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

CURATION NEWSLETTER No. 5 October 1982
American Society of Society of Ichthyologists and Herpetologists

PRELIMINARY OBSERVATIONS ON ACIDIFICATION OF ALCOHOL IN MUSEUM SPECIMEN JARS - Guido Dingerkus, Department of Ichthyology, American Museum of Natural History. Several years ago, after preparing a number of herring specimens via the enzyme clearing and staining process (Taylor, 1967), it was noted that the specimens had picked up very little of the alizarin stain. This did not seem to be due the lack Or alizarin in the staining solution, nor to the specimen not being in the staining solution for a long period of time. Rather, it appeared to be due to the bones themselves not being able to pick up the alizarin because of decalcification. Checking the pH Or the alcohol in the original jar with pH paper (Micro Essential Co.) revealed that it was about 3.5. When the alcohol was evaporated off a greasy brown deposit was left, which had a pH Or about 3. Since the isopropyl alcohol used at the AMNH as a pH of nearly 7 after being diluted to 50% with tap water, something extraneous had changed its pH so drastically.

The department's collection manager, Norma Feinberg, and I surveyed several hundred jars in the collection and found pH readings from 2 to 9. The jars with high pH readings contained specimens that had been fixed in formalin buffered with sodium borate, or in formalin made by adding sodium borate to paraformaldehyde. These specimens had a semi-cleared appearance. This condition has been noted as being an after-effect of buffering formalin with sodium borate (Taylor, 1977; also see Saul, 1981). The lowest pH readings (pH 2 to 3) were found in jars of specimens that had originally belonged to the Museum's comparative anatomy department, and had been fixed in a solution containing phenol (carbolic acid). In such jars the distinct odor of phenol could be detected. Other jars with no such odor or history had readings from PH 3 to neutral.

In other cases, jars that contained oily or fatty fishes (such as herrings, mackerels, cods, sharks, etc.) had lower readings than those of less oily fishes. Again, when the alcohol was evaporated off, a very greasy and acidic brown residue was left. Such jars could also be readily identified by heavy rusting of the metal lids. (It might be noted that even stainless steel tanks at the AMNH have been rusted in places by this acidic alcohol).

It was hypothesized that such acidity may be due to breakdown of the oils in these fishes. To test this, several freshly fixed herring were placed into fresh alcohol (50% isopropyl mixed with tap water pH 7). Within a year the pH of the alcohol had dropped from nearly 7 to 5.5, and the greasy brown residue could be extracted upon evaporation. Thus it appears that over long- time preservation in alcohol, body oils will dissolve out of the specimens into the alcohol. The breakdown of these oils into fatty acids may be the cause of the acidification of the alcohol.

In attempting to neutralize this acid it was decided against using a base, as this might lead to clearing of the specimens if too much base was added. In the case of some specimens that had a pH of

about 3, it took three changes of alcohol (for a week each) until the pH stabilized to around neutral for a week's time.

Another possible cause of acidification is the breakdown of formalin remaining in the alcohol or specimens into formic acid. Three tests wherein 1% formalin in 50% isopropyl (pH nearly 7 upon mixing) were allowed to sit in jars, with no specimens in them, yielded pH drops to 5.5 within a month. A control of just 50% isopropanol yielded no pH drop. Thus formalin left in the alcohol, or in the specimens, can also lead to acidification of the alcohol.

Although definitive proof does not exist at the moment, I believe that the brittleness of older specimens is also due to acidified alcohol, at least in part, as every jar of old specimens that had brittle specimens in it has been acidic (pH 3 to 5). However, the effects of historical curation of the collection (i.e., a change from ethanol to isopropanol, long term formalin storage, original fixation, etc.) can not be evaluated.

In alcian blue staining of the cartilage (Dingerkus and Uhler, 1977) of acidified specimens, the bones do not pick up the alizarin, but the cartilages are stained by the alcian blue as well as in a freshly preserved specimen. This is probably due to the fact that the chromophilic compounds in cartilage are acidic mucopolysaccharides. Being acidic, they are actually better preserved and not deleteriously affected by an acidic preservative.

From these findings I would make the following preliminary recommendations:

1. To prevent initial acidification of alcohol, distilled or deionized water should be used. If this is not feasible, use neutral tap water with no or little mineral or contaminant content.

2. Care should be used not to leave any formalin in the alcohol or in the specimens. However, extensive washing in water should not be done. Preferably several changes of alcohol are advised before specimens are placed in permanent storage.

3. Based upon observations above, oily or fatty fish specimens should be monitored periodically to determine if the alcohol is acidifying. If it is, alcohol should be changed until a neutral pH is maintained. (If the oils and fats are causing the acidity, it can be presumed that once all oils and fats are dissolved out of a specimen, no more acidification will occur.).

4. Specimens known to have been preserved or fixed in acidic solutions (including, but not limited to Bouin's, formalin-acetic-alcohol, phenol, and embalming solution) should undergo several changes of alcohol and be monitored until a neutral pH is maintained. It is also advisable that these specimens not be put with normally fixed and preserved specimens.

I would like to thank M. Norma Feinberg, Donn E. Rosen, C. Lavett Smith, Lance Grande, Harry Jacobson, and Terry C. DeFino for assistance in various aspects of this report.

References

Dingerkus, G. and L.D. Uhler. 1977. Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. Stain Technology, 52: 229-232.

Saul, W.G. 1981. Paraformaldehyde problems. ASIH Curation

Taylor, W.R. 1967. An enzyme method of clearing and staining small vertebrates. Proc. U.S. National Museum, 122: 1-17.

Taylor, W.R. 1977. Observations on specimen fixation. Proc. Biol. Soc. Wash., 90: 753-763.

JAR LIDS FROM KOLS - William H. Kreuger, Dept. of Zoology, University of Rhode Island. Curators with limited budgets should consider the 58 mm and 63 mm polyfoam-lined polypropylene jar lids distributed by Kols Containers, Inc. (1408 DeSoto Road, Baltimore, MD 21230). These lids fit the very useful 8 oz and 16 oz Paragon olive jars. Unlike the Kols closures reviewed in the ASIH Curation Report (Fink et al., 1979), these have knurled edges and are quite sturdy. (The 48 mm and 89 mm sizes lack knurled edges and have thinner walls). As the Report noted, lids of this type have excellent sealing properties and exhibit little or no "backing off" (although long term tests are needed to further monitor "backing off"). My repeated attempts to break them through forcible tightening have been totally unsuccessful. Prices as of August 16, 1982, are $.126 each for the 58 mm size ($.084 each for a case of 1400), and $.150 each for the 63 mm lid ($.100 each for a case of 1100). Minimum order is $25.00 --

TANK GASKET ADHESIVE -Alan Resetar, Collection Manager, Division of Amphibians and Reptiles, Field Museum of Natural History. One problem in dealing with metal specimen storage tanks is finding a suitable adhesive for fastening gasket material to tank surfaces that will not lose its adhesive capacities due to the effects of alcohol and time.

Three commercially available, automotive liquid adhesive products were tested to determine their effectiveness in bonding. The test consisted of bonding three two-inch long strips of closed cell neoprene gasket (0.5" x 0.5") to a flat piece of stainless steel with the adhesive, allowing them to set up for 4 hours and then immersing the test piece in a tank of discolored, used 70% ethanol. At the Field Museum, none of our tank gasket is immersed in ethanol for long periods so testing by immersion in ethanol is much harsher treatment than normal. Condensation is always evident on the inside of the lids of the tanks and presumably ethanol could come into contact with the adhesive in this way.

After three days in ethanol, the seal of the first product loosened up and the gasket came loose. The second product, a silicone based adhesive (similar if not identical to aquarium sealer) showed signs of loosening after 7 days. The third adhesive, a 3M product called Super Weatherstrip Adhesive (Part no. 08001) remained unaffected except for the loss of its yellow coloration which turned white. For two days after the immersion, the bonded gasket was stressed by pulling and pushing on it to try to dislodge it. Under these conditions it reluctantly began to pull loose. In some places the neoprene began tear before the bond between the adhesive and metal split.

I have used the 3M product in automotive applications and find it holds its bond under all temperature extremes and under mechanical stress.

In actual use on tank lids the adhesive is holding up very well.

I have found that the best way to install gasketing on a tank lid is to completely remove all debris and moisture from the gasket channel and put one generous bead of adhesive all around the channel opposite the surface that the edge of the tank contacts. Cutting the gasket into four strips and fitting these in snugly by bunching up the gasket slightly so that the corners are tightly filled formed a much better seal than taking one strip and trailing it around the corners of the gasket channel.

The 3M product is available in 5 ounce tubes which sell in the Chicago area for $3.75.

PRESERVATION NOTES: see Billy, A.J. 1982. The effects of formalin, and isopropyl alcohol on length and weight measurements of Sarotherdon mossambicus Trewavas. J. Fish. Biol., 21: 107-112.

Except where noted, this newsletter is written and compiled by the ASIH Ichthyological Committee on Curatorial Supplies and Practices and is intended for use by 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 (Committee Chairman), or Jose Rosado, MCZ, Harvard Univ., Cambridge, MA 02138; Janet Gomon, Susan Jewett or Leslie Knapp, Div. Fishes, Wr,-12, National Museum of Natural History, Washington, D.C. ,.'0560; William Saul, Academy of Natural Sciences, Philadelphia, PA 19103; Allan Resetar, Field Museum of Natural History, Chicago, IL 60605; or John Simmons, Museum of Natural History Univ. Or Kansas, Lawrence, KS 66045.