scopic studies of lipids (*8, 9, 29*) and the fact that the 1082 cm^{-1} peak is stronger than the 1068 cm^{-1} peak, we conclude that the opsin membrane phospholipids are in a fluid state at room temperature. This agrees with diffusion studies (*4, 30*). Further, it is likely that the membrane lipids remain fluid down to at least 15°C. Below 15°C the 1068 cm^{-1} *trans* peak becomes more intense relative to the *gauche* peak at 1082 cm^{-1}, an indication of a gel-liquid crystal transition. Such a low transition temperature could be consistent with the high degree of lipid hydrocarbon unsaturation present in opsin membranes (*30, 31*).

In conclusion, Raman spectroscopy of photoreceptor membranes can provide a sensitive probe of the membrane lipids and the membrane protein opsin (*32*). The main picture that has emerged from our study thus far is of an opsin protein which contains α-helical bonding but no detectable β-helical structure or random coil. Thus, the "dumbbell" model (*4*), which envisions significant β-helical structure such as found in gramicidin A (*17*), is unlikely. However, our data are consistent with the three-dimensional model recently obtained for purple membrane, which reveals a protein consisting of seven closely packed α-helical segments perpendicular to the plane of the membrane (*33*). We also find evidence for hydrogen-bonded tyrosines which may be present as Tyr . . . $^{-}$OOC bonds. In addition, the unusual configuration of disulfide bonds could be related to the proposed disulfide exchange during the bleaching process (*34*).

Note added in proof: Recent Fourier transform infrared absorption studies on photoreceptor membranes (*12*) indicate extensive α-helical structure and no detectable β structure for both opsin and rhodopsin.

KENNETH J. ROTHSCHILD
JAMES R. ANDREW
Harvard-MIT Program in Health Sciences and Technology and *Physics Department, Massachusetts Institute of Technology, Cambridge 02139*

WILLEM J. DE GRIP
Biological Laboratories, Harvard University, Cambridge 02138

H. EUGENE STANLEY
Harvard-MIT Program in Health Sciences and Technology and *Physics Department, Massachusetts Institute of Technology*

References and Notes

1. G. Wald, *Science* **162**, 230 (1968); T. G. Ebrey and B. Honig, *Q. Rev. Biophys.* **8**, 129 (1975); C. D. B. Bridges, in *Biochemistry of the Eye*, C. N. Graymore, Ed. (Academic Press, New York, 1970).
2. F. J. M. Daemen, *Biochim. Biophys. Acta* **300**, 255 (1973).
3. W. J. De Grip, thesis, University of Nijmegen, Netherlands (1974).
4. M. M. Poo and R. A. Cone, *Exp. Eye Res.* **17**, 503 (1973); W. T. Mason, R. S. Fager, E. W. Abrahamson, *Nature New Biol.* **247**, 562 (1974).
5. R. C. Lord, *Proc. Int. Congr. Pure Appl. Chem. 33rd* **7**, 179 (1971).
6. K. J. Rothschild and H. E. Stanley, *Am. J. Clin. Pathol.* **63**, 695 (1975).
7. H. E. Stanley, I. M. Asher, K. J. Rothschild, G. D. J. Phillies, E. B. Carew, R. D. Bansil, I. A. Michaels, in *Peptides: Chemistry, Structure and Biology*, R. Walter and J. Meienhofer, Eds. (Ann Arbor Science Publications, Ann Arbor, Mich., 1975).
8. J. L. Lippert, L. C. Groczycor, G. Meiklejohn, *Biochim. Biophys. Acta* **382**, 51 (1975).
9. D. F. H. Wallach and S. P. Verma, *ibid.*, p. 542; S. P. Verma, D. F. H. Wallach, R. Schmidt-Ullrich, *ibid.* **394**, 633 (1975); S. P. Verma and D. F. H. Wallach, *ibid.* **401**, 168 (1975).
10. A. Lewis, R. S. Fager, E. W. Abrahamson, *Int. J. Raman Spectrosc.* **1**, 465 (1973).
11. A. R. Oseroff and R. H. Callender, *Biochemistry* **13**, 4243 (1974).
12. K. J. Rothschild, J. Andrew, W. J. De Grip, H. E. Stanley, in preparation.
13. S. Futterman, *J. Biol. Chem.* **238**, 1145 (1963); J. J. H. H. M. dePont, F. J. M. Daemen, S. L. Bonting, *Arch. Biochem. Biophys.* **140**, 275 (1970).
14. H. Shichi, *Exp. Eye Res.* **17**, 533 (1973).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
16. K. J. Rothschild, I. M. Asher, E. Anastassakis, H. E. Stanley, *Science* **182**, 384 (1973); I. M. Asher, K. J. Rothschild, H. E. Stanley, *J. Mol. Biol.* **89**, 205 (1974).
17. K. J. Rothschild and H. E. Stanley, *Science* **185**, 616 (1974).
18. E. B. Carew, I. M. Asher, H. E. Stanley, *ibid.* **188**, 933 (1975).
19. M. Nakanishi, H. Takesada, M. Tsuboi, *J. Mol. Biol.* **89**, 241 (1974).
20. M. C. Chen and R. C. Lord, *Biochemistry*, in press.
21. H. Sugeta, A. Go, T. Miyazawa, *Chem. Lett.* (1972), p. 83.
22. S. K. Freeman, *Applications of Laser Raman Spectroscopy* (Wiley, New York, 1974).
23. M. N. Siamwiza, R. C. Lord, M. C. Chen, T. Takamatsu, I. Haroda, H. Matsuura, T. Shimanouchi, *Biochemistry* **14**, 4870 (1975).
24. M. C. Chen and R. C. Lord, *J. Am. Chem. Soc.* **96**, 4750 (1974).
25. R. C. Lord and N. T. Yu, *J. Mol. Biol.* **51**, 203 (1970).
26. F. Crescitelli, W. H. M. Mommaerts, T. I. Shaw, *Proc. Natl. Acad. Sci. U.S.A.* **56**, 1729 (1966).
27. R. Henderson, *J. Mol. Biol.* **93**, 123 (1975); A. Blaurock, *ibid.*, p. 139; see also (*34*).
28. F. S. Parker, Ed., *Applications of Infrared Spectroscopy in Biochemistry, Biology, and Medicine* (Plenum, New York, 1971), chap. 11.
29. J. L. Lippert and W. L. Peticolas, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1572 (1971).
30. R. A. Cone, *Nature New Biol.* **236**, 39 (1972); P. K. Brown, *ibid.*, p. 35.
31. E. W. Abrahamson and R. S. Fager, *Curr. Top. Bioenerg.* **5**, 125 (1973).
32. It is possible that the 1555 and 1161 cm^{-1} vibrations are due to residual retinal bound to the membrane and hence are resonance enhanced vibrations.
33. R. Henderson and P. N. T. Unwin, *Nature* **257**, 28 (1975).
34. B. H. McDowell and T. P. Williams *Vis. Res.*, in press.
35. We acknowledge helpful discussions with P. K. Brown, R. C. Lord, E. B. Carew, and B. Tokar. Supported by the NIH [with additional support for W. De Grip from the Dutch Organization for the Advancement of Basic Research (ZWO)].

7 November 1975

Shoot Initiation from Carnation Shoot Apices Frozen to −196°C

Abstract. *Aseptically excised shoot apices from* Dianthus caryophyllus *were frozen to −196°C and subsequently thawed to room temperature. Survival rates as high as 33 percent, determined by callus formation, growth, or chlorophyll formation, are reported; plant formation has been observed.*

During the past few years, several groups have developed techniques that expose plant suspension culture cells (*1*), callus (*2*), and both plant (*3*) and animal (*4*) embryos to temperatures as low as −196°C while maintaining cell viability. The objective of this work has been to obtain a reliable method for preserving specific cell and tissue culture lines for extended periods of time without the risk of microorganism contamination, equipment failure, or changes in chromosomal cytology and morphogenic potential (*5*). These problems are associated with the current practice of serial transfer. Plants have been grown from frozen cells after thawing, but initiation of organized development is difficult to achieve in cell cultures of many species even before freezing. Shoot tip or apex cultures, on the other hand, are routinely used to propagate many species (*6*) and, in addition, give rise to plants that are free of

Fig. 1. Shoot initiation from carnation shoot apices cultured in the light after being frozen to −196°C and thawed under the conditions described in the text. (a) Apex immediately after thawing (1 mm long). (b) Apex 2 months after thawing (7 mm long).

pathogens (*7*). The process of development in cell cultures also involves adventitious organogenesis of either shoots or asexual embryos, which can foster a high incidence of genetically aberrant plants (*6*). For these reasons, shoot apex cultures were chosen as the subject of investigation. I report both the successful recovery of viable plant shoot apices that were frozen to −196°C and the subsequent production of plants from surviving apices (*8*).

Carnation stock plants, *Dianthus caryophyllus* cultivars 'Scania' and 'Ellen Marie,' were maintained in a growth chamber with 8-hour days for at least 9 weeks. Shoot apices (the meristem and two-leaf primordia) were excised from stock plant cuttings and placed on a growing medium containing Murashige and Skoog salts (*9*), sucrose (30 g/liter), thiamine-HCl (0.4 mg/liter), myoinositol (100 mg/liter), indoleacetic acid (0.1 mg/liter), and kinetin (0.5 mg/liter). The *p*H of the solution was adjusted to 5.7 before the addition of agar (10 g/liter). After 4 days of incubation in the dark at 26° ± 1°C, apices were placed in a 4-ml vial containing 0.5 ml of a freezing solution consisting of 5 percent dimethyl sulfoxide in growing medium (without agar). The shoot apices were 0.5 to 1 mm long at this stage. Freezing was accomplished by pouring liquid nitrogen directly into the freezing vial and then dipping the vial into an open Dewar flask filled with liquid nitrogen. The cooling rate (between −10° and −70°C), measured with a copper constantan thermocouple at the liquid surface (most of the apices floated), was ≧ 1000°C min^{-1}. Shoot apices were thawed by plunging the vials into 37°C water. Such conditions gave an initial warming rate of 900° to 1200°C min^{-1}. Thawed apices were rinsed twice in liquid growing medium without dimethyl sulfoxide and then transferred to tubes of agar growing medium.

The rate of survival under the above conditions as determined by callus formation, growth, or chlorophyll formation was 15 percent (number frozen = 134) for 'Ellen Marie' and 33 percent (number frozen = 95) for 'Scania.' Surviving apices cultured in the dark formed callus. Shoot initiation and plant formation have thus far been observed (although in only about 5 percent of surviving apices) only when thawed apices were exposed to light (Gro-Lux fluorescent, 16 hour/day, 0.48 to 2.6 mw/cm^2). Figure 1 shows shoot apices at two stages of development after thawing. After the stage indicated in Fig. 1b, the shoots were rooted and subsequently transferred to soil as in Fig. 2. The plant on the right was grown from a shoot apex held at −196°C for 5 minutes. That on the left was grown from a similarly treated but unfrozen apex. Survival (callus growth) has been found in apices that have been stored at −196°C for as long as 2 months.

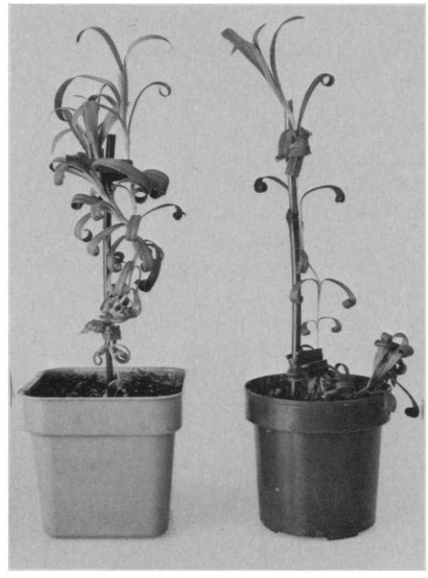


Fig. 2. Plants obtained from shoot apices of the 'Ellen Marie' carnation cultivar 4 months after treatment. The example on the left grew from an unfrozen control apex treated with dimethyl sulfoxide; that on the right grew from an apex that had been frozen.

My work demonstrates that a shoot apex can be frozen to −196°C and successfully thawed in a viable state. Furthermore, the fact that shoots and, subsequently, plants can be obtained from surviving apices means that the morphogenic potential of the organ is not necessarily destroyed by the freezing process. Finally, with additional refinement of the process it may be possible to establish plant organ banks to preserve plant genotypes without the problems associated with initiation of organized development, pathogens, and adventitious organogenesis.

MICHAEL SEIBERT
GTE Laboratories, Inc., Waltham, Massachusetts 02154

References and Notes

1. R. S. Quatrano, *Plant Physiol.* **43**, 2057 (1968); R. Latta, *Can. J. Bot.* **49**, 1253 (1971); K. K. Nag and H. E. Street, *Nature (London)* **245**, 270 (1973); D. K. Dougall and D. F. Wetherell, *Cryobiology* **11**, 410 (1974); Y. Sugawara and A. Sakai, *Plant Physiol.* **54**, 722 (1974); L. E. Towill and P. Mazur, in *Abstracts, American Society of Plant Physiologists Meetings* (Cornell University, Ithaca, N.Y., 1974), p. 10.
2. L. Bannier and P. L. Steponkus, *HortScience* **7**, 194 (1972); A. Sakai and Y. Sugawara, *Plant Cell Physiol.* **14**, 1201 (1973).
3. K. K. Nag and H. E. Street, *Physiol. Plant.* **34**, 254 (1975).
4. D. G. Whittingham, S. P. Leibo, P. Mazur, *Science* **178**, 411 (1972).
5. T. Murashige and R. Nakano, *Am. J. Bot.* **52**, 819 (1965); J. G. Torrey, *Physiol. Plant.* **20**, 265 (1967).
6. T. Murashige, *Annu. Rev. Plant Physiol.* **25**, 135 (1974).
7. ———, Second Dimock Memorial Lecture, Cornell University, Ithaca, N.Y., 1974.
8. M. Seibert and P. J. Wetherbee, in *Abstracts, Tissue Culture Association, 26th Annual Meetings* (Tissue Culture Association, Ann Arbor, Mich., 1975), No. 42.
9. T. Murashige and F. Skoog, *Physiol. Plant.* **15**, 473 (1962).
10. I thank P. J. Wetherbee for technical assistance and Dr. T. Murashige for discussion of this work.

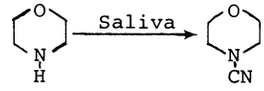
20 October 1975; revised 2 December 1975

Formation of Cyanamides from Secondary Amines in Human Saliva

Abstract. *1-Morpholinocarbonitrile (1-cyanomorpholine) was formed from morpholine when this amine was incubated in whole human saliva. Several other secondary amines appeared to form analogous products, and this transformation may therefore represent a general metabolic pathway for amines in saliva.*

Human saliva contains nitrite and is therefore an important source of man's exposure to this substance (*1*). The nitrite is formed via nitrate reduction by the normal oral microflora. Since nitrite is known to react with amines to form potentially carcinogenic nitrosamines, it seems logical that the microflora of the oral cavity would also participate in nitrosamine synthesis, thereby establishing a possible connection between environmental nitrate and human cancer.

To this end we have been conducting experiments in which secondary amines are added to whole saliva, which is then analyzed for possible nitrosamine formation. Morpholine (**1**) is one such amine that has been found to be converted to its nitrosamine (*2*); in the course of these experiments, however, we have discovered another type of product arising from the amine. In the case of morpholine, this new product has been characterized as the cyanamide (**2**).



(1)

1 **2**

A number of other secondary amines also form analogous products (*3*).

After searching the literature we have not found even a single reference on the occurrence of these well-known stable compounds in biological systems. Little information appears to be available on the biological activity of the dialkyl cyanamides.