TECHNICAL PAPERS

The Disappearance of Salt From Glass Ice During Low-Temperature Dehydration, and Its Implication in Electron Microscopy

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There are empirical observations in the literature that cells can be dried without the movement of salt only if they are quickly frozen to produce a glass ice, and then if the subsequent dehydration is accomplished at temperatures below about −55°C. (see the review of G. H. Scott. *Biol. Sympos.*, 1943, 10, 277). Ordinary lyophilizing temperatures greatly exceed this and cannot be expected to immobilize inorganic ions. These findings obviously have a very important bearing on the preparation of biological materials for the electron microscope.

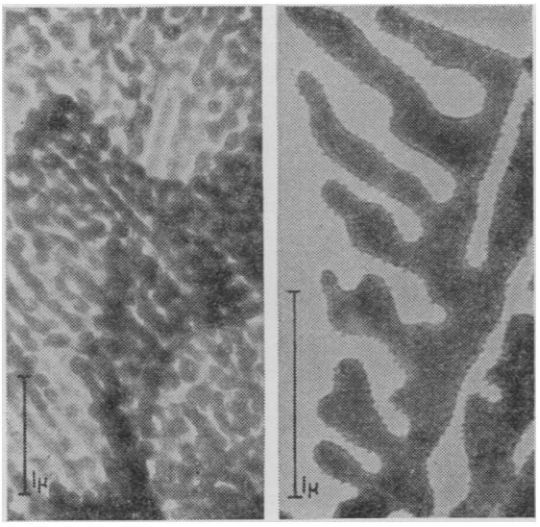

FIG. 1. The electron micrographs were made from a myosin sol originally dissolved in 0.5 M KCl. The aqueous film was frozen in liquid air and then dehydrated at −72°C. Note the total absence of any obscuring salt deposition.

It is for this reason that we have had in our laboratory a cryostat which is capable of dehydration at the temperature of a dry ice and alcohol mixture (−72°C.). This instrument has been in operation for more than a year, and we have prepared a considerable variety of biologically interesting specimens with it after first freezing them in liquid air. They have finally been examined with the electron microscope after such fragmentation as has been necessary.

A striking thing is that salt crystals have rarely been seen in material prepared by this method in spite of high tissue concentrations to begin with. Specimens which were relatively thick and bounded by membranes (as muscle fibers) undoubtedly retained their salt *in situ*, for even thin fragments had a high electron density. Single myofibrils, for example, were ordinarily quite opaque in spite of being less than 1 μ in thickness.

However, when one was dealing with a porous structure, the salt originally present disappeared in some undetermined manner. Thus, the intercellular "fluid" of loose connective tissue (perimysium has been most studied) showed a very low electron density. More dramatic and more definitive, though, were preparations made to study the structure of myosin sols. Aqueous films of purified myosin dissolved in KCl were frozen and dried as indicated. The salt concentrations were varied from 0.3–1.0 M. Although the details of the myosin structure will be described in a subsequent paper, it is necessary to point out here that the myosin was aggregated in long, fingerlike processes which often branched. Thus, a 3-dimensional network was formed, with a pore size that would be measured in only fractions of microns. That this mesh could offer delicate support is shown by Fig. 1. In these preparations there was never any trace of salt deposition, either in the interstices of the mesh or upon the myosin aggregates, and either in a crystalline form or in an amorphous mass. For all practical purposes the free and unbound salt had simply disappeared!

A special investigation would be necessary to determine where the salt goes under these circumstances. It would seem, however, that the phenomenon must depend upon the fact that one is dealing with ions that are fixed in a solid, yet are not associated in a crystal lattice. Conceivably the slightest jar after dehydration disperses them either as free ions or as molecules. But then one would expect that at least occasional masses would be caught and trapped in interstices such as are present in the myosin net. There was no evidence of this in porous structures, although there was within cells. The remaining possibility would seem to be that the ions (or perhaps molecules) literally evaporate from the regressing glass-ice surface during the dehydration. At first thought this latter suggestion would seem to run counter to physicochemical doctrine. But the author is not aware of any vapor-pressure work having been done on comparable systems in which isolated ions could be expected to appear on an evaporating solid surface. The attractive forces should be very different than at either a crystal face or a fluid surface, the ionic mobility being unimpaired in the latter. One may hazard the guess that the ionic or molecular vapor pressure may be quite appreciable at an evaporating glass-ice surface where isolated ions become exposed. If this latter hypothesis is the correct explanation of the observed phenomenon, it should be possible to find evaporated salt trapped or plated somewhere in the pumping system. It is likely that radioactive tracer techniques could be employed to good advantage to determine this. But in any case, aqueous systems containing high concentrations of salt can be prepared by this method for effective use with the electron microscope.