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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.

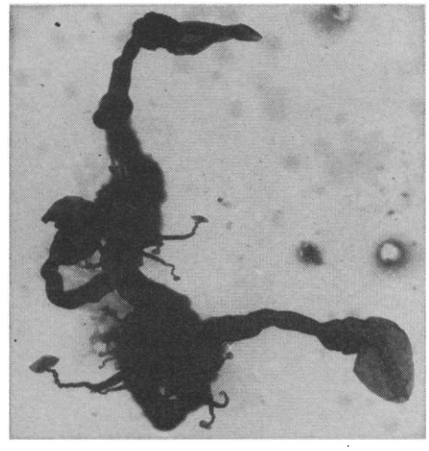

Fig. 1. Ice crystals and artifacts.

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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1 July 1968

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

glial cell" (*9*); and in well-defined areas of the diencephalon such as the nucleus arcuatus, the pars lateralis of the nucleus ventromedialis, the nucleus preopticus medialis, the nucleus stria terminalis interstitialis, and others, but was absent in such areas as the nucleus supraopticus, the nucleus suprachiasmatis and the mamillary nuclei (*10*). Glial cells were also unlabeled (*10*).

Therefore the important question arises: Are these conflicting results indeed authentic, that is, related to differences in dose, time, or individual animals; or are they artifacts to varying extent, that is, related to different and possibly invalidating technical steps employed during the preparation of tissue sections and autoradiograms?

Evidence has accumulated by centrifugal fractionation that estradiol is concentrated and retained for several hours in cell nuclei of tissues, such as uterus and induced mammary tumors of different mammalian species (*11, 12*). Although some cytoplasmic binding of estradiol is also found in such tissues, its extent is limited to only between 20 and 30 percent of the total uptake in vivo in immature and mature castrated animals (*13*). The nuclear ^{3}H-estradiol complex is unstable at *p*H's below 5.0 and above 10.0 (*11*), and if homogenization and fractionation are performed at room temperature the nuclear binding of the labeled hormone decreases, indicating its reversible nature.

Considering these biochemical data, it is likely that estradiol can be removed from its original binding sites by such histological procedures as liquid "fixation," embedding, wet section mounting, or the use of liquid emulsion at 40°C. Agreement exists between the biochemical data and the autoradiographic results only when all fluid treatments are excluded during the preparation of the tissue sections and the autoradiograms (*6*).

Diffusion, redistribution, and leaching of the label have been determined in our own autoradiographic studies with six different methods (*14*), using two diffusible compounds, ^{3}H-estradiol and ^{3}H-mesobilirubinogen. The extent of translocation artifacts was dependent upon employing such technical steps as liquid fixation, embedding, liquid emulsion coating, or thawing of frozen sections. For instance, in these experiments, diffusion of the labeled material into the epoxy resin could be demonstrated by liquid scintillation counting and by simultaneous photographic exposure of embedding material and tissue sections (*14*). The results obtained with each individual method were reproducible, although they deviated from each other. In autoradiography, reproducibility and minimum variability have been invoked to support authenticity of the results (*9*). If the data are reproduced by the same technique, however, this conclusion is unjustified (reproducibility pitfall) (*15*). With ^{3}H-estradiol, the divergent results obtained in these comparative studies were similar to those reported by the different investigators as already cited.

In the autoradiography of diffusible substances careful investigators have demonstrated radioactive material in all of the fluids used for tissue treatment. It is well established now that liquid "fixatives" not only extract varying amounts of tissue constituents but may also produce artificial binding of molecules which were previously unbound *in situ* (*16*). While diffusion artifacts are probably the most frequent and severe artifacts in autoradiography, many other artifacts are possible. The "estradiol story"—only one example of the many that could be quoted—may caution investigators in the use of autoradiographic techniques and the interpretation of the data derived from them and also arouse the attention of journal editors and referees.

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Serum Copper and Oral Estrogen

In their article on serum copper alteration after ingestion of an oral contraceptive (*1*), O'Leary and Spellacy state that "the mechanism of action of this increase is unknown but may represent a variation in the plasma proteins that bind the various metals." The effect of the principal steroidal constituents of the oral contraceptive used, mestranol and norethynodrel, as described by these authors is neither unexpected nor unique. The effect of estrogens on serum copper and ceruloplasmin has been recognized for over a decade, after a two- to threefold increase in serum copper and ceruloplasmin during pregnancy was reported in 1947 by Holmberg and Laurell (*2*). For example, Russ and Raymunt (*3*) found that serum copper and ceruloplasmin in a variety of patients was increased two to three times after the administration of 0.25 to 1.0 mg of ethinylestradiol per day for 3 to 4 weeks, an effect exceeding that noted by O'Leary and Spellacy. It has been postulated that this estrogen action is mediated through increased biosynthesis or secretion of ceruloplasmin or both by the liver. Thus, the changes in serum copper and presumably ceruloplasmin appear to represent a typical response to moderate estrogen or steroid treatment, an effect well known in the literature.

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Models of Pulsating Radio Sources

Linear polarization of signals from the pulsating radio star CP 0950 on 3 April 1968 indicates Faraday rotation of about 4 rad at 150 Mhz (*1*). This value "is comparable with the total rotation expected from the ionosphere" and sets an extremely sensitive upper limit on the weighted magnetic field through interstellar space. Smith comments "that there can be no appreciable Faraday rotation within the source of