TABLE I

PARTITION COEFFICIENTS OF ETHANOL AND HALOTHANE IN BRAIN SYNAPTOSOMES AND LIVER MITOCHONDRIA FROM ETHANOL-FED RATS AND CONTROL

	Ethanol, K_p	Halothane, K_p
Mitochondria		
Ethanol-fed	(8)1.17 ± 0.634	(5)21.4 ± 2.71
Control	3.60 ± 0.740	28.6 ± 5.58
Control/ethanol-fed	4.24	1.35
Synaptosomes		
Ethanol-fed	(5) 0.33 ± 0.11	(7) 21.07 ± 10.2
Control	(5) 1.00 ± 0.42	27.5 ± 11.3
Control/ethanol-fed	3.07	1.38

Partition coefficients were determined by incubating the membranes with a ^{14}C-labeled compound and ^3H_2O.

in the partition coefficients of the decane spin-probe (1.4 to fourfold difference). Since the latter are strongly correlated with the membrane order parameter, it is apparent that the changes in membrane rigidity are related to the binding of alcohol and anesthetics. Whether the reduced membrane binding is sufficient to explain in full the acquired resistance to the disordering effects of these compounds can only be answered by further refinement of these measurements. However, these data provide sufficient evidence to suggest that tolerance and cross-tolerance result in part from increased membrane rigidity, which decreases the partitioning of ethanol and other drugs into the membranes of chronic alcoholics. A preliminary short report of this work has been published (Rottenberg et al., 1981).

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REFERENCES

Chin J. H., and D. B. Goldstein. 1977. Drug tolerance in biomembranes: a spin label study of the effect of ethanol. *Science (Wash., D.C.).* 197:685.

Curran M., and P. Seeman. 1977. Alcohol tolerance in a cholinergic nerve terminal: relation to the membrane expansion-fluidization theory of ethanol action. *Science (Wash., D.C.).* 197:910.

Gaffney B. J. 1975. Fatty acid chain flexibility in the membranes of normal and transformal fibroblasts. *Proc. Natl. Acad. Sci. U.S.A.* 72:664.

Linden C. D., A. D. Keith, and C. F. Fox. 1973. Correlation between fatty acid distribution in phospholipids and the temperature dependence of membrane physical state. *J. Supramol. Struct.* 1:523.

Rottenberg H., D. Robertson, and E. Rubin. 1980. The effect of ethanol on the temperature dependence of respiration and ATPase activities of rat liver mitochondria. *Lab. Invest.* 42:318

Rottenberg, H., A. Waring, and E. Rubin. 1981. Tolerance and cross-tolerance in chronic alcoholics: reduced membrane binding of alcohol and drugs. *Science (Wash., D.C.).* 213:583.

Waring, A., H. Rottenberg, T. Ohnishi and E. Rubin 1981. Membranes and phospholipids of Liver Mitochondria from chronic alcoholic rats are resistant to membrane disordering by alcohol. *Proc. Natl. Acad. Sci., U.S.A.*, 78:2582.

MEMBRANE FLUIDITY CHANGES ACCOMPANY ACTIVATION OF SEA URCHIN EGGS

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The fertilization of sea urchin eggs is a visually dramatic example of cell activation. The resonance spectra of spin-labeled eggs have been used in several ways to analyze the membrane fluidity changes that accompany egg activation. We have consistently observed a decrease in order parameter, S, of eggs labeled with 5-doxylstearate associated with fertilization (1, 2). The average decrease in S was 2.1% for *Lytechinus pictus* and 2.5% for *Strongylocentrotus purpuratus*. This is a large change for a biological system. In pure phospholipid systems a change of ~ 20% is associated with the gel to liquid-crystal phase transition, but in biological systems changes of > 1% are quite rare.

RESULTS AND DISCUSSION

The change in S suggested that membrane fluidity increases upon activation. We have carried out a series of experiments to rule out likely sources of artifacts (2, 3). One potential source of artifacts concerns the changes in the surface structure of the egg at fertilization. In the first minute after fertilization the topography of the plasma membrane is drastically altered by fusion of cortical granules. Furthermore, a layer of protein, the vitelline layer, is removed from the exterior of the egg. Ammonia-activated eggs do not show these surface changes yet they still undergo the fluidity change. Experimental removal of the vitelline layer with dithiothreitol or protease had no effect on S. Indeed we found that the order parameter of eggs labeled with 5-doxylstearate did not respond to several treatments designed to alter the surface of the egg (2, 3).

Any interpretation of the spectral change induced by fertilization would certainly depend on the location of the spin probe within the egg. We approached the problem of localizing the probe in two stages. First we sought evidence that the probe might find its way to nonbilayer sites, such as neutral lipid droplets or protein binding sites. Knowing of no prior studies on the behavior of fatty acid spin labels in neutral lipid, we first turned to model systems. Here we found that the partitioning of spin label into neutral lipid

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from water was very low, and the label that did enter the lipid phase exhibited an isotropic spectrum far different from that observed in eggs. Furthermore, spectra of eggs labeled with 5-, 12- or 16-doxylstearate indicate increasingly disordered anisotropic motion characteristic of membranes. If there is a protein-bound component hidden in these spectra it must be quite small. We have used several parallel approaches to reveal the distribution of probe within the cell. Light microscopy autoradiography of cells labeled with [^{3}H] oleate under the conditions used for spin labeling revealed silver grains located throughout the cytoplasm and over the egg surface but not over the nucleus. [^{3}H] oleate was chosen instead of [^{3}H] doxylstearate because the double bond in oleate permits chemical fixation to unsaturated lipids within the membrane. As a second approach, 5-doxylstearate uptake was measured. From the absence of spin-spin interaction in the spectra an upper limit to the concentration of spin label in egg membranes can be calculated. This and the quantity actually taken up by the eggs make it clear that the label must be diluted throughout most of the egg membranes. Finally, we isolated a surface membrane fraction from fertilized and unfertilized eggs. This fraction yielded different spectra compared with whole eggs. All our data indicate that 5-doxylstearate distributes uniformly throughout egg membranes (2). The change in fluidity appears to be localized near the polar faces of the membranes because it is not reported by 12- or 16-doxylstearate.

We have localized the fluidity change within the well-defined sequence of events that occurs within 10 min after fertilization. This sequence includes changes in the membrane potential, intracellular calcium ion concentration, and intracellular pH. The bulk membrane fluidity change is not a consequence of the membrane potential change or the transient increase in intracellular calcium concentration; rather, the increase in cytoplasmic pH elicits the change in fluidity. Experiments are in progress to determine whether the fluidity change is a direct or an indirect consequence of the pH change.

The fluidity change in whole cells apparently reflects membrane activation averaged over the entire cell. We have isolated a surface membrane fraction from fertilized and unfertilized sea urchin eggs (4) in order to study fluidity changes in more homogeneous sample. The purification of this fraction, the cortex, as measured by marker enzyme specific activities or nucleic acid content, is a least 10-fold. When viewed by scanning electron microscopy or phase contrast microscopy, this fraction consists of nearly intact cortical hulls and smaller membrane fragments. Cortical granules, membrane-delimited spheres ~ 1 μm Diam, line the inner surface of the unfertilized egg cortex. These structures are absent from the fertilized egg cortex; the increase in cytoplasmic calcium concentration 30–60 s after fertilization triggers exocytosis of the cortical granules. A great advantage of the cortex preparation is that the structural response of cortical granules to calcium ion is preserved through the isolation. We and others have found that this structural change takes place in isolated cortices at 1–3 μM Ca^{++} (5).[1] This response offers a powerful tool to monitor the integrity of our in vitro system.

Cortices isolated from fertilized and unfertilized eggs differ in their S values by 3–4%. Cortices from fertilized eggs are less fluid than their counterparts from unfertilized eggs; in contrast, the bulk membranes of fertilized eggs are more fluid. There is no inconsistency in these results since the cortical membranes probably constitute only 5–10% of the total membrane in the egg. Results from photobleaching experiments on the surface of sea urchin eggs (6) and mouse eggs (7) are consistent with our own measurements on cortices. If the difference in S between the cortices of fertilized and unfertilized eggs reflects a difference in the fluidity of the plasma membranes of these eggs, then this difference is sufficient to alter the activity of membrane-bound enzymes by several fold (8).

The fluidity change in isolated cortices associated with fertilization can be mimicked in vitro by calcium. The addition of calcium to cortices from unfertilized eggs elicits an increase in S of ~ 4%, very close to the change associated with fertilization. We thus have an in vitro system that reproduces a change in fluidity that probably occurs in vivo. Isolated mitochondrial membranes, in contract, become more fluid after fertilization.[2] It seems clear that two distinct changes in membrane fluidity follow within 10 min of fertilization: the transient increase in cytoplasmic calcium concentration induces a decrease in surface membrane fluidity, and the increase in cytoplasmic pH causes internal membrane fluidity to increase.

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REFERENCES

1. Campisi, J., and C. J. Scandella. 1978. Fertilization-induced changes in membrane fluidity of sea urchin eggs. *Science (Wash., D. C.).* 199:1336–1337.
2. Campisi, J., and C. J. Scandella. 1980. Bulk membrane fluidity increases after fertilization or partial activation of sea urchin eggs. *J. Biol. Chem.* 255:5411–5419.
3. Campisi, J. 1979. Membrane lipid fluidity in the sea urchin egg. Ph.D. thesis, State University of New York at Stony Brook, Stony Brook, New York.
4. Campisi, J., and C. J. Scandella. 1980. Calcium-induced decrease in membrane fluidity of sea urchin egg cortex after fertilization. *Nature (Lond.).* 286:185–186.
5. Baker, P. F., D. E. Knight, and M. J. Whitaker. 1980. The relation between ionized calcium and cortical granule exocytosis in eggs of the sea urchin *Echinus esculentus. Proc. R. Soc. Lond. B Biol. Sci.* 207:149–151.

[1]Elhai, Jeff, and Carl Scandella. Unpublished results.
[2]Selak, Mary, and Carl Scandella. Unpublished results.

6. Wolf, D. E., E. Kinsey, W. Lennarz, and M. Edidin. 1981. Changes in the organization of the sea urchin egg plasma membrane upon fertilization: indications from the lateral diffusion rates of lipid-soluble fluorescent dyes. *Dev. Biol.* 81:133–138.

7. Johnson, M., and M. Edidin. 1978. Lateral diffusion in plasma membrane of mouse egg is restricted after fertilization. *Nature (Lond.).* 272:448–450.

8. Sinensky, M., F. Pinkerton, E. Sutherland, and F. R. Simon. 1979. Rate limitation of (Na^+ + Ka^+)-stimulated adenosinetriphosphatase by membrane acyl chain ordering. *Proc. Natl. Acad. Sci. U.S.A.* 76:4839–4843.

"AMBIQUITOUS" BEHAVIOR OF BRAIN HEXOKINASE

RAPID AND REVERSIBLE INTERACTION OF HEXOKINASE WITH THE OUTER MITOCHONDRIAL MEMBRANE

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Certain enzymes exhibit rapidly reversible associations with subcellular structures. These enzymes have been called "ambiquitous" (both places; cf., "ubiquitous," all places) to emphasize that their intracellular location may be variable, being a function of metabolic status in the cell (1). The most thoroughly studied ambiquitous enzyme is brain hexokinase (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1) (2). Hexokinase binds reversibly and specifically to the outer mitochondrial membrane, with the binding equilibrium being sensitive to physiologically relevant metabolites such as ATP and glucose-6-P. Variations in distribution of the enzyme between bound and solubilized forms are believed to be involved in regulation of catalytic activity, the bound form of the enzyme being more active. Variation in the soluble:particulate distribution of hexokinase has been reported to occur in response to in vivo perturbations of normal cerebral energy metabolism with increased proportion of mitochondrially bound enzyme found during periods of increased glycolytic rate (e.g., 3, 4).

Ambiquitous behavior has two fundamental requirements: there must be complementary recognition signals on enzyme and membrane which permit specific interaction; and there must be some mechanism by which the strength of that interaction can be modulated by parameters reflecting metabolic status of the cell. Current efforts

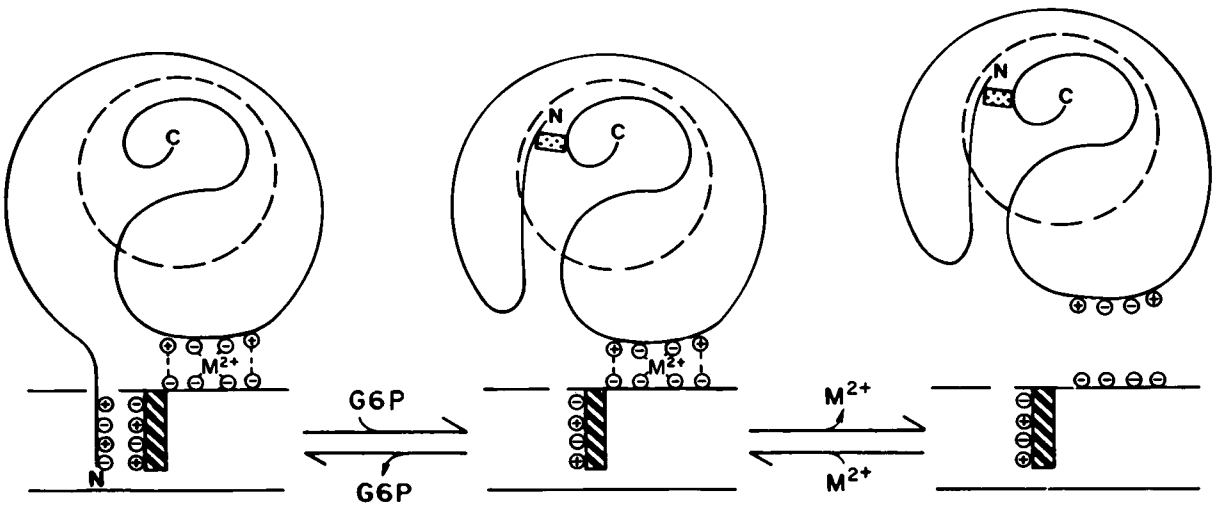

FIGURE 1 Speculative model representing interaction of brain hexokinase with the outer mitochondrial membrane. The enzyme is pictured as having two functional domains: one, enclosed by dashed circle, includes the C-terminus (arbitrarily) and the catalytic site; the other includes the N-terminal region and is responsible for interaction with the membrane. The N-terminal segment, led by the hydrophobic triplet, Tyr-Tyr-Phe, is inserted into the membrane and interacts with the hexokinase binding protein (crosshatched rectangle) in the membrane. Additional divalent cation-mediated interactions also occur (6). As a result of binding glucose-6-P (small stippled rectangle), the enzyme undergoes a conformational change which results in retraction of the N-terminal segment from the membrane (and also causes inhibition of catalytic activity). The enzyme is now bound less tightly, with only the divalent cation-mediated forces operational, i.e., the binding equilibrium is shifted toward increased amounts of "solubilized" enzyme. In the presence of 1 mM Mg^{++} the association of enzyme with the membrane can largely be maintained even in the presence of glucose-6-P. Since the intracellular concentration of Mg^{++} has been estimated at ~ 1 mM (7), it may be that, in vivo, the reversible interactions between hexokinase and the outer mitochondrial membrane are primarily restricted to the glucose-6-P sensitive states shown at the left of the figure, i.e., between tightly bound (N-terminal inserted) and loosely bound (N-terminal retracted) states. (Reprinted, with permission of Academic Press, from reference 2).

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