SPECIAL ARTICLES

AN ACCURATELY CONTROLLABLE MICRO-PIPETTE

A NUMBER of pipette devices have been employed for the injection or extraction of minute quantities, which have served their purpose quite satisfactorily. Among these may be mentioned the several methods described by Toldt,[1] Barber[2] and Chambers.[3] However, in certain recent work I was unable to use with the necessary accuracy any of these methods and so undertook to construct a micropipette which could be very reliably and precisely controlled.

The simple apparatus now being used serves my needs so surprisingly well that I offer this description of it hopeful that the method will be of service to others. By its use I have succeeded in extracting the micronucleus from the ciliate *Euplotes*, have injected very minute quantities of various solutions into the cytoplasm and macronucleus and have induced the formation of vacuoles near the contractile vacuole in such manner as to obtain significant data on the behavior and function of the latter structure. An account of these results will be published in later papers; I shall here only describe the method employed. I am indebted to Professor S. O. Mast for several important suggestions in the construction of the apparatus.

The general principle involved in the operation of this mechanism is the inducement at will either of large or of very delicate changes in a given volume of mercury by means of a small steel needle attached to a finely threaded thumb-screw.

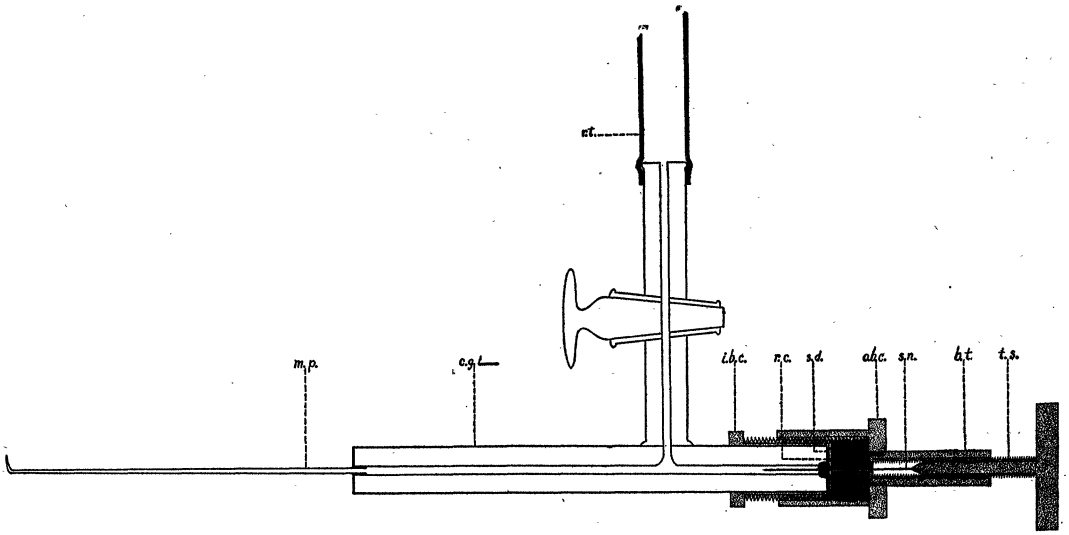

FIG. 1. *bt.*, brass tube; *c.g.t.*, capillary tube; *i.b.c.*, "inner" brass cap; *m.p.*, micropipette; *o.b.c.*, "outer" brass cap; *r.c.*, rubber cylinder; *r.t.*, rubber tube; *s.d.*, steel disk; *s.n.*, steel needle; *t.s.*, thumb-screw.

The mercury is contained in a capillary glass tube 7 cm. in length and 6 mm. in diameter with a bore of about 1 mm. Into one end of the tube is sealed the micropipette (*m.p.*) and over the other end an "inner" brass cap (*i.b.c.*), as shown in Fig. 1.

The end of this "inner" cap is covered and sealed by a thin steel disk (*s.d.*) having a central projection which inserts a short distance into an enlargement of the capillary bore. Through the center of the disk is a hole of

[1] Toldt, "Die Injection unter messbarem Drucke," *Archiv. f. Mikr. Anat.*, 1869, 5, 167, Taf. XI.

[2] Barber, M. A., "The Pipette Method in the Isolation of Single Microorganisms and in the Inoculation of Substances into Living Cells," *The Philippine Jour. Sci.*, Sec. B, Trop. Med., 9, 307.

[3] Chambers, R., "The Microvivisection Method," *Biol. Bull.*, 1918, 34, 121.

size just convenient to accommodate the entrance of the steel needle into the capillary tube. The needle, 3 cm. long and about 2/5 mm. in diameter, is soldered on to a finely-threaded thumb-screw (*t.s.*) which operates in a brass tube (*b.t.*). This tube is screwed firmly into the base of an "outer" brass cap (*o.b.c.*). In the inner end of the brass tube, the needle passes through a hole having a diameter the same as that in the steel disk. Into the "outer" cap is fit very closely a soft rubber cylinder (*r.c.*), in length one half that of the cap, through the center of which passes the needle. Inserting the needle into the hole in the steel disk, the "outer" cap is now screwed tightly on to the "inner" cap.

The device is supported and adjusted on the microscope stage by means of the Barber pipette-holder.

After the capillary tube and pipette are filled from a column of mercury contained in the rubber tubing (*rt.*), the system is then closed by the stopcock and is ready for operation. This is accomplished by regulating the thumb-screw which is threaded 60 turns to the inch. Very slight movements of it induce gradual changes of the meniscus of mercury in either direction in the tip of the micropipette (having a lumen of about five microns); these changes may be so delicate as to be almost inperceptible under a magnification of 400 diameters.

Two precautions are here worthy of note, viz., the use of glass tubing and mercury which are thoroughly clean, and the avoidance of air-bubbles anywhere within the system. To clean glass tubing, I have found the following method very effective: after sealing one end of the tube, put into it a few drops of 95 per cent. alcohol and a like amount of concentrated HNO_3. Upon adding a drop or two of H_2SO_4, an explosive reaction occurs which apparently oxidizes thoroughly any substances adhering to the surface of the glass. (The tube, of course, should be turned away from one's face before adding the H_2SO_4.) Break off the sealed end and wash the tubing well with distilled water.

To hasten the filling of the system with mercury and to remove air that may appear, it is advisable to fill nearly full the capillary tube (and add a drop of dust-free, distilled water which can be forced through the pipette point more easily than mercury) just before sealing in the pipette.

It is advantageous, also, to have the shank of the pipette fit fairly well the bore of the tube; air-bubbles are then less likely to appear in the sealing-wax between the shank and the surface of the bore.

The needle-pipette operates inside a moist chamber similar in design to that described by Chambers (*loc. cit.*). Distilled water or solutions of any sort for injection purposes may be drawn into the pipette after the mercury has been forced to the tip by turning the thumb-screw, then dipping the tip into a hanging drop of the solution and drawing a desired quantity of this into the pipette by reversing the movement of the screw. Obviously, cleansing with distilled water, which is sometimes essential, may be done in a similar way.

To extract cytoplasm or to remove a nucleus, a small amount of distilled water is drawn into the pipette, the tip then inserted into the organism and the operation completed by carefully manipulating the thumb-screw.

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THE AMERICAN PHILOSOPHICAL SOCIETY. III

SATURDAY, APRIL 24

Executive Session—9:30 o'clock

Stated Business.—Candidates for membership balloted for. Appropriations for the ensuing year passed. Annual address of the president.

Morning Session—10 o'clock

George Ellery Hale, Ph.D., Sc.D., LL.D., vice-president, in the chair

The problem of the evolution of the solar system: Ernest W. Brown, Sc.D., professor of mathematics, Yale University.

Certain aspects of recent spectroscopic observations of the gaseous nebulæ which appear to establish the relationship between them and the stars: W. H. Wright, astronomer, Lick Observatory.