INSTRUMENTAL INSEMINATION OF QUEEN BEES

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ABOUT THE HANDBOOK

This manual is intended to introduce instrumental insemination of honey bees to beekeepers, scientists, and technical workers in apiculture who want to learn the technique. A prior knowledge of bees, bee equipment, management procedures, and queen rearing is desirable for the student. However, an untrained person can master instrumental insemination and gain adequate experience in rearing queens and drones in a relatively short time, under the guidance of one who is already experienced.

This publication brings together current information on instrumental insemination in easily readable form. This information has been gathered from the technical literature, the most important of which is listed under "Bibliography," on page 27, for those who want to study the subject in detail.

CONTROL OF MATING

The purpose of instrumental insemination in honey bees is to control matings. Since queens in nature mate away from the hive and in free flight, natural mating cannot be controlled except where one has absolute control of the drones flying in the area in which the queen mates. The flying drone population can be controlled only where complete isolation from other bees can be achieved, as on small islands and in alpine meadows. The control of mating by confinement has been tried many times without repeatable success. Queen and drones do not mate within the hive, and most attempts to get queens to mate in enclosures have failed. By instrumental insemination any desired matings may be made.

DEVELOPMENT OF INSTRUMENTAL INSEMINATION

Instrumental insemination of queen bees did not succeed with the first attempt; rather it was paced by man's understanding of the structure and function of the reproductive systems of queens and drones. The first attempts at insemination, more than 180 years ago, probably failed because the mucus of the drone's ejaculate was thought to be semen. Not until 1920 was enough known of the reproductive organs of bees to start the development of a workable technique for instrumental insemination.

The techniques now employed had their beginnings in the late 1920's and early 1930's. In these first approximations, semen from one or two drones was taken up into a fine syringe and then part of the semen injected into a queen's vagina. Typical results were some partial inseminations: queens, tardy to begin laying, that produced at least some fertilized eggs. But, occasionally, inseminations were good enough to encourage further effort.

By the late 1930's it was recognized that the semen was not placed deeply enough into the queen's reproductive tract. Obstructing further penetration is a tonguelike structure, the valvefold. When it is moved out of the way, the syringe tip can be placed beyond it, and the semen discharged into the median oviduct. Placing the semen beyond the vagina and into the oviducts led to greatly improved results from instrumental insemination, even though partial inseminations were still too frequent, and queens were usually tardy to begin to lay.

It was soon discovered, during the early

1940's, that partial inseminations could be almost eliminated by injecting a larger volume of semen. Since that time, the semen of three to 10 drones per insemination has been used with consistently good results.

The problem of tardy onset of laying was solved about the same time. It was discovered that anesthesia of queens with carbon dioxide would hasten the onset of laying, not only of inseminated queens, but also of virgin queens. This discovery fits in well with insemination technique, because carbon dioxide is used to keep the queens still during instrumental insemination.

Along with the advances in technique, the equipment used for instrumental insemination was developed and improved into the two types of apparatus now used. Early means of holding the queen still by tying her with thread to a wooden support was supplanted by queen holders of the tubular or clamp type in which the queen is also anesthetized. The technique of holding the sting chamber open and the sting from over the opening to the vagina by hand-held forceps was replaced by using hooks manipulated through friction mounts or by rack and pinion. For collecting and injecting semen, the screw type syringe with a fixed glass tip and a plunger extending into the tip has been modified into a disassemblable metal syringe with a plastic tip and a plunger extending not into the tip but pressing against a rubber diaphragm, which activates a column of saline solution within the tip. A probe, designed especially to move the valvefold, has been used since the importance of the valvefold was recognized.

STRUCTURE AND FUNCTION OF THE REPRODUCTIVE ORGANS

A knowledge of the reproductive organs of queens and drones is a basic requirement for anyone learning the technique of instrumental insemination, just as it was for those who developed the technique. The information presented here should fill this need.

The reproductive organs of bees are located in their abdomens, along with several other organs concerned with other bodily functions such as digestion and breathing.

Female Reproductive Organs

The female reproductive organs are illustrated schematically in figure 1. Figure 2 shows a posterior exterior view of the genital opening with the sting chamber opened as in preparation for insemination.

A large part of the abdomen is occupied by two *ovaries (0)*, each consisting of long parallel egg tubules. Each ovary leads posteriorly into a *lateral oviduct* (*LOD*), which has accordion walls permitting great expansion for temporary storage of semen in newly mated queens and of mature eggs in laying queens. The two oviducts join posteriorly to form a short passage, the *median oviduct* (*MOD*), which in turn leads into the *vagina* (*V*). In instrumental insemination the end of the syringe must pass through the vagina and be placed in the median oviduct and the semen discharged into the two lateral oviducts.

Above the vagina lies the *spermatheca* (*SP*), a thin-walled spherical structure about 1 mm. in diameter, which is filled with a clear liquid in virgin queens. It is covered with a network of tracheae, which gives it a white appearance. It is in the spermatheca that the sperm are stored for the life of the queen. The *spermathecal duct* (*SPD*) leads from the spermatheca to the dorsal wall of the vagina.

A tonguelike structure, the *valvefold* (*VF*), projects into the vagina from its ventral wall. This structure can act as a valve to close the opening to the median oviduct when forced anteriorly against the forward wall of the vagina. It has transverse ridges which make it recognizable at times when it is exposed during instrumental insemination.

The vagina opens through the *vaginal orifice* (*VO*) into the anterior part of the sting chamber, the *bursa copulatrix* (*BC*), which is set off from the remainder of the sting chamber by a transverse fold in the ventral wall. In figure 2 this fold is stretched to form a triangle with the sting as its base. To either side of the vaginal orifice are two openings (*BPO*) that lead into a pair of pounches, the *bursal pouches* (*BP*). These openings can be mistaken for the vaginal

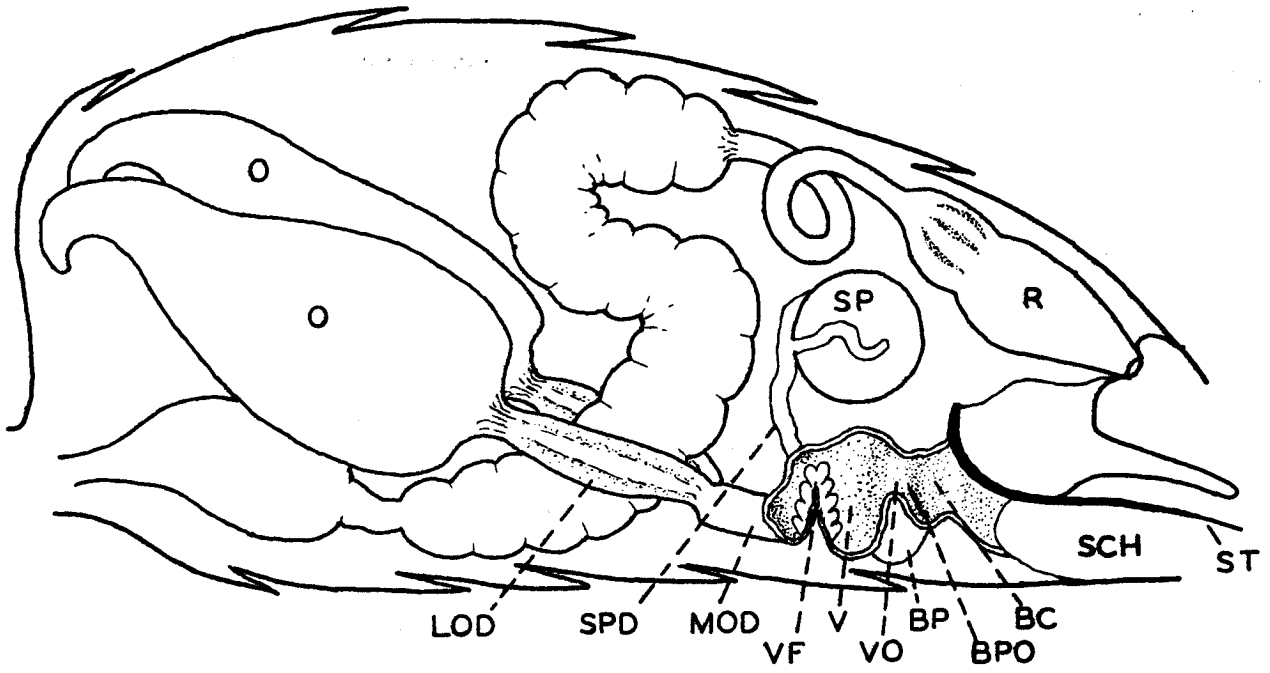

FIGURE 1.—Reproductive organs of the queen in approximately their natural position in the abdomen, with left side of vagina and bursa copulatrix cut away: *BC*, bursa copulatrix; *BP*, right bursal pouch; *BPO*, opening to bursal pouch; *LOD*, lateral oviduct; *MOD*, median oviduct; *O*, ovary; *R*, rectum; *SCH*, sting chamber; *SP*, spermatheca; *SPD*, spermathecal duct; *ST*, sting; *V*, vagina; *VF*, valvefold; *VO*, vaginal orifice.

orifice during instrumental insemination if the queen is not properly positioned.

In figure 2 the sting chamber is held open as in instrumental insemination by a *ventral hook* (*VH*) placed over the last *ventral plate* (*VP*) of the abdomenal exoskeleton and a *sting hook* (*STH*), which fits between the bases of the lancets of the *sting* (*ST*). Here the reproductive tract has been compressed, the bursa copulatrix opened wide to expose the vaginal orifice, and the vagina collapsed so that the valvefold lies immediately inside the vaginal orifice and occasionally is visible without any probing.

Male Reproductive Organs

The male reproductive system is illustrated schematically in side view in figure 3, with the right-hand members of the paired structures not shown. Its principal paired structures are *testes, vasa deferentia,* and *mucous glands.* The *ejaculatory duct* and *penis* are single structures. In a young drone the testes are very large, slimy appearing organs that occupy almost the entire upper half of the abdomen. They gradually shrink to small greenish-yellow structures in the sexually mature done. A testis consists of small tubules which empty into a chamber at the end of the vas deferens. The vas deferens has a coiled section where it joins the testis and a long enlarged section, the *seminal vesicle,* which joins and empties into the lower end of the corresponding mucous gland. The two mucous glands unite at their lower ends into the narrow ejaculatory duct which leads to the penis.

The sperm cells go through development in the tubules of the testes and then pass into the vesicles where they remain until ejaculated. In the meantime the testes shrink.

The seminal vesicles have muscular walls and are lined with secretory cells that provide nourishment for the sperm. At 3 to 4 days of age a few sperm are already in the seminal vesicles, and at 4 to 5 days there are about 5 million and this increases to 10 to 11 million at 8 days. It is very important that drones receive proper care

during this period. Sperm obtainable at any age are usable in artificial insemination; however, it is best to wait until all sperm have had a chance to mature. This is considered to be about 12 days of age. There seems to be no deterioration of sperm as aging continues.

The mucous glands also have muscular walls with secretory cells. As the drone matures sexually these glands become distended and white from the amorphous white mucus secreted into the lumen of the gland.

The penis is a soft membranous sack with a

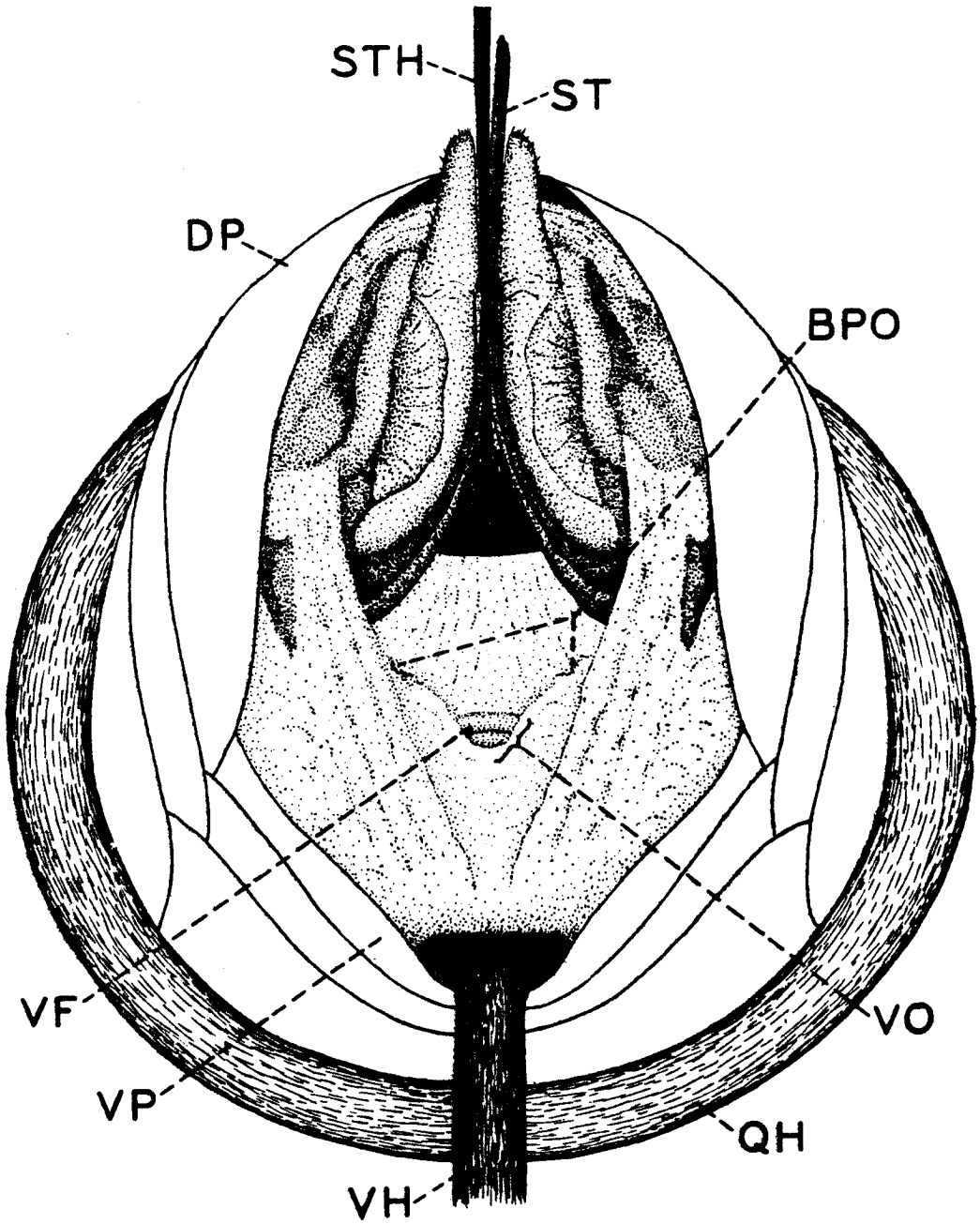

FIGURE 2.—Sting chamber of queen properly opened for insemination: *BPO*, opening of bursal pouches; *DP*, dorsal plate; *QH*, queen holder; *ST*, sting; *STH*, sting hook; *VF*, valvefold; *VH*, ventral hook; *VO*, vaginal orifice; *VP*, ventral plate.

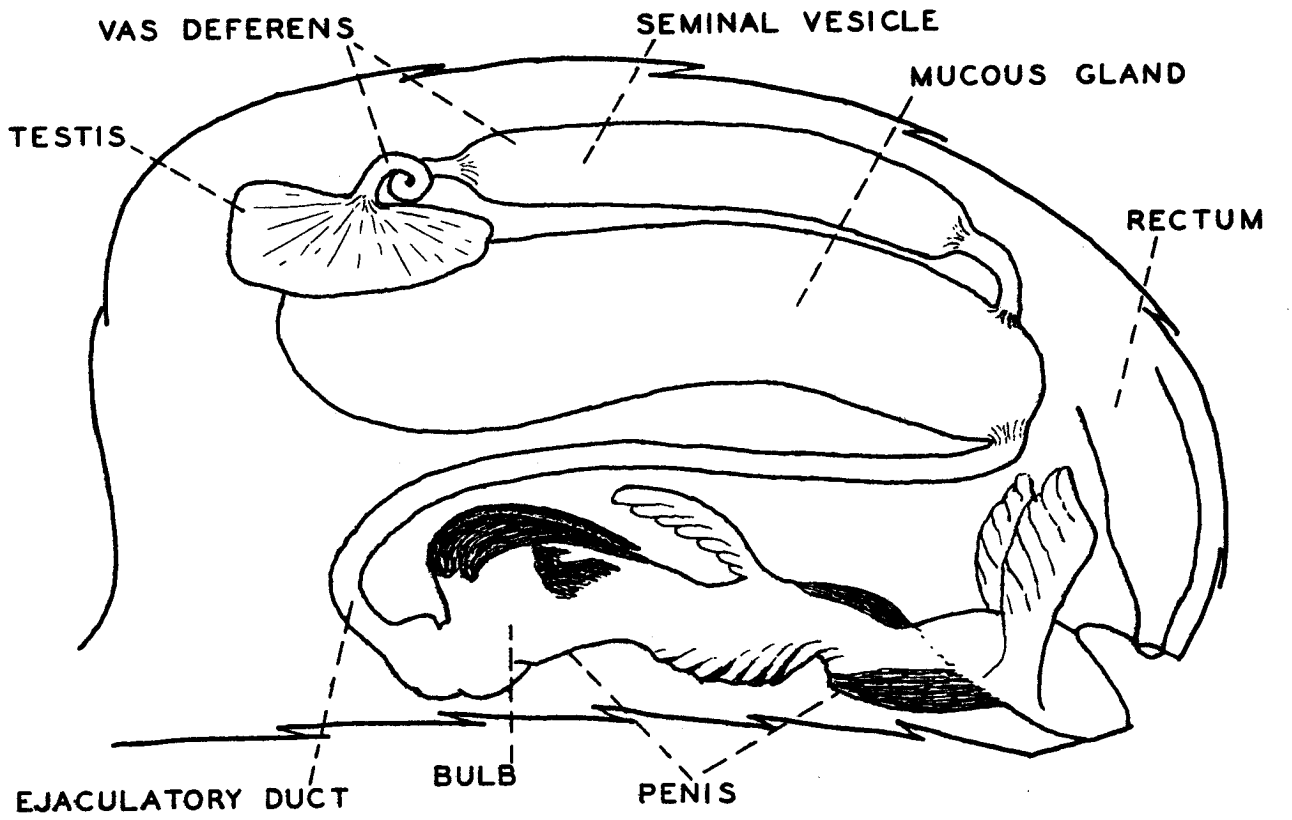

FIGURE 3.—Male reproductive organs in approximately their natural position in the abdomen. The right-hand members of the paired organs (testes, vasa deferentia, and mucous glands) are not shown.

number of bizarre processes and hairy areas. At its inner end it is enlarged into the *bulb* and provided with a pair of hard comma-shaped brown plates. The bulb is filled with a clear liquid which dilutes the sperm during ejaculation.

In mating the penis turns inside out to the outside of the body, pulling the ejaculatory duct through itself (fig. 4). This eversion is brought about by the simultaneous contraction of all the muscles of the abdomen. Ejaculation takes place during this eversion. A peristaltic contraction of the muscles of the seminal vesicles, beginning at their anterior ends, squeezes the sperm out through the ejaculatory duct; then the muscles of the mucous gland contract to push the mucus after the sperm into the bulb. These processes can be started by a number of artificial stimuli such as decapitation, pressure on the abdomen, chloroform fumes, or electric shock. When started in this way, the eversion usually stops at the stage illustrated in figure 4, *A*. With further pressure on the abdomen, which must be applied to complete the eversion, the bulb passes on through a narrow section of the penis with a jerk, the points of the comma-shaped plates appear at the very end of the everted penis, and the semen and mucus are ejected from the bulb to the outside (fig. 4, *B*). In natural mating the transfer of semen to the queen is thought to take place at this stage. Under the artificial conditions of mechanical pressure on the abdomen, the eversion often proceeds still further until the bulb and its plates are turned completely inside out (fig. 4, *C*). Sometimes the internal pressure becomes so great that the penis explodes. In the technique of inducing eversion and ejaculation artificially, the aim is to stimulate muscle contraction and not to force the action. The sperm and mucus then come out in natural sequence with minimum mixing of the two. It is impossible to obtain a normal ejaculation by mechanical means alone.

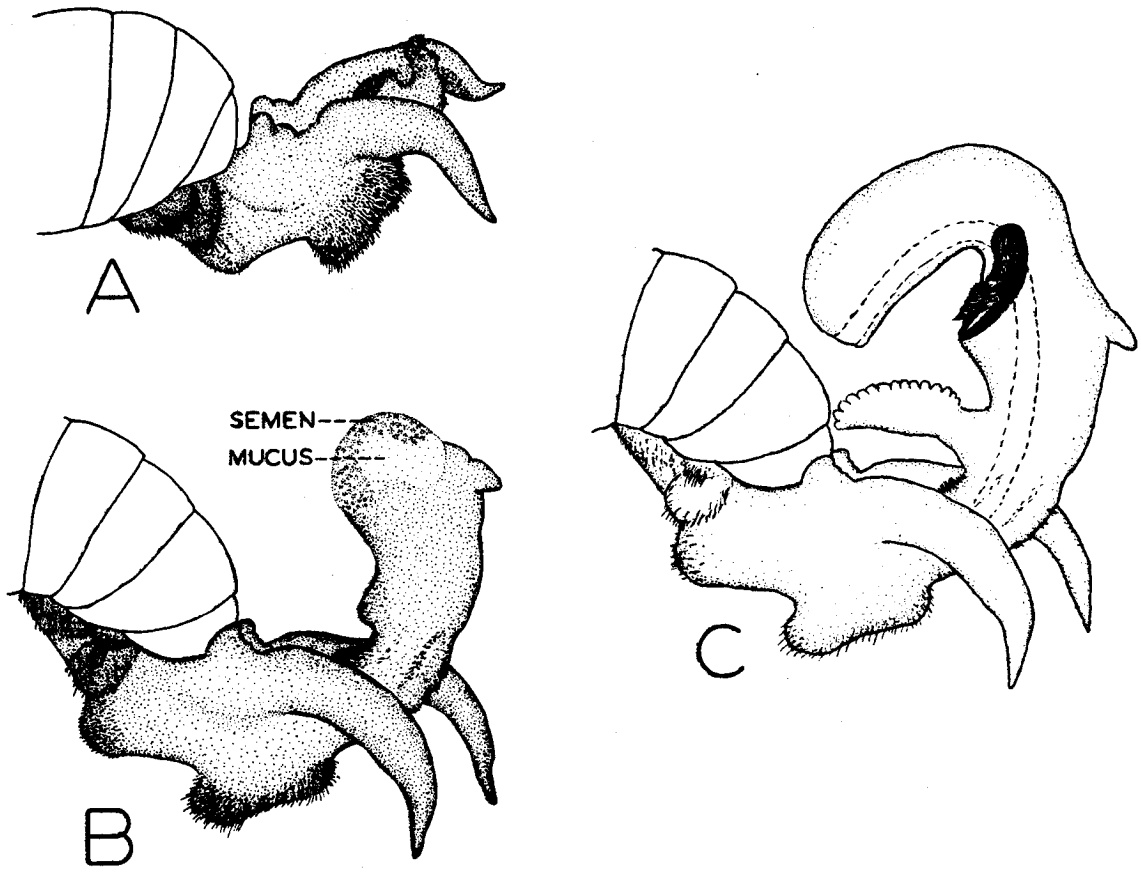

FIGURE 4.—Stages of the eversion of the drone's penis: *A*, Partial eversion usually encountered after initial stimulation; *B*, a more complete eversion usually obtained by squeezing the abdomen, with semen and mucus exposed; and *C*, a fully everted penis (semen and mucus not shown).

The semen is made up of the sperm plus liquids from the seminal vesicles and the bulb. The sperm are in bundles, giving the semen a cloudy or mottled appearance. This appearance together with its cream color makes semen easily distinguishable from the homogeneous snow-white mucus. The semen becomes darker and contains more sperm as the drone ages; the best quality semen of dark-cream color, not mixed with mucus, is obtained from drones at least 12 days old. The quality does not seem to deteriorate with further aging.

NATURAL MATING

The natural act of copulation has been difficult to study, but considerable information has been obtained by examining queens immediately after their return from the mating flight. The queen evidently cooperates actively by opening the sting chamber and lowering the valvefold voluntarily. The end of the penis enters the bursa copulatrix but not the vagina. Since the queen is not cooperating during artificial insemination, it may be necessary to lower the valvefold with a probe, to facilitate placement of the syringe tip in the median oviduct.

When the queen returns to the hive after mating, usually both oviducts are distended with semen, but often unequally. The anterior part of the vagina also contains semen, and some sperm have already reached the spermatheca. The bulb of the penis is found in the sting chamber or bursa copulatrix buried in mucus which also ex-

tends into the vagina, or the bulb may be absent.

During the course of the next 6 to 7 hours most of the sperm reach the spermatheca through the vagina and spermathecal duct, but some may remain in the oviducts 24 hours, especially after artificial insemination. How this translocation takes place is not yet perfectly understood. It has long been thought to be a process of active migration; however, while it is taking place, the queen frequently contracts her abdomen and little strings of dried semen are eliminated. From this evidence it has been assumed that the sperm is forced into the spermatheca during these contractions while the valvefold serves as an imperfect seal of the vaginal orifice. Perhaps both processes play a part. Whatever the procedure, it is as effective after instrumental insemination as after natural mating.

Naturally mated queens, on return from mating flights, contain an average of 11.6 microliters (μl.) of semen in their oviducts (maximum 28 μl.). From this it is estimated that queens mate with eight to nine drones on the average (maximum: 17). If the first mating does not satisfy, the queen will mate a second or even a third time.

EQUIPMENT FOR INSTRUMENTAL INSEMINATION

The major equipment needed to perform the insemination operation is illustrated in figure 5. It consists of the *manipulating apparatus* (fig. 6) to which the *queen holder* (*QH*), *ventral hook* (*VH*), and *sting hook* (*STH*) and *syringe* (*S*) are attached in movable fashion, a cylinder of carbon dioxide for use as an anesthetic, a stereomicroscope, and a source of light. A jar in which queens are given additional treatments with carbon dioxide is also shown.

Microscope and Light

The microscope should be of the stereoscopic type with a wide field. The most desirable magnifications will vary with the operator. An experienced operator may prefer to use a single magnification of 10 to 15 diameters for both the collection of semen and insertion of the syringe. A beginner may wish to have also a higher magnification of about 20 diameters available for the insertion; the experienced operator might find 20 diameters useful with difficult queens. The experienced operator may be able to use an even lower magnification (6 to 7 diameters) so that the two operations can be performed under a single field without moving the microscope.

Some type of illumination is essential. A microscope lamp with a concentrated beam of adjustable brightness is best, but any lamp that gives sufficient light to satisfy the operator is satisfactory. The beam should be large enough to cover both the semen collection and injection operations without moving the lamp, or the lamp should be attached to the microscope so the point of focus will always remain within the beam.

Carbon Dioxide Equipment

Carbon dioxide serves as anesthetic. It is obtainable in cylinders under high pressure which must be reduced to a few pounds per square inch by a reduction valve. If taken directly from the cylinder the gas will alternately freeze and thaw causing an irregular flow of gas. A needle valve permits adjustment of the flow to a very fine stream. A rubber tube carries the gas to the queen holder and another tube leads to a container in which queens are given additional anesthetizations.

Queen Holder

The queen holder (fig. 7) consists of an outer transparent plastic tube 1½ inches long and an inner stopper (also a tube) of the same length to which the carbon dioxide supply tube is attached. The stopper is made of two parts that screw together permitting the insertion of a felt washer. This washer provides friction to keep the stopper in place and prevents leakage of carbon dioxide back around the stopper. The friction is adjustable to a limited extent. Screwing the parts together more tightly tends to increase the diameter of the felt washer thus increasing the friction with the tube. The queen is induced to back into this tube and when her abdomen begins to protrude she is secured with the stopper. The carbon dioxide flows through

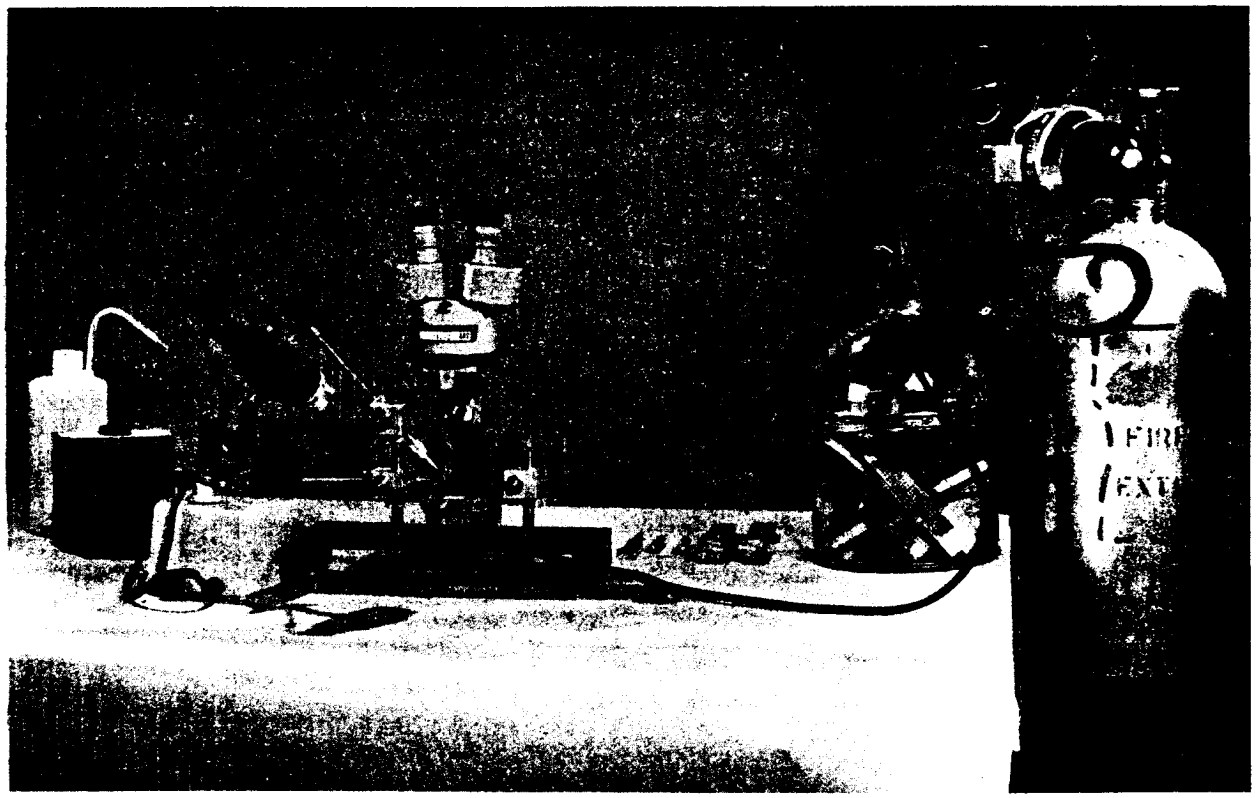

FIGURE 5.—Complete insemination equipment, showing manipulating apparatus under microscope with queen in place ready for injection of semen, jar for giving additional carbon dioxide treatments, and carbon dioxide cylinder with pressure regulator attached.

the hole in the stopper and bathes the queen as it passes out of the queen holder.

Syringe

Various types of syringes have been used during the development of the insemination technique. The Mackensen syringe (fig. 7) is the one most commonly used today. The barrel and plunger are made of stainless steel. The plunger is constructed in two parts so that when the screw plunger turns, the diaphragm plunger does not turn nor bore into the diaphragm. The diaphragm is made of rubber one-sixteenth inch thick. The tip and adapter are made of clear plastic. At its point the tip has an inside diameter of 0.005 inch and an outside diameter of 0.009 inch. This is about as small as the outside diameter can be made and still permit an inside diameter large enough for reasonably easy semen collection. Just inside the point, the inside diameter gradually enlarges for a distance of five thirty-seconds inch and then continues at a uniform bore of 0.031 inch. The tip is graduated in microliters and has a capacity of 10. With the 5/16-inch base, still larger capacities can be obtained by increasing the diameter of the uniform bore.

In operation the tip and adapter socket are filled with physiological salt solution, the tip is screwed into the adapter against the rubber diaphragm, and the adapter is then screwed into the syringe barrel socket. When the screw plunger is advanced, it causes the diaphragm plunger to push the diaphragm into the cavity at the base of the tip thus forcing the saline solution out. The semen is then taken in by retracting the screw plunger. Thus, the saline solution acts as a liquid plunger.

Originally the piece between the tip and barrel was the same diameter as the barrel and was

simply an extension that could be omitted. When the tip was screwed directly into the barrel a 1/2-inch-long diaphragm plunger was used. When it was found that the old style tips with 1/4-inch-diameter base did not have the capacity desired by modern workers, the base was enlarged to five-sixteenths of an inch and the connecting piece correspondingly. Thus, this piece became an adapter.

The adapter is a great convenience when sterilization is required as in a series of individual matings or to prevent the spread of disease. Several adapters and tips can be prepared, used one by one, and all cleaned and sterilized at the same time.

Sting Hook and Ventral Hook

Two hooks are used to hold the sting chamber open and the sting out of the way during the operation. Each hook is secured to a long handle, which slides within its hook holder. The critical parts of these instruments are shown enlarged in figure 8. The ventral hook is mounted to the left of the operator and is little more than a bent wire that hooks over the ventral plate. The sting hook is mounted to the right of the operator. It has an enlargement at the end which fits into the triangular area between the sting lancets and extends underneath them (fig. 2). It is used to pull the sting dorsally exposing the vaginal orifice. These hooks may vary considerably in dimensions and shape, and different workers in instrumental insemination have their preferences. One should be prepared to make these hooks out of spring temper brass wire. Suitable tools for this purpose are number six cut files of round, oval, and flat shapes, obtainable from any jeweler.

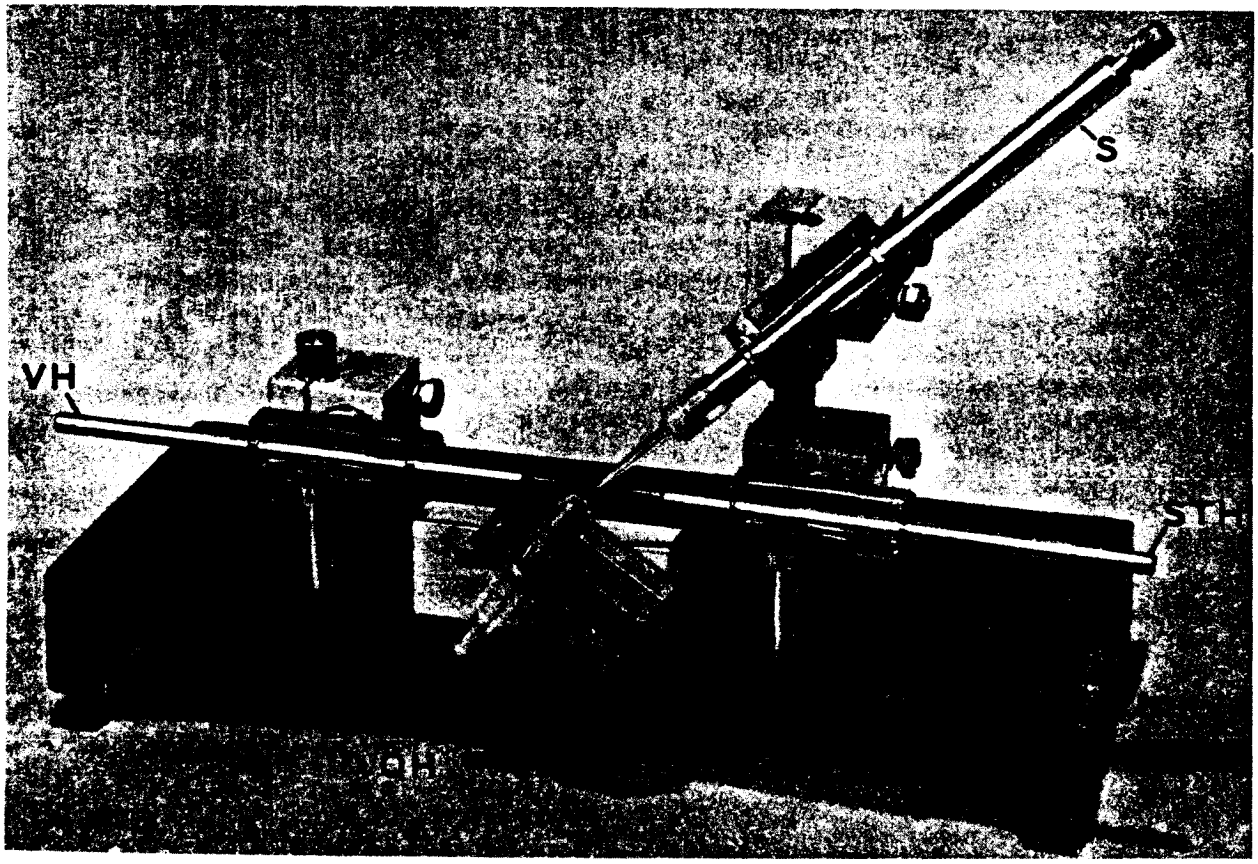

FIGURE 6.—Manipulating apparatus viewed from operator's side: *QH*, queen holder; *S*, syringe; *STH*, sting hook; and *VH*, ventral hook.

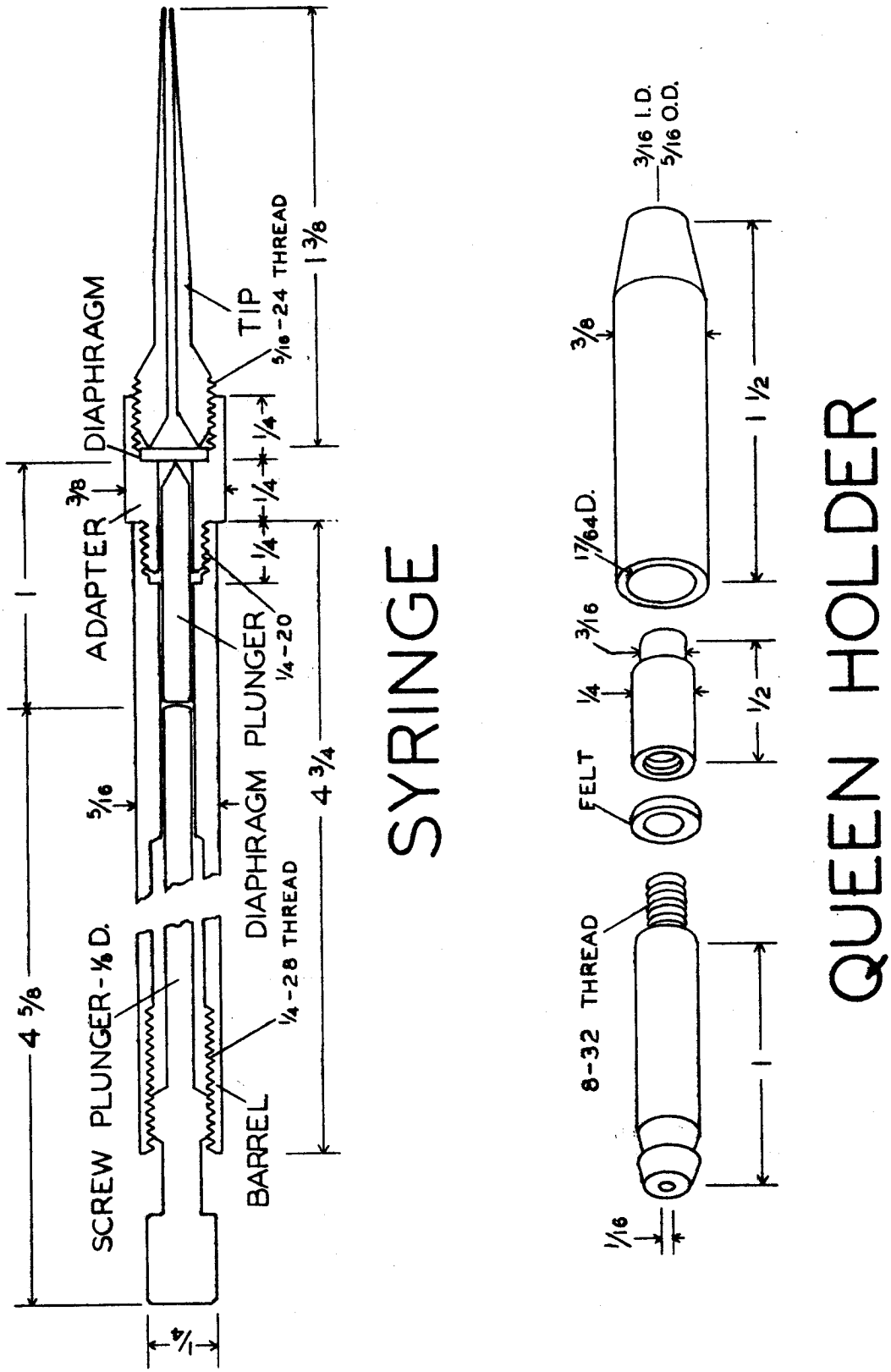

FIGURE 7.—Structural details of syringe and queen holder. Dimensions are in inches.

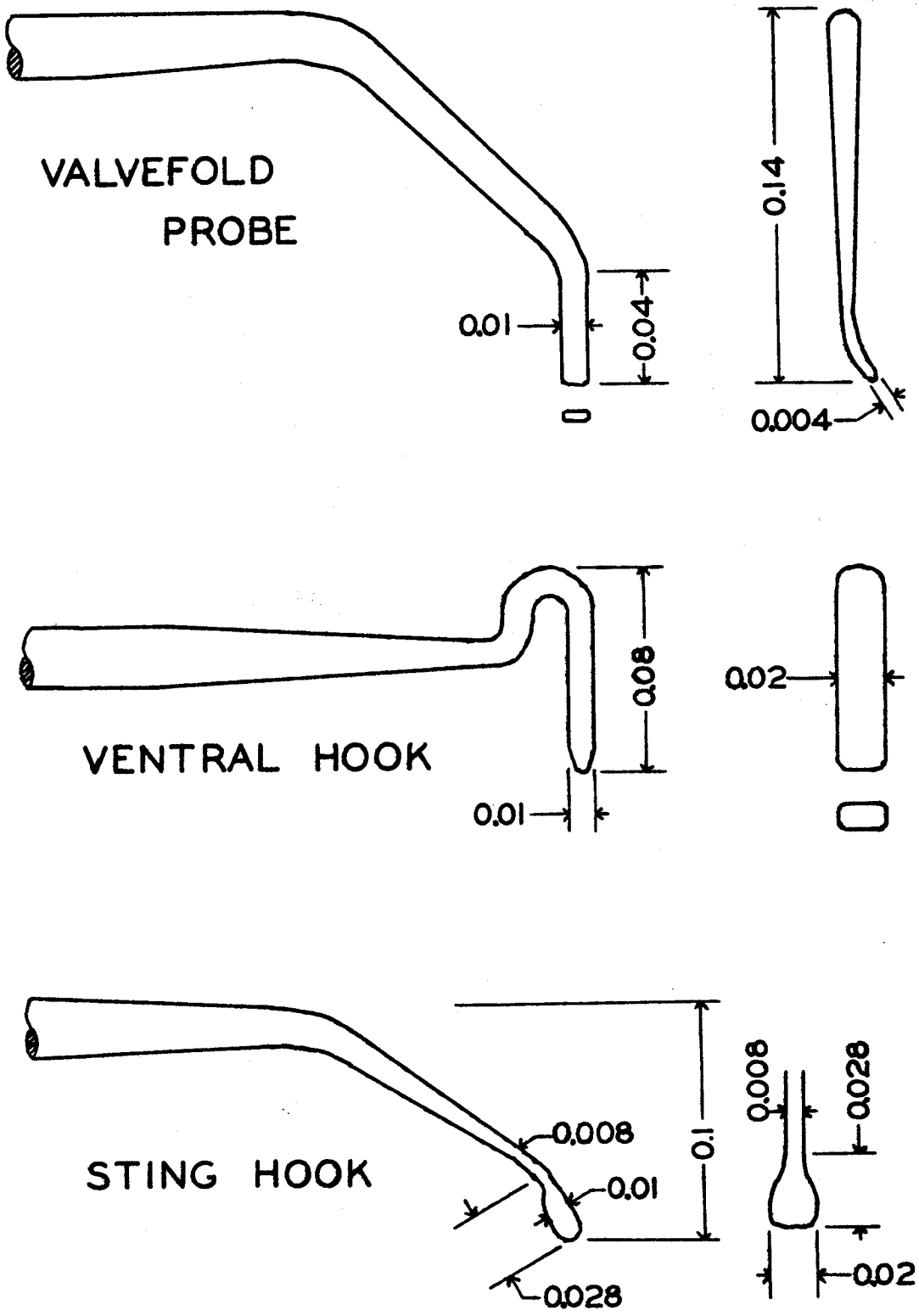

FIGURE 8.—Structural details of valvefold probe, ventral hook, and sting hook. Dimensions are in inches.