a green center. The same percentage of colorless colonies developed after the application of pressure for 2 hours as after application for 20 minutes.

Of the cells streaked on agar plates after exposure to 500 atm for 1/2 hour or 2 hours, only 1 percent showed color mutation, indicating that the phenomenon is pressure-dependent though apparently not time-dependent. Further study is required however, before definitive conclusions can be drawn. Although the mutant colonies appeared white on agar, they appeared yellow-orange when grown in liquid, thus reflecting synthesis of carotenoids (10).

Two additional "pressure" mutants, designated PR-2 and PR-3, were isolated. Mutant PR-2 synthesized about twice as much carotenoid as did PR-1 or PR-3 on the basis of the dry weight of the cells. This relationship is based on the $E_{\text{lem}}^{1/2}$ (11) value of 2500 for B-carotene (12) measured from the absorption spectrum of the total carotenoids extracted in 95 percent ethanol. The carotenoids have not been isolated or identified.

Neither in Cattell's review (13) nor in the studies cited by Johnson, Eyring, and Polissar (14) are there references to pressure-caused mutations. Whether the effect reported here is a reflection of a chromosomal or cytoplasmic gene change cannot be stated with certainty. However, there is evidence which suggests a lack of direct nuclear control over chloroplast formation. Furthermore, several studies show that both DNA and RNA are present in chloroplasts (15). Such evidence favors cytoplasmic mutation, rather than chromogenic mutation, as the probable mechanism upon which hydrostatic pressure acts. Also, Hedén's studies (16) suggest that nucleic acids are probably targets of hydrostatic pressure. It can be postulated that pressure acts on a stage of chloroplast formation in which there is an increase in volume (14) of either the structural organelle or some molecular phase vital to its development:

J. A. GROSS Life Sciences Research Division. IIT Research Institute. Chicago, Illinois 60616

References and Notes

- 1. S. H. Hutner and L. Provasoli, in Biochemistry and Physiology of Protozoa, A. Lwoff, (Academic Press, New York.
- 2, L. Ebringer, Nature 18, 606 (1961).
- J. A. Gross, T. L. Jahn, E. Bernstein, J. Protozool. 2, 71 (1955).
- E. G. Pringsheim and O. Pringsheim, New Phytol. 51, 65 (1952).
- M. DeDeken-Grenson, Arch. Intern. Physiol. Biochim. 67, 506 (1959); H. Lyman, H. T. Epstein, J. A. Schiff, J. Protozool, 6, 264 1959).
- Obtained from Dr. J. J. Wolken, Biophysical Research Laboratory, University of Pittsburgh Medical Center, Pennsylvania.

- 7. C. L. Greenblatt and J. A. Schiff, J. Protozool. 6, 23 (1959).
- 8. Pressure vessels were fabricated by the Experimental Mechanics and Materials section of HT Research Institute from 20-cm heat-treated round steel stock, from high strength steel forgings, or by modifying Mark II 40cm naval projectiles. Bores range from 10 cm to 40 cm; pressures can be controlled from ambient up to 10,200 atm in vessels of 10-cm bore, and up to 1080 atm in vessels of 40cm bore.
- J. A. Gross, S. K. Wirtschafter, E. Bernstein, T. W. James, Trans. Am. Microscop. Soc. 75, 480 (1956); R. A. Olson and E. K. Engel, Brookhaven Symp. Biol. (1958).
- Gross, paper presented at Annual Meeting, Midwest Sect., American Society of Plant Physiologists, University of Chicago, 11 June 1964.
- 11. E $\frac{1.\%}{1.\text{cm}}$ is the extinction (optical density) of 1-percent solution measured through a
- 1-cm light path.

 12. T. W. Goodwin and J. A. Gross, J. Protoool. 5, 292 (1958)
- Cattell, Biol. Rev. Cambridge Phil. McK. Soc. 11, 441 (1936).
- H. Johnson, H. Eyring, M. J. Polissar, e Kinetic Basis of Molecular Biology The Kinetic Basis of (Wiley, New York, 1954).
- (Wiley, New York, 1954).
 15. T. Iwamura, Biochim. Biophys. Acta 61, 472 (1962); E. H. L. Chun, M. H. Vaughan, Jr., A. Rich, J. Mol. Biol. 7, 130 (1963); A. B. Jacobson, H. Swift, L. Bogorad, J. Cell Biol. 17, 557 (1963); A. Gibor and S. Granick, Proc. Natl. Acad. Sci. U.S. 50, 1164 (1963); C. J. Pollard, Arch. Biochem. Blophys. 105, 114 (1964); Gibor and S. Granick, Science 145, 890 (1964), C-G, Hedén, Bacteriol. Rev. 28, 14 (1964).
- R. Cornish and associates, Experimental Mechanics and Materials Section, IIT Research Institute, in whose laboratory the high-pressure exposures were performed.
 This research was supported by IIT Research Institute under Projects L1000L1 and L1002.
- 14 October 1964