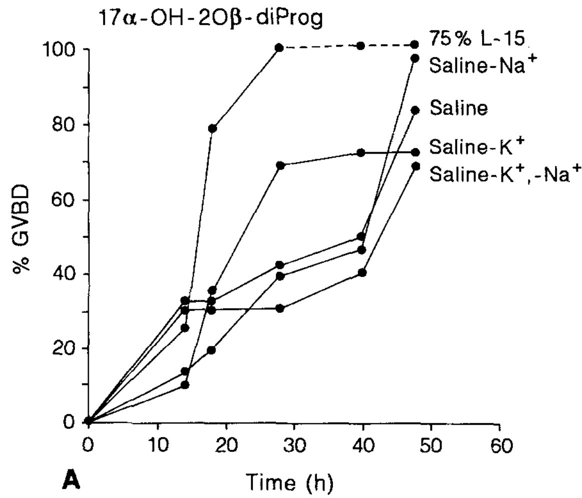


Fig. 5A, B. The relationship of follicle volume to changes in **A** follicle content and **B** concentration of Na^+ and K^+ relative to water during meiotic maturation in vitro. Lines fitted by least-squares linear regression, with the slopes and correlation coefficients indicated



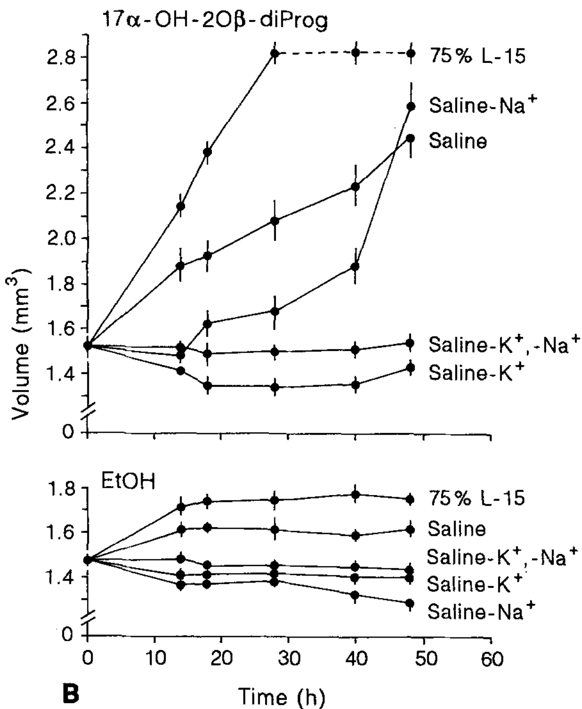


Fig. 6 A, B. **A** Extent of maturation (as indicated by GVBD) in steroid-treated follicles vs time in various media. Control follicles (not shown) treated with ethanol vehicle did not undergo maturation. **B** Volume of follicles undergoing maturation vs time in various media. The same group of oocytes was used for both sets of data in **A** and **B** *(above)*. Volume of vehicle *(EtOH)*-treated follicles vs time in various media is shown in the final graph. An equivalent amount of Na^+ was used to replace K^+, and an equivalent amount of choline$^+$ was used to replace Na^+ or $Na^+ + K^+$ in cation-deficient media. Means ± SD ($n = 20$–30 follicles per culture medium per time point) provided in **B**

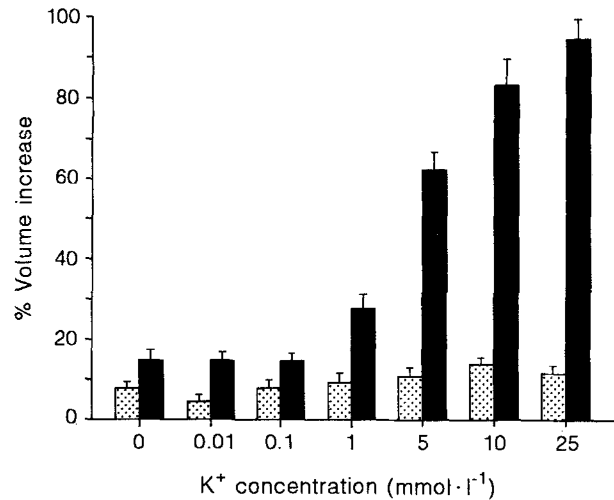


Fig. 7. Changes in volume of follicles incubated for 50 h in saline containing the indicated concentrations of K^+. The total molarity of Na^+ plus K^+ was kept constant in all cases. Follicles were incubated in the presence of either 17α-hydroxy-20β-dihydroprogesterone *(17α-OH–20β-diProg)*, to initiate maturation, or ethanol vehicle *(EtOH)*; in the latter case, no follicles underwent maturation. Means ± SD ($n = 19 \pm 1$ follicles per treatment group) ▨ *EtOH* ■ *17α-OH–20β-diProg*

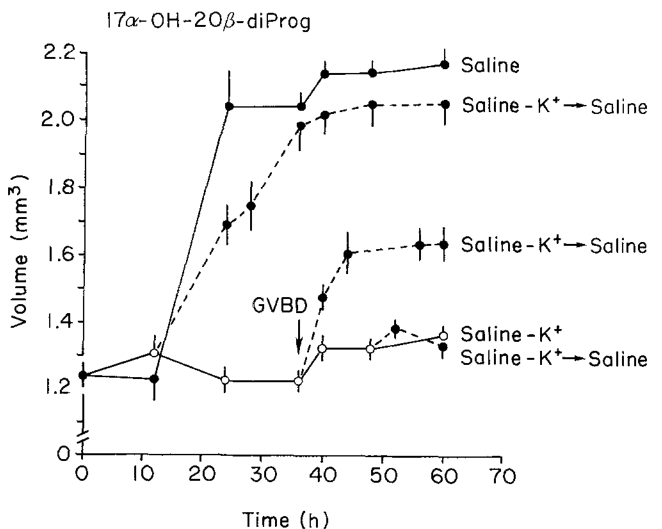


Fig. 8. Stage-dependent hydration of steroid-treated follicles incubated in saline (solution FO–R1; *filled circles*) or K^+-free saline *(open circles)*. In the latter case, follicles were also transferred to saline at various times. For reference, the time to 50% GVBD for follicles in K^+-free saline is indicated by an *arrow*. Presented as means ± SD ($n = 6$–10 follicles per treatment group per time period)

influx, maturation response, or water uptake of *F. heteroclitus* oocytes.

Finally, it was previously noted that oocytes failed to enlarge when associated somatic cells were removed (McPherson et al. 1987, 1989). In order to document this effect, more fully, the various cellular layers were removed from follicles (Greeley et al. 1987a; Petrino et al. 1989), and maturation was subsequently initiated by treating all preparations with steroid. Cultures were then incubated in L-15 medium for up to 86 h in order to assure that volume changes were complete. The results (Fig. 10) indicate that intact follicles enlarge by 75% during maturation, as expected (see Fig. 2), while the volume increase of defolliculated oocytes (most granulosa cells still attached) was somewhat less (51%). However, the average volume of denuded oocytes barely increased (12%)

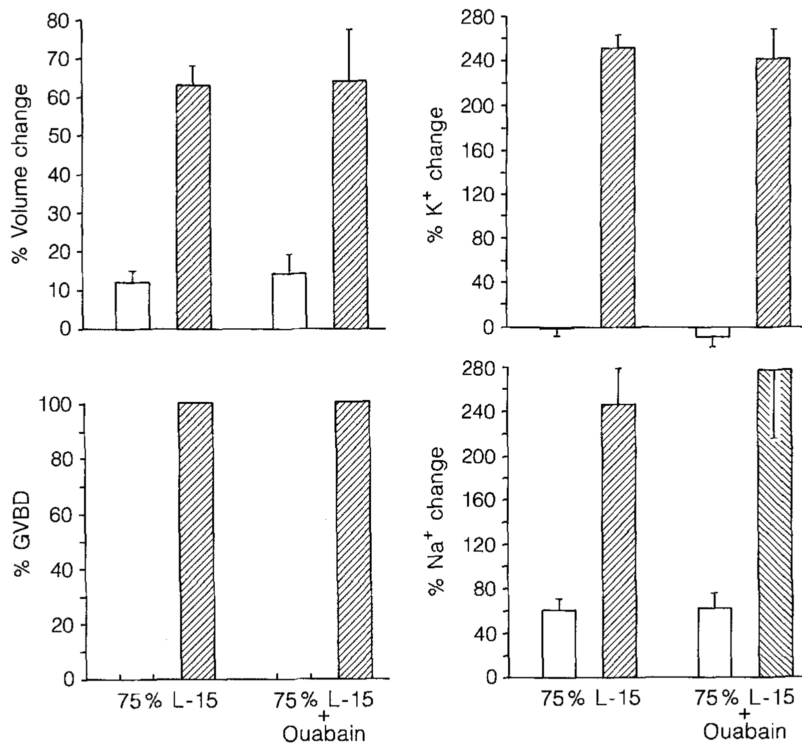


Fig. 9. Effects of ouabain on percent volume change, percent GVBD and percent change in total K^+ and Na^+ per follicle. Follicles were incubated for 40 h in L-15 medium with or without ouabain 10^{-5} mol · l^{-1} and treated with either 17α-hydroxy-20β-dihydroprogesterone *(17α-OH-20β-diProg)* or ethanol vehicle *(EtOH)*. No significant effects of ouabain were noted. Means ± SD of three trials (20 follicles per treatment group) □ *EtOH* ▨ *17α-OH-20β-diProg*

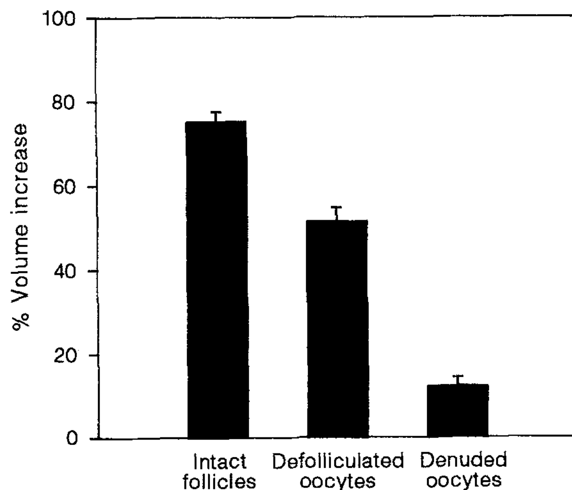


Fig. 10. Effect of somatic cell layers on hydration. The theca and surface epithelium were removed from intact follicles to give defolliculated oocytes, from which the granulosa cells were removed to provide denuded oocytes (Greeley et al. 1987; Petrino et al. 1989). The resulting preparations were then individually cultured in L-15 medium together with 17α-hydroxy-20β-dihydroprogesterone for up to 86 h (until no further volume increase occurred). All oocytes underwent maturation. Means ± SD of the final volume achieved (n = 45, 32, and 42 for intact follicles, defolliculated oocytes, and denuded oocytes, respectively)

during the lengthy culture period, similar to that generally observed for control (non-maturing) oocytes (Figs. 1A, 6B, 9).

Discussion

The present results (Fig. 1) demonstrate that the *F. heteroclitus* follicle undergoes a significant volume increase during meiotic maturation in vitro which is very similar to, although somewhat less pronounced than, that which typically occurs during maturation in vivo [Fig. 2; cf. Greeley et al. (1991)]. Water uptake accounts for 97% of the volume change observed in vitro (Fig. 3A), while continued deposition of yolk during the early stages of oocyte maturation provides an additional but minor contribution to volume changes in vivo (Wallace and Selman 1985). The lack of yolk deposition in vitro does not entirely account for the diminished volume increase, however, since the relative hydration achieved in vitro (78%) is also somewhat less pronounced than that achieved in vivo [81%; Greeley et al. (1991)]. Nevertheless, the ready availability of steroid-responsive follicles has allowed the investigation of physiological events of oocyte maturation in *F. heteroclitus* under experimentally controlled conditions.

Several mechanisms have been proposed to explain the sudden uptake of water by the maturing teleost oocyte. Earlier observations on the proteolysis of yolk proteins into smaller peptides in *F. heteroclitus* and a variety of other teleosts during the course of oocyte maturation and hydration first led to the suggestion that these abundant proteins might be involved in driving maturation-associated water movements (Wallace and Begovac 1985; Wallace and Selman 1985; Greeley et al. 1986b). It was thought that the internal osmotic pressure of the oocyte would rise and cause a compensating influx of osmotically obliged water as larger yolk proteins were proteolytically cleaved into more numerous smaller proteins and peptides during maturation. However, more recent studies have failed to provide any experimental evidence of such a role for the yolk proteins in the hydration of *F. heteroclitus* oocytes (McPherson et al. 1989).

Concomitant with yolk proteolysis, Craik (1982) and Craik and Harvey (1984, 1986) noted a pronounced loss of protein-associated phosphorus during oocyte matura-

tion in several marine teleosts and proposed that protein-linked phosphate may be utilized as an energy source during the massive water uptake that simultaneously occurred. These results implied, but did not indicate, that an ATPase/cation pump was involved. LaFleur and Thomas (1991) have also recently gathered evidence for a role of Na^+,K^+-ATPase in the oocyte hydration of two species of sciaenid fish. Our results demonstrate that a relatively high concentration of ouabain has no effect on any of the events associated with oocyte maturation in *F. heteroclitus* (Fig. 9), so either a typical Na^+,K^+-ATPase is not involved in this species, or the *F. heteroclitus* enzyme is insensitive to ouabain.

Various investigators have also suggested that an increase in intracellular free amino acids, resulting from either influx, de novo synthesis, or proteolytic degradation of yolk proteins, serves to drive water uptake by the oocyte (Oshiro and Hibiya 1981; Craik and Harvey 1984, 1987; Wallace and Begovac 1985; Wallace and Selman 1985; Greeley et al. 1986b; Watanabe and Kuo 1986). Experimental evidence for this possibility has been obtained by Craik and Harvey (1987) for the oocytes of cod and plaice, two marine species that spawn pelagic eggs. However, our own recent study of the various free amino acid levels in *F. heteroclitus* follicles undergoing maturation in vivo found no apparent relationship between these small organic solutes and the hydration of maturing follicles (Greeley et al. 1991).

However, the intracellular levels of K^+ and, to a lesser extent, Na^+ were found to correlate well with the volume and water content of *F. heteroclitus* follicles undergoing maturation in vivo (Greeley et al. 1991). This prompted another hypothesis: that an influx of cations into the follicle, particularly an influx of K^+, was the impetus for the water movements and volume increases associated with maturation (Hirose and Ishida 1974; Hirose 1976; Babiker and Ibrahim 1979; Craik and Harvey 1984, 1986; Watanabe and Kuo 1986; LaFleur and Thomas 1991). The present results support this proposal. As predicted by in vivo studies, hydration of *F. heteroclitus* follicles in vitro is accompanied by increases in the follicle content of both K^+ and Na^+ (Figs. 4, 5). In the case of K^+, the increase was less in vitro [reaching approximately 180 nmol per follicle in the preovulatory follicle (Fig. 4A)] than in vivo [270 nmol per follicle (Greeley et al. 1991)], which reflects the diminished volume increase (66%) observed in vitro (Fig. 2). Possible explanations for the reduced K^+ uptake in vitro include the disruption of normal circulatory routes for cation delivery and the presence of an enveloping epithelium restricting ion accessibility from the culture medium to the oocyte.

By itself, a correlation between cation uptake and the increased water and volume of maturing follicles, even when observed both in vivo and in vitro, can only suggest a causal relationship between the two sets of events. However, experimental evidence indicated that follicle hydration, but not maturation, is strictly dependent on the concentration of external K^+ (Figs. 6, 7). The enzymatic mechanism responsible for this K^+ dependency remains to be determined. Apparently, a membrane Na^+,K^+-ATPase is not involved, based both on the insensitivity of the hydration process to ouabain (Fig. 9) and the observation that Na^+, as well as K^+, accumulates within the follicle (Figs. 4, 5). Alternative mechanisms are therefore implied, such as the Na^+-K^+-$2Cl^-$ cotransport system (Hoffman 1985; Geck and Heinz 1986) that is believed to function in the volume regulatory mechanisms of various somatic cells.

F. heteroclitus oocytes are enveloped by several very thin somatic cell layers which, together with the enclosed oocyte, make up the ovarian follicle (Selman and Wallace 1983). It has been previously noted that these outer cellular layers make an insignificant contribution to the overall volume, water content, or ion content of the follicle (Wallace and Selman 1978, 1985; Greeley et al. 1991). Therefore, with regard to these parameters, the terms "follicle" and "oocyte" can be used interchangeably. However, when considering potential cellular mechanisms responsible for ion or water uptake, the distinction between these terms must be recognized.

In this respect, perhaps our most intriguing observation is that the ability of steroid-treated follicles to hydrate in K^+-containing medium is lost while they are undergoing maturation in K^+-free medium (Fig. 9). Several possible explanations may be offered for this observation, such as the gradual loss of follicle permeability, the degradation of the responsible enzyme(s), or the translocation of the responsible enzyme(s) to nonfunctional sites. Virtually nothing is known about any of these possibilities. However, one of the main events that is known to occur during oocyte maturation in lower vertebrates is the withdrawal of microvillar and macrovillar processes by the oocyte and overlying granulosa cells, respectively, so that intercellular contact is broken as the oocyte prepares for ovulation (Wartenberg 1962; Flügel 1967; Kobayashi 1985; Iwamatsu et al. 1988; Larabell and Chandler 1989). This process was artificially duplicated by first peeling off the outer surface epithelium and theca ("defolliculated oocytes") from intact follicles, followed by removal of the granulosa cells ["denuded oocytes"; Greeley et al. (1987a)]. When intact follicles and the various oocyte preparations were subsequently treated with steroid, all underwent maturation, but volume increases observed for the defolliculated and denuded oocytes were only 68% and 16%, respectively, of that of intact follicles. Assuming that the loss of the granulosa cells is entirely responsible for the loss of the hydration response, the somewhat diminished hydration of defolliculated oocytes can be explained by the observation that patches of granulosa cells are frequently lost from the surface of the oocyte during the preparation (Greeley et al. 1987a) of defolliculated oocytes.

Intercellular contact between the oocyte and overlying granulosa cells is mediated both by desmosomes, which anchor the cells to each other, and gap junctions, through which molecules with a molecular weight less than 1200 can pass from one cell to the other (e.g., Warner 1988). Such heterologous gap junctions have been well documented in teleost follicles (Toshimori and Yasuzumi 1979; Kessel et al. 1985; Kobayashi 1985; Iwamatsu et al. 1988; Iwamatsu and Ohta 1989). Therefore, the available evidence indicates that the mechanism for K^+ uptake by the follicle probably resides in the granulosa cells, and that sequestered K^+ may be translocated to the maturing oocyte via heterologous gap junctions which are dissociated as maturation reaches completion.

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