

longer periods of time than are now possible. The role of this regulator in sperm fertility is being studied.

References

1. BARKER, S. B., and SUMMERSON, WM. H. *J. biol. Chem.* 1941, **138**, 535.
2. CROSS, R., COVO, G., TAGGART, J., and GREEN, D. E. *J. biol. Chem.*, in press.
3. HENLE, G., and ZITTLE, C. A. *Proc. Soc. exp. Biol. Med.* 1941, **47**, 193.
4. HENLE, G., and ZITTLE, C. A. *Amer. J. Physiol.*, 1942, **136**, 70.
5. HOTCHKISS, R. D. *Adv. Enzymol.*, 1943, **4**, 153.
6. LARDY, H. A., and ELVEHJEM, C. A. *Ann. Rev. Biochem.*, 1945, **14**, 1.
7. LARDY, H. A., HANSEN, R. G., and PHILLIPS, P. H. *Arch. Biochem.*, 1945, **6**, 41.
8. LARDY, H. A., and PHILLIPS, P. H. *J. biol. Chem.*, 1941, **133**, 195.
9. *Ibid.*, *Amer. J. Physiol.*, 1941, **133**, 602.
10. *Ibid.*, 1941, **134**, 542.
11. *Ibid.*, 1943, **138**, 741.
12. LARDY, H. A., and PHILLIPS, P. H. *J. biol. Chem.*, 1943, **148**, 333.
13. *Ibid.*, 1943, **148**, 343.
14. *Ibid.*, 1943, **149**, 177.
15. LARDY, H. A., and PHILLIPS, P. H. *Nature, Lond.*, 1944, **153**, 168.
16. LARDY, H. A., and PHILLIPS, P. H. *Arch. Biochem.*, 1945, **6**, 53.
17. LOOMIS, W. F., and LIPMAN, F. *J. biol. Chem.*, 1948, **173**, 807.
18. REDENZ, E. *Biochem. Z.*, 1933, **257**, 234.
19. WINDSTÜSSER, K. *Klin. Wchschr.*, 1935, **14**, 193.
20. WOLF, C. G. *J. Physiol.*, 1921-2, **55**, 246.

Lack of Depolymerase Effect on Desoxyribonucleic Acid in Living Cells

J. O. Ely and M. H. Ross

Biochemical Research Foundation, Newark, Delaware

Desoxyribonucleic acid depolymerase has been reported to remove ultraviolet light absorbing constituents and Feulgen stainable material, from nuclei in sections of tissue, and from nuclei of dead cells (1, 2, 4). There has been no reference in the literature, so far as the authors know, to the use of this enzyme on living cells.

In some experiments in this laboratory, it was found that the enzyme removed desoxyribonucleic acid from nuclei of chicken erythrocytes in smear preparations. After treatment with the enzyme, the nuclei failed to absorb ultraviolet light and did not stain with Feulgen's reagent. Addition of the enzyme to freshly obtained chicken blood, however, failed to affect the nuclei. This result suggested that perhaps living cells were unaffected by this enzyme when it was added to the extracellular fluid; results of further investigation showed that this is so with chicken erythrocytes and cells of Walker Carcinoma No. 256 of the rat.

Smears of chicken erythrocytes were fixed by immersion in 95% ethyl alcohol; they were then placed into fresh chicken blood to which had been added an equal

volume of a solution of desoxyribonucleic acid depolymerase. The preparations were incubated at 37° C for periods of time up to 3.5 hr. In other experiments, suspensions of cells of Walker Carcinoma No. 256 of the rat in Ringer's solution were used in a similar manner. After incubation portions of the tumor cell-enzyme mixtures were transplanted into rats; if tumor growth followed it was assurance that the cells were living during the experiment.

The ultraviolet light (2654 Å) absorbing material and the Feulgen stainable material in nuclei of the smears of both chicken erythrocytes and tumor cells were removed gradually and completely; however, there was no apparent effect on the nuclei of supposedly living chicken erythrocytes in the enzyme-blood mixture, or on the suspension of tumor cells. The tumor cells produced tumors after subcutaneous inoculation into rats; assuredly, they were living during exposure to the enzyme.

The enzyme attacked cells killed by heat, formaldehyde, alcohol, Carnoy's fixative, and ultraviolet light. Apparently the method of killing the cell makes little difference; it appears to be only necessary that the cell be dead for the enzyme to act.

Inability of the enzyme to act on the desoxyribonucleic acid of living cells might be explained by: a) absorption of the enzyme by cell constituents other than nucleic acid; b) antienzyme action; c) impermeability of cell membranes; d) inability of the enzyme to attack nucleic acid in the state that it exists in the living cell.

The possibilities that absorption and antienzyme activity prevented action of the enzymes on living cells were excluded by the fact that dead cells were acted upon by the same enzyme solution which failed to act on living cells. The assumption that membranes of the living cell are impermeable to depolymerase offers a plausible explanation for the lack of effect on living cells; however, it cannot be proved indisputably.

Whether or not other enzymes added to the exterior environment of living cells would fail to act on the respective substrates in cells is not known. Northrop (3), in 1926, reported that trypsin and pepsin were not taken up by cells of living organisms (earthworms, *Euglena*, yeast, meal worms, gold fish, and *Fundulus*), whereas, when the organisms died the enzymes were taken up rapidly from solution.

Desoxyribonucleic acid depolymerase did not act on nuclei of living chicken erythrocytes or of living cells of Walker Carcinoma No. 256 of the rat; the enzyme acted on these cells after they were killed. Lack of effect of the enzyme on living cells apparently was not because of adsorption of the enzyme or antienzyme activity, but may have been because of cell membrane impermeability or inability of the enzyme to attack nucleic acid in the state that it exists in the living cell.

References

1. BRACHET, J. *Symposia of the Society for Experimental Biology. I. Nucleic acid.* Cambridge: University Press, 1947.
2. CATCHESIDE, D. G., and HOLMES, B. *Ibid.*
3. NORTHROP, J. H. *J. gen. Physiol.*, 1926, **9**, 497.
4. STOWELL, R. E. *Stain Technol.*, 1946, **21**, 137.