NATIONAL PARK SERVICE
DEPARTMENT OF THE INTERIOR

Conserve O Gram

May 1999 Number 11/3

Storage Concerns For Fluid-Preserved Collections

Fluid-preserved specimens may be plants, animals, or minerals (or any part) that are preserved in a liquid storage medium. Most fluid-preserved specimens are invertebrates, reptiles, amphibians, or fish. The most common fluid preservative is alcohol. The purpose of the fluid preservative solution is to stabilize the specimen and prevent it from deteriorating. The preservative solution and the storage container create a protective microenvironment around the specimen. If placed in a good, stable storage environment and maintained well, fluid-preserved specimens should last for hundreds of years.

Fluid preservation has the advantage of preserving the entire organism, including all tissues and gut contents. Fluid-preserved specimens can be dissected for anatomical studies. Some specimens can be removed from the preservative and prepared as dry mounts or skeletons. The disadvantage of fluid-preserved specimens is that the preservative chemically alters the tissues of the specimen, most obviously, by causing changes in coloration. Also, most preservatives cause physical changes to tissues, such as shrinkage.

This *Conserve O Gram* provides information on maintaining a collection of fluid-preserved specimens. It does not give complete information on collecting or preserving techniques.

Fixation

Most fluid-preserved specimens are first fixed in a fixative solution, usually formaldehyde. Fixation is a chemical treatment that prevents the breakdown of proteins into amino acids (autolysis) by forming chemical bonds (called crosslinks), and coagulates the contents of cells into insoluble substances. Fixation is necessary for specimens that are to be used for histological preparations. In some cases, specimens are placed directly into a preservative solution (e.g., ethyl alcohol) rather than being fixed. The preservative prevents autolysis but does not form chemical bonds, so although preserved, these specimens are not fixed. The oldest known fluid-preserved specimens were preserved in alcohol without being fixed.

Because the breakdown of tissue begins immediately after the death of the organism, collectors fix (or preserve) specimens as quickly as possible. The fixative solution is either injected into the tissues, or the specimen is cut open to allow the fluid to penetrate. Specimens to be preserved in fluid should not be frozen. Fixative cannot penetrate frozen tissue, and the freezing and thawing causes structural damage to the tissue.

The most common fixative is formaldehyde. Formaldehyde is sold as a 37% aqueous mixture of formaldehyde gas in water, with a little methyl alcohol to prevent the formaldehyde from forming a solid mass (polymerizing). One part of this formaldehyde solution is mixed with 9 parts of water to make a fixative or preservative solution called "formalin" or "10% formalin" (although it is actually a solution of 3.7% formaldehyde in water). Formaldehyde may present a health hazard and, when used long-term, removes calcium from bone. Work with formaldehyde in a fume hood or wearing a properly fitted respirator with a cartridge specific

for formaldehyde. Wear formaldehyde-resistant gloves. Formaldehyde is not commonly used as a long-term preservative for most whole animals. Whether used as a fixative or as a preservative, formaldehyde is acidic and must be chemically neutralized. The preferred method is to use four grams of monobasic sodium phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) and six grams of dibasic sodium phosphate anhydrate (Na_2HPO_4) per liter of formalin solution. Less suitable, but commonly used neutralizers include approximately one tablespoon of calcium carbonate or borax per liter of formalin solution.

Preservation

The preservative fluid is the solution that the specimen will remain in permanently. The preservative may be a fresh solution of the fixative solution, but usually it is an alcohol.

A fluid preservative has to stabilize the specimen, be germicidal, and prevent autolysis. The most common preservative fluids are ethyl alcohol (also called ethanol or grain alcohol) and isopropyl alcohol (also called isopropanol or rubbing alcohol). Denatured ethyl alcohol should only be used as a preservative if no other alcohol is available. Ethyl alcohol is denatured by adding a substance to it that renders it unfit for human consumption. These additives may affect the quality of specimen preservation. Neither methyl alcohol (wood alcohol) nor phenol should be used as preservatives.

Alcohols are good preservatives if used in solutions strong enough to kill bacteria and mold. The drawback to alcohols is that they dehydrate the specimens and dissolve certain pigments, proteins, and lipids out of the specimens. Ethyl alcohol is considered to be a superior preservative to isopropyl alcohol, which causes greater shrinkage of specimens and other undesirable physical changes. Isopropyl alcohol is also more toxic than ethyl alcohol. Ethyl alcohol is usually used in a 70% solution. Isopropyl alcohol has been used in solutions varying from 55% to 70%.

Formaldehyde may be used as a preservative, and for some specimens is better than alcohols, particularly for human anatomical specimens, fish and amphibian larvae, and some invertebrates. There are a number of proprietary preservatives on the market, such as Nasco-Guard™ and Caro-Safe™ which contain mixtures including alcohols and ethylene glycol. These products should be avoided, as there are no studies that demonstrate that these solutions are suitable as long-term preservatives.

Transfer Between Fluids

When a specimen is transferred from a fixative to a preservative fluid, or transferred between preservatives, the tradition has long been to simply remove the specimen from one solution, wash it in water, and place it directly into the other solution. This can cause osmotic imbalances and excessive shrinking or swelling of the specimens. To avoid these problems, the specimens should be staged through a series of graded concentrations (increasing by 10% to 20%) from one solution to another. For example, if moving a specimen from a 10% formalin fixative to 70% ethanol, it would be best to place it in staged solutions of 20%, 40%, and 60% ethanol for 24 hours each before placing it in the permanent fixative concentration of 70% ethanol. See *Conserve O Gram* 11/1 for detailed instructions for transferring specimens from formalin to other preservatives.

Housing

Fluid-preserved specimens must be stored in suitable containers (see *Conserve O Gram* 11/14, Storage Containers and Labels for Fluid-Preserved Collections). Shelves for jars must be flat and sufficiently wide that the specimen containers easily fit on them. Shelves should have a lip or rim to prevent containers from being knocked or pushed off. Shelf restraints (earthquake bars) are needed in areas prone to tremors or other vibrations. The shelving should be sturdy enough to accommodate the weight of the containers (for example, a one-gallon

container may weigh nearly nine pounds). The arrangement of shelving should allow safe access to the specimen containers. Heavy containers (those with a capacity larger than one gallon) should be housed on the floor or on low shelving. Aisles between shelving should be sufficiently wide (usually a minimum of 36") to allow for access to the containers and to comply with fire code.

Shelves should be spaced far enough apart to allow for easy access and removal of containers. For one-gallon jars, this means a distance of about 12" between shelves. Containers should not be crowded on the shelf. Ideal shelf storage density is achieved when shelves are 35% to 50% full; beyond a 50% container density, it becomes very difficult to access containers and to monitor the condition of the specimens without excessive movement or rearrangement of all the containers on the shelf.

The Storage Environment

Maintaining a proper storage environment is critical to the conservation of fluid-preserved specimens. Maintaining a stable temperature with very small fluctuations is best. In general, a temperature close to 18°C (about 65°F) is desirable. Below about 60°F, dissolved fats and formaldehyde in the preservative fluid may solidify. Relative humidity greater than 65% may allow mold to grow on the outer surface of containers, which can contribute to the deterioration of glass containers. Relative humidity that is very low (e.g., below 20% RH) for prolonged periods of time can dehydrate gaskets.

The biggest danger to fluid-preserved specimens comes from dehydration. Once dehydrated, it is very difficult to re-hydrate fluid-preserved specimens without destroying them. Fluctuating storage temperatures and high storage temperatures increase evaporation of the preservative fluid and cause stress to the specimens, leading to their destruction.

All light is damaging to fluid-preserved specimens. Light causes permanent, irreversible damage to specimens, including fading and embrittlement. Light damage is cumulative. Specimens should be kept in the dark whenever they are not being used, either in a dark room or preferably in closed cabinets. Specimens should never be exposed to sunlight; windows in the collection storage area should be completely covered. The collection storage area should have ultraviolet (UV) filters on all fluorescent or halogen lights (incandescent lights produce very little UV). Although glass containers do block the transmission of some UV light, they still allow the most damaging part of the UV spectrum to penetrate.

Pests are not often a problem for fluid-preserved collections, but their presence indicates that the storage area has environmental problems that should be fixed. Pests will damage or destroy external container labels, documentation, and exposed jar gaskets. Rubber or synthetic gaskets or stoppers are susceptible to damage from mold.

The storage environment should be routinely monitored for temperature, relative humidity, and pests. Containers should be checked regularly to detect fluid loss. When fluid loss occurs in a container, carefully inspect the container and closure to determine the source of the leakage. Correct or replace the container or closure as needed.

When it is necessary to replace lost fluid, begin by checking the concentration of the fluid in the container. Often the preservative will evaporate faster than the water, so a lowered fluid level may mean that more preservative loss has occurred than the total volume loss would indicate. Determine the concentration of the preservative solution with a digital density meter or a bulb hygrometer.[1] Add the appropriate concentration of preservative to maintain the desired strength in the container.

Because preservative solutions are usually solvents, they will become discolored over time

as various constituents of the specimens are leached out. The discoloration of preservative fluids (a change to a yellow or orange color) is usually the result of the extraction of lipids, proteins, and pigments from the specimen. There is no reason to change discolored solution unless it is so badly discolored that it might stain the specimen. If a preservative solution is badly discolored, becomes cloudy, or shows an accumulation of precipitate, the pH of the solution should be checked. This can be done with a pH test strip or with a pH meter equipped with a electrolyte dispensing electrode (non-dispensing pH electrodes quickly become clogged in preservative solutions).[2] If the solution has become too acidic (pH < 6), it should be replaced.

Storage Hazards

Most preservatives are flammable, particularly ethyl alcohol. Alcohol fumes accumulate at floor level and will ignite when there is an ignition source present such as a flame or spark. The storage area should have adequate ventilation so that fumes do not accumulate. Eliminate ignition sources from the storage area by positioning electrical plugs at least three feet above the floor and by restricting the use of electrical devices in the storage area. Clean up preservative spills immediately with absorbent pads. Smoking should never be allowed in or near the collection storage area.

Evaporation of fixatives and preservatives occurs constantly in a fluid collection, either from gas escaping from containers due to changes in temperature and air pressure, or when containers are opened to access specimens. Thus, there is always a certain amount of fixative and preservative fumes in the air. Be sure that ventilation is adequate in the collection storage area to disperse these fumes, and only open fluid-preserved specimen containers in a well-ventilated area.

Notes

1. Hygrometers are available through NPS *Tools of the Trade*. Digital density meters and hygrometers are available from major scientific supply companies.

2. Non-bleeding, plastic backed ColorpHast pH indicator strips are available from University Products, 517 Main Street, P.O. Box 101, Holyoke, MA 01041-0101; (800) 628-1912. Dispensing electrolyte electrodes are available from Hach Company, P.O. Box 389, Loveland, CO 80539; (800) 227-4224.

References

Jones, E.M. and R.D. Owen. "Fluid Preservation of Specimens." In *Mammal Collection Management*. Edited by H.H. Genoways, et al. Lubbock: Texas Tech University Press, 1987.

Levi, H.W. "The Care of Alcoholic Collections of Small Invertebrates." *Systematic Zoology* 15 (1966): 183-188.

Simmons, John E. "Storage in Fluid Preservatives." In *Storage of Natural History Collections: A Preventive Conservation Approach*. Edited by Carolyn L. Rose, et al. Iowa City, Iowa: Society for the Preservation of Natural History Collections, 1995.

John E. Simmons
Natural History Museum
University of Kansas
Lawrence, Kansas 66045-2454

The *Conserve O Gram* series is published as a reference on collections management and curatorial issues. Mention of a product, a manufacturer, or a supplier by name in this publication does not constitute an endorsement of that product or supplier by the National Park Service. Sources named are not all inclusive. It is suggested that readers also seek alternative product and vendor information in order to assess the full range of available supplies and equipment. The series is distributed to all NPS units and is available to non-NPS institutions and interested individuals by subscription through the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402; FAX (202) 512-2250 or on the Web at <http://www.cr.nps.gov/csd>. For further information and guidance concerning any of the topics or procedures addressed in the series, contact NPS Museum Management Program, 1849 C Street NW (NC 230), Washington, DC 20240; (202) 343-8142.