XVIII.—ON THE PRESERVATION OF EMBRYONIC MATERIALS AND SMALL ORGANISMS, TOGETHER WITH HINTS UPON EMBEDDING AND MOUNTING SECTIONS SERIALLY.

By John A. Ryder.

The question frequently asked of the investigator is, "How shall I preserve the materials I may collect for you?" This is a very important question, because, unless eggs, embryos, or small portions of animals which are to be studied microscopically by means of sections are properly prepared to begin with, it is often impossible for the embryologist or histologist to get satisfactory results. And from personal experience I may remark that there is nothing more mortifying to the student than to find a rare and valuable, or perhaps unique, specimen ruined beyond repair by the preliminary "preparation" it has suffered at the hands of persons either ignorant of proper preservative methods or indifferent in regard to their application. Alcohol, so universally used as a preservative, can be made to yield splendid results if properly employed, but if applied without discrimination or an understanding of the end in view, it may utterly ruin otherwise valuable materials if it is desired to use them in the prosecution of delicate anatomical or histological investigations. The shrunken, distorted, alcoholic specimens too often seen in our museums are a reproach to science, and it is high time that more care was exercised in the preparation of such objects, as it would not only be to the advantage of the systematist, but also to the anatomist and histologist, to say nothing of the better appearance which would be presented by such properly prepared collections of zoological materials.

The directions which follow are meant to be useful to those seeking information as to the proper mode of preparation to be applied to such delicate objects as embryos or small soft organisms which are to be transmitted to the National Museum in the best possible condition. Some suggestions under the heading of alcohol are also intended to apply to the care and preservation of large objects.

Vessels.—As receptacles for specimens, vials, jars, or bottles with conveniently wide mouths should be selected. Their form is of small consequence, provided they are sufficiently large to accommodate the object without distorting it, and with a mouth wide enough to admit of the removal of the specimen without injury after it has become indurated by

the preservative agent. They should also be capable of being closed securely so as to guard against leakage when packed for shipment, and also to prevent evaporation when stored away in the cabinet. Rubber corks are probably the best for small vials in which it is designed to preserve small embryos or small organisms.

Crowding a large number of small specimens into one vial is wrong, and abundant space should left besides the specimens for accommodation of a sufficient amount of the hardening reagent or the preservative fluid which may be used, otherwise it is not possible in most cases to harden the specimens uniformly and quickly, because there is not enough of the reagent around the specimens. Delicate embryos are often distorted in the process of killing and hardening, so as to be bent and twisted, which makes it difficult to get the axis of their bodies into a single plane, which is very desirable, especially if it is intended to prepare serial sections with the newer forms of the sledge microtome. In order to avoid such distortion as much as possible, if embryos or other small animals are killed and hardened immediately in the vials, it is best to cork the latter and lay them on their sides so that the objects, especially if slender, do not rest in a mass on the bottom, but on the undermost side of the vials. These precautions should be borne in mind in handling recently hatched fish embryos, the tails of the latter being especially liable to become bent and distorted by the weight of those overlying those on the bottom of the vial if the latter is placed upright while the process of hardening is in progress.

Packing and labeling.—It is of the utmost importance that small, delicate objects should be carefully packed in the bottles when it is proposed to ship them, so that they may not be shaken about, especially if moderately large; unless this is done important portions may be broken off and lost after the specimens have been hardened by the preservative. To avoid this, the specimen, if large, should be wrapped in pieces of cheese cloth, which may be secured around the object with string. If the objects are small wrapping them carefully in soft tissue paper will be found expedient, or if the bottle should not be full, soft tissue paper crushed into springy masses may be used to fill up the vacant space in the bottles, care being taken that the packing of paper is not forced in too tightly so as to injure the specimens. Paper is better for this purpose than cotton wool, which, in the case of specimens which have hooked teeth or processes projecting from the body frequently becomes entangled with such processes as to cause them to be torn off when the specimens are unpacked. Finally, it is a good rule in packing to fill the vials full of the preservative fluid, which keeps the specimens immersed and also prevents injurious shaking when shipped.

Not less important than the packing is the proper labeling of the specimens. Every vial, if containing only a single specimen or a single series, should be labeled with the date of collection, the locality, and the name of the collector. If a number are sent in the same vessel, each

distinct specimen or distinct series should be wrapped separately and have a label securely attached, giving the date of collection, locality, &c. In the case of embryos, care should be taken to record the age of the different series where this is known, and in the cases of fishes, amphibians, and some articulates, this may be known very exactly. To prepare a series of embryos of fishes, for example, and especially where their eggs are artificially hatched in some kind of hatching apparatus, it is very easy to prepare a series of specimens at intervals of, say, every twelve to twenty-four hours, and to place the separate lots of different ages in different vials, so that the investigator may use the material so prepared in a very complete study of the development of the form. In the preparation of such series it is important to give the date, and, if possible, the hour of the day when the eggs were impregnated, and to indicate upon a label within or pasted on the outside of the bottle, the exact age of the contents. The locality and collector's name should also be given. Labels placed within the vials so as to be immersed in the preservative agent should not be written in ordinary ink, but with a soft lead-pencil, as common writing ink is liable to become effaced from the paper by the solvent action of the preservative fluid. India ink, according to Semper, when dissolved in strong acetic acid, makes a black marking fluid which will remain perfectly black and legible in alcohol for years.

PRESERVATIVE AGENTS.

All of the best preservative agents tend to harden animal tissues.

Alcohol.—When alcohol of 95 per cent. is used it should almost always be diluted. One of the few cases in which 95 per cent. or absolute alcohol may be used to advantage is in the preservation of sponges, as I am informed by Dr. Benj. Sharp. These may be immersed in the very strongest alcohol as soon as they are removed from the water, a recent investigator having found that for the study of their minute structure this was the best preservative medium, after the unsatisfactory trial of a great many. The collared flagellate cells lining the respiratory and digestive cavities of these organisms are thus best preserved.

In almost all cases, however, the use of very strong alcohol is followed by more or less extensive and injurious shrinkage of the object, especially if it is very soft and watery, as in the cases of embryos, polyps, and mollusks, more particularly. In these cases the mixture first used should consist of alcohol, 1 part; water, 10 parts.

If the object is small or of moderate size it may be left in the above twenty-four hours, then transferred to a mixture of alcohol, 1 part; water, 3 parts, or to a mixture of alcohol, 1 part; water, 2 parts, according to the consistency of the object, and in which it may remain for two or three days and then be transferred to alcohol, 2 parts; water, 1 part, and, after a day or two, into 95 per cent. alcohol, if the specimen is intended for histological purposes.

With care and attention an organism as soft as the oyster may be hardened and preserved in common alcohol by gradually increasing the strength of the preservative solution, so that there is little or no perceptible shrinkage. The cause of shrinkage is the removal of the water of organization by the alcohol, and, in the case of badly prepared specimens, it will usually be found that the first bath of alcohol into which the object was plunged was too strong and had caused the withdrawal of the water of organization much too rapidly and produced an excessive shrinkage, much to the injury of the specimen. Some investigators recommend very strong alcohol for the preservation of brains, which it is desirable to harden as rapidly as possible and without distortion. In the case of such soft objects, especially if they are of considerable size, they should be suspended in the preservative fluid by a string attached to the cork, in order that the specimen's own weight may not distort it while the process of hardening is in progress, the distortion being aggravated by any curved or uneven surface upon which such a soft object may rest.

In the case of comparatively large objects, such as mammals and fishes, the body cavity should be filled with 40 per cent. alcohol by means of a syringe, so as to enable the preservative to act from within as well as from without. In the case of fishes, which are usually thickly covered with slime, especially such species as the eels, hags, and lampreys, the slimy coating should always be washed off before immersion in alcohol.

Dr. Whitman*, speaking of the use of alcohol says: "In the preparation of animals or parts of animals for museums or histological study, it is well known that the chief difficulties are met with in the process of killing. Alcohol, as commonly used for this purpose by collectors, has little more than its convenience to recommend it. Dr. Mayer has called attention to the following disadvantages attending its use in the care of marine animals:

"(1.) In thick-walled animals, particularly those provided with chitinous envelopes, alcohol causes a more or less strong maceration of the internal parts, which often ends in putrefaction.

"(2.) In the case of smaller crustacea *e.g.* Amphipods and Isopods, it gives rise to precipitates in the body fluids, and thus solders the organs together in such a manner as often to defy separation even by experienced hands.

"(3.) It fixes most of the salts of the water adhering to the surface of marine animals, and thus a crust is formed which prevents the penetration of the fluid to the interior.†

* Methods of Microscopical Research in the Zoological Station in Naples, Am. Nat. XIV, pp. 697–706 and 772–785, 1882.

† Dr. Mayer first noticed this in objects stained with Kleinenberg's hæmatoxylin, and afterwards in the use of cochineal, where a gray-green precipitate is sometimes produced which renders the preparation worthless. Such results may be avoided by first soaking the objects a few hours in *acid alcohol* (one to ten parts hydrochloric acid to one hundred parts seventy per cent. alcohol.)

"(4.) This crust also prevents the action of staining fluids, except aqueous solutions of the latter, by which it would be dissolved.

"Notwithstanding these drawbacks, alcohol is still regarded at the Naples Aquarium as an excellent fluid for *killing* many animals designed for preservation in museums or for histological work. In many cases the unsatisfactory results obtained are to be attributed not to the alcohol *per se*, but to the *method* of using it. Most of the foregoing objections do not, as Dr. Mayer has expressly stated, apply to fresh-water animals; and Dr. Eisig informs me that he has no better method of killing marine annelids than with alcohol. Judging from the preparations which were kindly shown to me, and which were all beautifully stained with *borax-carmine*, Dr. Eisig's mode of treatment must be pronounced very successful. The process is extremely simple: a few drops of alcohol are put into a vessel which contains the annelid in its native element, the sea water; this is repeated at short intervals until death ensues. After the animal has been thus slowly killed, it may be passed through the different grades of alcohol in the ordinary way (as described above), or through other preservative fluids. Objects killed in this manner show no trace of the external crust of precipitates which arises where stronger grades of alcohol are first used. The action of the alcohol is thus moderated, and the animal, dying slowly, remains extended and in such a supple condition that it can easily be placed in any desired position. The violent shock given to animals when thrown alive into alcohol of forty to sixty per cent, giving rise to wrinkles, folds, and distortions of every kind is thus avoided, together with its bad effects."*

Acid alcohol.—Dr. Whitman also says, "In order to avoid the bad effects of alcohol, such as precipitates, maceration, etc., Dr. Mayer recommends *acid alcohol*, 95 volumes, 70 per cent.; or, 90 per cent. alcohol, 3 volumes hydrochloric acid,† for larger objects, particularly if they are designed for preservation in museums. The fluid should be frequently shaken up, and the object only allowed to remain until thoroughly saturated, then transferred to pure 70 per cent. or 90 per cent. alcohol, which should be changed a few times in order to remove all traces of the acid. For small and tender objects, acid alcohol, although preferable to pure alcohol, gives less satisfactory results than picro-sulphuric acid."

"*Boiling alcohol.*—In some cases among the arthropods, Dr. Mayer has found it difficult to kill *immediately* by any of the ordinary means,

* [Embryo fishes may be killed without distortion by exposure to the fumes of chloroform. The embryos are placed in a watch-glass in water, or in a ring on the plate of a compressorium, when a few drops of chloroform are placed alongside of the watch-glass or ring filled with the water containing the embryos, and the whole covered with a larger watch-glass or bell-glass, and left a few minutes till the embryos are killed in their fully extended condition. The interesting collembolan, *Smynthurus*, may be killed in chloroform or ether with its remarkable bifurcate collophore fully extruded.]

† Acid alcohol as above prepared loses its original qualities after standing some time, as ether compounds are gradually formed at the expense of the acid.

and for such cases recommends *boiling absolute alcohol*, which kills instantly. For *tracheata* (insects) this is often the only means by which the dermal tissues can be well preserved, as cold alcohol penetrates too slowly."

It will be profitable to lay down the following general principles respecting the use of alcohol:

(1) *In almost all cases where soft tissues or objects are to be prepared, either for museum or histological purposes, it is best to at first immerse the object in weak alcohol, and to gradually increase its strength so as to extract the water of organization by degrees, and thus avoid the injurious shrinkage and distortion due to the use of too strong a mixture.*

(2) Sometimes it is desired to preserve a very large, soft object in alcohol, but, at ordinary temperatures, decomposition often begins in the center of the object before the alcohol has had time to completely saturate the object. Decomposition of this sort may be hindered and even prevented by placing the vessel containing the object in moderately strong alcohol in a refrigerator kept at a little above freezing. This prevents decomposition, and gives the alcohol time to saturate the object. Any other hardening agent may be applied in the same way to large objects, but in such cases care must be taken that the solution is not frozen during the process. Solutions of Müller's fluid or chromic acid may be frozen; alcoholic solutions not so readily, unless they are very dilute.

We have considered *alcohol* first as a *preservative*, *killing*, and *hardening* agent, because it is the best known and most generally used and has not yet been superseded by any of the many compounds which have been suggested as substitutes, mainly on the score of their cheapness.

Muller's fluid.—For convenience this compound probably ranks next in value to alcohol, as a temporary preservative agent and as a hardening fluid. The formula for its preparation is as follows:

Bichromate of potash	5 parts.
Sodic sulphate	2 "
Distilled water	200 "

This makes an orange-colored solution in which embryos, small animals, and pieces of tissue may be preserved for histological purposes. And it possesses one important advantage over chromic acid in this, that the object may be left in it for a month without injury, although it darkens albuminous substances permanently like chromic acid and may be objected to by some on this account. Nevertheless, it is a very convenient preservative compound in cases where the care and attention required in the use of better reagents cannot be applied to the preservation of such materials on account of their numbers or when the collector is traveling, with only occasional opportunities for overhauling and caring for his collections.

A convenient way for the traveling collector to carry the "stock" for this mixture is to have the solid ingredients put up in bulk by an apothecary. The bichromate of potash should be pulverized in a mortar, so as to dissolve the more readily, and mixed with sodic sulphate in the proportion of 5 parts to 2 of the latter. This mass can then be weighed out into parcels of 1 ounce, 1 dram, 1 scruple, which is exactly the quantity of solid material needed to mix with 1 quart of water (wine measure). These parcels containing the dry powder ready mixed can then be carried conveniently and the fluid mixed, to the amount of a quart at a time, whenever required.

After the objects have been kept in this solution for a month or so, they should be transferred to about forty times their own volume of water and the water changed every day for two or three days, in order to get rid of the bichromate of potash, when the specimens may be transferred to 70 per cent. alcohol. It has been objected that Muller's fluid produces precipitates in the cavities of objects, but there are few preservatives which do not, and on account of the convenience with which it may be used by collectors, it is, next to alcohol, probably, one of the most useful of all hardening and preservative compounds.

Chromic acid.—Solutions of this substance have been extensively used for the *hardening* of embryological and histological materials, and either alone, or, better still, in combination with other substances, is still recognized as one of the most useful reagents for this purpose. Whitman, speaking of it, says: "Chromic solutions have, in common with *osmic acid*, the peculiarity of hardening by virtue of the chemical combinations which they form with cell-substances, and all the consequent disadvantages with respect to staining. The use of chromic acid in the zoological station of Naples may be said to have been largely superseded by *picro-sulphuric acid*, *corrosive sublimate*, and *Merkel's fluid*, for it is now seldom used except in combination with other fluids. It is sometimes mixed with Kleinenberg's fluid, for example, when a higher degree of hardening is required than can be obtained by the use of the latter fluid alone. It is a common error to use too strong solutions of chromic acid, and to allow them to act too long. Good results are in some cases obtained when the objects are treated with a weak solution (one-third to one-half of 1 per cent.) and removed soon after they are completely dead." Weak solutions of one-half to one-fourth of 1 per cent., or even less, are also recommended by Semper, who allows it to act only for a short time, or until the cells are killed. But it is important in any case that as much as possible of the acid should be extracted by subsequent immersion of specimens treated with it in water or weak alcohol, since its presence often renders subsequent staining with carmine difficult. It interferes with the staining by means of the aniline dyes much less perceptibly, and in the case of some of those most diffusible and soluble in alcohol or water scarcely at all. Flemming uses exceedingly dilute solutions of chromic acid in order to fix the cleavage

figures and asters developed in eggs during their early stages of development. For this purpose he uses solutions of chromic acid of one-tenth of 1 per cent. to one-half of 1 per cent.

Dr. Whitman has recently discussed its unsuitableness when used alone in the treatment of pelagic fish-eggs in the American Naturalist for November, 1883. He writes: "The transparent eggs of various *Teleostei* floating on the surface of the sea present unusual difficulties in the way of hardening. I have had recourse to all the fluids commonly used for this purpose, and have failed to find any satisfactory method of hardening the yelk. Even the germinal disk cannot be well preserved by any of the ordinary hardening agents. Kleinenberg's picro-sulphuric acid, for instance, causes the cells all through the cleavage stages as well as the later embryonic stages to swell, and in many cases to become completely disorganized. The embryonic stages can be hardened in chromic acid (1 per cent.), but the yelk contracts considerably without becoming well hardened even after three days' immersion.

"All sorts of wrinkles and distortions are caused when the ova are transferred from the acid to the alcohol; my best results have been obtained with osmic acid and a modified form of Merkel's fluid. This fluid, as used by Dr. Eisig, consists of chromic acid (one-fourth per cent.) and platinum chloride (one-fourth per cent.), mixed in equal parts. Thus prepared it causes maceration of the embryonic portion of the egg. By using a stronger chromic acid (1 per cent.), and combining it as before with the same quantity of platinum chloride (one-fourth per cent.), everything may be well preserved and hardened except the yelk. Before transferring to alcohol, after one to two days' immersion in this fluid, it is necessary to prick the egg membrane in order that the alcohol may reach the egg readily, otherwise the membrane wrinkles badly and often injures the embryo.

"For the cleavage stages this fluid cannot be used with success unless the egg has been first killed by another agent; for eggs placed in this fluid continue to live for a considerable time, and may even pass through one or two stages of cleavage. It is therefore necessary to use some agent that kills almost instantly. For this purpose, I have found osmic acid the best reagent. The eggs are placed in a watch-glass with a few drops of sea-water, and then a quantity of osmic acid (½ per cent.), equal to that of the sea-water, is added. After five to ten minutes the eggs are transferred to the mixture of chromic acid and platinum chloride, and left for twenty-four hours or more. This fluid not only arrests the process of blackening, but actually bleaches the egg.

"After this treatment it is an easy matter to separate the blastoderm from the yelk by needles, and the preparations thus obtained can be mounted *in toto* or sectioned. As the blastoderm is quite thin during the cleavage stages, a whole series of these stages may be mounted and studied from the surface to advantage. After removal from the acid the

preparations may be stained at once, and then treated with alcohol and mounted in balsam."

The following directions for the preparation of the tongues of *mammalia*, for histological purposes, have been furnished by Mr. Edward B. Poulton, of Oxford, England, in a letter to Prof. G. B. Goode, from which the following extract is taken:

"It is well to cut out the organ, including the epiglottis. In the case of rare animals that cannot be obtained fresh, a tongue preserved in spirit is of great use, but is not so good as a chromic acid one of the fresh organ. The best method of preparation seems to be the following: Suspend the perfectly fresh tongue by a string in ½ per cent. solution of chromic acid,* about a quart, to which one-half pint of methylated † spirit has been added. (Three or four small tongues might be put in together; a very large tongue would need more.) Leave in this solution for a week, and then change it, and after another week place the organ in a pint of solution consisting of two parts water, one part methylated spirit or alcohol, and after a few days or a week place in a pint of fluid consisting of one-half water and one-half spirit; and then, after a week, in one pint of liquid consisting of two parts spirit and one part water, and then in strong spirit, which need not be so much as one pint. In this a larger specimen will keep any length of time, and is always ready for histological work. Some tongues which I worked at, given me by Professor Mosely, were in beautiful condition, and had been hardened nine years before. The first washes of spirit can be used many times for other tongues, but it is best to have a series of labeled jars with the various strengths of spirit (alcohol) in them, and pass the tongues from one into the other, from the lowest to the highest grade of spirit. This saves expense, even though it may call for an extra change of strong spirit at the end. The tongues should finally not cause the fluid to become yellow, but the first washes may be yellow (from the dissolved chromic acid). The tongues can then be packed together in one jar in plenty of strong spirit, each with a label tied to it, giving, if possible, the specific and generic names and date. Thus many can be sent together. If you ever get them, I should be very glad of spirit or fresh tongues (prepared in chromic acid) of any Edentate, Marmoset, and any South American monkey, tapir, peccary, or *Solenodon*. The chromic acid must not be used again." This last remark applies in all cases to the use of chromic acid.

Merkel's fluid.—Whitman gives the formula for the preparation of this as follows:

Platinum chloride dissolved in water	1:400
Chromic acid dissolved in water	1:400

"Professor Merkel, who employed a mixture of these two solutions in equal parts for the *retina*, states that he allowed from three to four days

* If it cannot be obtained fresh, it is still of great use to try the chromic acid up to some days after death, but not in the case of a regular spirit specimen.

† Alcohol will answer the same purpose.

for the action of the fluid. Dr. Eisig has used this fluid with great success in preparing the delicate lateral organs of the *Capitellidæ* for sections, and recommends it strongly for other annelids. Dr. Eisig allows objects to remain three to five hours in the fluid, then transfers to 70 per cent. alcohol. With small leeches I have found one hour quite sufficient, and transfer to 50 per cent. alcohol."

Whitman gives the following account of Kleinenberg's picro-sulphuric acid, now so much used in the Naples Aquarium. It is not a hardening fluid, and serves for *killing*, and thus prepares for subsequent hardening.

Perenyi's fluid.—This recently introduced hardening agent is compounded as follows:

Nitric acid (10 %)	4 parts.
Alcohol (90 %)	3 "
Chromic acid (¼ %)	3 "

Objects are left in this fluid from four to five hours, then transferred for twenty-four hours to 70 per cent. alcohol; then to 90 per cent. alcohol; and finally to absolute alcohol, in which they remain for four to five days.

Picro-carmine or borax-carmine are added directly to the fluid, so that the hardening and staining of the objects take place simultaneously. The precipitates which are produced when the reagent is mixed with the coloring solutions should be removed by filtration before the objects to be hardened are introduced. Eggs and embryos prepared in this mixture are said to cut like cartilage.

"*Kleinenberg's fluid.—Picric acid* (saturated solution in distilled water), 100 volumes; *sulphuric acid* (concentrated), 2 volumes. Filter the mixture and dilute it with *three* times its bulk of water.* Finally add as much creosote† as will mix.‡

"Objects are left in the fluid three, four, or more hours, and are then, in order to harden and remove the acid, transferred to 70 per cent. alcohol, where they may remain five to six hours. They are next placed in 90 per cent. alcohol, which must be changed at intervals until the yellow tint has wholly disappeared.

"*Summary of Dr. Mayer's remarks on Kleinenberg's fluid.*—The advantages of this fluid are, that it kills quickly, by taking the place of the water of the tissues; that it frees the object from sea water, and the salts contained in it, and that having done its work *it may be wholly replaced by alcohol.* In this latter fact lies the superiority of the fluid over *osmic* and *chromic* solutions, all of which produce inorganic precipitates, and thus leave the tissues in a condition unfavorable to staining. Picro-

* Dr. Mayer uses the fluid undiluted for arthropoda.

† Creosote made from beechwood tar.

‡ Dr. Mayer prepares the fluid as follows: Distilled water, 100 volumes; sulphuric acid, 2 volumes; picric acid (as much as will dissolve). Filter and dilute as above. No creosote is used.

sulphuric acid does not, like chromic solutions, harden the object, but simply kills the cells.

"As this fluid penetrates thick *chitine* with difficulty, it is necessary, in order to obtain good preparations of larger *Isopoda*, insects, &c., to cut open the body and fill the body-cavity with the liquid by means of a pipette. In larger objects care should be taken to loosen the internal organs so that the fluid may find easy access to all parts.

"The fluid should be applied as soon as the body is opened, so that the blood may not have time to coagulate and thus bind the organs together. *A large quantity of the fluid should be used, and it must be changed as often as it becomes turbid.* The same rule holds good in the use of all preservative fluids. It is well, also, especially with larger objects, to give the fluid an occasional stirring up.

"In order to avoid shrinkage in removing small and tender objects from the acid to the alcohol, it is advisable to take them up by means of a pipette or spatula, so that a few drops of the acid may be transferred along with them. The objects sinking quickly to the bottom, remain thus for a short time in the medium with which they are saturated, and are not brought so suddenly into contact with the alcohol. In a few minutes the diffusion is finished; and they may then be placed in a fresh quantity of alcohol, which must be shaken up frequently, and renewed from time to time until the acid has been entirely removed.

"The sulphuric acid contained in this fluid causes *connective tissue* to swell, and this fact should be borne in mind in its use with vertebrates. To avoid this difficulty, Kleinenberg has recommended the addition of a few drops of creosote, made from beechwood tar, to the acid. According to Dr. Mayer's experience, however, the addition of creosote makes no perceptible difference in the action of the fluid.

"This fluid must not be used with objects (*e. g.*, echinoderms) possessing calcareous parts which it is desired to preserve, for it dissolves carbonate of lime and throws it down as crystals of gypsum in the tissues. For such objects *picro-nitric acid* may be used. It is prepared as follows:

Water	95 parts.
Nitric acid (25 per cent. $N_2 O_5$)	5 "
Picric acid as much as will dissolve.*	

"Picro-nitric acid also dissolves carbonate of lime, but it holds it in solution, and thus the formation of crystals of gypsum is avoided. If much carbonate of lime is present, the rapid production of carbonic acid (gas) is liable to result in mechanical injury of the tissues, hence, in many cases, *chromic acid* is preferable to picro-nitric acid.

"Picro-nitric acid is, in most respects, an excellent preservative medium, and, as a rule, will be found to be a good alternative in those cases where picro-sulphuric acid fails to give satisfactory results. Dr. Mayer commends it very strongly, and states that with eggs contain-

* This mixture is used undiluted.

ing a large amount of yolk material, like those of *Palinurus*, it gives better results than nitric, picric, or picro-sulphuric acid. It is not so readily removed from objects as picro sulphuric acid, and for this reason the latter acid would be used wherever it gives equally good preparations."

This fluid is also said to be useful where the objects, such as arthropods, are inclosed in a chitinous skin.

For the preservation of infusoria, Blanc recommends a very dilute picro-sulphuric solution, which I give here as abstracted from the Zoölogischer Anzeiger, No. 129, by Whitman. The formula is as follows:

Picric acid, saturated solution in distilled water	100 volumes.
Sulphuric acid (concentrated)	2 "
Distilled water	600 "

"To this solution, which may be employed as it is for the larvæ of echinoderms, medusæ, and sponges, a little acetic acid (1 per cent.) is added for rhizopods and infusoria—two or three drops for 15 grams of the solution. The acetic acid is added in order to sharpen the outlines of the nuclei and nucleoli.

"This liquid is preferable to osmic acid, because it does not render the objects non-receptive to staining fluids.

"The entire process of hardening, washing, staining, and mounting can be more expeditiously performed under the cover glass than otherwise. The acid is allowed to work until the objects have become thoroughly yellow. The acid is then replaced by 80 per cent. alcohol, frequently renewed until the yellow color entirely disappears; 96 per cent. alcohol is next used, and then absolute alcohol.

"The hardened objects may be stained with picro-carmine, or, better, with an alcoholic solution of safranin. Five grams of safranin are dissolved in fifteen grams of absolute alcohol, the solution left standing a few days, then filtered and diluted with half its volume of distilled water.

"This solution of safranin is preferable to picro-carmine, because it colors more quickly, and because one can so regulate its action as to give a sharp definition to the protoplasm or the nucleus.

"After the object has been more or less deeply stained, according to the end in view, it is washed in 80 per cent. alcohol, which is renewed until a moment arrives when no visible clouds of color appear; at this moment the 80 per cent. alcohol is replaced with absolute alcohol, and this by clove oil.

"As safranin is soluble in alcohol, the process of washing will of course remove or weaken the color, but decoloration is gradual, so that one needs only to watch and apply the clove oil when the color has been reduced to the desired intensity. * * *

"The process of decoloration is not entirely arrested by the application of clove oil, contrary to Blanc's assertion, hence it should be replaced

by Canada balsam as early as possible. The same method is adapted to other microscopic animals."

In order to confine infusorians or very small embryos, so as to subject them to the foregoing treatment under a cover glass, a method which has given very good satisfaction is the following: Three very small pellets of bees-wax or bees-wax and olive oil are stuck to the one side of a cover near its margin, and arranged so as to form the angles of a triangle. The drop containing the organisms is then placed on the slide and the cover with its wax feet carefully laid upon it. A needle is then taken and its point pressed down upon the cover glass until it is forced downwards on its yielding supports and until its under surface is sufficiently approximated to the upper surface of the slide to clamp the living infusorians or embryos fast which have been placed between without actual compression. The application of the reagents may then be leisurely proceeded with without washing the objects away, as one reagent after the other is applied at one side of the cover and absorbed on the other by bits of bibulous paper.

Hertwig's method of preparing and cutting amphibian eggs.—Whitman has given the following condensed account of Hertwig's plan of dealing with amphibian ova, which would doubtless give advantageous results if applied to the treatment of fish ova which contain coarse yolk granules, which are apt to become detached from the sections in the process of mounting when the whole embryo has first been saturated with paraffine, which must be removed from the sections with warm turpentine or chloroform, or with cold benzole or xylol. The two first are the solvents which act most quickly when used for the purpose of removing the paraffine:

"Although the amphibian egg has long been a favorite object of study among embryologists—and quite as much so since section-cutting came into vogue as before—comparatively little progress has been made in overcoming the difficulties that attend its preparation for the microtome. The chief difficulties are found in freeing the egg from its gelatinous envelope, and in preparing it so as to avoid brittleness.

"The best method that has thus far been proposed for these eggs is unquestionably that of O. Hertwig, and I shall therefore give it in detail.

"1. In order to facilitate the removal of the gelatinous envelope the eggs are placed in water heated almost to boiling (90–96° C.) for five to ten minutes. The eggs are thus coagulated and somewhat hardened, while the envelope separates a little from the surface of the egg and becomes more brittle. The envelope is then cut under water with sharp scissors, and the egg shaken out through the rupture. With a little experience a single cut suffices to free the egg.

"2. By the aid of a glass tube the egg is taken up and transferred to chromic acid (one-half per cent.), or to alcohol, 70, 80, and 90 per cent. Chromic acid renders the egg brittle, and the more so the longer

it acts; therefore the eggs should not be allowed to remain in it more than twelve hours. While eggs hardened in chromic acid never change their form or become soft when transferred to water, those hardened in alcohol, when placed in water or very dilute alcohol, lose their hardness, swell up, and often suffer changes in form.

"3. Alcoholic preparations are easily stained; but chromic acid preparations are stained with such difficulty and so imperfectly that Hertwig omitted it altogether.

"There is an important difference between alcohol and chromic acid in their effect on the pigment of the egg. Chromic acid destroys the pigment to some extent, and thus obliterates, or at least diminishes, the contrast between pigmented and non-pigmented cell-layers. As the distribution of the pigment is of considerable importance in the study of the germ lamellæ, it is well to supplement preparations in chromic acid with those in alcohol, in which the pigment remains undisturbed.

"4. Eggs hardened in chromic acid were embedded almost exclusively in the egg-mass recommended by Calberla. The great advantage offered by this mass is that it supplies a sort of antidote to the brittleness of the egg. It glues the cell-layers together, so that the thinnest sections can be obtained without danger of breaking.

"5. As the dorsal and ventral surfaces and the fore and hind ends can be recognized in very early stages, it is important to know precisely how the egg lies in the egg-mass in order to determine the plane of section. In order to fix the egg in any given position in the embedding mass, Hertwig proceeds as follows:

"*a*. A small block of the hardened mass is washed in water to remove the alcohol, and in the upper surface of the block, which has been freed from water by the aid of filtering paper, a small hollow is made. This hollow is then wet with the freshly prepared *fluid* mass.

"*b*. The egg is washed in water to remove the alcohol, placed on a piece of filtering paper to get rid of the water, turned on the paper by a fine hair brush until it has the position desired; the point of the brush is next moistened and pressed gently on the upper surface of the egg, the egg adheres to the brush and may thus be transported to the hollow prepared for it in the block.

"*c*. After the egg has thus been placed in position a drop of absolute alcohol carefully applied will coagulate the 'fluid mass' with which the block was wet, and thus fix the egg to the block. The block is again washed, and finally embedded in the egg-mass."

My own experience with fish eggs, especially those of clupeoids, is that a 1 per cent. solution of chromic acid gives good results. The eggs or embryos should not be left in it more than twelve to twenty-four hours, according to their size, when, after repeated washings in water, in which they will not change even if kept in it for three or four days, they may be transferred to 30 per cent. alcohol, or even a weaker grade, then in a day or so to 70 per cent. In a few days a granular precipitate is

formed, however, which is objectionable, yet fish eggs so preserved retain their form and structural peculiarities in alcohol unimpaired for many months. For the early cleavage stages, however, I find that chromic acid is not so good; it tends to disorganize the cleavage spindles. For these, killing in some weaker reagent, such as a 1 per. cent. acetic acid solution, or the treatment suggested by Whitman in the case of pelagic fish eggs, would give better results.

In certain cases there is no need for the removal of the egg-envelope if the latter is pricked open, especially in such forms as have a large respiratory cavity around the vitellus; the envelope, as well as vitellus, may be saturated with paraffine dissolved in chloroform at about 120° to 150° F., and where the membrane is sufficiently transparent the object may be arranged in the paraffine with hot needles without difficulty, and the sections so prepared will thus not only be sections of the egg itself, but excellent sections of the membrane will also be obtained.

My method of embedding fish eggs which have been colored *in toto* with borax, carmine, or borax picro-carmine, is as follows:

a. After dehydration with about forty times their own volume of strong commercial or 97 per cent. alcohol, and afterwards saturated with oil of cloves, the embryos are placed in a watch-glass containing a melted mixture of chloroform and paraffine in equal parts, in which they may remain twenty or thirty minutes at a temperature not above 150° F. When saturation is complete the eggs have the same appearance in the melted mixture as in alcohol.

b. From the above they are transferred to another larger dish containing pure paraffine, which melts at 158° F., but which must, on no account, be allowed to boil. Here they remain for twenty to thirty minutes more.

c. The embryos are then transferred, one or two at a time, to a common slide, such as is used for mounting objects. The slide may be warmed over an alcohol lamp. A brass ring, 5 to 8 centimeters deep and .24 in diameter, is then placed on the slide around the object. This ring is then filled with melted paraffine, and the object arranged in it in the desired position with a hot needle, when the whole is left to cool.

d. After cooling the paraffine contracts within the ring, when the latter may be removed, and the discoidal block may then easily be loosened from the slide. The block may then be trimmed down with a scalpel into a shape suitable for fastening into the well in the carriage of a sledge microtome, or the block may be marked and laid away until it is wanted for use.

Fastening the block in the microtome.—This may be done by taking a hot needle and melting a cavity with it in the paraffine contained in the well of the carriage of the microtome, into which the block, with the object, is adjusted in the desired position and left till the paraffine has cooled around the block, when the operator is ready to commence cutting.

Osmic acid as a killing and hardening agent for infusorians and small embryos.—The use of osmic acid in the study of the development of *Amphiaxus*, by Hatschek, has given very good results. During their early stages the embryos of this creature are quite small, and swim about in the sea water in which they have been hatched. Hatschek killed the embryos and hardened them in the following manner: A few drops of a 1 per cent. solution of osmic acid was poured into the small vessel of sea water containing the embryos, and allowed to act only a short time. This killed the embryos and hardened them, and also afforded a ready means of collecting them, for as soon as they were dead they fell to the bottom of the vessel and were then easily picked up with a pipette, or the supernatant mixture of sea water and osmic acid was poured off and replaced with fresh sea water two or three times, so as to wash off the acid and arrest its further action. They were then transferred to absolute alcohol and finally to oil of cloves.

The embryos were embedded in wax and oil on a slide thinly coated with clove oil. A single drop of a mixture of wax and oil (beeswax and oil equal parts) is dropped on the embryo, when its position may he arranged on the slide by turning the congealed drop of wax. He covered the whole slide, which was greasy with oil, with a coating of the melted wax and oil. The position of the embryo is then carefully marked with needle scratches. The mass is then slipped off of the slide and covered on the opposite side with another coat of wax and oil. To prevent the two halves of wax from separating, it is a good practice to pass a hot needle through both at different points in order to bind them together. The sections are then cut by hand, which, judging from Hatschek's figures, was very successfully done.

M. Adrien Certes has used osmic acid in order to kill and precipitate infusorians and other minute organisms found in fresh and salt water with gratifying results. One cubic centimeter of a 1 per cent. solution of osmic acid he finds sufficient to kill the minute animal and vegetable organisms in 30 to 40 cubic centimeters of water, these organisms being precipitated to the bottom of the vessel and fixed in their form. The acid must not be allowed to act too long, and to prevent this an equal volume of distilled water is added to the mixture after the organisms have been killed.

In the case of some waters rich in organisms, microsopic examination of the deposit so obtained may begin after a few hours. In the case of very pure water it may be necessary to wait for twenty-four or even forty-eight hours before the supernatant liquid may be poured off and the precipitated organisms examined.

It affords a very ready way of killing and collecting very minute organisms from either fresh or sea water. This method may also be used to precipitate bacteria or other supposed hurtful organisms from suspected potable waters.

Henneguy's method of preparing and investigating the eggs of salmonoids.—"The ova of the *Salmonidæ* are usually employed by embryologists in the study of the development of the osseous fishes. It is difficult to examine them in the fresh state, either whole or by transmitted light, on account of the thickness of their envelopes, or after opening them, in consequence of the small consistency of the germ, especially at the commencement of segmentation. Chromic acid, the reagent most frequently employed to harden these ova, readily alters the young cells, and deforms the embryos by compressing them between the unextensible envelope of the ovum and the solidified vitelline mass. For the last two years I have employed in the laboratory of Comparative Embryology of the Collége de France a process which enables us to extract the germs and embryos from the ova of trout and salmon with the greatest facility, and without causing them to undergo the least alteration.

"I place the ovum for a few minutes in a 1 per cent. solution of osmic acid until it has acquired a light brown color, then in a small vessel containing Müller's fluid, and I open it with a fine pair of scissors in the midst of this liquid. The central vitelline mass, which is coagulated immediately on contact with water, dissolves, on the contrary, in the Müller's fluid, while the solidified germ and cortical layer may be extracted from the ovum and examined upon a glass plate.

"By treating the germ with a solution of methyle green and then with glycerine I have been able to observe in the cells of segmentation the very delicate phenomena lately indicated by Auerbach, Bütschli, Strasburger, and Hertwig, and which accompany the division of the nucleus, namely, the radiate arrangement of the protoplasm at the two poles of the cell, the nuclear plate, the bundles of filaments which start from it, and the other succeeding phases.

"This proves that the treatment undergone by the ovum does not at all alter the elements of the germ.

"In order to make cross-sections of the germs and embryos thus extracted from the ovum I leave them for some days in Müller's liquid and color them with picrocarminate of ammonia. After depriving them of water by treatment with alcohol of spec. grav. 0.828, and then with absolute alcohol, I put them for twenty-four hours into collodion. The embryo is then arranged upon a small slab of elder-pith soaked with alcohol, and is covered with a layer of collodion. When the collodion has arrived at a suitable consistency very thin sections may be made, including the embryo and the plate of pith, and these are to be mounted and preserved in glycerine.

"This process is applicable to all sorts of embryos which are not very thick, so that they may be colored *en masse*. It has the immense advantage of enabling one to see at what level in the embryo each section is made, to preserve each section in the midst of a transparent mass, which sustains all the parts and prevents their being damaged, as too

often happens when an including mass is employed from which the sections must be freed before mounting."

Binder's method of making permanent glycerine mountings.—Mr. Jacob Binder, of Philadelphia, commends the following simple method of mounting objects in glycerine, which may be found useful in conjunction with the preceding method of treating the eggs of salmonoids proposed by Henneguy, though it hardly seems probable that as good results can be got by the collodion method of embedding, which is recommended, as by some others.

Mr. Binder finds Bell's cement the best, and with it he draws a ring with a pencil upon the slide, which he allows to dry for twenty-four hours. Then another coat of the cement is applied on the top of the first ring with the aid of a turn-table. The mounting is then made with glycerine; the superfluous mounting material which is forced out from under the cover may then be washed off by holding the slide under a water-tap. The slide is then allowed to dry when the mounting is finished by the addition of another ring of Bell's cement around the edge of the cover, when the preparation is finished.

Mark's methods of treating the eggs of Limax.—For hardening the ova and fixing the nuclear structures this investigator used a 1 per cent. solution of osmic acid, the eggs being subsequently stained in Beale's carmine. He also used 1 to 2 per cent. solutions of acetic acid, in which the eggs were immersed for three hours or more and afterwards stained with Beale's carmine. Sections were made of eggs hardened in chromic acid.

E. Van Beneden's method of treating the ova of the rabbit.—For killing and hardening a 1 per cent. solution of osmic acid was used, when the eggs were transferred to *Müller's fluid* for two or three days, washed, and then mounted in glycerine.

Brass's method of killing Amœbiform Protozoa.—In order to cause these organisms to become comparatively quiet he recommends feeding them with pulverized organic matter; they are then very slowly killed on the slide by the use of the following solution, and while under observation beneath the cover-glass:

Chromic acid	1 part.
Platinum chloride	1 "
Acetic acid	1 "
Water, 400 to 1,000 parts.	

This solution, he claims, will kill monera and amoebæ without altering their organization. Osmic acid, he asserts, produces dendritic appearances in the plasma of such organisms which are abnormal to them. With this re-agent he has obtained evidence of a nuclear body in some of the Monera.

Brass also thinks that turpentine and paraffin, when used to saturate an object to fit it to be cut into sections, also produces abnormal alterations in the tissues, and he recommends treating a tissue which is to be

sectionized as follows: From absolute alcohol it is transferred to oil of cloves or lavender, and then to pure paraffine, brought just a very little above the melting point.

C. Weigert's rapid method of hardening the spinal cord.—Müller's fluid hardens the spinal cord in about eight weeks at ordinary temperatures, but this may be accomplished eight to ten days if the hardening is done in a warm chamber or oven kept at about 120° F. While this is in progress camphor water should be added to prevent the development of putrefactive organisms.

The hardening may be still more rapidly done if *Erlick's fluid* is used. This consists of—

Potassium bichromate	2½ per cent.
Copper sulphate	½ "

With the aid of heat this hardens the spinal cord in four days, without heat in eight to ten days.

The sections are stained with *acid fuchsin*,* which is used as follows:

The sections, not to exceed .025 mm. in thickness, are placed for one hour in a saturated solution of *acid fuchsin*, but the staining is greatly modified by the subsequent treatment, as the diffusely stained sections are next transferred to a large watch-glass and washed in water. They are then transferred to a third watch-glass and washed in the following solution:

One hundred cubic centimeters of absolute alcohol.
One gram of caustic potash.

This is allowed to stand for twenty-four hours, until the alcohol is saturated with the alkali. Ten cubic centimeters of this mixture are added to every 100 cubic centimeters of absolute alcohol, and in this mixture the colored sections are washed. This washing out process is the most important thing in the application of the method. As soon as the section is transferred to the alkaline alcohol on a spatula a cloud of the red coloring matter is set free. The section is then gently shaken, and as soon as the limits of the *gray matter* are defined it is transferred to a large watch-glass full of clean water. This last wash must contain no trace of acids; the traces of alkaline alcohol adhering to the spatula will do no harm, and the section must be washed in it till no more clouds of color are given off. It is then transferred to a fifth wash of clean water, when the operator should notice if the gray portions are the lightest. If this is the case and the section is still red the process has been successful. If the section is too pale it must be restained; or if the gray substance is not differentiated by a paler tinge it must be returned to the alkaline alcohol and then again washed in clean water twice in succession. The sections then dehydrated and treated in the usual way with clove oil and mounted in Canada balsam. Sections which have been embedded in celloidin should be treated with xylol instead of oil of cloves, and

* Fuchsin S. No. 130, made in the Baden Aniline and Soda Manufactory, may be obtained in small quantities from Dr. Grübler, Leipzig, 17 Dufour strasse.

these in order to be completely dehydrated must be transferred successively to two baths of absolute alcohol.

This method of staining sections of the nervous system is said to give results much superior to carmine, the anilines, or gold chloride, and to differentiate the fibers of the gray matter better than any other dye.

Embedding in celloidin.—Whitman, in the American Naturalist for October, 1883, describes the method as follows: "Very elegant results may also be obtained by an embedding mass originally invented by Duval and recently much improved by Merkel and Schiefferdecker.* This is collodion, or, preferably, a solution of so-called *celloidin.* If this substance cannot in general be cut to such extreme delicacy as the albuminous mass just described, it has a great advantage in being extremely pellucid. The original communication of the last-named author is easily accessible, so that Professor Thoma considers it superfluous to give a detailed account of it, but adds a few remarks on his own experience with it.

"According to the formula of Schiefferdecker, the embedding fluid consists of concentrated solution of celloidin in a mixture of equal parts of absolute alcohol and ether. The specimen is soaked successively in absolute alcohol and ether, and in the embedding fluid. This requires at least several days. After this time the embedding proper may be undertaken, and for this we have the choice of two methods.

"The even surface of a cork is covered with a thick solution of celloidin, so as to form by evaporation a strong collodion membrane on the cork. Upon this is put the specimen, covered layer by layer with fresh quantities of the solution of celloidin, each being allowed to dry only partially. When the object is thoroughly covered we immerse it in alcohol of 0.842 specific gravity. In twenty-four hours the whole is ready for cutting.

"The other method makes use of little paper boxes for the embedding. The specimen soaked in celloidin solution is fixed in the box by pins, the box filled with celloidin. The preparation is then placed on a flat piece of glass and covered with a glass cover which does not exactly fit the glass plate. In a few days the ether will have evaporated gently and slowly from the embedding mass, and the latter will shrink a little. If necessary more celloidin solution can be poured into the paper box to fill it again. It is only necessary to moisten the surface of the first mass with a drop of ether in order to allow of a perfect junction between the old and the new layers. The preparation is again exposed to slow evaporation below the glass cover, and a few days later the embedding mass will be consolidated to an opaline body, whose consistency can well be compared to that of the albumen of a boiled egg. The walls of the paper box can now be removed, and the embedding mass placed in very dilute alcohol, which will, in a very few days, produce a proper degree of consistency to admit of cutting.

* Arch. f. Anat. u. Physiol. (Anat. Abthiel.) 1882.

"This method differs in some degree from that which Schiefferdecker gives for embedding in paper boxes. As other observers have remarked, his method frequently gives rise to a great number of air-bubbles in the embedding mass. Consequent upon the altered manipulations of Professor Thoma, we have to adapt the embedded specimen to a cork for the purpose of cutting. This may be done in the following way: The even surface of the cork is covered by a thick layer of celloidin solution. This is allowed to dry up perfectly, so as to produce a hard membrane of celloidin. This is again covered with further celloidin solution. In the mean time the lower surface of the embedding mass is cut even and washed with absolute alcohol, and subsequently moistened with a drop of ether. This moist surface is adapted to the stratum of liquid celloidin on the cork, and exposed for a few minutes to the open air. After this the whole is placed in dilute alcohol, which in a few hours will unite the embedding mass solidly with the cork.

"In a great number of cases it may be regarded as a great advantage of the celloidin that it penetrates the tissues thoroughly, and yet remains pellucid, so as to be more or less invisible in the specimen. This quality can be made use of in another direction for the purpose of soaking specimens which are too brittle to be cut after hardening alone. We may make use of celloidin in a similar way to the gum arabic mentioned above. The minute, normal, and pathological anatomy of the lung in particular will derive great advantage from such a proceeding. Indeed, we are not able to get a perfect idea of the changes produced by pneumonia if we do not by this method or by the following (with paraffine) prevent the loss of a great part of the exuded substances which in this disease lie loose in the areolar cavities. The study also of micro-organisms in the lung will derive great benefit from the celloidin method, and it will be very welcome to many to know that the tissues embedded in celloidin may be stained with the different fluids, ammonio-carmine, alum-carmine, borax-carmine, hæmatoxylin, aniline colors, and various others. The reaction of acids and alkalies, particularly acetic acid and solution of potash is, moreover, not interfered with. And further, we are able to color the object before embedding with all staining fluids which are not soluble, or only little soluble, in alcohol or ether.

"After staining and cutting the sections may be mounted in glycerine and various other fluids. Mounting in Canada balsam requires, however, some precautions on account of the chemical character of the celloidin. Absolute alcohol and oil of cloves should be avoided and replaced by alcohol of 96 per cent., and by oleum origani. This is, at least the advice of Schiefferdecker, and Professor Thoma has had no occasion to be dissatisfied with the result."

The embedding mass, consisting of equal parts of chloroform and paraffine, used first by Bütschli, and which admits of subsequent embedding in pure paraffine, has given such excellent results in my hand, that

I do not see what else can be desired. The method which has given good results in the hands of the writer has already been described, and differs but little from the method commended by Brass. Sections of the most extreme thinness can be cut by its use, Bütschli having succeeded, with small specimens, in getting sections measuring only .002^{mm} or about $\frac{1}{12500}$ of an inch in thickness.

Combined killing, staining, and preservative agents.—To this category we may assign such combinations as ammonio-picro-carmine, borax-picro-carmine, and picric acid combined with nigrosin prepared according to the formula of Pfitzer. The preparation of ammonio-picro-carmine is attended with a good deal of trouble and takes a great deal of time and attention if some of the formulæ which have been proposed are followed in its preparation.

The readiest way of preparing a picro-carmine, I find, is to have a stock solution of borax-carmine on hand which may be poured into a saturated solution of picric acid in sufficient quantity to give a deep orange-red mixture which may then even be combined with a small percentage of alcohol. In this way we obtain a staining mixture which dyes small objects, such as embryos, with two colors in different parts, and is also a temporary preservative, killing, and hardening mixture. The objects after a day or two are removed from this mixture and put into 30 to 40 per cent., and finally into 70 per cent., alcohol.

Pfitzer's mixture of picric acid and nigrosin has been commended in botanical research; but it appears probable from a little experience which I have had with it that it will be useful in animal histology. A few drops of a watery solution of nigrosin are mixed with a saturated solution of picric acid; this mixture has an olive-green color; it kills quickly and stains the granules and nuclei beautifully, imparting to them a tint somewhat similar to that produced by hæmatoxylin.

Fixing sections upon the slide preparatory to mounting.—This I find may be very easily done by the aid of Schällibaum's mixture of oil of cloves and collodion.

Collodion	1 volume.
Oil of cloves	3 to 4 volumes.

The slides are thinly painted with this mixture over the center where the sections are to be placed with a perfectly clean camel's-hair pencil. The sections which are cut by the dry method are lifted from the upper side of the section knife as fast as cut, and laid on the slide in serial order. If a section stretcher or flattener is used on the knife the sections may be lifted off in short ribbons consisting of several consecutive sections sticking together, edge to edge. After the sections have been neatly arranged in successive rows, and in serial order from left to right, with the aid of a needle, the slide may be gently warmed over an alcohol lamp, when the paraffine will melt and let the sections drop down and sink into the film of collodion and clove oil. By warming the slide

for half a minute or more the clove oil is mostly vaporized or driven to the edges of the slide and around the border of the area which is occupied by the sections. When this is the case the sections will usually be found to be fixed. Then, before the slide has cooled very much, two or three drops of turpentine are poured upon the sections. The turpentine is warmed by the slide, and the paraffine from the sections is immediately dissolved away. Turpentine is again dropped on the sections and the slide turned on its edge and drained to wash away all that remains of the paraffine surrounding and included by the sections. Before the turpentine has quite dried upon the slide the mounting is done in Canada balsam dissolved in benzole. The balsam should be thin enough to run readily under a long cover-glass, and under which as many as one hundred and fifty sections may be mounted without getting any air bubbles included.

Such serial preparations enable the embryological investigator to study the morphology of embryos or small objects with the greatest ease and certainty, because none of the viscera or organs of even the smallest embryos are displaced or shoved out of their normal positions to the slightest degree in the sections if the object has been properly embedded and the process of mounting conducted with the proper care.

It was my intention at first to give the formulæ for the preparation and use of the most approved staining fluids, but the recipes for compounding these are accessible in a number of hand-books on microscopical technology, while Mr. Whitman has already given a very full account of those used with the best success in the zoological station at Naples, in his paper on methods, from which I have already drawn so largely, the title and place of publication of which I have given in the first portion of this paper. Those staining reagents which are given here are mostly such as are used in combination with some killing or preservative agent.

The principal object of this paper is to afford directions to collectors desiring to preserve the embryos of the lower vertebrates, fishes, and amphibians in such a condition as will enable the investigator to use them in his researches. As ordinarily preserved in alcohol such objects are next to worthless, either for figuring or dissection, as well as totally useless for microscopic preparations.