COMMENT: This electronic version of the newsletter was created by scanning the origional copy and then editing as needed. Apologies for any scanning errors that have not been corrected. Karsten E. Hartel, MCZ January 1994. hartel@mcz.harvard.edu

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ALCOHOLS: The following contribution is the beginning of what we hope will be a series of articles on the subject of alcohol as a preservative. Parts of Dr. Taylor's report might have been thought of before, but to our knowledge this is the first written attempt that distributes information on such a fundamental issue as the relationships of water in tissues, specimen volume, and alcohol concentration. In order to continue this series we invite additional articles, comments or rebuttals on the topic of alcohol as a preservative.

PRESERVATIVE PRACTICES: WATER IN TISSUES, SPECIMEN VOLUME, AND ALCOHOL CONCENTRATION - One of the purposes of using alcohol for preservation of specimens is to lower water concentrations in their tissues (i.e. dehydration) and thus place the specimens in a more inert environment. Protoplasmic (thus enzymatic) activity is associated with water and is sharply reduced with loss of water. Extensive dehydration will virtually but not completely eliminate the activity. The activity may continue at a low but imperceptible level for years, depending upon the degree of dehydration. The necessity for reduced activity, however, must be balanced against the fact that specimens may become distorted or shrunken during preservation. Such distortion may reduce the usefulness of the specimens or render them useless. We could thus look at preservation from the point of view of either the water loss or alcohol content of specimens. We normally think in terms of the latter. It is not the purpose of this discussion to define the best alcohol preservative. Instead, I wish to draw attention to factors affecting alcohol concentration that may be overlooked, resulting in weak preserving fluids.

It is well known that water from specimens will dilute any alcohol solution added to the specimens. The water content of animals is known to vary widely as does the water content of various tissues. Some of the water may be bound and not available for replacement with alcohol solutions. I have chosen theoretical percentages of water equaling 65% and 90% of the total body tissues for discussion herein. Both percentages are within the range of water concentrations found in whole animals. Bound water, if it exists, is ignored for the purpose of this discussion.

Another factor in alcohol dilution is the ratio of the volume of specimens to the volume of their alcohol preservative. The greater the volume of specimens added, the greater the volume of water available for dilution. The volume of specimens per container in many museums averages 25% or less. Relatively few containers contain 50% specimens by volume. Occasionally, especially where small specimens can be closely packed and the container filled, the volume of specimens occupies as much as 75% of the space.

TABLE 1.

Volume of specimens	25%		50%		75%	
Water in specimens.....	65%	90%	65%	90%	65%	90%

Alcohol percentages after the following changes:						
Add 40% alcohol.....	32.9	30.8	24.2	21.1	13.6	10.8
Change to 2nd 40% alcohol...	38.7	37.9	33.8	31.0	22.5	18.7
or						
Add 75% alcohol.....	61.6	57.7	45.5	39.5	25.4	20.3
Change to 2nd 75% alcohol..	72.6	71.0	63.4	58.2	42.2	35.1

Tables 1 and 2 present computed alcohol percentages based on specimens containing 65% and 95% water when occupying one-fourth, one-half and three-fourths by volume of the total. The percentages may be low when there is considerable bound water, tissues are impenetrable, or when there is shrinkage. We cannot duplicate these theoretical percentages in practice because of lack of knowledge about the actual amount of free water in tissues, but the calculated figures appear reasonable based on comparisons with random measurements of preservative density.

TABLE 2.

Volume of specimens	25%		50%		75%	
Water in specimens	65%	90%	65%	90%	65%	90%
Alcohol percentages after the following changes:						
Add 35% alcohol.....	28.8	26.9	21.2	18.4	11.9	09.5
Change to 55% alcohol.....	50.3	48.5	41.7	37.7	26.5	21.8
Change to 75% alcohol.....	70.6	68.9	61.9	57.3	42.9	36.2

Table 1 compares alcohol percentages resulting from the addition of specimens to 40% and 75% alcohol (as may be used with isopropyl and ethyl alcohols respectively). Note the low alcohol percentages after the addition of the first alcohol solution and improvement when the first alcohol is replaced with a second solution of-the original percentage is used. This improvement is greater than the difference in percentages resulting from the variable water content of the specimens. Of most significance is the extremely low alcohol concentrations in containers with a large volume of specimens. At 75% specimen volume the alcohol concentration is about one-third that of lots with 25% specimens whereas changes to a second alcohol solution improves the ratio to about one-half. Thus, whenever either 40% or 75% alcohol is added to a container with 75% specimens by volume the alcohol content is undesirably low.

Similar results are shown in Table 2. This table presents expected percentages when successive alcohol changes are made with increasing percentages of alcohol. It indicates that changes involving increasing percentages of alcohol generally do not result in a high a final percentage as the methods in Table 1. Most significantly, it shows that caution is advised when the specimen volume is near 75% or more. Specimens should either be placed in a larger container, divided into two or more containers, or processed through more changes of alcohol. If there is any question on the strength of the alcohol, it can be checked with a hydrometer after being left in temporary storage for some time.

Summary

1. The water content of specimens dilutes the alcohol of a preserving solution significantly.

2. Crowding of specimens or packing specimens into a container will result in a very low (perhaps-dangerously) alcohol concentration.

Instead of crowding or packing specimens in a single container, divide them among several containers or place them in a large container.

3. To approximate a-desired percentage of alcohol, for example 70% final alcohol using 75% alcohol, one or more changes of alcohol may be necessary unless the volume of the specimens is very small. - WILLIAM RALPH TAYLOR, National Museum of Natural History, Washington, D.C. 20560.

STORAGE TANKS FOR LARGE SPECIMENS - The AGRI-TAINER Corporation of Wenatchee, Washington, manufactures an inexpensive, easy-to-clean, polyethylene structural foam tank, equipped with a locking lid fitted with an "O" ring, and a threaded plug for drainage. These tanks are working well for us here at the College of Fisheries, University of Washington, as storage containers for large fish specimens. They come in two sizes: the larger COLOSSUS II, 42" x 44" x 29 3/8" currently priced at approximately $220, and the smaller COLOSSUS E, 42" x 29" x 25 1/2", approximately $170. For additional information write:- Agri-Tainer Corporation, P.O. Box 2004, Wenatchee, Washington 98801._ THEODORE W. PIETSCH, University of Washington, Seattle.

AVAILABILITY OF 32MM PLASTIC BOTTLE CAPS - The Smithsonian Oceanographic Sorting center recently received a sample of a 132mm black melamine (phenolic plastic similar to bakelite) unlined closure from Kol's Containers, inc., Baltimore, md. It fits the discontinued 132mm (2, 3, and 5 gallon) glass buckets which are in use in fish collections in large numbers. Although expensive (about $2.00 Plus 10 cents for a liner), it will probably be a better long-term closure than the metal caps that come with the buckets. Leslie W. Knapp, SOSC, National Museum of Natural History, Washington, D.C. 20560.

NEW STAINING TECHNIQUE- Ono, R.D. 1980. A silver impregnation technique to demonstrate muscle-bone-cartilage relationships in fishes. Stain technology, 55(2):67-70. The above paper describes a modification of the Winkelmann and Smith silver staining technique to demonstrate developing bone growth centers, and associated muscle origins and insertions in larval and small fishes. Although muscle, bone, and cartilage are stained, this technique may be impractical to many users due to the prohibitive cost of silver nitrate.

Except where noted, this newsletter is written and compiled by the ASIH Ichthyological Subcommittee on Curatorial Supplies and Practices and is intended for use of our membership. Comments are not to be construed as an endorsement of practices or products by ASIH. Correspondence should be addressed to: Karsten E. Hartel (subcommittee chairman), MCZ, Harvard U., Cambridge, MA 02138; Janet Gomon, Susan Karnella, Leslie Knapp, or Fran Irish, National Museum of Natural History, Washington, D.C. 20560; William Saul, Acad. Natural Sciences, Philadelphia, PA 19103; or Edward Wiley, Museum of Natural History, Lawrence, KS 66045.