

A Technique for Handling Stored Semen of Honey Bees^{1,2,3}

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ABSTRACT

A new method of handling and storing the semen of honey bees, *Apis mellifera* L., permits collection, storage, and insemination without the need to transfer semen from the insemination syringe to the storage tube and back again. A modified capillary tube serves as both an insemination tip and a storage container. When the

semen has been collected into this tube, the tube is detached from the specially designed syringe, both ends are sealed with petrolatum, and the tube is ready for storage. When semen is needed, the tube is replaced onto the syringe, the smaller petrolatum plug is forced out of the pointed end, and insemination proceeds.

The established method of handling and storing the semen of honey bees, *Apis mellifera* L., (Taber and Blum 1960, Taber 1961, Poole and Taber 1969), is to collect semen with the usual insemination syringe (Mackensen 1948, Mackensen and Tucker 1970) and transfer it to a capillary tube. About 4 syringe loads (10 μ l/load) are placed in a capillary tube, and this tube is then centrifuged, heat sealed, and stored. When needed, the tube is broken, and the semen is again drawn into the tip of the insemination syringe.

The primary purpose for designing a new method of handling bee semen was to make short term (1-10 week) storage practical. Bee semen can be stored (Taber and Blum 1960, Poole and Taber 1970), but if the procedures are not simple, short term storage is impractical. If semen could be temporarily banked, close coordination of queen and drone production would not be necessary, and matings could be planned more accurately. Storage could also permit more complete utilization of drones that are available.

The plan was to eliminate the centrifuging step and the transfers between the syringe and the storage tube. An insemination tip and syringe of new design were therefore needed. Most insemination syringes cannot be disconnected and reassembled. The syringe used by Velthuis and Sommeijer (1970) has a detachable tip, but the syringe is not capable of collecting a large volume of semen unless the operator periodically disconnects and re-attaches a modified tip.

EQUIPMENT

The new equipment has two key parts, a syringe and a tip. The tip is pictured in Fig. 1c and the syringe in Fig. 2.

The Tip.—The tips were made by heating and pulling a 75-mm Trident[®] capillary tube (ID = 1.1 - 1.2 mm). Each such tube was broken in half, and the pointed end of each of the resulting pieces was ground to an oblique point (Fig. 3a) with 400-grit silicon carbide paper and very briefly fire polished. The resulting tips are 35-50 mm long and can store about 30 μ l of semen (Fig. 1 b and c). After I had used the procedure to prepare a supply of tips, I marked them at intervals of 5 μ l and divided the sup-

ply into 4 groups on the basis of the width of the elliptical aperture as follows:

ID	OD 0.5 mm from the point
1. 0.13-0.17 mm	0.23-0.28 mm
2. 0.17-0.21 mm	0.25-0.33 mm
3. 0.21-0.25 mm	0.29-0.39 mm
4. 0.25-0.29 mm	0.34-0.43 mm

Tips in the 2nd size class were best because the inside apertures are large enough to permit a steady flow of semen, and the outside diameters are narrow enough for easy insertion of the point into the median oviduct. The Mackensen syringe tip (Fig. 1d) (Mackensen and Tucker 1970) has an ID of 0.13 mm at the point and expands rapidly beyond it; thus semen moves more readily through the Mackensen tip than through an equivalent-sized glass tip that expands more gradually. Nevertheless, I found that glass tips in the 2nd size class permit even easier passage of semen than the Mackensen tip because of the larger

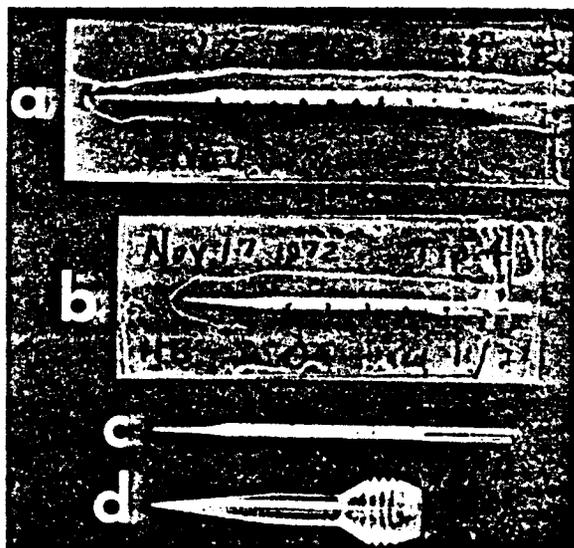


FIG. 1.—Glass and plastic insemination tips: a, glass insemination tip with a capacity of 60 μ l (filled with semen, sealed, and labeled); b, glass insemination tip with a capacity of 30 μ l (filled with semen, sealed, and labeled); c, glass insemination tip similar to b (filled with semen and sealed); d, Mackensen's insemination tip with a capacity of 10 μ l (empty).

¹ *Apis mellifera* L., Hymenoptera: Apidae.

² In cooperation with Louisiana State University.

³ Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the USDA. Received for publication July 12, 1973.

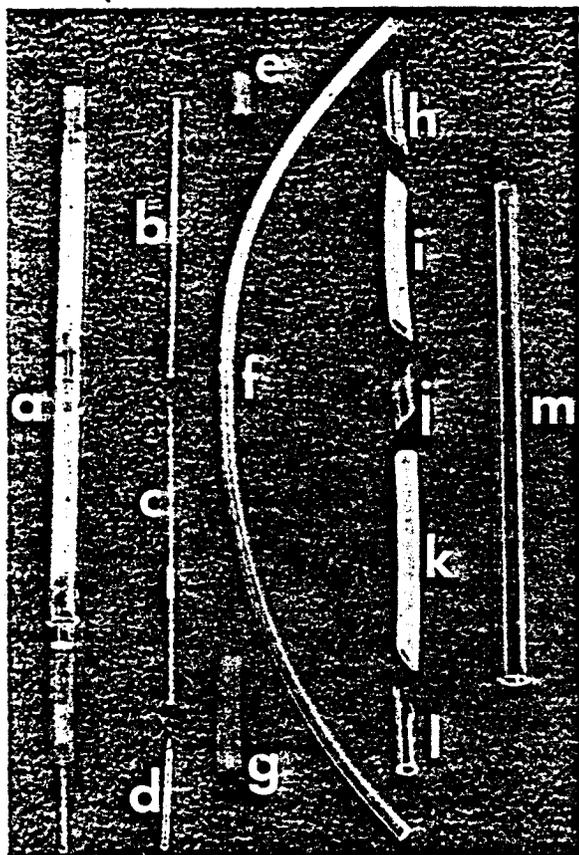


FIG. 2.—Insemination syringe and its component parts: a, the syringe; b-m, the individual pieces of the syringe. The innermost parts are on the left and the outermost on the right.

The following is a list of materials: b, c, and d—Corning® 5 μ l glass disposable micropipets; d is flame-sealed at both ends. e and g—amber latex tubing ID = $\frac{3}{64}$ in. (1.2 mm), wall = $\frac{1}{16}$ in. (1.6 mm). f—polyvinyl tubing (Tygon®), ID = $\frac{1}{16}$ in. (1.6 mm), wall = $\frac{1}{32}$ in. (0.8 mm). h, j, and l—polyvinyl tubing ID = 0.120 in. (3.05 mm), wall = 0.025 in. (0.6 mm). i and k—polypropylene tubing, OD = $\frac{3}{16}$ in. (4.76 mm), wall = 0.025 in. (0.6 mm). m—glass tubing, OD = 7 mm, wall = 1 mm.

ID. In Fig. 3, the size and shape of a glass tip are compared with that of a plastic Mackensen tip.

Actually, the size of the insemination point is really limited by the OD. Laidlaw (1944) stated that the median oviduct of a queen is ca. 0.33 mm in diameter. Mackensen's plastic tip has an OD of 0.23 mm at the point (Mackensen and Tucker 1970), and various other tips have apical OD's of 0.30, 0.30, and 0.27 mm (Van Laere in Ruttner 1969, Mackensen and Roberts 1948, Vesley in Ruttner 1969, respectively). The median oviduct is large enough to accommodate these tips, but truncate points are often difficult to insert because they are so blunt. An obliquely cut point permitted me to incorporate the easy insertion feature of a small point with the strength and smooth semen flow of a large point.

Also, I attempted to increase the storage capacity

of the tips. (The maximum storage capacity of a tip made from glass tubing with an ID of 1.1–1.2 mm is 35 μ l.) The glass tips cannot be more than 55 mm long because the distance from the queen holder to the syringe holder on the Mackensen-Roberts insemination apparatus is ca. 75 mm. Therefore, I made tips from capillary tubing having an ID of 1.4 mm. The result was a tip with a capacity of more than 60 μ l. Possibly even larger capillary tubes can be used, but with larger tubes it becomes increasingly difficult to maintain an air space in the tip between the semen and the saline solution.

The Syringe.—The new syringe (Fig. 2a) operates on a simple plunger and barrel principle. Parts 2c, d, and g make up the plunger, and 2f is the barrel. The other parts (except b and e) simply hold the barrel in position.

Although the design can be modified, there are 3 essential features:

1. The syringe must connect and disconnect to a capillary tube (a tip).
2. The syringe must provide strong suction and pressure (achieved by keeping the liquid capacity of the syringe very small and having tight fitting parts).
3. The syringe should have a mechanism that will open and close the system without disturbing the column of semen. The ability to open the system or reposition the plunger eliminates the possibility of losing suction or pressure while collecting semen or inseminating. For the syringe pictured in Fig. 2 and 4, only the plug (Fig. 2d) prevents it from being a hollow tube. Opening and closing the system, then, is simply a matter of removing and inserting this glass plug.

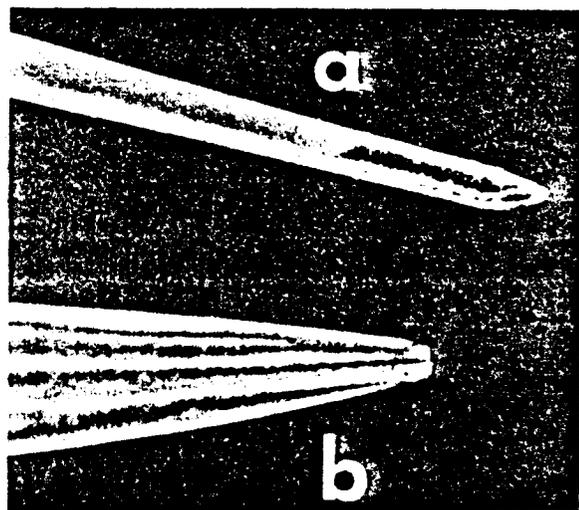


FIG. 3.—The points of the glass and plastic insemination tips: a, the diagonally cut point of a glass tip (OD = 0.27 mm, ID = 0.18 mm); b, the truncate point of a Mackensen plastic tip (OD = 0.23 mm, ID = 0.13 mm). Tip (a) contains a petrolatum plug (the clear area extending ca. 1.5 mm from the point) and semen; tip (b) is empty.

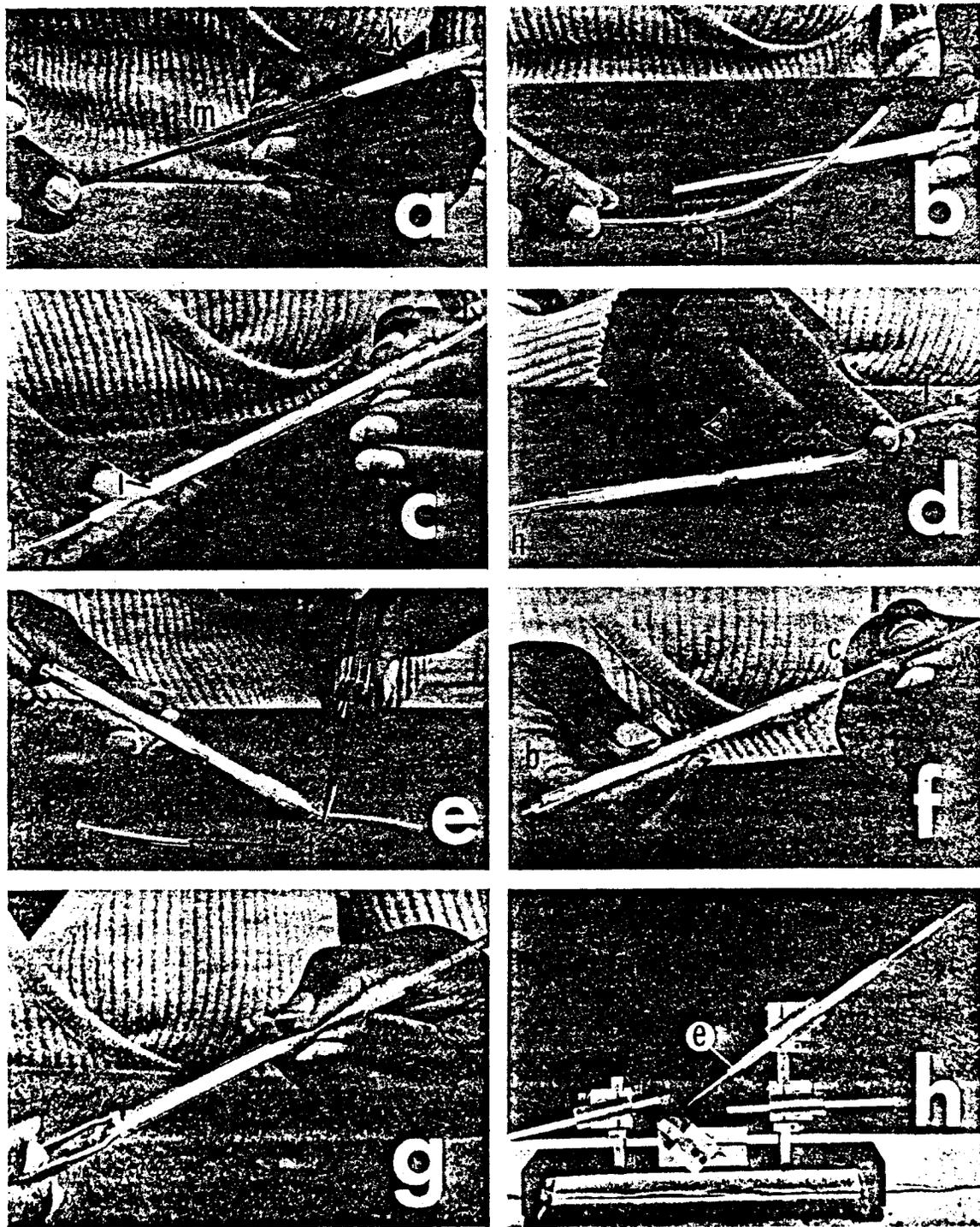


FIG. 4.—Assembling the syringe. The lettering of the parts corresponds to Fig. 2. A step-by-step method of assembly is as follows: (a) Tube *k* is poked into barrel *m* with a wire. Tube *m* was fire-polished until the aperture on the right (the plunger end) was 4.0–4.2 mm in diam. The opposite end was fire-polished too, but it must remain large enough to let the parts inside. (b) *j* is slipped over tube *f* until it reaches the approximate midpoint of *f*. (A drop of water helps *j* slide easier.) (c) Tubes *f* and *j* are positioned inside tubes *k* and *m*, and tube *i* is slid into tube *m*. (d) With tube *i* in place, *h* and *l* are slipped over tube *f*. (e) *h* and *l* are slid into place and the excess of tube *f* is trimmed at both ends. (f) Microbore capillary tubes (*b* and *c*) are then put into position. Piece *c* must be lubricated with stopcock grease. (Lubricating tube *b* is optional) (g) A band of latex tubing (the same diameter as 2*e* and *g*) is stretched over the protruding portion of tube *h*. This latex ring is not pictured in Fig. 2, but I found that it was necessary in preventing a pressure leak at that point. (h) The tip adapter (*e*) has been attached, and except for the latex ring the syringe is the same as shown in Fig. 2*a*. The syringe is positioned in a Mackensen insemination apparatus with a tip attached.

The insemination syringe (Fig. 2a) devised to be used with the interchangeable tips is made from glass and plastic tubing. The parts fit together without adhesive. Except for the layer consisting of e, f, and g, the 4 layers are arranged in rows in Fig. 2. A description of the tubing used in this syringe is detailed in Fig. 2. Fig. 4 gives assembly instructions.

PROCEDURE

The directions for using the new device are as follows:

Fill the syringe and tip with saline solution. A simple way to fill the syringe is to remove the plug (Fig. 2d) and insert the nozzle of a wash bottle containing saline solution. Fill the capillary tip separately and then attach it to the syringe. Establish a 5 μ l air space at the end of the tip to separate the saline solution from the incoming semen.

After the semen is collected, remove the tip from the syringe barrel. (The semen is collected directly from the drone's ejaculate into the tip.) Hold the base of the tip lightly against the tubing from which it has just been removed (Fig. 2e) so as to suck the semen to the extreme basal end of the tip. Remove the tip and push the basal end through petrolatum until a plug forms in the tube; allow no air between the plug and the semen. This plug is usually 4-7 mm long (see Fig. 1c). Again push the semen (followed by the plug) to the extreme point of the tip. Remove the capillary tube and create a similar, but smaller, petrolatum plug at the small end (Fig. 3a). The plug at the point is usually 1-2 mm long.

The semen is thereby sealed in the capillary tube, and no air remains between the semen and the petrolatum plugs. Sandwich the tube between 2 layers of Scotch[®] Magic Transparent tape that act as a label and give partial protection to the tip (Fig. 1a and b) and store.

When the semen is needed for insemination, remove the tube from storage, cut the tape away, and replace the tip on the syringe. The semen is still enclosed between seals of petrolatum, but these plugs are easily moved by pressure from the syringe. Collect the plug from the point on a paper towel, and proceed with insemination.

During insemination, the petrolatum plug at the basal end of the capillary tube moves directly behind the semen. It therefore acts as a barrier to separate the semen from the saline solution in the syringe. Normally, the petrolatum thickly coats the walls, and the plug is lost; however, when petrolatum follows semen, the plug remains intact. Also, the plug reduces the amount of residual semen left on the walls of the capillary tube.

After use, the tips can be cleaned and reused. An ultrasonic cleaner works well to clean the residual semen and petrolatum from the inside of the capil-

lary tubes. I stick the tips through styrofoam that floats in the cleaner. Soaking in concentrated acid also cleans them. After the tips soak in acid for a few days or are immersed a few minutes in an ultrasonic cleaner, they are rinsed, sterilized, and ready for re-use.

DISCUSSION

Recommendations of storage temperatures and semen diluents have been intentionally omitted. I selected 15°C (Poole and Taber 1970) and the streptomycin saline used by Mackensen and Tucker (1970), but work remains to be done in finding an optimum storage environment for bee spermatozoa.

The handling procedure has proved practical for short term storage, but may also be practical for long term storage and shipment. I have not checked storage beyond 5 months, and I have mailed these tips only twice. On those two occasions the tips were sealed in a 27x60 mm screw cap vial, then packed for mailing. Both shipments appeared to be in good condition when they arrived, and the one report on viability indicated that the semen had successfully inseminated queens.

As I mentioned earlier, the design of the syringe can be modified. The glass plunger is the part most vulnerable to breakage, so a metal replacement might be desirable. In a recent experiment, I filled 26 tips with semen and then inseminated 104 queens without breaking the glass plunger. Nevertheless, I have broken them. If the glass breaks on the inside of the barrel, the broken pieces must be pushed out and a new plunger inserted. One is wise to have an extra syringe in reserve, so an accidental break will not cause a serious interruption.

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