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9. Displays of the entire action potential were obtained from the original data channel by recording a delayed square pulse for each spike on a second channel. Reading the data channel backward on an oscilloscope triggered from the square pulses reproduced the entire action potential in reverse; rotating the photograph through 180° restored the original time sequence. Action potentials are shown with negative polarity up.
10. Supported by NIH grant FR 00166 and PHS 5T1 NB 5082-13. I thank Dr. E. Luschei for instruction in chronic unit recording techniques and Mrs. S. Barrow for assistance with the behavioral techniques.

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Ice Crystals

Odencrantz *et al.* (*1*) report that replicas of ice crystals prepared with the vapors of methyl-2-cyanoacrylate monomer exhibit thin whiskers (about 0.5 μ in diameter) over the surfaces of the replicas. They assume that these whiskers represent real ice whiskers present on the original ice crystals grown in their laboratory chamber, which led them to suggest that the breakup of these mechanically fragile whiskers could be a mechanism for the multiplication of ice crystals in the atmosphere.

Having had considerable experience in replicating crystals by this resin-vapor replication technique (*2*), I believe that the whiskers observed by Odencrantz *et al.* are artifacts produced during replication. For some time I had been mystified by the appearance of these whiskers on replication until I discovered that they could be entirely eliminated by (i) carefully removing excess moisture and other foreign materials from the surface on which the ice crystals were to be replicated, (ii) not overexposing the ice particles to the replicating vapor, (iii) making certain the cold-chamber atmosphere contained no residue of resin vapors from earlier replications before forming the ice fog, and (iv) adding a resin-polymerization catalyst (NH_3) to the chamber air before replication.

The reasons for these precautionary measures follow. To accomplish replication of an ice particle by the resin-vapor technique, the particle is exposed to the monomer vapor, which condenses and polymerizes over the particle surface to form a thin plastic shell or replica. The resin vapor, however, can quite readily react with moisture to produce globular or snake-like artifacts (often observed as "background" material deposited over substrate) (Fig. 1) as well as the whisker-like artifacts typified in Odencrantz's pictures. The substrate (glass slides) should be rinsed in ethanol and chloroform to remove surface water and foreign materials, especially acidic substances. The polymerization of the cyanoacrylate monomer is very sensitive to bases. Since water can serve as a weak base, the polymerization is initiated by the contact with the ice, but the addition of ammonia promotes more complete polymerization and therefore stronger, more artifact-free replicas.

For best results when replicating small ice particles, the following is suggested. About 5 cm^3 of ammonia gas should be introduced into the experimental chamber for every 10 liters of air, usually just prior to replication. The slide coated with the liquid monomer should be held 1 ml over the desired particles for about 10 seconds. This slide, initially at room temperature, should be backed with a thin slab of insulating plastic foam so that the temperature of liquid monomer does not decrease too rapidly during replication. (A critical amount of resin is needed to produce a complete replication, and the major force driving the resin vapor diffusion is the temperature gradient between the resin liquid and the ice.) All ice should be sublimed away from within the replicas before they are brought to room temperature.

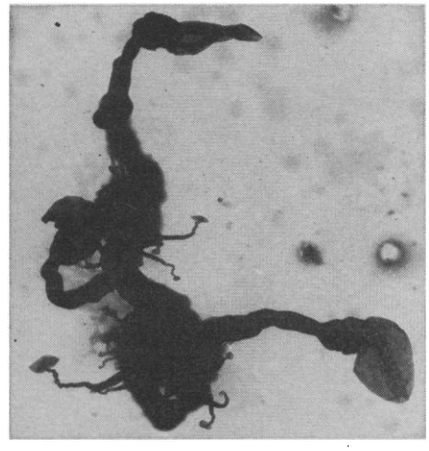

Fig. 1. Ice crystals and artifacts.

A more sophisticated procedure for replicating ice crystals with the cyanoacrylate monomer has been reported by Odencrantz and Humiston (*3*). However, the occurrence of artifacts was not considered, and the above remarks should be kept in mind while reading their paper.

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Too Much Noise in the Autoradiogram?

Reports regarding autoradiographic localization of noncovalently bound substances are very conflicting, and it appears that frequently such pictorial data are accepted without sufficient concern for their validity.

For instance, there are six reports of ^{3}H-estradiol localization in the uterus; not one of these agrees with another. Radioactivity was found to be concentrated in the lumen of glandular tubes in contact with the apical poles of cells (*1*); in the cytoplasm of the luminal epithelium (*2*); in the nuclei of endometrial and glandular cells (*3*); at the apex and base of luminal cells (*4*); in the cytoplasm preferentially at the cellular membrane of uterine eosinophilic cells in the connective tissue, while no nuclear labeling was detected (*5*); and in nuclei of luminal and glandular epithelium, the substantia propria, and muscularis as well (*6*).

Studies of the pituitary have yielded similarly conflicting results. ^{3}H-Estradiol was found to be concentrated over the nucleoli and at nuclear membranes of eosinophiles (*4*); in the cytoplasm of basophiles (*7*); and in nuclei of eosinophiles, basophiles, and chromophobes as well—however, not over nucleoli and at nuclear membranes (*8*).

In the brain, ^{3}H-estradiol was described as being localized in neurons of the nucleus supraopticus and nucleus paraventricularis (*7*); in neurons and glial cells throughout the brain as well as in the spinal cord, without "exclusive uptake by or absence of uptake from any particular type of nerve cell or