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IONIC CURRENTS THROUGH THE MEMBRANE OF THE MAMMALIAN OOCYTE AND THEIR COMPARISON WITH THOSE IN THE TUNICATE AND SEA URCHIN

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SUMMARY

1. The action potential and the membrane current of the mouse oocyte were analysed by current-clamp and voltage-clamp techniques and they were compared with those of other animal oocytes.

2. The matured and unfertilized oocyte of the mouse in standard medium with 6 mM-K showed the resting potential of $-23{\cdot}1 \pm 2{\cdot}9$ mV. The resting potential was relatively large in the medium with 20 mM-Ca or 10 mM-Mn, being $-35{\cdot}7 \pm 2{\cdot}6$ mV and further increased to $-46{\cdot}9 \pm 4{\cdot}8$ mV with replacement of Na in the medium by choline.

3. At the cessation of large hyperpolarization below -90 mV in standard medium, a regenerative potential was often elicited in the form of an off-response. The off-response depended upon the external concentration of Ca. In 20 mM-Ca medium it was constantly observed with hyperpolarization below -60 mV. Its critical level was -40 mV and its overshoot was $+15$ mV.

4. The time and potential-dependent inward current was observed both in standard and 20 mM-Ca media under voltage-clamp condition. In 20 mM-Ca medium the inward current was observed by depolarization beyond -40 mV and showed its maximum at -15 mV. It was greatly reduced by replacing the external Ca with Mn but retained by substituting Sr or Ba for Ca. The selectivity ratios among these alkali earth cations were Ca:Sr:Ba = 1·0:1·4:0·7.

5. The current–voltage relation in Ca and Na-deficient and 10 mM-Mn medium was linear from -200 to $+25$ mV. The hyperpolarization below -200 mV revealed an inward-going rectification. The depolarization above $+50$ mV under voltage-clamp condition induced the outward surge current with activation and inactivation processes.

6. In contrast to the mouse oocyte, the matured and unfertilized oocyte

of the sea urchin showed a large resting potential of −70 mV in 30 Ca ASW and the depolarization beyond −40 mV elicited an action potential with an overshoot of 20 mV. The action potential showed a notch in the rising phase and lasted about 1 to 2 sec.

7. Under the voltage-clamp condition both Ca inward current and the outward surge current were observed in the sea urchin oocyte membrane just as in the mouse oocyte membrane.

8. The selectivity ratios among alkali earth cations, Ca:Sr:Ba, for 'Ca channels' of the oocyte membranes were 1·0:1·4:0·7 in the mouse, 1·0:1·7:1·1 in the tunicate and 1·0:0·7:0·5 in the sea urchin. When the current density through Ca channels are revised in terms of the respective critical levels for Ca channels, the revised selectivity sequences become Ca > Sr > Ba, being common to all three species.

INTRODUCTION

The passive electrical phenomena of the membrane and the intracellular ionic concentration in the oocyte have been extensively studied in vertebrates and invertebrates (Tyler, Monroy Kao & Grundfest 1956; Morrill, 1965; Ito & Hori, 1966; Dick & McLaughlin, 1969; Palmar & Slack, 1970; Slack, Warner & Warren, 1973; Slack & Warner, 1973; Cross, Cross & Brinster, 1973; Powers & Tupper, 1974). On the other hand, no active electrical excitability had long been recognized in the egg cell membrane, although the fertilization potential has been considered to be analogous to action potentials (Peterfi & Rothschild, 1935; Tyler *et al.* 1956; Hiramoto, 1959; Maeno, 1959; Steinhardt, Lundin & Mazia, 1971; Higashi & Kaneko, 1971). However, recent studies on the oocyte membrane of the tunicate (protochordate) and of the starfish (echinoderm) have demonstrated the existence of the electrical excitability which is characteristic of the differentiated excitable membrane of nerve and muscle cells; Na, Ca and K channels (Miyazaki, Takahashi & Tsuda, 1972, 1974*a*; Okamoto, Takahashi & Yoshii, 1976*a*, *b*; Miyazaki, Ohmori & Sasaki, 1975*a*, *b*; Hagiwara & Takahashi, 1974; Hagiwara, Ozawa & Sand, 1975). The generalized existence of electrical excitability in the oocyte membrane in a wide range of animal species would provide a basis to understand the nature of the excitable membrane from the view point of ontogenesis and differentiation.

In the present paper we will mainly describe the generation of the action potential and the time and potential-dependent membrane currents in the oocyte membrane of the mammal. In addition, the comparison of properties of ionic channels in the oocyte membranes will be attempted among three different animal species: mouse tunicate and sea urchin.

METHODS

Eight-week-old female ICR and DDy mice were used. Pregnant mare serum (PMS) (4 i.u.) was injected into the mouse 48–50 hr before the injection of 4 i.u. human chorionic gonadotrophin (HCG). Sixteen to 18 hr after HCG administration, the oocytes were obtained by cutting the ampulla of the oviduct with two sharp needles. The follicular cells were removed with 150 i.u. hyaluronidase. The above procedures were similar to those reported by previous workers (Cross *et al.* 1973; Powers & Tupper, 1974). Unfertilized matured oocytes in the metaphase of the meiotic division were used. The ionic compositions of the bathing media are shown in Table 1. In a few experiments, 0·5 mM-$LaCl_3$ was added to 20 mM-Ca, or 10 mM-Mn, Na-free medium. All media contained 4 mg/ml. bovine serum albumin (BSA) and 1 mg/ml. glucose. Ca ions were replaced by Mn ions in the ratio 2:1, since the stabilizing effect of Mn ions was twice that of Ca ions in the mouse oocyte membrane. The oocyte was placed in a small hole on the floor of the bath in order to minimize mechanical disturbances during exchange of the solution (Okamoto *et al.* 1976*a*). The bath temperature was kept at 30–33° C. Two glass micro-electrodes of relatively high resistance (15–25 MΩ) filled with 3 M-KCl were inserted in the oocyte. One electrode was for potential recording and the other for current injection (Fig. 1). The current-clamp and voltage-clamp techniques for the egg membrane were described in detail elsewhere (Miyazaki *et al.* 1974*a*; Okamoto *et al.* 1976*a*). Under voltage-clamp conditions the holding potential was usually −90 mV.

TABLE 1. Ionic composition of media

A, mouse

	Na (mM)	K (mM)	Ca (mM)	Mg (mM)	Mn (mM)	Choline (mM)	Cl (mM)
Standard	139·0	6·0	1·7	1·2	.	.	150·8
20 Ca	111·5	6·0	20·0	1·2	.	.	159·9
10 Mn	126·5	6·0	.	1·2	10·0	.	154·9
20 Ca Na-free	.	6·0	20·0	1·2	.	111·5	159·9
10 Mn Na-free	.	6·0	.	1·2	10·0	126·5	154·9
5 Ca 7·5 Mn	122·8	6·0	5·0	1·2	7·5	.	156·2
10 Ca 5 Mn	119·0	6·0	10·0	1·2	5·0	.	157·4
10 Mn 30 K Na-free	.	30·0	.	1·2	10·0	102·5	154·9
10 Mn 133K Na-free	.	132·5	.	1·2	10·0	.	154·9

All media were buffered by 5 mM-piperazine-*N*-*N*′-bis(2-ethanesulphonic acid) (PIPES)-choline at pH 7·4 and contained 4 mg/ml. bovine serum albumin (BSA) and 1 mg/ml. glucose.

B, sea urchin

	Na (mM)	K (mM)	Ca (mM)	Mg (mM)	Mn (mM)	Cl (mM)
30 Ca ASW	430	10	30	50	.	600
60 Ca ASW	385	10	60	50	.	615
120 Ca ASW	295	10	120	50	.	645
10 Mn ASW	460	10	.	50	10	590

All media were buffered by 5 mM PIPES-choline at pH 7·4.

For the experiment on the oocyte membrane of the sea urchin, the matured female *Strongylocentrotus nudus* was used. By injecting 0·5 M-KCl into the body cavity of the sea urchin, unfertilized and matured oocytes were collected. The diameter of the sea urchin oocytes was 110–120 μm. The compositions of the artificial sea waters (ASWs) used for sea urchin eggs were similar to those described in case of the tunicate egg (Okamoto *et al.* 1976*a*, *b*; Table 1). The analysis of the electrical excitability was carried out by both current and voltage-clamp methods, as described above. In a few experiments the current electrode filled with 7 M-CsCl was used to inject Cs ions intracellularly to suppress the outward current (Adelman & Senft, 1966).

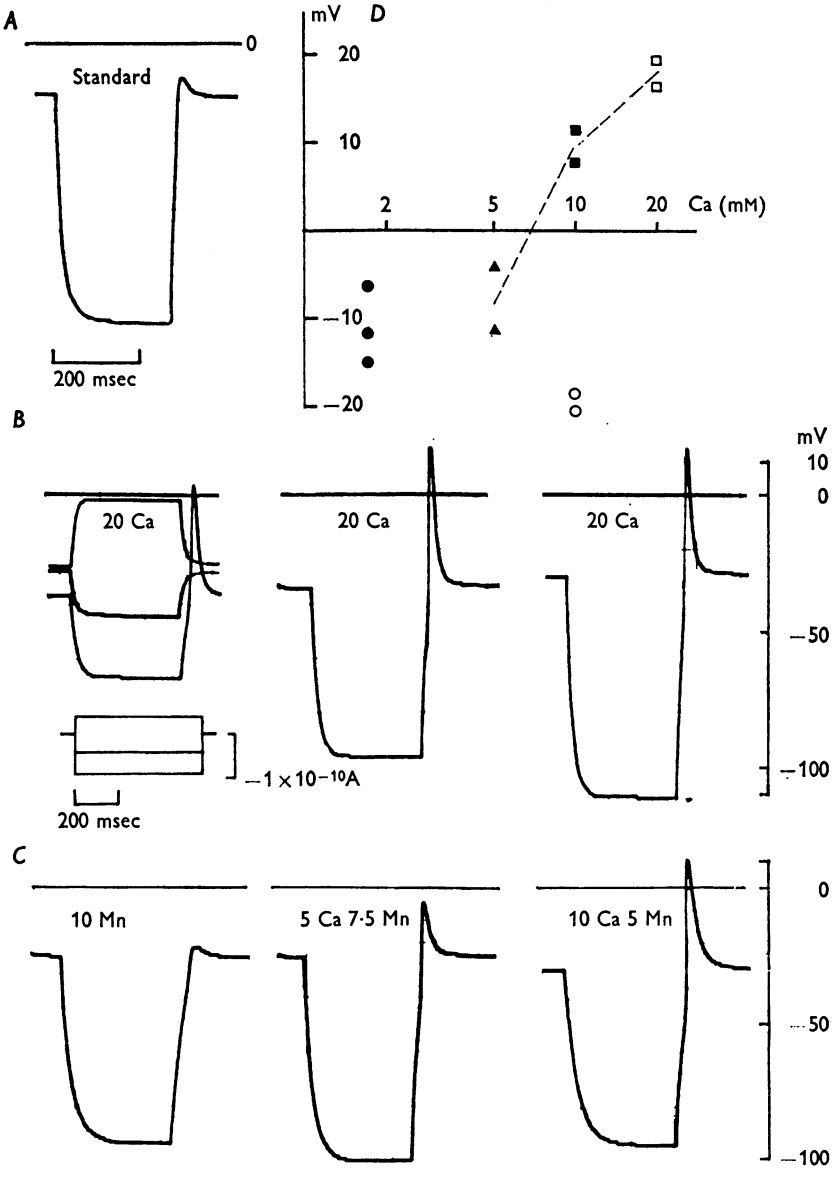
RESULTS

Current-clamp experiments of the mouse oocyte

Resting potential. The resting potential of the mouse oocyte in standard medium was $-23{\cdot}1 \pm 2{\cdot}9$ mV ($n = 10$), which was almost the same as that reported by Powers & Tupper (1974). In the medium of high divalent cation concentration, such as 20 mM-Ca or 10 mM-Mn, the resting potential was $-35{\cdot}7 \pm 2{\cdot}6$ mV ($n = 10$) and further increased to as much as $-46{\cdot}9 \pm 4{\cdot}8$ mV ($n = 10$) with replacement of Na by choline in the high divalent cation media, as described by Powers & Tupper (1974). The increase in the resting potential may be due to the fact that the stabilizing effect of the divalent cations reduces the leakage and that Na permeability plays a role in the resting or leakage conductance. In the standard medium with low Ca concentration of 1·7 mM, the membrane resistance was varied considerably and rapidly decreased after penetration of the microelectrodes. In the medium of high concentration of divalent cations it was kept at the relatively high value of 50–200 MΩ for several minutes. Most of the present experiments were therefore performed in the media of high concentration of divalent cations and the responses in the oocyte were observed within a few minutes after penetration.

Membrane capacity. The total membrane capacity was calculated from the time constant of the passive electrotonic response to a rectangular current pulse. It was $2{\cdot}5 \pm 0{\cdot}3 \times 10^{-10}$ F ($n = 5$). The specific capacitance was estimated to be $1{\cdot}5 \pm 0{\cdot}2$ μF/cm^2, assuming that the oocyte has a spherical surface with a diameter of 72 ± 3 μm ($n = 6$) (Pl. 1). This value of the specific membrane capacity was similar to that reported by Powers & Tupper (1974) and about one-and-a-half-times as large as that of the axonal membrane of the squid (Cole, 1968). Since the mouse oocyte was found to have a large number of microvilli on its surface in electron-microscopic studies M. Yoshii (personal communication), the estimated area of the membrane surface in the mouse oocyte may be smaller than the actual one.

Generation of off-responses. With depolarization or hyperpolarization less than 60 mV from the resting potential by constant-current stimulation,



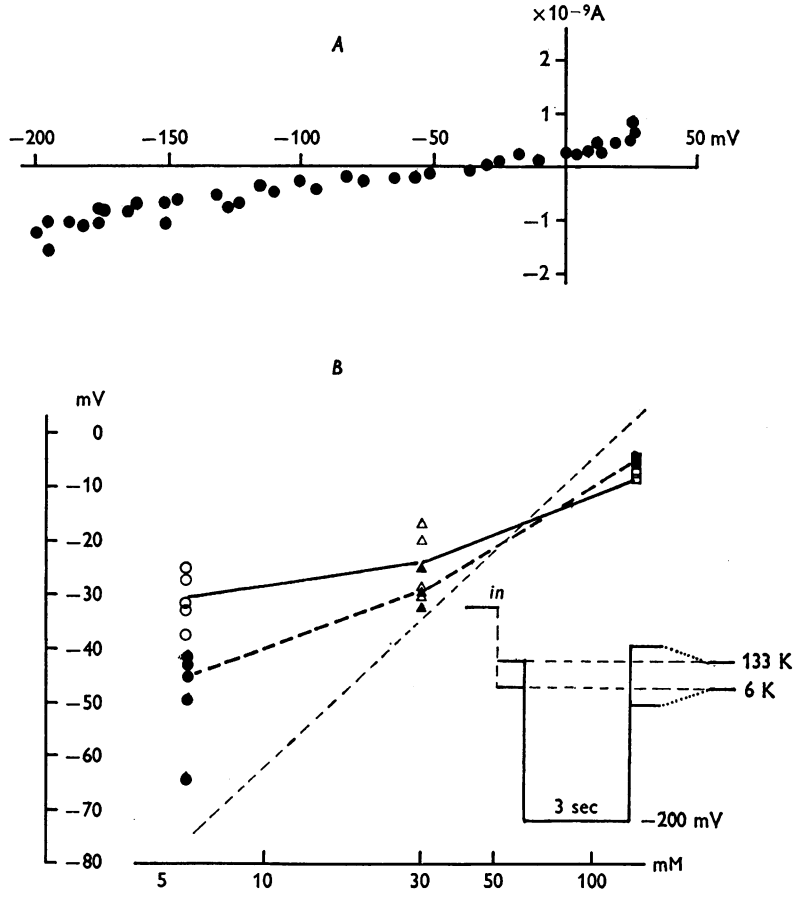
Text-fig. 1. *A-C*, records illustrate the potential responses of mouse oocytes to constant current stimulation in: *A*, standard (●); *B*, 20 Ca (□); and *C*, 10 Mn (○), 5 Ca 7·5 Mn (▲) and 10 Ca 5 Mn media (■). *D*, shows the relation between the external concentration of Ca ions and the saturated amplitude of the off-response. Symbols as shown; temperature, 33° C. Current intensities were: -4×10^{-10} A in *A*; $-1{\cdot}7 \times 10^{-10}$ A in the middle record in *B*, $-2{\cdot}3 \times 10^{-10}$ A in the record on the right side in *B*; and $0{\cdot}7 \times 10^{-10}$ A in all records of *C*.

the mouse oocyte membrane exhibited only a passive electrotonic change both in standard and high Ca media. However, at the cessation of hyperpolarization below −90 mV a regenerative potential was often produced in the form of an off-response in standard medium (Text-fig. 1*A*). In 20 Ca medium the off-response was always observed with hyperpolarization below −60 mV and had a definite critical level at about −40 mV (Text-fig. 1*B*, left). As the membrane hyperpolarization was increased, the off-response became larger in amplitude and it was saturated at approximately −90 mV in 20 Ca medium (Text-fig. 1*B*). A similar off-response has been reported in the unfertilized tunicate oocyte (Miyazaki *et al.* 1972, 1974*a*). It is suggested that in the mouse oocyte as well as in the tunicate oocyte the inward current system is more or less inactivated under the depolarized resting potential and that the hyperpolarization removes this inactivation. The off-response was accompanied by an overshoot of +15 mV in 20 Ca medium. Text-fig. 1*C* shows three samples of the off-responses obtained in 10 Mn, 5 Ca 7·5 Mn and 10 Ca 5 Mn media. Text-fig. 1*D* shows the relation between the external Ca concentration and the peak level of the off-response. It is clearly indicated that the peak potential of the off-response depends upon the concentration of Ca ions in the media. The replacement of Na ions by choline induced no significant change in the off-response. Thus, Ca ions appear to be responsible for generation of the off-response.

The steady-state current–voltage relation. As shown in Text-fig. 2*A*, the voltage-current (*V–I*) relation in the steady state in 10 Mn Na-free medium was linear in the potential-range from −200 to +25 mV. This finding is consistent with that of Powers & Tupper (1974), though the slope resistance was higher in the present experiment, being 50–200 MΩ probably because of high divalent cation concentration in the media.

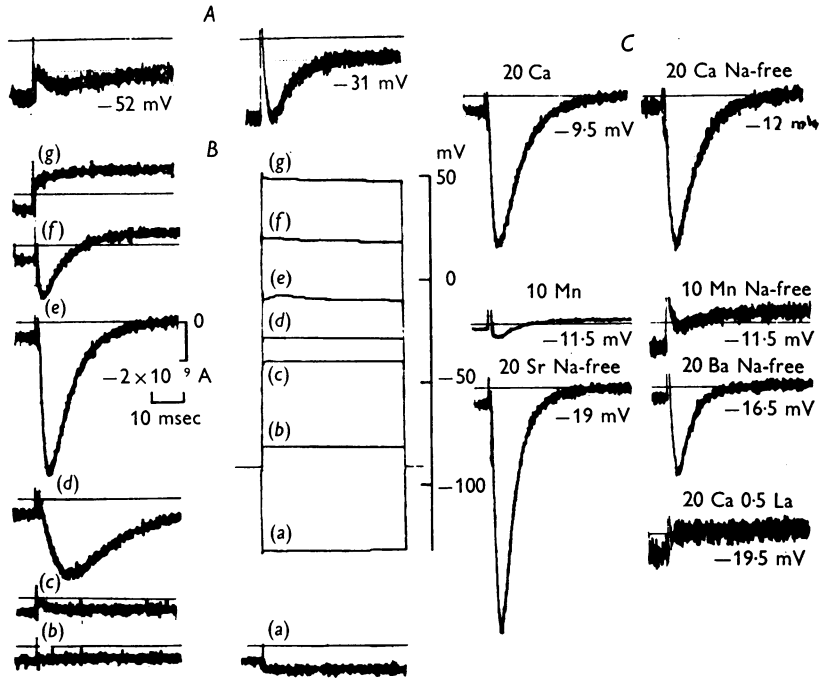
Effect of K ions on the resting potential. The continuous line in Text-fig. 2*B* shows the relation between the resting potential and external K concentration in 10 Mn Na-free medium. The membrane was only slightly depolarized with increase in K concentration. This indicates that the slope conductance mainly consists of the non-selective leakage.

The egg cell membrane of the tunicate and the starfish showed a marked K anomalous rectification and was a perfect K electrode in Na-free media (Miyazaki *et al.* 1974*a*; Miyazaki, Takahashi, Tsuda & Yoshii, 1974*b*; Hagiwara & Takahashi, 1974; Miyazaki *et al.* 1975*a*, *b*). In the mouse oocyte, after the membrane was hyperpolarized to −200 mV for 3 sec, it took a few seconds for the potential level to recover to the original level (inset of Text-fig. 2*B*). In 133 K medium the resting potential recorded immediately after the preceding hyperpolarization was more positive than the control level, while in 6 K medium it was more negative. The



Text-fig. 2. *A*, shows the voltage–current (*V–I*) relation obtained from four mouse oocytes in 10 Mn Na-free medium. Temperature, 32·5–33° C. *B*, shows the relation between the resting potential and the external concentration of K ions (in mM): 6, circles, 30, triangles; and 133, squares; in 10 Mn Na-free media. Each point corresponds to the datum of a mouse oocyte. Open symbols indicate the resting potentials immediately after the penetration of the electrodes and their means are connected by the continuous line. Filled symbols indicate the resting potentials a few seconds after the large hyperpolarization to − 200 mV for 3 sec and their means are connected by the heavy dashed line. The fine dashed line shows the relation for an ideal K electrode. The inset schematically illustrates the changes of the resting potentials after large hyperpolarization. *in*, indicates the time of penetration of the electrode.

relation between the external K concentration and the resting potential recorded immediately after the preceding hyperpolarization (heavy dashed line in Text-fig. 2*B*) approached to that of an ideal K electrode (fine dashed line in Text-fig. 2*B*). It may therefore be possible that the mouse oocyte reveals slight K selective permeability during hyperpolarization, which disappears gradually in a few seconds after returning to the resting condition.



Text-fig. 3. Membrane currents of the mouse oocytes under the voltage-clamp condition in the various media: *A*, standard medium; *B*, 20 Ca medium; and *C*, media as indicated on each record. Temperature, 33·0° C. Holding potential was −90 mV. The level of the potential step is indicated by the figure on each record in *A* and *C*. In *B*, each of current records corresponds to the potential record of the same alphabet. The dotted lines in *A* show the leakage current levels.

Voltage-clamp experiments of the mouse oocyte

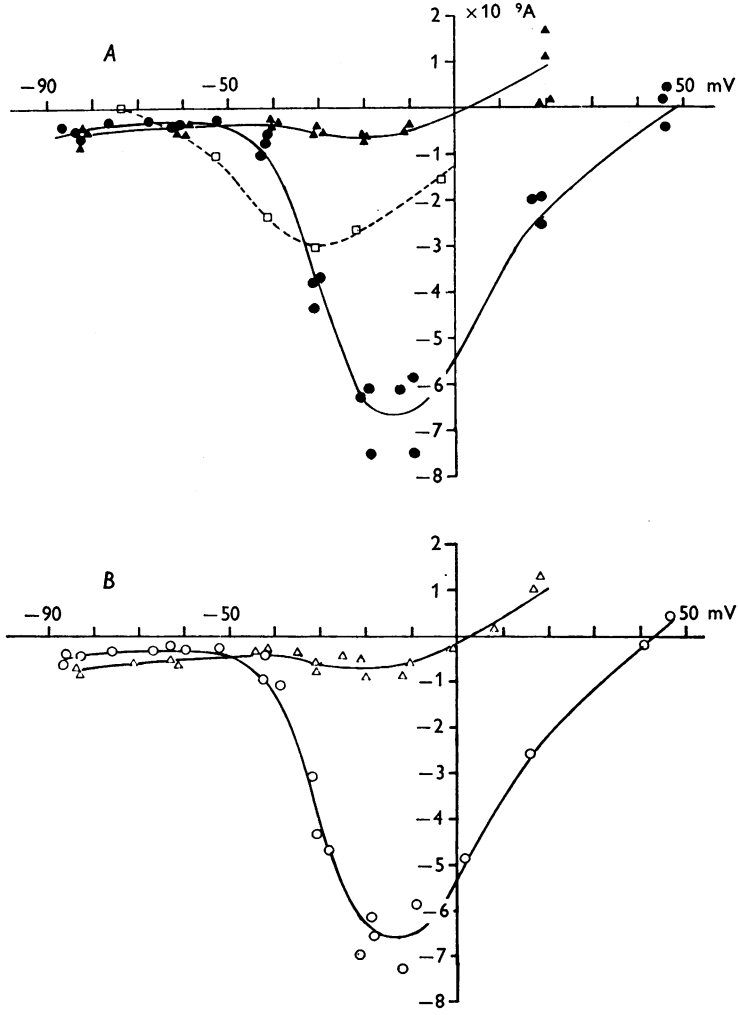
Ca inward current. Text-fig. 3*A* illustrates membrane currents in the mouse oocyte in standard medium at two potential levels of −52 and −31 mV. The record on the left side was obtained near the critical potential level for the off-response. These currents showed both activation and inactivation similar to those of other excitable membranes (Hodgkin & Huxley, 1952; Hodgkin, 1957; Hille, 1970). The leakage current in standard

medium was considerably large as seen in the records and rapidly increased soon after penetration of the electrodes. The following experiments were therefore performed in the media containing high concentration of divalent cations such as 20 mM-Ca or 10 mM-Mn. Advantages of these solutions were already described (see above).

A series of current records in 20 Ca medium is shown in Text-fig. 3*B*. Holding potential was -90 mV and the inward current appeared with a potential step at about -40 mV. The peak amplitude of the inward current increased with larger depolarizing steps until it became maximum at -15 mV. With the depolarization above -15 mV the peak amplitude of the membrane current decreased, probably because of a reduction in driving force for Ca. At the same time the leakage current usually changed its sign in the positive direction, the membrane current being outward at the steady state. In Text-fig. 4*A*, the *V–I* relation at the peak of the current in 20 Ca medium was estimated by averaging the current records in three oocytes (filled circles) and shown by a continuous line. The peak times of inward currents were 9–11 msec at -30 mV and 1·5–2 msec at $+19$ mV at 33° C, indicating that the time course of the activation became faster with larger depolarization and was potential dependent. This resembles the characteristics of Na or Ca inward currents of other excitable membranes (Hodgkin & Huxley, 1952; Hille, 1970; Hagiwara, Hayashi & Takahashi, 1969; Keynes, Rojas, Taylor & Vergara, 1973). Although the inactivation process was also accelerated by depolarization below -15 mV, it remained almost unchanged irrespective of the potential above that level. The Q_{10} of peak inward currents was 2–3 in the range from 20 to 35° C. Below 20° C the inward current was greatly reduced and was abolished at about 8° C. The reduction and abolition of the Ca current at low temperature were reversible. The Q_{10}s of time courses of activation and inactivation were about 4.

In standard medium containing 1·7 mM-Ca, the peak inward current was estimated by subtracting the steady-state leakage from the original current record at each potential level (Text-fig. 4*A*, open squares and dashed line). Since its maximum value was less than half of that in 20 Ca medium (Text-fig. 4*A*, continuous line), the contribution of Ca ions to the inward current was evident. In 10 Mn medium containing no Ca, the inward current was greatly reduced but the small current remained, the maximum peak current being $0{\cdot}84 \pm 0{\cdot}11 \times 10^{-9}$ A (the mean and S.D. of observation, $n = 3$; Text-fig. 3*C*, 10 Mn). The *V–I* curve in 10 Mn medium (Text-fig. 4*A*, filled triangles) showed that it attained to its maximum of inward direction at the same potential level as that in 20 Ca medium. In order to examine any possible contribution of Na to the inward current, membrane currents were measured in both 20 Ca, and

10 Mn, Na-free media (Text-fig. 3*C*) and the *V–I* relations were estimated (Text-fig. 4*B*). These curves were, respectively, very close to those obtained in the media containing Na ions in Text-fig. 4*A*. In 20 Ca medium the maximum peak of inward currents at 33° C was $-6{\cdot}64 \pm 0{\cdot}77 \times 10^{-9}$ A



Text-fig. 4. Averaged *V–I* relations at the inward peak of the membrane current of the mouse oocytes: *A*, in media containing Na ions (10 Mn, ▲; 20 Ca, ●; standard, □); and *B*, in media without Na ions (10 Mn Na-free, △; 20 Ca Na-free, ○). Temperature, 33° C. Holding potential was −90 mV in all media. In standard medium, the inward current is estimated by subtracting the leakage current from the peak of the current. All *V–I* curves are obtained by averaging the data from three oocytes except in standard medium, of which the *V–I* curve is obtained from an oocyte.

(the mean and S.D. of observation, $n = 4$). This value was not significantly different from $-6{\cdot}71 \pm 0{\cdot}57 \times 10^{-9}$ A in 20 Ca Na-free medium at 33° C ($n = 5$). It is concluded that the inward current of the mouse oocyte membrane is attributable to Ca current through Ca channels. These channels might allow a small amount of Mn ions to permeate (Yamagishi, 1973).

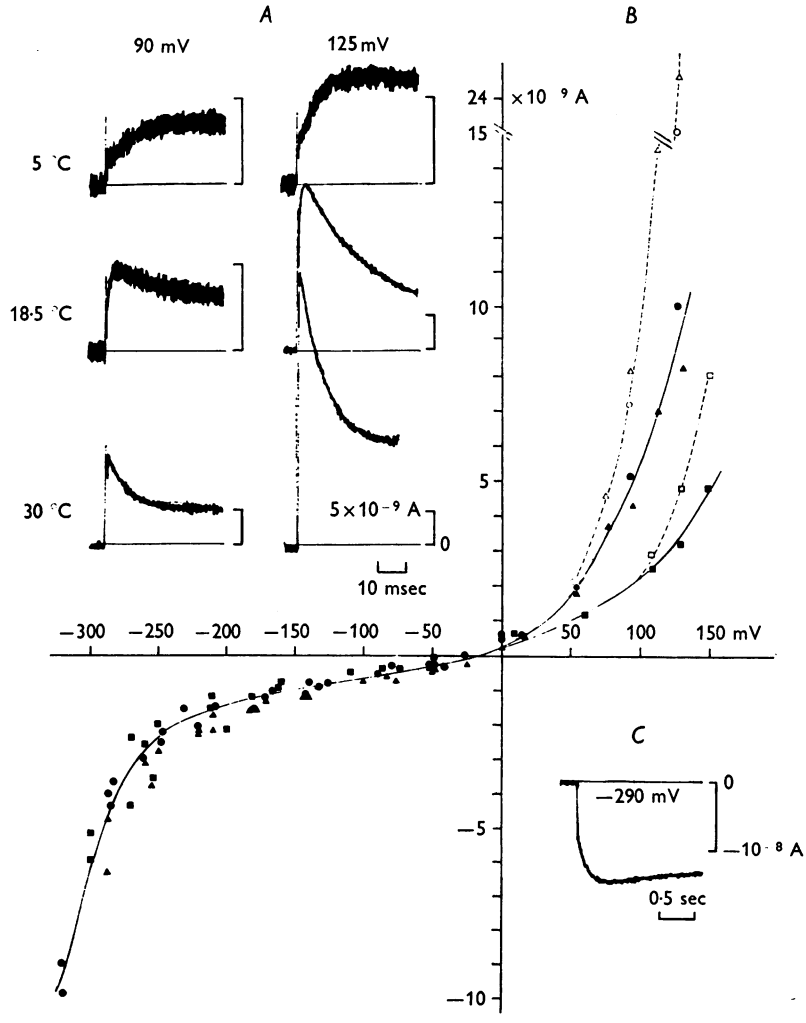
Selectivity ratios among alkali earth cations in 'Ca channels' of the mouse. For the Ca channels Sr or Ba can be substituted for Ca (Fatt & Ginsborg, 1958; Hagiwara, Fukuda & Eaton, 1974). The inward current of the mouse oocyte was larger in 20 Sr Na-free medium and was smaller in 20 Ba Na-free medium than in 20 Ca Na-free medium (Text-fig. 3*C*). The maximum peaks of inward currents in Sr and Ba media at 33° C were $-9{\cdot}70 \pm 1{\cdot}97 \times 10^{-9}$ A ($n = 5$) and $-4{\cdot}76 \pm 0{\cdot}62 \times 10^{-9}$ A (the mean and S.D. of observation, $n = 4$), respectively. Thus, the selectivity ratios of 'Ca channels' for alkali earth cations in the mouse oocyte were estimated as Ca (1·0):Sr (1·4):Ba (0·7). When 0·5 mM-$LaCl_3$ was added to 20 Ca medium, inward currents were completely abolished as shown in Text-fig. 3*C* (Hagiwara & Takahashi, 1967). These findings further confirm that the inward current of the mouse oocyte is due to Ca current through the Ca channels.

The stabilizing effect of Ca on the mouse oocyte membrane was also evident, because both the critical level and the potential level at which the peak inward current became maximum shifted in positive direction by 16 mV with an increase in the Ca concentration from 1·7 mM (standard medium) to 20 mM (Text-fig. 4*A*, dashed and continuous lines; Frankenhaeuser & Hodgkin, 1957). The stabilizing effects were not equal among alkali earth cations and their sequence was estimated as Ba $\geqslant$ Ca > Sr judging from the shifts of *V–I* relations of the peak inward currents.

Voltage–current relation of the steady-state current. In order to study the properties of leakage and outward currents, the membrane currents were measured in 10 Mn Na-free medium. When the voltage was clamped stepwise at a level between -200 and $+25$ mV, the current was changed instantaneously and was maintained at a steady level. The *V–I* curve in the steady state was linear in the range of these potential levels (Text-fig. 5*B*). These results indicate that the resting conductance of the membrane is mainly atrributed to the leakage current in agreement with the suggestion obtained by constant-current stimulation experiments (see above). The hyperpolarization below -200 mV from the holding level of -50 mV induced an instantaneous increase in the inward current and later activation and inactivation processes with very slow time courses (Text-fig. 5*C*). The *V–I* relation at the peak of the current showed a marked inward-going rectification. Since the extremely negative potential level was required to induce the rectification, the break-down of the

membrane may probably be responsible for the increase in this inward current. Both the leakage and the inward currents were not much dependent upon external K concentration (Text-fig. 5*B*).

Outward surge current. When the membrane was depolarized over +50 mV from the holding level of −75 mV, outward surge was observed with activation and inactivation processes (Text-fig. 5*A*). With larger depolarizing steps, the outward surge current was rapidly increased and at the same time its activation process was accelerated (Text-fig. 5*A*, 5° C). However, the time course of inactivation was almost independent of



Text-fig 5. For legend see facing page.

the potential level (Text-fig. 5*A*, compare 90 mV and 125 mV at 30° C). The *V–I* curve at the peak of the surge was shown by the dashed line in Text-fig. 5*B* and that of the steady state by the continuous line. When 0·5 mM-$LaCl_3$ was added to 10 Mn Na-free medium, the critical level of the outward surge shifted by 50 mV in the positive direction, probably due to the stabilizing effect of La ions on the membrane (Takata, Pikard, Lettvin & Moore, 1967; Blaustein & Goldman, 1968). The Q_{10} of the peak amplitude of the surge was about 2 and those of both activation and inactivation processes were 3–4 (Text-fig. 5*A*). All the above results suggest that the outward surge is due to an ionic current through a kind of ionic channel (Hille, 1970). An inward flux of Cl ions is not likely to contribute to this surge, because in Cl-free medium it remained unchanged. It is thus postulated that the outward surge represents the existence of a delayed rectification due to K channels. It is known that K channels in other excitable membranes also reveal more or less inactivation process (Nakajima, Iwasaki & Obata, 1962; Frankenhaeuser, 1963; Ehrenstein & Gilbert, 1966; Armstrong, 1969; Adrian, Chandler & Hodgkin, 1970). In Ranvier node of frog myelinated axon, the delayed rectification is suppressed by external TEA ions (Hille, 1967). In the mouse oocyte, however, the replacement of external Na ions by equimolar TEA (tetraethylammonium) ions or the injection of Cs into the oocyte made no effect upon the outward surge current.

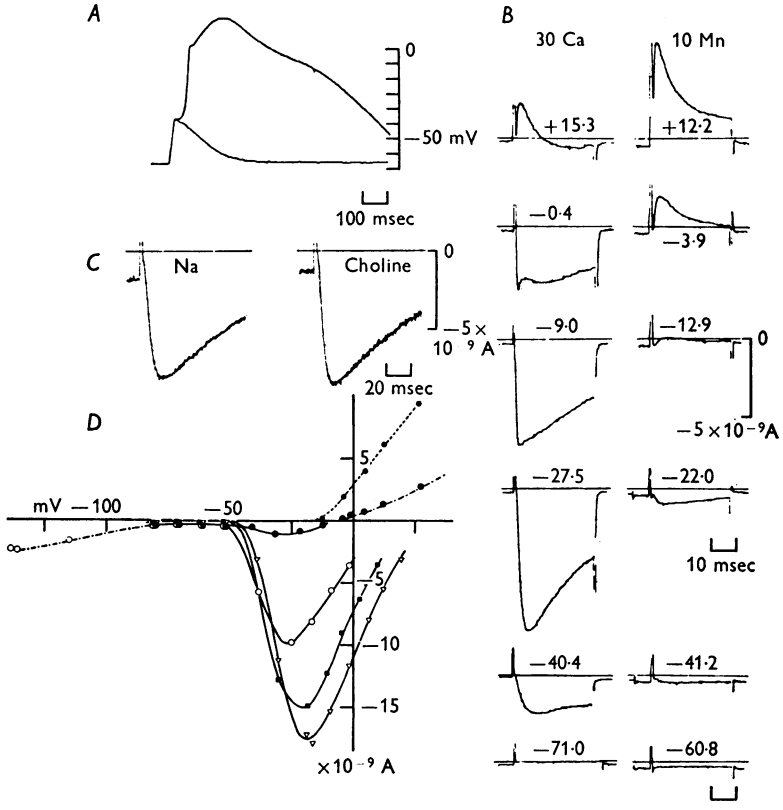
Text-fig. 5. *A*, outward surge currents of a mouse oocyte at two potential levels and at three different temperatures in 10 Mn Na-free medium. Holding potential was −75 mV. *B*, *V–I* relations of the leakage currents and the outward surges of the mouse oocytes in Na- and Ca-free media: 10 Mn 6K Na-free, circles; 10 Mn 133K Na-free, triangles; and 10 Mn 6K Na-free 0·5 La, squares. Temperature, 28–30° C. The holding potential was 0 or −50 mV for *V–I* relations below zero. It was increased to −75 mV in order to obtain the *V–I* relations at the peak and the steady state of the outward surge. Open symbols indicate the peak of the outward surge and filled symbols, the steady state of the outward surge and the leakage current. In the case of large hyperpolarization beyond −250 mV, the *V–I* relation was obtained at the beginning of the voltage step, i.e. just before the initiation of the activation (Text-fig. 5*C*). For the potential range below zero, data were obtained from three oocytes in 6 K medium, three oocytes in 133 K medium and three oocytes in 6 K medium with 0·5 mM-$LaCl_3$. For the potential range above zero they were obtained from three oocytes in 6 K, 133 K, and 6 K 0·5 La media. *C*, an inward current of a mouse oocyte at large hyperpolarization of −290 mV in 10 Mn Na-free 6 K medium. Temperature, 30° C. Holding potential was −50 mV.

Comparison of ionic channels in the oocyte membranes of three species: the mouse, the tunicate and the sea urchin

As described above, the Ca action potential which was found in the tunicate and the starfish oocytes (Miyazaki *et al.* 1974*a*, 1975*a*, *b*) also existed in the mouse oocyte as well. As shown in the following description, the ionic channels in the oocyte of the sea urchin, which belongs to the echinoderm as the starfish does, have considerable similarities to the channels in the mouse oocyte. Therefore, the comparison of Ca channels will be made among the oocytes of the three species: the mouse, the tunicate and the sea urchin.

Action potential and the membrane currents in the oocyte of the sea urchin. In contrast to the mouse and the tunicate oocytes, the sea urchin oocyte in 30 Ca ASW showed a resting potential of −70 mV which was comparable with those of other excitable membranes. An action potential was generated at the critical membrane potential of about −40 mV (Text-fig. 6*A*). Its overshoot was about 20 mV and its duration was 1–2 sec at 20° C. A notch was observed in the rising phase of the action potential. The above characteristics of the action and resting potentials are similar to those in the starfish oocytes (Miyazaki *et al.* 1975*a*, *b*; Hagiwara *et al.* 1975).

The identification of membrane currents in the sea urchin oocyte was performed under the voltage-clamp condition. A series of current records in Text-fig. 6*B* (left side) was obtained in 30 Ca ASW. The holding potential was −80 mV. The inward current appeared when the membrane was depolarized above −50 mV and its peak amplitude reached maximum at −25 mV. It was reduced with further depolarization. Both the rising and the falling phase of the inward current became faster with larger depolarizing steps. The peak time and the half-decay time at −25 mV were about 5 and 25 msec respectively at 30° C. In the potential range from −15 to +10 mV, the falling phase of the inward current was not simple exponential but included an outwardly directed notch. The membrane current became biphasic above +10 mV, being outward initially and inward later. In 10 Mn ASW which contained no Ca ions, the outward surge current was clearly observed with depolarization above −15 mV (Text-fig. 6*B*, right side), while the inward current was greatly reduced. The surge current showed the fast activation followed by the relatively slow inactivation processes. Its peak increased rapidly with increasing depolarization. It was suggested that the inward current was carried by Ca ions and that the outwardly directed notch on the falling phase of Ca current was the outward surge current superimposed upon the inward current. The contribution of Na ions to the inward current was probably negligible



Text-fig. 6. *A*, shows the potential responses to two rectangular current pulses of 2·9 and $3{\cdot}5 \times 10^{-10}$ A in a sea urchin oocyte. An action potential was elicited by the current of $3{\cdot}5 \times 10^{-10}$ A. 30 Ca ASW with Na. Temperature, 21° C. *B*, shows two series of current records under the voltage-clamp condition in a sea urchin oocyte in 30 Ca and 10 Mn ASWs with Na. The holding potential was −80 mV. Each potential level is indicated by a number at each record in mV. Temperature, 30·5° C. *C*, shows two inward currents of a sea urchin oocyte obtained in 30 Ca and 30 Ca Na-free choline ASWs. The clamped potential level was the same between two records, being −27·5 mV. Holding potential was −80 mV. Temperature, 21·5° C. *D*, shows *I–V* relations of a sea urchin oocyte in 10 Mn (filled circles), in 30 Ca (open circles), in 60 Ca (filled squares) and in 120 Ca ASWs (open triangles). The continuous lines indicate the *I–V* relations at the peaks of the inward currents. The dashed line above −15 mV indicate the *I–V* relation at the peak of the outward surge and the dash-and-dot line above −5 mV indicates the steady-state current. The dash-and-dot line below −80 mV shows the *I–V* relation of the anomalous rectification. Temperature, 30·5° C.

because replacement of Na in the ASW with choline kept both amplitude and time course of the inward current unchanged (Text-fig. 6*C*).

According to the recent report Ca currents in the starfish oocyte under voltage clamp condition do not seem to be exactly identical with those in the sea urchin oocyte, because Ca channels of the starfish show the activation with two steps and have specific sensitivity to external Na (Hagiwara *et al.* 1975).

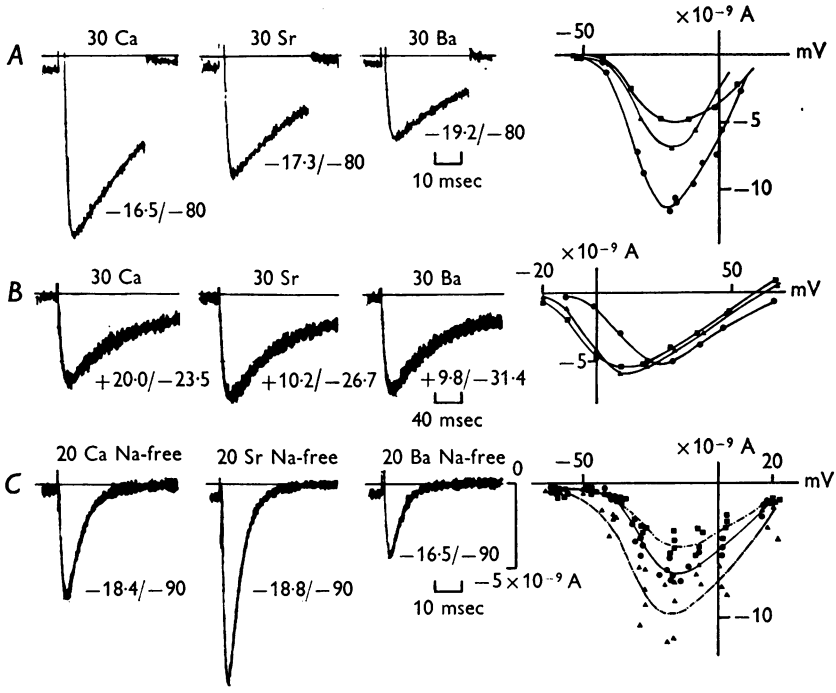
When Na ions in the ASW were replaced by Tris (2-amino-2-hydroxymethyl-propane-1, 3-diol), the inward current of the sea urchin oocyte was also reduced to about half of the control (0·52, 0·60, and 0·53 in three experiments). The sensitivity of the inward current to Na ions would seem to be confirmed by substituting Tris for Na. However, the inward current was unchanged by replacement of Na with choline, as described above, as well as with Cs or TEA. Therefore it is reasonable to assume that there was a pharmacological effect of Tris upon the Ca current.

Text-fig. 6*D* shows *I–V* relations of the sea urchin oocyte obtained in 10 Mn, 30 Ca, 60 Ca, and 120 Ca ASWs. The dashed line with filled circles indicates *I–V* curve at the peak of the outward surge current in 10 Mn ASW and dash-and-dot lines illustrate *I–V* curves at the steady state, while the continuous lines indicate the curves at the peak of the inward currents. With increase in concentration of external Ca ions, the inward current increased and the *I–V* curves shifted slightly to positive direction along the voltage axis. The former observation indicates that the inward current was carried by Ca ions. The latter represents the stabilizing effect of Ca ions. The small inward current remained in 10 Mn ASW, which suggested that Mn ions could permeate through Ca channels as in the mouse oocyte.

Comparison of the selectivity ratios among alkali earth cations for Ca channels. The selectivity of alkali earth cations for Ca channels was studied by substituting Sr or Ba for Ca in the oocytes of three species: the sea urchin, the tunicate and the mouse. The records in Text-fig. 7 *A*, *B* and *C* exemplify almost maximum inward currents in Ca, Sr, and Ba ASWs or media. In the diagrams of Text-fig. 7*A*, *B* and *C*, *I–V* relations of those three species at the peaks of the inward currents are shown in respective media or ASWs. From the maximum peak amplitudes of the inward currents in respective ASWs or media, the selectivity ratios were estimated. The values were not equal among three species: i.e. Ca(1·0):Sr(0·7):Ba(0·5) in the sea urchin; Ca(1·0):Sr(1·6):Ba(1·1) in the tunicate; and Ca(1·0):Sr(1·4):Ba(0·7) in the mouse (Table 2).

There were various shifts of the *I–V* curves along the voltage axis, partly because of difference in stabilizing effects among alkali earth cations and partly because of species difference in the thresholds for Ca currents. The shifts are conveniently represented by the changes in the potential level at which the peak inward current becomes half of its maximum; i.e. $V_{\frac{1}{2}}$ (Table 2, see Okamoto *et al.* 1976*a*, *b*). The sequences of the stabilizing

effects by alkali earth cations for Ca channels were not identical among three species, being: Ba ⩾ Sr > Ca in the sea urchin; Ca > Sr > Ba in the tunicate; and Ba ⩾ Ca > Sr in the mouse (Table 2). In the sea urchin oocyte, the shift of *V–I* curve on the replacement of Ca with Sr or Ba was only slightly observed. In Ba ASW the *I–V* curve was



Text-fig. 7. The inward currents and their *I–V* relations from the oocytes of three species obtained in alkali earth cation ASWs media. The inward currents and *I–V* relations of: *A*, were obtained in a sea urchin oocyte at 30·5° C; *B*, in a tunicate oocyte at 16° C; and *C*, in the mouse oocytes at 33° C. The figures in the left side show the inward currents in 30 Ca, 30 Sr, and 30 Ba ASWs (sea urchin and tunicate) or in 20 Ca, 20 Sr, and 20 Ba Na-free media (mouse). The holding and the test levels of the membrane potential are indicated by the denominator and the numerator of the figure in each record in mV. In the *I–V* relations filled circles indicate the data in Ca ASW or medium, filled triangles in Sr ASW or medium and filled squares in Ba ASW or medium. The continuous line in the right side figure of *C* of the mouse was an averaged *I–V* curve from the five records in 20 Ca Na-free medium. The interrupted lines were also averaged *I–V* curves from the data in Sr ($n = 5$) and Ba ($n = 4$) Na-free media with special attention to draw the curves through the points of the averaged $V_{\frac{1}{2}}$s (−35·5 mV in Sr medium and −31·6 mV in Ba medium) and the points of the averaged peak values (−9·7 and $-4{\cdot}8 \times 10^{-9}$ A in Sr and Ba media respectively).

TABLE 2. Permeability ratios for Ca channels of three species oocytes: mouse, tunicate and sea urchin

Soln.	Egg no.	Temp.	I_p[7] ($\times 10^{-9}$ A)	V_p[7] (mV)	$I_p/I_{p.Ca}$ Sr	$I_p/I_{p.Ca}$ Ba	V_i (mV)	I at (V_i + 20 mV)	$\triangle V_i$[1] Sr	$\triangle V_i$[1] Ba	I' (revised) Sr	I' (revised) Ba	P_{C+s}/P_{Ca} Sr	P_{C+s}/P_{Ca} Ba
							Mouse							
20 Ca	No. 1	33° C	7·25	−15·0	.	.	−32·5	7·20	.	.	.	.	.	.
	No. 2		6·65	−15·5	.	.	−32·0	6·50	.	.	.	.	.	.
	No. 3		6·70	−15·5	.	.	−32·5	6·50	.	.	.	.	.	.
	No. 4		7·25	−15·7	.	.	−30·0	6·82	.	.	.	.	.	.
	No. 5		5·70	−15·5	.	.	−33·0	5·55	.	.	.	.	.	.
	Mean		6·71	−15·4	.	.	−32·0	6·51	.	.	.	.	.	.
20Sr	No. 6	33° C	7·84	−17·0	.	.	−36·0	7·76	.	.	.	.	.	.
	No. 7		12·24	−19·0	.	.	−36·5	12·12	.	.	.	.	.	.
	No. 8		11·72	−17·5	.	.	−31·0	11·04	.	.	.	.	.	.
	No. 9		7·52	−17·5	.	.	−38·0	7·52	.	.	.	.	.	.
	No. 10		9·20	−19·0	.	.	−36·0	8·96	.	.	.	.	.	.
	Mean		9·70	−18·0	1·44	.	−35·5	9·48	−3·5	.	7·26	.	.	1·12
20Ba	No. 11	33° C	5·56	−13·0	.	.	−30·6	5·49	.	.	.	.	.	.
	No. 12		3·92	−14·5	.	.	−31·5	3·80	.	.	.	.	.	.
	No. 13		5·20	−15·5	.	.	−32·2	5·80	.	.	.	.	.	.
	No. 14		4·34	−14·5	.	.	−32·0	4·08	.	.	.	.	.	.
	Mean		4·76	−14·4	.	0·71	−31·6	4·61	.	+0·4	.	4·75	.	0·73
									$\triangle V_i$[2]		I'[3]		$P_{1·7}/P_{20}$[4]	
Standard (1·7 mM-Ca)	No. 15	33° C	3·05	−29·0	.	.	−48·5	2·90	.		.		.	
	No. 16		3·50	−28·0	.	.	−45·5	3·45	.		.		.	
	Mean		3·28	−28·5	.	.	−47·0	3·18	−14·5		12·4		1·91	

TABLE 2 (*cont.*)

Soln.	Egg no.	Temp.	I_p ($\times 10^{-9}$ A)	V_p (mV)	$I_p/I_{p,Ca}$ Sr	$I_p/I_{p,Ca}$ Ba	V_i	I at (V_i + 20 mV)	ΔV_i Sr	ΔV_i Ba	I' (revised) Sr	I' (revised) Ba	P_{C+s}/P_{Ca} Sr	P_{C+s}/P_{Ca} Ba
							Tunicate[5]							
30Ca	No. 1	16° C	5·30	+23·0	.	.	+ 8·0	5·00	.	.	.	.	.	.
30Sr			6·25	+14·0	1·18	.	− 3·5	6·10	−11·5	.	2·41	.	0·48	.
30Ba			5·60	+11·5	.	1·06	− 7·0	5·60	.	−15·0	.	1·67	.	0·33
100Ca	No. 2	12·5° C	7·00	+22·0	.	.	+ 3·6	6·60	.	.	.	.	.	.
100Sr			12·24	+ 6·0	1·75	.	− 8·5	11·96	−12·1	.	4·49	.	0·68	.
100Ba			6·64	+ 1·5	.	0·95	−12·5	6·16	.	−16·1	.	1·67	.	0.25
200Ca	No. 3	15° C	3·20	+31·0	.	.	+14·5	3·10	.	.	.	.	.	.
200Sr			5·32	+19·5	1·66	.	+ 3·5	5·19	−11·0	.	2·14	.	0·69	.
200Ba			3·42	+11·0	.	1·07	− 3·5	3·30	.	−18·0	.	0·77	.	0·25
200Ca	No. 4	16° C	11·5	+32·5	.	.	+18·0	10·9	.	.	.	.	.	.
200Sr			18·5	+19·0	1·61	.	+ 5·0	17·4	−13·0	.	6·13	.	0·56	.
200Ba			11·6	+15·0	.	1·01	− 1·5	11·3	.	−19·5	.	2·37	.	0·22
200Ca	No. 5	8° C	1·02	+27·5	.	.	+12·0	0·95	.	.	.	.	.	.
200Sr			1·78	+15·5	1·76	.	+ 0·7	1·65	−10·8	.	0·66	.	0·70	.
200Ba			1·41	+ 6·0	.	1·38	−10·0	1·36	.	−22·5	.	0·21	.	0·22
200Ca	No. 6	12·5° C	1·22	+32·2	.	.	+18·2	1·12	.	.	.	.	.	.
200Sr			2·06	+26·0	1·69	.	+ 9·0	2·01	−11·2	.	0·95	.	0·85	.
200Ba			1·26	+18·5	.	1·03	+ 3·0	1·18	.	−15·2	.	0·34	.	0·31
200Ca	No. 7	11° C	1·80	+34·0	.	.	+16·5	1·78	.	.	.	.	.	.
200Sr			3·34	+19·0	1·86	.	+ 3·7	3·32	−12·8	.	1·17	.	0·66	.
Mean					1·64	1·08			−11·8	−17·7			0·66	0·26

TABLE 2 (*cont.*)

Soln.	Egg no.	Temp.	I_p ($\times 10^{-9}$ A)	V_p (mV)	$I_p/I_{p.Ca}$ Sr	$I_p/I_{p.Ca}$ Ba	V_i (mV)	I at (V_i + 15 mV)	I/I_{Ca} (V_i + 15 mV) Sr	I/I_{Ca} (V_i + 15 mV) Ba	ΔV_i Sr[6]	ΔV_i Ba	P_{Ca+s}/P_{Ca} Sr	P_{Ca+s}/P_{Ca} Ba
						Sea urchin								
50Ca	No. 1	19·7° C	2·70	−20·0	.	.	−31·5	2·70	.	.	.	.	.	.
50Ba			1·70	−17·0	.	0·63	−31·5	1·70	.	0·63	.	0	.	0·63
50Ca	No. 2	20·0° C	6·10	−23·5	.	.	−35·5	6·05	.	.	.	.	.	.
50Sr			4·80	−24·5	0·76	.	−37·0	4·75	0·76	.	+1·5	.	0·86	.
50Ca			6·50	−26·0	.	.	−38·5	6·45	.	.	.	.	.	.
Mean of Ca			6·30	.	.	.	.	6·25	.	.	.	.	.	.
50Ba	No. 3	30·0° C	6·80	−10·0	.	0·60	−31·0	6·60	.	0·58	.	+3·0	.	0·73
50Ca			11·30	−19·0	.	.	−34·0	11·30	.	.	.	.	.	.
30Ca	No. 4	30·0° C	6·85	−23·0	.	.	−36·5	6·85	.	.	.	.	.	.
30Ba			3·30	−18·0	.	0·48	−34·5	3·30	.	0·48	.	+2·0	.	0·56
30Ca	No. 5	29·5° C	9·70	−19·5	.	.	−34·5	9·70	.	.	.	.	.	.
30Sr			6·90	−23·5	0·70	.	−36·5	6·85	0·70	.	+0·5	.	0·73	
30Ca			10·00	−25·0	.	.	−37·0	9·90	.	.	.	.	.	.
Mean of Ca			9·85	.	.	.	.	9·80	.	.	.	.	.	.
30Ca	No. 6	30·0° C	11·6	−15·5	.	.	−30·5	11·6	.	.	.	.	.	.
30Ba			5·05	−14·5	.	0·44	−30·0	5·05	.	0·44	.	+0·5	.	0·46
30Sr			6·95	−17·5	0·61	.	−32·7	6·95	0·61	.	+0·3	.	0·62	.
30Ca			11·25	−18·5	.	.	−33·0	11·25	.	.	.	.	.	.
Mean of Ca			11·43	.	.	.	.	11·43	.	.	.	.	.	.
Mean			.	.	0·69	0·54	.	.	0·69	0·54	+0·8	+1·4	0·74	0·60

[1] $\Delta V_i = V_{i.C+s} - V_{i.Ca+s}$ (20 mM). [2] V_i (1·7 mM-Ca) − V_i (20 mM-Ca).

[3] Revised Ca current in standard medium (1·7 mM-Ca). The revision was made with the following equation;

$$I(V_i + 20\text{ mV}) \times \exp(0{\cdot}0761\ \Delta V_i) \times (20\text{ mM})/(1{\cdot}7\text{ mM}).$$

[4] The ratio of Ca permeability between 1·7 and 20 mM solution.

[5] Some of the data were recalculated from the data in the previous paper (Okamoto *et al.* 1976*b*).

[6] ΔV_i for Sr in the sea urchin was obtained in comparison with V_i in Ca ASW after the recovery from Sr ASW (see text, p. 466).

[7] I_p; the maximum peak inward current where the membrane potential was V_p.

shifted to positive direction by a few millivolts reversibly. In Sr ASW there were various amounts of irreversible shift to the negative direction and the shift remained after the recovery to Ca ASW. With close inspection, however, the further negative shift of a millivolt was constantly observed on the recovery (Table 2, the sea urchin, $V_{\frac{1}{2}}$). Therefore, it is suggested that Sr ions also have a slightly higher stabilizing power than Ca ions besides an unknown and irreversible effect upon the critical membrane potential possibly due to their intracellular accumulation. It was difficult to determine the sequence, because the difference of the stabilizing power was not so significant. However, averaging the differences of $V_{\frac{1}{2}}$s in Sr and Ba ASWs from that in Ca ASW the sequence in the sea urchin was inferred as Ba $\geqslant$ Sr $>$ Ca (Table 2).

The peak inward current in 30 Ca and Sr ASWs in the sea urchin oocyte was always considerably reduced with an increase in the depolarization in the potential range above -15 mV (see I–V curves of the sea urchin in Text-fig. 7*A*). However in 30 Ba ASW the inward current was less reduced than in 30 Ca and Sr ASWs. This difference in I–V relations of the peak inward current among Ca, Sr, and Ba was unique in the sea urchin oocyte. It seems likely that the fairly large surge outward current was superimposed upon the inward current in Ca and Sr ASWs, while in Ba ASW the outward surge was suppressed, because the surge in the sea urchin oocyte was probably K delayed rectification and Ba ions could suppress the rectification, as suggested in other excitable membranes (Werman & Grundfest, 1961; Sperelakis, Schneider & Harris, 1967; Hagiwara *et al.* 1974). In the mouse and the tunicate oocytes, the shapes of I–V curves in Ba medium and ASW were not different from those in Ca and Sr media or ASWs. This may be due to the fact that the outward surges in these two species had the highly positive critical level of about $+50$ mV for their activation and thus they did not overlap with 'Ca channel' current.

Comparison of rectifications of the oocyte membranes in three different species. The dashed line in Text-fig. 6*D* illustrates the I–V relation at the peak of the outward surge in the sea urchin oocyte in 10 Mn ASW where Ca current was almost abolished. The I–V curve indicates that there is a marked outward-going rectification above -15 mV. In squid giant axon, the internal perfusion of Cs ions abolishes the delayed rectification (Adelman & Senft, 1966; Bezanilla & Armstrong, 1972). In the sea urchin oocyts, the outward surge at $+50$ mV level was also effectively suppressed up to an eighth by intracellular injection of Cs ions (not illustrated). As described previously, the surge was reduced by Ba ions in the external solution. These characteristics strongly suggest that the outward-going rectification in the sea urchin oocyte was K delayed rectification. This K rectification exists in the mouse oocyte, as described above, and also in the tunicate oocyte (Fig. 12 of Okamoto *et al.* 1976*a*), where the rectification had much more positive activation levels than that of the sea urchin oocyte.

The inward-going rectification below -50 mV in the sea urchin oocyte

(dash-and-dot line with open circles in Text-fig. 6*D*) seemed to be the same as the K anomalous rectification that has been analysed precisely in the tunicate egg (Miyazaki *et al.* 1974*b*) and in the starfish oocyte (Miyazaki *et al.* 1975*a*, *b*; Hagiwara & Takahashi, 1974), since the potential level for its activation in the sea urchin was common to those of the rectifications in these two species. However, an inward-going rectification found in the mouse oocyte (Text-fig. 5*B*) could not be identified as K anomalous rectification as seen in the tunicate or the sea urchin, because this was insensitive to external K ions and required an extremely negative potential (-200 mV) for its activation (p. 476).

DISCUSSION

Similarity of Ca channels in the mouse oocytes membrane to those in various animal species

Properties of Ca channels which induce Ca action potential have been analysed in the tunicate egg (Okamoto *et al.* 1976*b*). The Ca channels in the egg membrane of the tunicate have been confirmed to be essentially identical with those of other excitable membranes, such as crustacean muscle membrane (Fatt & Ginsborg, 1958; Hagiwara & Naka, 1964). It has been recognized that the starfish oocyte can also produce Ca action potential (Miyazaki *et al.* 1975*a*; Hagiwara *et al.* 1975). The present experiments have demonstrated that Ca channels exist in the mouse and the sea urchin oocyte membranes as well. The existence of Ca channels may therefore be a general characteristic of the egg membrane in the wide range of animal species.

The comparison of the egg Ca channels was attempted in this paper and revealed some differences in channel properties among the oocytes of three different species: the mouse, the tunicate and the sea urchin. These differences are: (1) critical membrane potentials for the activation; (2) sequences of stabilizing effects of Ca, Sr and Ba; (3) selectivity ratios of Ca channels for alkali earth cations: Ca, Sr and Ba; (4) the time courses of activation and inactivation.

The stabilizing effect of polyvalent cations upon ionic channels has been understood as the parallel shift in the I–V relation of the channels along the voltage axis (Frankenhaeuser & Hodgkin, 1957; Gilbert & Ehrenstein, 1969, 1970; Ehrenstein & Gilbert, 1973; Hille, 1968; D'Arrigo, 1973). Since the plasma membrane has been suggested to negatively charged at the surface, the diffuse double-layer potentials are very likely to exist in the vicinity of the surface (Gilbert, 1971). The stabilizing polyvalent cations in the external solution will reduce the effective negative charges at the external surface by both screening and binding

effects (McLaughlin, Szabo & Eisenman, 1971) and thereby shift the double layer potential to the positive direction. The above mentioned shift of the I–V relation along the voltage axis has been considered to be equal to the shift in the double layer potential (Gilbert, 1971).

The screening effect is defined as that induced by the electrostatic attraction of the polyvalent cations to the negatively charged sites and thus the effect will depend only upon the valency of the cations (McLaughlin *et al.* 1971; D'Arrigo, 1973). On the other hand, the binding effect is defined as that induced by the specific chemical adsorption of these cations to the negatively charged sites and thus the effect can be specific to the species of cations even within the cation group of the same valency (Gilbert & Ehrenstein, 1969; McLaughlin *et al.* 1971).

The critical membrane potential of Ca channels in the tunicate was about -10 mV in 30 Ca ASW and markedly positive in comparison with those of the mouse and the sea urchin. The above consideration on the stabilizing effect suggests that this relatively positive critical potential can be caused by increase in the binding power of the negative sites with the polyvalent cations or by a decrease in the surface negative charge density.

Different sequences of the stabilizing effects of Ca, Sr and Ba upon Ca channels were observed among three species, being: Ca > Sr $\geqslant$ Ba in the tunicate; Ba $\geqslant$ Ca > Sr in the mouse; and Ba $\geqslant$ Sr > Ca in the sea urchin (Table 2). These different sequences can be ascribed to different binding affinities of the surface negative sites to alkali earth cations because the screening effects could not produce the difference in the stabilizing effect when divalent cation in the external solution was exchanged in equimolar ratio. According to the recent theory on the ion selectivity of charged sites in ion exchangers, the negatively charged sites with the highest field intensity would prefer the cations with smaller diameters, thus the sequence being Ca > Sr > Ba within the alkali earth cations, while the sites with the lowest field intensity would prefer the cations with larger diameters, thus the sequence being Ba > Sr > Ca (Truesdell & Christ, 1967; Sherry, 1969; Diamond & Wright, 1969; D'Arrigo, 1974). Therefore, it may be inferred that the surface negative sites in the tunicate have the highest field intensity and those in the sea urchin have the lowest field. The sites in the mouse may have the field of middle intensity. It is suggested that in the sea urchin oocytes the stabilizing effect is mainly caused by the screening effect and the binding effect was probably of minor importance because there are only slight differences in the stabilizing effects among three alkali earth cations Ca, Sr and Ba.

According to the channel hypothesis for the ionic current in the excitable membrane, the selectivity ratios of the channels for permeating cations

are considered to be due to the intrinsic property of the negatively charged sites within the channels and not to be due to the property of the sites which are responsible for the stabilizing effect (Hille, 1970; Armstrong, 1975). Therefore, the differences in the selectivity ratios of Ca channels for Ca, Sr and Ba observed in the oocyte membranes in three species may indicate that the Ca channels are specific to animal species. However recent studies of the stabilizing effect on the ionic channels in the tunicate egg membrane have confirmed that the apparent conductance changes of channels occur in direct relation to the shifts of the I–V curves along the voltage axis (Okamoto *et al.* 1976*b*; Ohmori & Yoshii, 1977). The results suggest that the accumulation of the permeating cations at the orifices of channels is also regulated by the negativity of the surface double-layer potential. Therefore, the different sequences of the stabilizing effects of alkali earth cations upon Ca channels among three species could produce the apparent differences of the selectivity ratios of Ca channels for these cations.

Since the concentration of intracellular ionized Ca may be negligible compared with the external concentration in case of the oocyte as well (Baker & Warner, 1972), the constant field equation for Ca channels becomes as follows.

$$I_{Ca^{2+}} = P_{Ca^{2+}} \left(\frac{4F^2E^*}{RT}\right) \frac{(C^{2+})_s}{1-\exp(2FE^*/RT)},$$

where $(C^{2+})_s$ is a surface concentration of accumulated divalent cations permeating through Ca channels and E^* is an assumed acting transmembrane potential from the outer to inner surfaces. According to the theory of diffuse double layer, $(C^{2+})_s$ can be related with the double-layer potential at the outer surface, ψ, as follows.

$$(C^{2+})_s = (C^{2+})_o \exp(-2F/\psi RT),$$

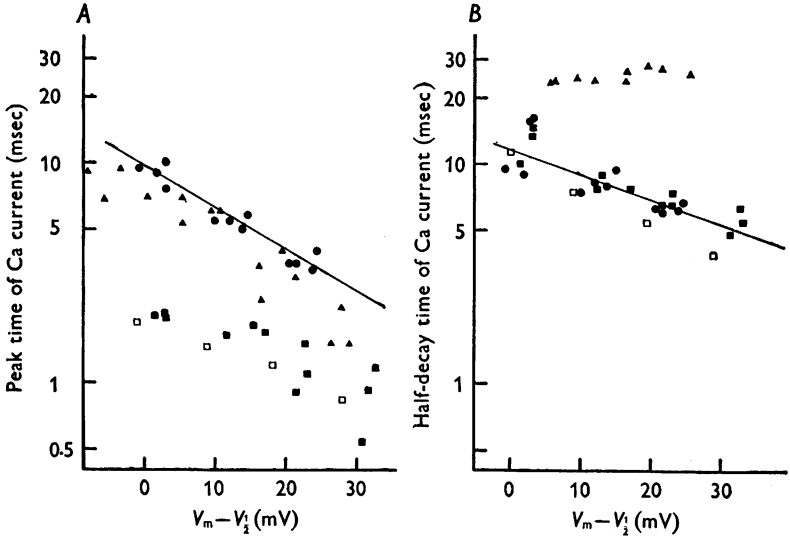
where $(C^{2+})_o$ is the bulk concentration. As described in previous papers (Okamoto *et al.* 1976*b*; Ohmori & Yoshii, 1977), the shifts along the voltage axis of all potential dependent parameters of an inward current including $P_{Ca^{2+}}$ were equal to that of a representative point on the I–V curve; $V_{\frac{1}{2}}$ where the peak of the inward current became half of its maximum. Therefore, although the stabilizing effects are different in the ASWs containing different divalent cations, the transmembrane potential E^* and consequently the fraction of opened channels are expected to be identical at the potential level (V_m) which had the same relation to respective $V_{\frac{1}{2}}$; $V_m = V_{\frac{1}{2}} + \alpha$. Thus the current at $(V_{\frac{1}{2}} + \alpha)$ represents the change of $P_{Ca^{2+}}$ if the current is revised by multiplying a Boltzmann factor in order to equalize the accumulation effects (Ohmori & Yoshii, 1977). The actual

permeability ratio was calculated as the ratio of the revised inward currents I' at $V_{\frac{1}{2}}$ +20 mV, where I' was defined as follows

$$I'(V_{\frac{1}{2}} +20 \text{ mV}) = I(V_{\frac{1}{2}} +20 \text{ mV}) \exp [2F/RT(\psi_t - \psi_c)] \times (C_c/C_t).$$

Here, ψ_t, ψ_c are the double-layer potentials at the outer surface in the test and control ASWs and C_t, C_c are divalent cation concentrations in the test and control ASWs. F, R, and T have the usual meanings. For example, $V_{\frac{1}{2}}$s of the tunicate egg, shown in Text-fig. 7*B*, are 8, −3·5 and −7 mV in Ca, Sr, and Ba ASWs and the peak currents I at ($V_{\frac{1}{2}}$ +20 mV) are 50, 61 and 56×10^{-10} A respectively. Considering that the shifts in the diffuse double layer potential are equal to the changes of $V_{\frac{1}{2}}$; $V_{\frac{1}{2}t} - V_{\frac{1}{2}c} = \psi_t - \psi_c$, the revised current Is at respective ($V_{\frac{1}{2}}$ +20 mV) are 50, 24 and 17×10^{-10} A. Thus the permeability ratios Ca:Sr:Ba = 1·0:0·48:0·33 were estimated. Similarly the ratios of the sea urchin eggs were estimated from the data illustrated in Text-fig. 7*A*, as Ca:Sr:Ba = 1·0:0·62:0·46. In the tunicate and the mouse oocytes, the level of ($V_{\frac{1}{2}}$ +20 mV) just corresponded to the potential where the peak of the inward current became maximum. In the sea urchin oocyte, however, the outward surge current was superimposed upon the inward current. Therefore, in order to minimize the contamination of the outward current, the measurement was done at ($V_{\frac{1}{2}}$ +15 mV) for sea urchin (Table 2, the sea urchin). Collected permeability ratios of the tunicate and the sea urchin eggs are tabulated in Table 2. In order to estimate the permeability ratios of Ca channels in the mouse egg, I–V curves of the Ca inward currents of four to five eggs were measured in each medium and the mean $V_{\frac{1}{2}}$s and the mean I' at ($V_{\frac{1}{2}}$ +20 mV) were calculated, as illustrated in Table 2. Average of the revised ratios of Ca:Sr:Ba are 1:0·66:0·26 in the tunicate egg, 1:0·74:0·60 in the sea urchin egg, and 1:1·12:0·73 in the mouse egg. Although the permeability ratio in the mouse egg seems to be different from the other two species, the ratio of Ba to Sr in the former is similar to that in the tunicate egg. There are points to be duly considered with respect to the relatively low Ca permeability in the mouse oocyte. It is well known that intracellular ionized Ca strongly inhibits 'Ca channel' current (Hagiwara & Nakajima, 1966). Since the surface to volume ratio in the mouse egg is extremely large as compared to the other two because of its smaller diameter, the relatively large leakage of Ca ions from outside to inside can be expected in high Ca medium. Actually the revised Ca current I' in standard medium with 1·7 mM-Ca is approximately 1·9-times the I' in 20 Ca medium (data from Text-fig. 4*A*, Table 2). Assuming that the Ca permeability decreases roughly half in 20 Ca medium and that the permeability in Sr or Ba medium does not depend upon the concentration, the permeability ratios are presumably Ca:Sr:Ba = 1·0:0·56:0·37, being close to those in the

tunicate egg. Relatively larger permeability to Ba ions was obtained in the sea urchin egg as compared to that in the tunicate egg. This can be explained by the fact that Ba ions in the external solution suppress the outward surge current of the sea urchin egg and consequently the apparent inward current increases. In summary it is concluded that the permeability ratios of Ca channels in the oocytes in the three species would essentially be the same, when the conductance changes due to the stabilizing effect and permeability changes due to above factors are taken into account.



Text-fig. 8. Dependence of the time courses of Ca inward currents upon the membrane potentials in the oocyte membranes of three species. *A*, the peak time (the time from the initiation of pulse to the peak of the inward current) against the membrane potential. *B*, the half-decay time (time from the peak to the half-decay) against the potential. The membrane potential levels are represented by the relative values to the respective critical membrane potential of the inward currents, that is: $V_m - V_{\frac{1}{2}}$. Filled triangles are the records obtained from four sea urchin oocytes at 30·5° C and filled circles from four mouse oocytes at 32° C. The continuous lines in both *A* and *B* were drawn by averaging the values in the mouse oocytes. Since most of the experiments of the tunicate oocytes were carried out at 16° C, the expected peak and half-decay times at 32° C were estimated by assuming Q_{10} of the time courses of Ca current as 4 in three oocytes (filled squares). Open squares indicate the expected peak and half-decay times at 32° C in one tunicate oocyte. They were estimated from those values at 16° C and 12° C by using the Q_{10} of 4·1 which was measured in the same oocyte. Solutions were 30 Ca ASW for the sea urchin and the tunicate oocytes and 20 Ca medium for the mouse oocytes.

The time courses of the membrane currents are highly sensitive to temperature in the egg membrane as well as other excitable membranes (Okamoto *et al.* 1976*a*). Inward Ca currents of the oocytes were recorded mostly at about 30° C in the mouse and in the sea urchin but at 5–25° C in the tunicate. Q_{10}s of both peak and half-decay times in the tunicate egg were calculated and their values at 32° C were estimated from data at 5–25° C. In Text-fig. 8 both peak and half-decay times in the three species are plotted against the relative membrane potentials in reference to $V_{\frac{1}{2}}$. The relations between the half-decay times and the relative membrane potential in the tunicate and the mouse oocyte were almost identical. However, the half-decay time in the sea urchin was exceptionally insensitive to the voltage. Since in the sea urchin oocyte the outward surge current was superimposed upon the Ca inward current above – 20 mV, the apparent long half-decay time may be expected. The relation of the peak time with the relative membrane potential in the sea urchin and the mouse oocyte were almost identical. The absolute values of the peak time in the tunicate egg was, however, less than half of those in other two at any potential level. At the moment we have no explanation for this short peak time.

The functional significance of Ca channels in the oocytes

In the present paper, it has been confirmed that similar types of Ca channels exist in the oocyte membranes of various animal species including mammals. In the mouse and the tunicate the resting potential of the oocytes are usually at depolarized level partly because of the leakage due to penetration of the micro-electrodes. At this level Ca channels are inactivated. It is possible that the real resting potential is at more hyperpolarized level and the endogenous depolarization such as fertilization potential at the invasion of sperms can evoke the Ca action potential or induce Ca influx into the cytoplasm. Actually it is suggested that the Ca action potential at the time of the fertilization prohibits polyspermy in the sea urchin egg (Jaffe, 1976).

It has been reported that Ca ionophore A 23187 can activate the unfertilized oocytes of the sea urchin, the starfish, the frog and the mouse by initiating the membrane depolarization, and by increasing cellular respiration and protein synthesis (Steinhardt & Epel, 1974; Steinhardt, Epel, Carroll & Yanagimachi, 1974). The activation by this artificial Ca ionophore, however, is independent of external Ca ions (Steinhardt & Epel, 1974). It is suggested that the Ca ionophore can also release the Ca in the intracellular stores to increase the intracellular ionized Ca level. Therefore it is highly probable that Ca channels, which are the intrinsic Ca ionophore, exist in both external and internal membrane structures and regulate the Ca influx into the cytoplasm.

As to the importance of Ca ions on the fertilization it has been suggested that the cortical granules in the sea urchin oocytes discharge their contents by an increase in the intracellular ionized Ca (Vacquier, 1975). There are several suggestions that the cell cleavage at early stages of the embryo is also regulated by intracellular ionized Ca (Baker & Warner, 1972). Actomyosin like materials have been extracted from the cortical regions of the oocytes (Sakai, 1968; Mabuchi, 1973). Thus the process analogous to the contraction of the muscle fibres has been proposed during the cleavage cf the early embryo (Kinoshita & Yazaki, 1967). Previous experiments on the tunicate eggs (Miyazaki *et al.* 1972) and the preliminary experiment on the mouse eggs indicated that Ca channels remained in the membrane after the fertilization. Therefore it is allowed to speculate that Ca channels play a role in the cleavage as well.

Recently Miyazaki & Hagiwara (1976) has reported that the external membrane of *Drosophila* egg does not show any regenerative response and we were also unsuccessful for demonstrating the action potential in the membrane of the frog oocyte (unpublished). Since in these animals the external medium around the oocytes is of very low ionic strength, it is reasonable for these oocytes to be unable to utilize the external Ca through Ca channels in the external plasma membrane. However, importance of Ca ions in the cleavage has been demonstrated in the amphibian oocytes (Baker & Warner, 1972). It is therefore possible that Ca channels exist in the internal membrane structures of the oocytes and play a role in the early process of the development.

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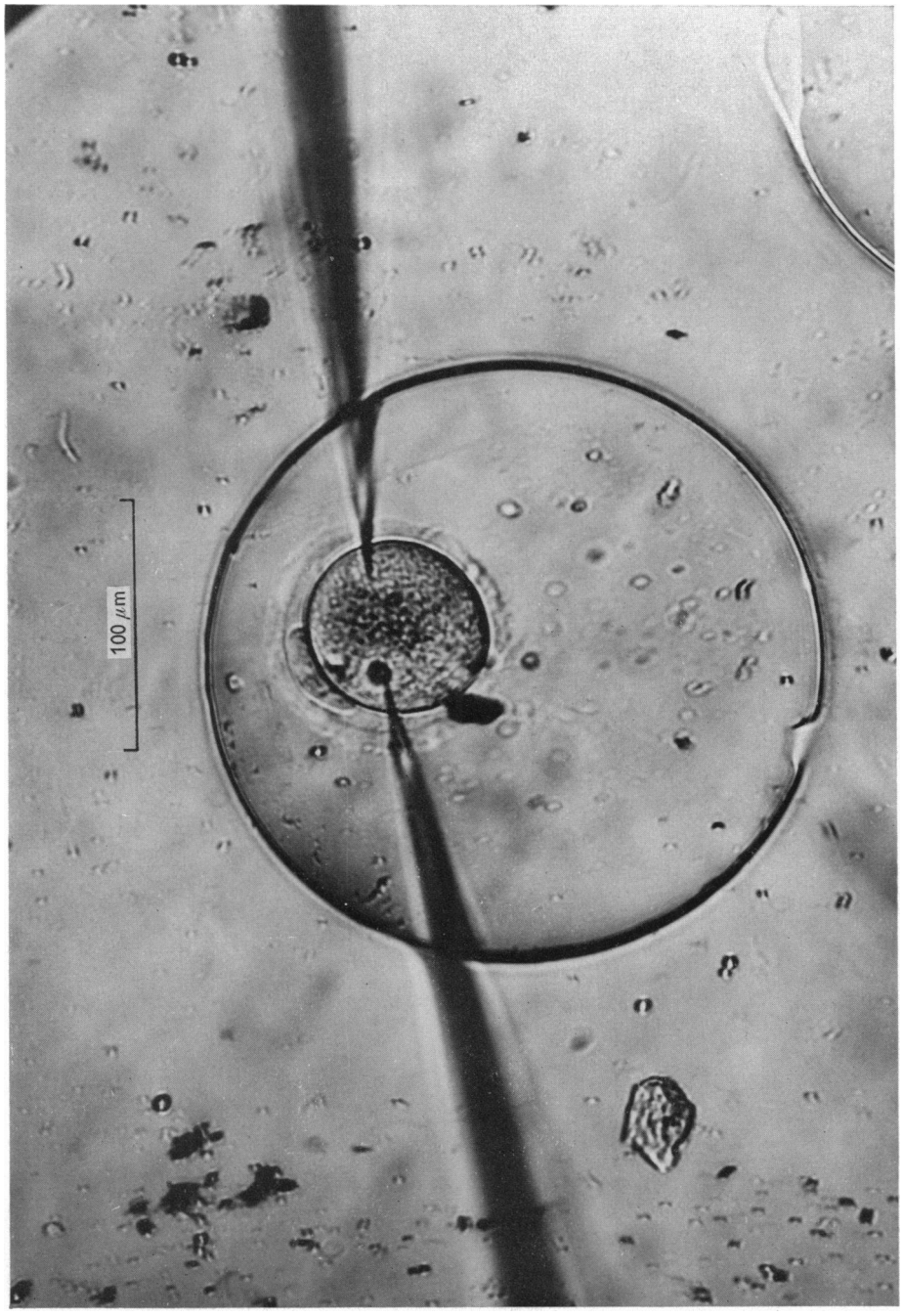
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EXPLANATION OF PLATE

The mouse oocyte penetrated by two micro-electrodes.