bleached out, but if the specimen is placed in strong alcohol, this is nearly if not entirely restored.

For preserving the blood color of specimens, Johres makes use of the following procedure and fluid: Sodium chloride, 1 part; magnesium sulphate, 2 parts; sodium sulphate, 2 parts; water, 100 parts. To this mixture are added from five to ten parts of a forty-per-cent. solution of formalin. After the specimen has become sufficiently hardened, the formalin solution is poured off, the specimen is washed in ninety-five-per-cent. alcohol, then kept in ninety-five-per-cent. alcohol until the blood color becomes restored, and finally is preserved in a mixture of equal parts of glycerin and water.

A ten-per-cent. solution of formalin is the best hardening reagent for eyes. The cornea remains translucent and the vitreous humor retains its natural transparency perfectly.

For hardening the central nervous system it is used in the strength of from four to ten per cent. Sections stain well with Weigert's hæmatoxylin method.

Formalin Mixtures.—For fixing purposes formalin is now used almost entirely in combinations with acids and chemical salts.

Lovdowsky's Fluids.—I. Distilled water, 20 c.c.; alcohol, ninety-five per cent., 10 c.c.; formalin, 3 c.c.; hydric acetate, 0.5 c.c. II. Distilled water, 30 c.c.; alcohol, ninety-five per cent., 15 c.c.; formalin, 5 c.c.; hydric acetate, 1 c.c.

These fluids are recommended by Lovdowsky for fixing the mitotic figures of cells.

Cox's Fluid.—Mercuric chloride, saturated aqueous solution, 30 parts; formalin, 10 parts; hydric acetate, 5 parts. Used by the author for fixing the nerve cells of the spinal ganglia.

Orth's Fluid (Formalin Müller's Fluid).—Potassium dichromate, 2.5 gm.; sodium sulphate, 1 gm.; water, 100 c.c.; formalin, 10 c.c. This fluid must be freshly prepared each time as it soon decomposes. When it is used in large quantities the salts may be made up into solution and the formalin added at the time of using; or a solution of the salts may be made of double strength and diluted with an equal volume of twenty-per-cent. formalin at the time of using.

Small pieces of tissue are fixed in this fluid for from twelve to twenty-four hours; then washed in running water for from six to twelve hours; and finally placed in eighty-per-cent. alcohol.

This fluid is the best general fixing fluid we have.

Methods of Decalcification.—Specimens to be decalcified should be reduced to pieces about 2 mm. in thickness. This can be done with a fine saw. They are then thoroughly hardened and placed in the decalcifying fluid. When they are decalcified, which may be tested by passing a needle into the tissue, they are thoroughly washed in running water until all the acid is removed; they are then hardened in alcohol.

Hydric Chloride.—One part of strong hydric chloride is mixed with ten parts of water, and ten per cent. of sodium chloride is added. Without the addition of the latter the acid will cause some tissues to swell. The specimen of bone is placed in a large quantity of this fluid, and a little fresh acid added day by day until the bone has become soft. Then it is washed in water until all traces of acid are removed, and preserved in alcohol.

Hydric Chloride and Palladium Chloride.—Waldeyer employs a one-thousandth-per-cent. solution of palladium chloride with one-tenth of its volume of hydric chloride for softening the cochlea. Small pieces of bone are placed in this fluid for twenty-four hours. If they are not softened at the end of this time the fluid is renewed, and they are allowed to remain another twenty-four hours, when, as a rule, they will be found completely softened. The specimens are then washed in alcohol, which is renewed at the end of twenty-four hours. This fluid does not cause the tissues to swell, nor does it interfere with the staining.

V. Ebner recommends a two-per-cent. solution of hydric chloride in a half saturated solution of sodium chloride. This fluid is slow in its action and should be changed daily until the specimens are thoroughly decalcified. The specimens are then washed in a half saturated solution of sodium chloride, to which a few drops of ammonium hydrate are added, until the reaction of the bone becomes neutral.

Hydric Chloride and Alcohol.—Hydric chloride, 2.5 c.c.; alcohol, 500 c.c.; water, 100 c.c.; sodium chloride, 2.5 gm.

Or hydric chloride, 1 to 5 c.c.; alcohol, 70 c.c.; water, 30 c.c.; sodium chloride, 0.5 gm.

Hydric Nitrate.—This acid is highly recommended by Busch. He employs a ten-per-cent. solution for adult bones and a one-per-cent. solution for fœtal bones. Fresh bones are placed for three days in ninety-five-per-cent. alcohol; then placed in the nitric-acid solution, which is changed daily until decalcification is complete. This requires from eight to ten days. The bones are then washed in running water for two hours, and finally placed in ninety-five-per-cent. alcohol, which is renewed at the end of three days.

Fœtal bones are first placed in a mixture of one-per-cent. potassium bichromate and one-tenth per cent. of chromic acid for one or two days; then decalcified in one-per-cent. hydric nitrate. When thoroughly decalcified they are washed in water and preserved in alcohol.

Sections of bone treated by this method stain well with eosin, but staining with hæmatoxylin is seldom successful.

Hydric Nitrate and Alcohol.—Mayer uses a five-per-cent. solution of hydric nitrate in ninety-per-cent. alcohol; Thoma 5 parts of strong alcohol and 1 part of hydric nitrate, renewing the fluid daily until the specimens are decalcified.

Phloroglucin and Hydric Nitrate (Haug).—Phloroglucin, 1 gm.; hydric nitrate, 10 c.c. The phloroglucin is placed in a porcelain dish and the hydric nitrate added drop by drop; it should be warmed carefully, as the action of the acid on the phloroglucin is very energetic. When all the acid has been added, the solution is diluted with 100 c.c. of distilled water. This should give a clear ruby-red colored fluid. Then there must be added 10 c.c. of hydric nitrate, the solution being afterward brought up to 300 c.c. by the addition of water.

The phloroglucin acts as a protector to the organic constituents and has no decalcifying action.

The action of this fluid is very rapid. Fetal and young bones become soft in half an hour; older bones, like pieces of the femur, require from four to five hours.

After decalcification the specimens are washed in running water for two or three days.

This solution may be made with hydric chloride instead of hydric nitrate, 30 c.c. of the HCl being used.

A slower acting fluid can be made as follows: Phloroglucin, 1 gm.; hydric nitrate, 5 c.c.; alcohol, 70 c.c.; water, 30 c.c.

These solutions should be made fresh as they do not keep well.

Ferri's Solution.—Dissolve 1 gm. of phloroglucin, by the aid of heat, in a mixture of 100 c.c. of water and 10 c.c. of hydric chloride; when cold add 200 c.c. of seventy-per-cent. alcohol.

Picric Acid.—This is used in saturated aqueous solution. It is slow in its action. The specimen should be small, and be suspended in a large quantity of the fluid. The latter must be kept in a saturated condition by the addition of fresh crystals of the acid and frequent agitation.

Schmol recommends the following mixtures: A. Formalin, 100 c.c.; hydric nitrate, 20 c.c. B. Müller's fluid, 100 c.c.; hydric nitrate, 3 c.c.

Trichloracetic Acid.—This acid has been recommended for decalcification. It acts slowly, and is used in five-per-cent. solutions.

Methods of Dissociation.—*Mechanical.—Scraping and Teasing.*—Which of these methods is to be employed will depend upon the nature of the tissue. Epithelium can be easily scraped off in large flakes by a knife, while fibrous parts must be teased apart with needles. Scraping is performed as follows: A small bit of the tissue is placed on a slide, with the epithelial surface uppermost, one end fixed with a needle, and the surface lightly scraped with the blade of a scalpel, the result of the scraping diffused in a drop of water, salt solution, or glycerin on a slide, and examined.

For teasing we employ needles mounted in handles. Various forms of the latter are supplied by instrument-makers, but a needle forced into the end of an ordinary pen holder will answer every purpose. In order to tease a specimen successfully, it is necessary to have some idea of its structure, whether its elements are arranged in parallel fibres or interlaced. In tissues arranged in parallel fibres, the needles are applied to one extremity and the specimen is torn into two parts; one of these is selected and the operation is repeated until it is impossible to separate the fibres any more. In other tissues a small bit is fixed by one needle, and with the other, commencing at one edge, the teasing is carried on in a deliberate manner until the tissue is reduced to the required degree of fineness. The operation of teasing is performed on a slide in a small drop of fluid. Unstained material is placed on a black background, stained material on a white background.

Brushing.—This operation is performed under water by gently brushing the surface with a camel's-hair brush.

Shaking.—Thin sections of tissue are shaken in a test-tube with a suitable fluid, then the contents of the tube are poured into a dish of water, the sections being floated on a slide.

Compression.—By making pressure on the cover-glass, especially after maceration of the specimen, one is often able to separate the elements. This pressure can be made with the handle of a teasing needle, but it is apt to be unequal, and there is danger of breaking the cover and thus destroying the specimen. It is better to use an instrument called a compressorium, constructed especially for this purpose. In Fig. 2670 is shown one form, invented by H. Jung, of Darmstadt.

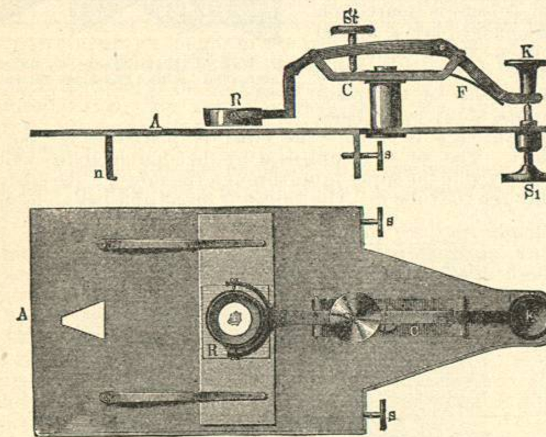

Fig. 2670.—Jung's Compressorium.

The plate *A* is attached to the stage of the microscope by the catch *n* and the two screws *s*, *s*. On this plate is a double lever, one arm of which has a movable ring, *R*, and adjusting screw, *St*, and the knob *K*. The two levers are so connected with the bent piece *C* that when *K* is pressed down the ring *R* is also pressed toward the stage plate. Conversely, an upward movement of the knob, produced by the spring *F*, raises the ring. For use as a compressor the screw S_1 is loosened, and *St* screwed down until the desired degree of pressure is obtained. This apparatus can also be employed for another useful purpose, viz., beating. The process of beating is often resorted to for the purpose of isolating cells, tissue, etc. In using the instrument for this purpose the screw *St* is so adjusted that the ring *R* lies close to a large and thick cover-glass covering the specimen, and S_1 is turned so that the lever can move but slightly. By quick and continuous movement of the lever, and the changing pressure on the cover-glass thus produced, tissues (after maceration) can be easily dissociated. When the cells are isolated they can be made, by a slower movement of the lever, to move about in all directions, so that they can be observed from all sides.

Interstitial Injection, or Artificial Œdema.—A hypodermic syringe is filled with salt solution or a warm solution of gelatin, the needle is inserted into the tissue—*e.g.*, the subcutaneous tissue of a freshly turned-up flap of skin—and the fluid is forced out. The fluid does not immediately diffuse itself, but forms a bulla covered with a thin film of tissue. A bit of this film is snipped off with scissors and examined. If gelatin be used, it soon solidifies, when thin sections are made with a razor.

Chemical Dissociation.—By submitting tissues to the action of different chemical solutions certain parts become dissolved or softened, while others remain unaltered. Consequently certain elements can be easily isolated by one of the mechanical methods.

The reagents used for this purpose are as follows:

Dilute Alcohol, "Alcohol au tiers" of Ranvier.—Alcohol of ninety-per-cent. strength is diluted with two parts of water. Bits of tissue are placed in this fluid for twenty-four hours; if at the end of this time the tissue is not sufficiently softened, the fluid is renewed and the action continued for twenty-four hours longer. Recommended highly by Ranvier for isolating epithelial cells.

Osmic Acid of the strength of one-tenth per cent. is recommended by Rindfleisch for the cerebral cortex. Small pieces of the cortex are placed in a considerable quantity of the fluid for a week or ten days, then in glycerin.

Chromic Acid is employed in the strength of two to three parts to one thousand of water. Recommended especially for nerve tissue and smooth muscle. The amount of fluid should be ten times greater than the piece of tissue. After macerating for twenty-four hours, the tissues are easily dissociated.

Potassium Dichromate is employed in the same strength as chromic acid, its action being similar. Müller's fluid, diluted with an equal volume of water, is also employed as a macerating fluid. Its action requires from twenty-four hours to a week.

Potassium Hydrate.—Used in twenty- to forty-per-cent. solutions in water. It acts very quickly on fresh tissues, and is used mostly for isolating the cells of nails, hairs, and epidermis. Sodium hydrate may be used in its place, but in more dilute solutions. Preparations made by this method cannot be permanently preserved.

For isolating histological elements S. H. Gage uses a thirty- to fifty-per-cent. solution of potassium hydrate. Perfectly fresh tissue is used. Pieces about half a cubic centimetre in size are placed in a large amount of the potassium hydrate solution until they can be easily shaken apart. The potash solution is then poured off and replaced by a sixty-per-cent. solution of potassium acetate containing one per cent. of hydric acetate. After a short time the tissue is washed in water; then stained in alum carmine for twenty-four hours; then washed in water and preserved in glycerin.

Sodium Chloride.—Used in ten-per-cent. solution in water for isolating white fibrous tissue. It requires from two to three days for its action.

Hydric Nitrate.—Used in twenty-per-cent. solution for muscle fibres. After being acted upon for twenty-four hours, the fibres are easily isolated by shaking the specimen in a test-tube with water. Schwalbe uses a twenty-per-cent. solution for isolating nerve fibres for measurement. He macerates the nerve trunk in the above solution at a temperature of 40° C. for twenty-four hours, and then washes in water. The fibres retain their normal diameter, but become very brittle.

Hydric Nitrate and Potassium Chlorate.—A piece of striated muscle is buried in crystals of potassium chlorate placed in the bottom of a vessel, and four times the vol-

ume of the crystals of hydric nitrate is poured on and allowed to act half an hour; the muscle is then shaken in a test-tube with water, when it will break up into isolated fibres.

Hydric Chloride is used mixed with an equal volume of alcohol for isolating the tubules of the kidney. A small bit of fresh kidney is placed in this mixture for twelve hours, then soaked in water for a few hours to remove the acid, and preserved in glycerin. The acid partially dissolves the intertubular tissue, so that by very careful teasing the tubules can be isolated. It is also employed in the strength of 1 to 250 of water for cleaving striated muscle tissue into discs.

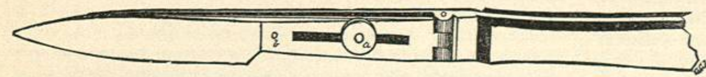

FIG. 2671.—Valentine's Knife.

ARTIFICIAL DIGESTION.—For the purpose of artificial digestion we make use of either pepsin or trypsin.

Brücke's Pepsin Fluid.—Glycerin extract of pig's stomach, 1 part; 0.2 per cent. HCl, 3 parts; thymol, a few crystals.

Kuskow's Pepsin Fluid.—Pepsin, 1 gm. is dissolved in 200 c.c. of a three-per-cent. solution of oxalic acid. The fluid should be prepared fresh each time.

Pancreatic fluid can also be made by dissolving six to seven per cent. of trypsin in water containing one per cent. of salicylic acid.

Schiefferdecker's Pancreatin Fluid.—A saturated solution of Pancreatinum siccum (Witte) is made in cold distilled water and filtered.

Pieces of tissue may be first digested in these fluids and then sections cut, or the sections may be first cut and then submitted to the action of the fluids. The digestive process is to be carried on at a temperature of from 37° to 38° C., either in an incubator or on a warm stage on the microscope.

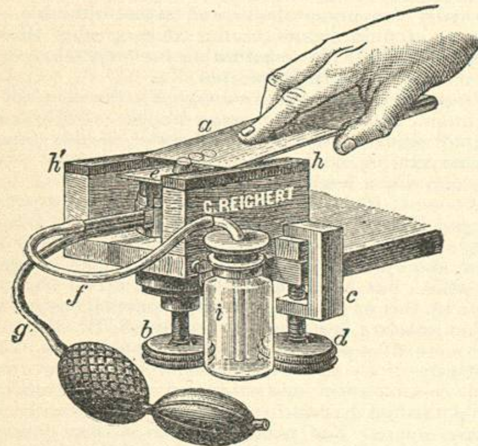

FIG. 2672.—Ether-Freezing Microtome.

Pepsin quickly dissolves connective tissue, muscle, most cellular elements, etc., but not elastic tissue or nerve fibres. Trypsin, on the other hand, dissolves elastic fibres and neuroglia, while connective tissue is not acted upon.

METHODS OF MAKING SECTIONS.—*Sections of Hard Tissues.*—Sections of undecalcified bone, teeth, etc., are made by cutting thin plates by means of a fine saw or an iron disc, on a lathe fed with flour of emery and water. These plates are then ground on a metal plate charged with flour of emery and water until transparent; they are then polished on a Turkey hone, washed in water, and dried in the air.

Sections of Fresh Tissue.—For making sections of fresh tissue Valentine's double knife (Fig. 2671) may be used, though the sections are generally too thick. This knife consists of two parallel blades attached to each other at their lower end by a hinge. At *a* is a screw sliding in a slot, by means of which the blades are approximated. For use, the knife is forced into the organ; cutting a thin slice which remains enclosed between the blades. The knife is then withdrawn and the blades are opened by sliding back the screw *a*; the slice of tissue adhering to one of the blades is floated off in salt solution.

At the present time one of the numerous forms of freezing microtomes is used for making frozen sections of fresh tissues. For freezing the tissue ether, rhigolene, or compressed carbon dioxide is used. Compressed carbon dioxide can now be obtained in steel cylinders, and is used to a considerable extent in place of ether.

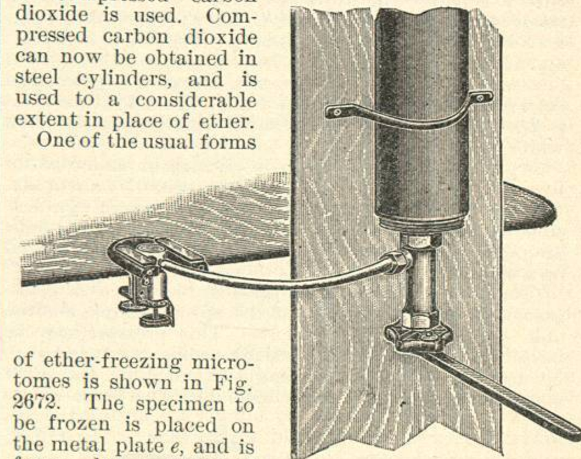

FIG. 2673.—Carbon-dioxide Cylinder Connected with Freezing Microtome.

One of the usual forms of ether-freezing microtomes is shown in Fig. 2672. The specimen to be frozen is placed on the metal plate *e*, and is frozen by a spray of ether delivered on the under side of the plate by the spray tubes passing through its side. The ether is placed in the bottle *i*. The air is compressed by the double bulbs and conveyed to the spray tubes through the tube *g*; the ether through the tube *f*. The knife *a* is broad and has a chisel edge; it slides on the glass ways *h'h*. The surface of specimen plate is raised by the micrometer screw *b*.

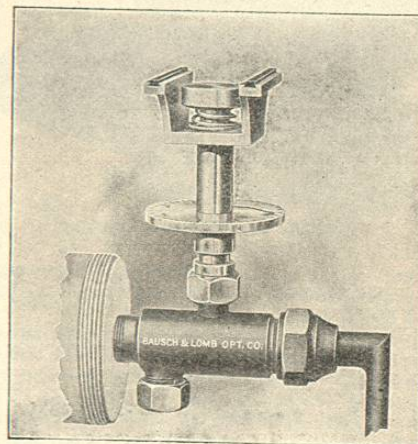

FIG. 2674.—Bardeen's Microtome.

In Fig. 2673 is shown one of the arrangements for using carbon dioxide for freezing. The steel cylinder is fastened to an upright plank so that its valve end comes on or about the level of the table. A thick rubber tube for conveying the gas passes to the microtome. The valve is opened by a long lever allowing the flow of gas to be easily controlled.

Professor Bardeen's freezing microtome is shown in Fig. 2674. This microtome is very simple in construction and is attached directly to the outlet tube of the steel cylinder The improvements introduced by Professor Bardeen, in both the microtome and the valvular arrangements of the cylinder, have resulted in a great saving of the carbon dioxide.

Specimens from which frozen sections are to be made should not be over 4 mm. thick. The freezing should be done slowly, and they should not be allowed to become too hard.

Several methods are now in use whereby sections of fresh tissue may be cut, stained, and mounted in a very short time. One of the earlier methods, that of Cullen (*Johns Hopkins Hospital Bulletin*, April, 1895, and May, 1897), has been modified and improved. Two of the principal modifications are as follows:

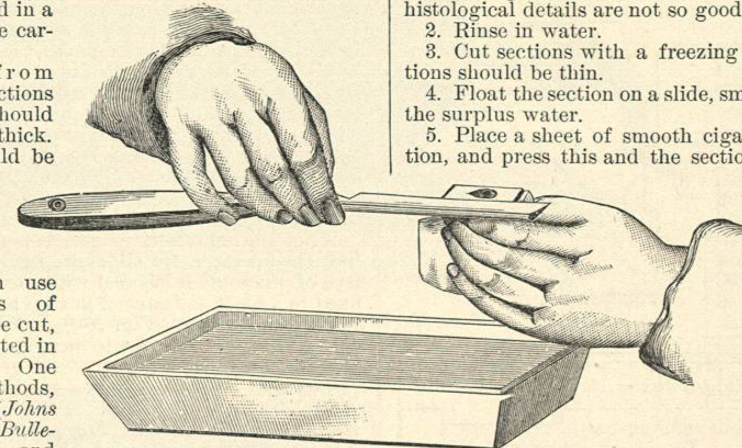

FIG. 2675.—Section-cutting.

Hodenpyl's Modification.—Any form of freezing microtome may be used. The technique is as follows:

1. Sections may be cut from perfectly fresh material, or from material which has been previously hardened one or two hours or longer in ten-per-cent. formalin. (It is convenient to drop bits of tissue into ten-per-cent. formalin at the time of the operation or during the post-mortem examination. By the time they reach the laboratory they are usually sufficiently impregnated to be cut and stained.)

2. Tissues already hardened in formalin should be soaked in water for a minute or two to remove the formalin before cutting.

3. Sections as they are cut may be dropped directly into the albumin solution, where they remain until needed. A solution of albumin which has been found to answer the purpose is prepared by adding to 50 c.c. by volume of egg albumen 150 c.c. of distilled water and sufficient of a solution (usually about 50 c.c.) of salicylic acid (saturated), which has been rendered slightly alkaline with lithium carbonate, completely to dissolve the albumen. The solution may be kept unchanged several weeks by adding a little gum camphor.

4. Unhardened sections should be placed in five-per-cent. formalin for from three to five minutes, after which they are soaked in the albumin solution for two or three minutes.

5. The sections are floated on cover-glasses, the excess of fluid being removed with filter paper when necessary. The sections are then blotted evenly, care being taken not to use pressure enough to cause them to bear the imprint of the cloth. (The best blotting material seems to be washed cheesecloth used in several layers. The use of filter paper, towels, muslin handkerchiefs, etc., for this purpose has not been satisfactory.)

6. The section must then be transferred immediately to alcohol, alcohol and ether (equal parts), osmic acid, or mercuric chloride, etc., in order to coagulate the albumin and to fix the section and complete the hardening.

7. Sections may be stained on the cover slip in various ways. For ordinary diagnostic work staining with hæmatoxylin and eosin answers well. The following is the method of procedure. (*a*) Stain for from two to five minutes in hæmatoxylin. (*b*) Decolorize by passing rapidly through acid alcohol—hydric chloride, 1 part; eighty-per-cent. alcohol, 99 parts. (*c*) Wash thoroughly in water. (*d*) Dehydrate and stain in eosin alcohol. (*e*) Clear in oil of origanum, oil of cloves, xylol, etc. (*f*) Mount in balsam.

Wright's Modification.—1. Place the fresh specimen in ten-per-cent. solution of formalin for about two hours. If time does not allow of this, boil the specimen in the same strength of formalin for two to three minutes. The histological details are not so good after boiling.

2. Rinse in water.

3. Cut sections with a freezing microtome. The sections should be thin.

4. Float the section on a slide, smoothe out, and remove the surplus water.

5. Place a sheet of smooth cigarette-paper on the section, and press this and the section down with a pad of soft, smooth filter paper, the face of which has been moistened with a few drops of ninety-five-per-cent. alcohol. Remove the filter paper and carefully strip off the cigarette paper, leaving the section adhering to the slide.

6. Flood the section, adhering to the slide, with absolute alcohol; after about thirty seconds drain off the alcohol.

7. Flow over the section and the adjacent surface of the slide a *thin* solution of celloidin; drain off immediately.

8. Flood the slide with ninety-five-per-cent. alcohol, and immediately place the slide for ten seconds in water. This hardens the celloidin film and prevents the section from curling.

9. Stain with hæmatoxylin or any combination of stains.

10. Dehydrate in ninety-seven-per-cent. alcohol.

11. Clear in oil of origanum, and mount in balsam.

Sections of Hardened Tissue.—Sections of hardened tissue may be made by free-hand cutting, or by the microtome. For free-hand cutting, the first requisite is a good razor or knife. An ordinary hollow-ground razor, with the lower side ground flat, will answer; but if one practises free-hand cutting to any extent, it is better to be provided with a larger and heavier one.

The section cutting is performed as follows: A shallow dish is partially filled with alcohol, with which the specimen and razor blade are to be constantly flooded, the blade being dipped into the alcohol. The specimen is held firmly between the fingers and thumb of the left hand (Fig. 2675), and the razor lightly with the right, the handle resting on the fingers, the thumb being used for changing the direction of the edge of the razor. The section is cut by drawing the razor blade diagonally

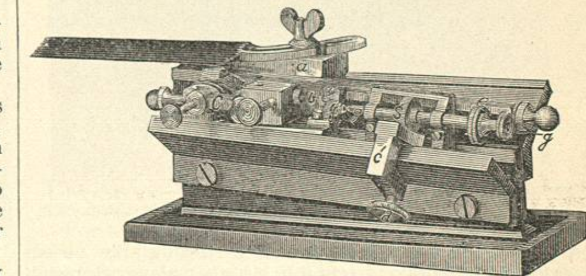

FIG. 2676.—Thoma's Sliding Microtome.

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through the specimen, from heel to point, and then floated off into a dish of alcohol. After cutting every third or fourth section the razor should be stropped, so as to keep the edge in the best possible condition.

Microtomes.—Thoma's sliding microtome (Fig. 2676) consists of a stand of cast iron, on which slide two car-

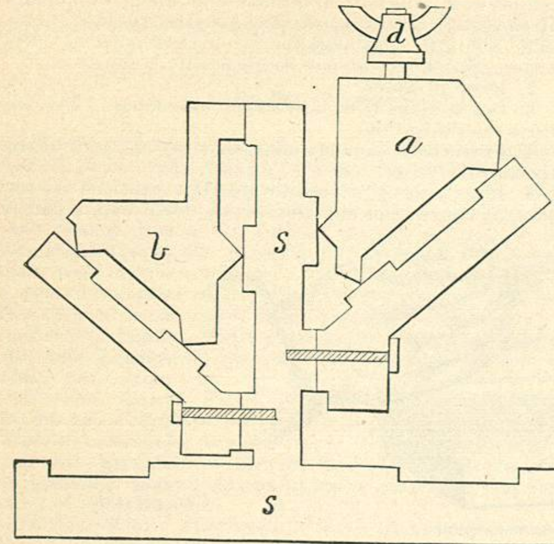

FIG. 2677.—Vertical Section, Thoma's Microtome.

riers. The section knife is attached to one of these, *a*, which slides horizontally. The other, *b*, holds the specimen to be cut, and moves on an inclined plane.

Professor Thoma found that a carrier moving on five points between two plane surfaces, will slide without difficulty between these planes, even if they are not absolutely geometrical planes, or if the angle included is not everywhere the same. Such a carrier will always take the same course, and consequently the knife will cut a

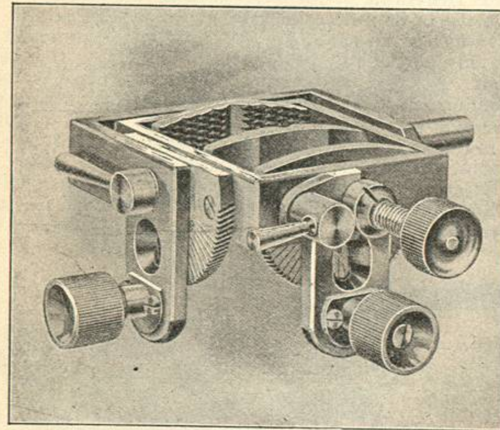

FIG. 2678.—Naple's Universal Clamp.

series of perfectly parallel sections through an object which is successively raised to a higher plane after each cut.

A cross-section of the instrument is shown in Fig. 2677. On the lower surface of the carrier *a* are shown three points, which give the geometrical projection of the five points of the carrier.

The inclination of the plane upon which the carrier *b* (Figs. 2676, 2677) slides is one in twenty; consequently, if the carrier *b* is moved 1 mm. on this plane it will raise the surface of the specimen .05 mm. A scale of millimetres, *e*, with a vernier *e'* (Fig. 2676), allows the operations to be easily regulated, and will be found sufficient for sections of 0.015 mm. in thickness. When sections of greater delicacy are required the micrometer screw *g*, *g* (Fig. 2676) is to be used, as it allows of the greatest exactitude in the management of the carrier *b*. The carrier *c'* slides on the same plane as the carrier *b*. In all positions of the latter it is possible to bring the point of the micrometer screw *g*, *g* in contact with the polished plate of agate, *f*, fixed on *b*. When in this position, *c'* should be firmly fixed to the stand of the microtome by the screw *d*. Every revolution of the micrometer screw *g*, *g* will then push the carrier *b* 0.3 mm., giving a thickness of section equal to 0.015 mm. The periphery of the drum, *s*, attached to the screw *g*, *g* is divided into fifteen equal parts; consequently, each division corresponds to a thickness of section equivalent to 0.001 mm. The first sections cut by this instrument have been 0.002 mm. in thickness.

When the microtome is used, it is placed on a table before the operator, the slides are freely oiled, and the surface of the knife is flooded with alcohol. The specimen fixed to a block is fastened in the clamp.

The Naple clamp (Fig. 2678) is the one now generally used and can be adapted to most of the microtomes. It

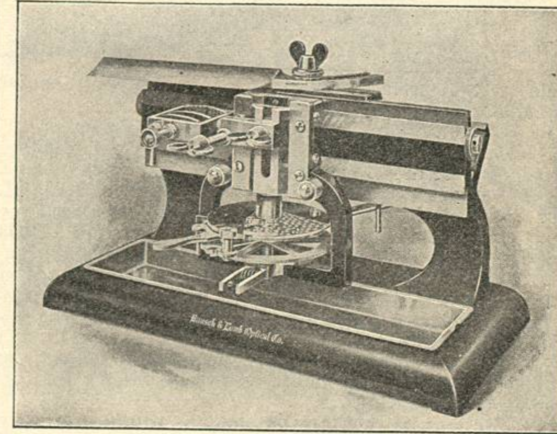

FIG. 2679.—New Form of Sliding Microtome with Automatic Feed Attachment.

is movable on two axes, one parallel to the long axis of the microtome, the other at right angles to it. This allows of the adjustment of the surface of the specimen to the plane through which the knife passes in cutting. The clamp is then fixed in a position as near the knife carrier *a* as possible.

The knife is so adjusted that it will be drawn obliquely through the specimen from heel to point. The sections, after being cut, are removed from the knife with a camel's-hair brush, and placed in a dish of alcohol.

In cutting sections of hard substances, such as the aorta and cartilage, it often happens that the sections, instead of being even, are thicker at one point than at another; or that the sections are striped, their thickness varying at different points. Professor Thoma has found that this is due to the fact that hard substances bend the edge of the ordinary knife. For preventing this he has introduced knives with stronger edges and shorter blades.

In Fig. 2679 is shown one of the newer forms of sliding microtomes with automatic feed attachment. It is adapted either for celloidin or paraffin cutting. The object clamp remains fixed during the cutting. The knife is clamped to the knife carrier, a triangular block, which slides on horizontal runways.

The automatic feed attached to the micrometer screw

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is operated by the motion of the knife carrier and is so adjusted that sections from 2 to 60 μ in thickness may be cut.

A larger form of automatic sliding microtome is shown

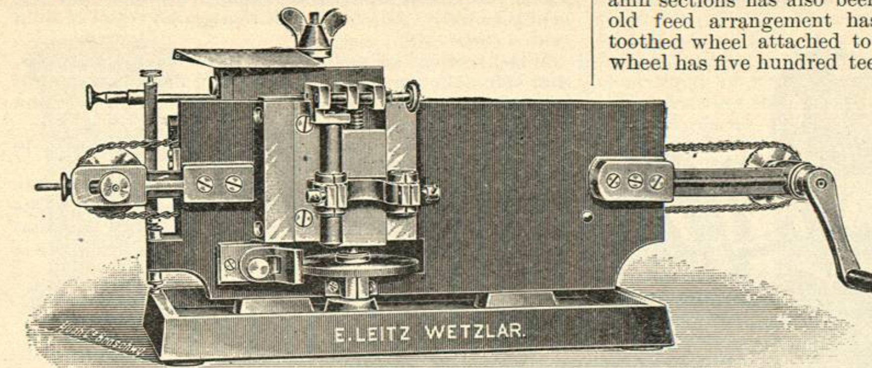

FIG. 2680.—Large Sliding Automatic Microtome.

in Fig. 2680. The knife carrier runs on a horizontal track and is moved by a chain attached to either end of it. This chain passes around two wheels, one of which is turned by a crank attached to it, causing a uniform sliding motion.

The specimen clamp is raised vertically by a micrometer screw to which is attached a large, toothed, circular disc, each tooth of which equals an elevation of 0.0025 mm.

The automatic feed is so arranged that each time the knife carrier reaches the left-hand end of the tract it strikes a lever which causes the circular disc attached to the micrometer screw to revolve. The lever being adjustable, it can be made to cause the disc to move any number of teeth from one to ten with each movement of the knife carrier, thus giving a thickness of section from 0.0025 mm. to 0.025 mm. in thickness.

The "Precision" microtome of Minot (Fig. 2681) is a

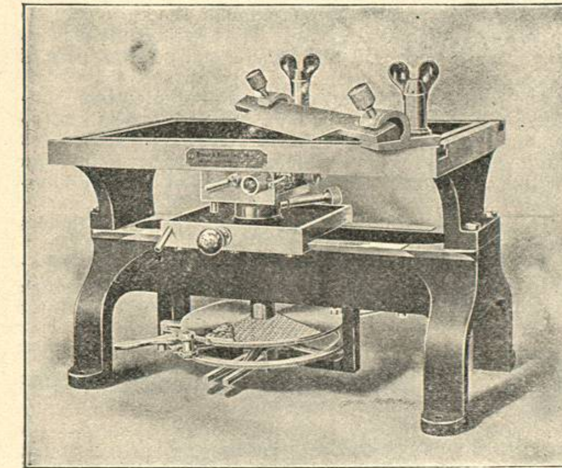

FIG. 2681.—Minot Automatic Precision Microtome.

departure from the usual form of sliding microtome in that the knife is fixed and the object clamp is on a sliding carrier.

The knife is clamped to the frame of the microtome, and the object clamp carrier runs on slides under the knife. There is an automatic feed attachment that may be regulated so that sections from 1 to 60 μ in thickness may be cut. This microtome may be used for celloidin or paraffin preparations.

The older form of Minot's rotary microtome for paraffin sections has also been improved (Fig. 2682). The old feed arrangement has been replaced by a large-toothed wheel attached to the micrometer screw. This wheel has five hundred teeth, and the micrometer screw has a pitch of 0.5 mm., so that each tooth of the wheel equals a feed of 1 μ. The feed of the micrometer screw is controlled by an adjustable cam, so that the thickness of section may be made to vary from 1 to 25 μ.

An adjustable carrier (Fig. 2683) for serial ribbons may be attached.

Microtome Knives.—The usual form of microtome knife is shown in Fig. 2684. Its upper surface is concave, its under one flat. Its cutting edge is of a double chisel form, and must be perfectly straight. The knife should be heavy and its length adapted to the size of the microtome used.

A knife for cutting paraffin should be heavier than one used for celloidin.

In order to obtain good sections the cutting edge of he knife must be kept in the best possible condition.

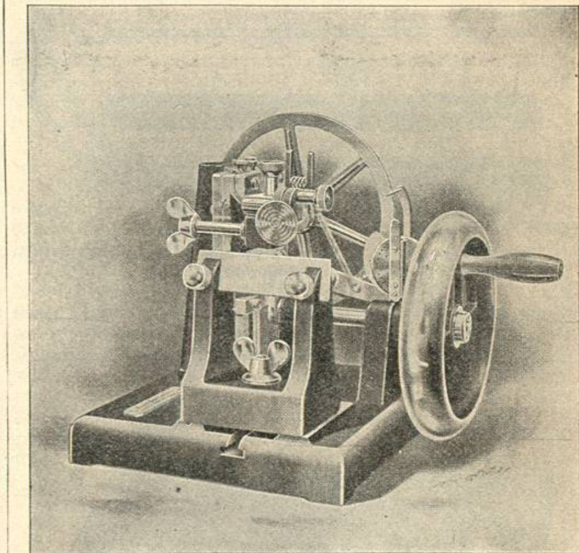

FIG. 2682.—Improved Rotary Microtome of Minot.

This is accomplished by stropping the knife frequently and honing it occasionally.

For honing, a yellow Belgian hone of large size is to be used. Its surface is thoroughly covered with a lather made with a pure soap and water. This lather is better than oil as it keeps the pores open and gives a better grinding surface. In honing, one of the backs (Fig. 2684) is fastened to the knife. This is for the purpose of keeping the angle of the edge of the knife constant. The knife is first sharpened on its concave side, then turned and sharpened on the flat side. The pressure on the knife should be light; it should be drawn across the hone from heel to toe, the edge being drawn against the sur-

face of the hone. The movements are illustrated in Fig. 2685.

For stropping the best form of strop is that known as the bow strop (Fig. 2686). The movements of the knife

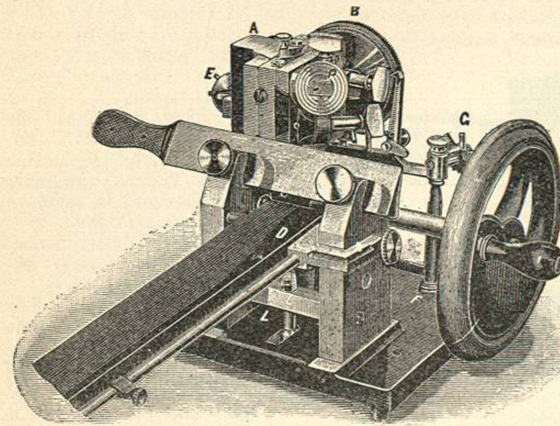

FIG. 2683.—Showing Carrier for Ribbon Sections Attached to Minot's Rotary Microtome (Old Form).

are the reverse of those of honing, and are shown in Fig. 2686. After use the knife should be carefully dried with a soft cloth.

After the sections are cut they are removed from the microtome knife with a camel's-hair brush and placed in

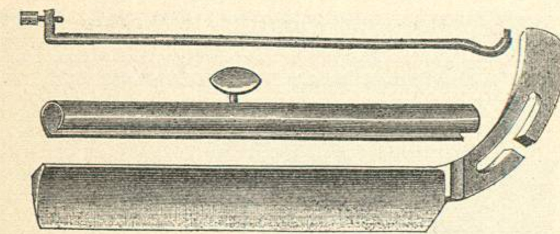
FIG. 2684.—Microtome Knife with Two Forms of Backs used in Honing and Stropping.

a dish of fluid. For sections of fresh tissue and frozen sections, salt solution should be used, the sections being submerged in it; for sections of alcoholic specimens, alcohol or water may be used.

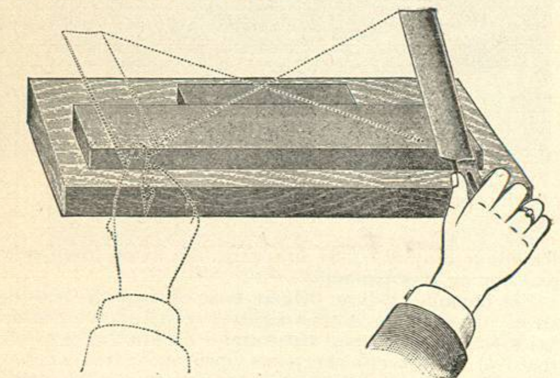
FIG. 2685.—Showing the Movements of the Knife in the Process of Honing.

In the further manipulation of the sections great care must be taken or they will be destroyed. For transferring them from the different reagents, stains, etc., a needle, camel's-hair brush, or nickel-plated steel spatula (Fig. 2687) must be used. In the majority of cases the latter instrument will be found most convenient. It is placed cautiously under the section floating in the fluid, gently raises it, and transfers it to a second vessel of fluid or to a slide.

When sections are transferred from alcohol to water, they spin about in a lively manner and finally spread out into thin laminæ. This is caused by the rapid diffusion of the alcohol.

Sections can be preserved indefinitely in eighty- to eighty-five-per-cent. alcohol.

Serial Section Cutting.—The preparations to be cut are to be embedded in paraffin. The melting point of this must have a certain relation to the laboratory temperature. A laboratory temperature of 17° C. requires a

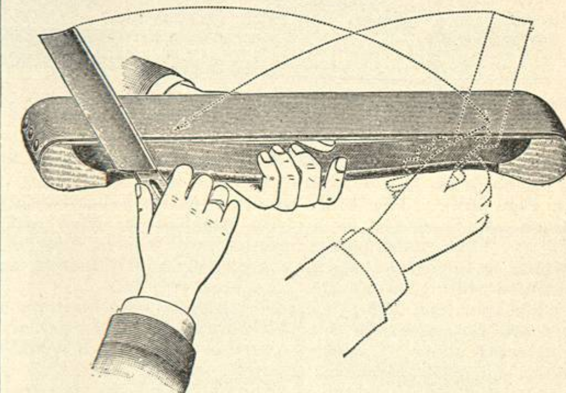
FIG. 2686.—The Bow Strop, and the Mode of Stropping.

paraffin melting at 45° C. At a temperature of 15° C. this paraffin would be too hard. At 22° C. a paraffin melting at 48° C. will be required.

After impregnation the specimens are embedded by one of the methods described under paraffin embedding.

The block of paraffin should be pared down close to the specimen, and should be cut so as to present a straight edge parallel to the edge of the knife, and the opposite edge parallel to this. If this precaution is not taken the sections will not adhere, and will not pass off in a straight line.

The sections can be cut on any sliding microtome, provided the knife be set square. Several special microtomes have been constructed for this kind of section cutting, viz., Caldwell's automatic microtome, Cambridge rocking microtome, and Minot's automatic wheel microtome.

The sections are fixed upon a slide by one of the following methods:

Water Method: The ribbons of sections are cut to proper lengths and floated on warm water, warm enough to soften the paraffin, but not to melt it, when the

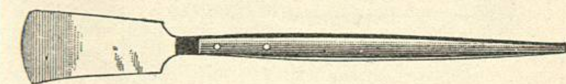
FIG. 2687.—Spatula.

sections will flatten out. They are then floated on a slide and arranged in position. The slide is then drained and allowed to become perfectly dry. To hasten the drying the slides may be placed in a water-oven at a temperature of 40° C. The paraffin must not be allowed to melt until the sections are perfectly dry. When perfectly dry the sections adhere to the slide, when the paraffin is melted and washed off with xylol.

Mayer's Method: Paint a mixture of equal parts of filtered white of egg and glycerin (and a few drops of

carbolic acid as a preservative) on a slide with a camel's-hair brush, and arrange the sections with this; then warm the slide on a water bath for a few moments until the paraffin is melted. As the paraffin in the sections melts it carries the albumen away from them. The paraffin is next to be dissolved out with turpentine or xylol, the latter removed with alcohol, and the sections stained.

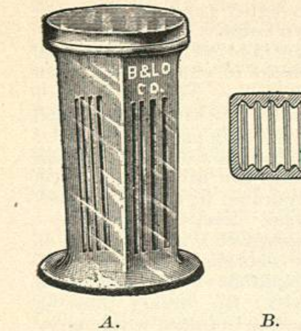

FIG. 2688.—Staining Jar for Serial Sections Mounted on Slides.

Schällibaum's Method: Mix one part of collodion with three or four parts of oil of cloves and shake well. Spread this solution in a thin layer, with a brush, on a slide; arrange the sections and evaporate them by gentle heat on a water bath. Then dissolve out the paraffin with xylol. The sections thus fixed may be stained in the usual way and mounted in balsam or glycerin.

Serial Sections of Celloidin-Embedded Specimens.—1. Prepare a series of glass plates; coat these with a thin layer of celloidin, as a photographer makes a moist plate, and allow them to dry.

2. Make sections and arrange ribbon-wise on strips of transparent porous paper, which should be double the width of the sections. Keep the strips, with the sections on them, moist by placing them on blotting paper in a dish with alcohol.

3. Transfer the sections to the celloidin-coated plate. Lay the strips, section side down, upon the glass plates, press them softly, and peel off the paper; remove all superfluous fluid with filter paper, taking care not to dry the sections.

4. Cover the sections with a thin layer of celloidin. When dry, the celloidin may be marked with a brush dipped in methyl blue, so that the series may be recognized.

5. Immerse the plates in water, and the celloidin will peel off from them, and we shall have the sections enclosed between two layers of celloidin, allowing them to be handled like a rag.

6. The celloidin plate may be cut up into as many pieces as there are sections, or it may be stained uncut and mounted in the usual way.

Staining.—The tissues may be stained *in toto* before being embedded in paraffin or after they are fixed to the slide. The latter is the method generally adopted, as a greater variety of stains may be employed.

In Fig. 2788, *A*, is shown a staining jar for holding ten slides. In *B* is shown a transverse section of the jar. The sides of the jar are grooved, the width of the groove being such that two slides, back to back, may be placed in it. These jars may also be used for dissolving out the paraffin, dehydrating, clearing, etc.

Born's Method of Reconstructing Objects from Microscopical Sections.—By the aid of a camera the outlines of the sections are transferred to wax plates, which are then cut out so as to correspond in outlines as well as in dimensions to the sections equally magnified in all three directions.

He makes three rectangular boxes of tin, 270 mm. × 230 mm. × 2.5 mm. The sections should be 0.04 mm. thick (never thinner than 0.02).

If we wish to construct a model of a specimen from sections $\frac{1}{30}$ mm. thick and magnified sixty diameters, the wax plates must be 2 mm. thick. Such a plate made in a box of the above dimensions contains 62.1 mm.; and its volume will be 124.2 mm. The specific gravity of beeswax is 0.96 to 0.97. It is melted with a small quantity of turpentine, which reduces its specific gravity to about 0.95; and the weight required to make one plate of the above size is, in round numbers, 118 gm. This quantity of wax is weighed out and melted. The tin box is filled 1.5 cm. deep with boiling water and the melted wax poured upon it. As soon as the plate has begun to stiffen, and while it is still soft, it should be cut away from the edges of the box, and, when the water has become tepid, removed to a flat support to stiffen.

For drawing the outlines of the section a piece of blue paper is placed, colored side down, upon the wax plate, and over this an ordinary sheet of drawing paper. The outlines are drawn on the latter, by the aid of a camera, and at the same time blue lines are traced on the wax. Then, with a small knife, the wax is cut out, the outlines being followed, the plates are placed together in the proper order, and fastened by passing the warm spatula along the edges.

METHODS OF EMBEDDING.—This process is divided into two classes: simple embedding, when the specimen, being small, cannot be held conveniently in the hand or clamped in the microtome, and is simply surrounded with an embedding mass to give it bulk; interstitial embedding, or impregnation, when the specimen is permeated with the embedding mass. The latter process is used when we have a specimen that lacks the necessary consistence for cutting (lung, etc.); or when the specimen contains cavities, the contents of which we wish to retain in their normal condition and position; or when the specimen is so brittle that it would be destroyed in the process of cutting.

Melted Masses.—Simple embedding in a melted mass (paraffin) is performed as follows: A small box is placed on a cork, and the specimen is placed in it and fixed in position by means of pins. The box is now filled with the melted mass and allowed to cool—when the pins and papers are removed, leaving the specimen enclosed in a solid block of the embedding material. Or a block of the embedding mass is taken, and a hole, a little larger than the specimen, is scooped out of one face; the specimen is washed in a melted mass of the same material and placed in the cavity, which is then filled.

The embedding boxes are made of paper. A plan which I have found very convenient is to have a series of wooden blocks, the faces of which correspond to the sizes of the boxes to be made. One of these blocks is taken, and the paper is folded over the face corresponding to the size of box wished for.

In Fig. 2689 is shown an adjustable metal embedding box for paraffin. It consists of two pieces, having the form of a carpenter's square—the long arm being 7 cm. the short 3 cm. in length, by 7 mm. high. On the outer side of the short arm is a block of metal, on the top of which is a spring (*a*, Fig. 2689), which curves over and presses against the long arm, thus holding the two pieces firmly together. The area of the box will vary with the position of the pieces. The area of the box having been fixed, it is placed on a glass plate, moistened with gly-

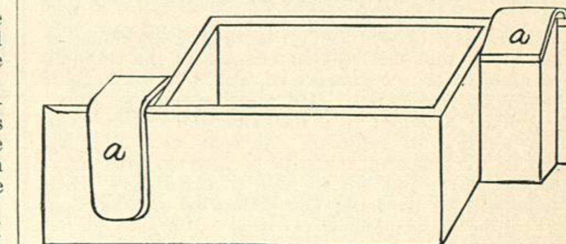

FIG. 2689.—Adjustable Embedding Box.

cerin, and a thin layer of the melted mass poured in. When it has cooled, the specimen, which has been washed in the same material, is fixed in position and the box is filled up. When the embedding mass has cooled the box is removed, and the specimen will be found embedded in a solid block of the material.