The Effect of Tracheal Interruption on the Spermathecal Wall of the Queen Honey Bee (36219)

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The honey bee queen, after mating with several drones within 2 weeks after emergence (1), can produce fertilized eggs for 2–4 years (2). The single spermatheca within which she stores spermatozoa after mating is an apparently simple sphere about 1.14 mm in diameter; its wall is a single layer of tall columnar epithelial cells; its lumen is lined with a thick mucinous cuticle; and its exterior surface is covered with an extensive tracheal network (3). It is attached to the common oviduct by a single spermathecal duct (2). The average spermatheca after mating contains 4.7 million spermatozoa in its 0.779 mm^3 volume (4).

The extent of the tracheal network has led to a widely held *a priori* assumption that its principal function is to supply oxygen to the spermatozoa within the spermatheca. For example, Koeniger (5) surgically removed 50–70% of the tracheal network from spermathecas of mated queens and found that they began laying unfertilized eggs within 14 days after tracheectomy. She did not examine her specimens histologically; but instead assumed that the infertility resulted from interruption of the oxygen supply to the spermatozoa.

The present report describes and discusses the effects of tracheectomy and ligation on the structure of the spermatheca itself.

Materials and Methods. All queens in these experiments had mated previously and were laying eggs in colonies. A few days before the operation, they were brought into the laboratory and placed in queen storage cages, which also contained attendant worker bees. [The workers received water and a 50% (w/v) aqueous sucrose solution with 26 mg of fumagillin and 88 mg of oxytetracycline–HCl added/liter of solution to prevent nosema disease and general septicemia, which can cause high mortality in caged bees.] The queens were separated from their worker attendants by wire mesh partitions which allowed only contact by mouthparts while feeding. They were removed from these cages for the operation and subsequently returned there to await autopsy at a later date.

Koeniger's surgical procedure (5) was used to expose and manipulate each spermatheca. Briefly, this involves restraining the queen under CO_2 anesthesia, cutting, and retracting a flap in the next to last tergite, and applying direct vertical pressure around the wound to move the spermatheca into the opening. A drop of 0.85% saline solution (also contained 30 mg/liter of penicillin G and 100 mg/liter of streptomycin sulfate) was placed over the spermatheca to prevent drying and minimize infection. Next, either part of the tracheal network was removed from the surface of the spermatheca, or a fine silk ligature was slipped around and under the spermatheca so that air movement to and from the tracheal network via its tracheal supply trunks was blocked. The ligature also constricted the spermathecal duct. Closure was accomplished by releasing the depressor, returning the flap to its normal position, and sealing the incision with wax.

Four types of spermathecas were produced by the operation: Group A, 14 control spermathecas from queens killed 4–22 days after sham operations (an incision but no tracheectomy or ligation); Group B, 14 spermathecas from queens killed 2–6 days after about 30% of the tracheal network was removed; Group C, 8 spermathecas from queens killed 8–11 days after about 10% of the tracheal network was removed; and Group D, 11 spermathecas from queens killed 2–16 days after ligation.

Each queen was killed within 3 weeks after her operation, her abdomen was carefully dis-

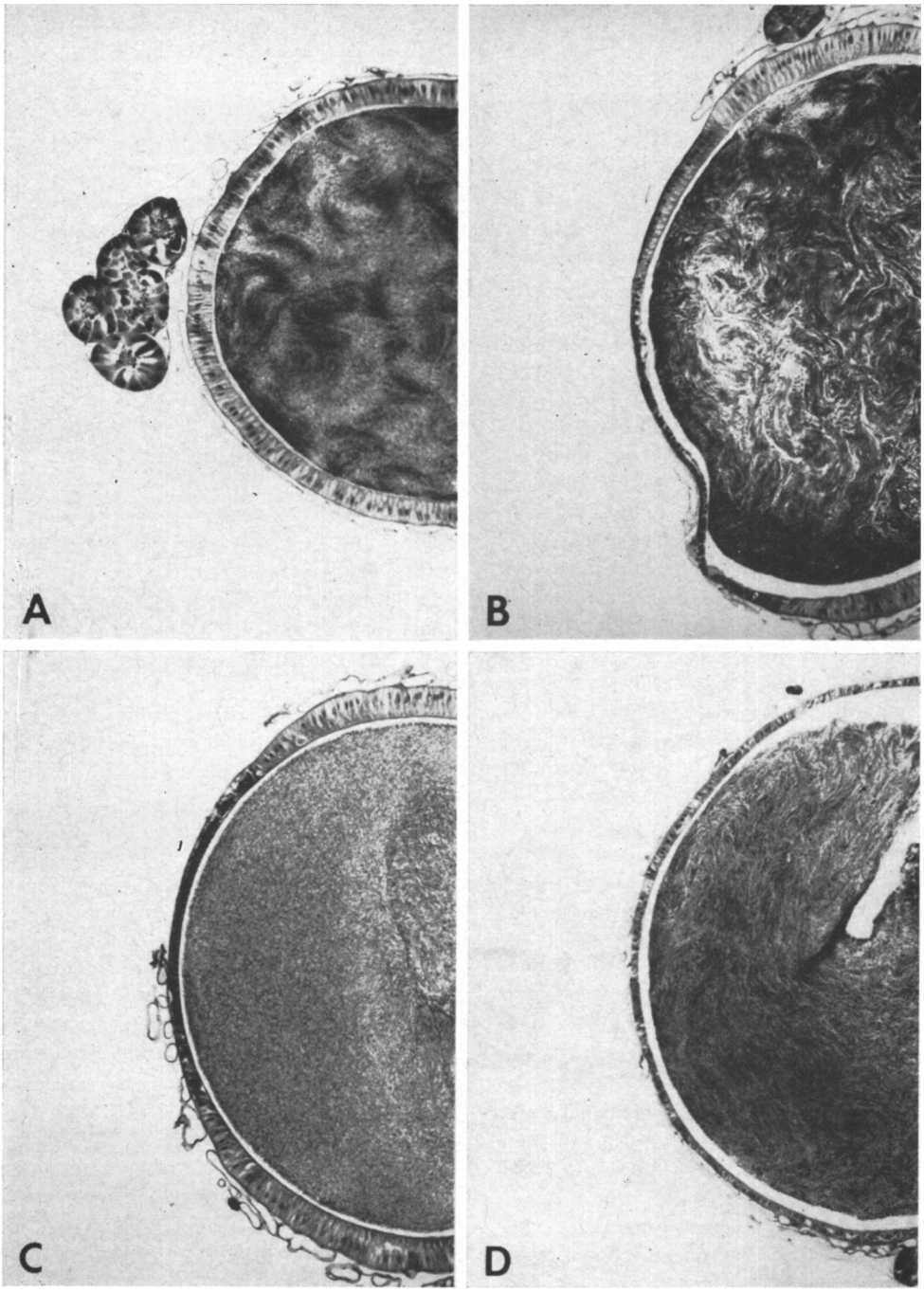

FIG. 1. Spermathecas from mated queen honey bees sectioned at 7 μ and stained with hematoxylin–eosin; 73×: (A) spermatheca 4 days after sham operation; (B) spermatheca 5 days after about 30% of tracheae was removed; (C) spermatheca 8 days after about 10% of tracheae was removed; (D) spermatheca 15 days after tracheal ligation. Note epithelial degeneration in all but the sham-operated control.

sected, and her spermatheca was removed and fixed in Bouin's fluid. All spermathecas were paraffin embedded, serially sectioned, stained with hematoxylin and eosin, and examined microscopically.

Results and Discussion. The spermatheca of each control queen (Group A) was indistinguishable from that of a normal unoperated queen (4) (Fig. 1A). The epithelial wall of each was composed of laterally compressed, tall columnar cells with basally oriented ovoid nuclei. The tracheal network, cell basement membrane, and cuticular lining of the lumen showed no visible effect from the sham operation. (The differences in appearance of the mass of spermatozoa within the spermathecal lumen, as they appear in Fig. 1, are within normal variation.)

Two clear-cut postoperative changes occurred in 30% tracheectomized spermathecas (Group B) (Fig. 1B). Epithelial necrosis typified by nuclear pyknosis, general eosinophilia, and, in extreme cases, disintegration of the epithelial wall was observed in the operated areas of 15 of 17 spermathecas in this group. In 4 specimens, the necrotic areas extended slightly beyond the operated areas; and, in 2, there were 1 or more small necrotic foci just outside the periphery. Reduction of epithelial cell height also occurred in the operated areas of all 17 specimens. This reduction was a gradual transition from normal or near normal height cells at the periphery to the shortest cells at the center of the operated area. Necrosis in the operated areas always occurred in the region of the shortest cells.

In the 10% tracheectomized group (Group C), 7 specimens had epithelial cell shortening and necrosis in the operated areas (Fig. 1C); and 1 specimen appeared unaffected by the tracheectomy.

Some epithelial necrosis and reduction in cell height occurred in each of the ligated spermathecas (Group D) (Fig. 1D). Extensive degenerative areas were found in 9 specimens, but in 2 specimens, the degenerative areas included only about 10% of the spermathecal surface. Microscopic examination of these 2 specimens suggested that, in each case, the ligature may not have been tight enough to occlude all tracheal trunks leading into the spermatheca tracheal network.

The results indicate it may be an oversimplification to assume, as Koeniger has (5), that the infertility of tracheectomized queens is caused directly by impairment of the O_2 supply to the spermathecal spermatozoa, especially since there is no evidence of direct respiratory connections between the spermathecal lumen and the tracheal network outside the wall (3). Instead, the epithelial cell necrosis, which develops promptly after ligation or tracheectomy (even when only a small portion of the tracheal network is removed), indicates that the integrity of the spermathecal wall is especially dependent on some function of its tracheal network. We can assume that dependence is principally respiratory since O_2–CO_2 transport is a function of the insect tracheal system (6, 7). The present findings support the concept of the spermatheca acting as an isolating structure in which spermatozoa can be stored, possibly in a very low metabolic state, for as long as its wall remains intact (3).

Summary. The spermathecas of honey bee queens were exposed surgically, and either a portion of the spermathecal tracheal network was removed or a ligature was placed to block air movement to and from the network. The queens were killed 2–22 days later, and a histologic appraisal was made of the operation's effect on the spermathecal wall. Both tracheectomy and ligation were promptly followed by severe shortening of epithelial cell height and necrosis of the spermathecal wall within the operated area. The findings support a concept of the spermatheca as an isolating structure for spermatozoa storage.

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