

exclusively worker brood in the worker cells. At the end of September only queens no. 9 and 43 were still producing only worker brood and these were the queens which were inseminated with more than 3 mm<sup>3</sup> semen.

### Conclusion

It is not difficult to inject the semen into oviducts of *A.c. indica* queens. Two smaller doses are preferable over one big dose. Therefore two or three inseminations with 3 mm<sup>3</sup> of semen each, are recommended. To obtain this amount of semen, one needs to collect semen from about 40—60 drones, and to provide this number from which the semen can be collected, 100—150 *A.c. indica* drones must be killed.

### LITERATURE CITED

- BÄHRMANN (R. (1961). — Über den Bau des Begattungsschlauches der vier Apis-Arten. *Leipzig. Bienenztg.* 75 (1) : 73—90.
- KAPIL R. P. (1962a). — Anatomy and histology of the male reproductive system of *Apis indica* Fab. (Apidae Hymenoptera). *Insectes soc.* 9 (1) : 73—90.
- KAPIL R. P. (1962b). — Anatomy and histology of the female reproductive system of *Apis indica* Fab. (Hymenoptera, Apidae). *Insectes soc.* 9 (2) : 145—163.
- RUTTNER F., WOYKE J. and KOENIGER, N. (1972). — Reproduction in *Apis cerana* 1. Mating behaviour. *J. apic. Res.* 11 (3) : 141—146.
- (1973). — Reproduction in *Apis cerana* 2. Mating behaviour. *J. apic. Res.* 12 (1) : 21—34.
- SIMPSON I. (1960). — Male genitalia of *Apis* species. *Nature, Lond.* 185 (4705) : 56 only.
- SHARMA P. L. (1960). — Observation on the swarming and mating habits of the Indian honeybee. *Bee Wld.* 41 (5) : 121—125.

### HANDLING STORED SEMEN OF HONEY BEES

E

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In 1960 Taber and Blum demonstrated that honey bee semen can be stored *in vitro*. Although the storage of bee semen is not yet as successful as that of bovine semen, POOLE and TABER (1970) were able to produce offspring from honey bee semen that had been stored *in vitro* for 8 months. In this paper I am proposing a simplified technique for handling stored bee semen.

In the established method of handling and storing honey bee semen (TABER and BLUM 1960, TABER 1961, POOLE and TABER 1969), the semen is collected in 10 µliter syringe loads with the usual insemination syringe (MACKENSEN 1956). About four syringe loads are placed into 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.

My plan was to eliminate the centrifuging step and the transfers between the syringe and the storage tube. This is accomplished by modifying a capillary tube to serve 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 (Fig. 1) (no air is allowed between the petrolatum plugs and the semen). When semen is needed, the tube is placed onto the syringe, the petrolatum plug at the distal end is forced out, and insemination proceeds.

### Equipment

Two pieces of equipment are needed: (1) a syringe that will deliver good suction and pressure (Fig. 2) and (2) insemination tips which can be easily detached from the syringe (Figs. 3 and 4).

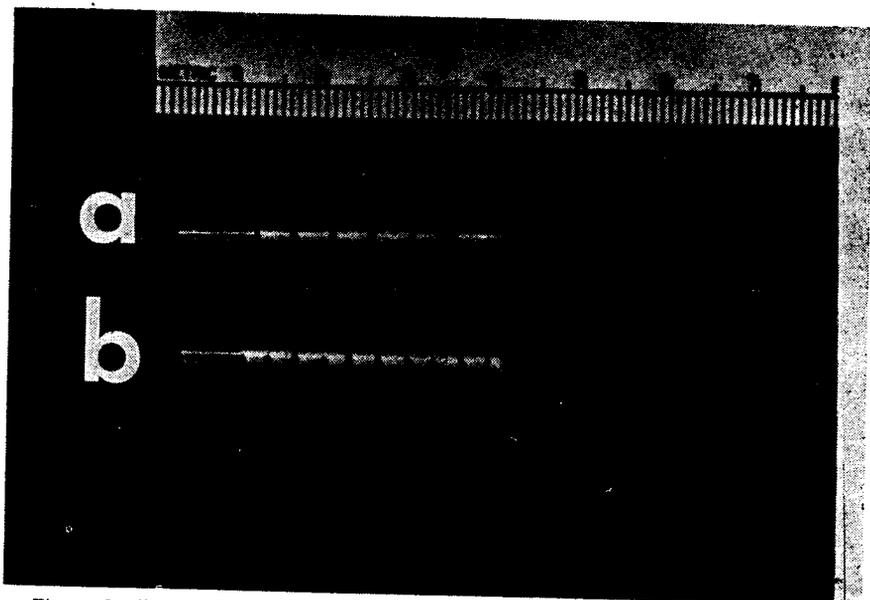


Fig. 1. Capillary tubes (insemination tip barrels) filled with semen and sealed with petrolatum plugs. The tubes are marked at  $5 \mu\text{liter}$  intervals. For semen collection and insemination it is necessary to attach a small adapter point to tube b (See Fig. 4d).

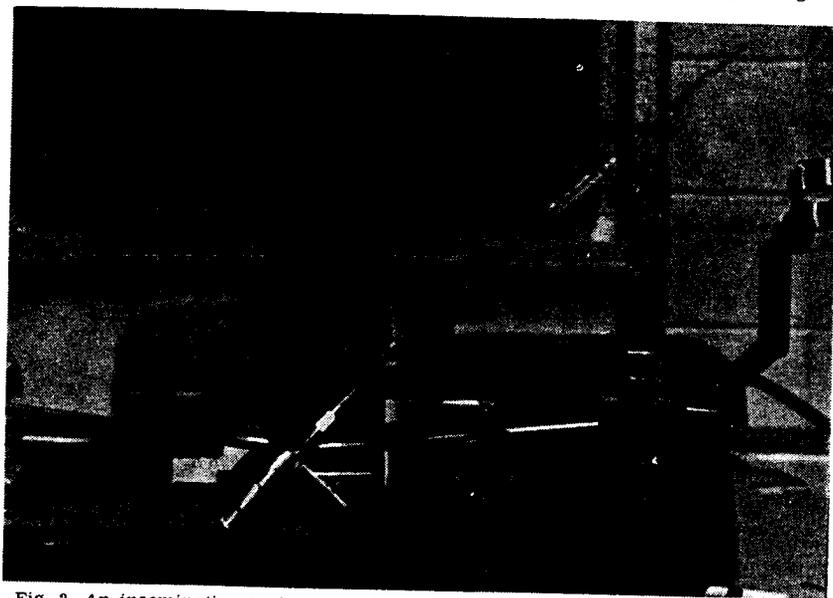
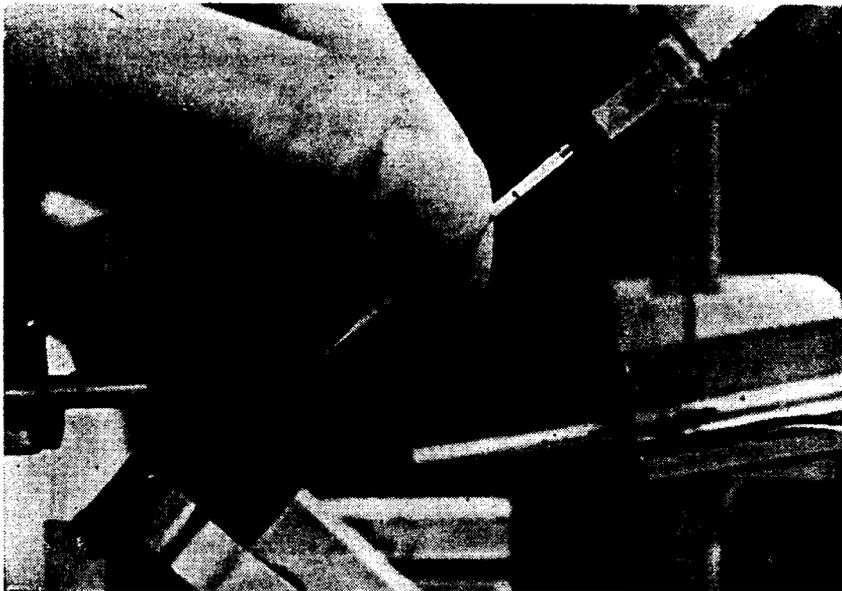
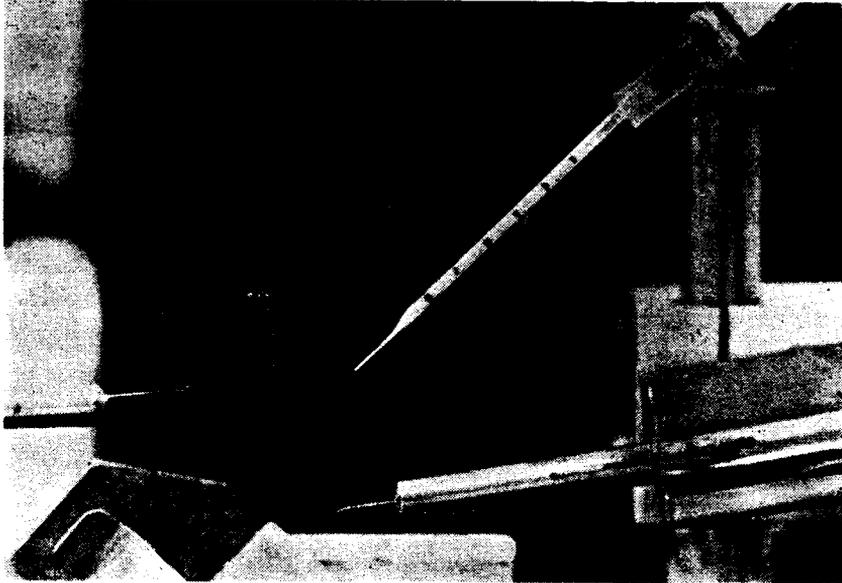


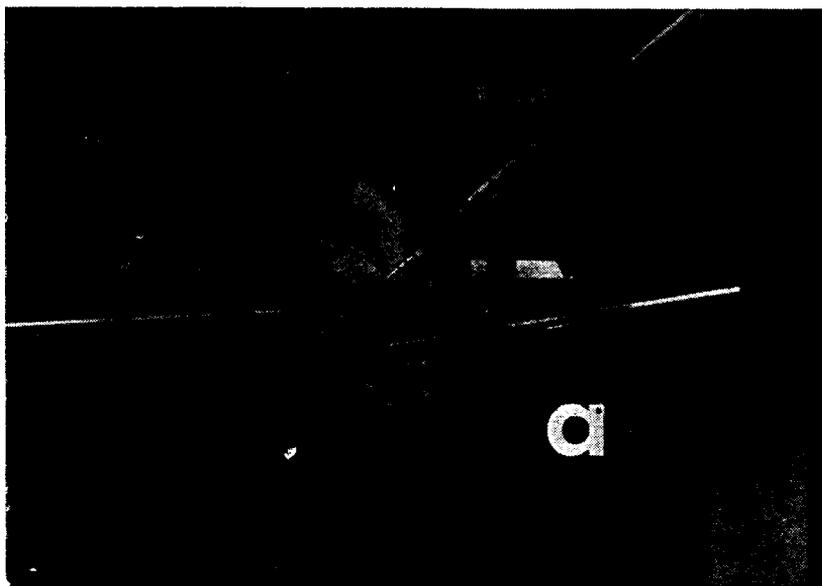
Fig. 2. An insemination syringe positioned in a Mackensen insemination apparatus. The syringe is constructed of a glass and plastic tubing. Specifications for the syringe are included in a paper that has been submitted to the Ann. Entomol. Soc. Am.

The only feature of the modified capillary tube that is not evident in Figs. 1 or 3 is the obliquely cut point. An oblique point enabled me to incorporate the easy insertion feature of a small point with the strength and smooth semen flow of a large point.

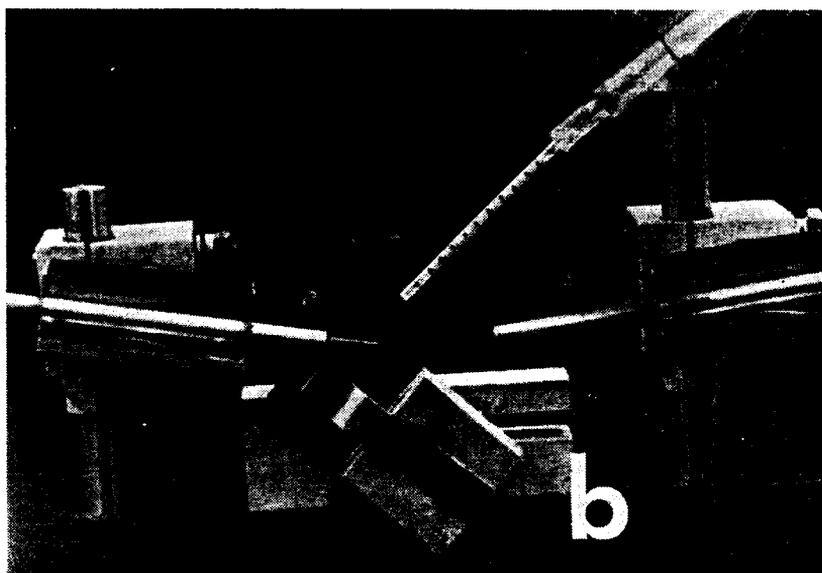


*Fig. 3. Steps for using semen that has been stored in a modified insemination tip. The petrolatum plug at the distal end is much smaller than if the storage tube had no point, but it is removed in the same manner (see Fig. 4c).*

The semen is stored either in the insemination tip or in a tip that has no point (Fig. 1). The fact that the small tube in Fig. 1 has the insemination point and the large tube does not, is not significant. Insemination points can be made on both 1.1 mm and 1.4 mm (ID) capillary tubing.



*Fig. 4a*



*Fig. 4b*

### **Methods**

Since I wanted to test the technique rather than the storage conditions, I used storage conditions that were similar to those with which POOLE and TABER (1970) had been successful. However, unlike POOLE and TABER, I diluted the semen with saline, making the

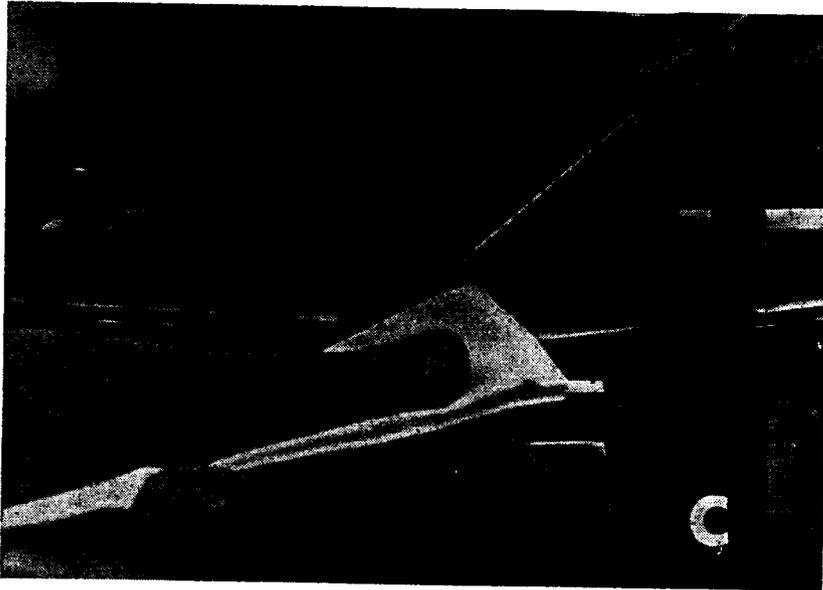


Fig. 4c

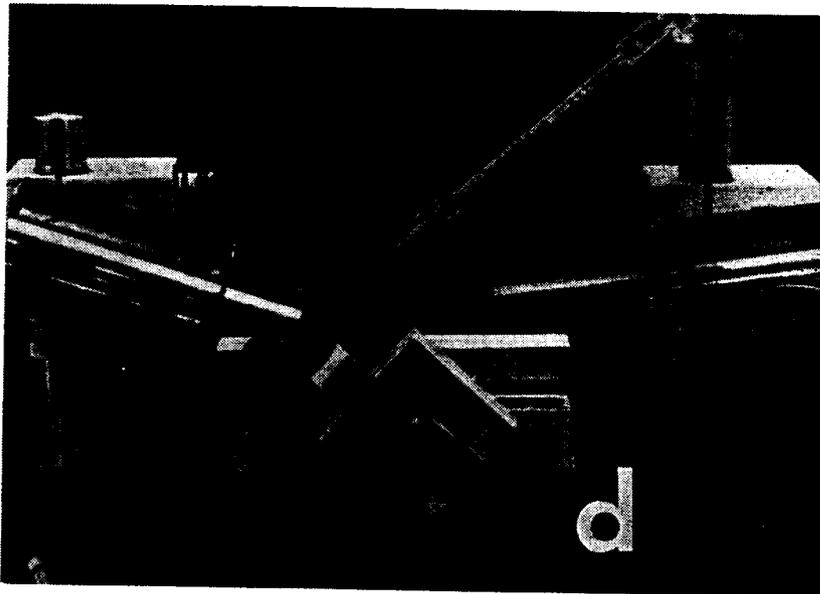


Fig. 4d

Fig. 4 a, b, c, d — Steps for using semen that is stored in a capillary tube. a and b — the tip is attached to the syringe ; c — the petrolatum plug is forced out and collected on a paper towel ; d — the point is attached and the semen is moved into the point, ready for insemination. (The petrolatum plug at the proximal end is easily moved by the syringe, and thus remains in the tube during insemination)

storage mixture about 85% semen and 15% saline (the saline was 0.85% NaCl and 0.25% dihydrostreptomycin sulfate). A storage temperature of  $14 \pm 1^\circ\text{C}$  was used.

The semen handling procedures are illustrated in Figs. 3 and 4.

### Results and discussion

The performance of 26 queens that were inseminated with stored semen is detailed in Table 1.

Of special interest are the queens that were inseminated with 150 day old sperm. Whereas the sperm in tube 6 did not successfully fertilize any queens, four queens produced worker

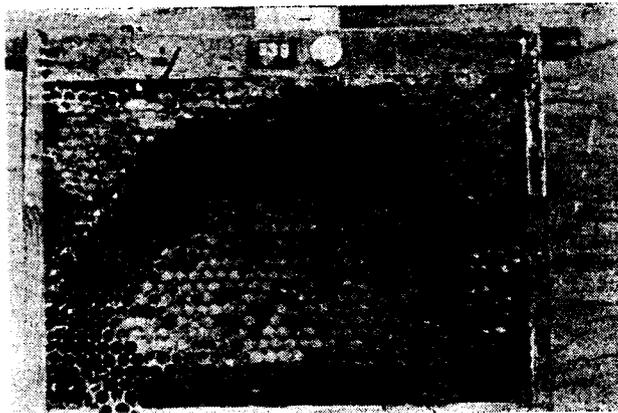


Fig. 5. The brood pattern of a queen which was inseminated with semen that had been stored for 150 days

offspring from the sperm in tube 7. One of these four produced only scattered worker brood, but the other three exhibited a solid brood pattern (Fig. 5).

When inseminating with stored semen I did not have problems with the plugging of the insemination point. On occasion, of course, the insemination point did become plugged,

Table 1  
PERFORMANCE OF QUEENS INSEMINATED WITH 6-7  $\mu$ LITERS OF STORED SEMEN

Tube No.	No. of days in storage	No. of laying ♀ produced	Date of initial oviposition	Evaluation (July 1973)
1	1	3	April 1973	None lost <sup>1)</sup>
2	3	5	April 1973	1 died (May 1973)
3	5	2	April 1973	None lost
4	25	6	June 1973	2 died (late June)
5	50	4	Nov. 1972	3 failing (Apr. 1973) <sup>2)</sup>
6	150	2	April 1973	Neither produced worker brood
7	150	4	April 1973	3 produced normal brood (1 was superseded July 1973)

1) Unless stated otherwise, all the surviving queens are producing normal worker brood.  
2) The good queen had 2.1 million spermatozoa in her spermatheca on April 9.

but the frequency of a plugged point didn't seem to be any greater than when fresh semen was used.

In combination with our present knowledge of sperm storage, this technique may enable one to employ short term (2—3 week) sperm storage, in a practical bee breeding program. In fact, we have begun to incorporate short term sperm storage in the Stock Center's breeding program to a limited extent. It has already proven itself as a convenience, and if the queens continue to perform well, we will increase our use of stored sperm.

My results also suggest that this handling technique may be practical for long term as well as short term sperm storage. I am not suggesting that research in sperm storage be restricted to capillary tubes with petrolatum seals, but successful long term storage may be "simply" a matter of finding a good additive, diluent, and or treatment to use in combination with an optimum storage temperature.

#### L I T E R A T U R E C I T E D

- MACKENSEN, O. 1956. — Some improvements in method and syringe design in artificial insemination of queen bees. *J. Econ. Ent.* 47 (5) : 765—768.
- POOLE, H. K. and S. TABER 1969. — A method of *in vitro* storage of honey bee semen. *Amer. Bee J.* 109 (11) : 420—421.
- POOLE, H. K. and S. TABER 1970. — *In vitro* preservation of honey bee semen enhanced by storage at 13—15°C. *Ann. Entomol. Soc. Entomol. Soc. Amer.* 63 (6) : 1673—74.
- TABER, S. and M. S. BLUM, 1960. — Preservation of honey bee semen. *Science* 131 (3415) : 1734—35.

### CHANGES IN THE MEIOSIS OF UNFERTILIZED BEE EGGS UNDER THE INFLUENCE OF TEMPERATURE

R

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The cytologic system governing diploid parthenogenesis, consists in most cases of the inhibition of one of the maturation segmentations. It is also this system which acts when the development of the unfertilized egg is stimulated artificially. In silk worms whose eggs are laid in the metaphase of the first maturation segmentation, high temperatures discontinue the development process. In amphibians, who lay eggs in the metaphase of the second stage, high temperature hampers the second maturation division. Instead of the four haploid derivatives, resulting usually from two maturation divisions, two diploid derivatives emerge.

Our experiment was intended artificially to stimulate the development of diploid parthenogenesis in the bee egg. By using the effect of temperature we influenced the maturation division and obtained two branches instead of four. Such a deviation was recorded in more than 60 cases.

After raising the temperature, changes were recorded as taking place in eggs of various ages, which makes us expect a diploid nucleus to form, and subsequently also diploid larva. This will be the object of our investigation in the future.