Resumen por el autor, George H. Bishop.
Universidad de Wisconsin.

La fecundación en la abeja.

I. Los organos sexuales masculinos, su estructura histologica y funcionamiento.

Los cambios que tienen lugar en la estructura histológica del aparato sexual mesodérmico en el zángano recién salido del huevo, indican que los zánganos jóvenes no pueden fecundar a las reinas a causa del estado no maduro de dichos órganos sexuales durante un periodo de nueve dias por lo menos. Los espermatozoides y el mucus permanecen en la vesícula seminal y el reservorio glandular mucoso, respectivamente, hasta el momento de la eyaculación. Los espermatozoides se insertan por sus cabezas en la pared de la vesícula seminal, cuya área superficial aumenta a causa de la formación de surcos y pliegues alternados que se disponen en espiral alrededor de su cavidad. La cavidad del conducto eyaculador ectodérmico, que se forma por invaginación del extremo anterior del pene, no se abre en la de la porción mesodérmica del aparato hasta que su extremo ciego quitinoso revienta al pasar los fluidos espermáticos. La musculatura de la base de la glándula mucosa está dispuesta de tal modo que su contracción bajo la acción del estímulo eyaculador separa esta región basal del reservorio mucoso distal, permitiendo el paso de los espermatozoides procedentes de la vesícula seminal a través de la base de la glándula y desde aquí al exterior por el conducto eyaculador. Durante la relajación que sigue a la primera contracción espasmódica, el mucus sigue al esperma, a causa de la presión ejercida por la contracción abdominal, de tal modo que obliga a penetrar a todo el esperma en los órganos femeninos. Después se coagula en contacto con el aire, cuando el pene se desprende del zángano. Varios estimulos artificiales causan una eyaculación normal en apariencia, y los mas seguros son la inyección de un ácido débil en el tórax y la decapitación durante la huida. La estructura de los órganos, naturaleza de los liquidos, y funcionamiento del aparato bajo la influencia de estímulos artificiales indican un papel diferente de los espermatozoides y mucus durante la copulación.

Translation by José F. Nonidez
Cornell University Medical College, N. Y.

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FERTILIZATION IN THE HONEY-BEE

I. THE MALE SEXUAL ORGANS: THEIR HISTOLOGICAL STRUCTURE AND PHYSIOLOGICAL FUNCTIONING

GEO. H. BISHOP

Zoological Laboratories of the University of Wisconsin

THREE TEXT FIGURES AND THREE PLATES

CONTENTS

INTRODUCTORY

The reproductive mechanism of the male honey-bee has been so often and so variously studied that one feels called upon to state at once the occasion for its further investigation.

The following study developed out of a series of unsuccessful attempts at the artificial fertilization of queen bees. Through a number of seasons this had been attempted by the methods which have been described by others as well as with newly devised apparatus. Two general classes of attempts were made. In the first, queen and drone were held in juxtaposition and the extrusion of the drone's organ brought about by pressure on the abdomen. In the second class of experiments, the seminal fluid of the drone was dissected out and injected with a pipette into the organs of the queen. From the first experiments (juxtaposition of queen and drone) it became more and more evident that extrusion of the drone's organ caused by artificial means did not necessarily, nor generally, duplicate the natural act of copulation, even when it seemed to do so. For the second (injection) experiments, it became necessary to know both what the character and functions of the components of the male spermatic fluid were and what disposal was made of them during and after the normal act of copulation. Finally, copulation in the bee has been witnessed so rarely and can be observed directly with such difficulty that a study of the structure and functioning of the reproductive organs is the most hopeful avenue of approach to the problem of fertilization in the honey-bee. Thus, while the anatomy of these organs has been worked over repeatedly, the physiological and functional aspect of fertilization in the bee has received inadequate attention.

To obtain the further data, regarding the functioning of the drone's organs, which seem a prerequisite to success in artificial matings, and to investigate the physiology and the mechanics of fertilization in the bee, work has been conducted along the following lines:

Histological and anatomical study of the drone organs and their respective secretions, by means of dissections, paraffin sections, and stained whole mounts and hemisections, mounted in balsam.

Manipulation of drones to cause extrusion of the penis, with ejaculation of the spermatic fluid; in the attempt to produce artificially a complete physiological duplication of the results of normal copulation.

The histological work was commenced in 1915 under Prof. Trevor Kincaid at the University of the State of Washington, after several years of independent and unsystematic attempts to induce controlled copulation between queens and drones by mechanical stimuli and technique. It was continued during the next two years in the zoological laboratories of the University of Wisconsin. Doctor Marshall of that laboratory has given particularly valuable help, not only by way of advice, but by assisting in those operations that required the attention of more than one person. Preparation for publication has been delayed by absence occasioned by the war.

HISTORICAL

Three papers[1,2,3] deal specifically with the development and histology of the drone sexual apparatus, and a larger number treat more or less comprehensively the gross anatomy, especially of the copulatory organ itself. The work of Bresslau[4] on the spermatheca of the queen and the accompanying mechanism has been checked by Zander[5] in a general account of the development of the organs in both male and female forms. Mating flights have been but rarely observed, and only incidentally, by bee-keepers, etc., and reported in their professional journals.[6, 7] Mating experiments have been reported frequently, but the very few cases of artificial or controlled matings reported as successful have not been sufficiently checked. There seems in this work to have been little attempt to take into consideration more than the superficial morphology with which the anatomical studies referred to have made us familiar.[8]

[1] Koschevnikov, G. Zur Anatomie der männlichen Geschlechtsorgane der Honigbiene. Zool. Anzeiger, Bd. 14, 1891.

[2] Michaelis, Geo. Bau und Entwickelung des männlichen Begattungsapparat der Honigbiene. Zeit. fur wissen. Zool., Bd. 67, 1900.

[3] Snodgrass, R. E. Anatomy of the honey-bee. U. S. Dept. of Agriculture, Bureau of Entomology, Technical Series 18, 1910.

[4] Bresslau, Ernst. Der Samenblasengang der Bienenkönigin. Zool. Anzeiger, Bd. 29, 1905.

[5] Zander, Enoch. Die Ausbildung des Geschlechts bei der Honigbiene, Zeit. der angewandten Entomologie, Bd. 3, 1916.

[6] Shuck, S. A. Note in American Bee Journal, 1882, p. 789.

[7] Pratt, E. A. Note in A B C and X Y Z of bee culture. A. I. Root Co.

[8] Shafer, Geo. D. A study of the factors which govern mating in the honey-bee. Michigan Agr. College Exp. Sta., Div. of Entomology, Technical Bulletin 34, 1917.

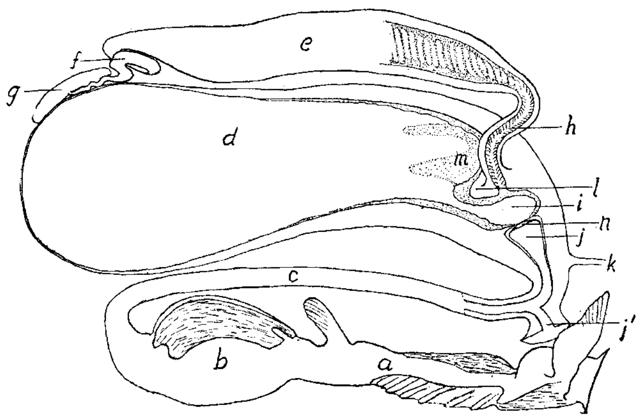
This bulletin furnishes an exception to the above statement. Concerned primarily with artificial fertilization experiments, it describes the superficial appearance of the queen's organs (oviducts) after normal copulation and of the drone's organs after extrusion of the penis has been brought about by pressure; there is also speculation on the nature of the stimulus that causes extrusion of the drone's organ in natural and in artificial conditions. He includes as well a valuable bibliography of experiments on artificial and controlled matings of drone and queen bees.

A detailed consideration of this literature, even of that part of it which deals specifically with the organ of copulation, will not be undertaken here. The morphology of the organs and the complex adaptations for mating have been adequately described, and in general there is no serious disagreement as to the position of the insects or the relation of their organs in copulation. However, the physiology of the process has been almost entirely ignored. The functioning of the 'mucous gland' has received little more than speculative attention; the disposal of the sperm in the queen's organs has scarcely aroused curiosity, and the intricacies of functioning of the internal sexual organs of the drone seem to have escaped notice for the most part. The present paper is rather an attempt to supplement the morphological data with physiological, than to controvert the facts established. The papers above cited are therefore not of immediate bearing on the work under consideration, other than as a point of departure. For a detailed description of the anatomy of the sexual apparatus the reader may be referred to any of the more recent papers (as Snodgrass). A brief and general summary will suffice to present the anatomical picture necessary to an understanding of the work which follows.

DESCRIPTION OF THE MALE ORGANS

The mating flight of the queen bee takes place at least five days after the emergence of the imago, and probably ten or more days after the emergence of the drone. The rapidly flying insects meet in the air, the drones in pursuit. According to the reports of eye witnesses and to the evidence from examination of the drone organ left in the queen's vagina after copulation, they clasp face to face and drop at once to the ground. The drone is stunned and soon dies. The queen twists the drone organ in two, by flying or crawling in a circle around the drone, retaining the portion broken off. This gradually dries up, and is pulled away by the bees in the hive some hours after the queen has returned thither.

The penis of the drone (text fig. 1, *a*) is elaborately adapted to this manner of mating. It is a hollow tube, ectodermal in origin, non-muscular, growing by invagination from the ninth segment of the abdomen. Three main functional regions can be identified; the penis tube proper (*a*), the enlarged bulb at its anterior end (*b*), and the ejaculatory duct (*c*), leading from the bulb to the mesodermal sexual organs. The penis tube, proximal to the external opening, is of relatively large diameter;

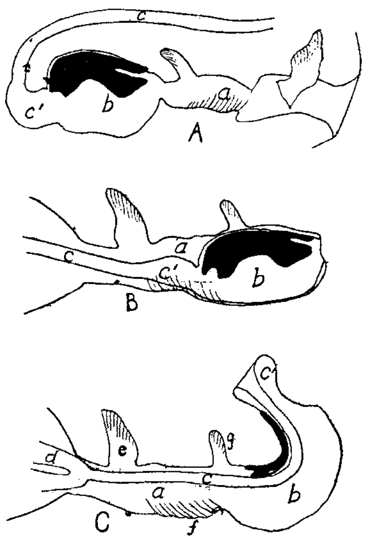


Text fig. 1 Diagram of one half of drone sexual apparatus, viewed from the medial side, showing the unpaired penis and ejaculatory duct, and the right members of the paired mucous glands, seminal vesicles, and testes of a mature drone. Internal anatomy of base of gland and vesicle shown as in optical section. The parts have been slightly displaced in mounting on the slide, in order to view them all in the horizontal plane; i.e., the anterior portion of the ejaculatory duct lies normally between the two glands, not below one of them; the tip of the other gland, joining the one figured at *j'*, extends dorsally at about right angles to this one, and not in the horizontal plane with it, and the vas deferens at *h* bends around the base of the gland so as to bring the seminal vesicle lateral to the gland rather than dorsal. *a*, penis proper; *b*, bulb of penis; *c*, ejaculatory duct; *d*, body of gland; *e*, seminal vesicle; *f*, vas deferens; *g*, testis; *h*, lower vas deferens leading to basal transverse pocket of gland, *i*, to which is applied the base of the cone-shaped end of the ejaculatory duct, *j*; *k*, slender muscle attaching the gland to the posterior abdominal wall; *l*, valve of muscle covered with glandular epithelium, which guards and partially surrounds the orifice of the vas deferens and partially divides the lumen of the gland proper from the basal pocket into which opens the vas deferens, and which upon contraction of the gland's musculature divides these regions completely; *m*, that portion of the gland's lumen which pushes out into the angle of the gland dorsal to the opening of the vas deferens; *n*, blind end of the ejaculatory duct.

it has a fairly stiff but elastic chitinized wall, and bears a series of complexly modified plates, bristles, and protrusions which appear to facilitate its entrance into, and secure its retention within, the vagina of the female. The medial portion, the bulb, is merely an enlarged and rounded part of this tube; it is on either side partially enclosed by a lateral shell-like plate, formed by the chitinous thickening of the wall of the bulb. This bulb tapers off into the third portion, the ejaculatory duct, a thin-walled, elastic, narrow-lumened tube leading to the seminal vesicles and the accessory glands (text fig. 1, *d* and *e*).

In copulation, this apparatus is everted from the drone's body into the vagina of the female. Since the penis itself has no muscles attached, its eversion is due to pressure from the muscular contraction of the abdominal walls. Starting at the region proximal to the genital aperture, the penis is gradually forced out from within, as one might force out a glove finger that had been turned inside out in stripping off, by blowing into the wrist of the glove. The eversion extends, according to Zander,[5] back to the median bulb, which, acting as a spermatophore, is kept from everting by its two lateral plates above mentioned (text fig. 2, *B*). Schafer[8] finds that the bulb also everts and concludes that it does not act as a spermatophore, but that its size merely enables these lateral plates, whose definite function is to hold the penis within the queen's organs, to turn inside out and lodge in their appropriate position like the gates of a canal lock (text fig. 2, *C*). The entrance of the ejaculatory duct into the bulb, according to this scheme, is thus brought through the everted bulb, and becomes the end of the everted penis. Either condition (*B* or *C*) may be produced artificially by greater or less pressure applied to the drone's abdomen.

The mesodermal portion of the sexual apparatus (text fig. 1, *d–h*) consists of three elements: 1) Paired testes, at the time of emergence of the imago, occupy a large part of the dorsal portion of the abdominal cavity; they undergo gradual diminution as the sperms are discharged, until at maturity only small triangular remnants remain (text fig. 1, *g*). 2) Passing posteriorly from each of these, leads a vas deferens, proximal to the testis sharply coiled (*f*), and distally expanded into a seminal vesicle (*e*). 3) Distal (posterior) to the vesicle again, the vas deferens curves sharply (*h*) to enter the third element, the accessory gland (*d*). This organ extends anteriorly to a region slightly beyond the testis, the rudiment of which in the mature drone is usually applied dorsally to the tip of the gland (text fig. 1, *g*).



Text fig. 2 Diagrams of drone's organs, showing uneverted, partially everted, and completely everted relationships of the various portions, in *A*, *B*, and *C*, respectively. *a*, posterior or proximal tubular portion of the penis with modifications for facilitating copulation, *e*, *f*, *g*; *b*, bulb portion of penis, with lateral chitinous plates shown in black; *c'*, proximal end of ejaculatory duct *c*, expanded where it joins the bulb of the penis; *d*, the mucous glands. For further explanations see text.

In development, each vas deferens grows back from the testis sheath until it meets, ventrally, a cup-like invagination of the ninth segment of the abdomen which is to form the penis and the ejaculatory duct. The vas deferens fundament then curves back on itself to form a hook like the letter J, later, becoming a U. The recurved tip of the J forms the mucous gland; the stem the seminal vesicle. A branch of the ejaculatory duct penetrates the lower portion of the U, at the base of the gland, thus uniting the ectodermal and mesodermal parts. Zander notes that the lumen of the ejaculatory duct does not become continuous with that of the gland until the contained fluids burst through the thin partition at the time of emptying of the secretions (see diagrams, pl. 1, and pl. 3, figs. 7–10). ("Die Berührungstelle, an der beide Kanalsysteme [of duct and gland], anscheinend bis zur Samenentleerung blind aneinander stossen," etc.) He leads one to infer that at the maturity of the drone (text fig. 2, *A*, *b*) the bulb of the penis, acting as a spermatophore, receives this secretion. Shafer, without noting this partition, infers that the sperms remain in the vesicle or the base of the gland until copulation, and do not pass into the penis bulb, but at the time of copulation are carried through the bulb in the ejaculatory duct (text fig. 2, *C*).

The accessory or mucous gland (text fig. 1, *d*, and pl. 1), developing from the blind recurved end of the vas deferens fundament, enlarges into a gourd-shaped body, lined with columnar glandular epithelium and enclosed by three muscle layers. These layers are an external longitudinal, a medial circular, and an inner layer which consists of three longitudinal bundles of fibers, extending from the base of the gland more than half way to its tip. The musculature is heaviest at the base, i.e., around the entrance of the ejaculatory duct, and attenuates toward the distal end. The whole is enveloped by a thin structureless membrane well supplied with tracheae. As the gland's lumen becomes filled with the secreted mucus, its distal end assumes a bulbous contour. The three muscle tracts of the inner layer cause an infolding of the glandular lining of the organ into three corresponding ridges, giving a cross-section of the lumen the shape of a clover leaf (pl. 1, fig. 3).

The seminal vesicle (text fig. 1, *e*, and pl. 1, *e*), like the gland, is lined with glandular epithelium, here thrown into ridges (Koschevnikov, "in Ringwalzen eingereiht"). There are two muscle layers surrounding it, an outer longitudinal and an inner circular layer. These correspond to the outer two of the three layers of muscle of the mucous gland. A membranous envelope covers the vesicle; this is continuous with the envelope of the gland on one side and of the testis on the other. Sperms, passing into the vesicle, tend to arrange themselves radially in its lumen, their heads attached to the wall, their free filaments toward the center (pl. 2, figs. 5, 5b). The testes, which mature their sperm some days before the emergence of the drone, and at this time occupy most of the abdominal cavity, rapidly degenerate thereafter; in old drones they are noticeable only as small greenish-yellow remnants applied dorsally to the accessory glands.

PRESENT INVESTIGATION

Attempts to obtain motile sperms from drones, by dissection or otherwise, demonstrate that they are not available in all drones. This fact has been variously interpreted. McLain[9] inferred that there were three classes of drones. One class yielded no spermatic fluid when extrusion of the penis was brought about by compressing the abdomen. A second class yielded only mucus from the accessory gland. A third yielded a seminal fluid containing sperm. Shafer,[8] in discussing McLain's work, agrees with him that the food which drones receive at mating time is important as a stimulant to the copulatory impulse. The writer sought to correlate the observed facts of McLain with the known fact that young drones (younger than an age variously stated to be from ten to twenty-one days) will not mate with queens. Drones of different ages were selected for study, ranging from pupae whose eyes were just becoming pigmented to mature insects three weeks after emergence. These stages are designated in the present paper by the letters A to H (table, p. 252).

The fact became immediately apparent that a definite and complicated histological development and growth of the organs, rather than a special food stimulant, was involved in the difference of functioning observed. This development takes place

[9] McLain. Description of experiments on artificial insemination of queen bees, in report of the entomologist, on Experiments in Apiculture, U. S. Commissioner of Agriculture's Report for 1885.

after the drone is of superficially mature appearance, and continues to the fifth day of imaginal life at least though the drone is probably not functional until later (table, p. 252) The contents of the organs, their mode of responding to such stimuli as cause extrusion of the penis, and the motility of the sperm are all correlated specifically with the age of the drone. The age can be determined by superficial examination of the degree of degeneration of the testis. This undergoes a gradual shrinkage, and changes from a creamy-yellow color and bean-like shape, of 5 mm. length, to greenish-yellow color, flat triangular shape, and 1.5 mm. length. This change in the testis is a convenient measure of the histological development, size, and maturity of the other organs.

A. Histology and anatomy of male organs

The vas deferens, seminal vesicle, and mucous gland are derived from a common fundament; when mature they are histologically similar; they perform their functions of secretion and contraction in essentially the same manner. A detailed description of the histological elements and of their physiological characteristics may apply, therefore, to all the parts derived from this fundament taken together.

The two histological elements chiefly concerned here are the columnar cells of the glandular epithelium which forms the continuous wall of the lumen of these organs and the muscle layers that continuously envelop them. Concerning the glandular cells, the important considerations are the manner and nature of their secretion and the qualities of the product; concerning the muscle layers, their arrangement and the effect of their contraction upon the contents of the organs at different stages of development.

1. The glandular epithelium. The glandular cells (pl. 2, figs. 5a, 6a) are extremely long and narrow, twenty or thirty times as long as thick, with oval nuclei, one to each cell, scattered through the basal half of the cell layer. The nuclei are so large relatively to the diameter of the cells that the oval form may be assigned to compression by the cell walls. Their scattered arrangement seems to be due to the fact that the nuclei bulge the walls slightly outward, and force nuclei of adjacent cells alternately upward and downward. The chromatin of the nuclei is mostly in three to five granules, the rest faintly scattering through the clear plasma. The chromatin stains more densely while secretion is taking place, and shrinks and takes the stain less densely when it ceases, but its disposition in the nucleus does not appear to alter. The cytoplasm is very finely granular (after fixation), slightly more densely staining around the nuclei, especially when the cells are functionally active after growth is complete. The distal ends of the cells contain larger and denser staining granules that give, in (cross-) section of the epithelium, the appearance of a dense granular band (pl. 2, figs. 5a, and 6a).

The cells of the glandular epithelium are modified according to the region of the lumen which they line. The cells lining the vas deferens between testis and seminal vesicle (text fig. 1, *f*) and those between the vesicle and the mucous gland (*h*) are more cubical than columnar. Here the nuclei are placed more evenly side by side and have a more rounded outline, but the characteristic structure and mode of secretion of the cells is identical. The cells of the seminal vesicle are about a half shorter than those of the gland, their nuclei are smaller and similarly disposed.

Secretion takes place by strangulation, with dissolution of the cell substance (pl. 2, figs. 5 and 6 b). The dense granular area at the tip of the cell widens, the granules increase in size, in refractiveness, and in density of staining, and finally vacuoles may appear among them. The end of the cell rounds up into a globule of secretion, which sloughs off into the lumen of the organ. This process is most pronounced in the gland, where the secretion retains its coarse granular character. These granules are transformed to highly refractive globules of somewhat larger size, as if by absorption of some of the fluid; the mass of the secretion at the same time becomes more viscous. In the seminal vesicle the granules are smaller and soon dissolve to a pale plasma (pl. 2, figs. 5 and 5 b). In the narrow portions of the vas deferens, at either end of the vesicle, neither the granulation nor the strangulation are apparent. This is possibly owing to the slowness of secretion and the small degree of dissolution of these short cells. In the distal region of the vas deferens adjacent to the gland, the lumen does not always, as elsewhere, become clearly defined, but may remain loosely stopped with a network of strands and membranes which appear to be remnants of the walls of cells that filled this space (pl. 3, fig. 10, and text fig. 1, *h*). These cells become shortened inside their former membranes, to form a thin epithelium against the muscular layer enclosing the vas deferens. The cytoplasm is dense, the nuclei shrunken. The picture closely resembles the final appearance of the basal portion of the gland into which this portion of the vas deferens serves to conduct the sperm (pl. 3, fig. 10).

As the drone approaches sexual maturity, this process of secretion and reduction of the glandular epithelium commences in the tightly coiled epididymis-like portion of the vas deferens leading from the testis (text fig. 1, *f*). It progresses from the tips of the cells back to the bases (pl. 2, figs. 5, 5 a, 5 b), and in the vas deferens as a whole, from the testis posteriorly through the seiminal vesicle. Shortly after the stage at which the cells lining the seminal vesicle start secreting, the cells lining the mucous gland commence to break down into secretion in the anterior end of the gland. The change progresses posteriorly again. Thus the cavities of these organs are enlarged through dissolution of their walls. This occurs earliest anteriorly, affecting last the posterior regions where the contents of both organs are to be evacuated into the ejaculatory duct (text fig. 1, *h* and *i*).

When this process has reached an advanced stage, it leaves the walls of the organs characteristically sculptured. In the gland (pl. 1, figs. *C*, *D*, *E*) the cells entirely disappear anteriorly, leaving a very thin membranous bulb-like sac which expands with mucous secretion. Posteriorly, the cell nuclei recede toward the basal region of the cells, the chromatin shrinks, the cytoplasm becomes heavily vacuolated, and the ends of the cells protrude into the gland's lumen in fringed and ragged patches (pl. 3, fig. 10, *m*). Vacuolization at the bases of the cells often appears to push whole areas of the cells out into the lumen, leaving their bases attached to the muscle layer by attenuated remnants of the cell walls. There is evidence that the cells tend to break down unevenly; during the early stages this leaves elevated circular ridges running around the long axis of the gland; but these are neither regular nor do they persist except in vague outlines in the final stages.[1]

In the vas deferens and seminal vesicle the effect is more elaborate (pl. 1, figs. C, D, E, *e* and pl. 2 figs. 5 and 5 b). Commencing at the anterior end of the vas deferens the cells break down unevenly and in such a manner as to leave the surface of the epithelium in very definite ridges. This is much more clearly defined and regular here than in the gland. This condition is described in the mature insect by Koschevnikov as "in Ringwalzen eingereiht"; but a close inspection of a cleared whole mount or hemisection reveals an arrangement as of a spiral screw with four successive threads. There are about seventy turns, each 'thread' making fifteen to twenty turns of the spiral, though occasionally one ridge ends and is replaced by a new one. As will appear later the function is apparently to increase the surface for attachment of the spermatozoa. The nuclei of the epithelial cells arrange themselves, not parallel to the basal membrane of the epithelium, but in a layer following the folded surface (fig. 5 b). The nuclei retain appearances of activity and do not show shrunken chromatin and clear plasma as do the remnants of cells in the epithelium of the gland.

The commencement of this secretory and erosive process in the vas deferens overlaps the period of spermiogenesis in the testis. As the lumen enlarges it becomes filled with fluid. The sperms pass into it and through it into the seminal vesicle; here as the sperms descend the cells also break down into a secretion. This process in the seminal vesicle serves three purposes: provides a medium for the spermatozoa by dissolution of the glandular elements, renders the rather firm glandular wall flexible and capable of considerable distention, and allows the enclosing muscles to act easily at the time of ejaculation of sperm.

The sperms, still grouped in bundles as they left the cysts of the testicular tubules, attach themselves by the heads to the ribbed surface of the vesicle, and the tails project into the lumen. When spermiogenesis is complete and all the sperms have become attached, a cross-section of the organ (fig. 5 b) shows, inside the muscular ring, first a ring of nuclei following the contour of the inner surfaces of the spiral ridges, then a distinct line of sperm heads at the surface of the epithelium, and finally the remainder of the lumen almost filled with the sperm filaments radially arranged, extending outward from a narrow central space. The spermatozoa even after attachment show a grouping into bundles.

Region of the ejaculatory duct. The development of the ejaculatory duct and its junction with the mucous gland-vas deferens fundament has been referred to above. The relation of the three parts, mucous gland (*i*), proximal part of vas deferens leading from the seminal vesicle (*h*), and the ejaculatory duct (*j*), requires a more detailed description (text fig. 1 and pls. 1 and 3).

The paired mucous glands lie parallel in the posteroventral region of the abdomen; the bulbous ends containing the mucous accumulation point anteriorly. The basal portion of each gland, with which both vas deferens and ejaculatory duct connect, bends at an angle of about 45° in the medioventral direction (text fig. 1). The tips of the two glands meet medially. The ejaculatory duct divides as it enters the junction of the two glands, and a branch penetrates the wall of each.

The vas deferens (*h*) makes a sharp curve from the seminal vesicle and enters the gland on the medial side, dorsal to the entrance of the ejaculatory duct (*j*). Around and particularly above the entrance of the vas deferens, the muscular wall of the gland is greatly thickened, and projects into the gland's cavity as a lip or valve guarding the entrance of the vas deferens (*l*). This valve partially cuts off from the body of the gland anterior to it the basal portion of the gland's cavity (*i*) into which lead both ejaculatory duct and vas deferens. It thus divides the cavity of the gland into two regions at the bend of the gland described above. One region of the gland's lumen becomes distally an elongated sac containing mucus, lying parallel to the main axis of the abdomen. Its posterior margin is the valve projecting from the dorsomedial side of the lumen. Below this valve, the second region consists of a small flat pocket lying across the base of the gland (text fig. 1 and pl. 1, *i*). Into this pocket and from the valve's posterior surface opens the vas deferens (*h*). Applied ventrally to this pocket is the expanded end of the corresponding branch of the ejaculatory duct (*j*). This flattens out into the base of a cone, whose wall does not break through into the gland's lumen, although the gland's wall is penetrated by the blind end of this duct (pl. 3, figs. 9 and 10).

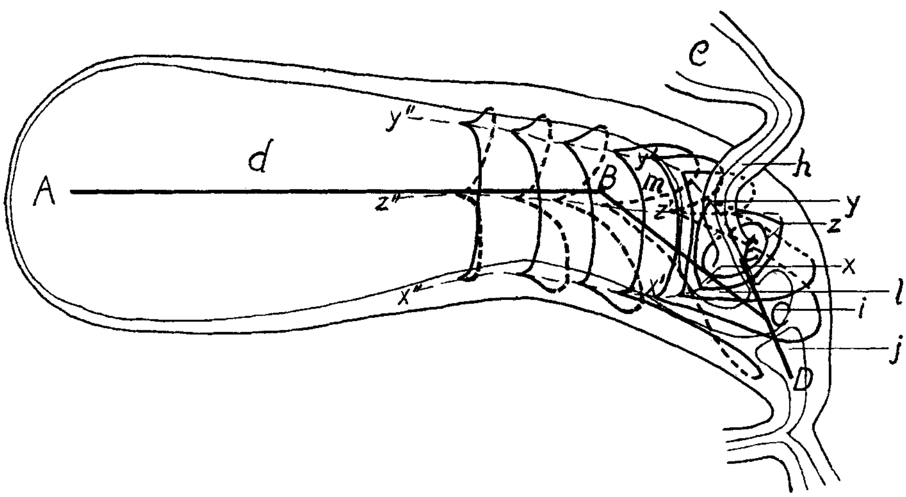
The relation of the parts therefore admits of the following hypothesis as to its functioning. If the flat pocket is collapsed, the edge of the valve is pressed close against the opposite side of the gland's lumen, shutting off completely the whole basal region of the gland from the sac full of mucus (pl. 1, fig. E, and pl. 3, fig. 10). The mouth of the vas deferens is applied at the same time exactly over the flattened blind end of the ejaculatory duct. If this be burst through, the result is a passageway through this system of organs extending through the vas deferens, seminal vesicle, lower vas deferens to the basal region of the gland, and out through the ejaculatory duct. It extends past the body of the gland as if the latter's content were not to be discharged with the content of the seminal vesicle; although in development the gland and vas deferens form a continuous tube, a tube whose lumen is closed off from the lumen of the ejaculatory duct by a membrane of chitin over the blind end of the latter. A consideration of the musculature of the region further points to this manner of functioning.

2. The muscle layers. The ejaculatory duct has no muscles in its wall; it is a single-layered tube of ectodermal origin invaginated from the hypodermis and chitinized on the inside. The vas deferens, seminal vesicle, and gland have two muscle layers, outer longitudinal and an inner circular layer, forming a continuous envelope over the whole of these organs. Running from the base of the gland half or more its length distally, a third or inmost muscular layer, consisting of three separate tracts of fibers, has been described (pl. 1, figs. 1 to 4, *x*, *y*, *z*). A closer analysis of the course of these fibers indicates that they do not comprise a distinct third layer, but that they consist of a modification or distortion in the arrangement of certain bundles of the inner or circular layer in this region, and that this rearrangement is the method by which the otherwise simple musculature of the gland's base is adapted to an involved and complicated manner of functioning. The change, during development, in the relationships of the gland to the vas deferens and seminal vesicle on the one hand and to the ejaculatory duct on the other gives a clue to the origin of this 'third layer.'

Recalling that the vas deferens grows posteriorly from the testis sheath as a J- and finally a U-shaped fundament, one arm of which forms the mucous gland, the musculature may be described more carefully. It consists of a relatively heavy circular layer of fibers which is not distinctly separable into fascicles, lying next to the glandular epithelium, and a relatively thin longitudinal layer collected into distinct fascicles, between which a connective-tissue network allows for distention of the organ (pl. 2, figs. 5 and 5 b). There are about thirty-five of these fascicles in a cross-section of the vesicle; in the gland they are not so distinct and the fibers are arranged in a less specific manner. Both layers are thinner over the narrow portions of the vas deferens adjoining either end of the seminal vesicle, and both taper off over the distal portion of the gland into a very thin and elastic connective-tissue membrane.

It is at the base of the gland, where the musculature is heaviest and whence originate the three bundles of fibers comprising the inmost muscle layer of the gland, that the ejaculatory duct becomes adjoined to the mesodermal portion of the sexual apparatus. Only in the light of the significance of this junction can the elaborate conformation of this musculature be adequately interpreted.

We may picture at the bend of the U-shaped gland-vesicle fundament one branch of the ejaculatory duct penetrating this muscle mass to reach the lumen of its respective gland. At this place the wall of the gland protrudes to meet the duct. This protruded portion becomes the basal end of the gland (text figs. 1 and 3, *i*). One arm of the U, representing the gland (*d*), increases in size, while the other, that representing the vas deferens (*h*) remains relatively small. The result is, first, that the vas deferens becomes a fine duct leading into the massive gland and, second, that as the gland protrudes toward the ejaculatory duct to form a definite basal pocket (*i*), the entrance of the vas deferens into the gland is left distal to and at one side of this secondarily formed basal region; that is, it comes to enter not at the end of the gland, but at some distance up its side. From a U shape, the lumen of the two organs, mucous gland and



Text fig. 3 Diagram to explain the derivation of the third or internal muscle layer of the mucous gland. Lettering as for text figure 1, with addition of the following: *A–B–C–E*, original direction of the lumen of the gland-vesicle fundament in development; *A–B–C–D*, direction of lumen in mature gland, whose base has enveloped the blind end of the ejaculatory duct *j*; *XYZ*, path of the circular muscle fibers around the end of the vas deferens in the wall of the gland; *X″Y″Z″*, fibers around the body of the gland; *X′Y′Z′*, fibers distorted out of the circumferential position by the protrusion of the gland's base to meet the end of the ejaculatory duct at *i*, and by the second protrusion into the elbow of the gland, *m*; *X–X″*, *Y–Y″*, *Z–Z″*, three inner longitudinal muscle tracts derived from fibers of the circular layer by change in shape of the gland's base at *i* and *m*. See text for further explanations, also text figure 1.

vas deferens, takes the form of a square-root sign (A╲B
C|——E,
D
text fig. 3), in which the perpendicular stem represents the gland, the horizontal arm the vas deferens, and the portion of the perpendicular below the arm, the protrusion which meets the ejaculatory duct.

This change in shape distorts the course of the fibers of the longitudinal and circular muscle layers. Their original course along and around the U-shaped axis of the original fundament is modified in conformity with the change from this axis to that of the mature gland. Where the base of the gland protrudes to meet the ejaculatory duct, the muscle layers are carried out in its wall and greatly thickened, investing the end of the duct and the base of the gland with a heavy and complexly arranged musculature (pl. 1 and pl. 3, fig. 10). Fibers of the outer longitudinal layer still pass longitudinally as before on one side, the dorsal and medial, as far as the vas deferens, and on the other side, the ventral, down to the basal tip of the gland whence leads the ejaculatory duct. From here they pass anteriorly again on the dorsal side to the vas deferens. On the lateral aspects of the base of the gland, the fibers of this layer must pass across in a transverse direction to reach the vas deferens. The fibers of the circular layer are correspondingly distorted. This layer still encircles the gland above the entrance of the vas deferens (text fig. 3, $x,''$ $y,''$ z'') and encircles the end of the vas deferens itself (x, y, z) at its juncture with the gland. But below the region of the vas deferens entrance, these circular fibers, whose fundamental course may be considered to have been in concentric rings about the end of the vas deferens, are distorted by the base of the gland having protruded toward the ejaculatory duct (x', y', z'). They extend toward the base of the gland in the same general direction as the longitudinal fibers of the same region, but criss-crossing them diagonally (pl. 3, fig. 10).

The fibers forming the valve that partially closes off the anterior from the basal or posterior portion of the gland (*l*) may be derived from a thickening of the circular layer around the end of the vas deferens. To form the valve these fibers protrude into the lumen of the gland anterior to the vas deferens and furnish posterior to it additional material for thickening the muscle mass about the end of the ejaculatory duct.

Contraction of the muscles encircling this region would close off the basal pocket in the gland by compressing the valve (*l*) against the opposite wall; contraction of the muscles extending along the base of the gland would collapse the flat pocket (*i*) cut off by this valve. In this manner the aperture of the vas deferens would be forced against the blind end of the ejaculatory duct, as the anatomical relations of these parts, previously described, indicates to be possible (p. 239; pl. 1, E, and pl. 3, fig. 10).

One more modification of the shape of the gland requires explanation. The vas deferens enters the mature gland on the median side; just anterior to its entrance the gland bends at an angle of 45° to meet the ejaculatory duct, so that its basal tip points ventrally and medially toward the tip of the other gland (text figs. 1 and 3). Thus the tips of the two glands come to point toward each other; in fact, are joined superficially. Anterior and dorsal to the entrance of the vas deferens and to the valve, the lateral portion of the gland's lumen protrudes out to form the elbow or bend (text fig. 1, *m*). This valve, which guards the aperture of the vas deferens, forms the posterior boundary of the protruded portion, and the muscle fibers of the gland's wall are carried out around the lumen (text fig. 3, y'–z'). This protrusion of the gland's wall thus causes a distortion of the muscle layers somewhat in the same manner as does the protrusion toward the end of the ejaculatory duct on the ventral side.

A cross-section of the gland just at this angle would show as a result of these changes a tripolar arrangement (pl. 1, figs. 1 and 2). At one pole is the entrance to the vas deferens (*h*), at a second, the base of the gland envelops the end of the ejaculatory duct (j), and at a third, the lumen of the gland swells out into the gland's elbow (m). In text figure 1 these regions may be identified between x and y, x and z, and y and z, respectively.

The three tracts of the inner 'third layer' may then be derived as follows (pl. 1, figs. 1 to 4, x, y, and z, and text fig. 3, x''–x, y''–y, and z''–z):

x. Fibers of the inner circular layer originating along the median side of the gland, from a region anterior to the opening of the vas deferens, and from the muscle mass in the valve guarding it, pass on the medial side of the gland toward its base, here penetrated by the ejaculatory duct (x''–x).

y. Fibers from the same region, but passing dorsal to the vas deferens, extend posteriorly around the protruding elbow of the gland (y''–y).

z. Both sets of fibers pass anteriorly on the opposite side of the gland's base, between the ejaculatory duct and the gland's elbow, to the region on the gland opposite to the end of the vas deferens (z''–z).

The anatomical findings suggest and bear out this derivation for these three muscle tracts, except that the muscles extend anteriorly further along the side of the gland than the mass of the circular fibers from which they are believed to have been derived. This may be considered a functional modification to afford that insertion of the fibers on the sides of the gland which would enable them to operate most effectively.

Along these three tracts and extending for about the same distance, the glandular epithelium is elevated into the lumen of the gland in such a manner as to give its cross-section a trilobed shape (pl. 1, fig. 3). One channel so caused (between two adjacent tracts) extends distally from the expanded elbow of the gland; along the lateral aspect, between y and z; a second, along the medial side from the valve anterior to the opening of the vas deferens, between x and y, and a third, between x and z distal to the end of the ejaculatory duct and passing opposite to the valve, is continuous ventromedially with the basal transverse pocket of the gland which receives vas deferens and ejaculatory duct. Distally all three merge into the uniformly rounded bulbous sac in which the gland ends.

3. The ejaculatory duct. The ejaculatory duct and the glandular lining of the basal portion of the gland with which it connects remain to be described (pl. 3, figs. 7 to 10). The blind end of the duct penetrates the gland's muscular coat; here it expands into a cone whose base becomes applied, as described before, to that aspect of the gland's basal region which is directly opposite the opening of the vas deferens. The hypodermal cells forming this cone become elongated around its base from cubical to a distinctly columnar form. The base of the cone becomes heavily chitinized, especally that part lying over the cells which are most elongated (pl. 3, fig. 9). At the center of the base the cells are shorter, and here the chitin is laid down in two layers over a very small area (pl. 3, figs. 7 to 10, *n*). Between these two layers is a small mass of material staining more densely (with iron-alum-haematoxylin) than the chitin, which later disappears or else shrinks greatly, leaving the two layers separated by a space. The layer toward the lumen of the gland is considerably thinner than the other (pl. 3, fig. 10), *n*); both together they form a weakened area in the base of the cone which may be likened to a drum.

The columnar cells of the base of the cone now recede laterally and decrease in length, finally leaving this double chitinized drum alone to close the end of the duct (pl. 3, figs. 9 and 10). At the same time the glandular layer of the mucous gland breaks down as described heretofore, and these gland cells over the center of the chitinized drum also withdraw laterally. This leaves the chitin drum exposed to the lumen of the gland, but unperforated, in which condition it can be demonstrated at all stages investigated, provided methods are used in killing which do not distort the organs to such an extent that the drum is burst. This fact, together with evidence to be submitted, indicates that both sperms and mucus remain in the seminal vesicle and gland, respectively, not only until maturity, but even until copulation.

B. Physiology

1. The secretions. The content of the mucous gland is elaborated first in the distal end, and tends to collect there throughout the process of elaboration; the thinning of the glandular wall allows of considerable distention of this distal end to its characteristic bulbous shape, and nutriment is evidently absorbed actively by the organ, for it increases in size until the stage F (nine days). The secretion changes in character from fluid to viscous, and aquires increasingly the property of immediately coagulating to a tough, cheesy or doughy mass. This happens on contact with air, water, Ringer's solution, alcohol, lymph from the drone's abdomen, or any bland reagent in which an attempt was made to mix or dissolve it. It shrinks markedly in fixation and dehydration, and, especially when taken from an old drone or from the exposed organ removed from the female after copulation, it becomes so hard as to nick the microtome knife. It is slightly alkaline in reaction.

Spermatic fluid removed from the seminal vesicle consists of very little lymph-like fluid densely packed with sperms. This is so dense that it will barely drop from a needle. The sperms being attached to the vesicle wall, it takes an appreciable time for them to loosen when the vesicle is freshly torn under a microscope. Up to the stage E (five days) they have to be squeezed loose; in later stages the stimulus of breaking open the vesicle causes them to release themselves readily, until at stage G (twelve days) they pour forth from the slightest cut of the vesicle in a writhing mass. Up to stage D or E the sperms, except for a very gentle beating of the filaments, are inactive when released. From this stage until apparent maturity (nine to twelve days) their activity when released increases, as well as the readiness with which they are expressed from the vesicle. It is concluded that in the vesicle the sperms are at all times at rest or nearly so, for if the vesicle is abruptly torn under the microscope the sperms attached along the torn edge appear quiescent for a moment. Also the spermatic fluid remains in the seminal vesicle until the time of copulation, not as Shafer suggests partly in the base of the gland, nor as Zander leads one to infer, in the bulb of the penis (this acting as a spermatophore). Inspection of the drone's abdomen, opened without fixing the organs, gives some appearance of support to these views, for then, due to disturbance in dissecting, the sperms are frequently found in the base of the gland or even in the penis bulb.

The spermatic fluid, in contrast to the mucus of the gland, mixes readily with any bland aqueous medium, salt, sugar, or lymph solution, but any dilution seems to decrease the activity of the sperms for a long time, though without necessarily killing them. Sperms on a slide under a cover-glass in salt solution were not killed by two hours' contact with ice, and fertile females have been frozen to − 2°C. for fifteen minutes without rendering subsequently laid eggs infertile.[10] The spermatic fluid and the glandular secretion are miscible in the penis before exposure to the air, and the sperms are intensely activated by the secretion of the gland. Particles of the vesicular wall stimulate them similarly. Whether this action is mechanical, as giving the heads of the sperms a firm hold for the exertions of their filaments (they collect around droplets of the secretion) or whether the action is chemical, as a stimulant, is not apparent, Contact with the mucus will not activate sperms that are too young to release themselves from the vesicular wall, and in the oviduct of the queen after copulation the sperms separate out of the mass of mucus and enter the seminal receptacle alone. The evidence seems to point, therefore, to the stimulus being a mechanical one, expecially since sperms are activated by the mechanical act of being torn loose from the wall of the vesicle.

The seminal vesicle when filled with spermatic fluid assumes a distinct yellow color, as contrasted with the pure white of the mucous gland. This contrast of color is noticeable whenever the transparent organs of the drone or queen are filled with these secretions. For instance, in freshly dissected drones the yellow

[10] Dzierzon has stated that queens can be rendered infertile, and hence 'drone layers,' in this way; but though the statement is widely quoted, the writer has not found a single other recorded instance of its being done, experimentally or otherwise; he was unable to produce the expected result by any temperature, either prolonged or extreme, from the effects of which queens would recover.

spermatic fluid can be seen passing through the base of the gland and the lumen of the ejaculatory duct, and the contents of these organs can be distinguished by the color. When the two fluids are loosely mixed in the bulb of the penis, the areas of yellow and white can be distinguished, and if the drone is stimulated to complete extrusion of the organ, with ejaculation, a rough determination can be made by color as well as by consistency as to whether sperm or mucus has been emitted. When a queen has been newly fertilized, the penis attached in her organs can often be seen to be distended with clear white mucus, while the oviducts are distinctly yellow when dissected out. This is found to be due to the fact that after copulation the sperms collect in a layer next the wall of the oviduct and conceal a central core of mucus.

Sperms when densely crowded exhibit a tendency to lie parallel in masses, the filaments beating in unison, giving a characteristic undulatory appearance. This grouping approximates their arrangement when attached to the walls of the vesicle (pl. 2, fig. 5 b). Free on the slide, the masses of sperms arrange themselves in whorls or undulating bands; after copulation a cross-section of the oviduct of a queen shows a wavy band next the oviduct wall, and in the spermatheca of a fertile queen the sperms again arrange themselves in whorls, with the densely staining heads massed and the lighter staining filaments extending parallel. When diluted on a slide or mixed (in the oviduct) with mucus, or when, in a newly fertilized queen, only a few sperms have entered the spermatheca, the arrangement is scattering and indiscriminate.

As for functions consistent with these characteristic qualities and behavior of mucous and sperm, respectively, actual results of copulation afford the final data. To anticipate a forthcoming paper dealing with this subject in detail, the sperms are received into the spermatheca of the female before the mucus is disposed of, and the latter is dissolved gradually from the distended oviducts of the queen bee into which both sperm and mucus are injected at copulation. The penis with which the female returns from the mating flight is distended with mucus alone, which has so hardened on contact with the air as to effectually stop and seal off the torn end of the organ. Having followed the sperm through the penis in ejaculation, the mucus has forced all the residual sperm out of the penis, so that whatever material is not injected into the female organs, and is thus to be lost when the penis is dropped, will not be the physiologically more valuable spermatic fluid.

2. Correlation of age with functioning. With the foregoing facts in mind, we may follow the differences in the response of the sexual mechanism, at different stages of development, to artificial or natural stimuli. In a young drone (up to four or five days) the chitinous blind end of the ejaculatory duct is still reenforced with layers of glandular and hypodermal cells; the walls of both mucous gland and seminal vesicle are stiffened with unresolved glandular epithelium; the sperms are either still in the testis tubules or are firmly attached to the vesicular wall, and are incapable of the activity which later characterizes them, and it is doubtful whether the valve which eventually occludes the gland's lumen is in the early life of the drone capable of doing so, for since the lining of the basal portion of the gland is the last to be resolved into secretion, this valve is still stiffened by a heavy glandular coat. The result of stimulating drones less than four or five days old is either no secretion at all when the organ is extruded or a secretion composed entirely or in large part of mucus, or if sperms are present, the glandular wall of the vesicle has pulled away with them, and the sperms are inert or vibrate their filaments but feebly.

After the fifth or sixth day the reaction is markedly different. The reinforcing cells over the end of the ejaculatory duct have withdrawn; the mucus is more viscous; the sperms release themselves more and more readily from the vesicle and are extremely active; the glandular walls of the organs are thin and pliable, and the sperm content of the vesicle is discharged through the ejaculatory duct ahead of the mucous content of the accessory gland. The whole reaction of the drone is also more violent and spasmodic. These conditions, while virtually established, as stated, at five or six days of age, seem to become accentuated up to the age of nine or ten days, although the morphological and histological changes after the sixth day are slight and although the variation in the physiological reactions concerned makes it difficult to measure accurately the degree of the response.[11] The following table will summarize the data correlating age of the drone with the histological and physiological findings.

3. Manipulation of drones. If a drone's abdomen is pinched sharply between thumb and forefinger, the pressure will generally cause partial or complete extrusion of the copulatory organ. The penis tube may evert throughout its length, as described heretofore, everting the two lateral chitinized plates that enclose its bulb, and also drawing the ejaculatory duct through the everted bulb (text fig. 2, *C*); in this case whatever fluid is expressed forms a drop at the end of the penis. Extrusion may stop, however, before this bulb has turned inside out (text fig. 2, *B*). The fluid will then remain for the most part in the bulb of the penis (*b*) and in the elastic and expanded end of the adjoining ejaculatory duct (*c*). There may be little or no spermatic fluid expressed, or the fluid may consist entirely of mucus, or it may consist of both mucus and sperm, rarely of sperm alone.

Selecting drones all of which were known to be old enough to function in normal copulation, experiments were undertaken to find what controlled the normal protrusion of the organ and the normal ejaculation of the secretions.

It was found almost impossible at first to dissect these drones without disturbance of the sexual apparatus. Drones held in the hand, without mechanical pressure being applied by the fingers, will often extrude the penis with a sort of explosive contraction of the abdomen. Even when extrusion does not occur, the mucous glands of dissected mature drones will generally be found to have burst at the expanded distal end, or else sperm and mucus will have been forced into the base of the gland, ejaculatory duct, or penis. If the drone's head is amputated a disturbance invariably occurs; frequently this goes as far as

[11] Whether functional maturity and ability to effectually inseminate queens is attained at the time of apparent histological and physiological maturity of the organs and secretions described, is a matter which only mating experiments can determine. Mr. F. W. L. Sladen, apiarist of the Canadian Department of Agriculture, informs me that queens mated to drones under two weeks of age produced a large percentage of infertile eggs. (See his forthcoming report for data.)

complete extrusion of the penis with ejaculation of spermatic fluid. Removing the abdomen from the thorax before dissecting lessens the effect; reducing the temperature also renders old drones less irritable (but young ones, three days old, more so). Slow injection of all fixatives containing acid causes contraction of gland and vesicle, with bursting of the gland or extrusion of contents through the ejaculatory duct.

More satisfactory results with Bouin's fluid finally led to the use of picric acid for killing, and the best results were obtained by injecting cold saturated aqueous picric acid solution through a fine-drawn pipette into the side of the thorax just beneath the wing, forcing the fluid in very slowly until the abdomen became slightly distended. This seemed to be effective partly through inhibiting the stimulation of the sex organs by the ganglia in the thorax, since indications of stimulation by these ganglia were observed before the irritant that was being injected could have reached the abdomen. Chloroform, ether, and cyanide were not satisfactory as anaesthetics to prevent distortion.

By rapidly opening a freshly cut-off abdomen under the low power of a binocular, the parts may occasionally be exposed quickly enough to allow of observation of the activity of the organs. The abdominal pressure that might force the penis to extrude is in this case eliminated by opening the abdomen, so that muscular contraction of the walls of gland and vesicle is the effective agent of the activity that follows. The typical observation under these conditions is twofold. First, a peculiar twitching contraction of the base of the mucous gland tends to straighten out the angle or elbow of this gland (pl. 1, fig. E), and often, by forcing the contents toward the distal end, bursts this through and releases the mucus in the abdominal cavity. Second the yellow spermatic fluid can be seen passing through the transparent vas deferens, base of the mucous gland, and down the ejaculatory duct to the penis. Mucus and sperm are thus separated, and microscopical examination of the organs killed immediately in this condition (pl. 3, fig. 10) shows that in the base of the gland the mouth of the vas deferens has been forced against the blind end of the ejaculatory duct. Generally the

Correlation between the age of drone and the stage of development and the functioning of the organs of sex

STAGE	AGE	SPERMATOGENESIS	SEMINAL VESICLE	ACCESSORY GLAND	EJACULATORY DUCT	RESPONSE TO STIMULI CAUSING EXTRUSION OF PENIS
A	4 days before emerging. Eyes lightly pigmented	Testes 5 mm. fill abdomen. Last stages spermatogenesis. First spermiogenesis	Secretion commences in vas deferens. Cells of ves. granular	Very little secretion distally. Length, $2\frac{1}{2}$–3 mm.	Branch has penetrated gland, chitin thin and colorless	
B	2 days before emergence. Eyes dark, body chitin low yellow	Spermiogenesis, sperms descend to upper part of seminal ves.	Secretion in vesicle, wall becomes corrugated in upper part	Distal cells strangulate, secretion granular. Length, 3 mm.	Blind end of duct thickens, two chitin layers separate	No appreciable secretions. Penis will not extrude on stimulating until just before emergence
C	Drone emerges	Spermiogenesis finished. Sperms half way down sem. vesicle. Attach to walls	Most of surface ridged, deeper at upper end. Granules of secretion dissolve	Cells of distal half active. Length, $3\frac{1}{2}$ mm.	Cells of end of duct begin to withdraw laterally. Chitin thickens	Drone will respond to strong stimuli by extrusion of organ, but usually only part way, and without ejaculation of secretion
D	3 days old	Vesicle full. Some sperms still free, most attached. Testis, 3 mm.	Vesicle enlarges with sperms	Distally wall is very thin, and gland cells resolved completely there. Bulbous contour. Length, $4\frac{1}{2}$ mm.	End of duct exposed to glandular epithelium of gland	Drone responds more readily than at stage C, but with no secretion, or with the ejaculation of mucus alone. Organ may be fully extruded if stimulus is violent and drone warm

E	5 days old	Few free sperms. Testis turning greenish in color, 2 mm.	Glandular wall thin and deeply ribbed. Vesicle full sized	Basal region resolving. Cells over ejac. duct recede. Apparent functional maturity. Length, 5–5½ mm.	Apparently mature	Organ may be caused to extrude with ejaculation of mucus and sperms. Latter are often not very active
F	9 days old	Same condition. Green tint more pronounced	Seminal vesicle, no change. Cells of vas deferens to gland vacuolated, lumen opened wider	Still slight secretion. No change in size	No change	Organ extruded more readily with characteristic explosive violence, and with ejaculation of sperms first, followed by mucus. Sperm active
G	12 days old	Testis 1½ mm. Same condition as above	No change	No change distinguishable	No change	Apparatus apparently mature after ninth day, though reaction seems to be more violent and more easily produced up to the 12th day. After that no change was detectable with methods used
H	21 days old	No change	No change	No change	No change	

chitinous drum will have been burst through; the valve guarding the entrance of the vas deferens will be pressed against the opposite side of the gland's lumen so as to occlude the latter, and the mucous content will often have burst through the anterior end of the gland's wall. If the organs are not killed at once, the musculature relaxes, and the gland assumes a somewhat more normal contour.

When the drone is opened more deliberately, especially after the injection of an acidified fixative or after stimuli that cause partial extrusion of the penis, the picture is different. The spermatic fluid will have passed down as before through the base of the gland and ejaculatory duct into the bulb of the penis (text figs. 1, *b*, and 2, *A* or *B*, *b*), but the mucous content of the gland will in this case have followed it down. The two are still unmixed. The sperm is invariably collected at the lower end, as if it had come down first, and the mucus lies behind it. From this it is concluded that after the first spasmodic contraction of the gland and vesicle that expresses the spermatic fluid through the gland's base, and shuts off the outlet of the mucus through the same channel, the muscles then relax enough to open the lumen again, and the mucus follows the sperm into the penis. The bursting of the end of the gland is in this case prevented by the increased and compensating pressure of the abdominal contraction, since the muscles of the abdomen contract in coordination with the sexual apparatus.

Very rarely, by careful opening of the drone, the gland does not burst upon contraction, and after relaxation its mucous content can be seen to follow the sperm down as has been inferred to be the normal manner.

With this manner of action in mind, various methods of stimulating the drones were tried, to find by what method could be brought about the most complete ejaculation of both mucus and sperm, together with complete extrusion of the penis, in the order above stated. The following treatments tested on mature drones are set down in order of increasing effectiveness in producing the desired effect.

1. Bouin's fluid, cold, or picric acid solution injected through the thorax caused no disturbance of the organs with careful handling. Cooling in general reduced irritability.

2. Slow pressure on the sides of the abdomen, especially if the drone was cold, often caused bursting of the abdomen between the sclerites, sometimes extrusion of the penis without ejaculation of fluid.

3. Allowing the drones to fly toward a bright window or artificial light and warming them to 40°C. often made pressure on the abdomen effective in causing ejaculation, but not always was the ejaculation complete.

4. Weak acid or fixative containing acid injected into the thorax, and especially into the abdomen, caused very violent contraction of the organs, but often killed the tissues without complete ejaculation of the fluids. Injection of these acidified reagents into the abdomen through the thorax caused ejaculation most frequently.

5. Sudden and slight pressure applied with the fingers to the sides of the abdomen of a warm excited drone generally caused a violent extrusion of the penis with complete ejaculation of both sperm and mucus. This was accompanied by an intense contraction of the muscles of the abdomen. This reaction of the drone was in close conformity with the result to be expected from anatomical considerations. The pressure is to be interpreted distinctly as a stimulus, and need by no means be sufficiently great to forcibly express the penis without a very pronounced reaction from the drone itself.

6. The most complete and most uniform results were obtained by holding a drone by the head, allowing him to use his wings as in flight until he was intensely excited, and the abdomen became distended as in rapid flight; the head was then deliberately pulled from the thorax. The drone reacted with what is believed to be substantially a normal orgasm as far as concerns the state of the organs of sex. It is inferred that the violent stimulus of decapitation under these conditions in some way duplicated the stimulus of sexual excitement, as far as the sexual mechanism is concerned.

Under this treatment, the penis everts throughout its whole length, including the bulb at its end (text fig. 2, *C*); the expanded end of the ejaculatory duct is brought through the bulb to form a cup-shaped disk at the extremity of the penis (fig. 2, C, *c'*), and from the central perforation of this cup (the ejaculatory duct) proceeds first a drop of yellow sperm, then a white mass of viscous mucus. The emptied and distorted sexual organs (mucous glands and seminal vesicles) are often forced into the base of the extruded penis by the violent contraction of the abdominal walls. (text fig. 2, *C*, *d*). The drone is paralyzed or stunned, but sometimes recovers enough to crawl about feebly, and may live for several hours.

CONCLUSION

It may be seen, therefore, that several lines of evidence point consistently to one specific manner of functioning of the sexual mechanism of the drone. First, the anatomical arrangement of the parts is such that the seminal vesicle is in more direct communication with the ejaculatory duct than is the mucous gland, and this connection is of such a nature as to suggest definitely a separate discharage, and therefore a distinctive separation of function, for sperm and mucus (p. 239 and pl. 1, E). Second, the arrangement of the musculature is such that its contraction brings about exactly that arrangement of ducts and apertures which will discharge first sperm, and then mucus, into and through the basal pocket of the gland and thence into the ejaculatory duct and penis (p. 243 text fig. 3, and pl. 3, fig. 10). Third, the physiological behavior of sperm and mucus is so characteristically different as to suggest a difference of function and disposal (pp. 246 to 247). Fourth, by actual observation, a disposal of sperm and mucus, entirely consistent with the anatomical and physiological findings, is induced by stimuli that may be considered closely to simulate, or even actually to duplicate, those stimuli that cause the normal reactions of the sexual organs. Under suitable conditions, the action of these organs, and the passage of the secretions through them in the expected order, may be observed under the microscope (p. 250). Finally, as will be shown in a subsequent paper, these secretions dispose themselves in the organs of the female at the time of copulation, and are disposed of by the female's reproductive mechanism after copulation, in a manner not only entirely consistent with the interpretation given above, but in an order that seems to preclude any interpretation which deviates materially from one herein set forth (p. 248).

SUMMARY

1. The drone is not sexually mature at the time of emergence of the imago, but undergoes a further growth period of at least nine to twelve days. The progress of this development is described in this paper for the sexual organs.

2. The sperm and the mucous of the accessory gland change both in character and in behavior as the process of development goes on, as does also the mode of functioning of the organs which elaborate and contain them.

3. Sperm and mucus each remain in their respective receptacles until copulation, and do not mingle before that time.

4. The partition closing these organs off from the ejaculatory duct, consisting of the chitinous lining of the blind end of that duct, does not break through until copulation. Then the secretions burst through it as they are forced out of their receptacles by contraction of the muscular walls of these organs.

5. The musculature of the whole base of the gland is so arranged as to cause, on violent contraction, the shutting off of the distal portion of the gland from the proximal by a muscular valve. The mucous content is thus closed off from its outlet through the ejaculatory duct; at the same time sperm is allowed to pass through the vas deferens and basal portion of the gland into the ejaculatory duct. This spermatic fluid is thus the first to be ejaculated.

6. The mucous content of the gland, upon relaxation of the muscles of the base of the gland, is then free to pass after the sperm, and forces all the sperm out of the organs. It also apparently forms a plug by coagulating on exposure to the air (e.g., when the penis is torn from the drone at the time of copulation).

7. The bulb and elastic end of the ejaculatory duct do not act as a spermatophore, although after copulation, and while still attached to the queen, they may serve to hold what mucus may not have been fully expressed into the oviducts.

8. The drone's organs may be inspected in an undistorted state only by the most careful manipulation, as they are easily stimulated to contraction and explusion of their contents. This contraction may be watched in a freshly opened drone. It is inhibited by injection of picric-acid solution into the thorax and thence into the abdomen. It is stimulated by injection of acids or of fixatives containing acids. The use of acidulated fixatives may be responsible for erroneous views that have been put forward as to the normal quiescent condition of these organs.

9. A response apparently duplicating the results of the normal act of copulation may be produced with considerable certainty by various means, as enumerated.

PLATES

PLATE 1

EXPLANATION OF FIGURES

C, D, E, and H Stages of development of the sexual organs of the drone (table in text, p. 252). Camera-lucida drawings of whole mounts, to show, in opitcal section, changes in size and conformation of the sex organs from emergence of the imago until sexual maturity. × 18.

Black shading, muscular envelope; lines and dots, glandular epithelium; broken lines, spermatozoa; dots, mucous secretion; cross-hatching, hypodermis of ejaculatory duct; lines, chitinous lining of same. *c*, ejaculatory duct; *d*, body of gland; *e*, seminal vesicle; *f*, vas deferens from testis; *h*, vas deferens leading from vesicle to gland; *i*, basal region of gland; *j*, cone-shaped end of ejaculatory duct, applied to *i*; *k*, slender muscle attaching gland to posterior abdominal wall; *l*, muscular valve guarding vas deferens orifice, and partially separating *d* from *i*; *m*, region of lumen of gland extending out into elbow of gland; *n*, chitinous drum over end of ejaculatory duct.

Legend on figure D applies alike to all. Numbered lines on C and E locate cross-section drawings of subsequent figures (q.v.).

C Drone at time of emergence. Secretion has just commenced in distal portions of gland and vesicle.

D Drone three days old. The bulbous expansion of distal portion of gland as a mucous reservoir is noticeable, and the spiral serration of the vesicular wall has extended throughout the organ.

E Drone five days old. Organs almost mature. Sperm attached to walls of vesicle, wall of gland largely resolved into mucus, chitinous end of ejaculatory duct attenuated.

The fixative has stimulated the basal musculature of the gland to slight contraction, without, however, discharging the content of either gland or vesicle. The transverse pocket, *i*, into which open vas deferens, *h*, and ejaculatory duct, *j*, is closed off from the distal reservoir of secretion, *d*, by the valve, *l*, and the ends of the vas deferens and ejaculatory duct are brought almost into apposition (compare pl. 3, fig. 10).

H Drone twenty-one days old, basal portion of gland and seminal vesicle. Later stages than five days show but slight further modification. The basal portion of the gland here is not distorted, as in E, but shows normal resting relationships of the parts. Even at this age the contents of the organs have not been discharged into the bulb of the copulatory organ.

1 to 4 Successive cross-sections of accessory mucous gland and seminal vesicle of drone at stage A, four days before emergence. × 24. Located on figure C, by lines numbered 1 to 4, respectively. Legend same as for figure D, with addition of *X*, *Y*, *Z*, regions of three inner muscle tracts.

1 Section through branched end of ejaculatory duct, and the basal portions of the two mucous glands.

2 Section through gland just above entrance of the vas deferens. Between the area representing the section of the distal portion of the gland's lumen, *m*, and that representing the basal portion, *i*, the black band, *l*, represents the edge of the muscular valve which, on contraction of the basal musculature of the gland, closes off the basal portion from the distal mucous reservoir (figs. C and E)

3 Section at middle of gland. Between *X* and *Y*, *Y* and *Z*, and *Z* and *X*, may be seen in cross-section the three channels into which the gland's lumen is modeled by the three muscle tracts, *X*, *Y*, and *Z*.

4 Section of the gland just anterior to the end of the seminal vesicle, through the region of the testis in the abdomen.

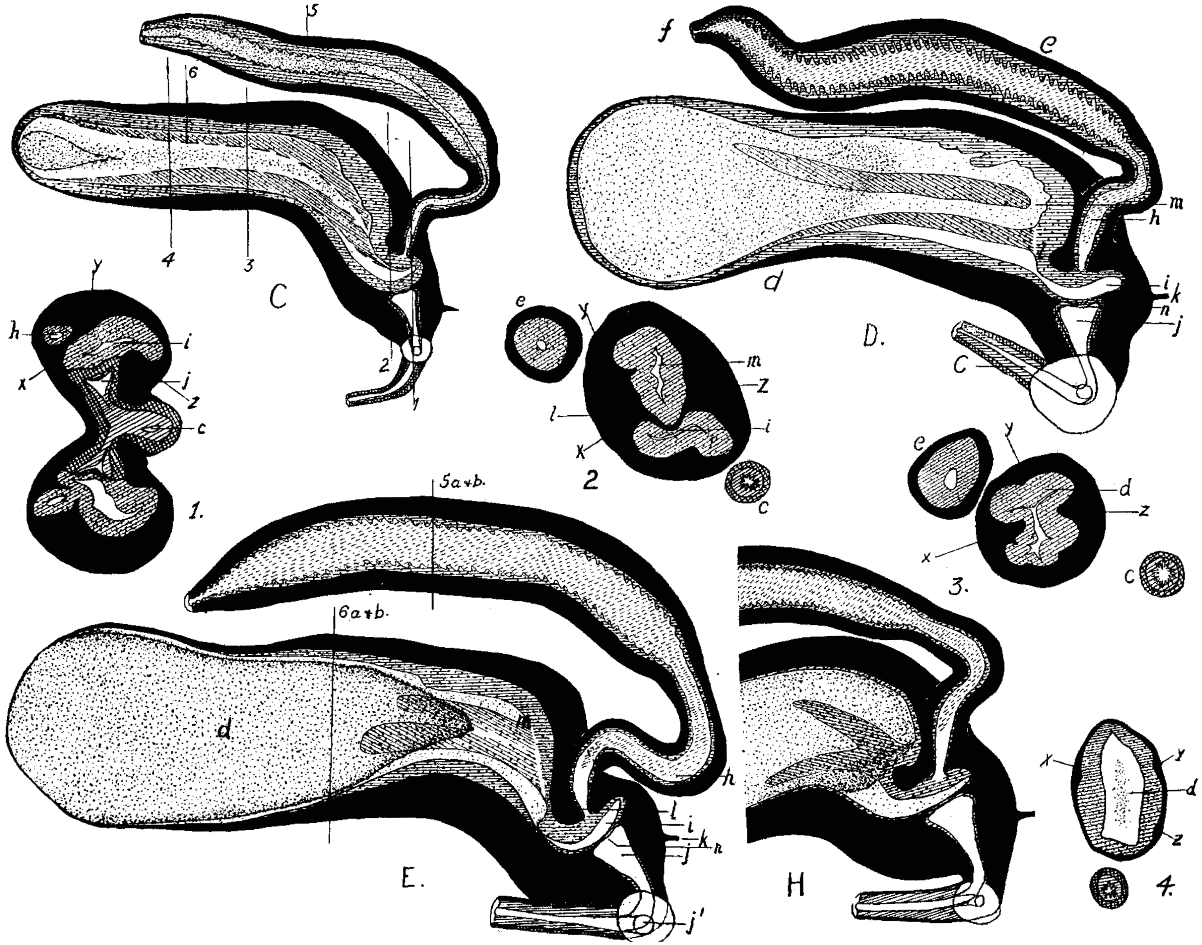

C
D.
E.
H
1.
2
3.
4.
5a+b.
6a+b.

PLATE 2

EXPLANATION OF FIGURES

Camera-lucida drawings of cross-sections of gland and vesicle, located by lines numbered 5 and 6 on figure C, and 5a and 6a on figure E, plate 1. All 10μ thick.

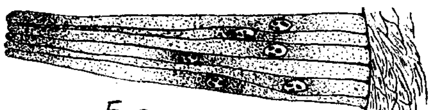
5 Cross-section of seminal vesicle, stage C $\times$ 135. *e*, loose membranous connective-tissue envelope of vesicle; *l.m.*, longitudinal muscle layer; *c.m.*, circular muscle layer; *g*, globules of secretion from the glandular cells, not yet resolved to a homogeneous fluid; *a*, *b*, *c*, *d*, sections of the four successive spiral ridges into which the wall of the vesicle is sculptured by uneven erosion of the glandular lining.

5a Enlarged view of epithelial cells of same. Cross-section of portion of seminal vesicle, glandular epithelium, stage A. $\times$ 340. Secretion has not commenced, but the granular band across the ends of the cells and the denser areas around the nuclei indicate the inception of the process.

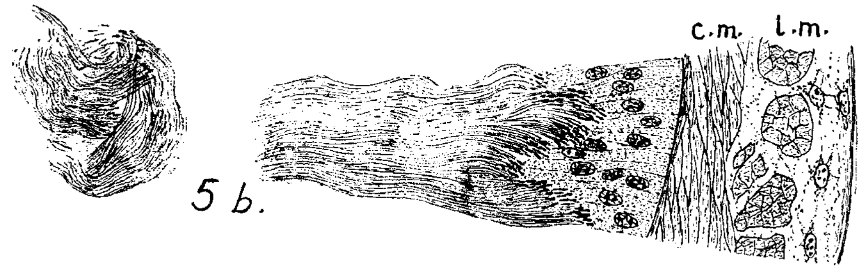
5b Cross-section of wall of seminal vesicle, glandular epithelium, stage E. $\times$ 340. Sperms arranged radially, heads attached to cells of the epithelium. Centrally a narrow lumen contains loose sperms in bundles just descended from the testis. The granular secretion of figure 5 has here resolved to a clear plasma, densely packed with sperms.

6a Cross-section of glandular epithelium of gland, stage A, before secretion has commenced. $\times$ 340.

6b Same as above, stage E, late stage of secretion. $\times$ 340. A mass of secretion, *s*, lies in the gland's lumen, globules of secretion, *g*, are strangulating off from the distal ends of the cells, which thereby decrease in length, cells heavily vacuolated at *v*.



5 a.



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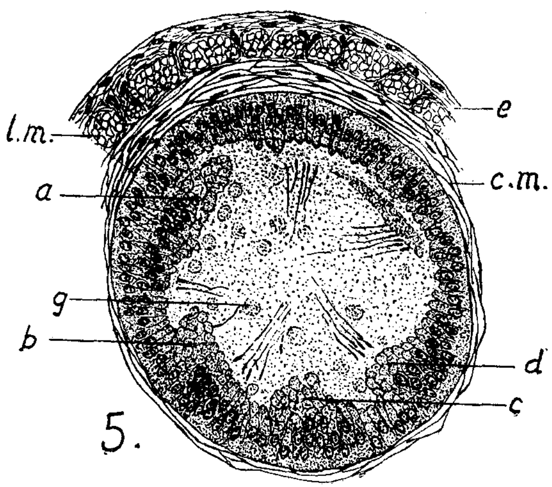
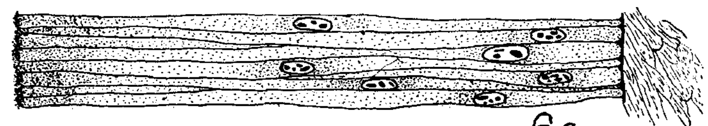
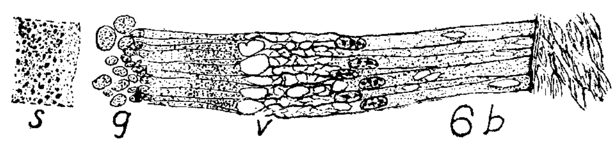


5.



6 a.



6 b

PLATE 3

EXPLANATION OF FIGURES

7 Diagrammatic cross-section through end of ejaculatory duct and base of gland. Stage C. × 37. Black, muscle; lines and dots, glandular epithelium; cross hatching, epithelium of ejaculatory duct; lines, chitinous lining of same. *h*, vas deferens; *j*, end of ejaculatory duct; *c*, section of middle portion of ejaculatory duct passing anteriorly along the gland; *i*, basal pocket of gland; *l*, valve partially dividing this from the distal portion *m*. (Compare text fig. 1.)

8 *a–g*. Serial sections of base of gland, to show the formation of the double chitinous partition over the end of the ejaculatory duct. Stage B. × 37. 10μ. Lettering as on figure 7. Dotted lines in *a* show area included in *b–f*. Figure *g* is that of a section through center of the end of the duct.

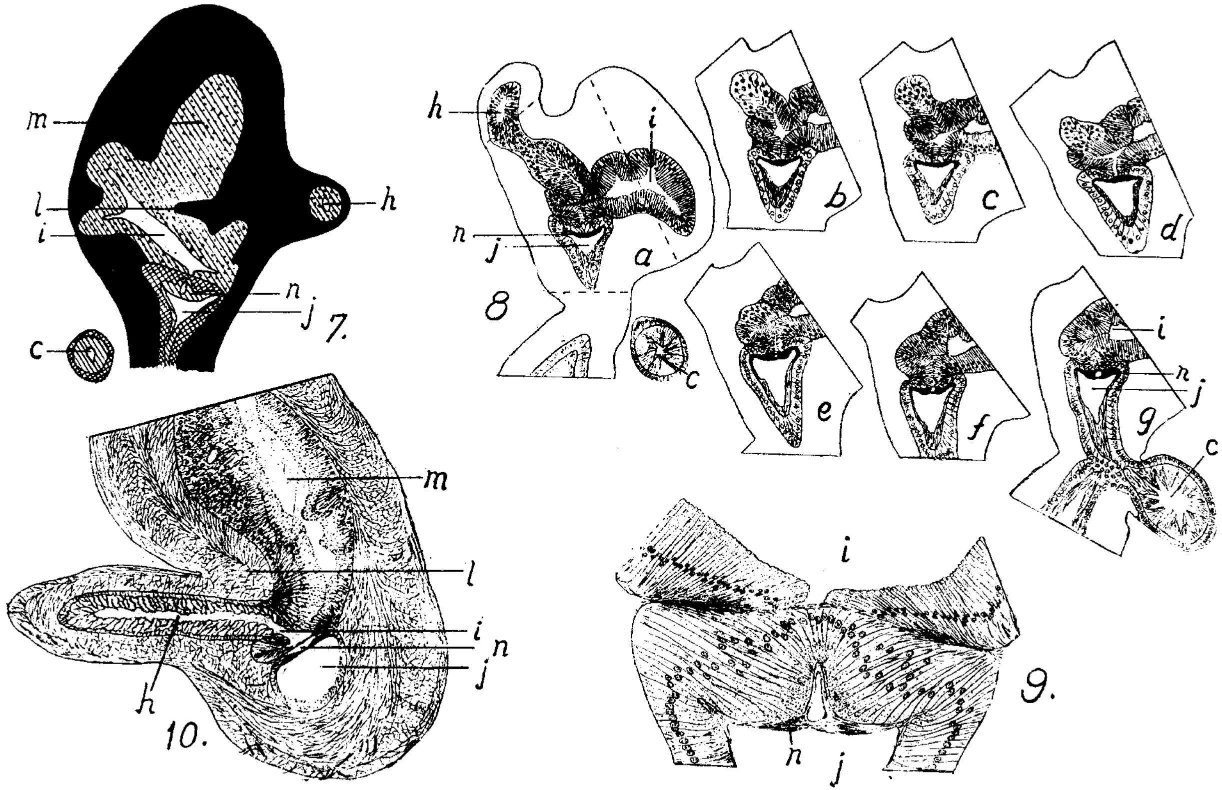
9. Higher magnification of the condition shown in figure 8g, but at later stage of development, C. × 180. 8μ. Lettering as on figure 7.

10 Section through lower part of vas deferens and base of gland, at a slight angle from the sagittal plane. Stage H. × 37. 10μ. Lettering as on figure 7.

The organ here figured, from a drone twenty-one days old, has contracted because of the injection of the fixative, so as to bring the vas deferens orifice and the end of the ejaculatory duct in apposition (at *n*), and force the valve *l*, against the opposite side of the gland's lumen, dividing this (*d*) from the basal pocket, *i*. The distal end of the gland (not figured) has burst, releasing the mucus into the abdomen. The seminal vesicle has not contracted, so that the drum of chitin, *n*, over the end of the ejaculatory duct, *j*, is left intact.

This condition of the basal portion of the gland therefore apparently duplicates the condition, during copulation, momentarily obtaining before ejaculation takes place. Spermatic fluid alone could enter the ejaculatory duct from the vas deferens, the mucus being retained in the distal reservoir of the gland by the closing off of the basal region of the gland.

An idea of the complexity of the musculature of the gland's base may be obtained from the figure, though the angle at which the section has been cut makes it difficult to trace the several layers distinctly.


m
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