SHORT NOTE

ESTROGEN STIMULATED PATHWAY CHANGES AND COLD-INACTIVATED ENZYMES

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It is well established that water near surfaces, including macromolecules and biological material, is very different from bulk water,[1] although the degree of ordering and the distance to which an effect is exerted are still in dispute. Shereshefsky,[2] Bernal,[3] Derjaguin,[4] Drost-Hansen[1] and others[5] have argued for an effect of surfaces on water structure at distances up to hundreds of Angstroms, or even farther. NMR studies have shown that tissue water is more restricted than bulk water, and that the water in young[6] or actively dividing tissue such as cancer[7] (which contains a higher than normal percentage of water) is more "bulk-like" than the water of more mature and stable tissue.

Temperature anomalies of enzyme activity have been attributed to solvent effects as water undergoes minor phase changes.[1] Cold-inactivation and cold-activation of certain enzymes probably represent extreme cases of such "anomalous" behavior, and at least for enzymes with more than one subunit, can be accounted for by the fact that hydrophobic bonds tend to be weakened by decreasing temperature.*[8-10]

In an earlier paper,[11] we suggested that estrogen may act through an effect on the energy charge and the water structure of uterine cells, and that this very general effect may explain why various non-specific insults (e.g., radiation,[12] possibly hypoxia,[13] and diverse substances such as carcinogens[14] and histamine[15]) have an estrogenic effect. Even age may have an effect similar to estrogen.[16-18]

It has recently been brought to our attention (A. R. Larrabee, personal communication) that fatty acid synthetase is cold-inactivated, and also that its activity in rat liver drops very sharply as early as two hours after feeding is stopped. This inactivation by cold has been attributed to dissociation resulting from weakening of hydrophobic bonds at low temperature.[19] Since lipid is the first substance to increase greatly in concentration in the estrogen activated uterus (although other components show very early incorporation of label following estrogen stimulation[20]), this fact is extremely interesting in relation to

* ". . . remember that thermal energy [kT] tends to disorder structures and, conversely, a lowering in temperature will increase the ordering." Drost-Hansen, Ref. 1, p. 186.

our proposal that estrogen increases the "structural temperature" of cell water, and so would tend to activate the cold-inactivated enzymes. The other cold-inactivated enzymes from various organisms and tissues include pyruvate kinase,[21] glutamate dehydrogenase, ATPase, arginosuccinase, glycogen phosphorylase, pyruvate carboxylase, glucose-6-phosphate dehydrogenase, 17β-hydroxysteroid dehydrogenase,[8] acetyl CoA carboxylase,[22] and the "muscle" or electrophoretically slow moving isozyme of lactic dehydrogenase,[23,24] which would also be suitable for regulating pathway changes involved in cellular activation.

For example, phosphorylase activity is promoted by estrogen treatment.[25] Glycogen breakdown can be rate-limiting for glycolysis.[26]

Estrogen stimulation, hypoxia, and carcinogenesis involve an increased proportion of the "muscle" isozyme of lactic dehydrogenase, which represents a useful adaptation to a glycolytic production of pyruvate that is larger than can be oxidized by the mitochondria or otherwise disposed of, since this isozyme can continue to oxidize NADH and reduce pyruvate even in the presence of high concentrations of pyruvate.[27]

Entropic activation of pyruvate carboxylase could tend to increase fixation of carbon dioxide[28] and production of oxaloacetate, which by transamination would increase aspartate concentration. According to Jervell et al.[29] the labeled aspartate pool (from $H^{14}CO_3$) is increased by estrogen to a greater extent than that of glutamate, suggesting that it, and its α-keto acid, oxaloacetate, are near the point of CO_2 fixation.

Barker and Warren[30] showed that the glucose-6-phosphate oxidation pathway is immediately activated by estrogen, yet the specific activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase decreased for the first six hours. Activation of these enzymes and raising of the NADP/NADPH ratio by the estrogen activated transhydrogenase, or by lactic dehydrogenase,[31] and even CO_2 fixation could contribute to the acceleration of this process.

It has been found that estrogen binding capacity of the uterine "receptor" molecule is considerably reduced at low temperature, and that the binding capacity is restored by raising the temperature,[20] and Talwar et al.[32] reported that the rate of binding of estrogen to a soluble uterine fraction increases with incubation time as well as with temperature, indicating a kind of cooperative interaction. It has been proposed that this change of binding might represent a temperature dependent transfer to the nuclear receptor, but Talwar's use of a "purified" extract argues against this view. The data relating to temperature effects on binding are consistent with the idea of a phase shift promoted by estrogen. Ling[33] has reported that progesterone increases the potassium selectivity of myometrium. Cone and Tongier[35] and Orr et al.[36] have found that the Na^+/K^+ ratio can very rapidly affect mitosis and DNA synthesis. Mueller et al.[37] observed that incubation in Eagle's medium can mimic estrogen stimulation. Such considerations suggest that it might be worthwhile to study the receptor protein as a possible initiator of phase transition, as an alternative to the view that it primarily serves to transfer estrogen to the nucleus.

Engel[38] has reported that one of his estrogen activated transhydrogenases has an affinity and specificity for estrogen comparable to that of the "receptor" protein, although the 17β-estradiol dehydrogenase has a lower affinity. If an intracellular enzyme binds estrogen and has its function modified by it, it is a receptor, but there is no evidence that the well known "9 S" receptor has enzyme activity. One of the seemingly well established "coenzyme" functions of estrogen is in the estrogen activated NADH oxidase function of peroxidase.[39] Peroxidase is "induced" in the uterus by estrogen treatment.[40] Temple et al.[41] found that the oxidase behaved "like an induced enzyme," except that "oxidase activity was stimulated by the administration of estradiol to oophorectomized rats in two hours, when net protein synthesis cannot be detected." "Receptor" protein is also "induced" by estrogen.[42,43] Whatever the relation of "receptor" to estrogen activated enzymes might be, the apparent oxygen wasting effect of the estrogen activated oxidase would help account for estrogen's ability to lower the Pasteur effect[44] and to lower the pO_2 of the uterine lumen,[45] and this effect would be compatible with the above mentioned diversion of pyruvate to oxaloacetate (which would tend to inhibit succinic dehydrogenase) and with the shift[45] toward M isozymes of LDH, which appears to correspond to hypoxia and would also divert pyruvate from oxidation. A consequent reduction of the energy charge might cause the phase change, or damage to structure as suggested by Warburg.[46] Racker[47] has recently proposed that increased temperature or altered pH may be involved in activation of the glycolytic pathway in cancer, and suggests increased hydrolysis of ATP as a possible cause. It was Racker who first observed that mitochondrial ATPase is cold-inactivated, and he has also pointed out that damage to mitochondria can reveal very high levels of ATPase activity.

Other physiological processes that might be accounted for by an entropic modification of protein association and enzyme activity include:

(a) ammonia formation by stimulated nerve,[48] muscle,[49] and uterus,[50] since glutamate dehydrogenase is among the known cold-inactivated enzymes and these tissues[51–53] seem to undergo a water phase change when stimulated;
(b) estrogen stimulation of water uptake, by a thermo-molecular pressure effect,[54] because of increased metabolic rate[55] and possible change of heat conductivity,[1] or by a more direct effect on gel structure;
(c) estrogen's effect of increasing ATPase activity of the uterus,[56] which might relate to the increased myometrial reactivity in the estrogen dominated uterus;
(d) microtubule formation, since these seem to be cold sensitive;[57] also, H. Nemetschek-Gansler,[58] in describing the ultrastructure of the myometrium under the influence of estrogen says that the appearance "suggests a high degree of depolymerization of the contractile proteins," and that progesterone produces something like syneresis, implying altered protein-water interaction;

(e) vernalization.[1] This is a process involving an actual and large temperature difference rather than merely a structural difference. Cold-activation of enzymes would be the expected mechanism.

Abdulla and McFarlane[59] suggest that since a pyrophosphatase is activated or "induced" on the surface of platelets by collagen, and inhibited by NaF, this enzyme may belong to a class of enzymes activated by solvent structure. It has been proposed that association in some cases blocks active sites. In such cases increased solvent structure would increase activity or lower substrate specificity, if it weakened hydrophobic bonds. (Xanthine oxidase is well known as a cold-activated enzyme,[60] possibly involving such a mechanism.) Since pyrophosphatases are involved in establishing equilibria favorable to synthesis of protein (amino acid activation), RNA, and DNA, and oxidation of fatty acids, the concept of activation by solvent structure would imply that these processes belong to a later period in cell activation, in which a certain degree of order has been restored. The known schedule of syntheses following estrogen stimulation is consistent with this view. Either androgen or progesterone, which can increase metabolic "efficiency,"[61,62] would probably be involved at this stage. Progesterone's thermogenic[28] and anesthetic[63] effects seem significant in this context. A theory recently proposed[64–66] for control of enzyme levels could account for apparent induction or repression by cytoplasmic stabilization of some proteins, and by destabilization of others.

This entropic and energetic interpretation presents dedifferentiation as a simple, stable, and general change of state. Differentiation remains as a highly individualized process, depending on a precise history and environment of each cell. However, the simplicity of this view of dedifferentiation suggests a relatively small number of points at which effective intervention might be made in altering the course of carcinogenesis. It also suggests possible new approaches to fertility, sterility, and aging.

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REFERENCES

1. W. Drost-Hansen, *Structure and Properties of Water Near Biological Interfaces*, Laboratory for Water Research, Coral Gables, 1970.
2. J. L. Shereshefsky, *J. Amer. Chem. Soc., 50,* 2966 (1928).
3. J. D. Bernal, *Symp. Soc. Exp. Biol., 19,* 17 (1965).
4. B. V. Derjaguin, *Symp. Soc. Exp. Biol., 19,* 55 (1965).
5. M. J. Tate and F. Franks, *Nature, 230,* 91 (1971).
6. C. F. Hazelwood, B. L. Nichols, D. C. Chang and B. Brown, *Johns Hopkins Med. J., 128,* 117 (1971).

7. R. Damadian, *Science, 171,* 1151 (1971).
8. J. Jaraback, A. E. Seeds, Jr., and P. Talalay, *Biochemistry, 5,* 1269 (1966).
9. A. A. Vazina and V. V. Lednev, in *Water in Biological Systems,* L. P. Kayushin, Ed., Consultants Bureau, New York, 1969, p. 70.
10. I. M. Klotz, *Fed. Proc., 24,* Suppl. 15, 5 (1965).
11. R. Peat and A. L. Soderwall, *Physiol. Chem. Phys., 3,* 401 (1971).
12. E. W. Hahn and W. F. Ward, *J. Reprod. Fertil., 20,* 151 (1969).
13. J. L. Boling, R. J. Blandau, J. G. Wilson and W. C. Young, *Proc. Soc. Exp. Biol. Med., 42,* 128 (1939).
14. C. D. Turner, *General Endocrinology,* Saunders Co., Philadelphia, 1966.
15. M. C. Shelesnyak and P. F. Kraicer, in *Delayed Implantation*, A. C. Enders, Ed., Univ. of Chicago Press, Chicago, 1963, p. 265.
16. L. Loeb, *Amer. J. Cancer, 35,* 159 (1939).
17. A. B. Schultz, *Proc. Soc. Exp. Biol. Med., 125,* 379 (1965).
18. W. B. Atkinson, H. Kaunitz and C. A. Slanetz, *Ann. N. Y. Acad. Sci., 52,* 68 (1949).
19. S. Smith and S. Abraham, *J. Biol. Chem., 246,* 6428 (1971).
20. G. C. Mueller, in *Biogenesis and Action of Steroid Hormones,* R. I. Dorfman and M. Dorfman, Eds., Geron-X, Inc., Los Altos, 1968, p. 1.
21. R. T. Kuczenski and C. H. Suelter, *Biochemistry, 9,* 939 (1970).
22. R. M. Dowben, *General Physiology, A Molecular Approach,* Harper and Row, New York, 1969, p. 304.
23. H. H. Kreutzer and W. H. S. Fennis, *Clin. Chem. Acta, 9,* 64 (1964).
24. H. A. Zondag, *Science, 142,* 965 (1963).
25. T. Takeuchi, M. Hayashi and W. H. Fishman, *Acta Endocrinol., 39,* 395 (1962).
26. E. Florey, *An Introduction to General and Comparative Animal Physiology,* W. B. Saunders Co., Philadelphia, 1966, p. 572.
27. D. M. Dawson, T. L. Goodfiend and N. O. Kaplan, *Science, 143,* 929 (1964).
28. R. L. Goodland, J. G. Reynolds, A. B. McCoord and W. T. Pommerenke, *Fertil. Steril., 4,* 300 (1953).
29. K. F. Jervell, C. R. Diniz and G. C. Mueller, *J. Biol. Chem., 231,* 945 (1958).
30. K. L. Barker and J. C. Warren, *Endocrinology, 78,* 1205 (1966).
31. E. W. Wilson, *J. Endocrinol., 44,* 63 (1969).
32. G. P. Talwar, M. L. Sopori, D. K. Biswas and S. J. Segal, *Biochem. J., 107,* 765 (1968).
33. G. N. Ling, *A Physical Theory of the Living State: The Association-Induction Hypothesis,* Blaisdell, New York, 1962.
34. A. W. Jones, paper presented at the New York Academy of Science International Conference on Physico-Chemical State of Ions and Water in Living Tissue and Model Systems, January 11, 1972.
35. C. D. Cone, Jr., and M. Tongier, *Oncology (Basel), 25,* 168 (1971).
36. C. W. Orr, M. Yoshikawa-Fukada and J. D. Ebert, *Proc. Nat. Acad. Sci. U.S.A., 69,* 243 (1972).
37. G. C. Mueller, A. M. Herranen and K. F. Jervell, *Recent Progr. Horm. Res., XIV,* 95 (1958).
38. L. L. Engel, *Endocrinology, 87,* 827 (1970).
39. K. Yokota and I. Yamazaki, *Biochim. Biophys. Acta, 105,* 301 (1965).
40. F. V. Lucas, H. A. Neufeld, J. G. Utterback, A. P. Martin and E. Stotz, *J. Biol. Chem., 214,* 775 (1955).
41. S. Temple, V. P. Hollander, N. Hollander and M. L. Stephens, *J. Biol. Chem., 235,* 1504 (1960).
42. A. J. Eisenfeld and J. Axelrod, *Endocrinology, 79,* 38 (1966).
43. R. J. Kraay and L. J. Black, *Proc. Soc. Exp. Biol. Med., 133,* 376 (1970).
44. J. P. Greenstein, *Biochemistry of Cancer,* Academic Press, New York, 1954, p. 348.
45. S. W. Clark and J. M. Yochim, *Endocrinology, 89,* 358 (1971).

46. O. Warburg, A. W. Geissler and S. Lorenz, in *Aspects of Yeast Metabolism*, A. K. Mills, Ed., Blackwell, Oxford, 1967, p. 327.
47. E. Racker, *Amer. Sci., 60,* 56 (1972).
48. S. Tashiro, *Amer. J. Physiol., 60,* 519 (1922).
49. S. Tashiro and O. P. Lee, *Amer. J. Physiol., 61,* 244 (1922).
50. R. Peat and A. L. Soderwall, inferred from observations in this laboratory.
51. O. G. Fritz and T. J. Swift, *Biophys. J., 7,* 675 (1967).
52. C. B. Bratton, A. L. Hopkins and J. W. Weinberg, *Science, 147,* 738 (1965).
53. R. Peat and A. L. Soderwall, NMR studies currently being done in this laboratory.
54. D. C. Spanner, *Symp. Soc. Exp. Biol., 8,* 76 (1954).
55. L. Martin, *J. Endocrinol., 20,* 187 (1960).
56. A. Csapo, *Amer. J. Physiol., 162,* 406 (1950).
57. O. Behnke, *J. Ultrastruct. Res., 31,* 61 (1970).
58. H. Nemetschek-Gansler, in *Cellular Biology of the Uterus,* R. M. Wynn, Ed., Meredith Publ. Co., New York, 1967, p. 353.
59. Y. H. Abdulla and E. McFarlane, *Biochim. Biophys. Acta, 258,* 337 (1972).
60. L. Robert and J. Polonovski, *Discs. Faraday Soc., 20,* 54 (1955).
61. Discussion by S. H. Sturgis following paper cited above, No. 21, p. 317.
62. A. B. Schultz, *Proc. Soc. Exp. Biol. Med., 116,* 653 (1964).
63. H. Selye, *In Vivo: The Case for a Supramolecular Biology*, Liveright Publ. Corp., New York, 1967.
64. P. J. Fritz, E. L. White, E. S. Vesell and K. M. Pruitt, *Nature New Biol., 230,* 119 (1971).
65. M. Rosenberg, *Nature New Biol., 230,* 12 (1971).
66. D. B. Miller, M. S. Summers and J. A. Niziolek, *Nature New Biol., 230,* 117 (1971).