SHORT NOTE

COLD-INACTIVATED ENZYMES AS METABOLIC CONTROLS

R. PEAT and A. L. SODERWALL
Department of Biology, University of Oregon, Eugene, Oregon 97403

(Received September 3, 1971)

There is now considerable evidence[1-10] that water is ordered, though not necessarily "bound," in the presence of macromolecules and in living cells. It has been suggested by A. Szent-Gyorgyi,[8,18] G. Ling,[5] and A. S. Troshin[11] that the state of water might exert control over metabolism, cell division, muscle contraction, etc. Some of the physical changes that we would expect to occur in ordered water are increased protonic conduction, stabilization of triplet states, stabilization of hydrated electrons, altered solubilities and ionic exchange, and weakened hydrophobic bonds. Anisotropic or ordered water (such as exists in narrow capillaries)[12] can have special properties, such as lowered vapor pressure, without being bound. Polanyi's adsorption isotherm, which assumes a potential acting through space, rather than only at the surface layer, seems to be an appropriate concept for understanding these long range effects.

It has been pointed out that lowered temperature weakens hydrophobic bonds, and that this may be the cause of cold-inactivation of certain enzymes.[13] The entropic contribution to exclusion of lipoids from the water phase would be similarly diminished in the highly ordered water of cells. Thus, we would expect the cold-inactivated enzymes, e.g., pyruvate carboxylase,[14] phosphorylase,[15] and estrogen dehydrogenase,[16] to be inactivated by the ordered environment of the resting cell, and to be activated by the relatively entropic state of cells in contraction,[17] division,[18] or anaerobiosis.[19] According to Drummond,[20] phosphorylase is in fact activated during electrically stimulated muscle contraction, without activation of phosphorylase kinase or increase in cyclic 3′,5′-AMP concentration.

If entropy changes in the cell water modify the activity of these enzymes, then this behavior of the proteins can be interpreted as an important control mechanism. Phosphorylase, and probably pyruvate carboxylase, would be homeostatic, or negative feedback controls, tending to provide energy in a state of low energy charge or physical activation. However, estrogen dehydrogenase, which uses estrogen as a coenzyme, may have an opposite effect, tending to destabilize the cell. If this is the case, another hormone system would be required to override the anti-homeostatic effect of estrogen. Progesterone, which must be present with estrogen in a precise ratio (but at a much higher concen-

tration) for reproductive success, may be the hormone with this function. This interpretation is consistent with observations that senescent animals may have high uterine metabolic rates,[21] high progesterone levels,[22] and that cancer tissue often responds to steroids; that is, any nonspecific irritation or insult that increased the entropy of cell water would promote the "estrogenic" state.

It seems significant that the known cold-inactivated enzymes are in important positions for the control of metabolism. It will be interesting to look for other cold-inactivated enzymes with this mechanism in mind. Also, it might be possible to learn something about cell water by studying these enzymes *in vitro*, their responses to surfaces, pressure, and other physical factors.

ACKNOWLEDGMENT

This study supported by USPHS Grant HD-04234-02.

REFERENCES

1. G. Chapman and K. A. McLaughlan, *Nature, 215,* 391 (1967).
2. F. W. Cope, *Physiol. Chem. Phys., 2,* 545 (1970).
3. R. Damadian, *Science, 171,* 1151 (1971).
4. O. G. Fritz and T. F. Swift, *Biophys. J., 7,* 675 (1967).
5. G. N. Ling, *Intern. Rev. Cytol., 26,* 1 (1969).
6. W. F. O'Brien, *Surface Sci., 19,* 387 (1970).
7. C. A. Rotunno, V. Kowalewski and M. Cereijido, *Biochim. Biophys. Acta, 135,* 170 (1967).
8. A. Szent-Gyorgyi, *Bioenergetics,* Academic Press, New York, 1957.
9. M. J. Tait and F. Franks, *Nature, 230,* 91 (1971).
10. H. E. Whipple, *Ann. N. Y. Acad. Sci., 125,* 249 (1965).
11. A. S. Troshin, *Problems of Cell Permeability,* Pergamon Press, Oxford, 1966.
12. J. L. Shereshefsky, *J. Amer. Chem. Soc., 50,* 2966 (1928).
13. F. Reithel, *Concepts in Biochemistry,* McGraw-Hill, New York, 1967, p. 241.
14. M. C. Scrutton and M. F. Utter, *J. Biol. Chem., 242,* 1723 (1967).
15. S. A. Assaf and D. J. Graves, *J. Biol. Chem., 244,* 5544 (1969).
16. L. L. Engel, *Endocrinology, 87,* 827 (1970).
17. L. Mandelkern, *J. Gen. Phys. (Suppl.), 50,* 24 (1967).
18. A. Szent-Gyorgyi, *Bioelectronics,* Academic Press, New York, 1968.
19. O. Warburg, in *Aspects of Yeast Metabolism,* A. K. Mills, ed., Blackwell, Oxford, 1968.
20. G. I. Drummond, *Amer. Zool., 11,* 83 (1971).
21. Unpublished observations of this laboratory.
22. B. C. Blaha, *Anat. Rec., 169,* abstract, 279 (1971).