Fertilization-Induced Changes in Membrane Fluidity of Sea Urchin Eggs

Abstract. *By use of a spin label fatty acid, 5-doxylstearate, an increase in bulk membrane fluidity was observed after fertilization of two species of sea urchin eggs. Eggs partially activated by ammonia showed a similar effect. The data suggest that a structural change involving membrane lipids accompanies activation.*

At fertilization the metabolically repressed ovum undergoes a dramatic change of state from quiescence to activation. The biochemical events subsequent to fertilization have been well characterized in the sea urchin egg (*1*). The primary stimulus for activation is a sperm-egg interaction of unknown nature that occurs at the exterior surface of the egg. The first measurable cellular responses occur before the fertilizing sperm has entered the egg (*2*); furthermore, parthenogenetic (*3*) and partial (*4, 5*) activation of these cells is possible in the absence of sperm.

For external stimuli such as sperm or activating agents to elicit an intracellular response, they must either cross or interact with the plasma membrane. A change in state of the membrane has been invoked as an obligatory step in cellular activation (for example, virus transformation of animal cells) (*6*). Spin label fatty acids have been used in lipid model membrane systems to detect changes in membrane structure (*7*). We have investigated the fluid state of bulk membrane lipids in unfertilized and fertilized sea urchin eggs, using the spin label fatty acid 5-doxylstearate (*N*-oxyl-4′,4′-dimethyloxazolidine derivative of 5-ketostearic acid; Syva, Palo Alto, California). Our results indicate that the bulk membrane fluidity of sea urchin eggs increases in response to fertilization and partial activation.

Gametes of *Lytechinus pictus* and *Strongylocentrotus purpuratus* were obtained by injection of 0.5*M* KCl into the body cavity (*4*). Eggs were washed with artificial seawater (Instant Ocean, Aquarium Systems, Inc., Eastlake, Ohio) and maintained at 15°C. Sperm were stored undiluted at 4°C. Eggs were fertilized by the addition of dilute suspensions of sperm, and fertilization was scored by the number of eggs that raised fertilization membranes, which were visible by phase-contrast microscopy. Unfertilized or fertilized eggs were incubated for 10 minutes with 5-doxylstearate solubilized with bovine serum albumin (BSA) [1 mg of fatty acid per milliliter of 5 percent BSA, essentially fatty acid–free (Sigma Chemical Co., St. Louis, Missouri) in seawater]. The eggs were washed to remove the BSA and unbound label, and then electron spin resonance (ESR) spectra were recorded on a Varian E-104 ESR spectrometer equipped with a variable temperature accessory. All spectra were recorded at 12°C. The order parameter, S, was measured from the ESR spectra graphically (*8*). By definition, $S = 0$ for an isotropically tumbling molecule and $S = 1$ for a molecule that is rapidly rotating about one axis only (as would be the case for a spin label fatty acid in a highly rigid membrane). Thus, a decrease in the order parameter may be interpreted as an increase in membrane fluidity.

Table 1. Change in order parameter, S, of spin label fatty acid on fertilization of sea urchin eggs.

Batch	S		Difference (%)
	Unfertilized	Fertilized	
	Lytechinus pictus		
1	.812	.798	1.72
2	.824	.811	1.62
3	.820	.803	2.19
4	.814	.792	2.72
5	.805	.775	3.78
6	.817	.767	2.57
7	.788	.781	0.883
	Strongylocentrotus purpuratus		
1	.818	.786	4.01
2	.840	.820	2.44
3	.869	.820	4.76
4	.824	.814	1.56

The order parameters for fertilized and unfertilized eggs of two species of sea urchin are given in Table 1. The batches of eggs were from different animals. Each batch showed a decreased order parameter after fertilization, indicating an increase in the fluidity of bulk membranes in response to activation by sperm. The average decrease in S was 2.21 ± 0.86 percent for *L. pictus* and 3.19 ± 1.45 percent for *S. purpuratus*. This change in the order parameter is two to three times larger than those reported for the effect of prostaglandin E_2 (*9*) or acetylcholine (*10*) on membrane fluidity of the human erythrocyte. Sea urchin eggs contain lipid droplets (*11*) which act as an energy reserve during development (*12*). Our preliminary data suggest that 5-doxylstearate does not partition into aqueous dispersions of neutral lipid.

The events after fertilization and before the first cell division may be divided into two biochemical sequences: early events, which occur within 2 minutes after insemination, and late events, which begin about 5 minutes after fertilization. Early events include Na^+ influx (*2*), cortical changes (*13*), and respiratory increases (*14*). Late events involve increased rates of protein synthesis (*15*); phosphate (*16*), nucleoside (*17*), and amino acid (*18*) transport; K^+ conductance (*2*); DNA synthesis (*19*); and chromosome condensation (*4*). Our ESR measurements require approximately 10 minutes to complete, so we cannot distinguish events that occur on this rapid time scale. Early events and late events can be resolved by using the technique of partial activation. Partial activation, which is achieved by incubating unfertilized eggs briefly in an ammonia-seawater mixture, triggers the late events while bypassing the early ones (*4*). Eggs thus treated are activated in the sense that they synthesize DNA and protein at elevated rates. Although chromosome condensation occurs in these cells, they fail to go through the first cell division (*20*).

Unfertilized eggs were activated by ammonia by incubation for 20 minutes in artificial seawater titrated to *p*H 9 with NH_4OH. Subsequently one portion of eggs was incubated with BSA-solubilized 5-doxylstearate, while the remainder was maintained at 15°C for 70 minutes longer, after which they were fixed in Carnoy's solution (95 percent ethanol and glacial acetic acid, 3:1) and stained for chromosome condensation with 1 percent orcein in 75 percent acetic acid. The results of these experiments (Table 2) showed that the membrane fluidity of partially activated eggs is increased (decreased order parameter) relative to that of unactivated eggs. The average decrease in S for ammonia-activated *L. pic-*

Table 2. Change in order parameter, S, of spin label fatty acid on ammonia activation of sea urchin eggs.

Batch	S		Difference (%)
	Unactivated	Activated	
	Lytechinus pictus		
1	.784	.776	1.00
2	.805	.778	3.90
3	.788	.780	1.00
4	.811	.781	3.87
5	.811	.787	3.02
	Strongylocentrotus purpuratus		
1	.834	.814	2.39
2	.833	.821	1.46
3	.832	.816	1.91
4	.838	.813	3.06

0036-8075/78/0324-1336$00.50/0

tus eggs was 2.45 ± 1.69 percent; for *S. purpuratus* eggs it was 2.21 ± 0.685 percent. Thus, the decrease in order parameter appears to be a late event.

Fertilized eggs grown to plutei, an advanced stage of development, in the presence of BSA-solubilized 5-doxylstearate were indistinguishable from controls. Unfertilized eggs, exposed to the solubilized fatty acid and washed, showed some inability to raise normal fertilization membranes: 20 to 30 percent of the eggs raised blebby fertilization membranes after insemination. By phase-contrast microscopy these abnormal fertilization membranes resembled the membranes raised after eggs were treated with soybean trypsin inhibitor (*21*). This effect did not depend on the presence of the spin label fatty acid and may be due to the titration by BSA of the protease necessary for detaching the vitelline layer from the plasma membrane (*22*). Partially activated eggs do not raise fertilization membranes, but under the labeling conditions they showed characteristic chromosome condensation. By these criteria, the presence of spin label fatty acid perturbs the membrane little if at all.

Taken together, these results indicate that metabolic activation of the eggs of these two echinoderms is accompanied by an increase in membrane fluidity. However, since spin label fatty acids have been shown to equilibrate among all the subcellular membrane fractions (*23*), our results may be interpreted only in terms of bulk membrane fluidity. There are two possible interpretations of the data. One is that activation of the ovum is accompanied by a change in the total cellular membranes to a more fluid state. Alternatively, one or more specialized membranes (such as the plasma membrane) enters a more fluid state on activation and the probe is showing the average change experienced by the altered plus the unaltered membranes.

JUDITH CAMPISI
CARL J. SCANDELLA

Department of Biochemistry, State University of New York, Stony Brook 11794

References and Notes

1. D. Epel, *Am. Zool.* **15**, 507 (1975).
2. R. A. Steinhardt, L. Lundin, D. Mazia, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2426 (1971).
3. L. W. Stearns, *Sea Urchin Development: Cellular and Molecular Aspects* (Dowden, Hutchinson & Ross, Stroudsburg, Pa., 1974), p. 66.
4. D. Epel, R. Steinhardt, T. Humphreys, D. Mazia, *Dev. Biol.* **40**, 245 (1974).
5. V. D. Vacquier and B. Brandriff, *ibid.* **47**, 12 (1975).
6. A. B. Pardee, L. Jiménez de Astía, E. Rosengurt, in *Controls of Proliferation in Animal Cells*, B. Clarkson and R. Baserga, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1974), p. 547.
7. J. Seelig, in *Spin Labeling, Theory and Applications*, L. J. Berliner, Ed. (Academic Press, New York, 1976), p. 373.
8. B. J. Gaffney, *Methods Enzymol.* **32**, 161 (1974).
9. P. G. Kury, P. W. Ramwell, H. M. McConnell, *Biochem. Biophys. Res. Commun.* **56**, 478 (1974).
10. W. H. Huestis and H. M. McConnell, *ibid.* **57**, 726 (1974).
11. P. R. Gross, D. E. Philpott, S. Nass, *J. Biophys. Biochem. Cytol.* **7**, 135 (1965).
12. H. Mohri, *Biol. Bull.* **126**, 440 (1964).
13. J. Runnstrom, *Adv. Morphog.* **5**, 221 (1966).
14. D. Epel, *Biochem. Biophys. Res. Commun.* **17**, 69 (1964).
15. ———, *Proc. Natl. Acad. Sci. U.S.A.* **57**, 899 (1967).
16. A. H. Whiteley and E. L. Chambers, *J. Cell. Physiol.* **68**, 309 (1966).
17. J. Piatigorsky and A. H. Whiteley, *Biochim. Biophys. Acta* **108**, 404 (1965).
18. D. Epel, *Exp. Cell Res.* **72**, 74 (1972).
19. R. T. Hinegardner, B. Rao, D. E. Feldman, *ibid.* **36**, 53 (1964).
20. D. Mazia, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 690 (1974).
21. V. Vacquier and D. Epel, *Nature (London)* **237**, 34 (1972).
22. E. J. Carroll, Jr., and D. Epel, *Dev. Biol.* **44**, 22 (1975).
23. J. Kaplan, P. G. Canonico, W. J. Caspary, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 66 (1973).
24. We thank D. Poccia for his assistance and for the use of his facility to maintain sea urchins. Supported by NIH grant GM23218 to C.J.S. and NIH training grant CA 09176-03.

13 October 1977; revised 12 December 1977

Immunity to Antigens Associated with Primate C-Type Oncoviruses in Pregnant Women

Abstract. *Cell-mediated and humoral immune responses against antigens associated with primate C-type oncoviruses were evaluated in humans by microcytotoxicity and radioimmunoprecipitation assays. Five of six women tested sequentially during pregnancy developed selective cell-mediated reactivity against baboon endogenous virus (BEV)–infected human fibroblasts. Responsiveness peaked during the second and third trimesters and corresponded temporally with elevated antibody levels to BEV antigens. Similar cell-mediated reactivity was not observed in nonpregnant individuals. Selective cell-mediated reactivity directed against cells infected with the simian sarcoma virus–simian sarcoma associated virus complex (SSV-SSAV) was observed in four of 20 healthy adults (three of 14 nonpregnant, one of six pregnant). These observations suggest that cell-mediated reactivity against primate C-type oncoviruses is occasionally detected in healthy nonpregnant adults, but that during pregnancy both cell-mediated and humoral reactivity against BEV may become selectively expressed.*

C-type oncoviruses are widely distributed in mammals and birds (*1*). They may be horizontally transmitted and induce neoplastic disorders; in some species they persist in latent form within the host's cells and are vertically transmitted from generation to generation. Such latent viral genetic material may become activated by treatment with certain chemicals or hormones, during aging, or during periods of immunological stress (*2*). Periods of rapid cell proliferation, such as occur during neoplastic growth or during gestation, may also be accompanied by the preferential expression of C-type oncoviruses (*3*).

Many investigators have detected C-type viruses by electron microscopy in placentas of human and nonhuman primates as well as other mammalian species (*4*). Although no human oncoviruses have been isolated from placental tissue, Kalter and co-workers (*5*) have isolated an endogenous C-type virus from a baboon placenta. The physiological function, if any, of these placental viruses is obscure. Whether or not the pregnant host responds immunologically to the placental viruses is also unclear.

In mice, both humoral (*6*) and cell-mediated (*7*) immune responses to endogenous C-type oncoviruses have been demonstrated. Cell-mediated reactivity against endogenous viruses has also been observed in rats (*8*). In man, humoral responses to primate C-type viruses have been observed by some investigators (*9, 10*), but not by others (*11*). We have studied the cell-mediated responses of peripheral blood lymphocytes against cells infected with primate C-type oncoviruses in both pregnant and nonpregnant humans, using a microcytotoxicity assay. Selective responsiveness developed during pregnancy against antigens associated with the baboon endogenous virus (BEV). Serological studies, in which a modified radioimmunoprecipitation (RIP) assay was used, demonstrated a concomitant increase in antibody production against BEV-associated antigens during gestation.

Human embryonic lung (HEL) fibroblast cultures were obtained from Flow Laboratories and maintained in our laboratory in Eagle's minimal essential medium (MEM) containing glutamine (2 m*M*) and a fourfold concentration of other essential amino acids and vitamins, 10 percent gamma-irradiated fetal bovine serum (FBS), penicillin (250 U/ml), and streptomycin (250 μg/ml) (complete MEM 4×). The HEL fibroblasts (passage 7) were infected with either the M7

0036-8075/78/0324-1337$00.50/0