J. Membrane Biol. 79, 203–210 (1984)

The Journal of
Membrane Biology

Regulatory Changes of Membrane Transport and Ouabain Binding during Progesterone-Induced Maturation of *Xenopus* Oocytes

Hans-Peter Richter, Dieter Jung, and Hermann Passow
Max-Planck-Institut für Biophysik, D-6000 Frankfurt/Main 71, Federal Republic of Germany

Summary. During progesterone-stimulated maturation of defolliculated full-grown *Xenopus* oocytes, the activities of the transport systems for L-alanine, thymidine, chloride, phosphate, and alkali ions decrease. Differences of the extent and time course of these changes suggest that they are controlled by at least partially independent mechanisms.

A closer investigation of the Na-K ATPase has shown that in unstimulated oocytes, ouabain produces maximal inhibition when 8–12 × 10^9 molecules are bound per cell. This number is bound during the first phase of a diphasic uptake process. Since this phase can be suppressed by increasing the concentration of external K^+ to 45 mmol/liter or more, it is concluded that it refers to binding to the Na-K pump in the plasma membrane. Ouabain bound prior to progesterone-induced germinal vesicle breakdown (GVBD) remains bound after the breakdown, although the Na-K pump loses the capacity to bind ouabain after GVBD in oocytes that had not been exposed to ouabain preceding GVBD. In the presence of Mg^{++} membranes isolated before regulatory inhibition of pumping and ouabain binding show a Na^+-dependent incorporation of ^{32}P from γ-[^{32}P]-ATP that can be reversed by the addition of K^+. The phosphorylation site migrates on LiDS-polyacrylamide gel electropherograms at about 98,000 daltons and can be identified as a Commassie blue-stainable band.

Key Words Na-K ATPase · ouabain · *Xenopus* · oocyte · maturation

Introduction

The final product of maturation of amphibian oocytes consists of cells that are ready to be shed into pond water whose osmotic pressure and electrolyte composition is totally different from that of the ovarian follicles where maturation took place. It is quite plausible, therefore, that oocyte maturation involves regulatory activity changes of the many transport systems in the plasma membrane (Pennequin, Schorderet-Slatkine, Drury & Baulieu, 1975; Bellé, Marot & Ozon, 1976; Ziegler & Morrill, 1977; Morrill & Ziegler, 1980; Carvallo, Albuja, Allende & Allende, 1981; Smith, 1981).

The present paper is concerned with the maturation of prophase-arrested, full-grown *Xenopus laevis* oocytes. In the ovaries these oocytes undergo germinal vesicle breakdown (GVBD), pass through the first meiotic division and finally are arrested in the metaphase of the second meiotic division. When the oocytes are shed and inseminated, the second meiotic division is completed. We induced maturation in defolliculated, full-grown oocytes *in vitro* by exposure to progesterone and we observed GVBD and the ensuing regulatory changes of a number of transport processes. Similar to the scattered observations published in the papers quoted above, in all instances, we found a decrease of the transport activities around the time of GVBD, amplifying the suspicion that a decrease of the activity of many, perhaps of all transport systems is an essential part of oocyte maturation. The Na-K pump activity disappears together with the capacity to bind ouabain.

At the final stage of this work, we became aware of a recent publication of Weinstein, Kostellow, Ziegler, and Morrill (1982) who, with respect to the Na-K ATPase, addressed similar questions, although with different techniques and in a different amphibian species, *Rana pipiens*.

Materials and Methods

Prophase-arrested oocytes were obtained by removal of portions of the ovaries of anaesthetized females of *Xenopus laevis*. They were released from the follicles by exposure to collagenase. Metaphase-arrested oocytes were obtained after hormonal stimulation of the female and dejellied with mercaptoethanol. To measure ouabain binding and uptake of rubidium, prophase-arrested or metaphase-arrested oocytes were incubated, if not stated otherwise, in large volumes of Barth's solution at 20–21 °C in the presence of ^3H-ouabain or $^{86}Rb^+$ (from Amersham). After suitable time intervals, 5–10 oocytes were removed, washed, and placed individually into separate vials for liquid scintillation

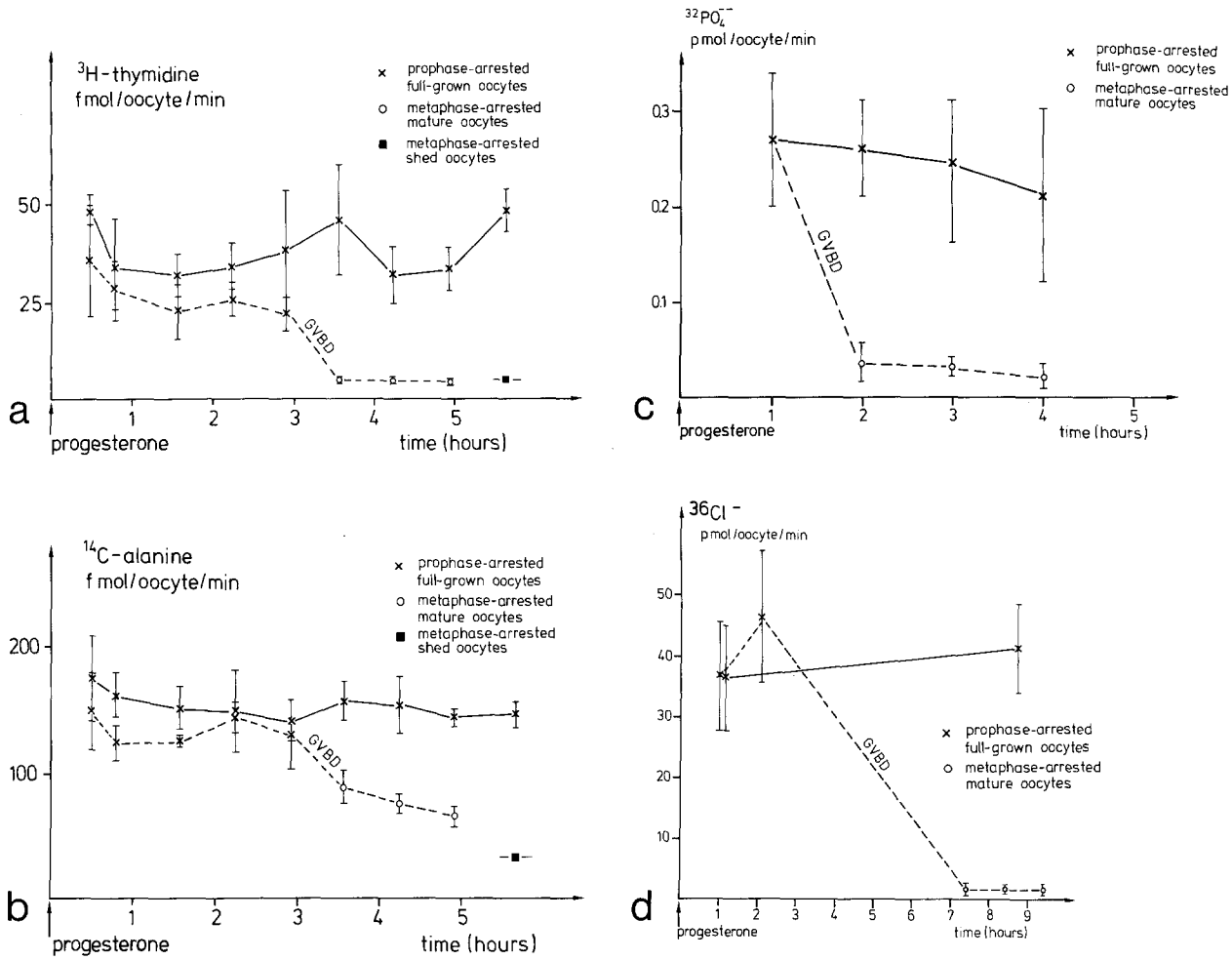

Fig. 1. Effect of progesterone-induced maturation on the rate of uptake of (*a*) thymidine, (*b*) alanine, (*c*) phosphate, and (*d*) chloride in defolliculated prophase-arrested, full-grown oocytes. For the times indicated on the abscissa, five oocytes were sampled and pulse-labeled for 30 min (*a* and *b*) or 60 min (*c* and *d*) in media containing the respective transport substrates in radioactively labeled form. Measurements were made in untreated control oocytes (——) and in oocytes exposed to 5 μmol/liter progesterone (---). x and o indicate, respectively, prophase- and metaphase-arrested oocytes, (-■-) refers to metaphase-arrested, shed oocytes. The ordinates indicate the rates of uptake. In this and all subsequent figures each data point and the corresponding "error bar" refer to the average of separate determinations in five individual oocytes. GVBD time of germinal vesicle breakdown

counting. The Na-K pump activity is calculated on the assumption that ^{86}Rb is transported at the same rate as potassium. Pulse label experiments were performed with defolliculated oocytes that were subdivided into two batches and resuspended in large volumes of Barth's solution. One batch received progesterone (final concentration 5 μmol/liter), the other served as a control. In suitable time intervals, five oocytes from each batch were transferred for 30–60 min for pulse labeling into media of the same composition as the original incubation media except that the radioactively labeled substrate was present, whose transport was to be measured.

Incorporation of ^{32}P from γ-[^{32}P]-ATP into isolated cortices was performed essentially as described by Knauf, Proverbio and Hoffman (1974). Cortices were obtained by differential centrifugation of homogenized oocytes in Ca^{++}-free De Boer's solution (Richter & Tintschl, 1983). The protein band pattern on Li-dodecylsulfonate (LiDS) polyacrylamide gradient gel electropherograms (pH 6.8, 10 °C) is essentially similar to the pattern observed with cortices that had been obtained through dissection by hand, as described by Richter (1980). Barth's solution: 88 NaCl, 1 KCl, 2.4 $NaHCO_3$, 0.82 $MgSO_4$, 0.33 Ca $(NO_3)_2$, 0.41 $CaCl_2$, 5 HEPES, pH 7.6. De Boer's solution: 110 NaCl, 1.3 KCl, 0.44 $CaCl_2$, pH 7.2 (in mmol/liter).

Results

Regulatory Decrease of Transport Processes in Progesterone-Stimulated Oocytes

We have studied the effect of progesterone on the Na-K Pump and on the transport of thymidine, L-alanine, chloride, and phosphate. The pulse labeling

experiments in Fig. 1 show that in the absence of progesterone, full-grown oocytes transport the various substrates with more or less constant rates for at least 4–9 hr. The differences of the uptake rates of individual oocytes that are depicted by the "error" bars represent predominantly biological variations of transport rates among the individual oocytes of a population (Jung & Richter, 1983). Uptake during the pulse-labeling period is expressed as fmol (10^{-15} mol) or pmol (10^{-12} mol) per oocyte per min. This can be converted into fluxes by division by the cell surface (about 4.5×10^{-2} cm^2).

When oocytes are exposed to progesterone, the transport activities decrease around the time of GVBD. The decrease of alanine transport begins at about that time, but is less pronounced and continues only slowly for the rest of the experiment.

The progesterone-induced inhibition of Rb^+ transport is accompanied by a decrease of the capacity of the oocytes to bind ^{3}H-ouabain, indicating that a reduction of the activity of the Na-K ATPase is involved (Fig. 2).

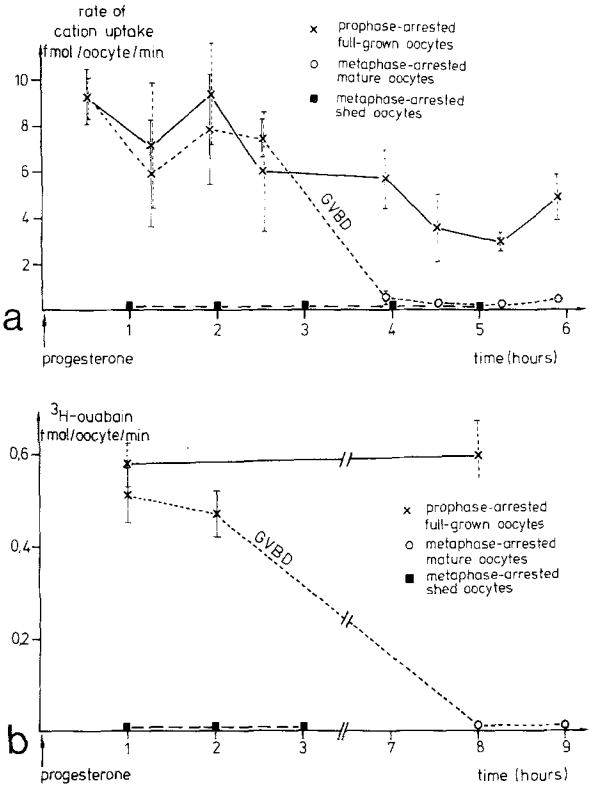

Fig. 2. Effect of progesterone on the capacity of full-grown oocytes to transport potassium (*a*) and to bind ouabain (*b*). The oocytes were incubated at 21 °C for the lengths of time indicated on the abscissa in the presence of 1 mmol/liter KCl. Samples were then exposed to ^{86}Rb as a tracer for K^+ or ^{3}H-ouabain (4.5 μmol/liter), and Rb^+ uptake and ouabain binding were measured by pulse labeling for 30 and 60 min, respectively. Measurements were made in untreated oocytes (——) and oocytes exposed to 5 μmol/liter progesterone (----). x and o indicate, respectively, prophase- and metaphase-arrested oocytes. -■- refers to metaphase-arrested, shed oocytes

The Na-K Pump

A first set of experiments characterizes the Na-K pump in unstimulated oocytes by measuring the ouabain-sensitive ^{86}Rb uptake and the capacity of the pump sites to bind ouabain. A second set deals with the regulatory changes of the transport activity of the Na-K pump. Finally, the activity of the Na-K pump *in situ* before and after progesterone-induced regulatory inhibition is compared with the capacity of the isolated membrane to accomplish the Na^+-dependent incorporation of ^{32}P from γ-[^{32}P]-ATP into the α-chain of the ATPase and to release the incorporated ^{32}P by the addition of K^+.

Alkali Ion Transport and Ouabain Binding in Full-Grown Oocytes

Pulse labeling experiments show that the capacity of full-grown oocytes for ^{86}Rb transport and ouabain binding persists for at least up to 58 hr after defolliculation (not documented). In oocytes of some females the transport rate may decrease (up to 50% in 6 hr, Fig. 2*a*); in others it remains essentially constant. In oocytes of the same female, the rates of both ^{86}Rb uptake and ouabain binding are somewhat variable. The standard deviations observed are, respectively, ±18% and ±14%. In addition, the mean values measured in oocytes of different animals may differ by a factor of two or more. Thus, comparisons of the behavior of the oocytes derived from different females need to take this biological variability into account.

The time course of ouabain uptake can be subdivided into 2 components, each of which can be described by a single exponential. The faster component is inhibited by external K^+. It nearly completely disappears at K^+ concentrations above 45 mmol/liter while the slower component remains virtually unaffected (Fig. 3). Since external K^+ is known to reduce ouabain binding to the Na-K pump (e.g., Prindle, Skelton, Epstein & Marcus, 1971; Bodemann & Hoffman, 1976), we attribute the faster component of ouabain uptake to ouabain binding to the Na-K pump.

The occurrence of ouabain uptake that is not related to binding to the pump does not only follow from the survival of a K^+-resistent uptake, but can also be demonstrated directly by manually dissect-

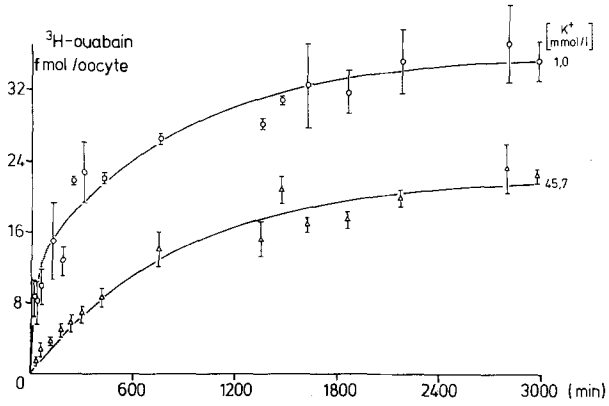

Fig. 3. Time course of ouabain binding to prophase-arrested, full-grown oocytes in the presence of 1.0 mmol/liter K^+ and 45.7 mmol/liter K^+ in the medium. Ouabain concentration 0.42 μmol/liter; 21 °C. The drawn lines represent nonlinear least squares fits to the data of the equation: $y = A \cdot (1 - e^{-\alpha t}) + B \cdot (1 - e^{-\beta t})$ where y = ouabain uptake. In the upper curve (1 mmol/liter K^+), A = 12.0 fmol/oocyte, α = 0.04 min^{-1}; B = 24.0 fmol/oocyte, β = 0.0012 min^{-1}. In the lower curve (45.7 mmol/liter K^+) the drawn line represents a single exponential (i.e., A = 0), where B = 22.2 fmol/oocyte and β = 0.0011 min^{-1}. Ordinate: Ouabain uptake

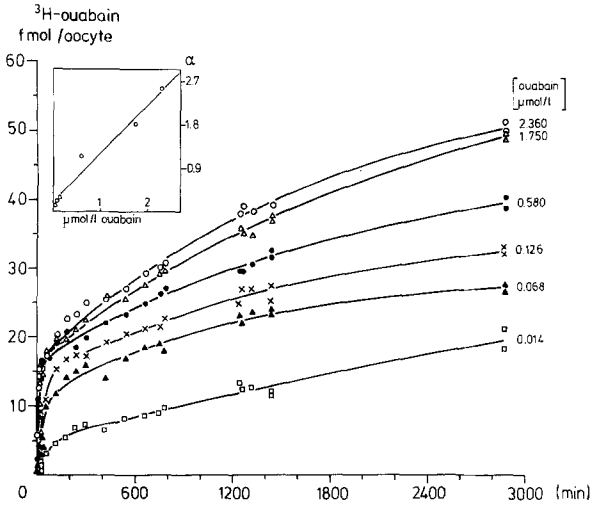

Fig. 4. Time course of ouabain binding to prophase-arrested full-grown oocytes as measured at the ouabain concentrations indicated (1 mmol/liter K^+; 21 °C). The drawn lines represent sums of exponentials as calculated by means of a nonlinear least squares fit (*see* the equation in legend to Fig. 3). In the inset, the rates α, of the initial rapid phase of ouabain binding (ordinate) are plotted against the ouabain concentration in the medium (abscissa). The straight line represents a computer fit by the method of least squares

ing the oocytes and measuring the ^{3}H-ouabain in isolated cortices and in the cell contents. Although the data leave no doubt about an internalization of ouabain into the cell contents, they were not accurate enough for quantitative determinations.

The rate of ouabain uptake has been followed at a range of ouabain concentrations in the medium at the fixed potassium concentration of 1.0 mmol/liter (Fig. 4). The parameter values derived from fits to the sums of two exponentials are listed in the Table. They represent the results of experiments with oocytes of two different females and illustrate qualitative similarity of the behavior of the two oocyte populations and considerable quantitative differences. The rapid component of ouabain binding to the Na-K pump increases approximately linear with the ouabain concentration in the medium (Fig. 4, inset). In oocytes of the two females the numbers of binding sites involved are rather similar while the rate constants differ by a factor of about 2, suggesting that the accessibility of the pump molecules differs.

The slow component of ouabain uptake that includes ouabain internalization continues far beyond the completion of binding to the pump. Rough estimates of the maximal amount of ouabain taken up by this process can be obtained by extrapolation of the pertinent exponential to infinite time. The results listed in the Table are, of necessity, inaccurate. Nevertheless, they illustrate that the internalization process involves a larger number of ouabain molecules than the binding to the pump.

Ouabain binding to the pump is associated with an inhibition of ^{86}Rb uptake. To study the relationship between ouabain binding and inhibition, oocytes were incubated for time periods up to 4 hr in media containing different ouabain concentrations. Subsequently they were removed to measure the amount of ouabain bound and the rate of the remaining alkali ion transport. Figure 5 shows decreasing alkali ion transport with increasing amount of ouabain bound, down to about 20% of the transport in the control. More ouabain binding produces no further inhibition, indicating a ouabain-insensitive component of rubidium flux. From the relationship between ouabain binding and the corresponding transport rate, the ouabain binding at maximal inhibition can be derived. We obtain about 20 fmol or 12 $\times$ 10^9 ouabain molecules per oocyte. This value is about 20% larger than the estimates based on the determination of the fast, K^+-sensitive component of ouabain binding (A in the Table and Fig. 5). The difference most likely reflects the ouabain internalization parallel to the ouabain binding to the pump that cannot be determined separately in experiments of the type shown in Fig. 5.

If one assumes $^{86}Rb^+$ as a suitable tracer for K^+ transport, it is possible to calculate from the rate of $^{86}Rb^+$ uptake and the known K^+ concentration in the medium the rate of K^+ influx. After deduction of the ouabain-insensitive flux, one obtains the pump-me-

Table. Parameter values derived by fitting the equation $y = A(1 - e^{-\alpha t}) + B(1 - e^{-\beta t})$ by a nonlinear least squares procedure to the experimental data on ouabain uptake by oocytes of two different females

Female I					Female II				
Ouabain	A	B	$\alpha \times 10^3$	$\beta \times 10^3$	Ouabain	A	B	$\alpha \times 10^3$	$\beta \times 10^3$
2.360	16.5	42.7	255.1	0.55	2.000	15.4	30.8	546.0	1.76
1.750	16.4	47.1	181.4	0.41	1.400	13.6	29.3	373.0	1.57
0.580	16.5	31.5	114.8	0.45	0.800	15.0	27.3	242.0	1.62
0.126	14.2	22.9	27.6	0.55	0.200	7.7	26.4	98.2	2.72
0.068	11.0	18.3	23.7	0.76	0.050	6.7	23.7	13.0	0.49
0.014	5.2	107.4	11.7	0.05	0.010	6.1	4.3	2.2	0.50
μmol/liter	fmol/oocyte		min^{-1}		μmol/liter	fmol/oocyte		min^{-1}	

The rate of ouabain uptake dy/dt can be assumed to represent the sum of binding to the pump (total binding capacity $\bar{A}$) and of incorporation into the cell interior (total capacity $\bar{B}$). The respective rates of uptake are:

$$dy_A/dt = k_1 \cdot s \cdot (\bar{A} - y_A) - k_1^* y_A \text{ and } dy_B/dt = k_2 \cdot s \cdot (\bar{B} - y_B) - k_2^* y_B$$

where y_A and y_B designate the amounts of ouabain associated with compartments A and B, respectively, and s the ouabain concentration in the medium. The k's represent rate constants for the respective forward and backward reactions. After integration with the appropriate initial condition, one obtains for $y = y_A + y_B$ the equation presented on top of the table. The parameters in this equation have the following significance:

$$A = \bar{A}\frac{k_1 s}{k_1 s + k_1^*} \text{ and } B = \bar{B}\frac{k_2 s}{k_2 s + k_2^*}$$

and $\alpha = k_1 s + k_1^*$ and $\beta = k_2 s + k_2^*$.

The ratio $k_1^*/k_1 = k$ represents the dissociation constant for ouabain binding to the Na-K pump. Our data suggest that K^+ is somewhere between 10 and 100 nmol/liter. This is the same order of magnitude of the probably more accurate value determined by Weinstein et al. (1982) in *Rana pipiens* by a totally different technique.

diated transport. Assuming that the number of ouabain molecules bound at maximal inhibition of K^+ influx is about equal to the number of pump molecules, and that 2 K^+ are transported per pump cycle, one can calculate an approximate value of the turnover number of the pump. The result is 5 sec^{-1} at 21 °C. Correction for ouabain internalization increases this figure by about 20%.

Alkali Ion Transport during Progesterone-Induced Oocyte Maturation

The successful action of progesterone on the oocyte is indicated by the GVBD. The length of time period up to GVBD is quite variable in oocytes of different females. During most of this period both the rate of ^{86}Rb uptake (as measured by pulse labeling) and the capacity to take up ouabain are more or less constant (Fig. 2). During the time around GVBD, a rapid decrease of both ^{86}Rb transport and ouabain binding is observed, indicating the regulatory inhibition of the Na-K pump. Although after GVBD very little ouabain is taken up, ouabain that had already been bound before GVBD is not released into the medium (not demonstrated).

The pulse-labeling technique used in the previously described experiments only allows one to tell whether a given uptake rate pertains to oocytes that had undergone GVBD or not (Fig. 1). To improve the time resolution for the determination of the change of the transport process relative to the time of GVBD we used a somewhat different technique (Fig. 6). After a progesterone pulse of 30 min we placed individual oocytes in Barth's medium containing $^{86}Rb^+$. Every 5 min each oocyte was removed from the radioactive medium. After washing the Čerenkov radiation was counted for 1 min in Barth's medium. The oocyte was then transferred back into the original radioactive medium. The incubation was continued for another 5 min until the next cycle of washing and counting was performed. In this manner, the change of the transport activity could be followed in the same oocyte for a long time before and after GVBD. Figure 6 shows the results obtained with three different oocytes from two females. It is obvious that the decrease of transport activity precedes the onset of GVBD. The decrease

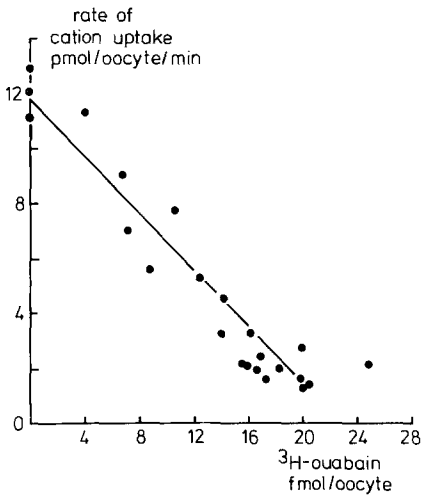

Fig. 5. Effect of ouabain binding on rate of potassium uptake in prophase-arrested, full-grown oocytes as inferred from measuring the rate of ^{86}Rb uptake. The ^{86}Rb uptake was measured at 21 °C by pulse labeling for 30 min after exposure of the oocytes to ouabain for 1–4 hr at a range of ouabain concentrations. Abscissa: Amount of ouabain bound per oocyte, at the beginning of the pulse labeling period for rubidium. Control experiments demonstrated that the release of ouabain is less than 10% during this period. The straight line was calculated by the method of least squares. The line ends at the maximal oubain binding that could be achieved at a range of high ouabain concentrations. The corresponding ordinate indicates the existence of a ouabain-insensitive component of ^{86}Rb flux

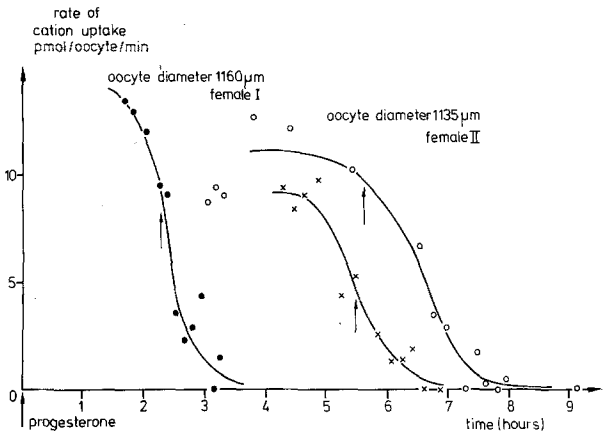

Fig. 6. Decrease of rate of potassium uptake during oocyte maturation as inferred from measuring the rate of ^{86}Rb uptake. Abscissa: Time at which the uptake rate was determined. Zero time indicates time of exposure to 5 µmol/liter progesterone. For each curve the uptake was determined consecutively in the same oocyte by measuring the Čerenkov radiation. The arrows indicate the times at which the unpigmented nuclear sphere (GVBD) was first observed near the animal pole. For description *see* text

continues for a considerable length of time after GVBD is complete. The half time for this decrease is around 60 min in two of the oocytes and about 120 min in the third oocyte. Values around 60 min had been obtained in a number of additional experiments. It should be noted that the time that precedes GVBD and the onset of the permeability change are quite different in the oocytes of the two females.

Localization of the ATPase on LiDS-Polyacrylamide Gel Electropherograms

The presence of the Na-K pump in the isolated membranes of full-grown oocytes of *Xenopus laevis* is demonstrated by phosphorylation with γ-[^{32}P]-ATP and subsequent LiDS-polyacrylamide gel electrophoresis (Fig. 7). Incorporation of ^{32}P is high when Na^+ and Mg^{++} are present, but it is low in the absence of either Na^+ alone or both Na^+ and Mg^{++} (not shown). Addition of K^+ to the phosphorylated enzyme reduces ^{32}P binding. These findings show that Na^+ is essential for the phosphorylation of the enzyme and K^+ for the dephosphorylation. On electropherograms the Na-K ATPase can be localized as a Coomassie blue-stainable band in the 98,000-dalton range and be identified with band IV_1 in the nomenclature of Richter (1980).

Discussion

The experiments described in this paper amplify what had been expected on the basis of other information in the literature. Maturation of the oocyte is accompanied by a decrease of the activity of many transport systems. Similar to the Na-K pump (Weinstein et al., 1982), the transport systems for thymidine, alanine, chloride, and phosphate show a regulatory decrease. These findings can be added to the previous observations on the transport of O-methyl-glucose (Carvallo et al., 1981), L-leucine (Ecker & Smith, 1971; Pennequin et al., 1975) and a passive component of K^+ transport that is independent of the alkali ion pump (Ziegler & Morrill, 1977; Morrill & Ziegler, 1980). The decrease of this latter component, together with that of the Cl^- permeability described here, may be responsible for the decrease of the electrical conductance of the membrane, which has been demonstrated previously (Bellé, Ozon & Stinnakre, 1977; Kado, Marcher & Ozon, 1981).

The observation that the activity of many, if not all, transport systems decreases during maturation, raises the question whether or not they are all regulated by the same metabolic intermediates or reactions. A comparison of the time course of the regu-

latory inhibition for alanine transport on the one hand (Fig. 1*b*) and the transport of alkali ions (Fig. 2*a*) or the anions chloride and phosphate (Fig. 1*c* and *d*) on the other shows that the alanine transport system does not shut down as abruptly or completely as the latter. In the case of alanine transport, the initial decrease after GVBD is followed by a rather gradual further decrease which continues until the oocytes are shed into pond water (Jung & Richter, 1983). Further pieces of evidence for differences of the timing of the regulatory decrease of different transport processes can be extracted from the literature; certain changes of membrane potential during maturation precede the onset of GVBD while the pump is inhibited thereafter (Ziegler & Morrill, 1977, Fig. 1). This suggests that the inhibition of passive ion movements (notably of K^+) is controlled independently of the alkali ion pump. It has also been reported that the regulatory changes of potassium permeability occur without concomitant changes of sodium permeability (Morrill & Ziegler, 1980), suggesting that the passive movements of the principal alkali ions are also regulated separately. Thus, many important transport systems shut down at different times and therefore either have different thresholds for responding to the accumulation of the same inhibitor or they are regulated by different processes.

The turnover number of the Na-K pump in unstimulated oocytes was found to be about 5 sec^{-1}*. This is lower than the value obtained for the highly purified ATPase from *Electrophorus electricus* which, for 21 °C, is about 14 sec^{-1} (Dixon & Hokin, 1974). The different value for oocytes is to be expected since, in contrast to the isolated enzyme, in intact cells the transport protein operates at an asymmetrical distribution of K^+ and Na^+ and in the presence of an electrical field across the membrane.

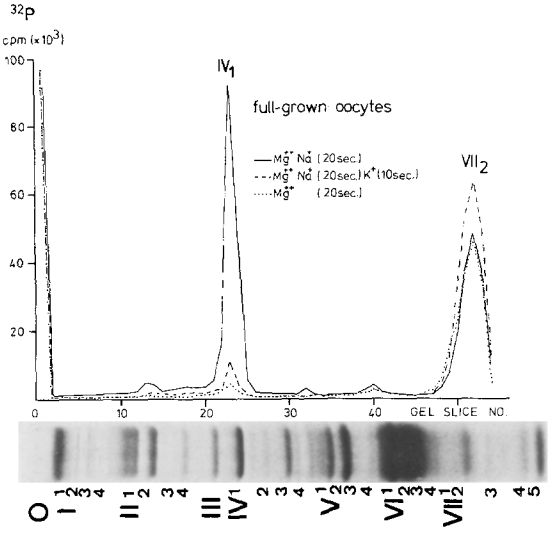

Fig. 7. Incorporation of ^{32}P into isolated cortices of prophase-arrested, full-grown oocytes after exposure to γ-[^{32}P]-ATP. Exposure in the presence of Mg^{++} alone (.....), Mg^{++} plus Na^+ (——), or Mg^{++} plus Na^+ and subsequent addition of K^+ (----). Final concentrations of Mg^{++}, Na^+ and K^+, 12 μmol/liter, 50 mmol/liter, and 12 mmol/liter, respectively; 0 °C. Times of incubation as indicated in the figure. The figure represents the ^{32}P labeling profile of a LiDS-polyacrylamide gel electropherogram of isolated cortices (gradient gels, 2–16% acrylamide, Tris-glycin-LiDS-buffer, pH 6.8, electrophoresis at 10 °C for 3 hr). The Roman numerals indicate the protein bands that correspond to the location of the radioactivity. Below the labeling profile a Coomassie blue-stained electropherogram

Under our experimental conditions, the extracellular concentration of K^+ was less than maximally stimulating and the conditions in the oocyte interior were unknown. Nevertheless, the data show that the Na-K ATPase in the unstimulated *Xenopus* oocyte seems to operate similarly as in other tissues[1].

From our data, the number of pump molecules per μm^2 of membrane surface was calculated to be about 2000. This is about two times higher than in *Rana pipiens* oocytes (Weinstein et al., 1982). Our value ranks among the highest values reported so far for any membrane. The density is in fact high enough to suggest that the Na-K ATPase belongs to the more abundant integral membrane proteins in

* Interestingly, the data for *Xenopus* oocytes reported here are more similar to those obtained with the isolated *Electrophorus* Na-K ATPase than with the observations on oocytes from *Rana pipiens*. For the latter, Weinstein et al. (1982) find a maximal strophantidin binding of 10 fmol/oocyte or 6×10^9 pump sites/oocyte and a strophantidin-sensitive current flow (attributed to the activity of the pump) of 91 ± 12/nA or 570×10^9 charges per second (20–22 °C). If we assume that each pump cycle is associated with the transfer of one charge we calculate from their data a turnover number of 95 sec^{-1}. This number pertains to a K^+ concentration in the medium of 1.9 mmol/liter, the data on *Xenopus* to 1.0 mmol/liter. Assuming that the stimulation of the pump is linearly related to the K^+ concentration, we arrive at a turnover number for *Rana* oocytes of 50 ± 6.6 sec^{-1}. This value exceeds that obtained with the isolated Na-K ATPase of *Electrophorus* (as calculated for 21 °C) by a factor of about 3.5, and the turnover number found in *Xenopus* by a factor of about 10. Thus, perhaps in *Rana* the Na-K pump is subjected to a stimulating influence that does not exist in *Xenopus*.

[1] The study of the Na-K pump in unstimulated oocytes showed a variation of the number of pump molecules per oocyte in different females (*cf.*, for example, Fig. 5 and the Table). It is not clear whether these differences indicate that the oocytes in some females were not yet fully grown, or if they reflect biological variations among the full-grown oocytes of the different females. Nevertheless, the calculated turnover numbers of the pump were rather similar, indicating that the activity of each pump molecule is always much the same, regardless of the number of molecules present per cell.

the oocyte membrane. This is confirmed by the finding that the α-chain of the Na-K ATPase could be detected at 98,000 daltons as a Coomassie blue-stained band on LiDS-polyacrylamide gel electropherograms of the isolated cortex. A compilation of ouabain binding data published by Benos (1981) suggests the existence of even higher pump densities in certain epithelial cells. However, considering this list one may suspect that in most of these cases a considerable fraction of the bound ouabain may have accumulated inside the cells, as was observed in Hela cells (Cook, Tate & Shaffer, 1982) and our experiments with *Xenopus* oocytes.

In *Rana* oocytes, the decrease of pump activity during maturation is accompanied by a similar decrease of Na-K ATPase activity as measured in homogenates of metaphase-arrested oocytes by Weinstein et al. (1982). In addition, we studied the activity of the Na-K ATPase in isolated cortical plasma membranes of prophase-arrested (Fig. 7) and methaphase-arrested oocytes by the phosphorylation technique[2]. Preliminary results indicate that there is no significant difference between both developmental stages with respect to the determination of the cortical membrane protein and binding of ^{32}P to the membrane. Since the pump activity of metaphase-arrested oocytes had decreased to virtually zero, the regulatory inhibition of the Na-K ATPase can obviously be reversed by resuspending the isolated cortical membranes in a medium free of cytoplasmic contents. Therefore, we believe that the inhibition of the pump neither involves proteolysis nor covalent chemical reactions like transamination, methylation, acetylation, and hydroxylation. More detailed characterization of the regulatory inhibition of ATPase activity during maturation is the subject of further investigations.

We thank Dr. L. Kampmann for his help with the curve fitting, Dr. H. Bodemann for support with the measurement of ouabain binding, Ms. A. Tintschl and B. Legrum for their collaboration, and Dr. W. Schwarz for reading the manuscript and his comments. This work was supported by the Deutsche Forschungsgemeinschaft.

References

Bellé, R., Marot, J., Ozon, R. 1976. Nature of progesterone action on amino acid uptake by isolated full-grown oocytes of *Xenopus laevis*. *Biochim. Biophys. Acta* **419**:342–348

Bellé, R., Ozon, R., Stinnakre, J. 1977. Free calcium in full-grown *Xenopus laevis* oocytes following treatment with ionophore A23187 or progesterone. *Mol. Cell. Endocrinol.* **8**:65–72

Benos, D.J. 1981. Ouabain binding to preimplantation rabbit blastocysts. *Dev. Biol.* **83**:69–78

Bodemann, H.H., Hoffman, J.F. 1976. Comparison of the side-dependent effects of Na and K on orthophosphate-, UTP- and ATP-promoted ouabain binding to reconstituted red blood cell ghosts. *J. Gen. Physiol.* **67**:527–545

Carvallo, P., Albuja, C.M. de, Allende, C.C., Allende, J.E. 1981. Hormonal regulation of glucose uptake by amphibian follicles. *Exp. Cell Res.* **136**:215–223

Cook, J.S., Tate, E.H., Shaffer, C. 1982. Uptake of ^3H-ouabain from the cell surface into the lysosomal compartment of Hela cells. *J. Cell. Physiol.* **110**:84–92

Dixon, J.F., Hokin, L.E. 1974. Studies on the characterization of the sodium-potassium transport adenosine triphosphatase. Purification and properties of the enzyme from the electric organ of *Electrophorus electricus*. *Arch. Biochem. Biophys.* **163**:749–758

Ecker, R.E., Smith, L.D. 1971. Influence of exogenous ions on the events of maturation in *Rana pipiens* oocytes. *J. Cell. Physiol.* **77**:61–70

Jung, D., Richter, H.-P. 1983. Changes of alanine-sodium co-transport during maturation of *Xenopus laevis* oocytes. *Cell Biol. I.R.* **7**:697–707

Kado, R.T., Marcher, K., Ozon, R. 1981. Electrical membrane properties of the *Xenopus laevis* oocyte during progesterone-induced meiotic maturation. *Dev. Biol.* **84**:471–476

Knauf, P.A., Proverbio, F., Hoffman, J.F. 1974. Chemical characterization and pronase susceptibility of the Na:K pump-associated phosphoprotein of human red blood cells. *J. Gen. Physiol.* **63**:305–323

Morrill, G.A., Ziegler, D. 1980. Na^+ and K^+ uptake and exchange by the amphibian oocyte during the first meiotic division. *Dev. Biol.* **74**:216–223

Pennequin, P., Schorderet-Slatkine, S., Drury, K.C., Baulieu, E.E. 1975. Decreased uptake of ^3H-leucine during progesterone-induced maturation of *Xenopus laevis* oocytes. *FEBS Lett.* **51**:156–160

Prindle, K.H., Skelton, C.L., Epstein, S.E., Marcus, F.I. 1971. Influence of extracellular potassium concentration on myocardial and isotropic effect of tritiated digoxin. *Circ. Res.* **28**:337–345

Richter, H.-P. 1980. SDS-polyacrylamide gel electrophoresis of isolated cortices of *Xenopus laevis* eggs. *Cell Biol. I.R.* **4**:985–995

Richter, H.-P., Tintschl, A. 1983. Cortex and plasma membrane proteins of *Xenopus* oocytes. *Cell Biol. I.R.* **7**:1105–1114

Smith, L.D. 1981. Frog oocytes and sea urchin eggs: Steroid hormones and sperm induce similar events. *In:* Progress in Developmental Biology. W. Sauer, editor. pp. 35–47 Gustav Fischer, Stuttgart-New York

Weinstein, S.P., Kostellow, A.B., Ziegler, D.H., Morrill, G.A. 1982. Progesterone-induced down-regulation of an electrogenic Na^+,K^+-ATPase during the first meiotic division in amphibian oocytes. *J. Membrane Biol.* **69**:41–48

Ziegler, D., Morrill, G.A. 1977. Regulation of the amphibian oocyte plasma membrane ion permeability by cytoplasmatic factors during the first meiotic division. *Dev. Biol.* **60**:318–325

[2] Weinstein et al. (1982, Table 3) demonstrated that the activity of the Na-K ATPase in homogenates of metaphase-arrested oocytes is much lower than in prophase-arrested oocytes. However, their work needs to be pursued further since their homogenates contained, besides the plasma membrane ATPase, microsomes and other cell organelles that may contain large quantities of the enzyme and thus obscure the effects on the plasma membrane. Our results refer exclusively to the latter.

Received 24 January 1984