INSEMINATION APPARATUS

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Equipment used for the instrumental insemination of queen bees can be divided into three categories: (1) beekeeping equipment (cages for queens and drones), (2) some standard laboratory equipment (tank of carbon dioxide, regulator, gas flowmeter, dissecting microscope with about 10X magnification, and a microscope light, and (3) specialized equipment (insemination stand, hooks and syringe). This chapter will deal only with the specialized equipment. This equipment varies throughout the world, so two authors, one from the USA and one from the FRG, will describe the equipment used in their respective countries.

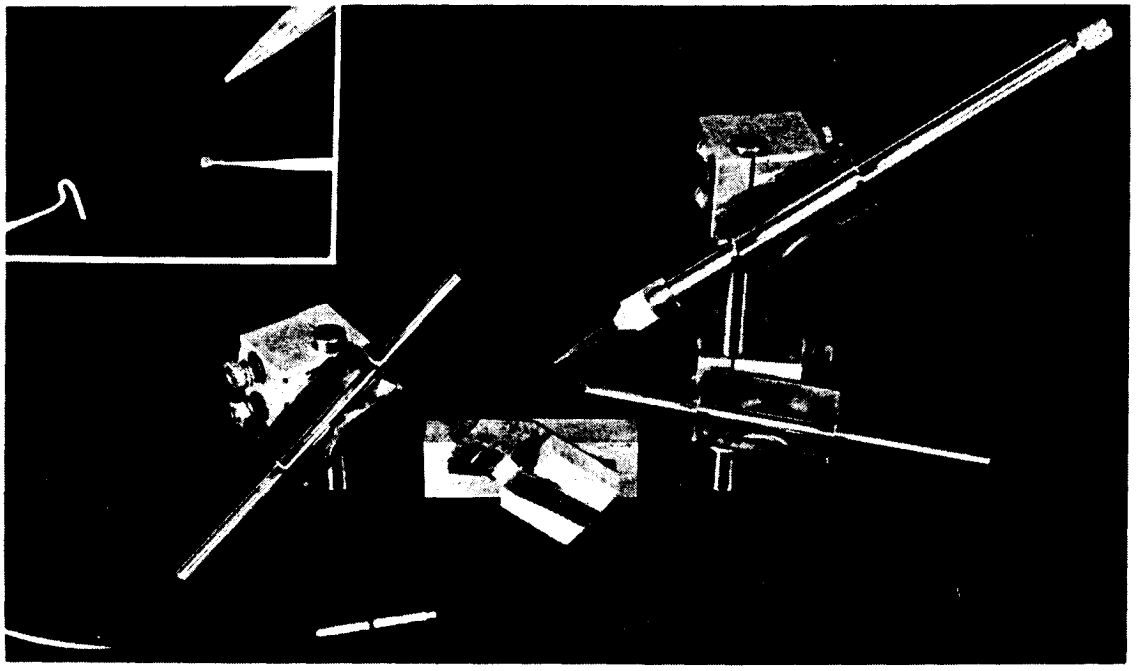

Fig. 15 — Mackensen insemination stand and syringe.
The ventral hook is on the left and the sting hook is on the post with the syringe. The posts can be reversed for left-handed use of the syringe. Upper left is an enlargement of the sting hook (top view), ventral hook (side view), and tip. The plug for the queen holder is attached to the end of the insemination hose. The mountings for the hooks and syringe are identical except for the size of rod that they will hold. These mountings allow for three types of movement: (1) a horizontal turning around the vertical post, (2) vertical turning around the horizontal axis where the mounting attaches to the box that holds the syringe or hook, and (3) in-out movement. Measurements and detailed mechanical drawings of all parts of the syringe and stand are given by Mackensen and Tucker (1970).

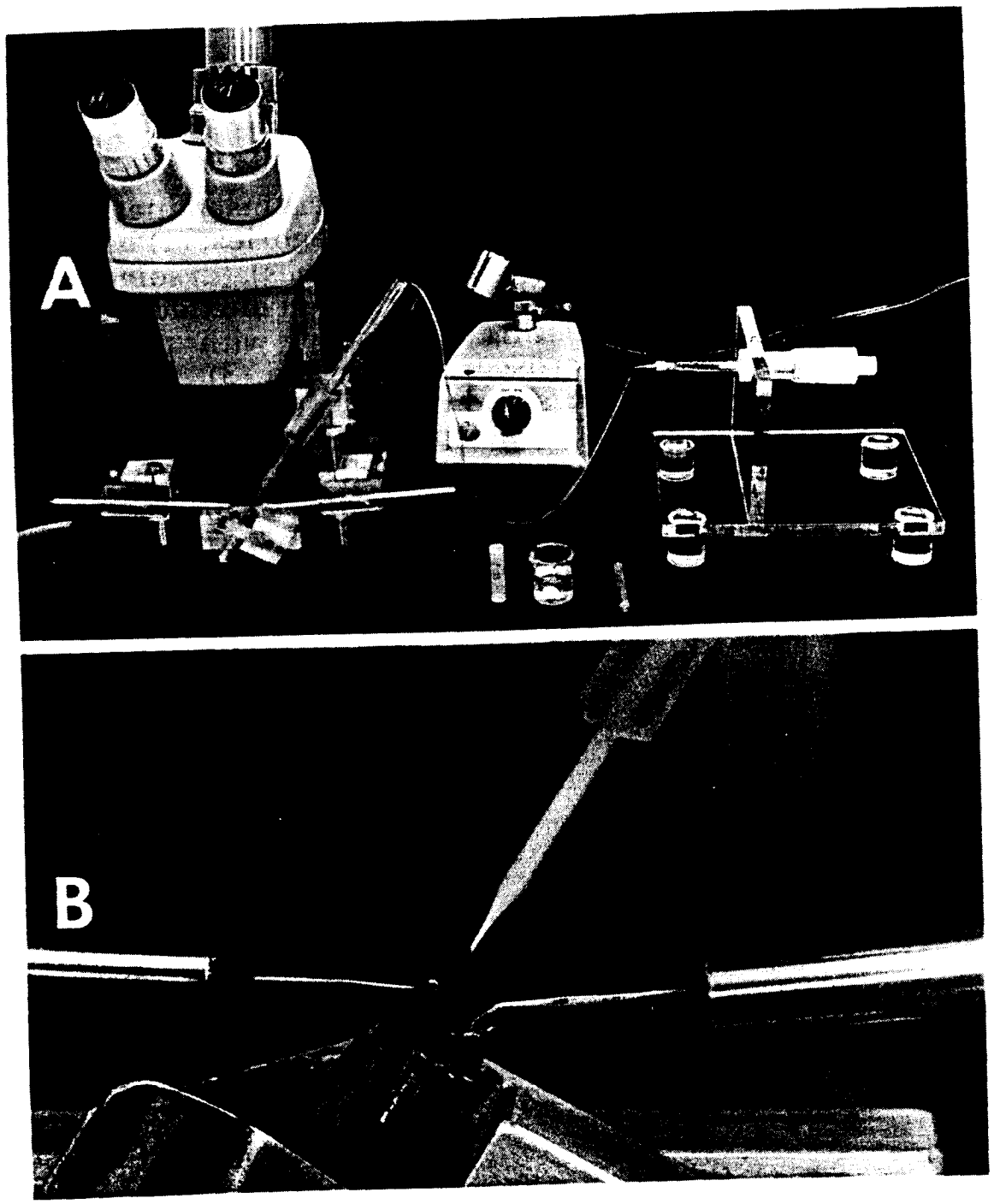

Fig. 16 — Insemination equipment in use:
(A) A Mackensen insemination stand holds the syringe shown in Fig. 3. The micrometer for the syringe (200 μl capacity) is mounted in a stand made of acrylic plastic. (B) Side view of a queen ready for insemination.

A. Equipment used in the USA

1. The stand

The MACKENSEN stand (Fig. 1) was first described by MACKENSEN and ROBERTS (1948). It was modified slightly by MACKENSEN and then redescribed in detail by MACKENSEN and TUCKER (1970). This stand is used by nearly everyone doing instrumental insemination of bees in the USA. The device aligns the holders of the ventral hook, sting hook, syringe and queen into one vertical plane. Within this plane, the operator can set the heights of the syringe and the hooks as well as the angle and horizontal placement of the queen.

The mountings for the syringe and hooks are similar and allow for horizontal and vertical rotation as well as in-out movement of the syringe and hooks (Fig. 15). The absence of gears enables the operator to feel the contact between the instruments and the queen. The mounts hold the syringe and hooks in place when the hands are removed.

Although separate pieces, the plug and queen holder (shown in Figures 1 and 2, respectively) are part of the insemination stand. After a queen backs into the acrylic holder, the plug positions the queen so that about 2 1/2 abdominal segments protrude (Fig. 16 B). Carbon dioxide gas (about 35 ml/min) passes through the plug to immobilize the queen. To keep each queen in the same position relative to the hook and syringe, one must align the end of the queen holder with the top of the block as shown in Fig. 16 B.

2. The syringe

Two types of syringes are described here, but there are many types used in the USA (MACKENSEN, 1954; KAFTANOGLU and PENG, 1980; HARBO, 1985; LAIDLAW, 1985). All can be used with the Mackensen stand.

The Mackensen syringe (Fig. 15) has been the standard syringe since 1954. It is made on a lathe and is composed of stainless steel and acrylic plastic (MACKENSEN and TUKKER 1970). The Mackensen syringe is used throughout much of the world and is the most commonly used syringe in the USA.

A syringe designed by HARBO (1985) is shown in Figs. 16 and 17. It is an hydraulic system that consists of a tip made from glass capillary tubing connected by plastic tubing to a

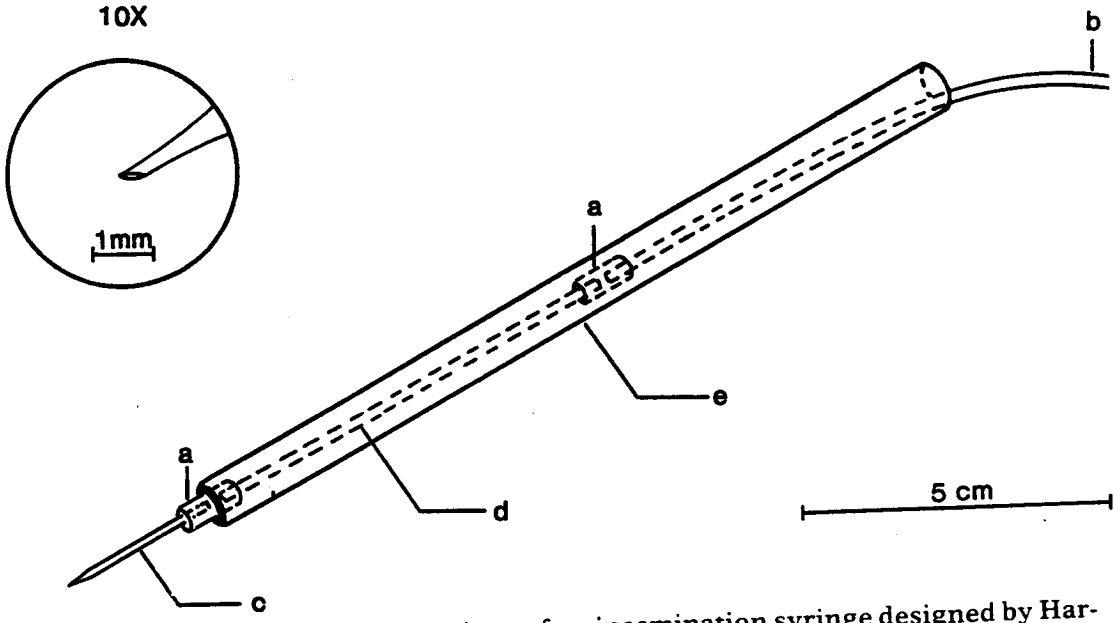

Fig. 17 — The barrel and tip portions of an insemination syringe designed by Harbo (1985)

The tip (c) is a glass capillary tube that was drawn to a point with heat and polished to an angular point (see enlargement). The inside diameter (ID) at the orifice of the point is 0.16—0.20 mm; the outside diameter (OD) at the orifice is 0.26—0.32 mm. The storage tube (d) is a glass or plastic tube (usually about 1 mm ID) that receives semen when large amounts are collected and that can be removed and stored, if desired. The syringe barrel (e) is glass tubing with an ID 5—6 mm and an OD 7—8 mm. The opening at the tip end of the barrel is reduced to a diameter of ca. 4.2 mm. This reduced orifice forms a tight fit around the latex connector that holds the tip. Thus the tip is held firmly for inseminations and yet is flexible enough to avoid breaking if bumped. The connectors (a) are sections of latex tubing ID 1.2, OD 4.5 mm). Tube b (polyvinyl tubing; ID 0.5 OD 1.5 mm) forms the hydraulic connection to the syringe micrometer shown in Fig. 2A. To assemble the parts, the storage tube, followed by tube b, is pushed into the barrel until the storage tube protrudes out the end where the tip attaches. The tip and the latex connector are attached to the storage tube, and then these parts are pushed back into the barrel until the connector fits as shown. The syringe and plastic tubes are filled with saline that has been boiled to remove dissolved air, thus keeping the hydraulic system responsive. However, the saline in the storage tube and tip and that used during insemination should not be boiled.

syringe micrometer. The system has a 200 μl capacity that can be expanded after this limit has been reached by disconnecting the tubing where it attaches to the syringe and discharging fluid from the micrometer. The syringe was originally designed for use in semen storage but is also useful when large quantities of semen are collected and mixed, or when an operator simply wants to collect all the semen before beginning the inseminations.

3. Insemination tips

The tip for the Mackensen syringe is made of acrylic plastic and has an inside diameter of 0.13 mm and an outside diameter of 0.23 mm at the point (MACKENSEN, 1954; MAKKENSEN and TUCKER, 1970). Unlike glass tips, the plastic tip enlarges rapidly from the point.

Glass tips for the syringe in Fig. 17 are described in the figure caption. The tip is filed to an oblique point (see 10X in Fig. 17) and put briefly into a flame to polish the cut surfaces

(HARBO, 1973). The angled point eases the insertion of the tip into the median oviduct and thus allows the operator to use a tip that has a relatively large outside diameter at the point.

B. INSEMINATION APPARATUSES USED IN EUROPE

The standard apparatus

When the insemination technique was introduced in Europe, the basic design of the insemination apparatus developed by MACKENSEN and ROBERTS was adopted. But the interest for easier handling resulted in many subsequent in-

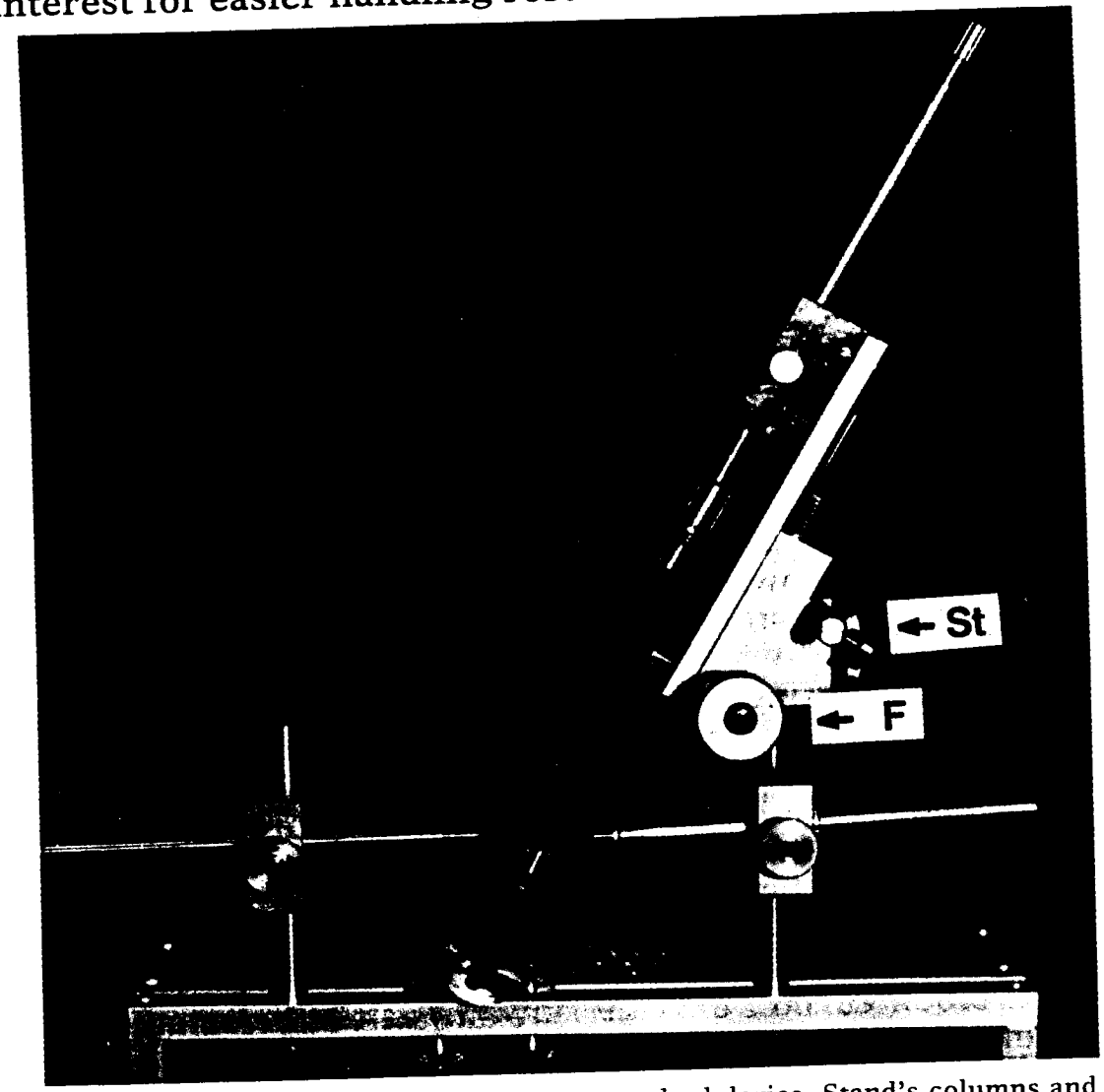

Fig. 18 — Ruttner, Schneider, Fresnaye standard device. Stand's columns and queen support are fixed to the longitudinal orifices from the foundamental plate. Their position can be changed. The handles for the ventral hook (left) and for sting's hook (right) are passed through certain balls and could be revolved anyhow. The fine F adjustment is moved through the syringe's support frontward and backward, and the St adjustment screw modifies the inclination. The syringe is provided with a larger adapter for glass points.

dividual developments. Almost all constituent parts have been substantially modified to provide for technical improvements (i.e. the queen holder devised by RUTTNER, including fine adjustments for alignment of the various parts). Special heed was paid to the introduction of the glass tips to replace the plastic tips used by Mackensen. The reason was their lower cost, but primarily providing for better hygienic conditions.

The "standard" apparatus (RUTTNER, SCHNEIDER, FRESNAYE, 1974) — brings together several of the above improvements. Since then, the standard apparatus has asserted itself for its performances many times. It will be described with its latest improvements.

The insemination apparatus (Fig. 18) consists of the following basic elements:

1. A stand with horizontal adjustment of the vertical supporters (posts) and of the queen block;
2. The queen block with the gas tube and the queen holder (Fig. 19);
3. The sting hook and the ventral hook, each of them fixed in a ball joint in the vertical support;
4. The syringe block with the rack-and-pinion drive and the adjustment screw;
5. The syringe (Mackensen type) with adapter for plastic or glass tips.

The queen holder developed by Ruttner

Figure 19 shows the position of the queen-holder in the queen block. The stopper is tightly fitted into the fixing plate. Gas supply is provided through the openings in the main component part and in the fixing plate. The queen block and the gas tube are fixed in the stand with tightening screws. The queen is first introduced into an auxiliary tube, with the same diameter as the queen holder, opened at one end only. She will walk inside the tube, and when she reaches the closed end of the tube she will go back, into the queen holder. The stopper will be pushed in before she has a chance to move forward again. When the queen holder is attached to the queen block carbon dioxide flows in at the prior adjusted. The inclination of the queen block can be afterwards adjusted, if necessary, after first loosening the nut M. For fine adjustment, the queen block can be moved or turned to the left or right, which is an important improvement as compared to the older types.

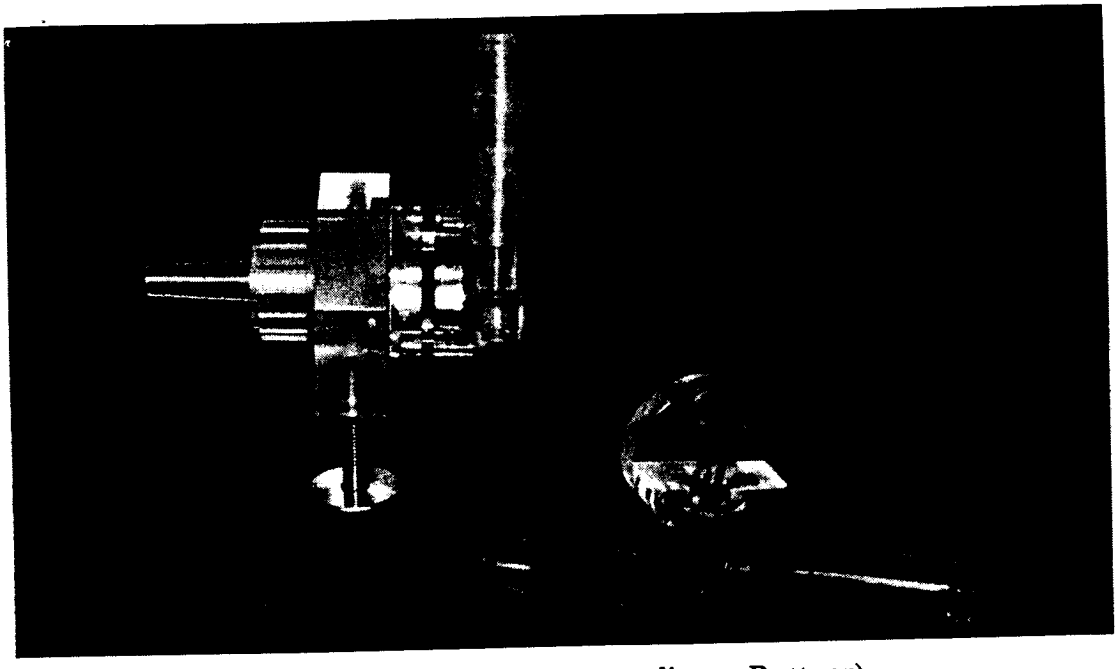

Fig. 19 — Queen's block (according to Ruttner).
Assembled on left, from lateral position. On right without stopper, having in front queen's support and entering tube.

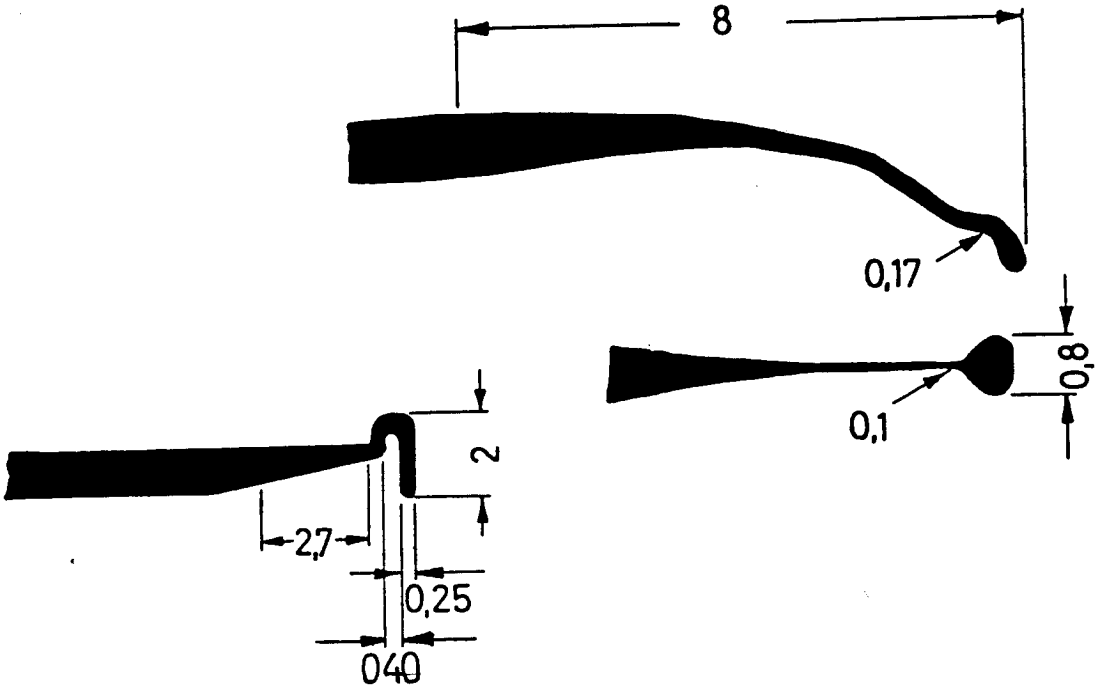

Fig. 20 — Ventral hook (left) and sting's hook (right)

Hooks and their mounting

The standard sizes of the hooks are given in Fig. 20. Particularly the sting hook must be made so accurately as to precisely fit into the sting chamber. Because its very thin neck it

may be easily broken, and must be handled with utmost care. He who cannot manufacture the hooks himself should make a sound choice among the products of several suppliers. Spare hooks must always be available.

The hooks are fitted into the handles either by plastic sockets or by soldering. The handles are mounted in ball joints and fixed into the hook holders of the two vertical supports. The tension on the ball is controlled by a tightening bolt.

SCHAFFERHANS (1987) developed a modified hook which, when in a more inclined position, provides for a more efficient opening of the sting chamber and the vagina. SCHLEY (personal communication) devised and made a ring-shaped holder which helps open the vagina even wider.

Mackensen syringe

The basic principle of the Mackensen syringe has asserted itself as the most adequate and it was therefore used also for the standard apparatus. Because the pressure of the pusher is exerted on a column of liquid with no air at all, by means of a rubber or silicon membrane, there is no mechanical play between the forward and backward motion. This is very important for an efficient collection of semen.

The adapter for glass tips devised by LAIDLAW and HAIDINGER is now made in general of metal to allow for sterilization in autoclaves. The larger adapter developped by SCHNEIDER (Fig. 18) has proved to be most efficient, enabling the drawing of approximately 70 μl semen into the larger glass capillaries. Pressure is very easily equalized so that the capillaries can be removed and shipped, stored or attached again to the syringe (publication in preparation).

Schley-plunger-syringe

In Europe, various suggestions have been made for drawing in large amounts of semen by plunger-syringes. The most adequate is that of SCHLEY (Fig. 21). The syringe can be readily removed for sterilization in the autoclave. The screwing plunger is particularly useful for semen collection. Additional facilities include a fast tensioning device and a dosimeter (for details see Schley's description, 1987).

The syringe block

The syringe block is attached to the right vertical supporter (stand post), above the hook holder. The syringe holder is a groove in which the syringe is easily and quickly introdu-

ced. It is fastened to a rack-and-pinion drive serial product of microscope mechanical stages screw enables fine adjustment of the inclination angle. When the tip is initially introduced incompletely and more inclined and is next slightly moved ventrally by means of the adjustment screw, it can be further inserted past the valvefold without using a vaginal probe (VESELY, 1967). To ease this operation, SCHLEY (1987) added another adjustment screw for parallel lateral control of the syringe movements. Another adjustment screw enables diagonal positioning, which previously could be obtained only by revolving the whole syringe block round the vertical support.

The tension of the rack-and-pinion drive of the syringe holder is adjusted so that the holder may be lightly moved and yet remain steadily in place. If it happens to slip down slowly, when the temperature is too high, the use of a harder vaseline is recommended for greasing the rack-and-pinion drive.

The distance between the insemination syringe and the queen block is relatively short in the standard apparatus so that the use of larger capillaries as syringe tips is not possible. But the distance can be slightly increased by attaching the syringe block to a third vertical support post (longer than the other two) (SCHNEIDER, publication in preparation). For this position the Mackensen syringe with a short barrel in current insemination laboratories is recommended to be used.

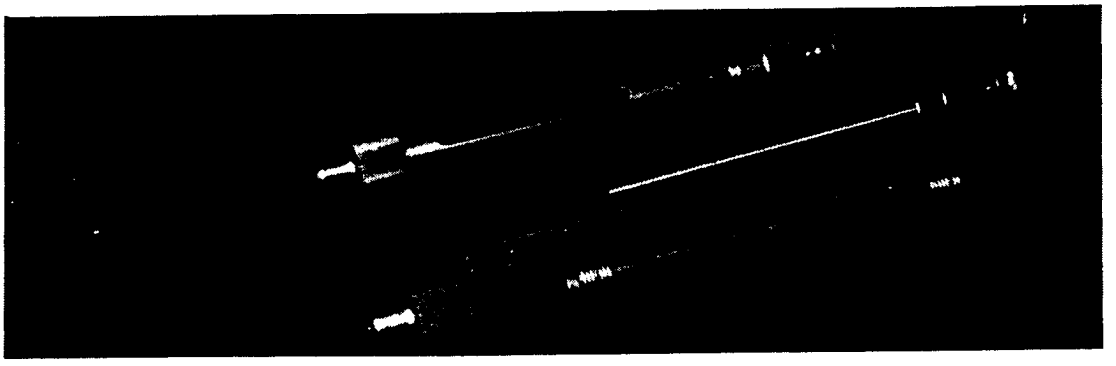

Fig. 21 — Piston syringe (according to Schley).
Assembled backward. On constituent parts frontward.

Glass tips

Glass tips are mostly used and are available on the market at fair prices. Their advantages are smooth surfaces which

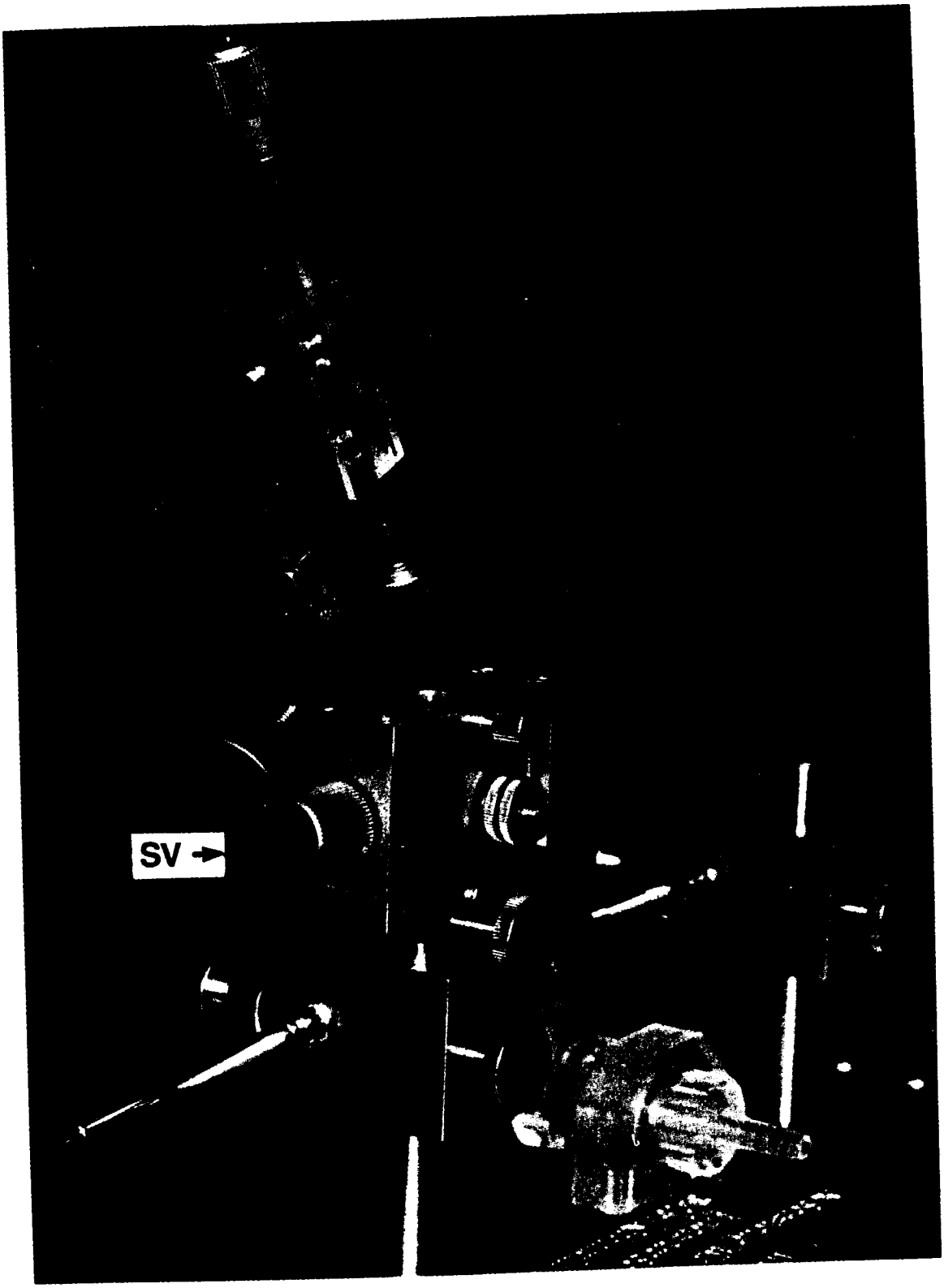

Fig. 22 — Syringe's block (according to Schley) (seen from the back)
Additional screw Sv makes the horizontal movement of the entire syringe's block easier, so that the point might be introduced without using the probe. Below is shown the ball for hooks' handles.

enable easier introduction, and efficient cleaning and sterilization.

SCHLEY (1981, a, b) devised a special apparatus for automatic stretching of the usual micropipettes into a desired tip shape which is also equipped with a device for re-sharpening the tips. The inner diameter at the end of the tip is 0.18 mm, and the outer diameter is 0.3 mm. The micropipettes of the initial, normal size may be used for storage or shipping of semen, being provided for this purpose with a cap made of a silicon or tygon tubing.

Other individually devised insemination apparatuses

In eastern Europe the apparatus devised by VESELY is widely used. A rack and pinion drive is directly attached to the syringe barrel and provides for back and forward movement.

A.and C.WINKLER (1981, 1986) integrated the insemination apparatus, the microscope and CO_2 supply into a single, portable unit. Plunger syringes are used, similar to the Schley syringes.

In Denmark, HOLM (1986) has developed an apparatus, initially devised by SWIENTY, in which the movements of hooks and syringe are controlled by micromanipulators. Finally we mention a Dutch model as representative for all original individual achievements. It is devised almost entirely with elements of the Fischer technique toy kit and works perfectly well.

Microscope, illumination and CO_2 supply

When making a choice from the very many stereoscopic microscopes available, special heed has to be paid to

— the height to which it can be adjusted during insemination,
— the magnification range,
— the field of view and
— the sharpness of spot focus.

At present, fluorescent lamps with glass fibres are preferred instead of the low-voltage lamps with heat-proof filters. As the light is projected from the end of the flexible "swan neck" it can be brought very close to the queen without requiring much space for that.

The carbon dioxide necessary for anesthetization is generally available in cylinders, the flow being adjusted by a valve. Small cylinders are easier to handle than the large ones and can be re-filled with gas from the large cylinder.

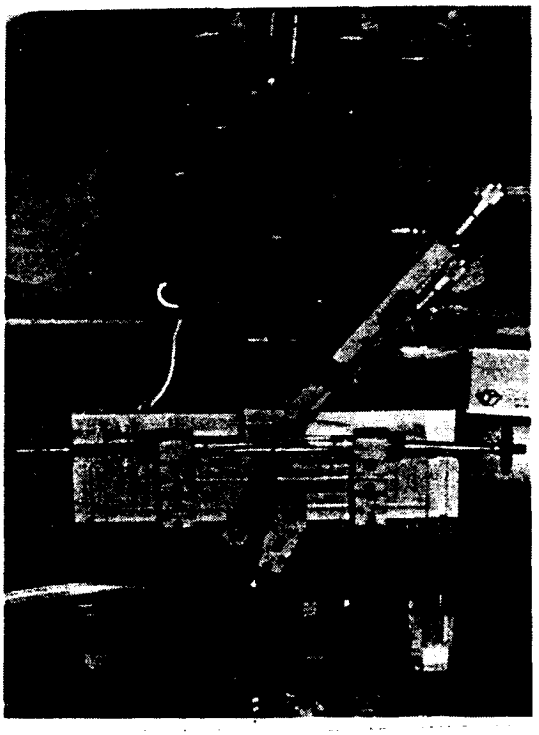

Fig. 23 — Insemination apparatus (anyone can make it), according to Fischer's technique

Addresses of suppliers of insemination apparatuses and auxiliary equipment

W. Seip, Hauptstr. 34—36, D-6 308 Butzbach-Ebersgöns; the standard apparatus with various modifications according to Schley, with plunger syringe, all component parts, and courses of instrumental insemination.

W. Uhl. Loherstr. 7, D-6 334 Aßlar; standard apparatus with Mackensen syringe (large adapter with threads in centimeters), and all auxiliary equipment and parts.

K. Burmeister, Bonebüttelar Weg 12, D-2350 Neumünster: glass tips.

Hessische Landesanstalt für Tierzucht, Abteilung für Bienenzucht, Erlenstr. 9, D-3575 Kirchhain: larger adapter for Mackensen syringes (with threads in inches); instrumental insemination courses.

Vaca Valley Apiaries G745 Buchtown Lane, Vacaville CA 95688 USA — Mackensen/Dowe apparatus, hydraulic syringe, glass tips, Schley instrument

Sweetwater Apiaries Box 449, Tylertown MI 39667 USA — Mackensen/Dowe apparatus, hydraulic syringe, glass tips

Honey Bee Genetics Box 1672 Vacaville CA 955696 USA — Modified Mackensen apparatus, glass & plastic tips with metric threads and threads in inch.