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Scope of Study: Many high schools cannot afford to purchase preserved materials that are invaluable as a teaching aid in biology; it is therefore necessary for the science teachers to furnish these materials. Local plants and animals give the student a better perspective of plant and animal associations in their natural environment. This report describes some of the methods of preserving botanical and zoological specimens, which may be used to create more interest and as a further teaching aid. It describes the methods of maintaining natural colors in the botanical specimens, methods of killing, fixatives or preservatives that may be used, protection from pest insects and different poisons that may be used. It describes some of the many ways in which to preserve the zoological specimens, some types of mounts that may be used, method of embalming, preparing skeletons, and how to prepare plastic corrosion preparations.

Use of the Study: These methods selected here are intended to be suggestive rather than comprehensive, and to be used as a guide to aid in preparing some of the rather inexpensive preparations of both botanical and zoological specimens.

ADVISER'S APPROVAL Jimmy V. Holt

METHODS OF PRESERVING BOTANICAL AND ZOOLOGICAL SPECIMENS USEFUL TO HIGH SCHOOL BIOLOGY TEACHERS

By

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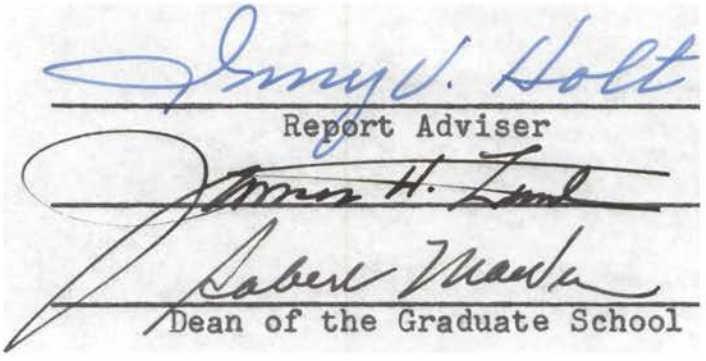

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C. D.

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

One of the greater problems, to a teacher of biology, is that of providing suitable materials for class use and having these materials on hand at the appropriate time.

Many schools cannot afford to buy the preserved specimens that are needed and used in biological studies; it is therefore necessary for the science teachers to furnish these materials. Too often the teacher regards the situation as hopeless as far as field work is concerned and may lack the requisite knowledge on collecting and preserving these materials.

A teacher who plans to do so will be able to substitute many local specimens for the standard ones of the biology texts. Local plants and animals give the student a better perspective of plant and animal associations in their natural environment. To preserve plants and animals will create more interest with student participation and the teacher soon realizes that much of the responsibility of collecting and preserving can be placed in the hands of the students. Too often teachers fail to realize that the main source

of supplies are at their very fingertips.

Many biology teachers will find their teaching will have more meaning and a greater enjoyment to them while pursuing this type of activity. The teaching of biology should and can be a pleasant experience to both teacher and student.

With the above facts in mind, this report is prepared as a guide to some of the simple and rather inexpensive methods of preserving botanical and zoological specimens.

The methods selected here are intended to be suggestive rather than comprehensive.

CHAPTER II

FIXATIVES AND FIXATION

One of the most critical operations in the processing of tissues is the killing of the protoplasm. The stopping of life processes within the cells should be accomplished with the minimum structural disturbance within the cells and minimum distortion of the arrangement of cells in the tissues. In addition to killing the protoplasm, the killing fluid or the subsequent processing must retain or fix the undistorted structure and render the mass of material firm enough to withstand the necessary handling.[1]

One of the simplest and most usable fixatives or preservatives for simpler plant and animal preparations is formalin-acetic acid-alcohol(F.A.A.). It is advantageous for the following reasons: materials are easily procured; it is a rapid penetrator; it will fix large volumes of tissue as compared to volume of liquid; it keeps indefinitely; tissues fixed in it do not have to be washed in water before dehydration and may be transferred directly to 70 per cent alcohol (ethyl or grain);

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[1]John E. Sass, Botanical Microtechnique, (Ames, Iowa, 1951) Iowa State College Press, p. 12.

tissues may be kept in it indefinitely without injury; and it is a very satisfactory preservative for museum specimens. Two formulas are in common use as follows:

Alcohol (ethyl or grain) 50 per cent.....90cc.
Formalin (commercial).....5cc.
Glacial acetic acid.....5cc.

Alcohol (ethyl or grain) 70 per cent.....85cc.
Formalin (commercial).....10cc.
Glacial acetic acid.....5cc.

Other variations of the above formulas are occasionally used. The addition of glycerin equaling about 5 per cent of the total volume will prevent complete drying out of specimens when the liquid evaporates because of an unsatisfactory stopper.

Propionic acid may also be used, the formula is then designated F.P.A.

Several modifications may be found in the literature. This fluid is stable, has good hardening action, and material may be stored in it for years. These properties make this formula suitable for large or impervious objects such as woody twigs, tough herbaceous stems, and old roots. The high concentration of alcohol is likely to produce shrinkage of succulent materials, although it is possible to develop a formula for some apparently tender subjects and even for filamentous algae. A balanced formula can be worked out by varying the acetic acid, which has a swelling action on protoplasm, from 2 to 6 per cent by volume. The formaldehyde

and alcohol, which have a shrinking action, should be held at the indicated concentrations.

Purpose of Fixation

1. That the material shall be preserved in the shape it had before fixation.
2. That the nuclear elements of the material shall be preserved.
3. That the cytological elements of the material shall be preserved.

All these functions can rarely, if ever, be secured from one solution.[2]

The time-honored liquid preservative for general taxonomic use is a 5 per cent aqueous solution of the commercial formaldehyde, or some combination of formaldehyde and alcohol.

When a preservative calls for ethyl or grain alcohol and this cannot be obtained, it is permissible to use rubbing alcohol, (70%) or isopropyl alcohol.

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[2]Peter Gray, Handbook of Basic Microtechnique, (New York, 1948), McGraw-Hill Book Company, Inc., p. 69.

CHAPTER III

PRESERVING BOTANICAL SPECIMENS

The preparation of plant specimens for botanical study is a simple matter and does not require previous experience. The most important points are: to make complete specimens, to dry them under pressure quickly and thoroughly and to label them properly.[1]

Probably most of the material to be collected will consist of flowering plants. These are classed as herbaceous or woody. Of the former, which include many plants that do not have showy blossoms, collect only those having flowers or fruit, in addition to leaves; if you can obtain both, so much the better.

The coarser seaweeds may be dried like flowering plants, but the delicate kinds with fine threadlike divisions must be patiently floated out on paper in sea water. Lichens and mosses should be taken only when in fruiting conditions, and dried under much less pressure than flowering plants and ferns.

The usual plant press consists of (1) two slightly flexible lattice-like frames measuring 12 by 17 inches,

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[1]Staff of the Smithsonian Institution, A Field Collector's Manual In Natural History, (Washington, D.C., 1944) The Smithsonian Institution, p. 73.

made from slats of ash or some other tough wood, (2) driers (thick blotting papers) of the same size, and (3) a pair of strong web trunk straps.

In some plants, such as hemlock and spruce, leaves shatter from stems soon after specimens are pressed and dried. This can be prevented by first preserving the specimens in the following solution for about 10 days or until the leaves show a normal green color:

Glycerin.....100cc.
Grain Alcohol.....100cc.
Formalin (commercial).....5cc.
Glacial acetic acid.....5cc.
Copper sulfate.....1 lump, size of fingertip.

After a week to 10 days, a more or less green color appears which is permanent. The specimens are then carefully rinsed in water, placed between driers and pressed in the usual way. Leaves and green color are retained by this treatment.

Dried specimens should be poisoned before attaching to the herbarium sheets, to protect them from insects. This may be done very satisfactorily with 70 per cent grain alcohol saturated with mercuric chloride. If the pure grain alcohol is not available, isopropyl alcohol may be substituted. The poison solution is applied with a paint brush, since spraying may cause inhalation of some of the poisonous mist.[2]

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[2]David F. Miller and Glenn W. Blaydes, Methods and Materials for Teaching Biological Sciences, (New York, 1938), MCGraw-Hill Book Company, Inc., pp. 248-249.

The largest part of the material collected by a high school teacher will be wanted for gross study in the laboratory. Such specimens may be preserved in alcohol, formalin-acetic acid-alcohol, or in formalin. A comparatively weak solution of formalin is suitable for plant tissues. For most plant specimens a 4 per cent solution of formalin will suffice, although certain very large, fleshy forms may require a 5 to 6 per cent solution. Plant material preserved in this way will keep indefinitely and will be available whenever needed for laboratory study.

The greatest disadvantage of formalin as a preservative, in addition to its somewhat disagreeable odor, is its ability to bleach plant specimens. Green leaves placed in 4 per cent formalin soon lose their green coloring matter and become yellowish or whitish. Alcohol presents the same difficulty, but the Uranium nitrate preservative will preserve the green color in plants:

Alcohol (50 per cent).....90cc.
Formalin.....5cc.
Glycerin.....2.5cc.
Glacial acetic acid.....2.5cc.
Copper chloride.....10 grams.
Uranium nitrate.....1.5grams.

The specimens are merely dropped into this fluid and left there until needed.

Lichens need never be preserved in solution. Simply dry them, and soak in water a few hours previous

to the time they are needed for study.

It is usually difficult to preserve the natural colors of flowers and fruits. The natural colors of some of these forms may be preserved to some extent, however, by adding ten per cent of pure cane sugar to the usual 4 per cent formalin solution. In some cases it will also be necessary to increase the strength of the formalin solution, even up to 15 or 20 per cent and then dissolve the cane sugar in this solution. Little work has been done in this field, and much experimenting is needed to determine the best solutions to use for various specimens.

Fruits such as apples may be preserved in a solution made as follows:

Distilled water.....4000cc.
Zinc chloride.....200gm.
Formalin (40 per cent).....100cc.
Glycerin.....100cc.

It is best to dissolve the zinc chloride in hot (distilled) water and filter while hot. Professor L. R. Hesler of the University of Tennessee reports that the solution preserves both the red and green colors in apples.

Many fleshy fungi (mushrooms) may be preserved in their natural colors in the following solution:

Formalin (commercial) 40 per cent.....6cc.
Alcohol (ethyl or grain) 50 per cent.....100cc.

In some cases it is necessary to use higher percentages of formalin, even as much as 50cc. of 40 per cent

formalin to 50cc. of 50 per cent alcohol. This solution is very good for the preservation of mushrooms for display purposes.[3]

Preserving Leaves

Several methods, none of them wholly satisfactory, are used to preserve the colors of autumn leaves. Each gives an interesting result.

The easiest way to preserve whole branches, with their leaves, is to place the freshly-cut branch in a dark, cool location, such as the cellar. This works well with beech leaves and with the foliage of the sumac and maple. It is unsuccessful with oaks. A second method consists of splitting the cut branch a few inches and then leaving the split end in a solution of four parts glycerin and one part water for about two weeks.

A third method involves drying the branches in warm sand. Clean, dry sand (screened, builder's sand is best) is heated in a metal can until it is very warm, but not painful to touch. While this sand is heating, another portion of sand, which need not be hot, is poured into a large box and smoothed out. The branches are then laid on this sand-bed in a natural position, and their outer ends carefully nailed to the sides of

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[3]Turtox Service Department Publication, Biological Field Work (Chicago, 1947), General Biological Supply House, pp. 33-35.

the box and supported by a few wires or sticks. The warm sand should then be sifted into the box, rather quickly and very evenly. The warm sand not only promotes evaporation of water from the leaf cells, but also presses the leaves evenly in a natural position. The excellence of the colors preserved by this method is surprising. Even the deep reds, among the most difficult to keep, lose very little of their natural beauty.

Single leaves can be preserved by coating them individually with paraffin. This may be done either by dipping the leaf into a pan of melted paraffin or by pressing it on each side with a warm flatiron upon which a little paraffin has been melted (a steam iron should not be used). The coating of paraffin should be thin, for a thick layer will dull the color of the leaf. This method preserves the color indefinitely. Leaves will possess remarkably lifelike colors.[4]

Killing, Color-Preserving and Drying Leaves

Most leaves of green plants can be killed successfully in the following solution:

6 parts water (distilled H_2O preferred)
3 parts 95 per cent grain alcohol
1 part commercial formalin.

Medicinal (rubbing) alcohol may be substituted for the pure grain alcohol, making the preservative less

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[4]Jack McCormick, "Preserving Leaves," Natural History, October, 1957, p. 432.

expensive and more easily obtainable. The formula must be adjusted as follows if this substitution is made, since medicinal alcohol is usually 70 per cent.

47 cc. water
43 cc. medicinal alcohol (70 per cent)
10 cc. commercial formalin (formaldehyde).

The green color of leaves may be preserved by saturating the above solution with finely divided copper sulfate crystals.

Procedure

1. The leaves of plants should be submerged in the above solution for a period of 10 hours at room temperature (30°C).
2. Remove the leaves and blot dry on absorbent paper or towels.
3. Place the leaves between clean newspaper and press firmly in a regular plant press until dry. (The process of drying can be speeded up by spacing the specimens with corrugated cardboard and placing the entire plant press over some heat source, such as a steam radiator, a light bulb or a warming plate). Do not overheat, if the temperature goes beyond 60°C for too long a period, the leaves will change to brown in color.

Preserving Algae

Specimens collected from the field should be put

in receptacles with just enough water to cover them, leaving ample space for air, especially if the sample is to be stoppered for some time before arriving at the laboratory.

Immediately upon returning from the field, vials or packets of material should be opened and poured into wide, shallow dishes so that they may be well aerated.

Some collectors prefer to spread algae on cards or stiff paper to dry, and then prepare them as herbarium specimens. In working with such specimens later, a few drops of water placed on the dried plants will soak up the material well enough that it can be lifted away for mounting on a slide. Specimens so treated, however, are not satisfactory for study unless one has had a long experience in examining algae and is familiar with their appearance in the undried condition.

If samples are to be preserved an amount of 6-3-1 preservative equal to the volume of the specimen (and its water medium) may be added to the vial. This preservative is composed of six parts water, 3 parts 95 per cent alcohol, and one part commercial formalin. If 5 cc of glycerin are added to each 100 cc of the preservative, a medium is produced which protects the specimen against total loss should the preservative evaporate. Cork-stoppered vials, as a rule, are much more serviceable than screw-cap vials which permit a

greater amount of evaporation of the liquid because the tops loosen upon standing for a time.

Formalin-acetic acid-alcohol (FAA) makes an excellent preservative and is especially suitable if material is later to be prepared for staining.

For general and incidental preserving, ordinary 3 per cent formalin may be used if the above ingredients are not available. (3cc of commercial formalin in 37cc of water).

If semipermanent microscopic mounts are desired, specimens may be placed on a slide, evenly spread out, in a large drop of 5 per cent glycerin. The slide should be set away under a dust-proof cover. Once or twice a day for two or three days other drops of the glycerin solution are added until, through evaporation of the water, approximately 100 per cent glycerin is obtained about the specimen. To this a small drop of melted glycerin jelly is added and the cover slip put in place. Care should be used to add just enough jelly to fill out the area under the cover slip so as not to allow leakage from beneath it. The cover may then be ringed with a sealing material such as balsam, colorless fingernail polish, Bismark Black, or Gold Size.[5]

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[5]G. W. Prescott, How To Know The Fresh-Water Algae, (Dubuque, Iowa, 1954), Wm. C. Brown Company, pp. 15-17.

CHAPTER IV

PRESERVING ZOOLOGICAL SPECIMENS

Preserving animal forms is a larger task than that of plant specimens. However, there is no reason why the teacher or student cannot successfully preserve many specimens obtained on field trips, and the following pages are to suggest simple methods of doing this.

Preserving Protozoa

Sometimes an unusually good or rare culture is discovered and it may be desirable to preserve some of it for future use. In general, however, a living culture is more instructive and should not be entirely replaced by preserved specimens.

Not all forms preserve equally well in all preservatives. A 10 per cent solution of formalin is about as good a universal preservative as any. Another good one is formalin-acetic acid-alcohol (FAA). This acts as a killing and fixing agent and if the protozoa are left in it they will keep indefinitely. The addition of a little glycerin tends to keep the animals from becoming too brittle.

Preserving Parasitic Flatworms and Roundworms

It is usually better to kill all forms of worms before preserving them. Some way of killing them in a relaxed condition is best. The two simplest ways of accomplishing this are as follows:

1. Place them in water and heat it slowly until the worms are limp and motionless. Then drop them into 6 or 8 per cent formalin.
2. A small amount of grain alcohol may be added to some water containing worms. After a few minutes add a similar amount of alcohol and continue until the worms are anesthetized. Then preserve in formalin.

Preserving Segmented Worms

Any annelid may be killed by slowly heating in water or by anesthetizing in alcohol as described above under the preserving of parasitic worms. To preserve large worms so that the internal organs will be in perfect condition and so that the worms will be fully extended, use the following procedure: heat the worms in warm water as in killing or stupefy them with weak alcohol until they become relaxed. Lay the worm on a table and insert a hypodermic needle or fine glass needle through the body cavity. Care should be taken not to strike the needle into the intestinal tract. Be sure that the needle is pointed well anteriorly (forward) before injecting. Slowly force the preservative into the body cavity (6 per cent formalin containing a

little glycerin). Maintain the pressure until the segments are fully extended. Withdraw the needle and insert it again about 10 segments posteriorly to (behind) the first point and inject again. When the entire worm has been injected lay it in a pan and pour over it enough of the preservative to cover it. Let this stand overnight and the worm will be in excellent condition for keeping or dissecting.

Preserving Mollusks

Snails may be killed by heating them in water. If done slowly this often causes them to protrude from their shells. They also may be dropped directly into 6 to 8 per cent formalin. Clams should be placed in warm water until the valves open. A small peg of wood can then be inserted between the valves to allow the formalin to penetrate thoroughly. Most of the other mollusks are usually preserved directly in formalin if desired for class work only.

Preserving Arthropods

Insects are usually collected for one of two purposes. First, the specimens may be desired for laboratory study and dissection, in which case they are usually preserved in formalin or alcohol. Second, specimens intended for display in the permanent insect col-

lection are usually pinned out in the regular manner and are "preserved" only by drying.

Another method of mounting insects for display should be mentioned. Large forms such as moths and butterflies can best be kept in good condition by placing in so-called Riker mounts, which are cardboard boxes with glass tops. The box is filled with cotton, which holds the insect in place and makes a contrasting background. Riker mounts are expensive. A good substitute can be made by taking narrow strips of wood and constructing a frame from them.

In preserving crayfish, first puncture the hard shell over the back with a small knife blade or sharp nail to allow the preservative to penetrate readily into the body. The best preservative is 6 per cent formalin. The isopods and water-fleas may be preserved in 4 per cent formalin or in alcohol.

If spiders are collected and killed by dropping them into 85 per cent alcohol they may be tightly stoppered and kept for any length of time. As suggested above, they also may be killed in the same manner as insects and placed in mounts, either Riker mounts or in cardboard or cellophane mounts.[1]

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[1] David F. Miller and Glenn W. Blaydes, Methods and Materials for Teaching Biological Sciences (New York and London, 1938), McGraw-Hill Book Company Inc., p. 179.

Preserving Sponges

For sponges which are to be used for exhibition, it is enough to immerse them directly in 70 per cent alcohol, renewing it when it becomes discolored. Those sponges which are to serve for study are best immersed in 90 per cent alcohol or in absolute alcohol, which should be renewed after three or four hours, and again after twenty-four to forty-eight hours.

If they are to be dried, they should be washed first in fresh water for a few hours, then should lie for about a day in ordinary alcohol, and then be placed in the air or in the sun.

Preserving Echinoderms

The hard-shelled echinoderms preserve readily in formalin solution which should be changed at least once before they are stored. The sea-cucumber must be anesthetized if the tenacles are to show and the body to be in any way expanded. This can be done with alcohol in water. Among the brittle-stars some species are so fragile that they can scarcely be handled at all without breakage. Others including the serpent-stars, do not break so readily. One method with these species is first to soak them in a 10 per cent formalin 10 per cent glycerin solution and then after ten days dry them in the sun. Specimens thus treated will withstand

considerable careless handling. The sea-urchins can be preserved in formalin or alcohol, preferably the latter. In either case the membrane about the mouth should be punctured in one or two places and the specimens then placed in a tray and the body fluids allowed to drain out. When thoroughly drained they may be placed in an alcohol-formalin solution. The sand dollars yield to formalin-glycerin treatment as described for the serpent-stars, but different species vary widely in their friability. It is best usually to store these specimens in the dry form.

Preserving Fishes

Practically all fish may be preserved in formalin, although specimens intended for the museum will keep in good condition longer if they are preserved in pure grain alcohol. Small fish may be dropped alive into full-strength formalin. These specimens will die with fins and body extended and they may then be preserved in eight per cent formalin or in 85 per cent alcohol. Larger fish must be preserved by injecting preservative into the body cavity.

The problem of preserving some of the beautiful natural colors of fishes is an extremely difficult one and so far no satisfactory preservative has been discovered. Some species (not many, unfortunately) will

retain their natural colors fairly well in a solution of six per cent formalin in which a considerable amount of cane sugar has been dissolved. The necessary percentage of sugar must be determined by experimentation and, in any case, results are rather temporary.[2]

Preserving Amphibians

Any of these animals are large enough that the body should be slightly opened to allow the preservative to enter quickly. A penknife blade stuck into the side is sufficient. Formalin, 7 per cent, is the best preservative. The addition of 15 per cent glycerin will help keep them soft for dissection.

Preserving Reptiles

To preserve turtles, first kill the specimens by drowning (which requires several hours), or by injecting about 10cc. of ether into the body cavity. As soon as the specimen is dead inject 10 per cent formalin into the body cavity, continuing the injection until the head is forced outward and the neck is distended with preservative. If the specimens are large the same preservative should be injected into the muscles of the legs. They may then be placed in the storage solution

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[2]Morris Miller Wells, The Collection and Preservation of Animal Forms (Chicago, 1932), General Biological Supply House, p. 51.

of 8 per cent formalin.

Snakes may be preserved by injecting 10 per cent formalin into the body cavity. If the specimen is a large one the preservative should be injected under the skin of the back and into the muscles of the tail. Harden in 10 per cent formalin and store in formalin of 8 per cent strength. If an injection needle is not present, use a knife to open places in the ventral side of the skin where the preservative may enter rapidly. Snakes may be skinned and the skin dried and preserved with an arsenic salt.

Preserving Birds

Birds, like other animals, may be preserved in formalin of about 7 per cent strength. However, this is satisfactory only for purposes of dissection; so much of the time is resorted to skinning the bird.

To skin the bird relax the legs and wings by pulling and stretching; then make an incision through the skin only from the lower breast bone to the anus. Carefully separate the skin on one side from the subjacent parts until the thigh is exposed and the knee is reached. Taking this leg in one hand push the knee up onto the abdomen and loosen the skin around it until, with scissors or knife, the joint can be separated or cut through. Apply corn meal between the skin and the

carcass to prevent adhesion and to keep the feathers clean and repeat with the other leg. Now loosen the skin at the base of the tail and cut through the vertebra at the last joint, taking care not to sever the bases of the tail quills. Loosen the skin from the rest of the body, peeling it forward in everted position with the raw side out, and when the wing is reached, loosen the skin about the base and cut the wing loose either at the body point or through the middle of the main wing bone. Draw the skin up over the neck until the skull is exposed, detach the delicate ear membrane from its cavity in the skull and loosen the skin, using the thumb nails, up to the eyes where extreme care must be exercised to avoid lacerating the eye-ball when the nictitating membrane is cut. Scoop out the eyes and by making one cut on each side of the head through the small bone connecting the base of the lower jaw with the skull, another across the roof of the mouth behind the base of the upper mandible, a third between the jaws of the lower and a fourth (horizontally) through the skull behind the orbits and parallel to the roof of the mouth, you will have freed the skull from all the accompanying brain and muscle tissue. In making the first two cuts be careful not to injure the bone (zygoma) which connects the bases of upper and lower mandibles on each side. Clean out every particle of muscle and

fat from the head to the very base of the bill. Skin the wing down to the wrist joint, detaching the roots of the larger feathers with the thumb or finger nails; remove the muscle from the bones, leaving all of the latter. Skin the legs to the lower thigh joint and remove all fat and muscle tissue. Remember throughout: (1) Do not stretch the skin, (2) do not soil the plumage, (3) use plenty of corn meal to keep the inner surface dry.

Once the skin is cleaned, apply the arsenic powder thickly to the raw surface, using a brush, a cloth or a rabbits foot. Do not let the skin dry before applying the arsenic. Shake off the excess powder and turn the skin right side out. Now with long forceps push cotton up into neck and wing sockets, down into the legs and into the body until the skin is held plumply in position. (Use gloves when applying poison).

The skin should now be bound lightly with thread to close the opening while the skin is drying. Such a preparation is known as a study skin and should be accompanied by data regarding season taken, locality, age, sex, colors, size and the collector.

Preserving Mammals

If it is wished, the entire body of a mammal may be preserved in the same manner as any other vertebrate.

The abdomen is opened by a knife or scissors just enough to allow the preservative to enter quickly. See that it does enter or the specimen will spoil quickly. Formalin, about 6 or 8 per cent, is the best and cheapest preservative. Always be careful not to overcrowd the preservative by placing too many specimens in one container.

If a skin specimen is desired, select a specimen that is in good condition and has not been torn by a trap or in shooting. Lay the dead animal on its back and make one straight cut in the posterior half of the median ventral surface. Be careful not to cut anything but the skin, and extend the incision to the base of the tail. Lift the edges of this incision and cut away the thin tissue that connects the skin to the muscles of the abdomen. Work the skin loose to one side until the base of the leg is reached. Dry corn meal is used freely between the skin and the flesh to absorb blood and to prevent sticking. Now cut down into the muscles at the hip and separate the bones at the joint. Pull the leg out through the opening in the skin, carefully removing the skin from the leg muscles. When the heel is reached, cut the muscles free from the leg bones. If the animal has much of a pad under its paw it will be necessary to cut through the bottom of the foot and remove the fleshy tissue. Remove the other leg in a similar manner. This leaves the skin from the hind legs

turned inside out with the leg bones protruding from the ends of the skin. Leave these bones attached. Remove the tail in a similar manner being careful not to tear the skin.

With the hind quarters free it is rather easy to pull the skin forward toward the shoulders. Cut whenever necessary, to avoid stretching the skin. At the shoulders proceed with the front legs just as with the hind legs and continue removing the skin until the ears are reached. Cut the ears loose from the skull leaving them in the skin and attached to it. As the eyes are reached unusual care must be exercised in order to get the lids loose from the skull without tearing or cutting them. The same care is necessary for the lips. The cartilage of the nose is cut close to the bone and left attached to the skin. When the skin has been freed from the head it is completely inside out. It should immediately be turned right side out again and any blood on the hair must be washed off at once.

This accomplished, it is again turned wrong side out and all fat, muscle, or other tissue scraped free from the surface. It is now ready to be preserved and poisoned. Use a rubber or leather glove for the handling of the poison which is equal portions of powdered alum and white arsenic. Take a handful of the arsenic alum mixture and scatter it thickly over the raw sur-

face of the hide. Rub this powder thoroughly into the skin. Do not forget the tail or the inside of the paws if they were cut.

The skull is to be returned to the skin so it must be detached and the tongue and jaw muscles cut off. The brain is mashed thoroughly by a wire inserted into the opening at the base of the skull and it may then be shaken out or washed out by a water jet.

The leg bones are now wrapped with thin strips of cotton until they have assumed the same proportions that they had before the muscles were removed. The cotton may be tied on with thread. The clean dry skull is inserted into the skin again and enough cotton added to the cheeks to replace the muscles removed. The skin is next turned right side out. Take a piece of soft iron wire and insert it into the tail. It may be necessary to wrap cloth about the wire to fill out the tail completely. The wire should be long enough to extend part way into the body where it ends in a small loop inside of the body filler which is next inserted. Make a bundle of cotton of as nearly the same shape and size as the trunk and neck as possible. Insert this into the skin through the slit and adjust. Do not attempt to stuff the body with small pieces, but bind them into a single form and tie with a thread. When the legs and head are all arranged properly sew the slit shut neatly. Lay the

animal ventral side down on a board and leave it to dry.

Embalming

When animals larger than a rat or guinea pig are to be preserved, especially if they are to be used for careful dissection they are best embalmed. This method requires freshly killed specimens, however, and it is well to remember that little time should elapse between the death of the animal and the embalming process.

The animal is slowly anesthetized until it is thoroughly limp. Proceed immediately to the work of embalming, stretching the legs out and the head up on the spreading board while the animal is limp. Cut the skin on the inner surface of the thigh and separate the muscle bundles carefully to expose the femoral artery along the bone, taking care to get the artery and not the vein. Expose the artery for an inch or two. Get the injection needle and syringe ready and then tie a thread loosely around the artery making a slip-knot for tightening later. Insert the needle into the artery pointing it toward the body. Some workers prefer to cut carefully through the wall of the thorax and inject directly into the aorta just above the heart. Apply the pressure on the syringe very slowly and steadily and continue it until the body assumes a swollen or turgid

appearance. Be sure to tie the thread tightly as soon as the needle is withdrawn.

A satisfactory embalming fluid which will preserve, and yet keep the muscles pliable is made as follows:[3]

Formalin (3 per cent).....880cc.
Glycerin.....100cc.
Carbolic acid (melted crystals).....20cc.

If heavy cloth is saturated with the solution and wrapped around the embalmed animal and the specimen kept in a closed can to prevent drying, it will keep indefinitely.

Preparing Skeletons

A specimen is selected that is large and perfectly formed and old enough so that the bones are well hardened and the teeth fully developed.

The fleshing of an animal is one of the most important operations in preparing a skeleton. Considerable care must always be taken not to cut through connecting ligaments which hold the bones in position. On the other hand, the flesh must be removed very thoroughly, for if too much muscle or flesh is left on the skeleton, maceration (decomposition) takes place too fast and in a very short time the bones will all become separated. First, the clavicles should be dissected out. These

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[3]David F. Miller and Glenn W. Blaydes, Methods and Materials for Teaching Biological Sciences (New York and London, 1938), McGraw-Hill Book Company Inc., p. 188.

small bones, when present, can always be located between the coracoid process and the episternum, but often do not articulate with either of these points. Next, dismember the four limbs and remove the skull by carefully cutting between the atlas and condyles. The next difficult part is to dissect out the hyoid or tongue bones. This group of nine bones, which lie in the muscles of the throat, should be dissected out intact. Now flesh the limbs, trunk, tail, skull, hyoid and clavicles. In working on the trunk take great care not to cut through the costal cartilages of the sternum. Pass a stiff piece of wire (12 or 13 gauge), 18 inches long, through the vertebrae, moving the wire back and forth to clean out the spinal cord. Next clean the skull, removing the brain with a brain spoon or similar instrument. The skeleton is now fleshed and ready for maceration.

Only fresh material should be used for making skeletons. Do not attempt to use specimens that have been preserved in formalin.

After the fleshing is completed examine the bones carefully to be sure that all of the natural cartilages and ligaments have been left intact, as they are essential to the proper setting of the bones.

Place the bones in a stone or earthenware container of suitable size and cover with water. (Never use acids or chemicals of any kind). Do not allow any foreign

substance such as metal, wood, and other materials to get into the maceration jar, as these will discolor the bones. The maceration jar should be kept in a warm room where an average temperature of about 78°F. can be maintained. At this temperature the maceration period is usually from six to eight days. Toward the end of this time the bones must be watched carefully, as the maceration of the flesh should be halted just in time to save the natural ligaments. When the flesh "jells" and sloughs off easily, remove the bones from the maceration bath and rinse under running water, going over them with a stiff brush to remove all remaining pieces of flesh. If the maceration has been timed correctly, the flesh should all wash off easily, yet the ligaments should remain. To make sure that all maceration has ceased, place the bones in a weak solution of caustic soda (about ½ ounce sodium hydroxide to a gallon of water) for one to two hours. After removal, wash in running water.

If the natural ligaments remain, the small bones are held together, while if the natural ligaments are destroyed it will be necessary to wire all of the small bones together, which usually results in a very unsatisfactory skeleton.

After all flesh has been removed and the bones thoroughly washed they are held in their natural posi-

tions for drying by running a stiff pointed wire through the vertebrae, forcing the point of the wire well into the sacrum.

Having accomplished this, bend the vertebral column into its natural shape. While the skeleton is still wet, space the ribs equally by tying and stringing them with thread. The limbs are dried in shape by pinning on a flat soft piece of wood or cork. After drying, should the bones still be greasy, place them in carbon tetrachloride for 6-8 hours. Then, on removal, plunge them into hot (not boiling) water for about 5 minutes, adding a little household ammonia, which will help to bring the grease to the surface. If it is desired to have a perfectly white skeleton, remove the bones from the water and place them in a 10 per cent solution of hydrogen peroxide for four hours. Hot chloride of lime is also a good bleaching agent but tends to destroy ligaments. Use it only on the large disarticulated preparations.

Mounting is largely a matter of experience and skill. Anyone with a little mechanical ability can work out methods of mounting on stiff wires or (in the case of large skeletons) on metal rods and tubes. In general, it is wise to enclose small skeletons in glass cases, as this protects them from dust and careless handling.

How to Prepare Plastic Corrosion Preparations

The process of making plastic corrosion preparations is surprisingly simple if one has had any experience in the injection of blood vessels. It involves only the forcing of colored plastic into the arteries and veins with a syringe of the type used by veterinarians.

The plastic used is vinylite which is especially prepared for injection purposes. It comes in three colors, red, blue and yellow and can be purchased from Ward's for $2.50 per pint. A 10-cc syringe is the best size to use and preferably it should be one with a rubber plunger. The size of the canula (needle) varies with the size of the bloodvessels to be injected. An 18 gauge will be found most useful although it may be necessary to go to the 19 gauge size for small vessels.

Before injecting the plastic, be sure the syringe and needle are perfectly dry inside and out, for if water comes in contact with the plastic the needle will clog. Fill the syringe with the plastic, slip on the canula, insert it into the bloodvessel and inject until the vessels are filled. Red Vinylite should be injected into the arteries, blue into the systemic veins and yellow into the hepatic portal.

The plastic hardens very quickly after injection

and no special treatment is necessary to accomplish setting. The soft tissue can be removed by cacterial maceration at 90°F. When the tissue is completely softened it can be washed away under a jet of water.

When the cast of the circulatory system is entirely free of soft tissue and dried, it may be mounted in a glass-topped case or a double-view mount, or it may be attached to a glass plate and mounted in a glass jar containing 5 per cent formaldehyde. It is not absolutely necessary to mount the cast since it possesses enough strength and rigidity so that it is not easily broken.

The following materials required for the making of plastic corrosion preparations are available from Ward's.*

Vinylite-Red, blue or green (Available only in pint cans)		$2.50 per pint
Injection syringe-10-CC capacity		$2.75
Injection Canula-18-gauge	each	$ .30

*WARD'S NATURAL SCIENCE ESTABLISHMENT, INC., P.O. Box 24, Beechwood Station, Rochester 9, New York.

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