In manipulating with melted embedding masses, it is necessary to have a constant and comparatively low temperature, as in many cases the specimen has to be soaked

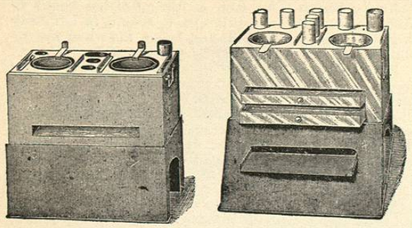

FIG. 2690.—Paraffin Water Baths.

in the melted mass for a considerable length of time (impregnation method). Two forms of water baths for this purpose are shown in Fig. 2690.

Impregnation Embedding.—In this process the specimen is soaked in the embedding mass until it is thoroughly permeated with it. The proceeding is then like simple embedding in a melted mass.

Embedding Masses: Celloidin.—This is a pure pyroxylin, manufactured by Schering & Company, Berlin; it is free from all foreign constituents, and makes a clear solution without sediment. It is soluble in a mixture of equal volumes of alcohol and ether, and the degree of concentration can be varied to suit any particular case.

For use, a five-per-cent. solution of celloidin is made in alcohol and ether, and a portion of this is diluted with a mixture of equal parts of alcohol and ether, so as to obtain a very dilute solution. The specimen is soaked in alcohol for twelve hours; then in alcohol and ether for the same length of time; then in the dilute celloidin for at least twelve hours; then in a five-per-cent. solution for one to eight days, according to the size and density of the specimen. When the specimen has become thoroughly permeated it is to be embedded in one of the following ways:

(*a*) Cover the smooth surface of a vulcanite block with a thick layer of celloidin solution and allow it to dry. Then place the specimen, which has been soaked in celloidin, on this and cover it, layer by layer, with a solution of celloidin, allowing each layer to dry partially before applying another. When the specimen is completely covered allow it to stand in the air for half an hour, and then immerse in eighty-per-cent. alcohol for twelve hours, when it will be ready for cutting.

(*b*) Embed in a paper box in the usual way, and allow the mass to stand until a pellicle forms on the surface; then immerse in eighty-per-cent. alcohol for twenty-four hours.

Viallanes uses chloroform for hardening the celloidin. He has found that this reagent coagulates the celloidin into a mass of the consistence of wax, but very elastic and perfectly transparent. He places specimens, previously soaked in ether, in a test-tube and covers them with a solution of celloidin. As soon as a film has formed on the celloidin, the tube is filled up with anhydrous chloroform and left for two to three days, when the mass will be hardened and somewhat shrunken, so that it can be shaken out of the tube. It is then placed in fresh chloroform for six days, when it will be ready for cutting. A. B. Lee found that a few hours' immersion in the chloroform gave the required consistence, and in no case was a longer time than three days required. The celloidin frequently becomes opaque on being put into the chloroform, but becomes transparent after a time.

Oil of Cloves Celloidin (Stepknow).—Celloidin (granular form), 1.5 gm.; oil of cloves, 5 c.c.; ether, 20 c.c.: absolute alcohol, 1 c.c.

The specimen, after being dehydrated in ninety-five-per-cent. alcohol, is placed in oil of cloves for from three to six hours; then in the above solution of celloidin for six hours. Then it is embedded, preferably, in a paper box, and allowed to stand in a warm place until the celloidin thickens; then placed in eighty-per-cent. alcohol or chloroform for from one to two hours.

In case the tissues can be cut into rectangular-shaped blocks they are mounted on blocks of vulcanite and then placed in chloroform.

Sections are cut with a knife wet with alcohol, and may be stained with the usual staining agents. Some of the aniline dyes stain the celloidin intensely, and are not removed by alcohol. If sections are to be mounted in Canada balsam, they are dehydrated in ninety-five-per-cent. alcohol — absolute alcohol dissolves celloidin — cleared in oil of bergamot, sandalwood, or origanum, as oil of cedar or cloves dissolves celloidin.

Paraffin.—For embedding, paraffins of a melting point of from 45° to 55° C. are used. By mixing these in various proportions, masses may be obtained whose melting point will range between these two. Paraffin should be chosen of a melting point suitable to the temperature of the laboratory. As a rule, a laboratory temperature of 18° C. requires a paraffin whose melting point is 48° C.

The specimen to be embedded is placed, from alcohol, in chloroform until the alcohol is replaced by the latter; this takes place quickly. The specimen is then removed to a dish containing fresh chloroform and shavings of paraffin are added, so that after the evaporation of the chloroform the specimen will remain covered by the paraffin. The vessel is now placed in a water bath (see Fig. 2690), at the temperature of the melting point of the paraffin, for from one-half to one hour. The paraffin melts very quickly, and the specimen, from the evaporation of the chloroform, is bathed in a concentrated solution of paraffin in chloroform, which soon penetrates the specimen, filling up all cavities. It is essential that all the chloroform should be driven off. When this has taken place, the specimen is embedded in a box in the usual manner and allowed to cool. After cooling, the block should be trimmed and clamped in the microtome, or mounted on a cork with a little melted paraffin.

A. B. Lee recommends that the specimens be soaked in oil of cedar. This clears them rapidly, and the paraffin penetrates quickly and thoroughly.

Sections are to be cut dry. If they have a tendency to roll, they can be held down by a camel's-hair brush, or by attaching a section-smoother to the knife. After cutting, the embedding mass is to be dissolved out before staining. For this purpose, of the many solvents recommended, turpentine, naphtha, and xylol are the best.

METHODS OF STAINING.—As a rule, the sections to be stained are placed in the staining fluid from water, and

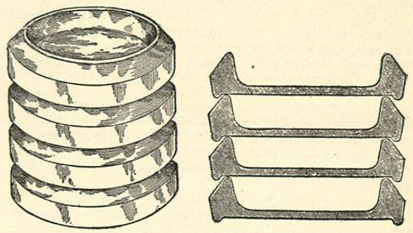

FIG. 2691.—Improved Syracuse Solid Watch Glass.

allowed to remain in the former for various lengths of time—from a few seconds to twenty-four or forty-eight hours. The time depends on the nature of the stain and the result to be accomplished, and will be noted under

the various stains. After the staining is complete, the sections are to be washed in water, to remove all the excess of the staining fluid, mounted directly in glycerin, or, after dehydrating and clearing, in balsam.

For holding the stains and for the operations of washing, dehydrating, etc., we use small dishes of porcelain or glass, watch glasses, etc. The improved form of the Syracuse solid watch glass (Fig. 2691) will be found very convenient. For transferring the sections, the spatula or lifter (Fig. 2687), needles, camel's-hair brushes, glass rods, etc., are used.

Innumerable substances are used for the purpose of staining, and each year adds a host to the list, many of which are of doubtful value. Only the most important stains will be treated of here.

Carmine.—This dye, when first introduced, was used in the form of an ammoniacal solution, and is still, but rarely, employed in this form. Ammonia solutions are not stable; they undergo various chemical changes upon standing, so that the solution cannot be depended upon. Unless all free ammonia is driven off, the staining is apt to be diffuse. Various other solvents are now used with better results.

Tissues that are to be stained with carmine must be free from acid. Preparations hardened in chromic acid or solutions of chrome salts, must be well washed in water, and then they stain very slowly, requiring days. Obersteiner hastens the process of staining by heating the fluid to 50° C., and keeping it at that temperature for an hour, when the staining will be completed. Henle and Merkle soak sections in a 1 to 500 aqueous solution of palladium chloride for ten minutes; then in the carmine fluid for a few moments, when they become stained of a deep-red color. If the stain is diffuse it can be removed by placing the sections in a mixture of one part of formic acid and two parts of alcohol for from five to ten hours. This removes the stain, in sections of the central nervous system, from everything except the axis cylinders (Ranvier).

Before mounting, sections stained in carmine should be washed in a one-per-cent. solution of acetic or formic acid to fix the stain.

Carmine stains deeply nuclei, protoplasm, smooth and striated muscle, the basement substance of bone tissue and decalcified bone, the axis cylinders of nerves, etc.

Ammonia Carmine Solutions.—Ranvier's: Carmine, 1 gm.; ammonia, 1 c.c.; water, 100 c.c. Rub up the carmine in a mortar with a little water and add the ammonia. When the carmine is all dissolved add the rest of the water. If there is an excess of ammonia heat the solution until the carmine begins to precipitate.

Heidenhain's: Carmine, 1 gm.; ammonia, 3 c.c.; glycerin, 96 c.c.; water, 96 c.c. Dissolve the carmine in the ammonia and add the glycerin and water. Heat on a water bath until the excess of ammonia is driven off, or neutralize with acetic acid. The sections are placed in a watch glass of this fluid, and this, with a second watch glass containing water with a trace of ammonia, is placed under a bell glass for twenty-four hours. The sections at the end of this time are washed in water and exposed, as above, to the vapor of acetic acid, and mounted in glycerin.

Borax Carmine.—Grenacher's: The author gives two formulæ. In the first he dissolves 2 to 3 gm. of carmine in a solution of 4 gm. of sodium biborate in 100 c.c. of distilled water and then adds 100 c.c. of alcohol; filters, and after allowing the filtrate to stand a week, filters again. This solution is used chiefly for staining *in toto*. Pieces of tissue, according to their size, are placed in the solution for from one hour to three or four days; then in acidulated alcohol (three to four drops of HCl to 100 c.c. of seventy-five-per-cent. alcohol) for from one hour to three or four days. His second formula, known as neutral borax carmine, is as follows: 0.5 to 0.75 gm. of carmine is boiled in a solution of 2 gm. of sodium biborate in 100 c.c. of water. The solution is filtered, and acetic acid added to the filtrate until the purple tint changes to that of ordinary carmine; it is then filtered again. Sections stain in this fluid, in from one to three minutes, an intense and diffuse red color; they are then washed in a mixture of one drop of HCl to a watch glass of seventy-per-cent. alcohol, when the color is withdrawn from everything except the nuclei.

Lithium Carmine.—Oth's: Carmine, 2.5 gm.; saturated solution of lithium carbonate, 100 c.c.

After staining, the sections are washed in a mixture of 1 c.c. of HCl, and 100 c.c. of seventy-per-cent. alcohol, when the color is withdrawn from all except the nuclei.

Picro-carmine.—Ranvier's: Add ammonia carmine to a saturated aqueous solution of picric acid, until a cloudy precipitate begins to appear. Evaporate to one-fifth of its original volume, allow to cool, and filter. Evaporate the filtrate to dryness, when a reddish powder is obtained. This powder is used in one-per-cent. solutions in water.

Gage's: Take equal parts, by weight, of carmine and picric acid. Dissolve the picric acid in one hundred times its weight of distilled water, and the carmine in fifty times its weight of strong ammonia, mix the two solutions, and evaporate three-fourths at a temperature of 40° to 50° C. Allow the fluid to cool, and filter through two thicknesses of paper. Evaporate the filtrate to dryness at a temperature of 40° C., and dissolve the residue in one hundred times its weight of water. Make 50 c.c. of such a solution and filter through absorbent cotton packed in the neck of a funnel; repeat the filtering four or five times, when a clear solution should be obtained. If this is not the case, dissolve the remainder of the powder in the proportion given above, allow the solution to stand for a few days in a tall glass cylinder, and then decant the clear fluid. When a clear solution is obtained, add to every 100 c.c. of the picro-carmine 25 c.c. of strong glycerin and 10 c.c. of alcohol. This solution can be kept clear by filtering once in six months.

Weigert's: Soak 2 gm. of carmine in 4 c.c. of strong ammonia for twenty-four hours in a closed vessel; then add 200 c.c. of a saturated solution of picric acid in water, and allow it to stand for twenty-four hours. Filter, and add acetic acid to the filtrate, drop by drop, until a slight precipitate appears even after stirring. Allow this mixture to stand for twenty-four hours, when a precipitate will form which cannot be wholly removed by filtering; add ammonia, drop by drop, at intervals of twenty-four hours, until a clear fluid is obtained. If the solution stains too yellow, add a few drops of acetic acid; if too red, a few drops of ammonia.

Hoyer's: Dissolve 1 gm. of carmine powder (see Hoyer's Carmine) in 5 to 7 c.c. of ammonia and 1 to 5 gm. of picric acid in 50 c.c. of water. Mix the two solutions, and add enough water to make the mixture measure 100 c.c., and add 1 gm. of chloral hydrate for preserving. If any free ammonia is present, warm on a water bath until it is driven off.

Oth's: Mix one part of Oth's lithium carmine with two to three parts of a cold-saturated solution of picric acid in water.

Sections stained in picro-carmine should not be washed long in water, or the picric acid stain will be washed out. They may be mounted in balsam or glycerin; if the latter, the glycerin should be acidulated with a few drops of acetic or formic acid.

Alum Carmine Solutions.—Grenacher's: Boil one-half to one per cent. of carmine in a concentrated solution of ammonia or potash alum for from ten to twenty minutes, filter, and add a few drops of carbolic acid to the filtrate. Balbiani adds a few drops of acetic acid to a watch glass of this stain with good results. Tizzoni adds a little sodium sulphate, thereby increasing its staining power. This fluid stains quickly, and nuclei stronger than other parts.

Cochineal Fluids.—Mayer's: Soak 1 gm. of powdered cochineal in 10 c.c. of seventy-per-cent. alcohol, for at least three days. Filter, and use the deep-red filtrate for staining. Sections are placed in this fluid from seventy-per-cent. alcohol, and they stain in a few moments, or it may require several days, the time depending on the size and nature of the sections. When stained, wash in sev-

enty-per-cent. alcohol until no more color comes away; then mount in the usual media. The results obtained by the use of this fluid vary with the nature of the tissue, and the presence or absence of certain salts—also upon the strength of the alcohol employed for solution. Over-staining may be removed by washing in one-tenth-per-cent. HCl, or one-per-cent. acetic acid.

Czoker's Alum Cochineal.—Boil 7 gm. of pulverized cochineal and 7 gm. of alum in 700 c. c. of distilled water, until the quantity of fluid is reduced to 400 c.c.; cool, add a few drops of carbolic acid, and filter. After allowing it to stand for a few days, filter again, when the fluid will be ready for use. Martinotti recommends the use of chrome alum in place of ordinary alum, and does not allow the temperature to go above 80° C. This fluid stains nuclei a hæmatoxylin color, other elements different shades of red. Alcoholic specimens stain quickly, in from three to five minutes; chromic-acid preparations require from three to five hours. After staining, specimens are washed in water and mounted in the usual media.

Carminic Acid.—The use of carminic acid in place of carmine was first suggested by Dimmock, but he used only aqueous or alcoholic solutions which gave but feeble stains. Mayer showed that in order to obtain suitable staining solutions of carminic-acid it must be combined with aluminum or calcium. I have found that the substitution of carminic acid for carmine in most of the formulæ given above makes a much better and sharper staining fluid.

Carmalum (Mayer).—Carminic acid, 1 gm.; alum, 10 gm.; distilled water, 200 c.c. Dissolve the carminic acid in the water and filter. Add a few crystals of thymol to prevent the formation of mould. After staining, wash out in distilled water, and if the plasma remains stained, wash in alum solution. This is a good fluid for staining tissues in bulk.

Hæmatoxylin.—This is one of the coloring substances contained in ordinary logwood. It is found in commerce in the form of reddish-brown crystals. Hæmatoxylin is not to be confounded with the ordinary crude extract which is sometimes sold under this name.

This dye is one of the best nuclei stains that we have, staining both alcoholic and chromic-acid specimens deeply and sharply. In chromic-acid preparations the color is likely to fade after a time, unless all the acid has been removed and the sections have been thoroughly dehydrated before mounting.

The clearest stainings are obtained with dilute solutions. After staining, the sections are washed well in water and mounted as usual.

Delafield's Solution: Saturated solution of ammonia alum, with an excess of crystals, 100 c.c.; to this is added 1 gm. of hæmatoxylin crystals dissolved in 6 c.c. of alcohol. This solution is allowed to stand in an unstoppered bottle in the light for four or five days, and then filtered. Then glycerin, 25 c.c., and wood naphtha, 25 c.c. are added; this is allowed to stand, as above, for from one to two days, and then filtered; the filtering is to be repeated at intervals, until all sediment ceases to form. It is to be diluted at least one-half with water for use.

Ehrlich's Acid Hæmatoxylin: Hæmatoxylin crystals, 1 gm.; strong alcohol, 60 c.c.; glycerin, 60 c.c.; water, 60 c.c., to which ammonia alum is added to saturation; hydric acetate, glacial, 3 c.c. This fluid is exposed to the light until it becomes of a deep-red color.

Gage's Hæmatoxylin: Distilled water, 200 c.c.; ammonia or potash alum, 7.5 gm.; this is boiled for five to fifteen minutes to destroy any germs in the water or alum; after cooling add 0.1 gm. of hæmatoxylin crystals dissolved in 10 c.c. of ninety-five-per-cent. alcohol and 4 gm. of chloral hydrate to prevent the development of germs.

For staining, this solution may be used full strength or diluted with alum water.

Up to a few years ago all formulæ for the preparation of hæmatoxylin solutions, especially those containing alum, ended with the following directions: "allow to stand in the light from eight to ten days before using." This was for the purpose of allowing the staining fluid to "ripen," as it is well known that freshly prepared solutions are of no use for staining.

This "ripening process" was found to be due to the oxidation of the contained hæmatoxylin and the formation of *hæmatein*, through the absorption of oxygen from the air. Paul Mayer and Unna found that this oxidation could be brought about immediately by the addition of a small quantity of hydrogen peroxide; Hansen, by the addition of potassium permanganate. I have hastened the process by passing air through the solution, using air compressed by the usual spray apparatus.

All hæmatoxylin solutions deteriorate in the course of time, the oxidation process continuing until precipitates form and the solution becomes colorless. Unna found that this over "ripening" could be delayed by the addition of a small quantity of sulphur. Paul Mayer used glycerin, and Gage, believing this to be due to the action of bacteria, sterilized his alum solution and then added chloral hydrate as a preservative. Owing to the investigations of Paul Mayer we are now in a position whereby *hæmatein* staining solutions can be quickly made, without the long "ripening" process.

Hæmatein, which Mayer uses in place of hæmatoxylin, comes in the form of dark greenish crystals or powder, and is freely soluble in alcohol and water. The commercial form is not always pure, and owing to this *hæmateate of ammonia* has been recommended in the place of hæmatein. This salt is also found in commerce, but of doubtful purity. It can be readily made in the laboratory as follows: One gram of hæmatoxylin crystals is dissolved, by the aid of heat, in 20 c.c. of distilled water and filtered. To this solution 1 c.c. of ammonium hydrate (sp. gr. 0.875) is added, and the dark purple fluid placed in a shallow porcelain dish, of such a size that the layer of fluid shall not exceed one-half a centimetre in depth. The dish is covered with filter paper, to exclude dust, and the solution is allowed to evaporate at the room temperature. The hæmateate of ammonia about equals in weight the hæmatoxylin used. In the preparation of this salt no metal instruments should be used until the powder is absolutely dry.

Mayer gives formulæ for three different solutions of hæmatein as follows:

Hæmalum.—Dissolve 1 gm. of *hæmatein* or *hæmateate of ammonia* in 50 c.c. of ninety-per-cent. alcohol, and add this to 1,000 c.c. of a five-per-cent. aqueous solution of *potash alum*, filter, and add a crystal of thymol to prevent the formation of mould. This fluid has a color resembling that of borax carmine. It may be used for staining in full strength, or it may be diluted with weak alum solution. In full strength it stains sections in about two minutes.

Acid Hæmalum.—This is prepared by adding two per cent. of hydric acetate to the above hæmalum solution. After staining, wash well in water to bring out the bluish tint of the nuclei. This solution is decidedly less diffuse in staining, and keeps better than the hæmalum.

Glycerin Hæmalum.—The above solutions of hæmatein do not keep well. To correct this, Mayer adds glycerin as a preservative. The solution is made as follows: Rub up 0.4 gm. of hæmatein in a mortar with a small quantity of glycerin; dissolve 5 gm. of potash alum in 70 c.c. of distilled water, and add 30 c.c. of glycerin. This mixture is gradually added to the hæmatein solution. This stain keeps well, but is somewhat slow in its action.

During the last year I have been using *hæmatein* in place of hæmatoxylin with the most satisfactory results. The staining fluid I have used is one made after a combination of Mayer's and Gage's methods. The solution is made as follows: A five-per-cent. solution of potash alum is made in distilled water and boiled, or what is better, placed in a steam sterilizer from one to one and a half hours. While this solution is warm, 0.001 per cent. of *hæmatein*, dissolved in a small quantity of alcohol, is added (it is my practice to keep a one-per-cent. alcoholic solution of hæmatein as a stock solution). After the fluid has cooled, 2 gm. of chloral hydrate should be added

for each 100 c.c. of the staining fluid. This stain keeps well, and in full strength stains sections in from two to three minutes. It may be used diluted with distilled water, when the staining requires a longer time.

Heidenhain's Iron Hæmatoxylin.—Sections are placed in a 1.5 to 4 per cent. aqueous solution of ammonio-ferric sulphate, $(NH_4)_2Fe_2(SO_4)_4$, for from one-half to four hours; then they are washed in water; and then stained in a 0.5-per-cent. solution of hæmatoxylin in water for half an hour. They are then removed from the stain and washed in water. The sections which are now deep black are decolorized in the ammonio-ferric sulphate solution. This acts slowly and its progress should be watched under the microscope. This is done by removing the specimens, from time to time, from the decolorizer and examining them in water. As soon as a proper differentiation has been obtained the sections are washed in water for at least an hour, the water being changed several times. All chromatin elements of the nucleus stain black and are sharply differentiated.

Aniline Dyes.—*Fuchsin, Rosaniline Hydrochloride.*—Soluble in alcohol and water. Used for staining fresh tissue, blood cells, and connective-tissue cells. Chiefly used for staining bacteria. Merkle has used it for staining the structureless membrane of the retina.

Acid Fuchsin, Rosanilin Sulphonate.—(Fuchsin S, No. 130 of the Baden aniline factory.) First used by Weigert for staining the central nervous system, and now used chiefly in combination stains.

Safranin.—An oxidation product of pure toluol, of which there are numerous preparations on the market. The form usually used is safranin O, which is soluble in alcohol and water. This is a nuclei stain best suited for chromic-acid preparations.

Flemming dissolves one-tenth to five-tenths per cent. in a mixture of equal parts of alcohol and water. He stains sections for twenty-four hours; washes in weak alcohol, which frees them from part of the color, then in absolute alcohol until no more color comes away; then in oil of cloves only long enough for them to become penetrated, as it extracts the colors; mounts in balsam.

Babes recommends the following methods:

1. Sections of alcoholic or chromic-acid preparations are stained for half an hour in a warm, concentrated aqueous solution of safranin, or in a mixture of equal parts of concentrated aqueous and concentrated alcoholic solutions. They are then washed in absolute alcohol quickly, as it withdraws the stain, cleared in turpentine, and mounted in balsam.

2. For demonstrating the details of cell structure. A supersaturated solution of safranin in water is warmed to 60° C. and filtered. After cooling the solution becomes muddy. A small quantity of this fluid is placed in a watch glass with the sections, and warmed slightly over an alcohol lamp for a minute, when the fluid will become clear. The sections are then washed in water, cleared in turpentine, and mounted in balsam.

Safranin is also a good reagent for amyloid substance, staining it orange-yellow, other tissue rose-red. It is also used for staining elastic fibres.

Eosin (Erythrosin, Primrose).—This dye is found in commerce in two forms, one soluble in alcohol (tetrabromofluorescin), the other in water (a potash salt of the first).

Fischer, who first introduced the dye, precipitates the coloring matter from an aqueous solution with an acid; filters, washes the precipitate with water, and then dissolves the precipitate in alcohol. He prefers the alcoholic solution for chromic-acid preparations.

Eloui dissolves the eosin in glycerin, and after staining, fixes the dye with a saturated solution of alum in glycerin.

For general use a 1 to 1,500 solution in alcohol of the precipitated eosin will be found most convenient. Sections are stained for a few moments and mounted in glycerin tinged with eosin, as pure glycerin withdraws the color. For mounting in balsam the sections are dehydrated in the alcohol eosin and cleared in oil.

This dye, in combination with hæmatoxylin, is the best for general work, the hæmatoxylin staining the nuclei of the cells purple, while the cell bodies and intercellular substance are stained rose-red.

Methylene Blue.—An oxidation product of pure aniline, soluble in both alcohol and water. It is used chiefly for staining bacteria: in combination with eosin as a nuclear stain; in a chemically pure state in Ehrlich's method for nerve fibres; and in Nissl's stain for nerve cells.

Unna's Alkaline Methylene blue.—Methylene blue, 1 gm.; potassium carbonate, 1 gm.; water, 100 c.c. This solution is diluted in the proportion of 1 to 10 with water for use. It is a good nuclear stain in combination with eosin.

Methyl Green.—The double zinc salt of the base pentamethyltri-p-amidotriphenylcarbinolmethylate. Used in one-per-cent. aqueous solutions, and is chiefly a nuclei stain.

Carnoy employs it in a concentrated aqueous solution, to which is added one per cent. of acetic acid and one-tenth per cent. of osmic acid for staining fresh tissue. Nuclei stain sharply. The excess of color is washed out with water.

Curshmann recommends this dye as a reagent for amyloid, which it stains violet. It is also used in several of the multiple stains.

Iodine Violet, Hoffman's Violet (*a*, Dahlia, bluish tint; *b*, Primula, reddish tint). The pentamethyltriamidotoluyldiphenylcarbinol.

Ehrlich employs a saturated solution in a mixture of 500 c.c. of alcohol, 100 c.c. of water, and 12.5 c.c. of acetic acid. He stains sections for twenty-four hours; then washes in water acidulated with acetic acid, and mounts as usual. Protoplasm and connective tissue are partially stained; nuclei stain blue-violet. Sometimes the fat of fat cells and goblet cells stain.

Jürgens employs an aqueous solution as a reagent for amyloid, which stains bright red, while the rest of the tissue stains blue-violet.

Flemming employs the dye in the same manner as safranin.

Gentian Violet.—Employed chiefly for staining bacteria.

Methyl Violet.—The pentamethyltriamidotriphenylcarbinol.

Cornil found that aqueous solutions stained the cells of hyaline cartilage violet, the basement substance reddish, connective tissue, fibrillated and elastic fascia violet.

This dye is also used as a reagent for amyloid, which it stains red. According to Capparelli this reaction is an optical instead of a chemical one. He examined unstained sections by light transmitted through a thin layer of this dye and found that the amyloid substance appeared as if had been stained. He then examined unstained sections on glass stained with the colors of the spectrum, and found that in the section on the violet the amyloid substance appeared red, while the normal tissue appeared blue. His conclusion was that the amyloid substance permits the red rays to pass, but stops the violet.

This dye is also used in staining bacteria.

Double Staining.—*Hæmatoxylin and Eosin.*—Sections are first stained in a solution of hæmatoxylin until they are of a distinct purple color, then washed well in water, and then stained in a dilute alcoholic solution of eosin. They can be mounted in either glycerin or balsam. If in glycerin, it is to be slightly tinged with eosin, or the color will be extracted from the specimen. If in balsam, the specimens are placed in alcoholic eosin, in which they become stained and dehydrated at the same time; then cleared in oil of origanum cretici, and then mounted in balsam or dammar. Nuclei stain purple, cell bodies and intercellular substance rose-red. This method is extremely useful for general work.

Eosin and Methylene Blue (Mallory).—Fix the specimens in mercuric chloride or Zenker's fluid.

Stain sections in a five to ten per cent. aqueous solu-

tion of eosin for from five to twenty minutes. Stain in Unna's alkaline methylene blue solution, diluted with from one to ten parts of water, for from one-half to one hour. Wash in water. Differentiate and dehydrate in ninety-five-per-cent. alcohol until the pink color returns in the sections. Clear in xylol and mount in xylol balsam or xylol dammar.

Nuclei stain blue, intercellular substance and cytoplasm red.

Special Stains.—*Biondi-Heidenhain Triple Stain.*—Saturated aqueous solution of orange G, 100 c.c.; saturated aqueous solution of acid fuchsin, 20 c.c.; saturated aqueous solution of methyl green, 50 c.c.

Prepare the above solutions and let them stand for several days with an excess of the dyes. Then mix the solutions and dilute with water in the proportion of 60 c.c. of the staining solution to 100 c.c. of water. This dilute solution should redden upon the addition of hydric acetate; a drop of the mixture placed on filter paper should show a bluish-green centre and an orange periphery. If the orange zone is surrounded by a red zone, the mixture contains too much fuchsin.

Sections should be stained for from six to twenty-four hours. Those of tissues fixed in mercuric chloride give the best results. After staining wash in water; dehydrate in alcohol; clear in xylol; mount in xylol balsam or dammar.

Picro-Acid Fuchsin (Van Gieson).—Van Gieson introduced this stain for staining the peripheral nerves and sections of the central nervous system. His solution was not made up with any definite proportion of the acid fuchsin.

As the results of my experiments I have succeeded in establishing the following formulæ for the staining fluid: For connective tissue the proportions giving the best results are 5 c.c. of a one-per-cent. aqueous solution of acid fuchsin to 100 c.c. of a saturated aqueous solution of picric acid. For sections of the central nervous system the proportion of the acid fuchsin has to be increased and that of the picric acid diminished. The elements in this tissue stain more slowly, consequently the prolonged action of the picric acid removes the hæmatoxylin from the nuclei. The proportions of the staining agents that have given me the best results are 15 c.c. of a one-per-cent. solution of acid fuchsin to 100 c.c. of a half-saturated solution of picric acid.

Sections of tissues hardened in Müller's fluid or mercuric-chloride solutions—alcohol hardening does not give as brilliant results—are first deeply stained in hæmatoxylin (v. Kahlden recommends alum carmine) and then well washed in water. They are then placed in the picro-acid fuchsin fluid for from one to three minutes. Sections of the central nervous system require fully three minutes, those from other organs, as a rule, require only one minute. After the sections are removed from the staining fluid they are washed in two alcohols. I find that the addition of a small amount of picric acid to the second alcohol increases the brilliancy of the yellow stain. The sections are cleared in oil of origanum cretici and mounted in balsam.

This staining fluid, when used as above described, stains connective-tissue fibres a bright red; protoplasm, muscular tissue, and red blood cells, yellow. The hæmatoxylin stain in the nuclei is usually changed in color; sometimes it shows purplish-red, at other times black. Axis cylinders, nerve cells, and neuroglia fibres stain red.

Kantorwicz uses 150 c.c. of a saturated aqueous solution of picric acid and 3 c.c. of a saturated aqueous solution of acid fuchsin.

He stains sections in Delafield's hæmatoxylin for half an hour and then in the picro-acid fuchsin for from three to five minutes. He recommends the stain for cell inclusions.

Weigert's Stain for Elastic Fibres.—Boil basic fuchsin 2 gm., resorcin 4 gm., in 200 c.c. of water; while this is boiling add 25 c.c. of liquor ferri sequichlorati and continue the boiling for five minutes. Allow to cool and filter. The dark precipitate remaining on the filter is dissolved in 200 c.c. of strong alcohol by boiling the filter in it. Bring up the solution to 200 c.c. by the addition of alcohol; cool and add 4 c.c. of hydric chloride.

Stain sections for from one-half to one hour. Wash in strong alcohol until the color ceases to come away, then clear and mount in balsam.

Elastic fibres stain bluish-black.

Unna's Stain for Elastic Fibres.—Orcein (Grubler's), 1 gm.; hydric chloride, 1 c.c.; alcohol (absolute), 100 c.c. Sections are placed in the stain and warmed at a temperature of 30° C. for from ten to fifteen minutes. The stain becomes thick from the evaporation of the alcohol. Wash in seventy-per-cent. alcohol; dehydrate in strong alcohol; clear in oil of origanum, and mount in balsam. Elastic fibres stain a deep brown color. If a nuclear stain is wanted stain in Unna's polychrome methylene blue.

Weigert's Stain for Fibrin.—Stain sections of tissue fixed in alcohol or Zenker's fluid in aniline-gentian-violet solution for five minutes; place the sections on a slide and absorb the surplus stain with filter paper; then treat with iodine, 4 gm.; potassium iodide, 6 gm.; water, 100 c.c., for one minute; wash with water; decolorize in aniline, 2 parts, and xylol, 1 part, wash in several changes of xylol; mount in xylol balsam. The fibrin threads stain blue.

Jenner's Blood Stain.—Grubler's water-soluble eosin 1.2-per-cent. aqueous solution, 100 c.c.; Grubler's *pure* methylene blue, 1-per-cent. aqueous solution, 100 c.c. These solutions are mixed, allowed to stand for twenty-four hours, and then filtered. The precipitate is dried on the filter at 55° C., then washed with distilled water; dried and powdered.

For use, a 0.5-per-cent. solution of the dried powder is made in *pure methyl* alcohol. Cover-glass preparations and slide smears of blood are stained by immersing them in this solution for from three to five minutes; they are then washed in water; dried thoroughly in the air, and mounted in balsam.

Metallic Impregnations.—*Silver Nitrate.*—This is used chiefly for staining the cement substance between cells, especially the endothelium of serous membranes, lymphatics, and blood-vessels. It also stains the basement substance of connective tissue and cartilage. It may be used either in the solid state or in solution. The tissues to be acted upon must be fresh.

For membranes, silver nitrate is used in solutions of 1 to 300 to 1 to 500. The fresh membrane (omentum or mesentery) is carefully removed and stretched over the rim of a porcelain dish, filled with distilled water, and is allowed to sag in the centre so that both sides of the membrane will be immersed in the water; it is washed carefully by agitating the dish, so as to remove any blood or albuminous substance that may be on the surface. Now pour off the water carefully, and fill the dish with a 1 to 500 aqueous solution of silver nitrate. Agitate the dish frequently, and after from twenty to thirty minutes—when the membrane has become semi-opaque—pour off the silver solution and wash carefully with distilled water. Now transfer to a dish containing alcohol and water (1 to 2) and expose to the sunlight until it becomes of a brown color. This requires from twenty minutes to half an hour. Preserve in glycerin and protect from the light. The silver is deposited in the cement substance between the cells as an albuminate, and after being reduced by the action of the light, shows as fine black or brownish lines.

The cornea may be treated with silver in solution or in the solid state. In treating the cornea with the solid salt proceed as follows: Remove the eye and rub the anterior surface of the cornea with a piece of silver nitrate. Excise the cornea and place in distilled water; brush with a camel's-hair pencil to remove the epithelium. Then expose the cornea to the action of the light until it has become brown. Preserve in glycerin.

Silver in solution is applied as follows: Submit the cornea of a freshly killed animal to the action of a jet of steam, and scrape off the opaque epithelium, gently, with a scalpel. Allow a five-per-cent. solution of silver nitrate to flow over it, from a pipette, for two or three minutes; then wash with a one-per-cent. solution of sodium chloride; excise and expose to the light in a mixture of alcohol and water (1 to 2) until it becomes brown. Preserve in glycerin.

By these processes the basement substance becomes stained yellowish-brown, the cell spaces appearing as clear, irregular-shaped openings.

For injecting the blood-vessels, a fourth-per-cent. aqueous solution is used. The animal is bled to death, and the vascular system is washed out with distilled water;* this is followed by the silver solution, which is allowed to act for ten minutes and is then washed out with distilled water. Bits of thin membrane are removed and exposed to the light until brown, and then mounted in glycerin. In warm-blooded animals the solution should be heated to 30° C.; in cold-blooded animals the injection may be made at the ordinary temperature.

Hoyer adds to a solution of silver nitrate, of known strength, concentrated ammonia until the precipitate which forms is seen to be just barely redissolved; then dilutes this solution with distilled water until it contains from 0.5 to 0.75 per cent. of silver nitrate. This solution stains the cement substance only, leaving the ordinary tissues unstained.

Instead of reducing the silver by light I have employed an aqueous solution of hydrochinon (1 to 1,000), to which is added a few drops of a saturated solution of ammonium carbonate to render it alkaline. The tissue, after being treated with the silver, is placed in this solution until it becomes brown, which requires from five to ten minutes; then it is washed thoroughly in water and preserved in glycerin.

Auric Chloride.—Gold is reduced in the bodies of cells of cartilage, connective tissue, cornea, and epithelium, leaving the intercellular substance free; also in the axis cylinders of nerve fibres. It is very uncertain in its action, but when successful beautiful pictures are obtained. Tissues submitted to its action must be fresh.

Cohnheim's Method: Cohnheim, who introduced this salt, proceeds as follows: Bits of fresh tissue are placed in a one-per-cent. solution of auric chloride until they become yellow, and are then exposed to the light in water acidulated with acetic acid until the gold becomes reduced—until it becomes of a deep purple color; this requires from one to three days. The specimens are preserved in glycerin.

Löwit's Method: Löwit employs the following method for nerve terminations in muscle: Bits of tissue are placed in a mixture of 1 part formic acid and 2 parts water, until they become transparent; then for ten or fifteen minutes in a one-per-cent. solution of auric chloride until they become yellow; then in the formic-acid mixture for twenty-four hours in a dark place; then for twenty-four hours in pure formic acid in the dark. After this they are washed in distilled water and preserved in glycerin.

Ranvier's Method: Bits of fresh tissue are placed in fresh filtered lemon juice until they become transparent; then are washed in water and placed in a one-per-cent. solution of auric chloride for fifteen to twenty minutes; then again washed in water, and placed in dilute acetic acid (2 drops to 50 c.c. of water), and exposed to the light for two to three days, when the reduction will become complete. In the case of muscle Ranvier places the tissue from the gold solution in a twenty-per-cent. solution of formic acid in the dark for twelve hours, when the reduction is complete.

He also employs the following mixture: One-per-cent. solution of auric chloride, 4 parts; formic acid, 1 part; boil and allow to cool. Bits of fresh tissue are placed in this mixture for twenty minutes; then washed in water, and placed in formic acid 1 part, water 4 parts, in the dark for twenty-four hours.

* Reich (Sitzber. d. Wien. Akad., 1873) uses a one-eighth to one-fourth per cent. solution of potassium nitrate in water for washing out the vessels.

Pritchard's Method: After the tissue has been treated with the gold solution, Pritchard reduces the gold with the following mixture: Amyl alcohol, 1 c.c.; formic acid, 1 c.c.; water, 98 c.c. The tissue is removed from the gold solution, washed in water, and placed in the above mixture for twenty-four hours in the dark, when it will probably have become of a violet color; if not, it is placed in a fresh quantity of the fluid for twenty-four hours longer. Wash in water and harden in alcohol.

Chrchtschonovitsch's Method: The fresh tissue is placed in a one-half-per-cent. solution of auric chloride for from thirty to forty-five minutes; then in distilled water for twenty-four hours; then in a saturated solution of tartaric acid, at a temperature of 50° C., until the gold is reduced; it is washed in water and hardened in alcohol.

Auric and Potassium Chloride.—This is of a more unvarying composition than the simple chloride; it is perfectly neutral in its reaction, and its solutions are more stable. It is used in solutions of the same strength as auric chloride. As a reducing agent Hoyer employs the ordinary pyrogallic acid developing solution used in photography. He adds one or two drops of this solution to 50 c.c. of water, and places the specimen in this for sixteen to twenty-four hours.

Osmic Acid.—The property possessed by osmic acid of staining fat black makes it a useful stain for the medullated nerve fibres. It also stains some other tissues various shades of black, but its action is uncertain. At present it is used chiefly for staining fat and nervous tissue.

It is best used in the form of vapor, as its penetrating power is then greater. The specimen to be submitted to the action of the vapor is pinned out on a cork fitted to a wide-mouthed bottle, in the bottom of which a few crystals, or a few cubic centimetres of a strong solution, of osmic acid are placed and left until the desired coloration is obtained.

It may also be used in solution in water or glycerin of the strength of from one-half to one per cent. Small bits of the tissue are placed in the solution for from one-half to twenty-four hours, and then washed thoroughly in water. Or a mixture of 1 part glycerin, 1 part alcohol, 1 to 3 parts water, and 1 part of a one-per-cent. solution of the acid may be used. Specimens may remain in this mixture for several days, until they are sufficiently stained.

All osmic-acid preparations should be preserved in glycerin.

Overstained specimens may be bleached by hydrogen peroxide.

Methods of Injecting.—*Self-injection of the Living Animal.*—This is accomplished by allowing a definite quantity of blood to escape from an opening in a vein of a living animal, and replacing the blood lost by some innocuous coloring matter, so that by the contractions of the heart the vessels are filled with much less injury than by an injection apparatus.

Chrzonszczewsky recommends a solution of 7.75 gm. of carmine in 3.6 c.c. of ammonia, to which is added 30 c.c. of distilled water. This fluid is to be filtered before injecting. Ten cubic centimetres of blood are removed from the jugular vein of a medium-sized rabbit, and 10 c.c. of the above fluid injected. If the larger vessels are now rapidly ligated, first the vein and then the artery, a physiological injection of the blood-vessels is obtained. Good results are obtained in the case of the kidney, spleen, etc. The injection may also be accomplished by placing the above fluid in the stomach, rectum, and abdominal cavity; and in amphibia by placing it in the lymph cavities. After the injection is accomplished the organs are placed in acidulated alcohol, to cause the fixation of the carmine.

This solution, as well as the sulphindilate of sodium, when injected in large quantities, is rapidly excreted by the kidneys, and the latter substance is precipitated in the biliary passages. If the ureter is ligated immediately after injecting a rabbit and the animal is killed at the end of an hour, the urinary tube will be found filled with carmine. In injecting the biliary passages it is not nec-