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THE MICROSCOPE,

AND

ITS APPLICATION

TO

CLINICAL MEDICINE.

BY

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in a cell, the cover of which is fixed on with this substance, without danger of the glass cracking in consequence of the alteration of the volume of the fluid by variations of temperature. Another advantage is, that when pressed upon wet glass, the thin layer of fluid is expelled by the pressure, and the cement adheres firmly. In order to fix on the cover of a preparation jar with this cement, all that is necessary is to roll a small piece out between the hands, and lay it all round the top of the jar or cell. By pressing it gently with the finger and thumb, it adheres firmly to the glass; the cell is then filled with the preservative solution, and the cover applied (§ 93). The edge may be covered with any coloured varnish, or paper, or it may be bronzed or gilt, according to taste. This cement will not answer where strong spirit is used, but if only weak spirit be employed, or some aqueous preservative solution, the joint will last for almost any length of time. I have several preparations which have been placed in the creosote and naphtha solution (§ 97) in large cells, and they are now perfectly air-tight, although upwards of five years have elapsed since they were first put up. The lime and India-rubber cement answers well for fixing on the glass tops of large preparation jars, and looks exceedingly neat; but, if spirits be used, a little air must be permitted to remain in the jar, or the cover may be cemented on the cell according to the plan described in § 93.

The apparatus, cements, &c., used in microscopical investigations, may be obtained, arranged in a case, of Mr. Highley, 32, Fleet Street.

Upon subjects treated of in the preceding chapter, the following works may be referred to:—Translations from "Het Mikroskoop," Harting, Utrecht, in the Edinburgh Monthly Journal for March, 1852, New Series; "Anatomical Manipulation," Tulk and Henfrey; Papers by Dr. Goadby, in Silliman's "American Journal of Science;" Dujardin "Nouveau Manuel de l'Observation au Microscope," Paris, 1843; Strausdurkheim "Traité pratique et theorique d'Anatomie Comparative," Paris, 1842.

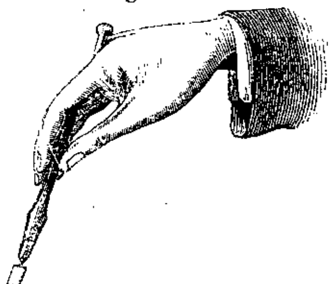
CHAPTER V.

OF MAKING CELLS FOR PRESERVING PREPARATIONS. THE VARIOUS FORMS OF CELLS EMPLOYED.

64. Of Cutting Glass.—In making the cells which are intended to contain anatomical preparations we often require to be able to cut very thick plate glass. For this purpose a large plate-glass diamond is necessary. The use of the diamond is easily learned with a little practice. The most important points to bear in mind are—

1st. To hold the diamond firmly in its proper position, by placing the upper part of the handle between the first and middle fingers, the fore-finger resting on the anterior, and the thumb pressing upon the posterior flat surface (fig. 59).

Fig. 59.



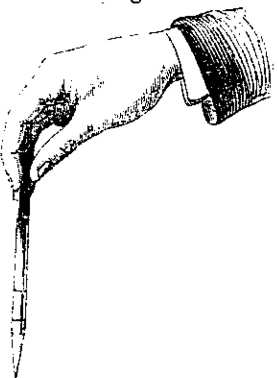
2ndly. To be careful not to bend the wrist, but to allow the entire movement, when the diamond is drawn over the glass, to take place in the elbow and shoulder joints; and,

3rdly. Always to keep the instrument inclined at the same angle. The diamond should not be pressed strongly upon the glass, for in this case it frequently produces only a mere scratch. It should never be attempted to go over any part of the same line twice; as in this way the diamond would soon be injured. For cutting plate glass of ordinary thickness, a common glazier's diamond only will be required.

65. Of cutting the thin Cylinder Glass.—The thin glass which is used for covering preparations is cut with what is termed a writing diamond (fig. 60): a small instrument which

is used for scratching marks on glass. In order to cut the thin glass into small squares, the sheets must be placed upon a very flat surface; as, for instance, a piece of plate glass, or a board planed perfectly smooth. The diamond being held upright, straight lines are marked with the aid of a flat ruler, according to the size of the squares to be cut. When all the lines are made, the pieces of glass can be readily broken off. They should be kept in a box with a little starch powder, to prevent them from being broken in smaller pieces.

Fig. 60.



A piece of thin glass of any shape can be readily cut with the writing diamond, taking care not to lean too heavily.

66. Method of cutting circular pieces of Thin Glass.—Thin glass circles have been cut with a beautiful instrument on the principle of a pair of compasses; but the following method is very simple and efficacious. Common brass curtain rings of various sizes are obtained; and on each side of a ring is soldered a straight piece of wire, or a round hole is made in a perfectly flat piece of brass, as shown in fig. 61. By placing a finger on each side, the ring can be maintained in a proper position, while the circle is marked out with the diamond. These rings may be obtained of Mr. Matthews; or small pieces of cardboard, or gutta percha, in which circular holes of various sizes have been cut out, may be substituted for them.

Fig. 61.

67. Of grinding Glass for making Cells, &c.—For the purposes of grinding, it is essentially necessary to obtain a perfectly-flat surface. A large flat piece of thick cast iron will answer the purpose very well, or a perfectly-flat stone may be employed, which should be about a foot square, or larger, if cells of considerable size are required.

Perhaps, however, the best substance to make a plate for

grinding glass upon is pewter. A flat slab of pewter can be readily obtained, and as the emery becomes imbedded in the metal, a most efficient grindstone is obtained. This, I believe, was first suggested by Dr. Goadby. Common sand, of a fine quality, or emery powder, will be required. The grey sand, which can be purchased at the oil-shops, answers very well for coarse glass-grinding. Water is poured upon the slab, and the glass rubbed very gently round and round. If rubbed too forcibly, there is danger of breaking it, or of chipping off small pieces from the edge. Care must be taken to keep the sand constantly wet during the operation. After being ground upon a stone with sand, it may be rendered more smooth by being further rubbed down with emery.

Sometimes it is merely required to roughen the surface of the glass, as in making the thin cells. In this case, it is better to use fine emery powder moistened with water or turpentine, and placed upon a small piece of thick plate glass; or a common hone may be used. The surface of glass may also be ground by rubbing it well with a rag covered with emery powder.

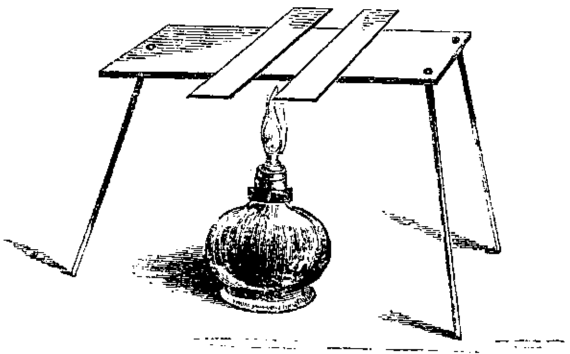
68. On fixing Cells to the Glass Slides.—Before referring to the various kinds of cells, it will be better to describe the method of fixing them to the glass slides. This may be effected by gold size, Canada balsam, or by varnishes of various kinds; but the method which is to be preferred in most instances, and that which is now in constant use, is to cement them with marine glue, which forms a most perfect joint. To effect this, it is necessary that the glass should be heated some degrees above the boiling-point of water, in order that the glue may run upon it freely. When the glue is thoroughly melted upon the slide, the cell is applied, gently pressed down, and the whole allowed to become cool.

The most convenient manner of making the slides sufficiently hot is to place them on a flat brass or iron plate, to which heat may be applied by a lamp placed below (fig. 62).

If a considerable number of cells are to be mounted, it is better to have a large and perfectly-flat cast-iron plate, which can be heated by a gas or oil lamp, upon a proper support, or

by a small stove. The slides may be placed in a row in one part of the plate, and the cells in another part. As soon as the slides are hot, a few small pieces of marine glue may be laid upon the slide in the position in which it is intended to place the cell, or the glue may be first melted in a pot and applied to the warm

Fig. 62.



glass with a stick. When the glue is thoroughly melted, the cell is moved to its place with a pair of forceps, and gently pressed upon the slide until it is found that the glue has wetted it in every part, forming a thin and almost invisible layer between the glass surfaces. It is frequently necessary to press the cell firmly on the glass slide, in order to break down little gritty particles often present in considerable quantity in marine glue. The whole can then be removed from the hot plate, and allowed to cool gradually. In moving the cell or slide, forceps, the ends of which have been protected with small pieces of cork, or covered with thread, pieces of stick, and a cloth will be found of service. The sudden application of the cold finger often causes the glass to crack. The greater part of the glue which has run over the slide may be removed with any small instrument having a sharp square end. A large brad-awl (fig. 63) answers very well. If a little solution of potash be rubbed upon the glass with the aid of a stick, the superfluous glue can easily be removed from the glass, or the

Fig. 63.

cells may be soaked in dilute potash for half an hour, and the softened glue removed with a hard brush and soap and water. After cleaning the slides with potash, they should be well washed in soap and water, and afterwards thoroughly cleaned by being rubbed with a little weak spirit and a soft cloth.

CELLS FOR PRESERVING PREPARATIONS.

In order to preserve a preparation for any length of time, it becomes necessary to place it in a cell or air-tight vessel, more especially if it be preserved in a liquid. In order to effect this object, several precautions must be observed, the nature of which will be considered in the next chapter. In this place I propose to describe the most useful cells to the microscopical observer, and the method of making them.

The forms and thickness of cells for microscopical preparations must of course vary according to the nature and size of the object to be mounted.

Thin cells may be made of various substances. Even paper answers exceedingly well in some cases, and is well adapted for dry preparations. A thin layer of white lead, which has been allowed to dry, has also been employed for the same purpose. White lead, made into a thick liquid with linseed-oil and turpentine, has been recommended by some observers. Various varnishes have likewise been used; but where it is required to keep the specimen in some preservative solution, glass is the substance which in all cases forms the best material for making cells.

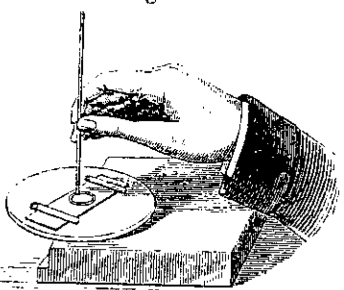
THIN GLASS CELLS.

Sometimes preparations are of such extreme tenuity that it is only necessary to place them on the slide with a drop of some preservative solution, and then to cover them with a square of thin glass, the edges of which have been anointed with gold size or other appropriate cement. The superfluous fluid is next absorbed with bibulous paper, and the slide allowed to dry for a few minutes. A layer of gold size or other cement is then applied round the edges of the thin glass in order to fix it to the slide. In this way an excessively-thin cell may be formed; but preparations mounted in cells made in this manner can

seldom be kept for any length of time without the entrance of air-bubbles. This arises from the outer layers of the gold size drying more rapidly than the more internal layers. By the contraction thus produced the edges of the cement are drawn off from the glass, to which, however, it does not adhere with great tenacity, in consequence of the surface being highly polished. It is, therefore, always better to make very thin cells of glass or other material, which can be cemented to the glass slides with marine glue or other cement; or else to make the cell by painting the slide with a ring of varnish, marine glue, or Brunswick black, and allowing this to dry thoroughly before the preparation is placed in it. In this manner the thinnest cells which can be required are readily made.

69. Cells made of Brunswick Black.—Perhaps Brunswick black (§ 59) is, for the purpose just mentioned, the best. It is painted upon a glass slide with a fine camel's-hair brush, and allowed to dry perfectly, when, if the cell be not sufficiently thick, another layer may be applied. If the cell be required immediately, it is better to warm the slide slightly before applying the varnish. If too great a degree of heat, however, be employed, the varnish becomes brittle and the cell unfit for use. Very neat circular cells, composed of Brunswick black, are readily made by the little instrument designed by Mr. Shadbolt, and figured in the margin (fig. 64). The glass slide is placed under the springs on the circular table, which is then made to revolve, while a brush containing the varnish is held about three-eighths of an inch from the centre of the slide. These cells are very useful for mounting the most delicate urinary deposits, such, for instance, as casts of the renal tubes, &c.

Fig. 64.

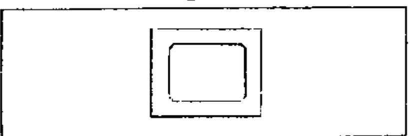


By this method the thinnest cells that can be possibly required may be readily made in a few minutes.

70. Very thin Cells made of Tinfoil.—This may be easily accomplished by cutting with a pair of scissors a piece of thin tinfoil the size of the cell which it is desired to make. A hole is cut in the centre of the tinfoil sufficiently large to hold the preparation which is to be preserved, and the tinfoil is then attached to the glass slide with marine glue. When cold the cell may be filed perfectly flat with a very fine file, or rubbed with a little emery upon a piece of plate glass, and the marine glue should be afterwards removed from the centre with a little solution of potash. The cover may be fixed on with gold size or varnish, as in other cases. Thin cells have also been made of gutta percha, but there is great difficulty in fixing the cell firmly upon the glass slide. This, however, has been effected by some observers; but in consequence of the difficulty, it is a method not generally employed. Preparations, however, mounted in cells, composed entirely of gutta percha, keep very well for a length of time. For the cement adapted for attaching gutta percha cells to glass, vide § 60.

71. Cells composed of very thin Glass.—These cells are very convenient, and will be found useful for preserving many preparations. They may be obtained of different degrees of thickness, and are made usually by perforating the thin cylinder glass which is used for covering the cells, or by grinding sections of a thick glass bottle to the required tenuity (fig. 65). Round cells of thin glass are made as follows:—A great number of squares of thin glass are cemented firmly together with marine glue, and, when cold, a hole of the required size is drilled through them all. They are next separated from each other by heat, and after being cleaned with potash, may be fixed on the glass slides with marine glue in the usual way, and kept ready for use. It is a good plan to roughen the surface of these cells, which renders the subsequent entry of air less likely, as the gold size adheres much more firmly to a ground, than to a polished, surface. This is readily effected by rubbing the cell, after it has been fixed upon the glass slide, up and down a narrow hone or strip of plate

Fig. 65.



glass, on which some moistened emery powder has been placed. In this way also the thickness of the cell may be reduced if required.

72. **New method of making thin Glass Cells.**—For some time, however, I have been in the habit of making these thin glass cells as follows :—

One of the thick glass rings (fig. 68) is heated on the brass plate, and one side covered with marine glue. As soon as the glue is thoroughly melted, a small piece of the thin glass is carefully applied, and the whole allowed to cool (fig. 66). When quite cold, the point of a semicircular, or round file is sharply thrust through the centre of the thin glass, which is carefully filed to the size of the interior of the ring, and then taken off by heating it a second time on the plate, when it may be cleaned with potash, and is ready to be fixed to the slide. The success of this simple process depends upon the very intimate adhesion of the thin glass to the ring; and this is so firm, that however roughly the file may be used, any crack which is made, never runs across that part of the thin glass which is fixed to the ring. In this way, thin glass cells may be made of any shape without the slightest trouble; and by having many of the rings on the hot plate at the same time, and taking them in rotation, a very short time only is required to make a great number. Very large thin glass cells can thus be readily formed (fig. 67), which could not be made at all, or only with great labour, by any other process.

Fig. 66.

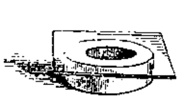
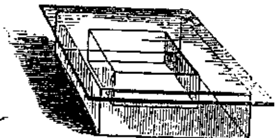


Fig. 67.



THICK GLASS CELLS.

73. **Deep Glass Cells.**—The deep glass cells which are used for mounting injections and other preparations of considerable thickness, are made either by cutting sections of thick glass

bottles, or round tubing (fig. 68); or by drilling holes of the required size, in pieces of plate glass, gutta percha, &c. (fig. 69.) All these of course require fixing to plate-glass slides, as above described.

Fig. 68.

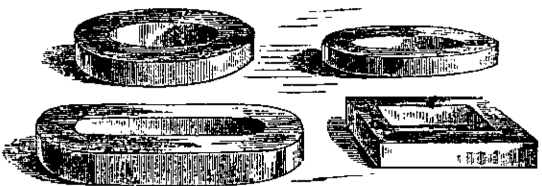
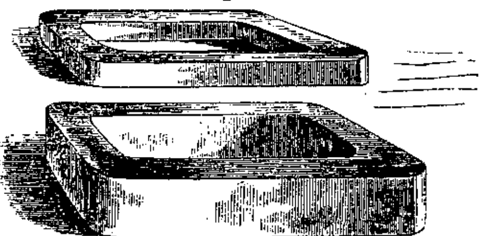


Fig. 69.

Cells made by these methods may be obtained of various shapes and sizes;* the small round, or square cells are very convenient for mounting small pieces of injection. When larger preparations are to be preserved, the oval or square-shaped cells, (figs. 69, 70,) may be employed. Mr. Storer has succeeded in making cells on this

Fig. 70.



* The following table gives the prices and dimensions of some of these cells previous to being mounted upon the glass plates—

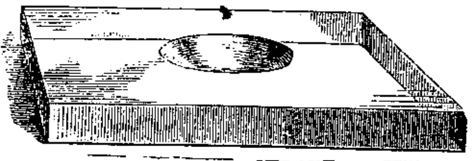
Length. In.	Breadth. In.	Depth. In.	Price.
6	3	½	2*s.* each.
3	3	½	6*d.* each.
1	1	¼	2*d.* or 3*d.* each.

Smaller cells from 1*s.* 6*d.* to 2*s.* per dozen.

plan, as large as six inches by three, and half an inch deep. The glass is cast in a mould, and thin sections are cut off of the required depth.

74. Concave Glass Cell.—Another form of cell which has lately been much used for mounting injections, is made by grinding a deep concavity (which may either be circular or oval), in the centre of a strip of very thick plate glass. (Fig. 71.)

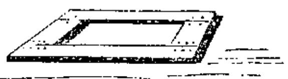
Fig. 71.



Cells of all the forms above mentioned, varying from a quarter of an inch in diameter to six inches, and of any required depth, may be obtained of Mr. Dennis, Mr. Topping, or Mr. Matthews, and when fixed upon the slides and ready for use, vary in price from threepence to two shillings or half-a-crown each.

75. Shallow-built Glass Cells.—Very large glass cells cannot be made by any of the above processes; and when only one cell of a particular size is required, it will be better to make it according to one of the following plans. The simplest method of constructing such a cell, provided it be not required of great depth, is the following:—A piece of thick plate glass, of the proper dimensions, is selected, and portions of equal width are cut off from the four sides, as shown in the annexed diagram (fig. 72); these are afterwards fixed in their proper places on the slide, with marine glue, care being taken to place the surface which has been cut by the diamond downwards, so that the furrows thus made may become filled up with marine glue. The upper surface is then to be ground rough with a little emery powder.

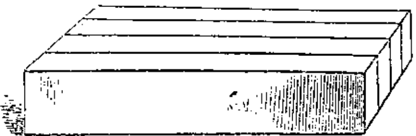
Fig. 72.



76. Deep-built Glass Cells.—If the cell be required of considerable depth, it must be made by joining together four strips of plate glass, the edges and ends of which have been ground perfectly flat and smooth. The following is the method of pro-

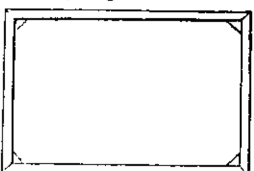
ceeding:—Four strips, of any depth and length required, are carefully cut off a piece of thick plate glass (§ 64); the surfaces of the four pieces are then joined together with marine glue, in the usual manner, and are adjusted as evenly as possible (fig. 73). When cold, both edges are ground perfectly square on a very flat stone, with common sand and water (§. 67). Time, of course, will be saved by using a grindstone, but the above method answers very well.

Fig. 73.



By joining the slips of glass in this manner, we must necessarily obtain the ground surfaces perfectly square, a point of great difficulty if each piece be ground separately. The ends of the slips of glass must now be ground perfectly square; all together, if the sides of the cell are to be equal; but if two are to be longer than the others, they must be separated and ground two and two. When the cut surfaces are ground perfectly even, the glass slips may be separated from each other, and the ends, after being cleaned, joined together with marine glue. The junctures are rendered stronger by fixing a small triangular piece of glass in each corner, as shown in fig. 74. The triangular piece is prepared by grinding down one of the angles of a very narrow strip of plate glass, which may then be cut into the proper lengths with a file. To join the ends we must proceed as follows: the ends of two of the sides are first warmed on the hot plate and joined with marine glue, taking care that they are placed perfectly square, and that the triangular piece is in its place; when cool, a third side is added with the same precautions; and lastly, the fourth is joined to the others: if the slips have been properly ground, this is easily effected, but if the ends be

Fig. 74.



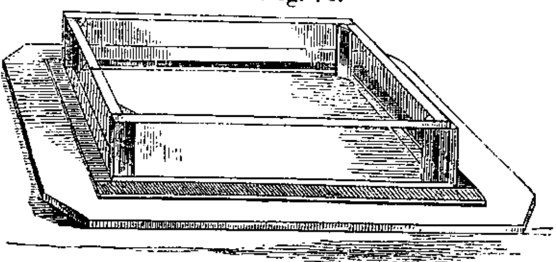
not perfectly square, it is quite impossible to make a good cell.

Mr. Dennis, who makes most excellent cells of this kind, has lately bevilled the ends of the sides before joining them together, as shown in fig. 74. By this excellent arrangement, the junctures are much firmer, and the future collapse of the sides, which sometimes happens after the preparation has been put up for some time, is rendered impossible.

When the angles are all joined, and the glue hard, it becomes necessary to grind both surfaces of the cell again, in order that they may be perfectly flat before it is attempted to fix the cell on the glass slab. This last grinding must be conducted very cautiously, particularly if the cells be large; one surface may be ground, and this fixed to the slab in the usual way, taking care not to raise the temperature too high, for fear of melting the glue by which the corners are joined together. The slab of plate glass should be cut rather larger than the cell, and the edges ground smooth.

When the slab is sufficiently heated, small pieces of glue are to be placed upon it, and, when these are melted, the cell may be applied, by aid of forceps, as described in § 68; narrow strips of glass should then be fixed all round the cell, as shown in the figure, care being taken that every point of the cell is connected with the slab by the glue. After the whole has become quite cold, the other surface of the cell may be ground smooth and the superfluous glue afterwards scraped off, and the cell thoroughly cleaned with potash, naphtha, or ether. (Fig. 75.)

Fig. 75.



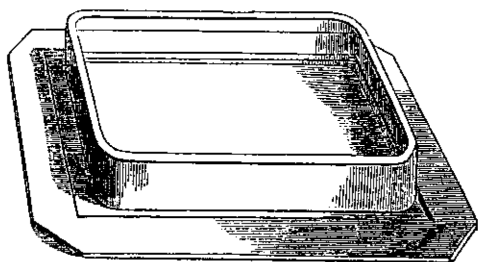
Cells made in this manner occasionally leak after a time, in consequence of the numerous joints which they contain; and

this is more particularly liable to happen if tolerably strong spirit be employed to preserve the preparation, because marine glue is softened by it.

For the method of making the built glass cells, and indeed most of the cells mentioned in the present chapter, we are indebted to Dr. Goadby; to whom also the thanks of practical observers are due, for the many improvements suggested by him in several branches of manipulation, especially in making minute and delicate dissections.

77. New method of making deep Glass Cells.—A method of making these large glass cells, which I have adopted for some time past, to a great extent, obviates some of the defects of the built glass cell, and, at the same time, the cell is made in much less time. Instead of joining the angles of separate pieces of glass together with marine glue, I take one long strip of plate glass of the proper depth, and with spots of ink accurately mark the length of the sides of the cell. At these marks the glass is carefully bent at a right angle in the flame of the blowpipe, taking care to keep each side perfectly square as it is bent; the ends are joined together in the blowpipe flame, and the surfaces are afterwards ground even on the stone. The heat must be applied very gradually, commencing below. In this process there is some difficulty in preventing the glass from cracking as it cools. This may, to a certain extent, be avoided by allowing the cell to cool very gradually, or, what is better, by placing it in an oven for a short time. If flatted flint glass could be obtained, the process would be very easy of execution, and cells of considerable depth might be readily made. When the surfaces have been ground flat, the cell is fixed to a slab in the usual way (fig. 76).

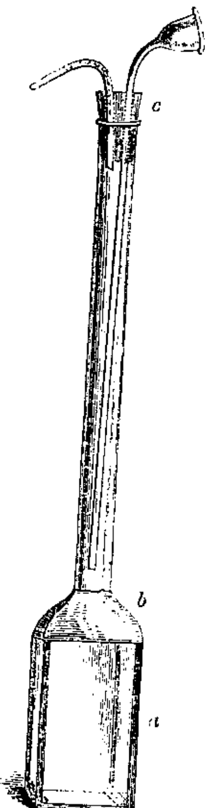
Fig. 76.



78. Cells made with the aid of Gutta Percha.—Occasionally

cells may be required of very peculiar shapes, wide perhaps in one part, and narrow in another; or of a form which it would be very difficult to make with glass only. Some time ago I required a cell of such a form that it would contain a proteus, while the circulation in the branchiæ could be observed under the microscope. The part of the cell containing the branchiæ and head must necessarily be perfectly flat, otherwise the object would not be sufficiently distinct.

Fig. 77.



A cell of a form which would enable the animal to be kept quite steady, and which allowed the water to be frequently changed while he was under observation, was made as follows:—A built cell of the form shown in fig. 77 *a*, was made in the usual manner (§ 67), one end only being left open. A piece of tube, of rather less diameter than the cell in its shortest dimensions, was then fixed to the open end with gutta percha, made soft by soaking in boiling water (fig. 77 *b*). Two pieces of glass tube of the form shown in the figure at *c* were then inserted into a cork, which accurately fitted the mouth of the tube. The total length of this cell was about twelve inches. The gutta percha joint was found to be quite firm, and the cell answered the purpose for which it was designed. The flat part of the cell, containing the head and branchiæ, was placed upon the stage of the microscope, and the animal supplied with fresh water from time to time by the funnel tube. In this way the

proteus could be kept under observation for upwards of two hours. The same plan may be successfully followed in making cells of other shapes.

On the subject of making cells, Papers by Dr. Goadby, in Silliman's "American Journal of Science and Arts," 1852, and "Quekett on the Microscope," may be also consulted with advantage.

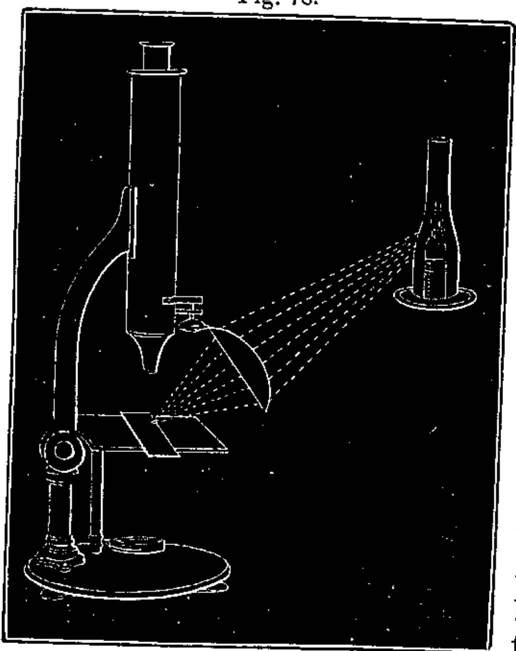
CHAPTER VI.

EXAMINATION OF OBJECTS BY THE MICROSCOPE—REFLECTED LIGHT—TRANSMITTED LIGHT—LAMPS—SUBSTANCES EXAMINED IN DIFFERENT MEDIA—DISSECTION UNDER THE SURFACE OF FLUID.

REFLECTED LIGHT.

79. Examination of Objects by Reflected Light.—Reflected light is employed for the examination of the surface only of substances placed in the field of the microscope, these objects being often too thick to admit the passage of light through them. In those cases in which the surface of transparent objects is to be examined, a piece of black paper, or cloth, or other opaque substance must be placed behind in order to prevent the passage of any rays of light through them. The light is usually rendered more intense by being condensed upon the object by means of a bull's-eye condenser (fig. 79). This is the method usually resorted to for

Fig. 78.

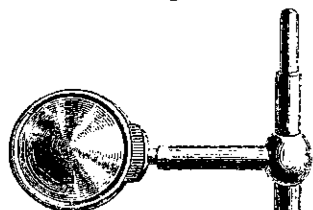


the examination of objects by reflected light. The microscope, light, and condenser are arranged as shown in fig. 78.

TRANSMITTED LIGHT.

80. Examination of Objects by Transmitted Light.—This mode of investigation is by far the most important with which the microscopical observer will be concerned. All transparent objects are examined in this manner, and it is that which more especially concerns the medical practitioner, for it will be according to the facility which he acquires in making thin sections of various tissues which he will subject to examination by transmitted light, that his success in this branch of clinical inquiry will depend.

Fig. 79.

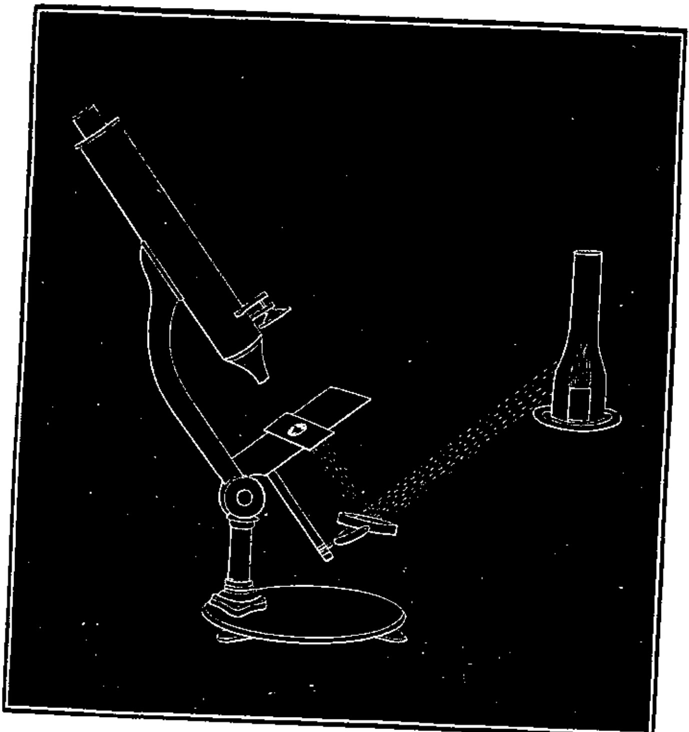


The illumination of objects which are to be examined by transmitted light may be effected in two ways, either by reflecting the light from a mirror situated beneath the stage (fig. 80), or by arranging the instrument in such a manner that the direct light from the lamp may be transmitted through the object. The former plan is the one commonly adopted, and it is the only one that need occupy attention here.

The different appearance of the same objects under the influence of transmitted and reflected light is represented in § 82.

81. Illumination of Transparent Objects — Lamps. — The best light for microscopical examination is ordinary daylight. If the sun be shining, the light should be obtained from some other part of the sky, as the direct rays of the sun are too dazzling to enable the observer to examine the minute structure of objects, and should never be employed. It has been said that the best light for microscopical examination is to be

Fig. 80.



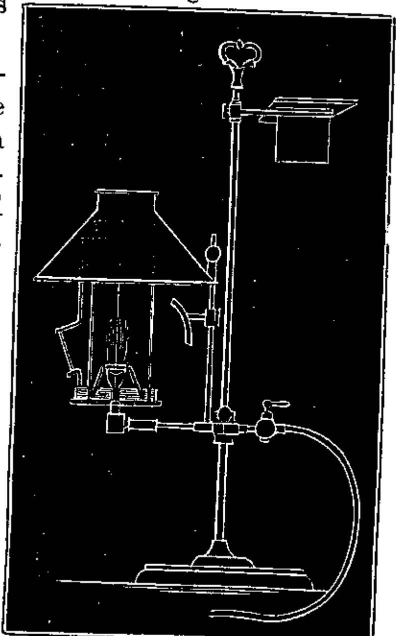
obtained from a cloud opposite the sun. Of artificial lights, the gas-lamp shown in fig. 81 is to be preferred.

This lamp was devised by Mr. Highley, of Fleet-street, and a description of it will be found in the second number of the "Microscopical Journal," p. 143.

The burner is an Argand, with very small holes. This is covered with a Leblond's blue-glass chimney; and the light

before passing to the mirror is transmitted through a circular piece of neutral-tint glass (which is fixed in the brass shade), as well as through the blue-glass chimney. Thus an unusually white light is obtained, which is very powerful and quite steady.

Fig. 81.

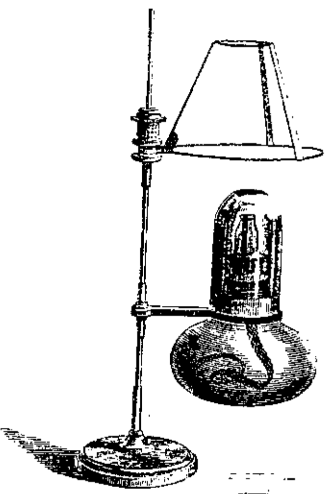


The lamp is also fitted with a small water-bath, and brass plate for mounting cells, &c.

An oil-lamp, constructed upon the same principle as the gas-lamp just described, is an excellent substitute. A common fish-tail gas-burner covered with a ground glass answers exceedingly well, and so also does the white light produced from a gaslamp covered with one of the opaque white bell-glasses. The small camphine lamp made by Messrs. Smith and Beck, fig. 82, forms a very convenient, and a most efficient means of illumination to those who do not possess the advantages of gas. The French moderator lamps, which are now coming into general use, are excellent lamps for the microscope. An ordinary Cambridge reading-lamp also answers very well; and where nothing better can be obtained, the light derived from a common wax candle may be employed, more particularly if it be protected with a glass in order to prevent flickering. In all cases the observer will find it a great comfort to protect his eyes from the direct glare, either by covering the lamp with a tin or paper shade, or by placing a piece of thick cardboard around the eye-piece of the microscope, in such a manner as to prevent his eyes being dazzled by the lamp, while at the same time he is enabled to look through the instrument without inconvenience.

It cannot, however, be too much insisted upon, that microscopical examination should be as much as possible avoided at night; and there cannot be a question that artificial illumination, however perfect it may be, is highly injurious to the eyes; and if persisted in for any length of time will lead to serious impairment of vision. Every observer who has had any experience with microscopical examination by candle-light, can testify to the fact that working for a few hours fatigues the eyes greatly, although by daylight no such effect is produced.

Fig. 82.



For the examination of opaque objects, such as injections, &c., artificial light is advantageous, particularly if the bull's-eye condenser be used, in order to concentrate the rays of light more fully upon the object.

EXAMINATION OF TRANSPARENT OBJECTS.

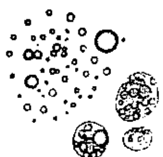
When an object is to be examined by transmitted light, the mirror should be so inclined as to reflect the rays directly through it, except in those cases in which exceedingly delicate structures are subjected to examination; when minute lines or points, which were previously invisible, will be brought into view if the mirror be placed so as to throw the rays obliquely through the object. If sufficient light can be obtained in that way, a plane mirror is the best, but if a powerful light is required, a concave reflector must be substituted. The larger microscopes are furnished with both, but to some of the smaller instruments a slightly concave reflector alone is attached. Mirrors made of porcelain, plaster of Paris, and perfectly white

writing paper, have been used with certain advantages, but they are not commonly employed, and are only necessary for certain special investigations (§ 20).

The well-known appearance of air-bubbles and oil-globules when examined in a fluid medium, is due to the difference of their refracting power, and should be familiar to the eye of every microscopic observer, for in the course of his investigations he will be constantly meeting with these bodies. Figs. 83 and

Fig. 83.

Fig. 84.



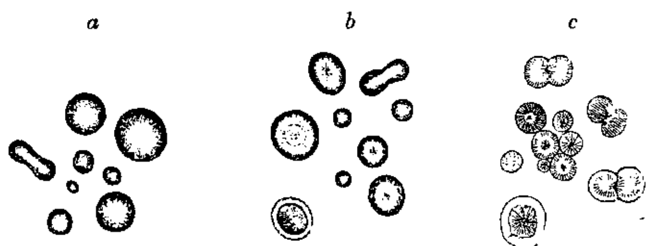
84 show the appearance of air-bubbles and oil-globules of different sizes, in water, when examined with a quarter of an inch object-glass.

82.—Influence of the medium in which the substance is immersed upon its appearance in the Microscope.—The microscopical appearance of a particular substance will be found to differ very much according to the nature of the medium in which it is immersed. This, therefore, is a point especially worthy of attention. The thickness of the outline of any specimen subjected to examination depends upon the different refracting powers of the substance itself, and of the medium in which it is immersed. The greater the difference of refrangibility between the object and the surrounding medium, the thicker will the outline appear; but in those cases in which the refracting power is nearly equal, the outline of the specimen will appear as a very thin line. Fig. 87 *c*. If the refracting power of two substances is quite equal, one cannot be distinguished from the other, except by variations in colour, &c.

If we treat blood corpuscles with a drop of acetic acid, or of a solution of an alkaline carbonate, we shall find the membrane of the corpuscle swell up, become more transparent, and appear to dissolve. This solution is rather apparent than real, for if a little solution of iodine be added, the cell wall will become again visible, and it probably depends upon a change taking place in the refracting power of the corpuscle, by which it approximates very nearly to that of the liquid in which it floats. After the addition of strong acetic acid to a drop of blood, the globules will still be faintly visible with a dull light, although by a strong light they are quite invisible.

Many substances should be examined in two or three different media, for in this way we are often enabled to discover peculiarities of structure which could not be recognised by examining the substance in one medium only. The student will gain much practical information by subjecting the same substance to microscopical examination—first, in the dry way; secondly, in water; and, thirdly, dried and afterwards mounted in turpentine, oil, or Canada balsam. The most instructive specimens to examine in this manner are crystalline substances and hard tissues, the intimate structure of which is not destroyed by drying and by being subsequently moistened with different fluids.

Fig. 85.



In fig. 85 is represented the appearance of some crystals of carbonate of lime from horses' urine, examined in different media; *a*, in the dry way; *b*, in water; *c*, in Canada balsam; and under the influence of reflected light (fig. 86 *a*).

In fig. 87 are represented some crystals of oxalate of lime under the same circumstances.

Fig. 86.

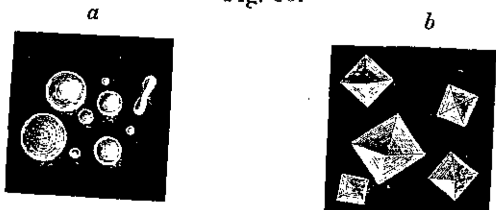
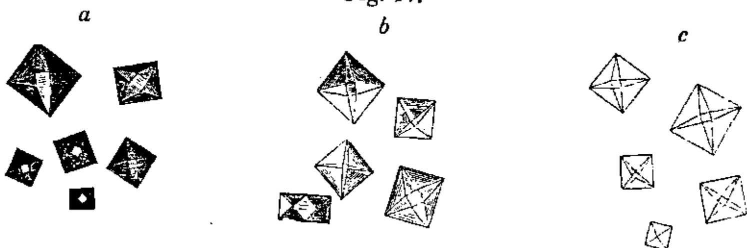


Fig. 86 *b* represents some of the crystals of oxalate of lime viewed by reflected light.

Fig. 87.



It is extremely difficult to lay down rules which will enable the observer to tell which method is best adapted to display the microscopical characters of any particular substance to the greatest advantage.

Substances having naturally a very smooth or polished surface, or to which such a surface can be artificially given, if sufficiently transparent, may be put up as dry preparations.

Thin sections of bone and teeth, specimens of hair and several crystalline substances, may be mounted in this manner.

Substances found moist in their natural state will usually require to be mounted in fluid. In certain cases, however, where outline and general form only are required, the objects may be dried; as, for instance, blood corpuscles or spermatozoa, which exhibit their general characters very well when mounted in this manner. Specimens of epithelium, muscle, nerve, and most of the tissues in a healthy or morbid state, require to be immersed in fluid.

If the substance be very dense and opaque, or contains small

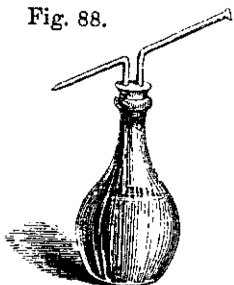
cavities filled with air, which are rendered indistinct by the opacity of the surrounding texture, it will be advantageous to examine it in Canada balsam, which will render the intervening substance more clear, in consequence of approaching it in refracting power, while the little spaces filled with air will appear quite dark and well defined. If, however, the walls of the cavities are to be examined, it is better to use turpentine, or thin balsam, which will penetrate the cells and drive out the included air. Moderately-thick sections of bone, teeth, or shell, and substances generally, the structure of which is not affected by drying, such as hair, nails, horn, &c., may be mounted in Canada balsam.

In order to remove adhering particles of fatty matter, it becomes necessary to wash the substance in ether, or in a mixture of alcohol and ether. It may afterwards be dried over the water-bath (§ 47) at a gentle heat, or allowed to dry spontaneously, and then moistened with water. Portions of certain textures, siliceous substances, &c., may be freed from particles of phosphate or carbonate of lime, by soaking them for a few minutes in moderately-strong acid. White fibrous tissue may be rendered clear and transparent by the addition of a drop of acetic acid. This is an operation which is frequently necessary in examining various textures, particularly if we wish to ascertain the presence of a nucleated structure, or of yellow elastic tissue. These substances having been completely obscured, in consequence of the abundance of the white fibrous tissue, which becomes perfectly transparent after the addition of a drop of acetic acid. Certain other processes are requisite in special cases, which will be more particularly dwelt upon when the methods of examining the different textures and deposits are considered.

83. Importance of cleaning Specimens for Microscopical Examination, and of separating them from other substances with which they may be mixed.—Substances intended for examination should always be carefully separated from impurities, such as dust, &c., which would render their structure indistinct. Washing in water, or other liquid, is often very necessary. In order to effect this object, the substance may be simply held

in forceps, and shaken about in a glass of water, or a small stream of water may be projected upon it from a wash-bottle (fig. 88), or from a common syringe, to which a small jet has been attached.

Fig. 88.

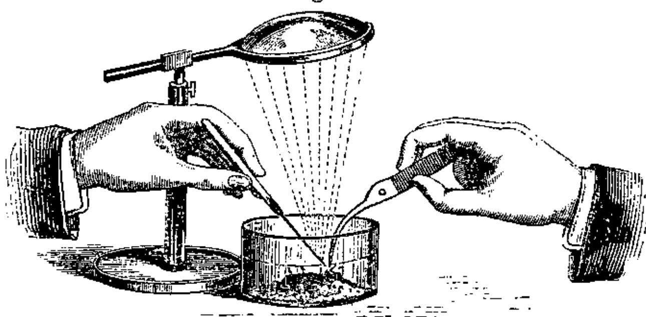


Deposits may be separated from dust and lighter particles by treating them with water, and after subsidence, pouring off the supernatant fluid, and replacing it with fresh, as often as may be necessary.

Large particles may be picked out with the aid of forceps or with needles.

84. Dissection under the surface of Fluid.—In making minute dissections of certain tissues, great advantage is often gained by dissecting under the surface of water; or of alcohol, in those instances in which the substance has been for some time previously immersed in the latter fluid. In this way, many delicate tissues may be separated and floated off, as it were, from the adjacent textures (fig. 89). When it is required to

Fig. 89.



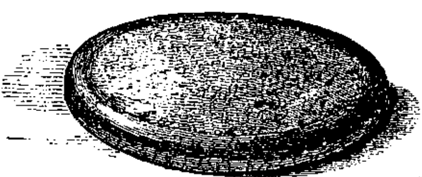
separate from each other the coats of an artery or mucous membrane, this becomes a very convenient method of proceeding. In certain cases, however, in which tissues are intended for microscopical examination, a certain amount of change of structure must be expected; the epithelium will often be abraded, and the cells much distended from endosmosis, so that such tissues should be immersed as short a time

as possible. In tracing sweat, or other minute ducts, or delicate branches of nerves, more especially in the lower animals, dissection under the surface of fluid is the only method by which success can be anticipated.

85. Glasses for dissecting under Water.—The vessel for containing the fluid should be proportioned to the size of the preparation to be dissected, and it should be of sufficient depth for the complete immersion of the object, fixed upon a loaded cork in its proper position; but at the same time the vessel should not be too deep, because greater difficulty would be experienced in following out the delicate branches of nerves, &c. The upper surface of the object should not be more than a quarter of an inch under the surface of the fluid (fig. 89). Vessels for this purpose may be made of glass, zinc, or earthenware. For small dissections, circular glass cups, similar to those sold in water-colour boxes, will be found most convenient; but for larger preparations a square zinc trough may be employed. The square-built glass cells (page 64) used for mounting anatomical preparations will often be found very convenient for dissection in fluid.

86. Method of fixing the Object.—The object intended for dissection must be fixed to some soft substance by means of small pins. For general purposes, pieces of cork, about a quarter of an inch in thickness, attached to a piece of sheet-lead, of sufficient weight to sink the cork (fig. 90), answers exceedingly well; but, in some cases, wax, or a preparation made by mixing wax and gutta percha, will be more convenient. A small plate of this mixture may be placed at the bottom of the cell in which the dissection is to be made, and may easily be fixed in its place by being a little pressed down at the edges, after it has been placed in the cup; or it may be poured while hot into the bottom of the cell, and there allowed to cool.

Fig. 90.



The preparation is to be fixed by means of small pins, or small pieces of thin silver wire, in a convenient position for dissection; and as the fluid becomes turbid from the accumulation of small portions of tissue which have been removed, it is to be poured off and carefully replaced by fresh. From the cell in which the dissection is made, the preparation, intended for mounting in a glass cell, may be removed by allowing it to float with some of the fluid into a watch-glass or tea-spoon, and in this manner it may be transferred.

87. Preparation of Wax and Gutta Percha for fixing Objects to, for Dissection, mounting in Cells, &c.—This preparation is readily made by heating about equal parts of white or yellow wax and gutta percha in a pipkin, over a coke fire, or gas lamp (care being taken to prevent the mixture catching fire, as it is very inflammable), and stirring with an iron rod until an uniform fluid mass is obtained. In order to give a darker colour to the mixture, lamp-black or indigo may be added. Cakes of this substance may be readily made, by pouring it while hot into a tin tray, which has been slightly wetted to prevent the wax adhering too firmly. When cold, this mixture forms a material which will hold a pin firmly, and which is not so brittle as wax. A very thin layer will be found sufficient for most purposes. If required very tough, a larger quantity of the gutta percha than that mentioned in the text may be added. The operation of making this substance should always be performed in the open air, as a very offensive smell is evolved from the melted gutta percha.

CHAPTER VII.

OF PREPARING OBJECTS FOR MICROSCOPICAL EXAMINATION, AND OF PRESERVING THEM—MOUNTING OBJECTS IN A DRY STATE—IN FLUID. PRESERVATIVE SOLUTIONS. MOUNTING OBJECTS IN CANADA BALSAM. ARRANGING PREPARATIONS IN THE CABINET.

SUBSTANCES require different methods of preparation, in order to display their minute structure to the greatest advantage. As already indicated (page 74), the nature of the medium in which the body is immersed, determines, to a considerable extent, its microscopical appearance. The appearance will also be modified according as the examination is made with transmitted or reflected light (§ 79, 80). In many cases it is important to subject a specimen to examination in two or three different ways, as described in page 74.

The mode of examination and the methods of preserving objects, may be arranged under three heads, each of which will now be separately considered:—

1. Preservation of objects in a dry state.
2. Preservation of objects in aqueous fluids.
3. Preservation of objects in turpentine, oil, Canada balsam, or some highly-refracting medium.

OF MOUNTING OBJECTS IN A DRY STATE.

88. Examination and Preservation of Objects in a dry state.—If it is only required to examine the character of a specimen in a dry state, it may simply be laid upon a glass slide, and placed in the field of the microscope; if, however, the substance be of a very delicate structure, or in a minute state of division, it is

better to place a piece of thin glass over it in the usual manner, in order to protect it.

Dry objects may be mounted in a thin glass cell (§ 71), or in a paper cell, or, if of extreme tenuity, they may simply be placed on a glass slide, and covered with thin glass, which should be fixed to the former by a small piece of gummed paper (rather larger than the glass cover), in the centre of which a hole has been cut of sufficient size to permit the entire object being seen. The paper may of course be of any colour, or ornamented according to the taste of the operator.

When objects are to be examined by reflected light, they may be placed in little glass or cardboard cells, or in pill-boxes; or they may be put up in glass cells. The preparation should be placed upon a dark ground, which may be effected either by cutting a piece of dark blue or black glazed paper, of the exact size of the cell, and placing it within; or the black paper may be fixed on the posterior surface of the slide; or this surface may be covered with black paint or black varnish (§ 59).

OF THE PRESERVATION OF OBJECTS IN AQUEOUS FLUIDS.

89. Examination of Objects in aqueous Fluids.—There are various methods by which preparations may be subjected to examination, and preserved as permanent objects in a moist state, and the different value of the various preservative solutions which are in use, entirely depends upon the nature of the substance to be mounted. Distilled water forms a very good fluid for some objects, while for the preservation of most, it is necessary to immerse them in water impregnated with some antiseptic agent, which is not volatile at ordinary temperatures. Many, again, are best preserved in spirit, or in a solution of some salt. It is very difficult to lay down rules which will enable the observer to choose a preservative fluid for any particular specimen. A little experience, however, will soon enable him to judge which solution is best adapted for the purpose. In those cases in which special solutions are of advantage, it will be stated.

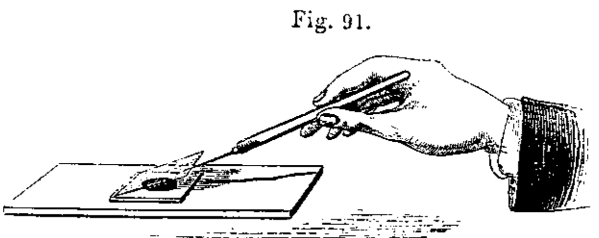
90. Of mounting Objects immersed in fluid in Cells.—Cells

of any form or size may be used in mounting objects in the moist way, according to the size and nature of the preparation. Some objects require to be pinned down to a piece of wax (§ 87), others may be allowed to float in the fluid in which they are placed. Many require a certain amount of pressure between the glasses in order to display them well. The operation, however, is performed in much the same way in each case, and the same general rules will apply to all moist preparations.

After the object has been allowed to soak for some time (a day or two) in some of the fluid in which it is to be preserved, it may be removed by means of forceps, or a pipette, to the cell, which should be previously filled with the solution. After all air-bubbles have been carefully removed by shaking the preparation a little in the fluid, or by conducting them to the surface by means of a camel's-hair brush or a fine wire, the object may be placed in the proper position. The cell should then be quite filled with solution, which may be caused to rise above its walls by pouring it in very carefully, or by letting it gradually flow from a pipette.

91. Of placing on the thin glass cover.—The thin glass cover which should of course be rather less than the external dimensions of the cell, and previously cleaned with a little spirit of wine, may then be taken in the forceps, and, after gently

Fig. 91.



breathing upon the lower surface, one edge is allowed to touch the fluid, and the glass cover may then be permitted to fall gradually, until it floats on the convex surface of the solution; this may be conveniently effected with the aid of a needle, as shown in fig. 91. Gentle pressure is then applied in order to force out the superabundant fluid, which must be sucked up by

a cloth, soft sponge, or piece of blotting paper; or, if in great quantity, it may be removed with a pipette.

The edges of the cell may be allowed to dry for a minute or two, and the thin glass cover may be fixed in its place by putting on a thin layer of gold size, Brunswick black, or solution of shell-lac, with a small camel's-hair brush, or with a small piece of soft wood cut to a blunt point, and hammered in order to separate the fibres a little, which in many instances is more convenient. A very thin layer of the gold size or varnish should be first applied, and this should be allowed to dry thoroughly before a second is put on; if a large quantity be painted on at once, some of it will probably run into the preparation, or the surface of the varnish, in consequence of becoming hard before the inner portion, will contract; the cement being gradually drawn from the surface of the cell, a little of the fluid evaporates, air enters, and the preparation requires remounting. It is a very good plan to put a little gold size round the edges of the thin glass before placing it on the fluid, as by this method air is not so likely to be admitted into the cell while the edges are drying, previous to the application of the varnish externally. The best way of preventing the ingress of air after the preparation has been mounted for some time, is by first applying a few layers of gold size or black carriage varnish, and when these are thoroughly dry, painting them with a solution of shell-lac or sealing-wax in spirit, taking care that each subsequent layer of varnish is a little broader than that previously applied.

92. Of mounting Preparations in large Glass Cells.—The method of mounting preparations in the large glass cells is somewhat different to that employed in putting up small microscopical preparations. In consequence of the great size of the cover, it is more difficult to apply than those of the small cells, and gold size or Brunswick black are not very well adapted to fix it to the walls of the cell. After the vessel has been well cleaned with a little weak spirit, the preparation may be placed in it, either floating in the preservative solution, or fixed to a piece of mica, which is cut to fit the cell exactly. Or, perhaps, it may be displayed to greater advantage by being pinned to a tablet of wax, or of the composition described in

§ 87, cut so as to fit the cell exactly. The preparation may also be kept in its proper place by strings attached to loops of thread, fixed in various parts of the cell. The loops may be placed in any situation, by attaching them first to a small piece of glass, which is to be cemented to any part of the cell with a little marine glue, and the aid of a hot iron. This was the method employed by Mr. Goadby in mounting his beautiful dissections of the nervous system of the lower animals, many of which are now in the Museum of the College of Surgeons. When the preparation is placed in its proper position, the cell should be filled with fluid. The cover, after being carefully cleaned, may be put on, by allowing one end first to touch the fluid, and then gradually inclining it until it falls in its proper position, the cell being quite full of fluid, and free from air-bubbles.

93. Methods of fixing the Glass Cover on the large Cell.—The next operation is to fix the cover in its place. All excess of fluid round the edges must first be removed with a soft cloth, piece of sponge, or blotting-paper, and then a layer of thick solution of shell-lac (§ 58) should be immediately applied round the cover, to connect it to the walls of the cell. The edges of the cover may be advantageously anointed with a little of the shell-lac solution before it is applied.

Mr. Goadby fixed on the cover of his large cells with marine glue. This he effected as follows:—The cell being quite clean, and the preparation having been arranged in the proper position, a little of the preservative fluid is to be poured in. The cover, with a hole already drilled in one corner, was applied, and fixed in its place with marine glue, which was melted with a hot iron. When the top was thoroughly fixed with marine glue, the cell was filled with fluid through the hole. After all bubbles had risen to the surface and had been replaced with fresh fluid, the hole was stopped up with a cork, and a small piece of glass was cemented over it to keep the cork in its place. The hole should not be closed for a day or two, in order to permit all the air-bubbles to rise to the surface.

The method of which I have had the greatest experience is

the following:—Some of the French cement (§ 63) is rolled out into thin strings, which are pressed all round the top of the cell with the finger and thumb, care being taken that there is not more cement in one part than in another. This operation must be performed before the preparation is placed in the cell. One small space, about a quarter of an inch wide, is left uncovered with cement at one end of the cell.

The preparation is next put in, and the cell filled with the preservative solution up to the brim. One end of the cover is slightly pressed into the cement at the opposite end of the cell to that in which the vacant space was left; the cover is then allowed to fall gradually, until it everywhere touches the cement. The cell is now quite full of fluid. After the cover has been pushed sufficiently into the cement, and a certain quantity of fluid has escaped, the hole may be stopped up with a small piece of cement, and the preparation should then be allowed to lie still for a time, when it may be wiped dry, all superfluous cement removed with a knife, or smoothed down with a hot iron, and the edges painted with gold size or some kind of varnish. This plan is exceedingly simple and easy of execution, and appears to last quite as well as other methods. The great advantage of it is, that the cement never thoroughly hardens, but always retains sufficient elasticity to allow for change of volume of the fluid, when exposed to the extremes of temperature. If the preparation be preserved in strong spirit, however, the cell will not remain air-tight, as the spirit insinuates itself through the cement and evaporates,—air of course rushing in to supply its place.

The different cements employed for fixing the thin glass cover to the cell have been already described (§ 56-63).

Various other cements have been recommended for the purpose of fixing the cover upon large glass cells. Roman cement has been used by some observers: and my friend Mr. Stewart, who has had considerable experience with it, tells me it answers perfectly well.

PRESERVATIVE SOLUTIONS.

94. Spirit and Water.—Mixtures of spirit and water of various strengths are required for preserving different preparations. In diluting spirit, distilled water only should be employed; for if common water be treated with spirit, a precipitation of some of the salts dissolved in it not unfrequently takes place, rendering the mixture turbid and unfit for use. Proof spirit will be strong enough for all general purposes, except for hardening portions of the brain or nervous system, when stronger spirit must be used. Two parts of rectified spirit, about sp. gr. ·837, mixed with one part of pure water, makes a mixture of sp. gr. ·915-·920, which contains about 49 per cent. of real alcohol, and will therefore be about the strength of proof spirit. One part of alcohol, 60 over proof, to five parts of water, forms a mixture of a sufficient strength for the preservation of many substances.

95. Glycerine.—A solution of glycerine adapted for preserving many structures is prepared by mixing equal parts of glycerine with camphor water. The latter prevents the tendency to mildew. It may be used as other preservative solutions.

Glycerine is obtained by boiling oil with litharge. The oleate of lead remains as an insoluble plaster, while the glycerine is dissolved. It may be rendered free from lead by passing a current of sulphuretted hydrogen through it; and the clear solution, after filtration, may then be evaporated to the consistence of a syrup.

96. Thwaites' Fluid.—This fluid has been much employed by Mr. Thwaites for preserving specimens of desmidiæ; but it is also applicable to the preservation of animal substances.

Water	16 ounces.
Spirits of wine .	1 ounce.
Creosote, sufficient to saturate the spirit.	
Chalk, as much as may be necessary.	

Mix the creosote and spirit, stir in the chalk with the aid of a pestle and mortar, and let the water be added gradually.

Next add an equal quantity of water saturated with camphor. Allow the mixture to stand for a few days, and filter. In attempting to preserve large preparations in this fluid, I found it always became turbid, and therefore tried several modifications of it. The solution next to be described was found to answer very satisfactorily. Water may also be impregnated with creosote by distillation. It should be remarked that M. Strausdurkheim has succeeded in preserving preparations in camphor water only.

97. Solution of Naphtha and Creosote.

Creosote . . .	3 drachms.
Wood naphtha .	6 ounces.
Distilled water .	64 ounces.
Chalk, as much as may be necessary.	

Mix first the naphtha and creosote, then add as much prepared chalk as may be sufficient to form a smooth thick paste; afterwards add, very gradually, a small quantity of the water, which must be well mixed in a mortar. Add two or three small lumps of camphor, and allow the mixture to stand in a lightly-covered vessel for a fortnight or three weeks, with occasional stirring. Pour off the almost-clear supernatant fluid, and filter it if necessary. Preserve it in well-corked or stoppered bottles.

I have some large preparations which have been preserved in upwards of a pint of this fluid, for more than five years, and the fluid is now perfectly clear and colourless. Some dissections of the nervous systems of insects have kept excellently; the nerves keeping their colour well, and not becoming at all brittle. Two or three morbid specimens are also in an excellent state of preservation; the colour being to a great extent preserved, and the soft character of the texture remaining. I have one preparation mounted in a large gutta percha cell, containing nearly a gallon of this fluid.*

98. Solution of Chromic Acid.—A solution of chromic acid will be found well adapted for preserving many microscopical

* Mr. Quekett recommends a mixture of one part of naphtha to seven or eight of water as a good preservative solution. Op. cit., p. 281.

specimens. It is particularly useful for hardening portions of the nervous system previous to cutting thin sections. The solution is prepared by dissolving sufficient of the crystallized acid in distilled water, to render the liquid of a pale straw colour.

The crystallized acid may be prepared by decomposing 100 measures of a saturated solution of bichromate of potassa, by the addition of 120 to 150 measures of pure concentrated sulphuric acid. As the mixture becomes cool, crystals of chromic acid are deposited, which should be dried and well pressed on a porous tile, by which means the greater part of the sulphuric acid is removed, and the crystals obtained nearly pure.

99. Preservative Gelatine.*—

Gelatine	1 ounce.	Spirits of wine	½ ounce.
Honey	4 ounces.	Creosote	6 drops.

Soak the gelatine in water until soft, and to it add the honey, which has been previously raised to the boiling-point in another vessel. Next, let the mixture be boiled, and after it has cooled somewhat, the creosote dissolved in the spirits of wine is to be added. Lastly, filter through thick flannel to clarify it. When required for use, the bottle containing the mixture must be slightly warmed, and a drop placed on the preparation upon the glass slide, which should also be warmed slightly. Next, the glass cover, after having been breathed upon, is to be laid on with the usual precautions, and the edges covered with a coating of the Brunswick black varnish. Care must be taken that the surface of the drop does not become dry before the application of the glass cover; and the inclusion of air-bubbles must be carefully avoided.

100. Goadby's Solution.—

Bay salt	4 ounces.
Alum	2 ounces.
Corrosive sublimate	4 grains.
Boiling water	4 pints.

* Mr. H. Deane, Transactions of the Microscopical Society, quoted in Quekett's Treatise on the Microscope.

Mix and filter. This solution may for most purposes be diluted with an equal bulk of water. For preserving delicate preparations it should be even still more dilute.

101. Burnett's Solution.—This fluid has been patented, and may be obtained in a concentrated form, in bottles of different sizes, 53, King William-street, London-bridge.

Its preservative properties appear to depend upon the chloride of zinc. A strong solution of chloride of zinc forms a very powerful antiseptic, and also possesses the property of absorbing noxious odours, &c.

102. Other saline solutions.— Many other saline solutions have been employed by different observers. Of these, a saturated aqueous solution of chloride of calcium, free from iron, has been much recommended for preserving specimens of bone, hair, teeth, and other hard structures, as well as many vegetable tissues (Schacht). A solution of alum in the proportion of 1 part of alum to 16 of water has been found to answer pretty well for some substances. Gannal's solution, which consists of 1 part of acetate of alumina dissolved in 10 parts of water; solutions of common salt (1 part to 5 of water, with a little camphor), corrosive sublimate, persulphate of iron, arsenious acid, sulphate of zinc, and solutions of several other salts, have been recommended as preservative solutions, but their employment has not been always attended with the most satisfactory results.

Arseniuretted hydrogen gas has also been recommended for the preservation of animal substances, but it is not adapted for microscopical preparations.

The particular preservative fluid adapted for the preservation of the different textures will be indicated when the method of preserving these is brought under consideration, and at the same time any special processes adapted for demonstrating the structure of different textures will be adverted to.

MOUNTING OBJECTS IN CANADA BALSAM.

103. Methods of drying the substance previous to its immersion in the balsam.—It is of the utmost importance that substances which are to be mounted in Canada balsam should be thoroughly dried before being placed in this menstruum, otherwise the preparation will always be covered with little bubbles of steam, which will prevent it from being seen distinctly. Many substances may be dried by leaving them exposed to the air, especially in the sun; but usually it will be better to expose them over the water-bath (fig. 47) for a short time, before putting them up. If a slight elevation of temperature does harm to the structure, it may be thoroughly dried by exposing it for some time over a dish containing strong sulphuric acid, the whole being covered with a bell-jar, or placed under a receiver, which may be exhausted by connecting it with the air-pump.

104. On moving air from the interstices of a tissue.—This may be effected by placing the substance, previously carefully dried in a little fluid balsam, under the receiver of an air-pump; any air in the tissue is then forced out as exhaustion proceeds, and its place becomes occupied with balsam.*

When membranes and thin sections of tissues, such, for instance, as sections of the spinal cord, muscular fibre, &c., are to be preserved in balsam, they must be very slightly washed, and then spread out upon the glass slide with the aid of needles and forceps. They should be allowed to dry spontaneously, and afterwards it is better to expose them for a short time over sulphuric acid, and the section may then be put up in the usual way, but without removing it from the slide. Frequently it will be found advantageous to wet the surface very slightly with turpentine before the balsam is applied. If the sections appear too thick when dry, the surface may be scraped with a sharp knife, or very thin shavings may be removed.

* "Dr. Golding Bird has applied this method for mounting the polypidoms of zoophytes with great success."—Microscopical Journal, 1853, p. 85.

105. Precautions to be observed in applying the Balsam.—Canada balsam (§ 61) forms a most useful agent for mounting various substances; and the structure of many can only be clearly made out when they are examined in this menstruum.

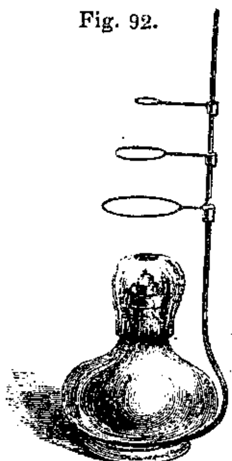
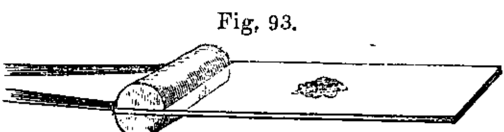


Fig. 92.

In this method of mounting objects no cells whatever are requisite. The balsam should be pale and old. The glass slides must be warmed before the balsam is put on, and for this purpose the glasses may be held in a pair of wooden forceps, or in a pair of common forceps, the legs of which are covered with cork (fig. 93) and heated over the spirit-lamp (fig. 92) or upon the brass plate (§ 46). The latter plan is the most convenient when several preparations are to be mounted at the same time, because they may be arranged in a row along the plate, and the balsam placed upon each slide as it becomes hot.

The Canada balsam may be heated after it is placed upon the slide, or the tin vessel, fig. 94, may be kept warm for some little time before the balsam is taken out, in order to allow the air-bubbles entangled in it to rise to the surface before it is applied.

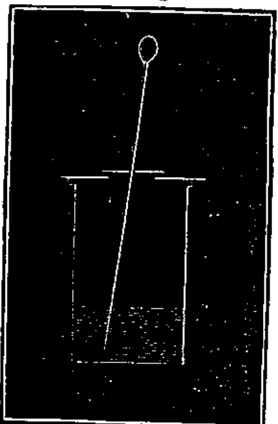
Fig. 93.



The slide being warm, and the small quantity of Canada balsam sufficient to contain the preparation having been placed upon it, it must be gently moved about while the balsam is hot and quite fluid, until all the air-bubbles have floated to the surface and collected together towards one spot. A pointed wire or needle should then be taken, and all the bubbles either drawn out upon the end of it, which may be readily effected, or broken by the wire after it has been heated. In those cases in which

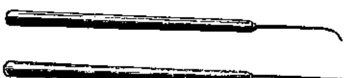
the preparation is not detached from the glass slide upon which it has been allowed to dry, it is only necessary to place the drop of balsam upon it and gently warm it, following the usual precautions; afterwards the thin glass cover may be applied. When the preparation has been dried separately over the water-bath and cleaned, it may be taken in a fine pair of forceps, gently warmed, and carefully placed in the hot and perfectly fluid balsam. After it has been thoroughly wetted by the balsam, and all adhering air-bubbles removed (§ 104), it may be placed in the position it is intended to occupy. The thin glass cover, adapted to the size of the preparation, having been previously cleaned and warmed, may then be taken in a pair of forceps, and, after being held over the warm balsam for a minute, allowed to fall gradually upon the preparation (beginning at one side), until it becomes perfectly wetted with the balsam. The glass may now be slightly pressed in order to force out the superfluous balsam, and the preparation allowed to cool.

Fig. 94.



106. Of removing the Air-bubbles from the Balsam.—The only difficulty of mounting preparations by this method consists in getting rid of all adhering air-bubbles completely. This may generally be effected by carefully heating the balsam on the slide, and the preparation, first separately, and then together, and by removing the bubbles after they have been made to collect at one spot, with the aid of a fine-pointed wire. A needle, which should be stuck into a small wooden handle, is a most convenient instrument for this purpose (fig. 95).

Fig. 95.



If the balsam be heated to too high a temperature it becomes hard and resinous before the preparation can be placed in the proper position; in such a case it is better to clean the slide

and use fresh balsam. Any balsam may be removed from the preparation by soaking it for some time in turpentine. Some preparations require boiling in the balsam, in order to drive out air contained in cavities in their interior. As a general rule, however, care must be taken to prevent the balsam from boiling, because many organic substances curl up and become entirely spoiled at this temperature.

107. Of mounting Preparations of considerable size in Fluid Balsam.—It is sometimes necessary to mount opaque objects of considerable size in liquid Canada balsam, in which case we must either place it in a glass cell or between two pieces of window-glass, which are joined together at the edges with sealing-wax; a plan followed by many continental anatomists. In this case the balsam should be perfectly fluid, but not thinned with turpentine, for Mr. Quekett has observed that if this be done, air-bubbles appear to be developed some days after the preparation has been finished, although when first put up it was perfectly clear. This result is occasioned by the turpentine not being thoroughly mixed with the balsam at first. After some days a complete admixture occurs, and little cavities are formed in consequence of the two bodies occupying a larger bulk when separate, than when intimately mixed with each other.

When the preparation has been placed in its proper position, and the thin glass has been applied, it only remains to remove any superfluous balsam from the slide. This is readily effected by the aid of some rags, an old knife, or the large bradawl, (fig. 96,) and a little turpentine. The greater quantity can be scraped off with the knife, and the remainder may be removed by carefully moistening it with turpentine, and then cleaning it off with a rag. The preparation is now finished, and may be left plain, the edges of the balsam varnished over, or the slide may be covered with ornamental paper, according to the taste of the operator.

Fig. 96.

NAMING PREPARATIONS AND THEIR ARRANGEMENT IN THE CABINET.

108. **Placing the Name on the Glass Slide.**—Every preparation should be named as soon as it is finished. The name is generally written at one end of the slide with the writing diamond, fig. 49, but a small paper label answers every purpose; and it is a good plan, when long descriptions are necessary, to affix a number to each slide corresponding to the number referring to the description of the preparation in a catalogue.

In this catalogue all the particulars having reference to the object should be entered, including a note of the date of mounting, and also describing the fluid in which it was preserved.

Cabinets for Preparations.—Microscopical preparations should be kept in drawers or in boxes prepared for the purpose, in order to preserve them from dust and from the influence of light. Dry preparations and those mounted in Canada balsam may be arranged in vertical grooves cut in the partitions of the drawer. The grooves should be wide enough to allow the slide to fall in easily, and should be about a quarter of an inch apart. Preparations mounted in fluid, however, must be kept in a horizontal position, although they necessarily occupy much space; but otherwise it will be found that the tendency for the cells to leak is much increased. Large preparations can only be kept lying perfectly flat in shallow drawers.

Boxes and cabinets of all kinds for keeping collections of microscopical preparations may be purchased of Mr. Topping, Messrs. Smith and Beck, and of Mr. Matthews.

Upon the subjects treated of in chapters VI. and VII., besides those works referred to in the text, the following have been consulted:—Translations from "Het Mikroskoop," Prof. Harting, Utrecht, in Edinburgh Monthly Journal; "Lectures by Dr. Goadby," in Silliman's Journal; "The Microscope, in its Special Application to Vegetable Anatomy and Physiology," Dr. Hermann Schacht, translated by Currey; "Ralph's British Desmidiæ;" Papers in the "Microscopical Journal," and "Transactions of the Microscopical Society;" "Traité pratique et théorique d'anatomie comparative," Paris, 1842, Strausdurkheim; "Nouveau Manuel de l'observateur au Microscope," Dujardin, Paris, 1843; Quekett, op. cit.

CHAPTER VIII.

ON INJECTING. INSTRUMENTS EMPLOYED IN INJECTING—COLOURING MATTERS—OF THE OPERATION OF INJECTING.

In order to examine the different arrangement of the ultimate divisions of the blood-vessels in various parts of the animal structure, it becomes necessary to fill them with some opaque substance, by which their distribution may be rendered distinct, and their general arrangement readily observed, when subjected to examination by the low powers of the microscope. When the capillaries are empty, they are scarcely visible, and frequently it is quite impossible to distinguish them from the tissues in which they ramify. The process by which these minute vessels are rendered distinct, is called *injecting*, and portions of structure which have been treated in this manner are spoken of as *injections*, or *injected preparations*. Injections are of two kinds,—natural and artificial. Natural injections, as the name implies, are obtained from the dead animal without any preparation whatever, the capillaries being gorged with blood, and thus rendered distinct. Artificial injections are always prepared by forcing some opaque or coloured liquid into the small vessels from a large one.

109. **Natural Injections** are frequently found very perfect, and in some instances show the arrangement of the minute vessels more perfectly than they can be exhibited by any artificial process. This condition results from the capillary vessels being distended with blood at the time of death, or perhaps soon afterwards, and the general redness which is always observed in a tissue in this condition, is said to be due to a state of congestion. Patches of the mucous membrane of the intes-

CHAPTER IX.

PREPARATION OF OBJECTS FOR EXAMINATION BY TRANSMITTED LIGHT—METHOD OF EXAMINATION—EXAMINATION OF SOFT TISSUES—KIDNEY—LIVER.

129. **Methods of Examination.** — The most advantageous method of examining particular tissues, and the best manner of preserving them, can only be learnt by long practical experience. It will only be attempted here to introduce some of the methods which are most frequently employed to demonstrate the minute anatomy of those textures which most frequently come under the notice of the practitioner.

In order to examine the structure of many tissues, it is necessary to obtain a section sufficiently thin to permit the transmission of the light readily, and so evenly cut, that the minute structure of the tissue may be submitted to examination in every part of the section. The difficulty of making thin sections of many textures is often very great, and, to effect this object satisfactorily, a knowledge of certain mechanical operations becomes necessary. Sometimes we require to cut a thin section of a soft pulpy texture, which can scarcely be touched without injuring its delicate structure, and altering the position of its constituents; while, in other instances, we must obtain a very thin transparent section of a substance so hard, that steel tools will scarcely scratch it, such as the enamel of teeth, fossil teeth, &c.

The method of making sections of soft tissues will first be briefly described, and afterwards the processes adapted for cutting hard textures will be alluded to. Other mechanical operations, such as tearing up the tissue with fine needles,

pressing it between glasses, removing soft pulpy portions by washing, &c., are often necessary, and will also require to be considered.

Chemical reagents are frequently very useful in elucidating the nature of minute structures, either by destroying or altering some of the component parts of the tissue, or by simply rendering the substance more transparent. Much may frequently be learnt concerning the nature of the inorganic portion of the tissue by ignition upon platinum foil, and burning off the carbonaceous portion of the residue by exposing the specimen for a length of time to the action of a red-heat.

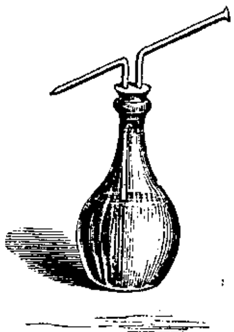
By simply drying a tissue we are sometimes able to make out a point in its structure, which had entirely eluded our observation when the tissue was examined in a recent state, and there are other processes of practical importance in the demonstration of minute structures, which should also be considered.

130. **Boiling the Tissue previous to Examination.** — This operation is often of great service in enabling us to demonstrate the structure of a tissue. For instance, the fibres of which the crystalline lens is composed, are best shown after boiling the lens in water. The branched muscular fibres in the tongue of the frog, and in other situations, may be made out very readily by boiling the organ in water for a few moments, and then tearing up small portions with fine needles. Beautiful sections of muscular fibre can often be obtained after the texture has been boiled in water. Various glands and other textures often require to be boiled for some time in water, in order to harden them sufficiently to enable us to cut thin sections; but in all cases the microscopical characters of the recent texture should be examined, as well as that which has been hardened by boiling. Small portions of tissue can be readily boiled in a test-tube over the spirit-lamp.

131. **Washing, soaking, or pressing the Tissue.**—Not unfrequently we wish to get rid of the soft and more pulpy part of a tissue, in order to subject the more dense and fibrous portion to examination. This object is usually effected by soaking the tissue in water for some little time, and then placing it under a running stream of water, by which means the softer portions

are gradually washed away. Soaking in water frequently enables us to tear up a tissue very readily with the aid of needles, and thus to demonstrate its structure. Occasionally it is found necessary to press the tissue, and rub parts of it together, before the soft pulpy portions can be got rid of. In this way we may demonstrate the supporting or trabecular tissue of the spleen, and the areolar and vascular tissue of the liver, &c. Thin sections of kidney, liver, and other glandular organs, may be thus treated when the matrix is to be subjected to examination separately.

Fig. 108.



In these operations the wash-bottle (fig. 108) will be found useful. Generally it will be better to make a thin section of the tissue first, and then soak and wash carefully, when the parts may be seen *in situ*.

132. Drying the Tissue previous to Examination.—Thin sections of various tissues can frequently be obtained only by first drying the substance thoroughly, and then cutting off a thin shaving with a sharp knife. In this way specimens of skin, mucous membrane, and many other tissues, are often most advantageously prepared. The tissue is stretched on a board with pins, and then allowed to dry, when a very thin section can be cut off and examined in Canada balsam; or it may be placed in water for a short time, in which case, when subjected to examination, it will often be found to have regained its first appearance. Portions of muscular fibre, the tongue, skin, and many other tissues, may be allowed to dry in this manner, and then we may with a sharp knife readily obtain exceedingly thin sections, which could not be procured in any other manner. The drying may be effected in a warm room, or in a current of air. A high degree of artificial heat should be avoided.

133. Application of Chemical Reagents.—In the examination of various substances, much information may frequently be

gained with respect to their nature by exposing them to the action of various chemical agents. The action exerted by alcohol, chromic acid, corrosive sublimate, &c., is often very useful in enabling us to cut thin sections for microscopical examination. When we require to make a tissue very hard, we need only soak it in alcohol or some other chemical reagent which has the property of coagulating albumen. By resorting to the use of chemical reagents we are frequently enabled to make out the true nature of a body, which has entirely baffled our powers of observation, when subjected to microscopical examination only. Chemical reagents assist us in microscopical investigation, by rendering parts of the texture transparent, so that we are enabled to see structures which were previously obscured, or by making the tissue itself darker, or by causing certain alterations in its general characters which are not easily explained. The method of applying chemical reagents, and a general description of the changes which ensue, will be found in Chapter XVI.

In describing the methods of demonstrating the minute anatomy of the most important animal tissues in a healthy or morbid state, I shall have frequently to refer to the assistance which is derived from the application of chemical tests.

134. Igniting the Substance in order to remove Organic Matter.—When the inorganic portion of a tissue which we wish to examine is not altered by exposure to a red-heat, recourse may be had to ignition, in order to get rid of the animal matter. In this way crystals of carbonate and phosphate of lime, and granules of siliceous matter, may be separated from the organic material with which they were combined. The beautiful siliceous shells of the diatomaceæ may be separated from organic matter by a similar process. The ignition should be performed in a small platinum capsule, or upon a small piece of platinum foil. The carbonaceous residue must be exposed to the dull red-heat of a spirit-lamp (§ 43) for some time, until only a pure white ash remains, which will be found to contain the objects of our search in a very perfect state. If the siliceous matter only is wanted, the ash should be treated with strong nitric acid, which will dissolve any carbonate or phosphate. The insoluble

residue may then be washed and dried, and subjected to microscopical examination while immersed in turpentine or Canada balsam. In many cases, this method is superior to that of boiling in nitric acid, in order to remove the organic matter. Both processes may, however, be employed where only the siliceous residue is wanted, but if we require the salts of lime, ignition at a dull red-heat is alone applicable.

135. Of cutting thin sections of Soft Tissues. — There is no more important operation in microscopical investigation than the present. The student is continually requiring thin sections of different textures, and whether he pursues the study of vegetable or animal physiology, or morbid anatomy, it will often be necessary to make a very thin section of the tissue which is to be examined; and upon the amount of skill he displays in cutting these sections, will the success which attends his investigation mainly depend. The darker and more complicated the tissue may be, the more important does it become to obtain a section of extreme tenuity, for otherwise sufficient light cannot be transmitted through the tissue to enable us to see its structure; moreover, in a thick section, the objects occupying different planes so much interfere with each other as to prevent the possibility of any one being defined clearly.

Cutting a thin section of a soft tissue may at first sight appear a very simple process, but it will be found to require considerable skill on the part of the operator. Sections of the large glands, and other soft tissues, may be made with an ordinary knife which should be very sharp. A clean surface is first cut, and then a thin slice is removed with a slow sawing motion of the knife, which is much facilitated by the application of a drop of water; indeed, whenever we require a very thin section of a soft tissue, the blade of the knife should always be well wetted with water.

The most important instruments for making thin sections of soft tissues are the following: scissors of different sizes (§ 53), Valentin's knife, double-edged scalpels, or lancets mounted in handles, and a few other instruments, such as forceps (§ 55), and needles of different sizes (§ 52), mounted in handles, are often required in demonstrating minute structure.

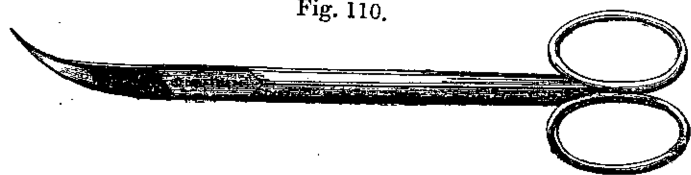
136. Scalpel specially adapted for Cutting thin sections of Soft Tissues.—*Double-edged Scalpel.*—For cutting thin sections, a knife of the form represented in fig. 109, will be found very useful, and, where only sections of small dimensions are required, this will answer all the purposes of Valentin's knife. In cases however, where a section is wanted of considerable size, the latter instrument must be used. The double-edged scalpel is made after the fashion of a common lancet; it is not so wide, but should be quite as thin. When employed for making a section (after cutting a clean surface), the point is made to perforate the surface, and carried along at a proper depth, so as to cut its way out. The width of the section may then be increased by carrying the knife first to the right, and then to the left, until a section of the desired width is obtained.

Fig. 109.

Common Lancets mounted in handles will be found convenient for cutting thin sections, but each side of the blade should be sharpened down to the point of insertion into the handle.

Scissors are also very useful instruments for cutting small thin sections of different tissues. The most convenient form for this purpose is that shown in fig. 110. When only very small portions of a tissue are required for examination, they will be more readily removed with the scissors than with any other instrument.

Fig. 110.



137. Valentin's Knife.—This instrument is of the greatest value in making thin sections of soft tissues, but it requires care

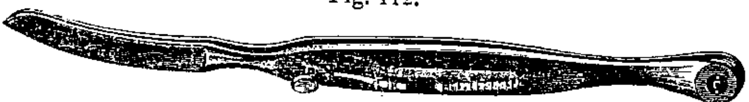
to keep it in good order. It is very easily made blunt if used for cutting fibrous or cartilaginous textures. By its aid most beautiful sections of the kidney, liver, and other soft glandular organs may be obtained with the greatest facility. The blades

Fig. 111.

should always be dipped in water just before use, for, if wet, the operation of cutting is much facilitated, and the section is more easily removed from between the blades. Immediately after use the blades should be washed in water, and dried with a soft cloth or a piece of wash-leather. If a drop of water gets into the upper part of the knife where the blades meet, the screw must be taken out, and each blade cleaned separately. With care the knife may be kept in use a long time.

Two forms of Valentin's knife are used; in one of these the blades are sharp on both edges and of a lancet-shape, and in the other, which I much prefer, of the form represented in the figure (fig. 111). The best form of Valentin's knife that I have used is that represented in fig. 112, which has lately

Fig. 112.



been made by Mr. Matthews. The blades of this knife can be completely separated from each other and easily cleaned. Moreover, the distance between the blades is regulated by a little screw *a*, which is a most convenient arrangement.

EXAMINATION OF SOFT TISSUES.

Before examining morbid tissues the student should acquire a thorough knowledge of the appearances which the textures present when in a state of health; for, without being familiar with the minute anatomy of healthy tissues, he will hardly be in a position to appreciate the changes which have been brought

about by disease. In this work it will be quite impossible to discuss, even in the most cursory manner, the healthy and morbid condition of the various tissues, and the methods of subjecting them to examination; nor can I attempt to describe the morbid alteration which may have taken place in any one gland or texture. At the same time it is my desire briefly to refer to the mode of investigating the healthy and morbid appearances of those textures which most frequently engage the attention of the physician. Where any particular method of investigation is required to demonstrate the minute anatomy of a tissue, it will be my endeavour to give an illustration of it, so that the student will, I hope, be enabled without much difficulty to fill up for himself at leisure, by reference to standard works, those deficiencies which limited space will not permit me to supply.

138. Method of submitting a portion of Tissue to Microscopical Examination.—In order to subject a portion of tissue or other substance to examination by transmitted light, we usually proceed as follows:—One of the glass slides (§ 49) is carefully cleaned, and the thin section of tissue which has been removed by the aid of forceps and scissors, or a scalpel, placed in the centre; a drop of clean water is then added, and the whole covered with a square of thin glass (§ 50), also perfectly clean. If the under surface of the thin glass be gently breathed upon it becomes wetted more easily. The substance may be unravelled with needles, or, if necessary, any other operation performed before covering it with the thin glass. If the substance be covered with too much soft pulpy matter, it may be slightly washed in water before being placed upon the slide, or a jet of water from the wash-bottle (fig. 108), may be forced upon it. Thin sections will require to be laid flat upon the slide, with the assistance of needles and forceps.

139. Great caution necessary in drawing inferences from Microscopical Appearances.—The difficulty of making out the structure of many organs and tissues is very great, and considerable experience is often required to demonstrate distinctly the anatomical characters of a healthy texture. These difficulties are much increased in the examination of morbid growths. When chemical reagents are applied, the effects

must be very carefully observed, otherwise there is danger of mistaking the change of character produced by the application of the reagent for a morbid alteration. Even the addition of a drop of water often materially alters the microscopical characters of a tissue. It is only by very frequent and careful examination of morbid growths that the observer can hope to recognize and interpret their characteristic appearances, and it should only be with the utmost caution, and after long familiarity with microscopical examination generally, that he should attempt to pronounce an opinion with reference to the nature of a morbid growth; for without extensive observation and great care, he will run the risk of bringing discredit not only upon himself as an observer, but also upon microscopical investigation generally.

Every observation should be carefully recorded in a note-book at the time it is made, and drawings added, if necessary; and it is very important that the student should take every opportunity of describing, in the simplest manner possible, the appearances which he has observed under the microscope.

The examples of tissues and morbid growths which will be brought forward have been introduced more especially for the purpose of referring to some particular process of manipulation which is important in the investigation.

As examples of glandular organs the kidney and liver may be referred to, because these are very frequently the subjects of investigation in cases of disease, and the changes which they undergo in structure has received a large share of attention. For a detailed description of the anatomy of these organs in a state of health I must refer to the different treatises on physiology and physiological anatomy, and I shall only here mention such points as should be especially taken notice of in a microscopical examination.

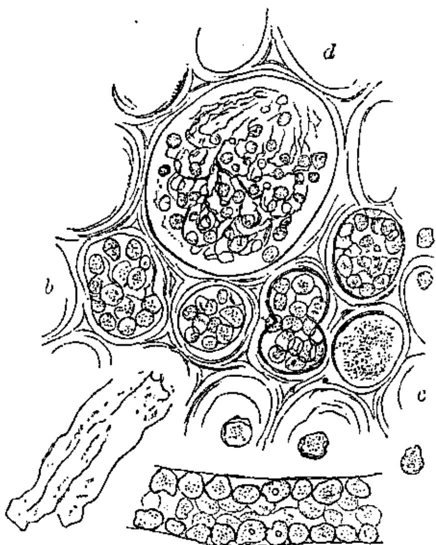
140. Kidney.—The kidney may be examined by making very thin sections (§ 135), or by simply scraping a freshly-cut surface with a knife; in which case small portions of the tubes, isolated Malpighian tufts, and cells of epithelium will be obtained, but the relative position of the structures will of course be lost. A thin section may be obtained either with a sharp

knife, or more advantageously with a Valentin's knife (§ 137), by which means a section including both the cortical and medullary portion of the organ may be made. After washing the section very slightly, it may be placed with a drop of water between two pieces of glass, and examined in the microscope, first using a low power (an inch glass), by which the general arrangement of the tubes will be seen, and afterwards a quarter of an inch object-glass, by the aid of which the different characters of the epithelium in the straight and convoluted portions of the tubes may be demonstrated.*

Fig. 113.

141. Basement Membrane and Epithelium.—Just at the edge of the specimen, a portion of a tube stripped of epithelium, and exhibiting the basement membrane (fig. 114*a*) very distinctly, may often be observed. The thick glandular epithelium of the convoluted portion of the tubes (fig. 113) should be compared with the more scaly form of that which occupies the straight part. It will be found that in the latter the central channel is wider than in the former position, although the total diameter of the tube is less. This arises from the

Fig. 114



* In this, and in the following chapters, unless stated to the contrary, the appearances described are observed with a quarter-of-an-inch object-glass (about 200 diameters); but, as already indicated, it is always important to subject specimens to examination with low as well as with high powers.

tained pus," would lead to a very different inference from that derived from the statement that "cells having all the characters of pus globules had been found in the blood," or that the "casts of the tubes contained cells resembling those of pus." The former will be true in extremely few cases; the latter in a vast number that fall under the observation of every practitioner. If, however, we find a considerable number of globules under the field of the microscope, of nearly uniform size, agreeing in general characters with the pus globule, and upon the addition of acetic acid exhibiting the characteristic reaction, we shall seldom be wrong in inferring that they are really pus cells.

In examining the blood in cases in which the white corpuscles are enormously increased in number, there can be no difficulty in deciding, since we have every reason to believe that pus globules could not possibly exist in the blood under the same circumstances.

CHAPTER XVI.

THE APPLICATION OF CHEMICAL ANALYSIS TO MICROSCOPICAL INVESTIGATION.—METHOD OF APPLYING TESTS.—EFFECTS OF REAGENTS UPON ANIMAL STRUCTURES.

278. Importance of Chemical Analysis in Microscopical Investigation.—By microscopical examination alone we are enabled very readily to distinguish with certainty the presence of exceedingly minute quantities of substances presenting definite crystalline forms, or exhibiting special anatomical characters. If the composition of a crystalline body has been once made out, by resorting simply to the microscope we are enabled by observing the form, even of a single crystal, to indicate its nature and properties. By the aid of the microscopical examinations of some of the secretions, we have seen that the course of pathological changes may be investigated, and morbid alterations of structure detected, which cannot be rendered appreciable to the senses by any other known methods of investigation.

By an acquaintance with the behaviour of certain substances with particular chemical reagents, and the application of this knowledge to microscopical investigation, we are often enabled to distinguish peculiarities of structure, to ascertain the chemical composition of minute quantities of matter, and to demonstrate clearly the existence of compounds in the animal frame with the greatest certainty, which would entirely escape our observation if we subjected them separately to the most careful chemical analysis, or to the most searching microscopical examination.

The application of chemical analysis to microscopical investigation, and the examination of crystalline forms in the microscope, is fast throwing a new light upon the physiological

changes which are constantly taking place in organized bodies in health, and the modifications which these changes undergo when influenced by circumstances interfering with or counteracting healthy actions; the consideration of which must especially interest practitioners, in the various forms of disease which are constantly being brought under notice.

The laboratory has already become a most necessary adjunct to the dissecting-room, the museum, the post-mortem room, and the clinical wards of our hospitals; and he who would wish to apply all the means at present at our disposal to unravel the mysteries of disease to aid him in forming a correct diagnosis, or to assist him in the investigation of those changes which occur in different organs, and are familiar to him in the post-mortem theatre, will do well to make medical and pathological chemistry, with microscopical examination, essential parts of his studies.

The works of Vogel, Schmidt, Scherer, Hœfle, and others, which have been published within the last ten or twelve years, have done much to advance this branch of investigation; while those of Golding Bird, Schwann, Robin and Verdeil, Lehmann, and Gorup-Besanez, and the excellent Atlas of plates by Dr. Funke, show the vast importance which the combined methods of chemical and microscopical investigation are very fast assuming.*

It is not within the compass of the present work to do more than refer to the general principle upon which such examinations are conducted, and to give examples of those processes

* "Anleitung zum Gebr. des Mikroskopes zur Zooch. Anal. u. zur Microscop. Chemisch. Untersuch," Dr. Julius Vogel, 1841.—"Chemische und Mikroskopische Untersuchungen zur Pathologie," Dr. J. J. Scherer, Heidelberg, 1843.—"Entwurf: einer Allg. Untersuchungsmethode der Säfte u. Excrete des Thierischen Organismus," Dr. Carl. Schmidt, 1846.—"Chemie und Mikroskop am Krankenbette," Dr. Hœfle, 1850.—"Physiological Chemistry," Dr. Lehmann, translated by Dr. Day, Cavendish Society, 1851.—"Atlas of Physiological Chemistry," Dr. Otto Funke, Cavendish Society, 1852.—"Traité de Chemie Anatomique et Physiologique," Robin et Verdeil.—"Urinary Deposits," Dr. Golding Bird, 1853.—"Manual of Zoo-chemical Analysis," Gorup-Besanez, translated by J. W. Slater.

which appear to be of the greatest importance to the student in medicine, and which he will be frequently called upon to perform.

As an instance of the great advantage of the application of a few simple tests to microscopical investigation, I may refer to the effects of ether upon fat globules, which are so commonly found in different tissues, and crystalline bodies composed of phosphate or carbonate of lime, which sometimes resemble them so nearly in refractive properties, in form, and in general appearance, as to have led to mistakes with reference to their nature. The application of a drop of ether has no effect whatever upon the latter, but instantly dissolves the former, so that by this very simple plan we are enabled at once to decide a very important question, and one which has led to much discussion, in consequence of the solubility of the globules in ether not having been ascertained in certain cases, which have been since considered as of a doubtful nature.

The detection of the presence of mere traces of urea, lithic acid, and other substances, by the application of reagents, and subsequent microscopical examination, will be referred to in Chapter XVII.

ON THE CHEMICAL AND MICROSCOPICAL EXAMINATION OF ANIMAL SOLIDS AND FLUIDS.

Preliminary Operations.—After having noted carefully the general characters which the substance exhibits, with reference to form, colour, size, weight, hardness, &c.; and fluidity, transparency, tenacity, &c., in the case of liquids; and after portions of solid textures, and the deposit from fluids have been subjected to microscopical examination, we may proceed to ascertain the reaction.

279. Reaction.—The reaction of any moist substance is ascertained by testing it with a piece of blue and reddened litmus or turmeric paper. If the substance be dry, or the reaction of a vapour is to be tested, the paper must be first moistened with a drop of distilled water. The blue paper is reddened by acids, while the red is rendered blue, and the

turmeric brown, by alkalies. As the change of turmeric is only visible when the alkaline reaction is very decided, it is not much employed in animal chemistry.

If the acid reaction is due to the presence of carbonic acid, the blue colour will be restored upon gently warming the paper over a lamp, upon a glass slide, or upon a warm plate.

An alkaline reaction may depend upon the presence of *volatile* or *fixed alkali.* The red colour is restored upon warming the paper which has been rendered blue by the presence of volatile alkali (ammonia or carbonate of ammonia), while it is not restored if the change is produced by the presence of a fixed alkali (potash, soda, or their carbonates, or an alkaline phosphate, &c.)

280. Specific gravity—Solids.—The specific gravity of animal solids may be taken in two ways.

First. By weighing in air, and afterwards in water, which is the process usually followed, and that which affords the most accurate results. The precautions necessary to be observed in carrying out this process will be found in "Gorup-Besanez' Zoo-chemical Analysis," "Bowman's Practical Chemistry," and other analytical works on chemistry.

Secondly. The specific gravity of solids may be obtained by placing small portions in certain solutions, the specific gravity of which has been previously ascertained by experiment: this latter method has been employed lately for ascertaining the specific gravity of the brain in different cases.*

The solutions are prepared in considerable quantities at a time, and kept in large bottles numbered according to the specific gravity.

Several glasses are nearly filled with the solutions from different bottles, and arranged in regular order. The piece of tissue is thrown into one, and, if it sinks, it must be placed in the fluid of the next higher specific gravity, and so on, until it neither sinks towards the bottom nor rises to the surface, when

* Dr. Bucknill "On the Specific Gravity of Cerebral Substance," Lancet, 1852.—Dr. Sankey in the "British and Foreign Medico-chirurgical Review," Jan. 1853, p. 40.

the specific gravity marked upon the bottle will correspond to that of the substance itself, since a solid will only displace an equal bulk of a solution which is of the same density as itself.

The soluble substances employed for making the solutions may be syrup, various salts, glycerine, and other compounds, which do not exert any chemical action upon the tissue, the specific gravity of which we wish to determine.

281. Specific Gravity—Liquids.

First. By the converse of the last operation, namely, by placing little glass bulbs, the specific gravity of which is marked upon them, into the solution, the density of which we wish to know, until one is found which neither sinks nor swims. This will indicate the specific gravity of the fluid. This method is not so correct, nor so easily applicable to general purposes as the two following.

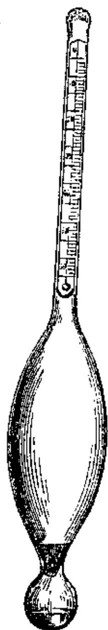


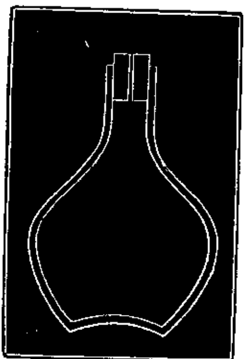
Fig. 207.

Secondly, by the hydrometer or urinometer. The number which is on a line with the surface of the fluid, when the instrument comes to rest, indicates its specific gravity. This method is tolerably correct, if the observer is careful to obtain the best instruments; but many which I have examined, indicated a specific gravity eight or ten degrees from the truth. The hydrometer or urinometer should always be tested by the specific gravity bottle. It may be remarked that the degrees marked upon the stem should gradually diminish in length, from above, downwards. If they are equal, as in the figure, the instrument may at once be pronounced as incorrect, without resorting to an experiment. Fig. 207 is an exact copy of one of the *imperfect* instruments commonly sold.

Thirdly, by the specific gravity bottle, which consists of a small glass flask. When quite dry it is accurately counterpoised in a delicate balance, and filled up to a certain point with distilled water, and weighed. The distilled water is then poured

out, and it is filled up to the same point exactly with the liquid to be tested, and again weighed. The specific gravity is then readily calculated from these data.

Fig. 208.



Some bottles are made to hold exactly one thousand or five hundred grains of distilled water, and are provided with a perforated stopper, through which the excess of fluid escapes, after the bottle has been filled, care being taken not to include air-bubbles (fig. 208). The outside of the bottle is wiped dry, and the whole weighed. The weight shows the specific gravity at once, upon deducting the weight of the thousand-grain bottle; or, when a five hundred-grain bottle is employed, the amount only requires to be doubled.

282. **Evaporation and Drying.**—The evaporation of animal fluids, and the desiccation of animal solids, must always be conducted over a water-bath, otherwise there is great danger of decomposition occurring. For operations upon small quantities, the water-bath, described in § 47, will suffice, or the cans of the injecting apparatus (fig. 104) may be removed, and basins placed over the holes.

In endeavouring to obtain crystals of organic substances, it is always advantageous, to evaporate the solution over the surface of sulphuric acid under a bell-jar, or, what is better still, in vacuo. In some instances, the evaporation may be conducted by simply exposing the liquid placed in a basin or watch-glass, and covered lightly with paper, to the air; or, where very slow evaporation is necessary, the watch-glass may be covered over with a bell-glass.

When quantitative analysis is to be performed, much greater care must be observed in the process of drying. For a description of the apparatus required and the cautions to be observed, see "Gorup-Besanez' Zoo-chemical Analysis," &c.

283. **Incineration.**—By incinerating a small portion of any organic substance, upon a piece of platinum foil, we are enabled to ascertain whether it contains inorganic salts, or con-

sists entirely of organic matter, in which case the substance leaves only a black residue, which burns off entirely after a short time.

The destruction of organic matter, by exposing the substance to a red heat, upon platinum foil, or in a platinum capsule, has been already adverted to. In order to obtain the inorganic constituents perfectly free from carbon, it is sometimes necessary to keep the mass, for a considerable time, at a dull red heat. If the temperature be too high, the process is often much retarded, in consequence of the fusion of some of the salts, as the phosphates and chlorides, and the inclusion of small masses of carbon, which are thus protected from the action of the atmosphere.

The platinum basin or foil may be supported over the lamp upon a piece of wire, bent in the form of a triangle, or upon one of the small rings attached to the spirit-lamp (fig. 147). It may be removed from the lamp with the aid of an old pair of forceps.

284. **Apparatus required.**—The chemical apparatus which is necessary in the course of microscopical investigation is very simple, and the greater number of instruments have already been referred to. The following are among the most important pieces of apparatus :—

A few conical glasses of different sizes (§ 217). Test-tubes of various sizes (§ 215) arranged on a stand. Spirit-lamps, with various supports (§§ 43–45), or, where gas is laid on, the gas-lamp (fig. 81). Small porcelain basins, watch-glasses; a simple water-bath (§ 47), or the injecting-can (§ 116), may be used, if several evaporations are to be conducted at once. A small platinum capsule, a strip of platinum foil, a blowpipe, pipettes (§ 216), and glass stirring-rods.

REAGENTS.

The reagents necessary are not very numerous;—they should be perfectly pure. Of the greater number only very little is required; but of alcohol, ether, and one or two others, it is necessary to have a moderate quantity.

The reagents should be kept in stoppered bottles, of about the capacity of two ounces.

285. **Alcohol.**—Alcohol of different strengths will be required for the purpose of dissolving certain animal substances, and for separating them from other constituents, which are insoluble in this reagent.

Alcohol should always be diluted with distilled water, and it is better to prepare a considerable quantity at a time. It is convenient to have two or three bottles which will hold about two quarts each. The strength of each specimen should be written upon a label and attached to the bottle.

The importance of alcohol, as a preservative solution, has been already referred to (§ 94).

286. **Ether.** — An ounce or two of ether will be quite sufficient for microscopical purposes. It should be kept in a stoppered bottle, provided with a glass cap, to prevent loss by evaporation. A little should also be kept in one of the small glass bottles with capillary orifices (§ 300), for the convenience of applying to cells containing highly refracting globules, resembling oil, &c., under the microscope.

287. **Nitric Acid** should be kept of two different degrees of concentration: one the strongest that can be procured, and another containing about 20 per cent. of the strong acid. This last is the acid most used by the microscopist, especially in separating muscular fibre cells. It is prepared by mixing one part of the strong commercial acid with five parts of water.

288. **Sulphuric Acid** is sometimes required undiluted, but a small bottle of diluted acid (one of acid to five of water) should also be at hand. The pure colourless acid should always be procured;—it is to be purchased for about 1*s.* 6*d.* a pound, but only very small quantities are required.

289. **Hydrochloric Acid** may be obtained perfectly colourless. It may be kept in the pure state and diluted as required.

290. **Acetic Acid.**—Two specimens of acetic acid will be found convenient. One, a solution of the strongest acid which can be procured; the other, containing about 20 per cent. This is prepared by dissolving one part of the strongest liquid acid, or of the pure glacial acetic acid, in five of water.

291. **Chromic Acid** is usually required very dilute. For the purpose of hardening tissues, a watery solution of a straw-colour will be found strong enough. It is easily prepared by dissolving a little of the crystallized chromic acid in distilled water. For the method of preparing the crystallized acid, see § 98.

292. **Solution of Potash** should be kept of two or three different strengths. One, the strongest which can be procured; another, made by mixing one part of the strong with three or four of water; and a solution consisting of one part of liquor potassæ to eight or ten of water will be found of a useful strength for the examination of many preparations.

293. **Solution of Soda** is generally required very dilute. It may be made by mixing one part of the strong solution of the shops with five or six of water; but this, for many purposes, will require to be still further diluted. Or, about twenty-five grains of the fused soda may be dissolved in an ounce of distilled water.

294. **Ammonia.**—Solution of ammonia, made by mixing one part of the strongest liquor ammoniæ with three of water will be found sufficiently strong for all the purposes for which this reagent will be required.

295. **Nitrate of Barytes.**—A cold saturated solution of the salt forms a test solution of convenient strength. It should be filtered before use.

A solution of nitrate of barytes is employed as a test for sulphuric and phosphoric acids. The precipitated sulphate of baryta being insoluble both in acids and alkalies; while the phosphate of baryta is readily soluble in acids, but insoluble in ammonia.

296. **Nitrate of Silver.**—A solution of nitrate of silver is prepared by dissolving one hundred and twenty grains of the crystallized nitrate in two ounces of distilled water, and filtering if necessary.

Nitrate of silver is employed as a test for chlorides and phosphates. The *white* precipitate of chloride of silver is soluble in ammonia, but insoluble in nitric acid. The *yellow* precipitate of tribasic phosphate of silver is soluble in excess of ammonia, as well as in excess of nitric acid.

297. Oxalate of Ammonia.—Some crystals may be dissolved in distilled water, and, after allowing time for the solution to become saturated, it may be filtered.

Oxalate of ammonia is used as a test for salts of lime. Oxalate of lime is insoluble in alkalies and in acetic acid, but soluble in the strong mineral acids. In testing an insoluble deposit for lime, it may be dissolved in nitric acid and excess of ammonia added; the flocculent precipitate is readily dissolved by excess of acetic acid, and to this solution the oxalate of ammonia may be added. The precipitation of oxalate of lime is favoured by the application of heat. Many deposits of phosphate are with great difficulty soluble in acetic acid, hence the necessity of first adding nitric acid, as above directed.

298. Iodine Solutions.—An aqueous solution of iodine is easily prepared, by dissolving a few grains of iodine in some distilled water, until it acquires a brownish-yellow colour. A solution of iodine is sometimes useful for colouring certain substances which are so transparent as to be scarcely distinguishable upon microscopical examination.

A darker solution may be obtained by employing a solution of iodide of potassium to dissolve the iodine (one grain of iodine and three grains of iodide of potassium, to one ounce of distilled water). For testing bodies suspected to consist of starch, the following solution is recommended by Professor Schultz. Zinc is dissolved in hydrochloric acid;—the solution is permitted to evaporate in contact with metallic zinc until it attains the thickness of a syrup; and the syrup is then saturated with iodide of potassium. The iodine is next added, and the solution, if necessary, is diluted with water.*

It will be found convenient to keep small quantities of those solutions, in most frequent use, in the small capillary tubes or bulbs (§ 300). A small box containing twelve bulbs will be quite sufficient for all ordinary purposes. For the examination of the urine, not more than six or seven will be necessary.

* "The Microscope, in its special Application to Vegetable Anatomy and Physiology," Schacht, translated by Currey.

METHOD OF APPLYING TESTS TO SUBSTANCES INTENDED FOR MICROSCOPICAL EXAMINATION.

299. Tests kept in Glass Bottles.—The matter to be tested may be placed upon a glass slide, and, if necessary, a drop of water added, to moisten or dissolve it, as the case may be.

In these operations we usually require only a small drop of a solution, and it will be found most convenient, in applying it to the object, to take a drop from the bottle by dipping a stirring-rod into it, and withdrawing it immediately. Enough will be found adhering to the stirring-rod for the purpose required. The rod should not be dipped in a second time, without being first well washed in water,—for if this be not scrupulously attended to, there is great danger of conveying some of the substance intended for examination into the test bottle, in which case the whole contents are spoiled.

Without great care in all our manipulations, we shall be in danger of removing a portion of one substance from a glass slide and carrying it to a deposit which is subsequently examined;—a result which might lead to great inconvenience. Claws of echinococci, and other minute bodies, in themselves highly characteristic, may thus be transported, and find their way into deposits in which we should not expect their presence; and from such an accident we might be led to infer, very erroneously, the existence of hydatids when the presence of the claws of the echinococci really resulted from accident. Without the greatest attention to cleanliness, the microscopical observer will be constantly led into error, and thereby bring discredit upon himself and upon the science.

Nothing is more common than to find a specimen which we are examining in the microscope covered with a vast number of starch granules, which have been introduced from without. Usually they are derived from the squares of thin glass which are commonly kept in a little starch powder to prevent fracture.

Accidents of this kind can always be avoided, by not allowing the drop of the reagent to touch the deposit until the rod has been removed. This can be effected by placing the drop near the substance intended for examination, and then allowing it to come in contact with it, either by inclining the

glass slide, or by leading it with a glass rod or other small instrument to the matter to be tested.

300. Tests kept in Glass Bulbs with Capillary orifices.—By far the most convenient method with which I am acquainted, of applying chemical reagents to minute quantities of matter, consists in allowing a drop to issue from a small glass vessel, having a capillary orifice, by which means a quantity even much less than a single drop can be readily obtained, and there is no chance of any portion of the preparation being introduced into the test solution.

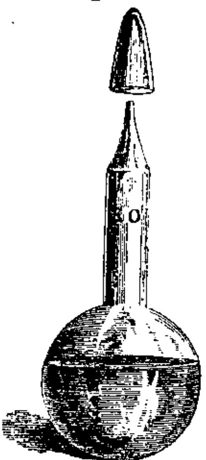


Fig. 209.

With this view a small bulb, about an inch in diameter, was blown at one end of a piece of glass tube, the other was drawn out to a moderately fine capillary point, and a small cap, made either of glass or gutta percha, was adapted to the end (fig. 209). These bulbs were easily filled, by expanding the air within them, by the heat of a spirit-lamp, and then inverting them in a small vessel containing the solution which was to be introduced. As the bulb cooled, the liquid rushed into it, to supply the place of the previously expanded air. A small bubble of air should, however, be retained in the bulb, by the expansion of which some of the fluid can afterwards be expelled. The bulbs containing the strong acids and alkalies should be furnished with glass caps, but gutta percha will be sufficient for the other tests. When it is required to expel a drop of the solution, the bulb is taken in the hand, and the air in the interior being expanded by the warmth, a small quantity of the solution is forced out.

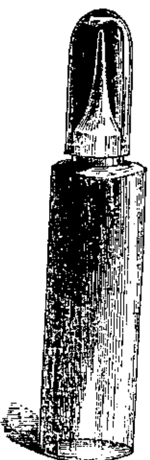


Fig. 210.

Mr. Highley, of Fleet-street, has had some small bottles made of the form shown in the accompanying figure (fig. 210). These are all capped with glass, and, as the bottom is flat, they stand very readily. They are fitted up in small cases, and will be found

exceedingly convenient to the microscopical observer. It is better to have the cap made of a conical shape, corresponding to that of the end of the bottle, otherwise, a little of the fluid is liable to collect between the cap and the neck, which runs down the sides when the cap is removed.

301. Application of the Reagent to minute quantities of matter.—With the aid of the bulbs just referred to, the most minute traces of different substances may be readily detected. The solution of the substance, consisting perhaps of only one drop, is placed upon a glass slide. This drop may be very readily divided into four or five smaller drops, if necessary, to each of which a separate test may be applied. For instance, suppose we have a minute quantity of the ash of an animal tissue, or of the solid residue of an animal fluid, to examine, and we wish to ascertain if it contains carbonates, sulphates, chlorides, and phosphates, and whether phosphate of lime and magnesia are present, we may proceed as follows:—the portion of ash, which may, perhaps, be half the size of a pin's head, or even less, is removed from the platinum foil, upon which it has been ignited in order to remove organic matter, and placed upon a glass slide. It is moistened with the smallest quantity of water, and then treated with a minute drop of nitric acid. If effervescence takes place, a carbonate is present. The acid solution is then divided into three portions, with the aid of a small stirring-rod, and the solutions tested as follows:—

1st portion.—If a drop of a solution of nitrate of silver gives a cloudy precipitate, chlorides are present.

2nd portion.—If nitrate of barytes produces a white precipitate, sulphates are present. Upon the addition of excess of ammonia, the precipitate produced by nitrate of barytes will be increased if phosphates exist in the solution. The precipitate of phosphate of baryta is flocculent, and readily distinguished from that of sulphate of baryta (which is dense and granular), by its solubility in acids.

3rd portion.—If lime or magnesia are present, in the form of phosphate, a precipitate will be produced upon adding excess of ammonia to the nitric acid solution. The mixture may be stirred a little, with a fine piece of glass or platinum wire, and

then allowed to stand for some time. A piece of thin glass is now applied, and the precipitate subjected to microscopical examination. Phosphate of lime occurs as a granular amorphous sediment, while the ammoniaco-magnesian, or triple phosphate, is usually found crystallized in a beautiful stellar form, or as minute prismatic crystals.

302. Testing for Carbonates. — As carbonates are often present in very minute quantity in the ash of organic substances, a slight modification of the plan above given may be pursued, and, in this way, the smallest traces may be detected. If only a few bubbles of carbonic acid are given off upon the application of the acid to the substance; or if, in consequence of the solubility of the carbonate present, they are evolved very rapidly, they frequently elude observation.

In testing for minute traces of carbonates we may proceed as follows:—The portion of ash, deposit, or tissue (as the case may be), is placed upon a glass slide, and lightly covered with a piece of thin glass. A minute drop of nitric or acetic acid, not too strong, is then allowed to escape from one of the bulbs. This is drawn by capillary attraction between the glasses, and soon comes in contact with the substance to be tested. Any bubbles which may be given off are thus confined, and they may generally be seen clearly enough. In some instances, however, advantage is derived from subjecting the specimen to microscopical examination, when the formation of the gas can be seen; and the bubbles set free cannot possibly be mistaken for air-bubbles, which had been included in the interstices of the tissue previously, and afterwards expelled upon the addition of the fluid, because they may be seen gradually to increase in size and number as the action of the acid continues. In testing for carbonates, the possibility of this occurrence, however, must always be borne in mind, and the fallacy carefully guarded against.

EFFECTS OF REAGENTS UPON ANIMAL STRUCTURES.

303. Effects of Acids.—The effects of the application of cold strong acids to animal textures is very variable; in some instances the tissue is completely destroyed, while in others

scarcely any effect seems to be produced. The mineral acids generally coagulate albuminous tissues, and render their microscopical characters confused and indistinct. Tribasic phosphoric acid, however, is an exception to this rule. Acetic acid dissolves many of the substances allied to albumen.

The appearance of some structures is scarcely altered by the application of a strong acid; for instance, the blood corpuscles exhibit their usual form and general character for some time after the addition of strong nitric acid, and the cells of the epidermis and nail, although turned of a yellow colour, are not destroyed; the latter are separated somewhat from each other, but their outline may often be seen beautifully distinct upon microscopical examination. On most of the mineral constituents of the body, insoluble in water, the acids act as direct solvents.

304. Acetic Acid.—Acetic acid is one of the most useful reagents to the microscopical observer. It has the property of rendering the cell-wall very clear, while the nucleus is often rendered darker and more distinct. In many instances the action of the acid upon the cell-wall probably arises from endosmosis; the cell becomes much larger, and the wall more pulpy and thicker, and approaches more nearly in density and refracting power to that of the solution in which it is immersed. In numerous instances, by adding a saline solution to cells which have been previously rendered transparent by acetic acid, they again contract, and the outline becomes distinct. In some cases, however, the cell-wall is actually dissolved by the acid, and its contents set free. Acetic acid will be required of various strengths, the most useful proportion being one part of the strong acid to three or five of water. Acetic acid is very frequently used to make epithelial structures transparent, in order that the arrangement of the minute vessels and nerves in papillæ, &c., may be demonstrated, as in the case of the tongue, skin, &c. Sections of preparations which have been hardened by maceration in alcohol, often require boiling slightly in acetic acid before they can be rendered transparent. The action of acetic acid on white fibrous tissue is very characteristic, as it converts it into a transparent jelly-like mass, in which a few

nuclei are visible. Upon the yellow element, on the other hand, this reagent exerts no action whatever.

The action of acetic acid upon pus-globules has been already adverted to (§ 276).

Acetic acid may also be employed for testing crystalline bodies as phosphates and carbonates. It distinguishes phosphate or carbonate of lime from oxalate of lime (all of which are insoluble in water), by dissolving the two former, while it does not affect the latter even if boiled with it.

The action of acetic acid upon any particular tissue, upon any form of cells, fibres, &c., that are subjected to examination, should always be specially noted.

305. Dilute Nitric Acid is much employed in microscopical research.—An acid composed of one part of acid to two or three of water forms a good solution for hardening some structures, previous to cutting thin sections. The thin sections may sometimes be rendered very transparent by being treated afterwards with dilute caustic soda. For demonstrating fibre-cells in organic muscle, nitric acid is a valuable reagent. For this purpose the solution should contain about twenty per cent. of strong acid, and the muscular fibre should be allowed to macerate in it for some time, when small pieces may be removed with scissors, and after being carefully torn up with fine needles, subjected to examination.

By boiling animal tissues in strong nitric acid, they become destroyed, while any siliceous constituents remain behind. In this manner, the siliceous skeletons of the *Diatomaceæ* may be separated from any organic matter with which they may be combined. This is one of the processes employed for obtaining these beautiful objects from guano.

306. Sulphuric Acid.—Hydrochloric Acid.—The pure concentrated acids only should be used for microscopical investigation. They may be obtained at most of the operative chemists.

Concentrated sulphuric acid causes epidermic structures to swell up very much, and the cells to separate from each other so as to be readily isolated. Boiling acid completely dissolves them. In the examination of hair, strong sulphuric acid will be found to render the outline of the cells very distinct.

Hydrochloric acid is usually employed for dissolving out the mineral constituents of certain tissues, such as bone or teeth. As a rule, it is better to use dilute acid (one of acid to three or four of water), in which case, however, a longer time must of course be allowed, than when the acid is concentrated.

307. Effects of Alkalies.—The action of alkalies, even when cold in a very dilute state, is to dissolve most animal textures. Cell-membranes are frequently almost instantly dissolved, while the nucleus appears to be acted upon with greater difficulty.

Alkalies are also employed for dissolving certain crystalline substances which are occasionally found in animal tissues, such, for instance, as deposits of alkaline lithates, which are not unfrequently met with in the form of considerable deposits in the tissues of gouty persons.

308. Potash and Soda.—The action of potash and soda upon animal structures is very similar. Both very readily dissolve substances of an albuminous nature, but the effect of soda is more gradual, and it has been found that for most purposes in microscopical research, this reagent possesses advantages over potash.

These reagents are usually employed to dissolve the layer of epithelium covering mucous membranes, in order to examine the arrangement of the structures beneath the basement membrane; and in investigating the termination of the nerves and vessels in papillæ and other structures, they, especially the latter, are very valuable.

Dilute solution of caustic soda is also a valuable reagent in pursuing investigations on the nerves and nervous centres.

For the purposes above mentioned, the alkalies should be diluted with water. One part of solution of caustic soda to eight or ten parts of water, will be found a convenient strength. The changes are expedited by the application of heat, which, however, must not be too great, for fear of complete solution taking place. Where the structures are hard and dry, the action is much facilitated by warming the substance with the reagent in an ordinary test tube,—a plan which is much recommended by Kölliker.

Carbonates of Potash and Soda.—Some animal textures become

hardened by prolonged maceration in carbonate of potash, but this plan does not appear to be so generally useful as others previously indicated. Epidermic structures are not much altered by these salts. Gurlt recommends skin to be hardened in solution of carbonate of potash for the examination of the sweat ducts.

309. Effects of Alcohol and other Substances in hardening Animal Structures.—There are several reagents which render soft tissues quite hard and firm, so that very thin sections may be cut off easily with a sharp knife; of these alcohol is most commonly employed. Alcohol permeates animal tissues readily, and coagulates the albuminous structures very firmly: it may be used of different strengths according to circumstances.

In hardening structures with alcohol, it is better to add weak spirit first, and then to replace this by stronger, increasing the strength each time the spirit is changed. By proceeding in this way, the tissue contracts very gradually, and its form and general characters do not become so much changed as when it is plunged at once into strong alcohol.

A weak solution of chromic acid hardens parts of the nervous system and other tissues very effectually, so that thin sections may be readily removed from structures hardened in this manner.

Solutions of soluble salts are also used for hardening animal textures, especially Goadby's solution, and solutions of common salt, or nitrate of potash. Animal textures may also be hardened in wood naphtha.

CHAPTER XVII.

OF OBTAINING CRYSTALLINE SUBSTANCES FROM THE FLUIDS AND TEXTURES OF THE ANIMAL BODY, AND OF THEIR MICROSCOPICAL EXAMINATION.

310. Formation of Crystals in Animal Fluids.—Some crystalline bodies are deposited from their solution in animal fluids by simple evaporation; others less soluble may be deposited by allowing the fluid to stand still for a short time, when certain changes occur in some of its constituents, which lead to the precipitation of bodies in a crystalline form, such, for instance, as lithic acid, or crystals of triple phosphate. In other cases it becomes necessary to add some reagent before the crystals are thrown down, while not unfrequently a long and often complicated chemical analysis is required, in order to isolate some of the substances which were previously held in solution, and obtain them in a crystalline form. The addition of water in some cases causes the most rapid crystallization, especially when the crystallizable material is contained in a cell, as when water is added to blood, in order to obtain blood crystals. Instead of water, in other instances, it becomes necessary to add alcohol, in which fluid the crystals may be much less soluble than in water.

311. Influence of other constituents upon the Crystallization.—In many instances it is exceedingly difficult to separate some crystalline bodies from other constituents of the animal substance, the presence of which much increases their solubility and prevents their crystallization. The extractive matters exert this influence in a marked degree, and it is only of late years that several new bodies of definite chemical composition have been isolated. Creatin and creatinine may be instanced amongst the number, for these were not very long ago included