Neither buccal nor post-buccal annuli united on the ventral side. Both genital orifices in somite XI., and behind them a group of prominent copulatory glands.

Macrobdella decora Verrill.—*Hirudo decora* Say 1824, *H. decora* Leidy 1868. Jaws with about sixty-five teeth, copulatory glands opening by four pores in a quadrate figure on the last annulus of XIII. and the first two of XIV. The first annulus of XXVI. is divided marginally and sometimes also dorsally into two annuli.

This species is widely diffused in fresh waters of the United States, having been reported from Maine to Minnesota and from Pennsylvania to Kansas. It is frequently used instead of imported leeches by physicians and is said to be equally efficacious, although its capacity is somewhat smaller, only about 5 gm. It is so powerful, however, that serious results have followed its attacks upon the legs of children wading in its haunts.

Among the land leeches which form a special section, Reptantia, of this family, and are distinguished by the absence of an eyeless ring between the third and fourth pair of eyes, a few forms deserve brief mention.

Hæmadipsa Tennent. Body almost round, sucker separated only by a slight constriction. Near the sucker auriculæ with the pores of the last pairs of nephridia. Typical metamere with five annuli.

Hæmadipsa zeylanica Moq.-Tand.—*H. japonica* Whitman. Length 20 mm., or in extended form 60 mm., diameter of body behind 6 mm., in front 2 mm.; sucker 5 to 7 mm. in diameter. Annuli ninety-seven; male genital pore between annuli 30 and 31, female between 35 and 36. In each jaw ninety incurved teeth.

The animal, when gorged, has the form of a flask of about 10 mm. in diameter at its largest part, while the neck measures only 2 or 3 mm. in thickness. The color is exceedingly variable, and yet five or six varieties may be recognized, one of which, var. *japonica* Whitman, known in Japan as the mountain leech, is common in the entire Indomalayan region, China, and Japan. It has long been known on the island of Ceylon, where it forms a veritable pest to natives and Europeans alike. The leeches appear in immense swarms, particularly in moist regions and during the rainy season, and at all levels from the coast up to 4,000 feet, although they occur even as high as 15,000 feet in the Himalayas. During the dry season they hibernate in the earth; at other times they live in moss among stones and on shrubs and trees. The approach of large animals brings them out in myriads; they move with surprising activity, and springing on the unfortunate passer-by they suck often for hours before falling off. No clothing is close enough to protect man from their attacks, and during army manœuvres they have inflicted large losses on European troops, attacking the soldiers even when asleep. Accounts of travel in Ceylon abound in narratives of the ferocity of these pests, and recent writers from the Philippines comment on their activity in that country in equally forcible terms. It is probably this species, and very likely the variety *japonica*, which is reported by Blanchard as abundant in the Philippines, to which the trouble is due, although other species of *Hæmadipsa* manifest, no doubt, similar habits of life.

The bite is not poisonous, as often maintained, but the large number of wounds and careless treatment afford abundant opportunity for secondary infection of a serious character. Among the natives of Ceylon one may see many deformities induced by these leeches, which are accordingly feared more than serpents and carnivores.

An allied form, *Phytobdella Meyeri* R. Bl., is also recorded from Luzon.

The family of the Rhynchobdellidæ, or proboscis leeches, is characterized by a protrusible pharynx, while the typical metamere consists of three annuli and jaws are lacking. Few of these forms are able to penetrate the human skin, but among those of sufficient power are especially American forms, which appear as occasional parasites of man and in some regions are used for therapeutic purposes. The two species of importance are both members of the following genus, which is restricted to the warmer regions of the American continent:

Liostoma Wagler 1831—*Hæmenteria* de Fil. 1849. Body broad and flattened; back covered with many prominent papillæ; the first and third ring of each somite from VI. to XXII. inclusive split on the ventral surface so that on the ventral surface the space occupied by five pseudo-annuli corresponds to that occupied by the three adjoining dorsal annuli. Male genital pore between annuli 26 and 27, female between 28 and 29. Two pairs of eyes so closely set that they appear as a single pair.

Liostoma Ghilianii R. Bl.—*Hæmenteria Ghilianii* de Fil. 1849. Length of alcoholic specimen 190 mm., breadth 100 mm., corresponding to a probable length while living of 350 to 400 mm. Annuli seventy, together with three preocular; the first seven annuli form the oral sucker.

This giant form inhabits the basin of the Amazon and the swampy regions of Guiana. Blanchard reports that it attacks horses and cattle, and a few individuals are sufficient to kill even a full-grown animal.

Liostoma coccineum Wagler 1831—*Hæmenteria officinalis* de Fil. 1849, *H. mexicana* de Fil. 1849, *Glossiphonia*

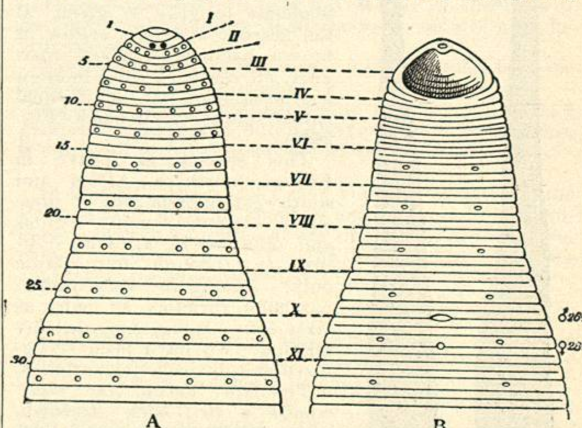

Fig. 2658.—*Liostoma coccineum*, or Mexican Leech. Diagrammatic view of anterior somites, *A*, from dorsal surface with eyes and sensillæ, *B*, from ventral with nephridial and sexual pores. (Modified from Blanchard.)

granulosa Jimenez 1865. Length 80 mm., breadth 22 mm. Annuli seventy, with two preocular; the first six annuli form the oral sucker (Fig. 2658).

The range of this species extends from Mexico to Paraguay through Central and South America. In Mexico it is universally used as the medicinal leech. In some instances, however, its bite appears to be accompanied by serious results. There are noted almost immediately a feeling of general lassitude and an unpleasant itching and twitching, succeeded soon by general urticaria, and in a short time apoplectic symptoms are manifested; but cases occur in which the cerebral congestion or the urticaria is wanting. Most authors incline now to the belief that these symptoms are due to the salivary secretion poured into the bite by the leech, and yet it seems also clear that some predisposition on the part of the individual is also a prerequisite. Brandes suggests that the symptoms of poisoning are manifested only in persons exhibiting hyperæsthesia, and attributes the rare cases of serious illness following the application of *Hirudo medicinalis* in Europe to the same sensitive constitution. (Compare in this connection the effects produced by the bite of *Argas*, as reported by Brandes and noted in the article *Arachnida*, Vol. I., p. 437.)

In spite of these accidents this is actually the only species used for medicinal purposes in Mexico, sufficient evidence of its generally satisfactory character. In some provinces (Guadalajara) the leech is regularly cut in two after it has taken hold in order to measure definitely the quantity of blood extracted. *Henry B. Ward.*

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HISTOLOGICAL TECHNIQUE.—Methods of Studying Living and Fresh Tissue.—For observations on living tissue, we now make use of the cold-blooded ani-

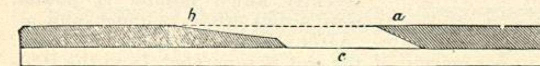

Fig. 2659.—Schulzer's Larva Holder.

mals almost exclusively; and, on account of the convenience, use the tails of amphibian larvæ, and small fishes and frogs. For holding these small animals, while under observation, the larva holder of Schulzer (Fig. 2659) will be found very convenient. The head of the animal is placed under the edge, *a*, the tail is spread out on the bevelled plate, *b*, and covered with a cover-glass. The cell holds sufficient water to cover the animal. To keep the animal quiet, we wrap it loosely in a piece of cloth, leaving the gills free, or add a few drops of ether to the water. If the observations are to be continued for any length of time, provision must be made for the renewal of the water. This can be accomplished by any of the usual methods of irrigation.

For observations on the frog, it is necessary to paralyze the animal with curare. A slight nick is made in the skin over the posterior part of the head, and two or three drops of a one-twentieth-per-cent. solution of curare* is injected into the dorsal lymph sac by means of a long, slender pipette introduced through the above nick. The exact amount of curare to be used will depend upon its quality and the size of the animal, and can be determined only by experiment. In the course of a few hours the animal will become completely paralyzed, while the vegetative functions continue, the necessary amount of oxygen being supplied by cutaneous respiration.

We utilize for these observations:

1. *The Web.*—The advantage of this part of the animal is that we do not inflict any injury, consequently we are not likely to meet with any disturbances of the vital processes; but, on account of it not being very transparent, it is inferior to other parts. A frog poor in pigment should be selected, and after being wrapped in a moist cloth it is laid on an oblong sheet of cork, in one end of which a hole, at least 15 mm. in diameter, is made; at the edges of this hole four or five pins are stuck, to which bits of soft string attached to the toes are tied, spreading the web out over the hole. The cork is now placed on the stage of the microscope, and the web is moistened at intervals to prevent its drying.

2. *The Tongue.*—For observations on this and other organs of the animal, Professor Thoma has invented a series of frog plates, which are shown in Fig. 2660. The one in the centre of the figure is for the tongue; that on the left for the mesentery; that on the right for the lung and bladder. These plates consist of a bed plate, *a*, of brass, covered by a thin sheet of hard rubber. At *B* is an opening, which varies in the different plates, covered with a thick glass plate on which the organ to be examined is placed. At some distance from this glass plate runs the brass rim, *c*, *c*, *c*, 7 mm. high, which by a proper inclination conveys the irrigating fluid, as it flows off the organ, to the tubes, *d*, *d*, to which are attached rubber tubes leading to a vessel for receiving the waste fluid. The supports, *t*, are for holding the irrigating cannula, *g*. They are pivoted to the plate, and move on a perpendicular axis; to the upper end is attached a short split tube, which is tightened by a small screw; this is connected with the support by a hinged joint, allowing it to be moved on a horizontal axis. In this tube is placed the glass irrigation cannula, *g*. It will be noticed that in the tongue and mesentery plates two supports are provided. This is to allow of the use of two cannulæ, one for irrigating the upper, and the other the under, surface of the organ. At *e* is a perpendicular rod for supporting the ring holding the cover-glass. At each side of the plates (in the tongue plate it is at the end) is a notched support, *k*, for holding the rubber tube attached to the cannulæ introduced into the different organs for inflation, etc. Between the rim, *c*, *c*, *c*, and the plate, *B*, bits of cork are wedged for pinning out the organs.

For examining the tongue, the animal is placed on the plate belly down, and the nose is brought close to the edge of the glass plate; the tongue is drawn out over the plate, and fastened to the bits of cork by pins which are cut off short.

3. *The Mesentery.*—Male frogs are to be used, so that the examiner may not be embarrassed with the ovaries. An incision is made through the skin, on the side, from the pelvis nearly to the axilla. After all hemorrhage has ceased, the abdominal cavity is opened by an incision of 10 to 20 mm. in length; a coil of intestine is drawn out carefully over the glass plate so that it will fall upon the bits of cork, to which it is pinned, leaving the mesentery spread out in a thin layer on the glass plate.

4. *The Bladder.*—A glass cannula (*B*, Fig. 2661) is filled with a three-fourths-per-cent. salt solution, and the rubber tube closed with a bit of glass rod. The cannula is now inserted into the cloaca, and directed forward into the bladder; it is held in place by a thread passed through the skin over the sacrum and tied around the cannula. An incision, similar to the one for the mesentery, is made in the side of the animal. The glass rod is removed from the rubber tube, and the latter is raised slightly so

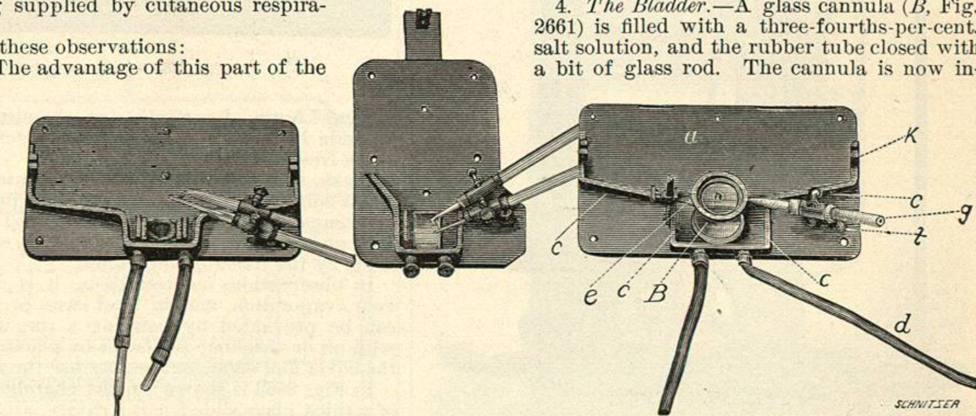

Fig. 2660.—Thoma's Frog Plates.

* S. H. Gage recommends the following solution: Curare, 0.2 gm.; ninety-five-per-cent. alcohol, 20 c.c.; water, 20 c.c. Grind up the curare in a mortar with the water and alcohol. Do not filter.

as to cause the fluid to flow into the bladder, distending it. The animal is now placed on the frog plate, and by gentle manipulation with the handle of a scalpel the distended organ is brought upon the glass plate and fur-

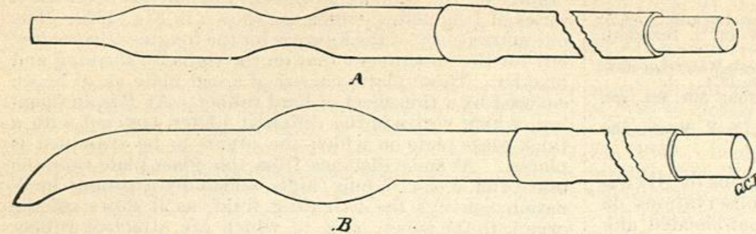

FIG. 2361.—Inflation Cannulæ.

ther distended if necessary; the glass rod is now replaced in the rubber tube, and the latter fixed in the support (*k*, Fig. 2660). The animal is covered with a bit of moistened filter paper, and the frog plate is placed on the microscope.

5. *The Lung.*—The cannula (*A*, Fig. 2661) is introduced through the epiglottis, and held in place by a thread passed through the nose and tied around the constriction of the cannula. An incision, carried well into the axilla, is made through the skin on the side of the animal, and when all hemorrhage has ceased the thoracic cavity is opened. The operator should now remove the bit of glass rod from the end of the rubber tube attached to the cannula, and gently blow into the same, when the distended lung will be forced through the incision. The animal is placed on the frog plate, and the distended lung brought upon the plate *B*. In Fig. 2662 is shown the arrangement of the frog plate on the microscope, and the irrigating bottle. The stage of the microscope is to be inclined so as to cause the irrigating fluid to flow away. The bottle attached to the ring stand, filled with a three-fourths-per-cent. salt solution, is closed tightly with a rubber cork,

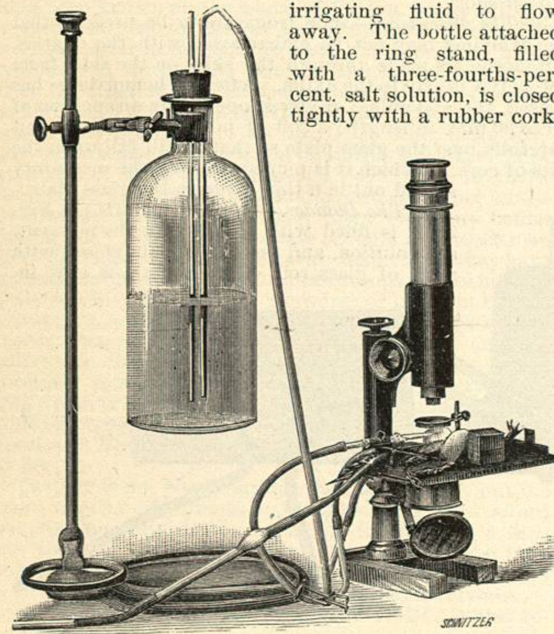

FIG. 2662.—Frog Plate and Irrigating Apparatus.

through which pass two glass tubes. To one is attached a rubber tube, which is connected with the glass cannula (*g*, Fig. 2660) and conveys the irrigating fluid. The other is for regulating the pressure, which can be varied by raising or lowering the tube. The flow from the end of the irrigating cannula should be by drops, at short intervals, and is regulated by the pressure in the bottle, the size of the opening in the point of the cannula, and, if necessary, by a spring clip placed on the rubber supply-tube.

Fresh tissues are to be examined in the fluid that bathes them during life, or in a fluid that will change them but little if at all. Such fluids are known as indifferent fluids, and resemble in composition the natural fluids of the body. They are as follows:

Aqueous Humor of the Eye.—Obtained by puncturing the cornea of a recently killed animal, and allowing the aqueous humor to escape.

Blood Serum.—The blood of a recently killed animal is poured into a tall glass cylinder and allowed to coagulate. After coagulation has taken place, the upper margin of the clot is separated from the sides of the vessel, to permit it to sink; the vessel is allowed to stand for twenty-four hours, and the clear serum is then drawn off with a siphon, care being taken not to disturb the clot.

Iodized Serum.—Prepared by adding to every 1,000 c.c. of blood serum, obtained as above, 10 c.c. of tincture of iodine. This fluid alters the tissues slightly and stains them yellow. Instead of blood serum, amniotic or pericardial fluid may be used, but it must be absolutely fresh.

Artificial Serum.—This is to be used only when the natural serous fluids cannot be obtained. It is prepared by dissolving 2 gm. of sodium chloride and 28 gm. of egg albumen in 250 c.c. of distilled water, and adding 2.3 c.c. of tincture of iodine; the solution is then filtered.

Salt Solution (three-fourths per cent.).—Made by dis-

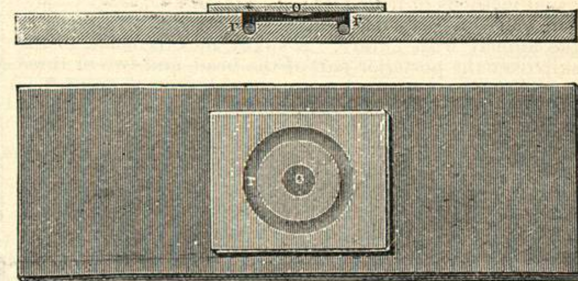

FIG. 2663.—Moist Chamber.

solving 7.5 gm. of perfectly dry, chemically pure sodium chloride in 1,000 c.c. of distilled water. This solution alters fresh tissues but slightly.

Fresh, thin membranes can be examined in one of the above solutions without any previous preparation. Bits of organs, fibres, etc., are to be teased on a slide, in a drop of one of the indifferent solutions, or sections can be made by the freezing microtome.

In observations on fresh tissue it is necessary to prevent evaporation, and in most cases pressure. The first can be prevented by painting a ring around the cover with oil or vaseline; the latter by placing a hair between the cover and slide; or we may use the moist chamber.

In Fig. 2663 is shown a moist chamber. In the centre of a thick glass slide is cut a cavity, around the circumference of which is a groove (*rr*). The latter is filled with water; the specimen is placed on the cover-glass (*o*), or in the middle of the cavity.

METHODS OF APPLYING REAGENTS.—*Application of Fluids, Irrigation.*—A drop of the reagent is placed on the slide in contact with the edge of the cover-glass, and on the opposite side of the cover a bit of filter paper. The latter sucks out the fluid, which is replaced by the reagent flowing in on the other side. This process of ir-

rigation can be continued for any length of time, if the reagent be renewed as it becomes exhausted, and the filter paper as it becomes saturated.

Application of Vapors and Gases.—A simple way of applying a volatile reagent to a specimen is to put a drop

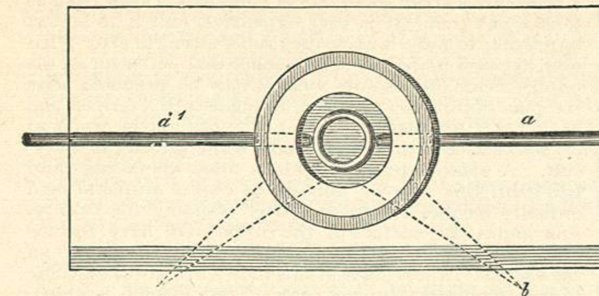

FIG. 2664.—Stricker's Gas Chamber.

of such reagent in a cell on a slide, and invert a cover-glass, on which the specimen has been placed, over it. For gases it is necessary to use a gas chamber. Such a piece of apparatus is shown in Fig. 2664.

It consists of a thick glass slide, in the upper surface of which is ground out a circular cavity. Two glass tubes are cemented into the two half-canals. The tube

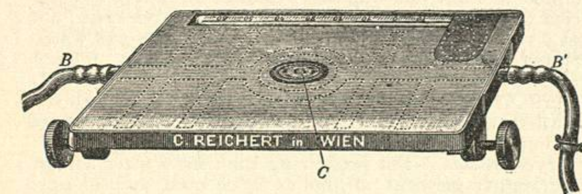

FIG. 2665.—Warm Stage.

a is connected by an india-rubber tube, a^2, with the gas generator, and conducts the gas to the chamber; the other, a^1, serves for its exit.

Application of Heat.—Fig. 2665 shows the latest form of warm, stage in which hot water, used for heating, or which, by the use of ice water, may also be converted into a refrigerating slide. The central opening *C* (Fig.

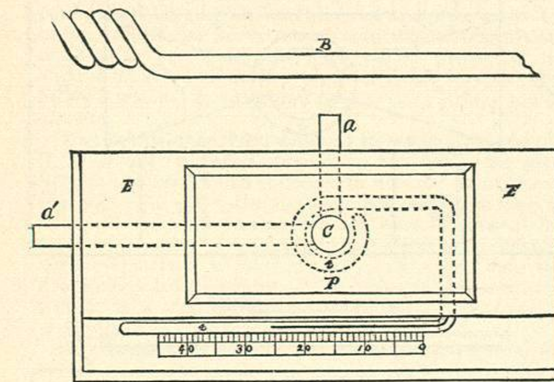

FIG. 2266.—Stricker's Warm Stage and Gas Chamber.

2665) permits the passage of light. At each end of the slide a small tube, *B*, *B'*, is inserted, by means of which it is connected with a vessel of water.

In Fig. 2666 is shown a combined warm stage and gas chamber. It consists of a rectangular piece of ebonite, *E*, *E*, fixed to a brass plate that rests on the stage of the microscope. On the upper surface of the ebonite is a brass plate, *P*, with an opening in the centre, *C*, leading into a brass tube, closed below by a piece of glass. For heating, the copper wire is placed on the tube *a*, the temperature of the plate being indicated by the thermometer *t*. The gas is conducted into the chamber by the tube *a'*, while *a* serves as an exit.

For long-continued observations at an elevated temperature Zeiss' warm chamber (Fig. 2667) for enclosing the microscope should be employed.

Application of Electricity.—For the application of the electric current to specimens, the slide shown in Fig. 2668 will answer as well as a more complicated piece of apparatus. This can be made as follows:

Take a glass slide 27 × 127 mm., and cover the surface with gold size; press the moist surface firmly down on gold leaf or tinfoil; allow it to dry, and then scrape away the metal so as to leave the two triangles *e*, *e'*, with an interval, *a*, between their apices, of about 5 mm. for the object. The specimen is placed at *a*, and covered with a cover-glass. For transmitting the

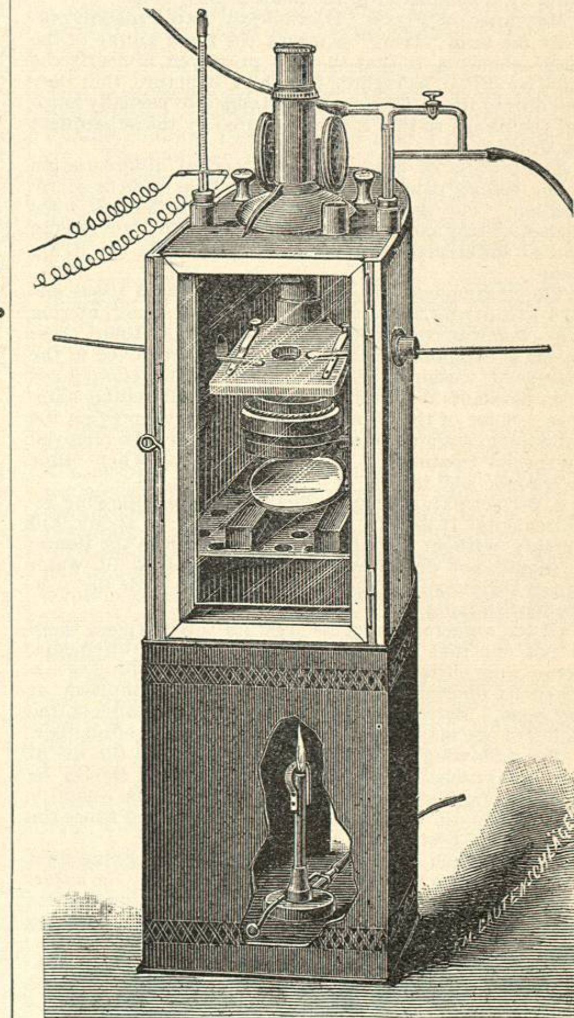

FIG. 2667.—Zeiss' Warm Chamber.

current the ends of the slide are clamped by the clips *c*, *c'*, to which the wires leading from the battery are attached.

In Fig. 2669 is represented a combined moist chamber and electric slide. The slide, *s*, is covered with tinfoil, *f*, on its upper and lower surfaces, the foil on the upper surface being carried to the upper edge of the glass cell, *c*, which is cemented to the slide. The slide is placed on the copper supports, *k*, *k*, that are attached to the stage, *p*, if it be a glass one; if not, they must be insulated by

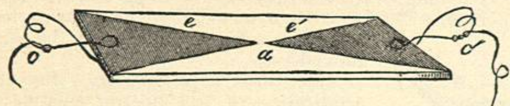

FIG. 2668.—Gold-leaf Electrodes.

strips of hard rubber. Two small strips of foil are cemented to the cover-glass, leaving a space of 5 mm. between the ends. The bottom of the cell is filled with a few drops of water; the specimen is placed on the cover-glass, in contact with the tinfoil, and the cover is inverted and placed on the cell, *c*, so that the foil comes in contact with that covering the cell. The electric current is conducted to the specimen through the metal supports by wires placed in the holes in their ends.

METHODS OF FIXING, HARDENING, AND PRESERVING.—By the term "fixing" is meant the rapid killing of the tissue elements, so that they are preserved in nearly the same condition, as regards form and structure, that they had during life. The process of fixing also partially hardens the tissue, so that it is not injured by the subsequent manipulations.

The process of fixing produces optical differentiation in the tissues causing alterations in their indices of refraction. The index is raised in varying degrees, some elements being more affected than others, thus producing optical differences that were not present in the living tissue.

The fixation causes a coagulation of certain tissue elements rendering them insoluble, the fixing agent entering into chemical combination with them. At times these chemical compounds are soluble and are removed in the process of washing. Some of the insoluble compounds form mordants to certain stains, giving differential stainings. Some of the chemicals used for fixing prevent the subsequent staining of the tissue unless they are removed by special treatment. This is especially so when osmic or chromic acid is used.

A perfect fixing reagent should meet the following requirements: It should penetrate rapidly; it should kill quickly, without having an injurious action on the tissue; it should not dehydrate, as the withdrawal of water causes shrinkage; it should not interfere with the subsequent staining.

Of the numerous reagents used for this purpose, there is not one that meets all of the above requirements. Some cause shrinking, others more or less swelling, while others fix nuclear structures well but not cytoplasm, or *vice versa*. By combinations of several fixing agents, the faults of one may be counterbalanced by those of another.

The following rules should be observed in the use of fixing solutions: The size of the specimen should be *small*, averaging about 0.5 c.c. in bulk, and the quantity of fluids should be *large*, as much as a hundred times the bulk of the specimen.

The specimen should be removed from the fixing fluid as soon as it has been thoroughly penetrated by the latter. This depends upon the fixing agent used, and on the size and density of the specimen. Usually from twelve to twenty-four hours is a sufficient time for proper fixation.

In some instances it is desirable to fix an entire organ. In such cases the fixing fluid is injected through the artery and allowed to flow from the vein for from one to two hours, after which the entire organ is immersed in a large quantity of the fixing fluid.

After the specimen has been fixed it is to be washed in running water until all of its fixing agent has been removed. The specimen is then further hardened in graded alcohols.

Hardening.—After the fixative has been thoroughly removed from the specimens they are then hardened by passing them through graded alcohols, beginning with sixty per cent. and increasing the strength by about ten per cent. at the end of each twelve hours until strong alcohol, ninety-five per cent., is reached.

Absolute alcohol as found in commerce varies from 98 to 99.5 per cent.; it is very expensive, and it is almost impossible to keep it up to the proper strength after it has been exposed to the air. An alcohol that will meet all the requirements of absolute alcohol may be prepared after the plan of Ranvier: Cupric sulphate is pulverized and heated at a temperature of 100° C. until all the water of crystallization is driven off, and a white powder is the result. A glass-stoppered bottle is filled about one-third full with this powder, anhydrous cupric sulphate, and ordinary alcohol is added. After standing for twenty-four hours, the surface of the copper will have become decidedly blue, from the absorption of the water from the alcohol. A test made at this time will show the alcohol to be over ninety-nine per cent. Fresh alcohol is added, from time to time, until all of the copper has become blue in color, when it is no longer of any use for dehydration. The supernatant alcohol is now poured off, and the bottle placed in a hot-air sterilizer at a temperature of 100° C. until the cupric sulphate is again rendered anhydrous.

Commercial alcohol usually tests about ninety-five per cent. and is known as strong alcohol. The graded alcohols used in hardening are prepared by diluting this strong alcohol with water. The amount of water to be added to each one hundred volumes of ninety-five-per-cent. alcohol is as follows: For making 60-per-cent., add 41.5 volumes of water; 70-per-cent., 21 volumes of water; 80-per-cent., 6.5 volumes of water; 90-per-cent., 5.5 volumes of water.

All fixing agents if allowed to act long enough will harden the tissues, but as a general rule their prolonged action renders the tissues brittle.

Preservation.—For preserving the tissues after hardening, eighty-per-cent. alcohol is to be used, as prolonged immersion in strong alcohol interferes with the subsequent staining of the specimens.

Osmic Acid is a very volatile substance, its vapor being extremely irritating to the conjunctiva and nostrils. It

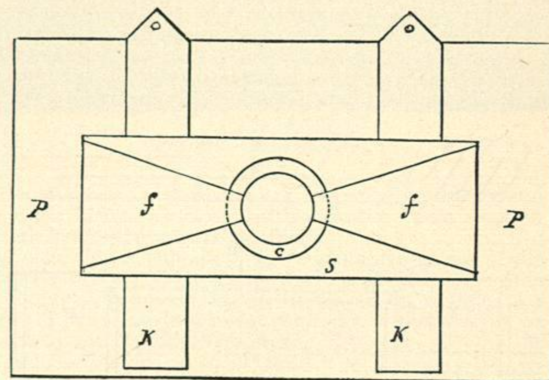

FIG. 2669.—Electric Slide and Moist Chamber.

is found in commerce in glass tubes, each containing 1 gm. or 0.5 gm. of the crystals. It is used in aqueous solutions of the strength of 0.1 to 2.0 per cent., or in combination with other acids in the proportion of 1 to 10,000.

For making a solution, a bottle is thoroughly washed with sulphuric acid, then with distilled water, that all traces of organic matter may be removed; then the label is removed from the tube, and the tube is washed in sulphuric acid and distilled water; the tube, its two extremities being broken off, is now put into the bottle, and the proper amount of distilled water is added to make a solution of the required percentage. The solution is to be kept in a well-stoppered bottle protected from the light. According to Cori this solution will keep perfectly, if enough potassium permanganate be added to give the fluid a slight rosy tint. As the fluid becomes colorless small quantities of the permanganate should be added to restore the tint.

Solutions that have deteriorated by the reduction of the osmic acid may be restored by the addition of a few drops of hydrogen peroxide.

Osmic acid is more valuable as a fixing than as a hardening agent. It fixes cytoplasm perfectly, nuclei badly. Its penetrating power is but slight, so that exceedingly small pieces of tissue must be used, and even then its action will be confined chiefly to the superficial parts. The vapor of osmic acid has a much greater penetrating power.

Tissues to be submitted to the action of the vapor are pinned out on a cork, fitted to a wide-mouthed bottle, in which there are a few cubic centimetres of a one-per-cent. solution of the acid, and allowed to remain until they become of a brownish color. After fixing, the excess of the osmic acid must be thoroughly removed by washing in running water. Any acid remaining will cause an over-blackening and it will also interfere with the subsequent staining.

Any over-blackening is best removed by immersing the tissue in a mixture of one part of hydrogen peroxide and twenty-five parts of seventy-per-cent. alcohol (Overton).

Osmic acid stains fatty matter black and is used as a stain for fat.

For increasing the penetrating power of osmic acid, Kolossow uses a one-half-per-cent. solution in a two- or three-per-cent. aqueous solution of uranium nitrate. Lately he has recommended the following mixture: Absolute alcohol, 50 c.c.; distilled water, 50 c.c.; hydric nitrate, 2 c.c.; osmic acid 1 to 2 gm. This mixture, he states, keeps well.

Chromic Acid.—Chromic acid is an exceedingly deliquescent salt, and in the presence of organic matter is readily reduced to the sesquioxide. It should be kept in ten-per-cent. aqueous solution, which may be readily diluted to the required strength for use.

It is used in aqueous solutions of one-tenth to one per cent. as a fixing agent, and allowed to act for a few hours only. For fixing nerve tissue, solutions of one-fiftieth to one-eighth per cent. are used. The pieces of tissue should be small, and after removal from the fluid should be well washed in water and preserved in eighty-per-cent. alcohol.

Mayer rinses the fixed material in water and then places it in seventy-per-cent. alcohol in the dark; the alcohol is changed daily until it ceases to become colored by the chromic acid; then the tissue is hardened in strong alcohol. Any chrome compound that may be present in the sections is removed by soaking the sections in 100 c.c. of seventy-per-cent. alcohol, to which is added four to six drops of hydric chloride. After being in this mixture for a short time the sections become perfectly white and stain well with the usual stains.

As a hardening agent, chromic acid is used in aqueous solutions of one-sixth to one-half per cent. It is very slow in its action, taking weeks, and even months if the specimen is large. As a rule, the pieces of tissue must be small, 2 to 3 cm. on a side, and the quantity of fluid large, 200 c.c. for each cubic centimetre of tissue. The fluid must be renewed at the end of the first, third, and fifth days. After the hardening is completed the specimen is placed in water for a day or two, to remove all traces of the acid, and then preserved in alcohol. After partial hardening in chromic acid, the action can be completed in alcohol, care being taken thoroughly to wash out the former before placing the specimen in the latter. Two or three weeks' immersion in the acid will be sufficient if the hardening is to be completed in alcohol.

The action of chromic acid seems to be analogous to that of tanning. The acid enters into combination with the tissues, rendering them tough like leather. A prolonged action of the acid will render them brittle.

Chromic acid has the property of softening bone, and is often used as a decalcifying agent. The acid is an excellent hardening agent for the central nervous system, nerve tissue, glandular tissue, and epithelium.

Chromic Acid and Alcohol.—Klein used a mixture of two parts of a one-sixth-per-cent. solution of chromic acid and one part of alcohol for his investigations on cells, and claims that it gave better results than the ordinary reagents, even osmic acid.

Urban Pritchard uses the following mixture: Chromic acid 1 gm., water 20 c.c., alcohol 180 c.c. The chromic acid is dissolved in the water and added to the alcohol. This was used for hardening the cochlea.

Chromic and Picric Acids.—A mixture of ten parts of a saturated solution of picric acid, twenty-five parts of a one-per-cent. solution of chromic acid, and sixty-five parts of water makes an excellent hardening fluid. Its action is slow.

Chromic Acid and Platinic Chloride (Merkel's Fluid).—This is recommended by its originator as an excellent fixing agent, especially for the retina. The formula is: Platinic chloride, 1 gm.; chromic acid, 1 gm.; water, 800 c.c. The specimen is to be kept in this fluid for from two to three hours, then preserved in alcohol. Eisig allows specimens to remain three or four hours in this fluid, and then preserves them in seventy-per-cent. alcohol. Specimens so treated stain well with almost any stain.

Chromic and Osmic Acids.—Flesch recommends the following mixture: One-per-cent. solution of osmic acid 10 parts; one-per-cent. solution of chromic acid, 25 parts; water, 65 parts. Specimens remain in this mixture for from twenty-four to thirty-six hours. The hardening is completed in alcohol and the specimen is preserved in the same.

Chromic and Acetic Acids.—One-per-cent. solution of chromic acid, 20 to 25 c.c.; one-per-cent. solution of hydric acetate, 10 c.c.; water, 70 c.c. This solution is recommended by Flemming when the after-staining of the specimen is to be effected with hæmatoxylin. These preparations do not stain well with aniline dyes.

Chromic and Formic Acids.—Ralb adds from four to five drops of formic acid to 200 c.c. of a 0.33-per-cent. solution of chromic acid. This mixture must be prepared fresh at the time of using. Small pieces of tissue are fixed for twenty-four hours and then washed in water.

Flemming's Fluid.—First Formula: Chromic acid, 0.25 gm.; osmic acid, 0.1 gm.; hydric acetate, 0.1 c.c.; water, 100 c.c. Second Formula: Chromic acid, one-per-cent. aqueous solution, 15 parts; osmic acid, two-per-cent. solution, 4 parts; hydric acetate, 1 part.

Small pieces of tissue are fixed in this fluid for from twelve to twenty-four hours, then well washed in running water and hardened in graded alcohols. These fluids were used by Flemming in his investigations on cell division. They fix the nuclear structures perfectly, but the cytoplasm not so well.

Fol's Modification.—Osmic acid, one-per-cent. solution, 2 parts; chromic acid, one per cent. solution, 25 parts; hydric acetate, two-per-cent. solution, 8 parts; distilled water, 68 parts.

Carnoy's Modification.—Osmic acid, two-per-cent. solution, 16 parts; chromic acid, two-per-cent. solution, 45 parts; hydric acetate, 3 parts.

Hermann's Modification.—Chromic acid, one-per-cent. solution, 12 to 15 c.c.; hydric acetate, 1 to 2 drops; osmic acid, 0.25-per-cent. solution, 8 to 10 drops.

Vanlair's Modifications.—I. Osmic acid, one-half-per-cent. solution, 40 parts; chromic acid, one-half-per-cent.

solution, 140 parts; hydric acetate, 10 parts. II. Osmic acid, one-half-per-cent. solution, 10 parts; potassium dichromate, one-half-per-cent. solution, 10 parts; hydric acetate, 2 parts.

Hermann's Fluid.—This is a modified Flemming's fluid in which platinic chloride is substituted for the chromic acid; its composition is as follows: Platinic chloride, one-per-cent. aqueous solution, 15 parts; osmic acid, two-per-cent. solution, 2 parts; hydric acetate, 1 part.

Potassium Dichromate.—The action of this salt is similar to that of chromic acid. It is used in aqueous solutions of one to two per cent. for fixing, and two to five per cent. for hardening. Its action is slow, and it has the advantage of never rendering the tissues brittle.

Müller's Fluid.—This fluid was first introduced by H. Müller for hardening the retina. It is an excellent hardening fluid for the majority of tissues, especially for the central nervous system. Its action is slow, but its penetrating power great. It requires a month to six weeks to complete the hardening of a spinal cord. If the temperature of the fluid is kept at 30° to 40° C., the time is reduced to from eight to ten days (Weigert).

The composition of the fluid is as follows: Potassium dichromate, 2 to 2.5 parts; sodium sulphate, 1 part; water, 100 parts. It should be used in large quantities, renewed at the end of twelve hours, then every third day until the hardening is completed. Then wash well in water and preserve in alcohol. (See Orth's fluid under Formalin Mixtures.)

Kultzschitzky recommends the following for fixing the gastro-intestinal canal: Potassium dichromate, 2 gm.; mercuric chloride, 0.25 gm.; hydric acetate, two-per-cent. solution, 50 c.c.; alcohol, 50 c.c. Some of the dichromate precipitates out, so the fluid must stand for a few days and then be filtered. The specimen is to be fixed for from three to five days; then washed in water and hardened in graded alcohols.

Dichromate-osmic-platinic Mixture (Lindsay Johnson). Potassium dichromate, 2.5 gm.; osmic acid, two-per-cent solution, 10 c.c.; platinic chloride, one-per-cent. solution, 15 c.c.; hydric acetate, 5 c.c.; water, 70 c.c. Dissolve the dichromate in the water and then add the other ingredients. This mixture is an excellent fixing fluid for the most of tissues, though used first by Johnson for the retina.

Erlicki's Fluid.—This is also an excellent hardening fluid for the central nervous system. It consists of potassium bichromate, 2.5 parts; cupric sulphate, 0.5 part; water, 100 parts. At a temperature of 30° to 40° C. it hardens the spinal cord in four days.

Tellyseniczky's Fluid.—Potassium dichromate, 3 gm.; hydric acetate, 5 c.c.; water, 100 c.c. Small pieces of tissue in this fluid are fixed for from one to two days and then washed well in water. This is an excellent fixative for cytoplasm and fair for nuclear structures.

Ammonium Dichromate.—This is used in one to two per cent. solution in water, in place of the previous salt, for hardening the central nervous system. If 1 c.c. of ammonia be added to each 100 c.c. of potassium dichromate solution, the color changes to a bright yellow, and its power of penetration is increased.

Ammonium Chromate (Neutral Chromate).—As a fixing agent this salt is used in five-per-cent. solution, and allowed to act for twenty-four hours. The specimen should afterward be washed well in water and preserved in alcohol.

Picric Acid.—Used in cold saturated solution in water, 1 part of picric acid to 86 parts of water at a temperature of 15° C. The bits of tissue must be small—1 to 2 cm. on a side for each 100 c.c. of fluid. The solution must always be in a state of saturation; to accomplish this a few picric-acid crystals are placed in the bottle, and the latter is shaken occasionally. After fixing, the tissues *should always be washed in alcohol and not in water.* A few drops of a saturated solution of lithium carbonate in water, added to the alcohol, will hasten the process.

This reagent acts by gradually transposing the albumin into an insoluble compound, so that tissues harden in it entirely without shrinking. As the tissues become less solid than with other reagents, it is necessary to complete the hardening in alcohol. The majority of tissues are stained yellow, but the color is withdrawn by alcohol, water, glycerin, etc.

Picro-acetic Acid.—Bolles Lee recommends a saturated solution of picric acid in a one-per-cent. solution of hydric acetate.

Boveri dilutes a saturated aqueous solution of picric acid with two volumes of water and adds one per cent. of hydric acetate.

These solutions are recommended for fixing cellular elements.

Picro-chromic Acid (Fol).—Saturated aqueous solution of picric acid, 10 parts; chromic acid, one-per-cent. aqueous solution, 25 parts; water, 65 parts. At the time of using add 0.005 gm. of osmic acid.

Picro-hydrochloric Acid (Mayer).—Water, 100 parts; hydric chloride, 8 parts; picric acid to saturation.

Picro-nitric Acid (Mayer).—Water, 100 parts; hydric nitrate, 5 parts; picric acid to saturation.

Picro-sulphuric Acid (Kleinenberg).—Distilled water, 100 parts; hydric sulphate, 2 parts; picric acid to saturation.

Picro-mercuric Chloride (Mann).—Picric acid, 4 gm.; mercuric chloride, 15 gm.; tannic acid, 6 to 8 gm.; alcohol, 100 c.c. Recommended for all animal tissues. Fix for twenty-four hours at the body temperature of the animal, then wash in iodine alcohol.

Picro-platinic-Osmic Acid (von Rath).—Saturated aqueous solution of picric acid, 200 c.c.; platinic chloride, 1 gm. dissolved in 10 c.c. of water; hydric acetate, 2 c.c.; osmic acid, two-per-cent. solution, 25 c.c.

Picric Alcohol (Altman).—Picric acid, 2.5 gm.; alcohol, ninety-five per cent., 35 c.c.; water, 70 c.c.

Picric Alcohol (Gage).—Picric acid, 2 gm.; alcohol, ninety-five per cent., 500 c.c.; water, 500 c.c.

Fix in the above solutions for from one to two days. Wash out in graded alcohols.

Picro-formalin (Graf).—Graf recommends the following solutions for cytological work:

1. Sat. aq. sol. picric acid,	1 vol.,	Formalin, 5 per cent.,	1 vol.
2. " " " " "	1 "	" 10 " "	1 "
3. " " " " "	1 "	" 15 " "	1 "
4. " " " " "	95 vols.,	" full strength	5 vols.
5. " " " " "	90 "	" " "	10 "

Small pieces of tissue are fixed for thirty minutes; then washed for one hour in a large quantity of thirty-per-cent. alcohol; then in fifty-per-cent. alcohol; seventy-per-cent. alcohol; eighty-per-cent. alcohol; and finally preserved in ninety-five-per-cent. alcohol.

Picro-Formalin—Platinic Chloride (Bouin).—Platinic chloride, one-per-cent. aqueous solution, 20 parts; picric acid, saturated aqueous solution, 20 parts; formalin, 10 parts; formic acid, 5 parts.

Palladium Chloride.—Employed by Waldeyer in one-thousandth-per-cent. aqueous solution, with the addition of a few drops of hydric chloride to aid in the solution, for softening the cochlea. The specimen is placed in this solution for twenty-four hours, and then in absolute alcohol for the same length of time.

F. E. Schulze uses this salt in 0.01 to 0.2 per cent. solutions. As the penetrating power is slight, small pieces of tissue are to be used—a piece the size of a bean to 30 c.c. of the solution. The hardening is completed at the end of thirty-six to forty-eight hours, but the specimen may remain in the fluid for months without harm. This solution stains protoplasm dark yellow, striated muscle brownish yellow, smooth muscle straw yellow, medullated nerves black. Hyaline membranes and elastic fibres stain slight yellow and remain transparent. Interstitial substance of connective tissue remains colorless, and will stain deeply with carmine and other stains, while the tissue colored by the palladium remains unstained. Sections are to be well washed in water and mounted in glycerin.

Fraenkel recommends the following as a fixative for connective tissue: Palladium chloride, one-per-cent. aqueous solution, 15 parts; two-per-cent. solution of osmic acid, 5 parts; and a few drops of acetic acid.

Mercuric Chloride is an excellent fixing agent. It kills rapidly and does not interfere with the subsequent staining of the tissues. It is slow in penetrating so that small pieces of tissue must be used. As a fixative it is used in saturated aqueous solution (about seven per cent.); or in saturated solution in three-quarters-per-cent. solution of sodium chloride. The simple aqueous solution does not keep well, depositing after a time a fine white precipitate. This precipitation can be prevented by the addition of a slight amount of hydric nitrate (Bolles Lee).

A better fixation is obtained if a small quantity of hydric acetate, about one per cent., be added to the aqueous solution. Bolles Lee speaks highly of a saturated solution of mercuric chloride in five-per-cent. hydric acetate. Van Beneden uses a saturated solution in twenty-five-per-cent. hydric acetate.

The specimens should be left in the fixing fluid as short a time as possible. A prolonged immersion renders them brittle.

After fixation, the tissues are washed in seventy-per-cent. alcohol, to which tincture of iodine is added until the alcohol becomes of a mahogany color. If the alcohol becomes decolorized more iodine is added, and this is repeated until the alcohol ceases to lose color. Finally the specimens are washed in eighty-per-cent. alcohol to remove the iodine. This procedure must be followed with all specimens that have been fixed in a fluid containing mercuric chloride. They are then preserved in ninety-per-cent. alcohol, in which after prolonged immersion they become very brittle.

Alcoholic Solutions of Mercuric Chloride.—Mercuric chloride is more soluble in alcohol than in water, so that by the use of this menstruum a stronger solution may be obtained.

Gilson's Fluid.—Absolute alcohol, 1 part; hydric acetate, glacial, 1 part; chloroform, 1 part; mercuric chloride, to saturation. Small specimens are fixed for from twenty-five to thirty seconds, then washed in alcohol (see above).

Ohlmacher's Fluid.—Absolute alcohol, 80 parts; chloroform, 15 parts; hydric acetate, glacial, 5 parts; mercuric chloride, to saturation (about twenty per cent.). Small pieces of tissue are fixed for from fifteen to thirty minutes, and then washed out in iodine-alcohol.

Lang's Fluid.—First Formula: Mercuric chloride, 3 to 12 gm.; sodium chloride, 6 to 10 gm.; hydric acetate, 6 to 8 c.c.; distilled water, 100 c.c. Second Formula: Saturated solution of mercuric chloride in picro-sulphuric acid, to which is added five per cent. of hydric acetate.

Carnoy's Modification of Lang's Fluid.—Mercuric chloride, 5 gm.; sodium chloride, 5 gm., hydric acetate, 5 c.c.; water, 100 c.c.

Pacini's Fluid.—Mercuric chloride, 1 gm.; hydric acetate, 2 c.c.; water, 300 c.c.

Zenker's Fluid.—Mercuric chloride, 5 gm.; potassium dichromate, 2.5 gm.; sodium sulphate, 1 gm.; hydric acetate, 5 c.c.

The hydric acetate is added just before using. The specimen is fixed for from two to forty-eight hours; washed well in water, then in graded alcohols, and finally in ninety-per-cent. alcohol to which is added from one-half to three-quarters per cent. of tincture of iodine. This is an excellent fixing fluid, especially for muscular tissues. Tellyseniczky states that it is equal to Flemming's fluid for cellular structures.

Apathy's Fluid.—Alcohol, fifty per cent., 100 c.c.; sodium chloride, 0.5 gm.; mercuric chloride, 3 to 4 gm.

Rath's Fluid.—Alcohol, 200 c.c.; mercuric chloride, 1 gm.; hydric acetate, glacial, 2 c.c. Fix for from four to five hours.

Alcohol is used for the purpose of fixing tissues either in the form of one-third alcohol, "alcohol au tiers" of Ranvier, or absolute alcohol. The one-third alcohol consists of 1 part of ninety-per-cent. alcohol and 2 parts of water. Tissues are placed in this fluid for twenty-four hours; then stained in an alcoholic staining fluid. They should never be treated with an aqueous medium, except picrocarmine, alum carmine, or methyl green.

Absolute alcohol penetrates quickly and fixes fairly well, but there is apt to be some distortion on account of shrinkage. It hardens at the same time. Except in a few special cases alcohol should be discarded as a fixative and its use restricted to hardening and preserving.

Alcoholic Mixtures.

Carnoy's Fluids.—I.* Hydric acetate, glacial, 1 part; absolute alcohol, 3 parts. II. Hydric acetate, glacial, 1 part; absolute alcohol, 6 parts; chloroform, 3 parts.

The chloroform in the second formula makes its action more rapid.

Zacharias' Fluid.—Hydric acetate, glacial, 1 part; absolute alcohol, 4 parts; one-per-cent. solution of osmic acid, 2 to 3 drops to each 10 c.c. of the above.

Fix for from one to three hours in the above mixtures and then wash out in alcohol. These fluids give good fixation of mitotic figures and of the central nervous system.

Alcohol and Osmic Acid (Vignal).—Alcohol and one-per-cent. osmic acid, equal parts. After fixing wash in eighty-per-cent. alcohol.

Formalin.—Formalin is a forty-per-cent. solution in water of the gas formaldehyde (HCOH), an oxidation product of methyl alcohol. It is also known in commerce as "formol" and "formalose." The commercial product rarely tests over thirty-eight per cent. When exposed to the air a partial decomposition takes place with the formation of a white deposit, paraformaldehyde. The vapor of formalin is irritating to the mucous membranes. It also has an irritating effect on the skin, hardening it and causing the formation of annoying cracks which do not heal unless the hands are kept from coming in contact with the formalin.

Formalin penetrates rapidly and kills quickly, but unfortunately in most instances does not fix the tissue elements sufficiently to enable them to withstand the subsequent manipulations without change. On account of this fault pure formalin has been almost entirely abandoned as a fixative, but the agent is quite extensively employed in mixtures.

As a fixative it is used in the strength of from one to five per cent. In five-per-cent. solutions I have found it to be a good fixative for cystic tumors of the ovary, also for the mucous membrane of the uterus.

Bolles Lee has called attention to what he considers an inaccurate manner of stating the percentages used. He claims that the proper way of stating the strength of the solutions is to say "formalin diluted with so many volumes of water." It is now understood that when a percentage is given it means the stated percentage of commercial formalin and not of the gas formaldehyde.

The following is a table for formalin solutions:

100% formalin	= 40% formaldehyde		
50% "	= 20% "	= 1 vol. formalin	+ 1 vol. water.
25% "	= 10% "	= 1 " "	+ 3 " "
12½% "	= 5% "	= 1 " "	+ 7 " "
10% "	= 4% "	= 1 " "	+ 9 " "
5% "	= 2% "	= 1 " "	+ 19 " "
2½% "	= 1% "	= 1 " "	+ 39 " "
1% "	= 0.4% "	= 1 " "	+ 99 " "

As a preservative agent for gross specimens, formalin is used in the strength of from two to ten per cent., a five-per-cent. solution usually giving the best results. The weaker solutions cause more or less swelling of the tissues.

The quantity of the fluid should be large—one hundred times the volume of the specimen—and the fluid should be renewed at the end of twenty-four hours. In cases of large specimens it is well to renew the fluid a second or even a third time.

When used in this manner, formalin preserves the form and to a certain extent the natural color of the specimens. In some cases the blood color appears to be

* This is also known as Van Gehuchten's fluid.