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.

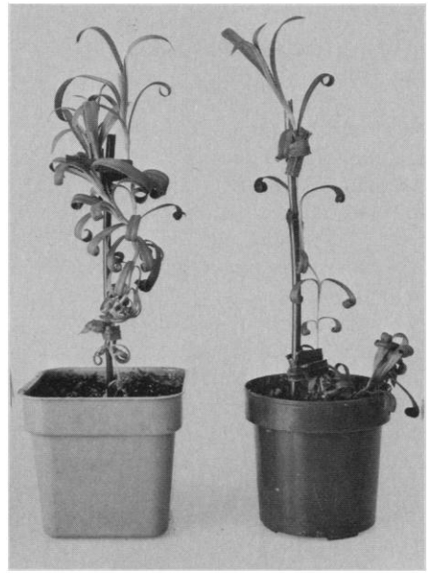

Fig. 2. Plants obtained from shoot apices of the 'Ellen Marie' carnation cultivar 4 months after treatment. The example on the left grew from an unfrozen control apex treated with dimethyl sulfoxide; that on the right grew from an apex that had been frozen.

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.

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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.

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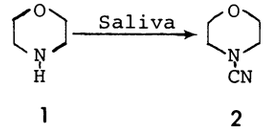
Formation of Cyanamides from Secondary Amines in Human Saliva

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.

The parent compound, cyanamide itself, which is probably atypical of the series, is listed as "irritating and caustic" (*4*).

Our original investigation concerned the metabolism of morpholine (**1**) in human saliva. Whole saliva was collected without stimulation over a period of less than 30 minutes and then incubated with 500 mg of added morpholine per liter for 4 hours at 37°C (*5*). Under these conditions the nitrite naturally present in human saliva (*1*) reacted with morpholine to yield concentrations on the order of a few micrograms of *N*-nitrosomorpholine (NNM) (*2*) per liter of saliva. Analysis of the saliva was carried out by extraction with CH_2Cl_2 followed by gas chromatography and mass spectroscopy (*6*). In addition to the expected NNM (with $m/e = 116$), a peak with a longer retention time was detected with $m/e = 112$ for its molecular ion (M^+). In many cases the concentration of this compound was on the order of several milligrams per liter. Since its mass spectrum also contained a conspicuous ion at M-30 (a frequent characteristic of *N*-nitrosamines), we initially believed that this compound might be *N*-nitrosooxazine, the 2,3,5,6-dehydrogenation product of NNM. This assumption was shown to be incorrect, however, when the compound failed to produce any response from a sensitive, nitrosamine-specific detector (*7*) and, later, when high-resolution mass spectrometry (*8*) indicated that this molecular ion at $m/e = 112.0631$ had a molecular formula of $C_3H_8N_2O$ rather than $C_4H_4N_2O_2$. Formation of analogous products from secondary amines (such as diphenylamine) with no reactive sites other than the amine hydrogen suggested that the unknown compound was formed by the substitution of a [CN] moiety at this position.

An authentic sample of compound **2** was prepared by established procedures (*9*) and was found to have chromatographic retention times (*6*) and mass spectrum indistinguishable from that of the unknown morpholine metabolite. Consequently, we believe that this metabolite was the cyanamide shown in Eq. 1.

1-Cyanomorpholine did not form in saliva that was centrifuged to remove bacteria and other cellular material. It was not detected in any suspensions of several pure strains of nitrate-reducing bacteria when morpholine was present during the course of growth or nitrate reduction (or both). Moreover, the addition of $^{15}NO_2^-$ or $^{15}NO_3^-$ caused no incorporation of ^{15}N into the 1-cyanomorpholine formed in whole saliva. Hence, this reaction seems to represent a novel method of secondary metabolism by salivary microorganisms and is apparently unrelated to concurrent nitrification.

Although cyanamides appear to be unknown as biological products, the occurrence of the cyano group—attached to carbon or sulfur—is well known. Organic cyanides are common constituents of plant materials (*10*) and include β-cyanoalanine, β-aminopropionitrile, and several cyanoglucosides. In microorganisms and animals, these compounds are known to be metabolized, in turn, to thiocyanate (*11*), a common constituent of human saliva.

Several possibilities can therefore be suggested to explain the biotransformation of amines to cyanamides in saliva. For example, it may be caused (i) by the enzymatic transfer of a cyano group from thiocyanate or an organic cyanide to a secondary amine, or (ii) by a nonenzymatic reaction, such as a direct reaction of the secondary amine with thiocyanate. The potential biological activity of these materials and the mechanism of their synthesis remain to be determined.

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References and Notes

1. S. R. Tannenbaum, A. J. Sinskey, M. Weisman, W. Bishop, *J. Natl. Cancer Inst.* **53**, 79 (1974).
2. S. R. Tannenbaum *et al.*, in *Analysis and Formation of N-Nitroso Compounds* (International Agency for Research on Cancer, Lyon, in press).
3. Compounds tested include the following secondary amines: dibutylamine, diisobutylamine, diphenylamine, pyrrolidine, and piperidine. All yielded products with an m/e corresponding to the loss of H and the addition of CN.
4. *The Merck Index*, P. G. Stecher, Ed. (Merck, Rahway, N.J., ed. 8, 1968), p. 306.
5. Saliva was collected in sterile test tubes: saliva from a single individual was used for almost all experiments. A total of seven individuals provided saliva. Incubation was conducted aerobically in Erlenmeyer flasks on a shake table. Reactants were added to the saliva in a manner that avoided dilution of more than 5 percent, and the *p*H was checked and adjusted to 7.2, if necessary. Each individual's saliva had activity in forming the cyanamides, and individuals were tested more than once during the course of the experiments. Since the population of individuals tested is both small and homogeneous, we cannot arrive at any conclusion on the generality of this reaction in the overall population.
6. Analyses were carried out on a gas chromatograph–mass spectrometer combination (Varian-Aerograph series 200: Hitachi–Perkin-Elmer RMU-7E) equipped with a precolumn solvent stripping system [J. M. Essigman and P. Issenberg, *J. Food Sci.* **37**, 684 (1972)]. The precolumn was 0.6 cm by 0.3 m stainless steel packed with 20 percent Carbowax 20M on 40/60 Chrom W; the analytical columns were 2 cm by 150 m stainless steel coated with a Carbowax mix (20M and 4000, 1 : 1) or 0.3 cm by 1.8 m stainless steel packed with 4 percent OV-1 on 80/100 Chrom W.
7. D. H. Fine, F. Rufeh, B. Gunther, *Anal. Lett.* **6**, 731 (1973).
8. The high-resolution gas chromatography–mass spectrometry was performed by Dr. Peter Roller of the National Cancer Institute, Bethesda, Md.
9. D. Martin and A. Weise, *Chem. Ber.* **99**, 3367 (1966); W. L. Garbrecht and R. M. Herbst, *J. Org. Chem.* **18**, 1003 (1953); L. F. Fieser and M. Fieser, *Reagents for Organic Synthesis* (Wiley, New York, 1967), vol. 1, p. 174.
10. G. W. Butler and R. W. Bailey, *Chemistry and Biochemistry of Herbage* (Academic Press, New York, 1973), vol. 2, pp. 35 and 457.
11. R. T. Williams, in *Biogenesis of Natural Compounds*, P. Bernfeld, Ed. (Pergamon, London, 1963), p. 589.
12. Supported by PHS contract NO1-CP-33315 from the National Cancer Institute.

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Specificity of Naturally Occurring Antibody in Normal Gibbon Serum

Abstract. *Gibbon natural antibody examined by immunoelectron microscopy reacted with the entire envelope of type C virus and with areas on the cell surface equivalent to or smaller than the diameter of a virion in gibbon and human culture cells infected with or releasing type C viruses. The antibody activity was absorbed completely by two cell cultures infected with gibbon ape leukemia virus and by the virus itself, and partially by normal gibbon spleen cells and dog thymus-derived cells infected with baboon endogenous type C virus, and fresh white blood cells obtained from a patient with chronic myelogenous leukemia in acute blastic crisis.*

It has become evident that natural antibodies against RNA type C virus-associated cell surface antigens (CSA) and viral envelope antigens (VEA) are present in the serum of healthy animals. The occurrence of humoral antibodies against CSA and VEA as well as the induction of cell-mediated immunity was reported in mice (*1, 2*), and natural antibodies against type C viruses of gibbon lymphosarcoma and granulocytic leukemia were found in 15 out of 133 serums from healthy gibbon apes by immunofluorescence microscopy and neutralization tests (*3*). In earlier studies of gibbon type C VEA by neutralization tests and immunoelectron microscopy xenogeneic antiserum was used (*4*), so that the interpretation of specificity was complicated by the possibility of interfering xenogeneic antibodies other than specific antibodies.

The determination of antigen or antibody specificity of nonhuman primates presents special problems because of their relatedness to humans; RNA-directed DNA polymerase (reverse transcriptase) in cells from human patients with acute myelogenous leukemia (AML) was shown to be antigenically related to the reverse transcriptase of two oncogenic type C viruses from nonhuman primates—the woolly monkey simian sarcoma virus