MANUAL OF MICRURGY

BY

RUTH B. HOWLAND

AND

MORRIS BELKIN

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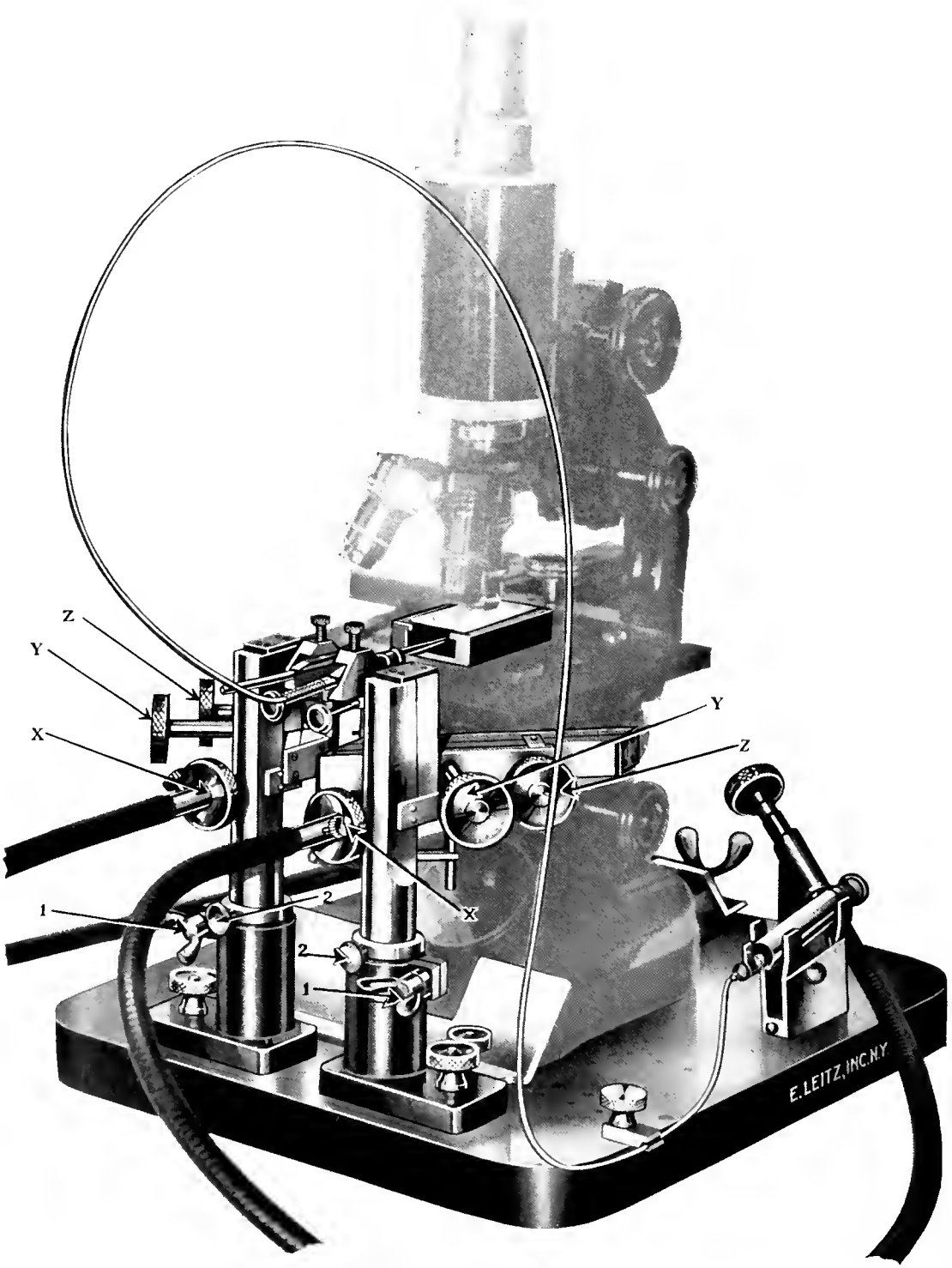

Model 1: Consisting of Two Manipulators

Designation of numbered parts:

1—Clamping screws to hold pillars firmly in place;

2—Clamping screws to arrest pillars at proper height;

X—Knurled heads for micrometer movement of needles in perpendicular axis;

Y— } Knurled heads for micrometer movement
Z— } of needles in horizontal axis.

PREFACE

This manual was written in response to a need for an outline of instruction in the technique of microdissection and microinjection. All directions have been made especially simple and detailed so that students who have no recourse to personal instruction may find it easy to learn the technique. In preliminary form, the manual has been used for the past two years at Washington Square College, and at Woods Hole.

Although it deals chiefly with the Chambers apparatus, the principles described are fundamental, and may be applied, with suitable minor modifications, to any other type of micromanipulative apparatus now in general use.

It is felt that the bibliography is an important component of the manual, since the scope of this book permits us to deal but briefly with special methods and techniques. Investigators interested in greater elaboration of any special problem will find it necessary to refer to original publications. The bibliography also serves to familiarize the reader with the range of application of the micrurgical method.

We are greatly indebted to Miss Helen Holt for invaluable aid in assembling the manuscript and in compiling the bibliography. Our thanks are due to Mr. C. G. Grand for aid in preparation of the drawings, many of which are original, and others redrawn from various sources. We also desire to express to E. Leitz our appreciation of their kindness in contributing the photograph used as the frontispiece.

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New York, February 27, 1931.

TABLE OF CONTENTS

SECTION I.

LABORATORY DIRECTIONS FOR THE ASSEMBLING AND USE OF THE CHAMBERS MICROMANIPULATOR.

Index of Complete Equipment for Micromanipulation

The various parts and attachments which comprise the complete equipment are as follows:

1. Base plate.

   Heavy rectangular metal plate, having attached to it, at one end, two round pillar sockets. This is the front end of the base plate. On the base plate there are three metal wings with screws. Two of them are at the foot of the pillar sockets; one is at the rear of the base plate. At one side there is a screw-clamp holder for a syringe, on the other a holder for the flexible shaft.

2. Two micromanipulators.

3. A glass syringe.

4. Injection apparatus composed of (a) hypodermic needle; (b) tube proper; (c) adapter for holding pipette.

5. Two metal adapters for holding needles.

6. One glass moist chamber.

7. One mechanical stage.

8. One flexible shaft. (Use of this optional).

9. One microscope with accessories:-
   a) "split" condenser. (Top lens removed).
   b) objectives
      1) #1b
      2) #3b
      3) #5
      } Leitz
   c) oculars
      1) 10x Leitz

10. Lighting system.
    a) Light shed.
    b) 100 Watt lamp in socket with cord.
    c) 500 cc Florence flask for heat filter.
    d) One Gage daylight glass.

11. Microneedle and Micropipette block with cover. (For holding reserve needles and pipettes).

Accessory Equipment

Lens paper
Filter paper cut into strips for moist chamber
Vaseline
Drop bottle of distilled H_2O
Petri dish with chemically cleaned cover slips
1 small beaker, 150 cc with soap suds
1 dish for used pipettes and needles
2 Petri dishes with damp filter paper to serve as moist chambers

Chambers' Micromanipulator. Construction and Working Principle.

This is a mechanical device whereby very fine movement of microneedles, micropipettes, etc., suitable for manipulating microscopic objects under high magnification may be effected. The apparatus consists of two instruments, right and left, which are mirror images of each other. The right hand instrument is the one which, if held in such a position that the free end of the heavy cylindrical pillar points downard, the three large screws will be in a horizontal position to the right. Each instrument is mounted by inserting the pillar into a pillar socket attached to the heavy supporting base plate. The parts of which an instrument is composed and the principle on which it operates are as shown in figure 1.

Three bars of rigid metal are connected at their ends to form a "z-like" figure by resilient metal acting as a spring-hinge. By the action of the micrometer screws, the bars can be forced apart; on reversing the screws, the bars return to their original position owing to the spring action at the end of the bars. By this means, arc movements may be imparted to the tip of a needle when mounted in its holder on the free end of the third bar.

The needle, or any other suitable device, the tip of which is to be manipulated, is held in a carrier called the adapter fastened to the free end of a bar "A" at "X". The needle is made to extend so that its tip is at the apex of an imaginary triangle at "D". In order to obtain two movements at right angles to one another and in the horizontal plane, the tip of the needle must be at the apex "D" of a right-angled triangle the base of which is a straight line joining the centers "E" and "F" of the two springs holding the three bars, "A", "B" and "C" together. The shank of screw "G" passes through a large hole in bar "C" and is screw-threaded in bar "B". Turning it spreads apart bars "A" and "B" and imparts an arc movement to the needle-tip at "D" at right angles to that procured by turning screw "H".

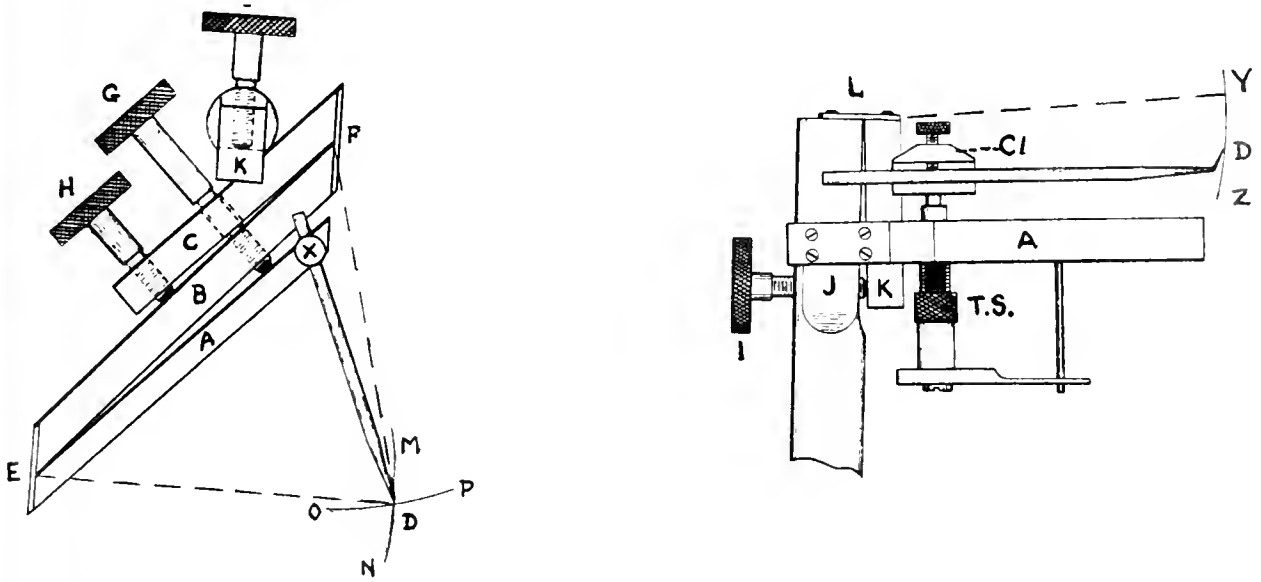


Fig 1

The movement in the vertical plane at right angles to the afore-mentioned movements is produced by screw "I" and abuts against a vertical extension "K" of bar "C". The extension "K" is parallel to the bar "J" and is connected to it at its top by means of a solid spring-hinge. Turning screw "I" spreads apart bars "J" and "K", and lifts the whole combination "A", "B" and "C" and imparts an arc movement in the vertical plane to the tip of the needle at "D". To procure a vertical movement, the tip of the needle at "D" must lie in the same horizontal plane "L" - "D" with the spring fastening "K" and "J" together. When screw "I" is turned, the needle tip will then move in an arc "Y" to "Z" more nearly vertical than any other arc on the same circumference of which the point "D" is the center.

Pick up the right hand instrument, and carefully keeping in mind the description of the structure and working principle just read, turn each screw (G, H, and I) clockwise until the bars are engaged, and slightly separated. NEVER FORCE THE BARS APART TO THE LIMIT OF THEIR MOVEMENTS. THIS PUTS UNDUE STRAIN ON THE SPRING-HINGE, AND IN TIME WILL TEND TO WARP THEM PERMANENTLY. ALWAYS RELEASE ANY TENSION WHEN THROUGH MANIPULATING.

Note that in addition to the parts already described the unit has also a clamp (Cl) into which may be fastened the metal holder (adapter) for micropipette or microneedle. You have already moved this clamp vertically by turning screw "I". This gives the finest vertical movement of which the instrument is capable. Two other ways of moving the clamp are possible. First, a coarse vertical movement may be imparted to it by turning the screw "T.S.", which is located on the inner side of the bars, attached to a square frame. Turn this screw and note the relative magnitude of motion.

Finally by loosening the small screw which is at the base of this clamp, the clamp may be rotated freely from side to side, or may be moved up and down in its socket. This is the coarsest and most rapid way of adjusting the position and height of the micropipette and needle. It is used to adjust the height of the carrier for different sized chambers. When once adjusted it is not used further. The carrier may be rotated without changing its vertical height.

Pick up the left hand instrument. Note that the three screws must also be turned clockwise to engage the bars. Do not attempt at present to mount these instruments. Instructions for mounting them on the metal base are given in a later section.

Choice of Microscope and Microscope Accessories

MICROSCOPE STAND. Any good compound microscope may be used if it meets the following requirements. First, due to the fact that a special condenser must be used, the opening in the microscope stage must be large enough to allow the top of the split condenser to be flush with the surface of the stage. Second, the mechanical stage must have sufficient size and range to accommodate a moist chamber (fig. 2, x) constructed on a 2" by 3" base and allow of its being moved back so that the cover slip roofing the moist chamber completely clears the condenser lens. To insure this, it is advisable to use a microscope with a handle curved outward. This permits the rocking back of the moist chamber to a greater distance so that this clearance of the condenser lens is possible.

MOIST CHAMBER. The moist chamber (fig. 2, x) just mentioned is constructed as follows:

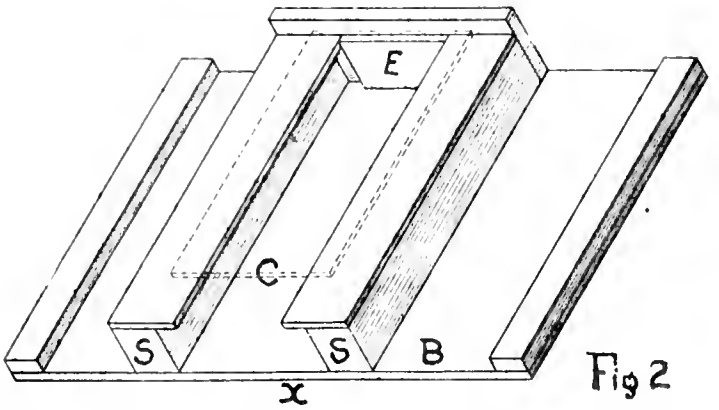
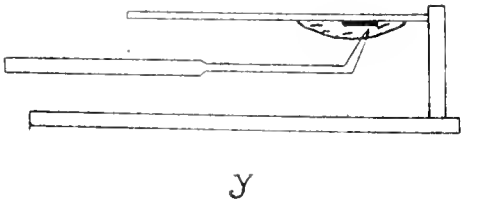


Fig 2



The base, B, is a thin glass-slide about 2 x 3 inches in size. The sides, S, consist of strips of plate-glass about 2 inches long and $\frac{1}{4}$ inch wide, and of a height determined by the available condenser, usually 3/8 inch. One end, E, of the chamber is closed with a strip of glass of the same height as the sides and backed by another strip a fraction of an inch higher. The trough of the chamber should be at least 7/8 of an inch wide. Wet strips of blotting paper should be placed along the sides of the trough. This moist-chamber is designed for cover slips of a size 22 x 40 mm. The cover slip, C, is sealed on the chamber with a thin layer of vaseline.

The moist-chamber is open at one end to permit the entrance of microneedles or pipettes for operating the tissue or cells which are suspended in hanging drops from the under surface of the cover slip. See figure 2, y.

MECHANICAL STAGE. A mechanical stage is used to clamp and move the moist chamber. Any make may be used provided it be sturdy and accurate, and provided it racks back sufficiently to permit complete clearance of the condenser lens by the open end of the moist chamber.

LENS SYSTEM. A. "Split" Condenser. (Three lens condenser with top lens removed). The reason for splitting the condenser is that operating in the hanging drop suspended from the cover slip necessitates raising the focal point from the stage level to the under side of the cover slip. Therefore, the complete three lens condenser ordinarily supplied would focus the light too far beneath the operating field; and the intensity of light would therefore be insufficient. Hence the top lens is removed, giving a floodlight of increased brilliance. This "split" condenser is raised until the top lens is flush with the upper surface of the microscope stage. The large diameter of this "split" condenser lens is the reason why a larger aperture in the microscope stage is needed.

B. Objectives and Oculars. The objectives and oculars used, will, of course, vary with the magnification desired. In general the rule is: for best illumination use the lowest ocular with the highest objective. For very low power work, such as rapidly searching a field, etc., the #1b Leitz objective and 10x ocular are a serviceable combination. A somewhat higher and very workable combination is a #3b Leitz objective and a 10x ocular. For still higher power a #5 Leitz objective may be used. This #5 objective furnishes considerable depth of focus for its degree of magnification. High dry and oil immersion objectives may also be employed when desired; and the magnification may be further increased by using oculars of the highest power. The Leitz 8x Periplan ocular is recommended for critical definition of cell constituents.

LIGHTING SYSTEM. It is very desirable that the lighting equipment of a laboratory be uniform. This is especially necessary when color work, such as pH determination is being done by a group. A simple, yet serviceable, lighting unit, (fig. 3) may be constructed by

placing a 100 Watt Mazda C spotlight bulb, B, within a light shed made of metal, Sh. The bulb should be placed in a horizontal position and attached to a socket, Sk, which can be rotated so that the two large filaments within the bulb face down. This is done to avoid overheating and melting of the large filaments of the bulb. To avoid either displacing the bulb or socket, a switch, Sw, is interposed in the circuit. The shed is made of galvanized iron with a window cut out in one face -- the "front" face. Metal prongs are soldered around the two sides and lower edge of the window to form a slot into which may be inserted a 3" x 3" daylight glass. The "back" edge of the roof should be open to permit ventilation. An arched opening is cut at the base of one side (either right or left) to accommodate the light socket.

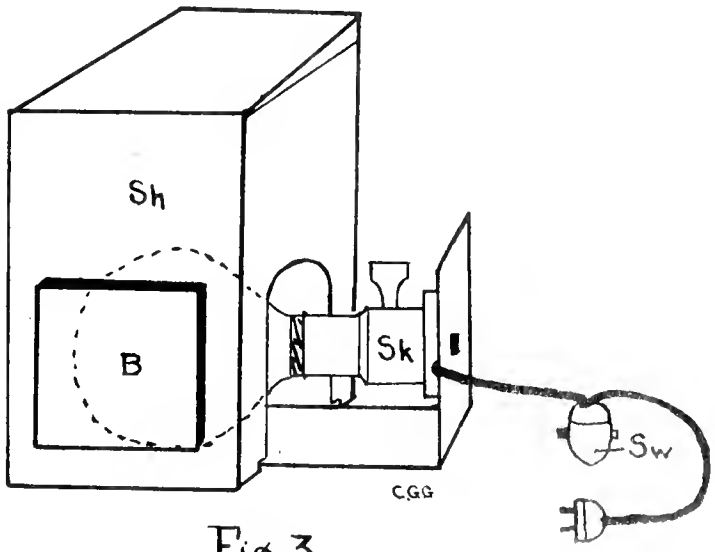


Fig 3

To absorb the heat of the lamp and concentrate the light on the mirror of the microscope a 500 cc Florence flask filled with distilled water should be placed between the microscope mirror and the source of light. A convenient distance is about three inches from the mirror. The light shed may be placed about 18 inches from the mirror.

Assembling of Complete Apparatus for Microdissection

Place the heavy metal base on your table so that the pillar sockets are away from you. Pick up right and left instruments and insert the pillars in these holders in such a position that the bars form the two sides of a triangle with the open base toward you. Clamp the instruments loosely in place by means of the screws in the side of the sockets. Arrange their positions so that the coarse adjustment screws are clear. Place the microscope on the base and push it CAREFULLY forward so that the stage and its attachments easily clear the bars. If any part of the microscope sub-stage apparatus comes in contact with the bars, the latter must be moved outward slightly or the entire instrument must be lowered in the pillar socket just enough so that all parts of the microscope are free from contact. The instruments may now be firmly clamped in place. Continue moving the microscope forward until the ends of its U-shaped base are firmly in contact with the two metal wings which are screwed into the manipulator base at the foot of the pillar sockets. When the above manipulations have been successfully completed, fasten the microscope firmly into place by means of the third metal wing found at the rear of the base plate (the end nearest you). It may be necessary to adjust this

wing by moving it forward or backward over one of the holes in the base plate which receives its fastening screw. It may be that you are using a microscope so built that the stage hits the bars even when the two instruments are lowered to their limit in the pillar sockets. If so, raise your microscope off the manipulator base plate by slipping under it a block of metal, bakelite, wood or any firm material. Slip the moist chamber in the holder of the mechanical stage. Then in each of the clamps, place one of the needle holders, with the screw end toward you; the holders being parallel to each other, and raised sufficiently so that they are about $\frac{1}{4}$ inch above the microscope stage. The apparatus is now assembled for mounting the microneedles.

Making and Mounting of Microneedles

1. The Microburner. To make microneedles, it is necessary to have a very small source of intense heat. A source of this kind is most readily constructed by pulling out a 6 inch piece of 3 mm bore glass tubing in a Bunsen flame, so that one end tapers quickly to a very fine point. If the point is sealed, open it by gently breaking off the tip. Attach this tubing to a source of illuminating gas, and light the flame. The jet thus obtained will probably be about 3 inches or more in length. The opening must be reduced by gently approximating the lighted end to a Bunsen flame. The aperture in the glass tubing will gradually diminish in size, reducing the size of its flame. Continue until the flame is about $\frac{1}{4}$ inch long, with the gas turned full on. To secure finer control of the gas flow, a Hoffman pinch-cock is clamped on the rubber tubing. A right angle bend in the glass tubing is now made about three inches from the fine open end. The glass tubing, point up, may now be fastened to a table by means of plasticine.

2. The Microneedles. Over a Bunsen flame draw out a six inch piece of glass rod to capillary fineness. The proper size of this capillary may be checked by inserting a segment of it into the opening of the needle holder. The optimum diameter is that which just permits easy fitting into the needle holder. When a sufficient supply of capillary rod has been made, break it up into six inch lengths, for ease of handling. From these lengths of capillary rod, the microneedles are made by pointing them over the microburner, holding the capillary horizontally with the greatest part of its length in the left hand between thumb and index finger. Grasp the right end of the rod with a pair of flat forceps, resting your closed hands on the outer, fleshy part of the palm. Bring the rod over the microflame, which should be about the size of a pin head, so that the rod is heated about $\frac{1}{2}$ inch from the forceps. As the rod melts pull horizontally and smoothly until the rod parts. This pulling action should at the same time remove the glass from the flame. (See figure 4).

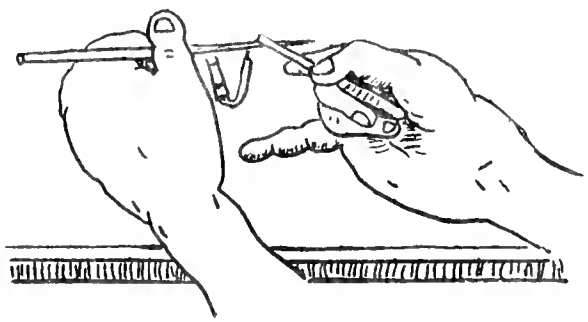


Fig. 4

Examine the needle point under the compound microscope by laying it very carefully on a clean glass slide and bringing it into the field. A well made needle will have a fine, sharp, rapidly tapering point. If the heat has been too intense, or the pull has been too rapid, an extremely long and fine flexible "hair" will result. If the heat has not been sufficient, the glass will break, giving a blunt, jagged, unserviceable point. A typical, well made needle is shown in figure 5.

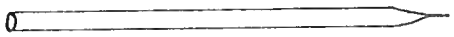


Fig. 5

Make one dozen of these microneedles in this manner leaving a three inch shaft, and place in the needle block. The block may be made of any wood about $\frac{1}{2}$ inch thick, and convenient size, (6 inches by 3 inches) in which holes have been drilled, 1/8 inch in diameter, $\frac{1}{2}$ inch deep, and $\frac{1}{2}$ inch apart. Bring the needles to your instructor for criticism and inspection.

3. Bending the Microneedles. Needles which have been approved should now be bent at an angle. This is done by carefully applying heat not more than 5 mm from the tip and pushing upward with a steel needle or fine forceps. The angle of bending varies with the use in view. It has been found satisfactory to work with needles of two types. (Figure 6).

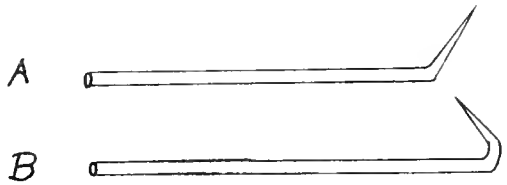


Fig. 6

In type A, there is a single bend of about 60 degrees. In type B there is a double bend, the second one carrying the point back over the shaft. CARE MUST BE TAKEN DURING THE BENDING OF NEEDLES TO PROTECT THE SHARP POINT FROM MELTING IN THE FLAME.

4. Steps Preliminary to Mounting and Use of Microneedles.

(a) Turn the three screws (G, H and I) of both manipulators four or five revolutions until the bars are slightly separated. This will allow movement in two directions in each of the three planes.

(b) Use your #1b Leitz objective with 10x ocular.

(c) Make sure that your lighting system gives you sufficient illumination and that a heat screen is interposed.

(d) Wet with distilled water the strips of blotting paper along the sides of the moist chamber. Grease the tops of the side bars sparingly with vaseline, and put a cover slip in place. Rack the moist chamber back until the condenser lens is cleared.

5. Mounting the Microneedles. Test the operation of the screw on the end of a needle holder. Insert a needle of type A in the left holder. If the needle is clamped in too tightly the snaft may be broken. Should this occur run a wire through the lumen to clear it of broken glass. Slide the needle holder forward until the needle tip

is just over the condenser. By gently moving the needle from side to side locate the position of the needle under the microscope and center its point. With the point continually under observation slowly rack the moist chamber forward. Stop the movement as soon as the edge of the cover slip comes into view, and determine the relative positions of needle point and cover slip. If the point is above the cover slip, lower it by means of the coarse adjustment until it is definitely beneath it. If the point is below, raise it slightly. The fine screw should not be used for these preliminary adjustments. Now rack the moist chamber completely back. Mount a needle of type B in the other holder, and slowly rotate the point inward. With the naked eye approximate this point as closely as possible to the other point. THIS REQUIRES GREAT CARE. Under the #1b, find and check the position of the second needle point with respect to the first. Be certain that both points are in the same plane. Rack the moist chamber forward over the needles. Change to the #3b objective. Center the needles with respect to the microscope field under this magnification.

Making and Use of Micropipettes

1. Making Micropipettes. Using six inch lengths of glass tubing draw a supply of capillaries as outlined above. Always use chemically cleaned glass of at least 4 mm bore and with thin wall. Have a supply with the ends fused to keep the interior clean and dry. To make a pipette, take a capillary and break off the fused ends. As in the directions for making microneedles, heat and pull the capillary tubing over the microburner. Examine under the microscope. The kind of pipette point will again depend on the amount of heat, and rapidity and smoothness of pull. A fairly wide variety of pipettes will undoubtedly result. They may be roughly classified into four categories. (See figure 7).

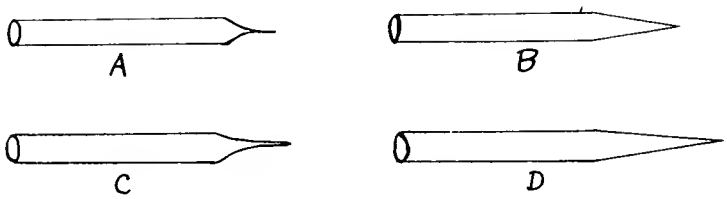


Fig. 7

A. Coarse quick taper, usually of little value.
B. Coarse gradual taper, may be used for injecting viscous fluids in large cells.
C. Fine quick taper, best for general use.
D. Fine gradual taper, best suited for fluids of low viscosity.

2. Bending the Micropipettes. Bend the micropipettes using the same technique as for microneedles. The two most convenient angles are 45° and 90°. Make a dozen micropipettes with shafts about three inches long, place these in your pipette block according to the classification given above, and present to the instructor for inspection and criticism.

3. Setting up Injection Apparatus. Clamp the metal injection tube to the base-plate by means of the metal square attached to the tube. The end carrying the hypodermic needle should be toward the syringe holder. Fasten the syringe in its holder. The length of tubing between the square metal clamp and the hypodermic needle will be found to exceed the distance between the clamp and the syringe holder. This length must be reduced by careful bending or coiling until the metal tip of the syringe can be securely fitted into the hypodermic needle without strain. Next fasten the

adapter in the right hand holder. It is a metal tube, fitted with rubber washers and screw caps at both ends. One end, A, carries the pipette, the other, B, forms a junction with the injection tube. At A there is also a metal washer, W, one end of which is concave. Care must be taken to see that the rubber washers fit snugly; and that the rubber washer at A is inserted first. The concave end of the metal washer must always face the rubber. The adapter is constructed as shown in the accompanying diagram. (Figure 8).

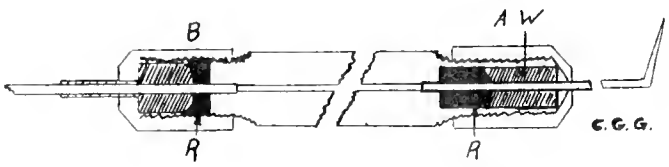


Fig. 8

To prevent tension on the adapter and clamp, loop the tubing back of the adapter, until all strain is removed. Fill the entire system with dust-free distilled water, which has been recently boiled to reduce air content. Fit into the adapter a piece of sealed off capillary and screw in tightly. Exert enough pressure on the plunger to be certain that there are no leaks. Leaks may occur (a) at the point where the syringe fits into the hypodermic needle, (b) at the point where the metal tubing enters the adapter, (c) where the capillary enters the adapter. If there is leakage at (a), work syringe and hypodermic together until they fit more tightly. If there is leakage at (b) or (c) it may be due to the absence of the rubber washer which should be in this position, or due to an imperfect fit of these washers. Remedy all defects until you have a water-tight system.

Mount a microneedle in the left hand holder. Center and focus as usual. Empty the syringe until it is but three-quarters full. Then insert a micropipette in the left hand holder, and fasten securely. Press on the plunger. If the system is water tight, water will partially fill the shaft of the pipette. If the pipette tip has been broken by any chance, this water will be driven out of the pipette tip. Discard such a pipette. If the tip is sealed the water will recede into the adapter on release of pressure. Now set the pipette into position using the microneedle as a guide.

Place a drop of distilled water on a cover slip, invert and put in place on the moist chamber. Rack the moist chamber forward. Using objective #3b, focus on the edge of the hanging drop. Carefully raise the pipette into the drop with the fine adjustment, until the pipette tip touches the cover slip with sufficient force to break only the tip. Attempt filling the pipette by pulling back gently on the plunger. Then lower the tip, move it out of the hanging drop, and see whether fluid can be expelled by exerting slight pressure on the plunger. If the tip is not broken, repeat this procedure until successful. Rinse the tip by filling and emptying it several times to rid it of free alkali. Always break the tip of the pipette at the magnification under which it is to be used.

Your apparatus is now ready for operation. Compare and check your set-up with the one shown in the frontispiece. It is advisable to read the following references to familiarize yourselves with the general morphology and behavior of the organisms to be used for experiments.

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MICRODISSECTION EXPERIMENTS

I. On Amoebae.

A. Amoeba proteus. 1. Make a small hanging drop on a cover slip containing a half dozen amoebae.* Bring up one needle and transfix an organism against the cover slip. Bring up the other needle, pierce the animal. Lower both needles. Describe the effect of puncture on the amoebae.

2. Pierce another amoeba with two needles as above. Tear the amoeba by moving one needle laterally. Describe the effect.

3. Without injuring it, move an amoeba to the edge of the drop with one needle. Hold the amoeba against the cover slip until it attaches itself and begins to crawl. Then press the other needle firmly against the amoeba, and by moving the needle backwards and forwards, cut the cell against the cover slip. Cutting may sometimes be accomplished by using only the point of the microneedle, though usually it is more easily done by raising the needle until the tip is flattened against the cell.

4. Dissect out a nucleus and impale it on a needle point.

B. Amoeba dubia. Repeat the above. Note the specific differences in these two organisms as to:

a. relative ease of puncturing wall.
b. relative ease of severing in two, etc.
c. variation in form, consistency, or reaction of nuclei.

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*Footnote: A simple transfer pipette may be made, and the amoeba handled by one of the following methods: (1) Mouth Pipette: Draw out a five inch piece of clean glass tubing over a Bunsen burner and point it off. Attach to the other end six inches of soft rubber tubing. Practice drawing up several amoebae in a small drop of culture medium and depositing them on the cover slip.

(2) Hand Pipette: Draw to a fine point a three inch length of clean glass tubing of small diameter (3 mm). Attach to the large end two inches of soft rubber tubing of about the same bore. Fold back on itself about half an inch of this tubing and tie it securely in this position with a bit of string. With this small hand pipette practice picking up amoebae as before.

II. On a Ciliate. Blepharisma, Spirostomum or Paramecium.

A. Successful handling of a ciliate calls for a much smaller drop than was used for an amoeba, since the ciliate moves so rapidly. The smaller the drop the greater the ease in catching one.

1. Impale and cut animals against the cover slip.

2. Attempt cutting animals against the water film. This is done by raising the needle into the drop above the animal and lowering it by a quick movement when the animal swims directly beneath it. Which of these ciliates cuts with the greatest ease?

III. Human tissue cells.

A. Mount, separate and tear cells obtained from the lining of the cheek.

1. Compare with other cells as to ease of cutting and reparability.

2. Are contiguous cells easily separated?

MICROINJECTION EXPERIMENTS

I. On Amoebae.

A. Amoeba dubia.

1. A typical oil - Olive oil. Fill a pipette which has been bent nearly to a right angle with oil by the method described above for aqueous solutions. By means of the mechanical stage, center a firmly attached amoeba over the pipette tip. Focus on the under surface of the organism. Slowly bring up the pipette; pierce the under surface and slowly expel a drop of oil of about the diameter of the nucleus. Withdraw the pipette quickly and smoothly. Observe the following points: (a) the immiscibility of the oil with the protoplasm and (b) the circulation of the globule. (See references number 1 and 2).

2. Injection of a salt solution: Fill the pipette with some M/50 $CaCl_2$ solution. Inject successively increasing amounts of solution beginning with a volume equal to that of the nucleus. Observe: In what striking way does the reaction differ from that of the injection of pure water? (See reference number 3, p. 379).

3. Injection of pure water into dyed amoeba: Isolate a few amoebae in 10 cc of spring water, in Syracuse watch glass and add a drop of 1% neutral red solution. Allow to stand for 5 - 10 minutes. Transfer into the usual hanging drop for operation. Fill the pipette with glass distilled water and inject. Notice that the injected liquid disperses the granules at the site of injection, leaving a clear zone. This dispersal of particles may be used as a criterion of volume and rate of injection. Vary the quantity of water injected - from a volume equal to that of the nucleus to a volume one-half as large as that of the amoeba. Observe the effects following the injection of increased quantities of water. (See reference number 3, p. 376 and reference number 4).

4. Injection of pure water into undyed amoeba: Repeat the experiments outlined in section 3 until you gain facility in estimation of injection volumes.

5. Injection of an indicator dye. Phenol Red. Fill the pipette with the dye in the usual manner. Expel a drop on the cover slip and note whether there is a difference in color between the expelled drop and the large drop of dye. If the expelled drop is bluish, it indicates that free alkali has been picked up from the walls of the pipette. If so, empty and fill until no color change is observed in the droplet. Inject as above, gradually increasing the amount. (See reference number 5). This dye has a range of from pH 6.8 - 8.6, with one turnin point from yellow to orange at about 6.8, and another from orange to pink at about 7.9. Observe whether the dye permeates the cytoplasm; is picked up by any cytoplasmic inclusions; diffuses through the plasma membrane; or undergoes any color changes.

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SECTION II.

APPLICATIONS OF THE MICROMANIPULATIVE METHOD AND DESCRIPTIONS OF VARIOUS ACCESSORIES USED IN SPECIAL PROBLEMS.

SECTION II

1. MACHINE FOR PULLING MICRONEEDLES AND MICROPIPETTES

In designing a machine for this work a study was first made of the hand movements of an expert in making needles and pipettes. The essential movements seemed to be a removal of the glass from the heater at the correct temperature followed by a rather quick horizontal pull-out.

In the machine as designed, a parallel motion was devised which, when the glass becomes plastic enough to stretch under a light pull, lifts the glass up and out of the heater and, at the same time, increases the pull by an increase of leverage. The result is a rapid pull-out at the correct instant.

The machine as shown in figure 9 is in its final position after pulling two needles, and one needle (a) is still held in the machine by the clamp (b) though the locking nut (c) has been unscrewed, (much more than is necessary to release the latch (d)). It is obvious from the drawing that the clamps (b) remain always parallel to the base due to the hinged side bars (e) but perhaps it is not so clear that the alignment rod (f) passes with a sliding fit through the cross bars (g) of the clamps (b) so that the two clamps must move up and down together, always remaining in alignment with each other. To "set" the machine the clamps are pressed down until, by turning the knurled knob (h), the retaining bar (i) may be swung over the alignment rod (f). The clamps are then opened and a glass rod or capillary of double length is dropped between the guides (j). This rod or capillary will rest on the leather pads (k) and when the clamps are closed and latched will be held firmly by the rubber pads (l). The retaining bar (i) may now be swung off, without changing the position of the machine, though the glass will now be under the tension of the springs (m). In this position the glass rod or capillary will pass through the heater (n) and upon switching on the current the glass will become softemed. It will pull out, first slowly and then, as the clamps rise, the action will become rapid ending with a quick pull-out, and two needles or pipettes will be pointed and ready for bending.

This machine has a number of elements which can be adjusted to give any form of needle or pipette point desired. This is necessary as different types of points are often required, but no change in adjustment is usually necessary for glass of different diameters.

The heating element is a platinum wire in a V-shaped holder of mica. A resistance must be used in series with the heater with a snap switch to turn the current on and off. If the platinum wire is too small it may burn out. At least size #26 should be used. The best form of the wire is shown in the diagram. The width of the heater determines the length of glass softened and so controls the length of the tapering shank of the point. With the form shown this will be about correct, but the shank can be lengthened by spreading the loops of the wire if desirable. The temperature of the heater depends upon the current which flows and this can be adjusted by the series resistance to give a safe temperature which will not burn out the platinum.

At this temperature, the color of the wire is a bright yellow. A current of 7½ amperes may be used for #26 platinum wire.

The heater is adjustable in position and should be set squarely under the glass rod. The heater can be raised and lowered and the loops bent in and out. If the heater is too low or too open at the top of the loops the heat will not be maintained long enough as the glass rises, and the final taper may be too short or the tip broken. On the other hand if the heater is too high the final taper will be too long or the tip fused.

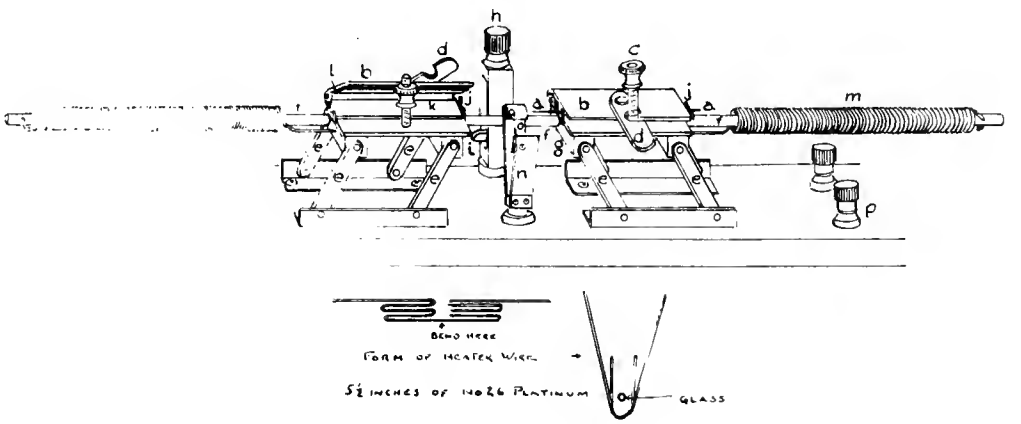


Fig 9

One other adjustment that can be made is the position, up and down, of the bar that locks the machine while the glass is being clamped in place. The lower the bar is adjusted the less will be the initial tention on the glass. It should be kept fairly low.

It will be seen that each of these adjustments affects the others. Thus an increase of heater current or a raising of the heater, or a lowering of the locking bar will all act to increase the heat delivered to the glass. The main thing seems to be a rather intense heat at the start, maintained as the glass rises until just before the pull-out. The exact setting however, can only be made experimentally.

With the machine adjusted to give the type of point desired, it is possible to turn out pipettes or needles at the rate of four to six per minute. This machine is now in use in Dr. Chambers' laboratory. Du Bois, 1931.

2. EXTENSION ARMS FOR CARRYING MICROMANIPULATORS

A pair of accurately slotted metal extension arms, capable of carrying the manipulators may be attached to the base of the Chambers machine. These arms, devised by Wright and McCoy, permit the manipulator to slide back from its initial position, and return to precisely the same position. This sliding device also facilitates the rapid exchange of micropipettes and other microapparatus. A single arm, A, with its central slot, S, is shown in figure 10. A manipulator, M, is in position, and by releasing the screw, Sc, may be moved back and forth. The screw Scr fastens the arm firmly to the manipulator base. Wright and McCoy, 1927.

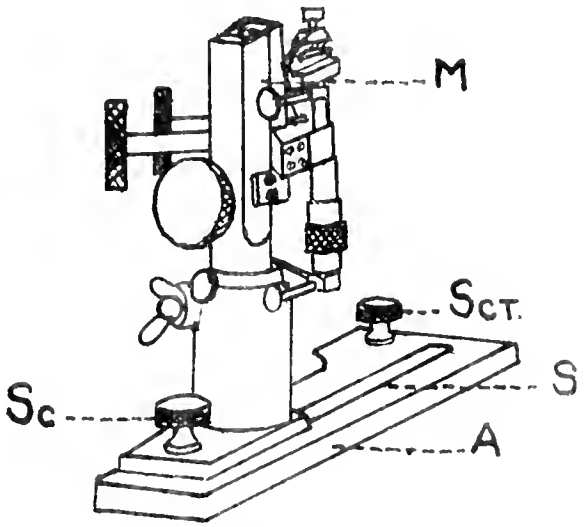
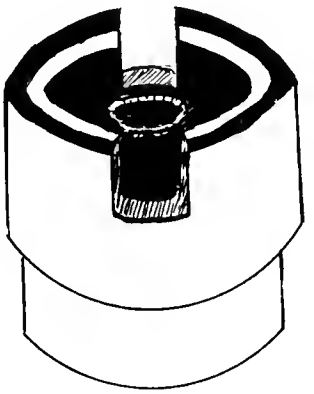


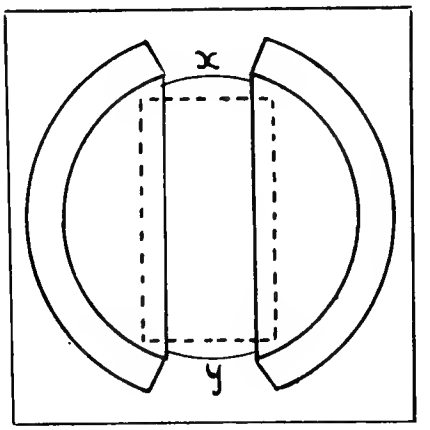
Fig. 10

3. SPECIAL DARK FIELD CONDENSER AND MOIST CHAMBER.

A new dark field condenser (fig. 11 a) has been especially designed for use with the Chambers micromanipulator. This condenser is of the bicentric type. It has a focal length of 10.7 mm and a working distance of 14.0 mm. The range of aperture of the illumination rays is from 0.85 mm to 0.99 mm, a remarkably large aperture, necessary for use with oil immersion objectives. The condenser has a diameter of 49 mm, a height of 42.5 mm, and is provided with lateral slots 15.5 mm wide and 10 mm high to allow ample room for the vertical and horizontal movements of the microneedles. A depression in the base may be filled with water to prevent evaporation of hanging drops during operations. In order to use this condenser, the opening in the microscope stage must be 50 mm in diameter.



a CG.G.



b

Fig 11

A special moist chamber for use with this condenser is shown in figure 11 b. The base is an 80 mm square of glass, with a circular hole cut in the center. This hole is bordered except at two opposite regions, x and y, by two curved glass walls, 18.5 mm high. The enclosed chamber is roofed with glass in such a manner as to leave an exposed space across which a cover slip with hanging drops may be laid as shown in the diagram. The walls should be lined with moist cotton or filter paper, and the chamber fitted over the condenser. For the best results a strongly concentrated light should be used and the condenser should be racked up and down to bring to focus the most intense light on the tip of microneedles. Hauser, E.A, 1930, Ueber Mikromanipulationen an Latex im Dunkelfeld. Kolloid Zeitschrift, 53, 78.

4. HERMETIC MOIST CHAMBER.

The hermetic moist chamber for injections under anaerobiosis was made of glass, the joints being cemented together with balsam (see Figure 12). It consisted of two troughs, one to hold mercury for sealing the chamber and the other to serve as the moist chamber proper. The latter contained a gas inlet and an outlet. Through the axis of the inlet there passed a capillary tube used for delivering the reduced dye into the moist chamber under complete exclusion of oxygen. The chamber was 30 x 40 mm and 15 mm high; and the total volume, including the gas space over the mercury seal, was about 25 cc. Purified nitrogen was passed through at a rate varying from 50 to 250 cc per minute. The chamber was roofed over by a coverslip (40 x 60 mm) and sealed by strips of mica, all heavily coated with vaseline and pressed firmly on the broad contact surfaces. The seal was tested for tightness and found to withstand a pressure of at least 2 to 3 cm. of water. The diffusion of oxygen through the vaselined seals was of a negligible order, or else it was effectually eliminated by the constant stream of purified nitrogen passing through the chamber, for drops of reduced indicator often remained uncolored on the coverglass for several hours during an experiment.

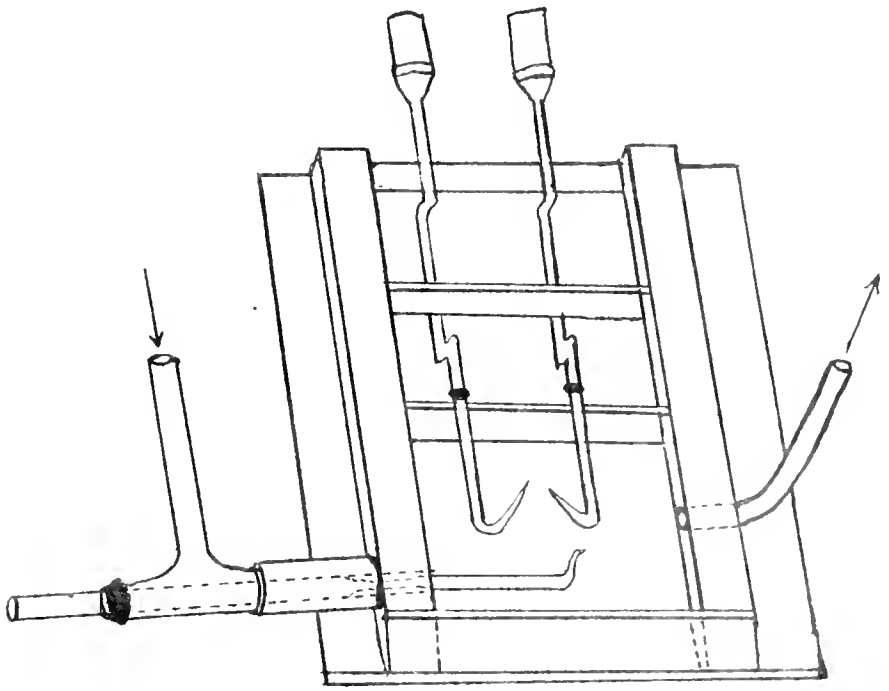


Fig. 12

Purification of nitrogen. - An essential part of the experimental technic is the particular care to eliminate leakage of atmospheric oxygen into the anaerobic system. Unprotected rubber connections are dangerous because oxygen diffuses through the rubber in quantities sufficient to upset conditions and lead to erroneous conclusions. This point needs emphasis. Cohen, B.,Chambers, R., and Reznikoff, P., 1928.

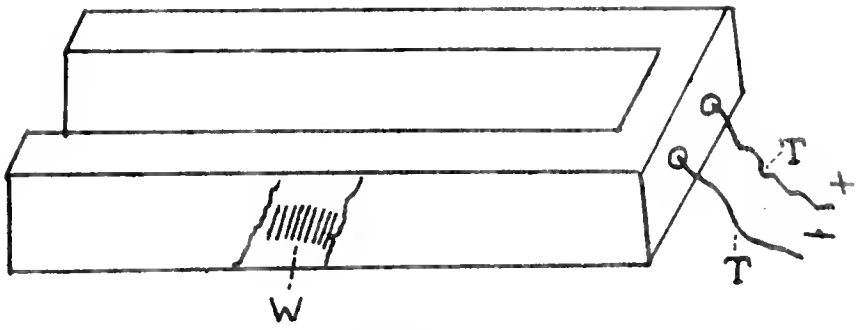


Fig.13

Hecker has devised a jacket, heated by electricity, which can be placed around the moist chamber and hence maintain within it any constant temperature. The jacket is made by coiling wire about a frame which surrounds the chamber on three sides. The terminals of the wire are connected to a rheostat by which the amount of heat may be controlled. The construction of the device is indicated in Figure 13, where (w) is the wire fastened about the internal frame, and (T) the terminals leading to the rheostat. Hecker, 1916.

6. ISOLATION AND TRANSFER OF BACTERIA.

Kahn has applied the micromanipulative technique to the isolation of single bacteria. His method, now generally in use in bacteriological laboratories, is as follows. Micropipettes are drawn on the ends of 4 mm ~~of~~ glass tubing cut into six inch lengths. It is more convenient to have the moist chamber with its open end so placed that the pipette may project into it from the left side. This facilitates the frequent interchange of pipettes so necessary in isolating bacteria. For this purpose the pipette holder is attached to the left side of the microscope. A deeper and wider moist chamber than that used by Chambers for his cytological work is found more convenient for rapid changes. The method of procedure for the isolation of a single bacterium may be summarized as follows: (1) Prepare a young liquid culture from a subculture not more than 18 hours old. (2) Insert the tip of a sealed pipette into a tube of liquid culture and open it by gently rubbing it against the wall of the tube. Then with a rubber tube on its shank suck up a small amount of the culture. (3) Mount this on the instrument and bring the tip into focus in the center of the microscopic field. Raise the pipette until its tip touches the undersurface of the coverslip and expel an appreciable droplet. This may have to be diluted with sterile fluid if the culture is too dense. (4) After securing a moderately dilute preparation fill the same or a new pipette to a little below its bend. Lower the pipette, and with the aid of the mechanical stage, bring another portion of the cover slip into view. By alternately raising and lowering the pipette a series of minute droplets will be found to contain a single microorganism. (5) Replace this pipette with a new sterile one containing a small amount of sterile liquid medium which must not run below the elbow. This new pipette is now brought directly under a droplet containing a single microorganism. The pipette is then slowly raised and as soon as it touches the surface the droplet with the contained organism will flow into it. This occurs by capillary attraction and no suction is required. (6) This droplet, which is known to contain only one microorganism, is carefully removed from the apparatus

and its tip inserted into a tube containing a suitable sterile medium. The entire contents of the pipette are now to be expelled by blowing. As an added precaution it is well to break off the tip of the pipette in the culture medium. The blowing may be done by mouth or by a rubber bulb operated either by the hand or by the foot. For continued observation of colony growth, bacteria are isolated on cover slips which are then inverted over depression slides and sealed with a vaseline paraffine mixture. The bottom of the depression slide contains a few drops of 1% agar. This maintains excellent conditions of moisture over a period of several days to several months. Barber 1911 a; 1914. Chambers 1922 a. Kahn 1922,1929. Kahn and Schwarzkopf 1931. Schouten 1905, 1911. Gee and Hunt 1928.

7. MEASUREMENT OF CAPILLARY PRESSURE.

A. LANDIS METHOD

Landis has adapted the Chambers technique for measuring pressures in small vessels such as capillaries. His microscopic instruments consist of blunt rods and micropipettes. The rods are made by fusing small balls 20 u to 50 u in diameter, on the ends of glass microneedles. Pipettes used vary in diameter from 4 u to 8 u at the tip and are introduced directly into the capillaries. Pipettes and rods are introduced into the microscopic field from one side and the pipette is directed downward instead of upward as in a hanging-drop preparation. The rods are used to occlude the walls of the vessels and thus stop capillary flow temporarily or permanently by pressure; or to hold the mesentery firmly as the capillary is introduced into the resistant walls of larger vessels.

The set-up employed is diagrammed below. (Fig. 14) The accompanying description is from Landis.

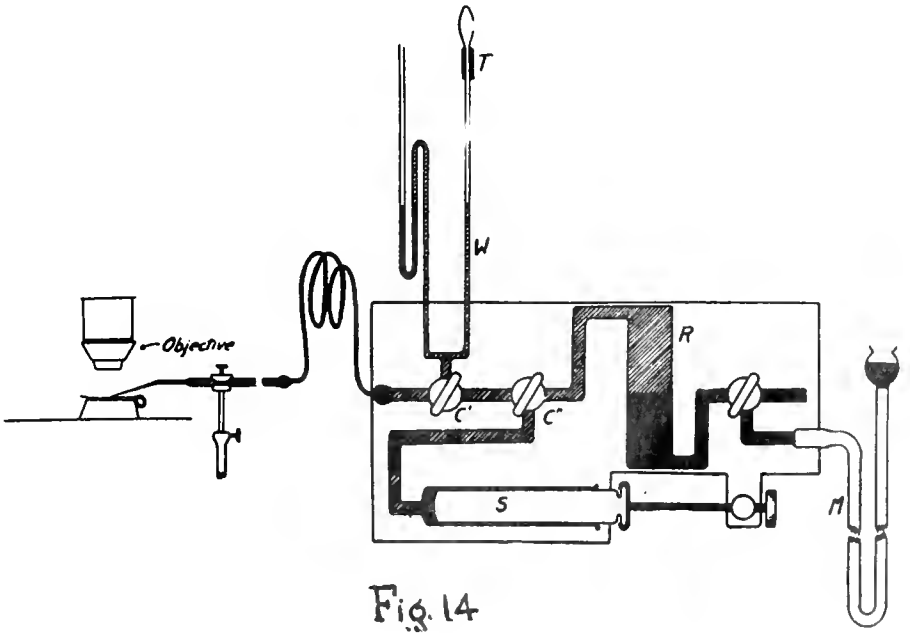


Fig. 14

The apparatus consists essentially of a reservoir, R, a syringe, S, for exerting large positive or negative pressures, and a column of water, W, by which measured amount of pressure up to 30 cm of water may be applied to the contents of the micropipette. A dye solution may be drawn into the tip of the micropipette by a column of mercury, M, or by carefully withdrawing the plunger of the syringe when

the two stopcocks, C', C", are properly arranged. For accuracy of control the plunger of the syringe is moved by a screw adjustment.

After the pipette has been introduced into the capillary the stopcocks are so turned that the pressure is applied to the dye in the pipette by the column of water, W, the height of which can be changed rapidly by the movement of the syringe plunger. To correct for capillarity in the upright tube the instrument has been calibrated by balancing the water column against known pressures.

For measuring the higher pressures found in the arteries a mercury column is more convenient. This is connected through a U-tube with the water manometer. If the opening at the tip of the tube, W, is open the whole system functions as a column of water, but if it is closed by a ringlet of rubber tubing, T, the pressure applied is measured by the mercury manometer providing correction be made for the column of water between the surface of the mercury and the tip of the pipette. This arrangement allows a more rapid succession of pressure determinations in arterioles and arteries than would otherwise be possible.

The material used by Landis consisted of mesenteric capillaries of the frog. A loop of the intestine was brough through an incision in the abdominal wall and the mesentery laid loosely over a transparent glass stage. The preparation was kept moist and clean by a slow constant drip of saline solution.

B. FLOREY METHOD

A method by means of which fine blood vessels are made accessible to manipulation is also described by Florey. This method seems equally well adapted for study and manipulation in vivo of any group of tissue cells thin enough to allow the penetration of sufficient light for critical observation.

Florey's method is as follows: "A trough made from sheet tin was fashioned to accomodate the body of the rat. In one side of the trough, at the appropriate place, a rectangular piece of metal was cut away so that the gut, g, with its mesentery, m,

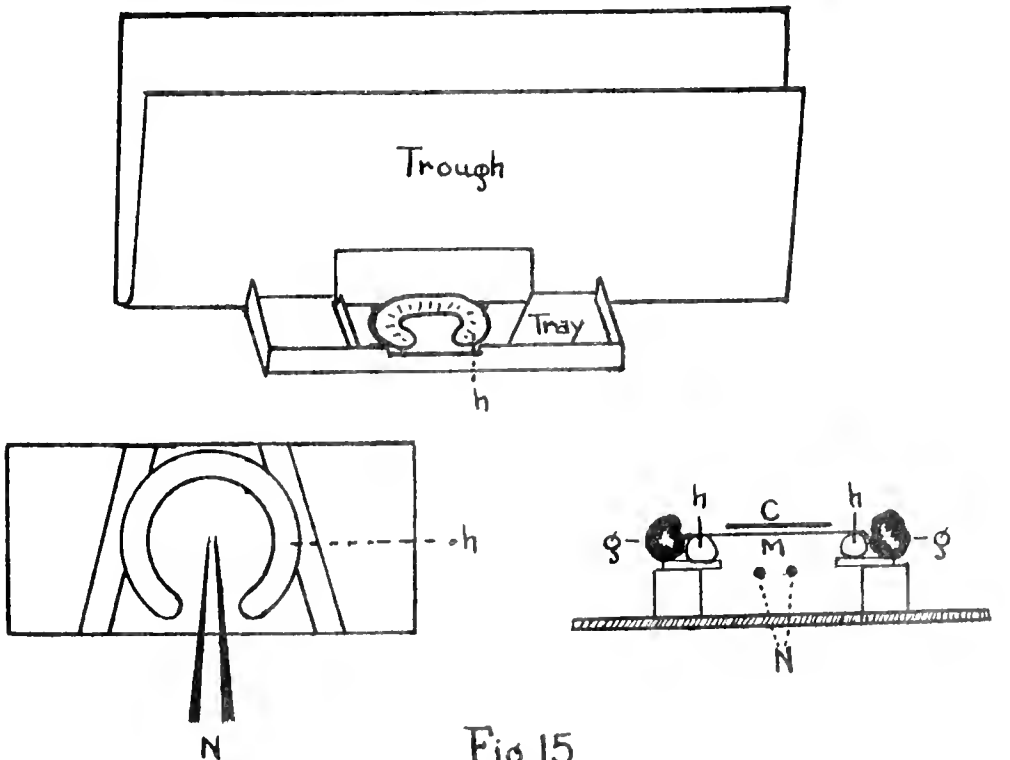


Fig 15

attached could be withdrawn through it. To the large trough a tray of the same material was soldered. The tray was furnished with a circular hole about 1.5 cms in diameter, in the bottom, the hole being covered with a glass slip cemented to the metal by means of de Khotinsky's cement. A moist chamber consisting of two parallel strips of glass was placed above this opening. To the top of these strips was cemented a small glass tube bent into the shape of a horseshoe, h. (See figure 15).

The whole apparatus was then mounted on a mechanical stage of a microscope by which means it could be moved accurately. The circular hole in the tray was so arranged as to be over the lens of the condenser. This sub-stage condenser had an especially long focal distance for use with hanging drop preparations. The typs of microscope used had a large curve in the supporting arm so that the trough containing the rat fitted in snugly between the objectives and the microscope arm. The rat was anaesthetised with urethane, subcutaneously injected, and an incision made through the right flank through which the gut and its attached mesentery could be withdrawn. The animal was then placed in the trough and the incision arranged opposite the rectangular hole in the side of the trough. The gut, g, was withdrawn with padded forceps and the mesentery spread over the glass ring. Some drops of warm saline were then placed on top of the spread mesentery and a round cover slip, c, applied. The surface tension at the interface between the mesentery and the glass caused the former to be flattened out on the under side of the coverslip. The tray was filled with saline, which also ran into the moist chamber so that the mesentery was kept moist from above and below. The whole apparatus was placed in a warm box during the period of observation. This apparatus was constructed so that the capillary vessels could be manipulated from below by means of a Chambers' micro-dissection apparatus. (See figure 15). Florey, 1926. Landis 1925-26. Wearn and Richards, 1924-25. White and Schmitt 1926.

8. MICROELECTRODES.
A. ETTISCH AND PETERFI.

A non-polarisable microelectrode has been developed by Ettisch and Peterfi. Its construction is as shown in figure 16, where (1) is the agar-filled capillary with micro-tip, (2) the holder for capillary, (3) the agar filled T-tube, (4) the calomel electrode, (5) the Bakelite holder, and (6) the wire leading to the electrometer. The micro-electrode is fastened as pictured in the diagram. The capillary itself is constructed and filled with agar as shown in figure 16, A-F, in which (A) is the empty capillary, (B) the agar (in N/10 KCl) sucked in to fill central portion of the capillary, (C) one end sealed in flame, (D and E) the other end pulled out to form a micro-tip, (F) agar forced to tip by warming. The shank end is then broken off. Major difficulties which beset the preparation of the

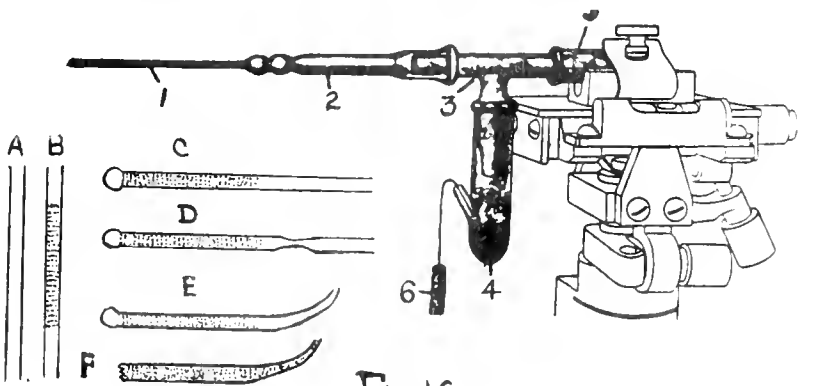


Fig. 16

capillary are: contamination, non-uniform filling with agar, air bubbles, inequalities in the electrolyte concentration of the agar.

B. TAYLOR (MICROELECTRODES AND MICROMAGNETS)

Two kinds of microelectrodes and one micromagnet have been perfected and used with very good results. First, it was found that a platinum wire (No. 35, C.P.) inserted into a close-fitting quartz capillary can be drawn over a minute oxy-acetylene flame to a perfectly insulated needle-point less than one micron in diameter. Indeed, the platinum core in this exceedingly fine point may closely approximate the limits of microscopic vision.

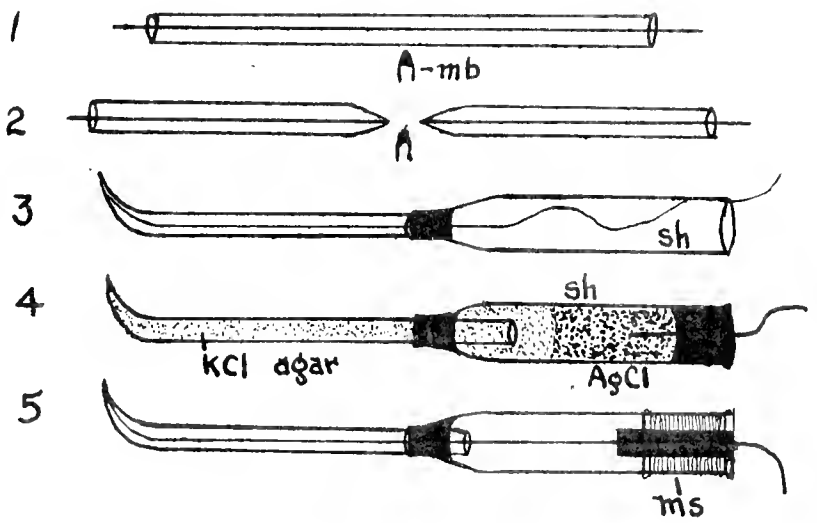


Fig. 17

The platinum wire at the opposite (undrawn) end extends a few mm beyond the quartz capillary in order that the former may be annealed to an insulated copper wire of about the same diameter as that of the platinum wire and two feet in length. The drawing of the electrodes (see Fig. 17, 1 and 2) and the annealing are readily done over the oxy-acetylene microburner (m.b.). About 3 mm. of the needle-tip of the electrode is bent over the flame, at right angles (Fig. 17, 3, sh). This shank fits into the instrument-holder of a micromanipulator. By means of the latter the electrodes are operated with finest precision in a moist chamber on the stage of the microscope.

The second type of microelectrode is non-polarizable. For many purposes the platinum electrode would appear to be entirely satisfactory, but certain experiments make necessary the use of this second type.

A micropipette, made preferably from a quartz capillary of about 1 mm in outside diameter and 50 cm long, is filled by means of a good Luer syringe with a melted 0.5 per cent KCl solution of carefully dialyzed agar. When the agar solution has thoroughly hardened, the micropipette is sealed with White's dental cement (of low melting point) into a glass shank (Fig. 17, sh.) similar to that described above for platinum electrodes. The inner end of the pipette, which should extend only a few mm into the "bowl" of the shank, is now well covered with a few drops of the melted agar solution. When the agar has thoroughly cooled, the bowl of the shank

is almost filled with slightly moist, well pulverized AgCl (C.P.). Into the silver chloride is inserted a No. 20 silver wire of convenient length (about 1 ft.). The end of the "bowl" is then filled and completely sealed with de Khotinsky cement which thereby firmly holds in place the silver wire. It is advisable to insulate the exposed portion of the silver wire by coating it with de Khotinsky cement or by means of glass beads which can be quickly made by cutting a capillary tube into lengths of 1 cm each.

After mounting the two electrodes of this type in the micromanipulator they are "shorted" for some time previous to use, in order to obviate any difference of potential.

The E.M.F. is provided by a dry cell of about 1.5 volts (the voltage being precisely determined by means of a Weston Standard Cell). Included in the circuit are two resistance boxes totalling 12,000 ohms and one of 40 ohms, a nitrogen-mercury key and a specially designed Leeds and Northrup galvanometer having a sensitivity of 35,714 megohms.

The micromagnets, consisting of quartz with a soft iron core, are drawn in the same manner and quite as fine as the platinum electrodes described above. Into a close-fitting quartz capillary 30 cm long is inserted a No. 30 soft iron wire with free ends extending 20 cm beyond either end of the capillary tube. The magnets are then drawn over the microburner as illustrated in Figure 17, 1. The very tip of the iron core is completely enclosed by the quartz. After slightly curving the drawn ends (Fig. 17, 5) each magnet is thereupon mounted and temporarily cemented into the glass shank such that the free end of the iron wire projects 3 or 4 cm beyond the base of the shank. This permits the soldering of the wire onto one end of the soft iron core (i.e., Fig. 17,5) of the magnet spool (m.s.). This spool, made of wood, is 2.5 cm long and 1.5 cm in diameter - a size sufficient to accomodate the winding on of 1500 ft. of No. 36 triple insulated copper magnet wire (m.w.). One end of the soft iron core (4 mm in diameter) should extend about 3 mm beyond the spool in order to provide support for the glass shank. Having soldered the iron wire to the projecting core, the glass shank, now unsealed from the micromagnet, is slid over the free end of the iron core whereon it is firmly cemented by means of the de Khotinsky wax. In turn, the micromagnet is permanently cemented in the shank (Fig. 17, 5).

The micromagnets, like the microelectrodes, are supported and operated in the moist chamber by means of the micromanipulator.

It is evident that these electrodes and magnets afford a means for the study of the electric and magnetic properties of protoplasm in the interior of a living cell, thus eliminating an unknown factor - the cell-membrane. But they also provide means for an independent study of this unknown factor. It is believed that other uses also may be made of these microinstruments.

C. TAYLOR AND WHITAKER.

A hydrogen microelectrode has been designed by Taylor and Whitaker. Gases or liquids may be substituted in place of hydrogen for either the saturation or the protection of the platinum electrode. The microelectrode is non-polarizable, and is sheathed within a quartz micropipette. Its construction is as follows:

A platinum wire is tightly fitted into a quartz capillary and the two together are drawn to microscopic dimensions over a minute oxygen-gas flame. The electrode tip is then cut off under the microscope at a suitable point with a microdiamond.

Since platinum contracts more on cooling than does quartz, there appears a space between the platinum core and its quartz insulation of a size sufficient to allow any electrolyte to enter by capillarity. The platinum tip is melted to a bulb which by contraction of the cooling platinum perfectly seals the surrounding space. A coat of platinum black is then deposited electrically on the platinum tip and the electrode is cleaned in dilute H_2SO_4 and thoroughly washed in hot distilled water. In order to control the gas content on the electrode and protect it from contamination the electrode was mounted and used as follows:

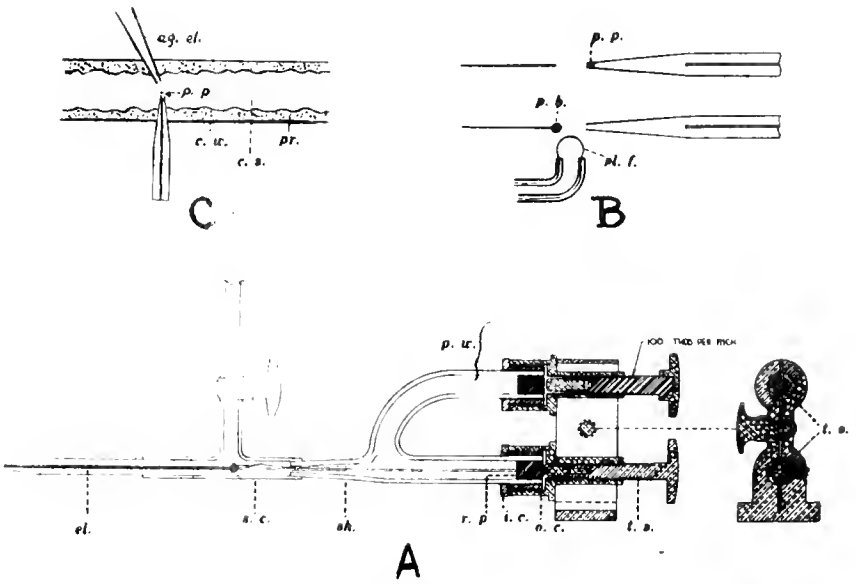


Fig. 18

A two-armed Pyrex glass shank is fitted by cement with two rubber plugs (Fig. 18, A. r.p.) and filled with mercury. Opposed to each plug is a finely threaded thumb-screw. Into the rubber of the straight arm of the shank has been cemented a section of a small brass tube. This supports one end of the electrode (e.l.). The platinum wire of the electrode is of sufficient length to extend back into the mercury of the shank. To afford electrical contact with the mercury, a platinum wire (p.w.) is sealed in the wall of the upper arm of the shank. Onto the single end of the shank is cemented a right-angled stop-cock (s.c.). The tip of the pipette is then sealed tight with hard paraffin and simultaneously the stop-cock is turned off. To effect this seal, a small ball of paraffin mounted on the tip of a quartz rod is brought into contact with the tip of the electrode (Fig. 18, B). A platinum filament is used to melt the paraffin, The tip of the micropipette is pushed into the melting paraffin through which the gas continues to bubble until the stop-cock is closed. Thereupon the melted paraffin enters the tip by capillarity and with cooling produces a gas tight seal. The device is then fixed in place on the micromanipulator.

Another micropipette is filled with saturated KCL in agar, and so serves as a "salt bridge". The tips of the two micropipettes are inserted into the cell, and the platinum (hydrogen) electrode is moved forward by means of the thumb screw, pushing out the paraffin plug. (Fig. 18, C).

D. GELFAN.

Another type of non-polarizable electrode has been designed by Gelfan. It consists of three parts, the micropipette (Fig. 19, b), the tip of which penetrates into the cell; the main pipette (Fig. 19, a), into the shank of which the micropipette is sealed, and the AgCl electrode (Fig. 19, e). The latter is sealed into a glass plug (Fig. 19, c) which is ground to fit perfectly the other end of the main pipette. The main pipette and the capillary micropipette are both filled with the same agar which is impregnated with KCl. The system is then Ag (Fig. 19, d), AgCl (Fig. 19, e), KCl in agar (Fig. 19, f).

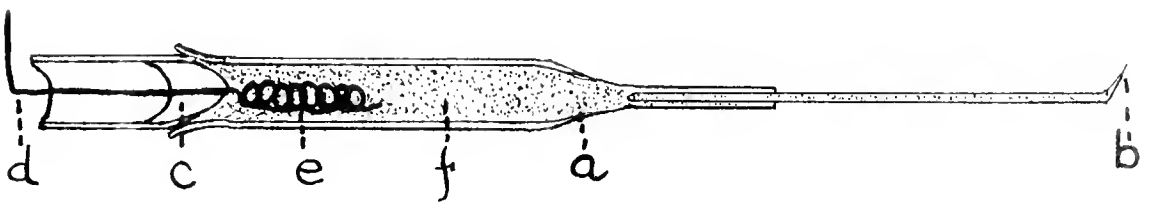


Fig. 19

The main pipette (Fig. 19, a) is drawn out from glass tubing to a suitable size for the pipette-holder of the micromanipulator. For the micropipettes, capillary pipettes are drawn out from clear quartz tubing to about 0.5 mm. in diameter. These in turn are drawn out at one end to minute points over an oxygen microflame. The tips of the pipettes, which have openings of about 1-2 microns in diameter, are bent at an angle of almost 90 degrees.

The author further discusses the preparation of the AgCl electrode, the preparation of the agar, and describes the method of filling the micropipette with agar.

The electrodes when used are connected in series with two or three dry cells, a two-way switch, and a sensitive Leeds and Northrup galvanometer. The wires of the system must be well insulated.

E. SEN.

In the type of electrode devised by Sen, the conducting medium is a continuous film of silver, gold, or platinum for stimulating electrodes; and silver-silver chloride for non-polarizable electrodes, on the outer surface of glass or quartz microneedles. Any desired number of microneedles is drawn out according to the method of Chambers, the shank ends of the needles are warmed in a micro-flame and embedded vertically in a block of paraffin. The needles are then cleaned by immersing vertically down in a beaker of cleaning fluid for some hours, and then thoroughly washing them in running water. They are then silvered by Brashear's method, washed and dried in a hot oven. Electric contacts with these silvered needles are made by inserting them in the electrode holder (Fig. 20, G, glass tube, W, Wood's metal, E, microelectrode, L, copper wire lead).

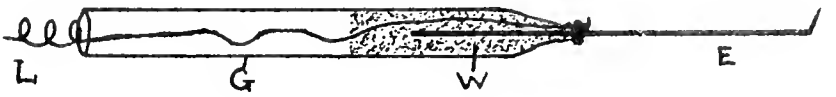


Fig. 20

Wood's Metal previously placed in the tapering end of the holder, is melted by gentle heat and the needle thrust in. On cooling of the metal a good contact is secured. After mounting the coated needle in this way, thicker coats of silver or any other metal can be deposited electrolytically. The electrodes are washed and kept in distilled water for some hours before use. When necessary the metallic film of the shank can be insulated by applying shellac with a fine brush. The insulation of the micro-tip is done under a microscope. The tip pointed upward is illuminated in the dark field of the microscope and its shank is gradually lowered by rack and pinion into a small cup of shellac solution. By this method one can insulate to within 5-10 microns from the tip. Ettisch and Peterfi, 1925a, b, Gelfan, 1927, Taylor, 1925 a, Taylor and Whitaker 1926, '27, Wright and McCoy 1927.

9. OTHER MICROTOOLS.

A. MICROHOOKS AND MICRODISTILLATION APPARATUS.

Microchemical experiments on unknown particles of microscopic dimensions are described by Titus of the Eastman Kodak Company. These tests are carried out principally for the purpose of identifying the unknown substances, and the technique is directly comparable to that used on large quantities of material in chemical laboratories. For this work Titus has devised such microscopic apparatus as a microdistillation apparatus (figure 21, x) with support, condensing cell, and crucible with heating element. The assembly of this apparatus and its relative size is further shown in diagrammatic section in figure 21,y. The microcrucible C, is in position beneath the condensing cell CC, directly under the high power lens of the microscope. Quartz microhooks for carrying particles during chemical analysis are shown in figure 21, z. These are drawn out by means of a hot point, 1, and successive steps in the preparation of the hooks are shown pictorially in 2, 3, 4, with the completed hook at 5. Titus, R.N. and Gray, H. LeB., 1930.

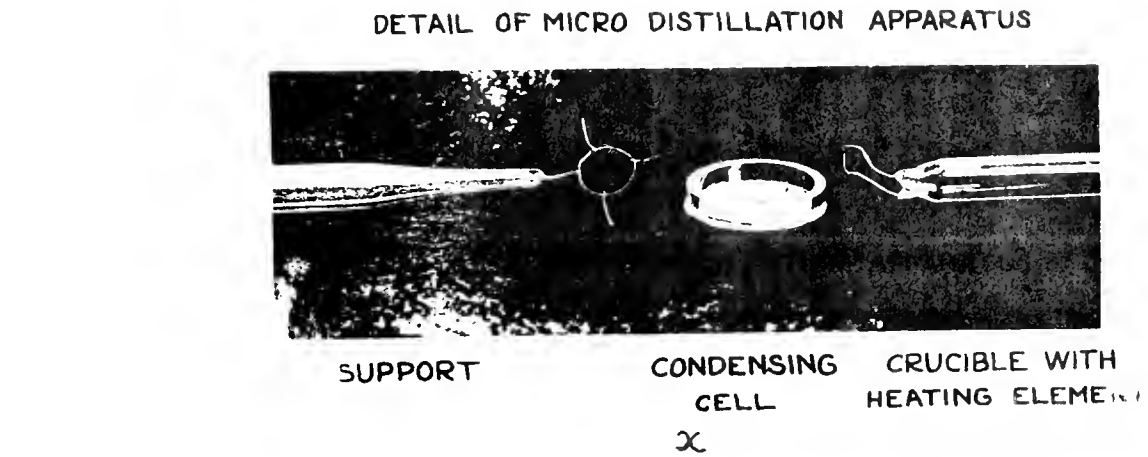


Fig. 21

B. MICROGUILLOTINE.

This instrument was designed to effect at any desired place, and with a high degree of precision, the breaking of fine glass objects and instruments such as microneedles or micropipettes. The device consists of a frame made of a strip of brass with a thin groove milled out along its center and folded to form a square with the groove inside. (Fig. 22).

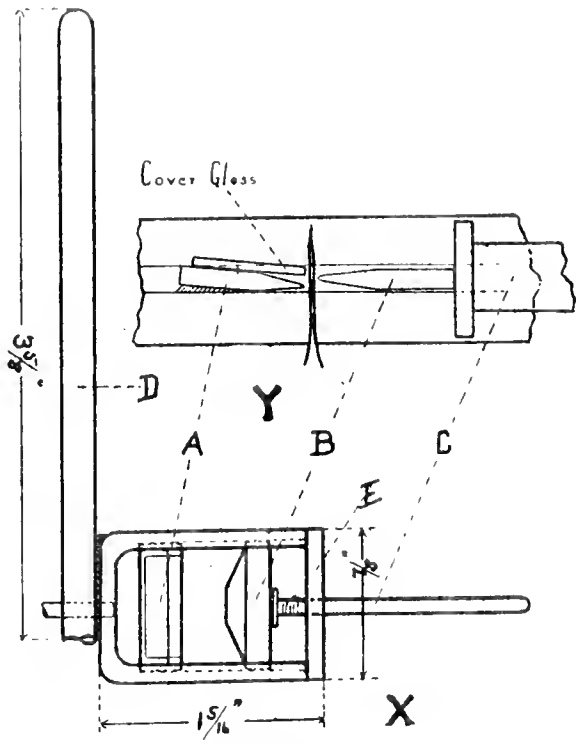


Fig. 22

Into this groove are fitted (A) a piece of Gillette safety razor blade, and (B) another piece of blade, cut to form a wedge front and attached to a holder (C), by which it may be drawn back and forth. On top of blade A is a thin piece of cover glass so placed as to have a space between it and the blade just wide enough for the edge of blade B to enter (see Fig. 22, Y). The edges of this cover glass and blade A, which face blade B, must be accurately lined up, one above the other. The entire frame is soldered to a rod, as indicated.

In operation, the guillotine is supported by its rod in one of the holders of the micrurgical machine under the microscope and the object, held by the other holder, is introduced between the blades. The guillotine and the object are then approximated to each other until the object rests against blade A at the place where the break is to be made. Blade B is then pushed in gently but firmly against the object and the break is made. If hollow objects, such as micropipettes are to be broken, the blades must be scrupulously clean or the pipette may clog.

If greater delicacy in manipulating blade B is desired, the sliding holder to which it is attached may be changed for a screw, and a hole may be tapped in cross-bar E to receive it. Also, the position of blade B, and blade A with its superposed cover slip, may be exchanged, making the latter the movable, cutting unit. Belkin 1928.

C. MICROPINCETTE, MICROKNIFE, MICROSPATULA, AND MICROCAUTERY.

Peterfi has devised various microtools made of glass, a few of which, (a) a micropincette, (b) a microknife, and (c) a microspatula, are shown in the accompanying diagram (Fig. 23).

Fig 23

The micropincette may be mounted in a double needle holder (Fig. 24). The two microtips are approximated by means of the screw A, and further adjusted in vertical or horizontal planes by the screws V and L. Peterfi 1923.

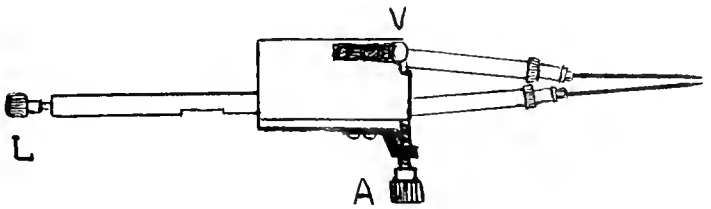


Fig. 24

Figure 25 is a schematic representation of a microcautery (4) after Peterfi, showing also the electrical hook-up, in which (1) is the connecting plug, (2) the resistance, and (3) the switch. Peterfi 1923.

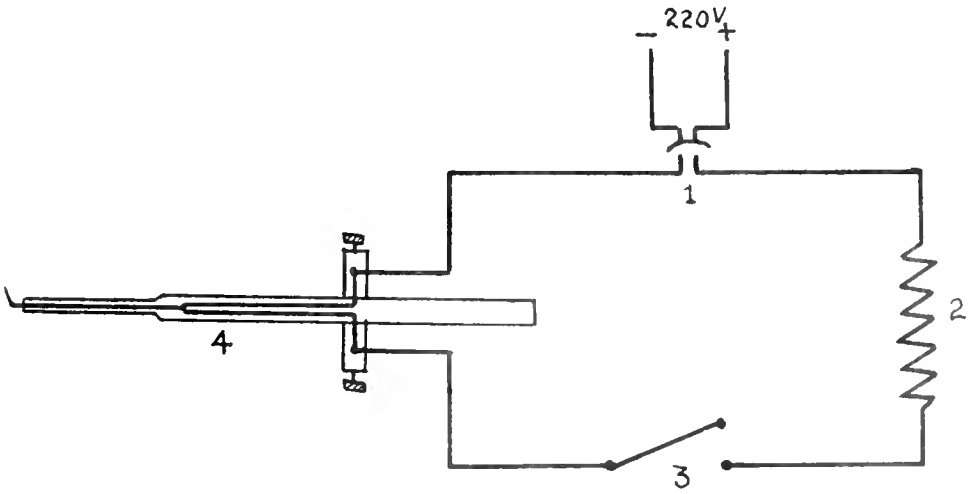


Fig. 25

SECTION III.

1. SPECIAL BIBLIOGRAPHY

A few group indices compiled with respect to certain major fields of micromanipulative work are given below.

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Wright and McCoy, 1927.

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Pollack, 1928.
Reznikoff, 1926, 1927, 1928.
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