Natural Color Preservation

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Specimens are preserved, primarily, in order to demonstrate or study at some future time, their appearance, shape, and structure as found in the living state. Heretofore, no one method of procedure or preservative has completely fulfilled all of these requirements. The development of such an ideal preservative has been unsuccessfully sought since the time of the ancient Egyptians, when the now lost art of embalming mummies was in vogue. The writer recalls reading some of the scientific articles dealing with natural colors in tissues, at about the same time an article appeared in one of the leading popular journals. This article described how the Russians had so ably preserved the body of Nicolai Lenin. It was stated that only one or two Russian scientists were allowed to know the method used. After 24 years of preservation, Lenin's body is still seen in its original lifelike appearance by the hundreds of Russians who daily file by his mortal remains.

An adequate method of preserving color and tissue detail has long been needed in fields other than that of embalming, for example, in preservation of pathology and museum specimens, preservation of minnows for artificial bait, and preservation of tissues for histological sections. A review of the literature concerning preservatives and methods for retaining natural colors reveals that most of them were empirically formulated. This was made necessary by the incompleteness of the knowledge, at the time, of the chemistry involved. Recent scientific studies of the involved chemistry concerning the formation of the natural colors in living tissues of man and animals have made possible the development of an entirely new method of preserving these natural colors. Since this new method has been perfected, sufficient confirmatory work has been done to justify its use in a number of different fields.

The use of this method is especially applicable to the retention of natural colors in normal or pathological specimens. Veterinary and medical colleges have always been handicapped by being unable to easily, quickly and inexpensively preserve the natural lifelike appearance of specimens. It has often been a question of using either fresh material, or specimens preserved in formalin or alcohol. Fresh material is frequently expensive or unobtainable when needed. Specimens preserved in formalin or alcohol are usually colorless, repugnant in appearance, and elicit impressions of death and decomposition. Conversely, specimens which retain their natural colors tend to favorably impress a mental picture in the memory of the student or observer. Naturally colored specimens retain the appearance of living tissues and convey an impression of the way the specimen looked in the living animal.

Nationally recognized psychologists have stated that 90 percent of the knowledge gained by the average person is learned through recognition, while only 10 percent is learned by repetition. During World War II thousands of soldiers were rapidly trained in the complicated intricacies of modern warfare by means of visual education. This experience indicates that students in veterinary and medical colleges can be more easily, quickly, and competently trained by supplementing reading material with visual observation of normal and pathological specimens mounted in natural colors.

Veterinary and medical scientists en-

gaged in research work occasionally find that the preservation of specimens is invaluable in substantiating scientific proof of some discovery or development. Such specimens are much more convincing scientific evidence if their natural colors are preserved. Other conditions wherein the use of natural color preservatives are invaluable are for refresher courses for doctors in all fields; the presentation of papers at veterinary meetings, where adequate specimens could take the place of detailed yet inadequate descriptions during the presentation of papers; and the dissemination of information of newly identified diseases, or of diseases now considered eliminated but against which the professions must be constantly on guard. In cases such as these, it is often difficult for the practitioner who has been out of school for some time to accurately visualize the detailed description of a condition. A well preserved example of the condition to be memorized is inestimably more effective than are the words used to describe it.

During the past several months, several veterinarians and physicians, who have seen the writer's collection of naturally colored specimens, have expressed a desire to start a collection of their own. Numerous veterinary and medical pathologists, as well as instructors in other courses, are planning to or have already started to use this method of preserving natural color in specimens. They are all especially attracted by the simplicity of the technic, ease of application, inexpensiveness of materials, and practicability of use, whether for display, visual education, or merely as a hobby. Largely because of the numerous questions asked about the method, it was deemed advisable to write a complete description of the technic, so that the information would be made available to everyone desiring to use it.

After selection of the specimen to be preserved, it is first hardened in formalin with added preservative. Great care must be taken to exclude any oxygen from the solution, both by removal of that in solution, and by the elimination of the air from the vessel. When the specimens are sufficiently hardened, they are washed and mounted. The solution into which the specimens are permanently placed is composed of special preservatives and either distilled or tap water. If the tissue to be perserved is quite large, add from 0.5 to 1 percent formalin to insure against any possibility of decomposition.

The water to be used in making both the formalin solution for hardening, and the final preservative solution may be either distilled or tap water. Distilled water is preferable, because it is freed from salts and chlorine. The oxygen content is also likely to be lower, especially if it has been kept in a stoppered container. When using tap water or distilled water containing dissolved oxygen, it is necessary to remove it to prevent its combination with the more sensitive color pigments in the tissue. To remove the oxygen from the water, it may either be boiled, or subjected to a negative pressure of at least 25 in. of mercury by use of a vacuum pump or an inexpensive water aspirator.

Formalin is an excellent fixative for tissues. It possesses high germicidal efficiency, thus preventing bacterial decomposition. As a fixative, it polymerizes the tissue cells. Inasmuch as it is first necessary to harden the tissue before preserving it, formalin is the agent of choice, since hardening is essentially polymerization of the cells. In the use of this fixative, there are several points to be considered. Formalin has a tendency to become tinged with brown upon standing. Only the clear U.S.P. grade should be used. By first neutralizing the formalin with calcium carbonate in the form of marble chips, it becomes crystal clear, and remains so for a long time. To a container one-fourth full of marble chips, add enough formalin to completely fill the vessel. Be sure that the container is tightly closed. For best results, allow this solution to stand for several days to insure complete neutralization.

Some precaution must be taken to tie up any oxygen which cannot be entirely excluded from either the fixative or the final preservative solution. If no special agents are added to the solutions, the oxygen will combine with the natural

pigments of the tissues, and render them pale and indistinguishable. At the time of this writing, the only adequate agents for this purpose are assembled in two solutions to be used in conjunction. These preservatives of natural color are called Natural Color Preservative No. 1 and Natural Color Preservative No. 2. To use these preservatives, all that is necessary is to add 10 cc. of each per liter of the fixative solution, regardless of the strength of the formalin. For the solution used in the permanent mounting of the specimens, which may be water or formalin solution of any strength, add only the Natural Color Preservative No. 2 at the rate of 10 cc. per liter.

Fixing Tissues

The recommended procedure for fixing tissues with formalin and preserving their color with the Natural Color Preservatives is as follows: Select only fresh specimens whenever practicable, because this is an important factor in retaining natural colors. Upon exposure to atmospheric oxygen some of the natural pigments are oxidized. Color preservatives may restore some of these colors to their full intensity, and many more almost to their original intensity, but the colors thus restored still have a tendency to fade when exposed to excessive light. Place the freshly collected specimens into a formalin solution of the desired strength, to which has been added 10 cc. of each of the Natural Color Preservatives per liter of solution of neutralized formalin. Before placing the specimens in the fixing solution, wash off all the blood clots and foreign material. The washing should not be too vigorous.

Different types of cells require varying concentrations of formalin for fixation, therefore some experience is desirable in determining the strength of the solution to be used. A few experiments in mounting specimens soon demonstrate that the more delicate specimens require weaker solutions of formalin to avoid distortion. After 24 to 48 hrs. of gradual hardening, stronger solutions of formalin may be used. Large and firm specimens may be placed directly into a 10 to 20 percent formalin solution. Thus, delicate specimens that have a tendency to shrink or twist should be tacked to a board and placed in a 2 to 4 percent formalin solution containing the Natural Color Preservatives No. 1 and 2. Organs such as kidneys, which have a tendency to shrink or wrinkle, should also be gradually hardened. For best results, organs of this type should be injected either per artery or per vein with the preservative solution. While this is not absolutely essential, most organs or parts of an animal body retain their natural shape much better if time is taken to inject the arteries or veins with the preservative. After 24 to 48 hrs. of partial fixation, delicate tissues are usually hardened sufficiently to permit transfer to a stronger formalin solution.

Fixation entails hardening the tissues, and causes them to permanently assume an unchangeable shape or position. Therefore, care must be taken in placing the specimen in the container so that it will retain the desired shape during the process of fixation.

When the tissues are completely hardened, they are ready for permanent mounting. To accomplish this, remove the specimen from the fixing solution and rinse in cold running water. The easiest way to do this is to attach a rubber tube to a faucet; using the full force of the water pressure, work the end of the tube around in the container in such a manner as to remove all the debris and dissolved albumin from the tissue. In making the final solution in which the specimen is to be permanently placed, warm the water to the highest temperature to which the specimen will be exposed when on display. Otherwise, the solution in the completely filled container might expand and cause the container to leak. To each liter of the warmed water, add 10 cc. of the Natural Color Preservative No. 2. If the specimen is large or thick, it is advisable to add 0.5 to 1.0 percent of formalin. Completely fill the container with the permanent solution to exclude all air from the sealed container.

Many pathological tissues from animals

and man are rare or possess some unusual feature which makes their preservation as specimens highly desirable. Extra care should be taken in preserving their natural colors. It is advisable to remove the air from specimens to eliminate any possibility of oxidation of the color pigments. The air may be removed by covering the specimens to a depth of two in. with the permanent solution in a vacuum desiccator. Weight down with a perforated desiccator plate and draw a vacuum with a negative pressure of 25 in. until air bubles cease to rise from the specimens. Rinse the specimen in running cold water and immediately mount in fresh, slightly warmed, permanent solution.

If a vacuum desiccator is not available, one can easily be made by using a gallon-sized pig's feet jar. Place a rubber vacuum cup from an automobile top carrier over a hole punched or bored through the center of the lid of the jar. By means of a rubber tube connect a large hypodermic needle inserted into the center of the rubber vacuum cup to an inexpensive water aspirator or a vacuum pump. The rubber vacuum cup and the rubber tube or air pressure hose may be purchased at any auto supply store.

Oxidized Pigments

Should the age of the specimens be such that some of the color pigments were oxidized or air was present in the water or specimens, some of the colors may start to fade a few days after mounting. The fading starts in the upper part of the container and gradually extends downward. At the first sign of fading, discard the mounting solution and replace with the fresh permanent solution. Draw a vacuum to remove the undissolved air or uncombined oxygen.

It is quite difficult to preserve the full natural colors in liver specimens. The presence of bilirubin, biliverdin and perhaps glutathione may offer some explanation for the appearance of a brownish or golden brownish color in the permanent solution. By using a 20 percent formalin fixative solution and changing the permanent preservative solution three or four times, most of the brownish tinge can be eliminated.

There are several fine collections of specimens in the United States mounted under watch glasses. This type of mounting is generally restricted to specimens of small size or thin sections of tissue due to the limitations imposed by the size of watch glasses. An excellent description of the watch glass method of mounting may be found in Circular No. 454, published by the United States Department of Agriculture. It fully describes the preparation and mounting of animal tissues under watch glasses.

By using Natural Color Preservatives No. 1 and No. 2 many of the detailed steps described in Circular No. 454 can be either eliminated or simplified. In place of the modified Kaiserling fixing solution, known as Kaiserling No. 1, use the following solution: Natural Color Preservative No. 1, 10 cc.; Natural Color Preservative No. 2, 10 cc.; potassium acetate, 30 Gm.; formalin U.S.P., 200 cc.; and distilled water, 1,000 cc.

This substituted solution not only fixes the tissues but preserves the natural colors at the same time. As a result, the procedure of restoring the colors with two grades of alcohol and its later removal by means of boiling with a vacuum are all eliminated. After fixation, wash the specimens and mount in the Kaiserling preservative solution, known as Kaiserling No. 2, following the directions given in Circular No. 454, remembering to remove the air from the solution and tissues with a vacuum.

The addition of Natural Color Preservatives No. 1 and No. 2 at the rate of 10 cc. of each to 1 liter of formalin solution is a decided improvement over the usual method of hardening tissues for histological work. The retention of the natural colors in tissues improves the differentiation of the cellular structures.

Recently, the writer added 1 part of paraformaldehyde to 99 parts of boric acid as a powder for dry packing some tissues to be mailed to a laboratory for a histological examination. Another mixture containing 2 parts of paraformaldehyde

and 98 parts of boric acid was also made up to determine if a stronger mixture of paraformaldehyde would be necessary to prevent decomposition. In a third container, tissues were packed in boric acid.

Upon observing the tissues after 10 days it was found that those in the two containers with the paraformaldehyde had no odor and showed no evidence of decomposition. However, it was noticed that there was a hard shell around the tissues. Tissues packed in boric acid had an offensive odor and were partially decomposed.

Fresh mixtures containing 1 and 2 percent paraformaldehyde were then made. To these were added Natural Color Preservatives No. 1 and No. 2 in the concentrated form. After five days, tissues packed in these mixtures were found in good condition with fair retention of the color pigments. Apparently there is good argument in favor of adding paraformaldehyde to some other powder for dry packing tissues to avoid the possibility of decomposition. Laboratory diagnosticians are frequently handicapped in making a satisfactory diagnosis when they receive laboratory specimens partially decomposed. It is suggested that someone might try paraformaldehyde or some of the other new fixative chemicals as an agent which would improve the boric acid method of dry packing specimens.

Substitute Glassware

Some of our more fortunate colleges and museums are financially able to purchase standard museum jars for mounting specimens. Museum jars are crystal clear, uniform in size and make very attractive display containers. However, the present scarcity and almost prohibitive cost practically eliminates their use by the average college or by the individual.

Those who are unable to purchase museum jars may substitute less expensive glassware. Inexpensive glassware can be obtained that is uniform in size and shape, and reasonably clear as well as attractive in appearance. As an example of the price and size of inexpensive glassware, the following may be obtained from the Hazel-Atlas Glass Company, who has branches in several of the larger cities: square gallon-size glass jars with caps, about 18 cents apiece in gross lots; widemouth, straight side, quart glass jars, $6.87 per gross; and widemouth, straight side, pint glass jars, $4.91 per gross. Probably other companies can supply similar glassware.

Tubular Containers

Discarded fluorescent tubes may be used in mounting such specimens as intestines affected with enteritis, nodule worms, or full length tapeworms. Fluorescent tubes come in many different sizes and lengths, making it posible to arrange a variety of displays that are attractive and spectacular. They offer an inexpensive, easily obtainable source of glassware which can be readily converted into suitable containers for certain types of specimens.

Converting fluorescent tubes into display containers is quite simple. Heat the metal cap on the end of the tube where the trade mark is located. Some manufacturers use plastic caps but heating them with a low flame Bunsen burner will melt the adhesive used to stick the cap to the tube. Remove the metal or plastic cap, and with a pair of pliers break the glass tip used to draw a vacuum. The thin glass plate covering the end can then be broken and the edges smoothed with a rat-tail file. The white powder on the inside of the tube can be removed with warm, soapy water and a cloth attached to the end of a long wooden ramrod.

Institutions or individuals who cannot purchase museum jars might possibly induce some plastic manufacturer to make a screw-type clear plastic cap or lid for covers for the less expensive glassware. The use of a clear, plastic cap would permit light rays to penetrate the container from above, increase the area of vision of the container, and add to the attractiveness of a display.

Summary

An entirely new method of preserving the natural color pigments in animal tissues has been developed. The retention of these colors in tissues is of special importance in the mounting of specimens,

making it possible to preserve their natural appearance. It is a practical method, requiring only a few chemicals with no unusual skill in its use. As a result, the usual cost of mounting specimens can be materially reduced.

Editor's Note: The preservatives discussed in this article under the name of Natural Color Preservatives Number 1 and 2 are prepared and sold only by Dr. Coon. Inasmuch as the patent is pending, the constituent chemicals cannot be revealed at this time. The preparations may be obtained by writing directly to Dr. Coon at Sioux Falls, South Dakota. We have seen his museum, and feel that his system of preservation is sufficiently outstanding to warrant its serious consideration by anyone interested in the preservation of tissue specimens.

Expensive Meat

Djelal, a high-strung French stallion went mad with fright on the first leg of an air trip to the United States and killed himself in frantic efforts to kick his way out of the plane. The 4-year old horse, winner of races in both France and England, was sold to an American syndicate of breeders for $250,000.

Seventeen dogs, all barking frantically, were cooped in the plane with the frenzied Djelal and were still barking when the pilot took off again after making an emergency stop to get rid of the body.

The United States lamb crop is estimated at 20,467,000 lambs, or about 8 percent and about 1,700,000 head smaller than the 1947 crop. This is the smallest lamb crop in 24 years of record. The 1948 lamb crop is 17 percent or 4,070,000 head below the 1946 crop of 24,540,000 lambs.

Rats cost Iowa farmers 40 million dollars a year. The United States Fish and Wildlife Service estimate that it cost $2.00 a year to keep one rat on a farm.

Cattle Diseases

The greatest disease threat to cattle production in America is brucellosis. This fact was pointed out to the American Veterinary Medical Association by its committee on diseases of food producing animals.

With brucellosis listed as the No. 1 disease in cattle, the American Veterinary Medical Association's committee on diseases of food-producing animals has listed other major cattle ailments.

Mastitis still ranks as one of the biggest problems of the dairy industry, despite the increasing use of penicillin to treat the disease.

Losses from calf scours and pneumonia continue to be excessively high; often due to makeshift methods of housing and feeding calves.

Shipping fever, the major disease problem of feeder stock, is probably linked to the pneumonia rate among cattle and hogs in packing centers.

Anaplasmosis is being reported in new territories every year and is fast becoming a disease of major importance.

Several states have reported an increase in tuberculosis of cattle in the past year.

Scabies (itch) of cattle is prevalent in several New England and western states. A 3-year campaign may be necessary to clean up some range herds.

Vitamin Preparations

Cod liver oil as a source of vitamin A for pets may have little value if the oil has been standing on the shelf for weeks. The same is true for other fish oils.

Dr. M. L. Morris, New Brunswick, N. J., indicated this fact while addressing the small animals section of the American Veterinary Medical Association.

"Although vitamin A is one of the most important vitamins in the daily rations of dogs and cats ,it is really a very unstable substance," Dr. Morris declared.