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Structure and Development of the Testes in the Adult *Sitophilus zeamais* Motschulsky (Coleoptera : Curculionidae)

by

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(With six plates)

INTRODUCTION

Although detailed studies have been done on the structure of the testes of the Coleoptera in general (Snodgrass, 1935 ; Zacharuk, 1958 ; Imms, 1964 ; Wigglesworth, 1965 ; Chapman, 1969 ; Gerber et al. 1971), very little is known about the structure of the testes of the Curculionidae. Khan (1949), while studying the organ systems of *Sitophilus oryzae* (L.) devotes only some general attention to the structure of the testes. There is no detailed study on the development of the testes in any adult Coleopteran.

This investigation was undertaken to study the morphological and histological structure of the testes and the associated glands of the adult *Sitophilus zeamais* Motschulsky (Curculionidae), and also to determine the changes which occur in the course of the adult life.

MATERIALS AND METHODS

The bionomics and the economic importance of *S. zeamais* have been dealt with in earlier communications (Ganesalingam, 1972 ; 1974). The weevils were reared on rice grain under laboratory conditions as described previously, except that small holes were made in the plastic caps of the rearing bottles. The average temperature during the experimental period ranged from 17.9°C to 30.8°C, and the average relative humidity was 76.3 %.

The procedures in dissection of the weevil and preparation of the whole mounts and histological sections of the testes, were similar to those described earlier (Ganesalingam, 1974).

OBSERVATIONS

S. zeamais has a pair of testes ; each testis consisting of a pair of white globular and shiny kidney-shaped follicles apposed to one another (Plate I). All the four follicles are covered by a common sheath, the tunica which holds them together (Plate II, 1), but each follicle has also its own sheath. There is no connection between the follicles in each testis. A short vas efferens arises from the inner side of each follicle and on joining its fellow

expands to form the vesicula seminalis (Plate II, 2). The vas efferens is short, narrow and thin walled (Plate II, 3). The vas deferens passes posteriorly from the seminal vesicle and is then joined by the duct of the first accessory gland, which runs a short distance alongside it, and at the point of junction opens the second accessory gland (Plate II, 4). From this point a common duct passes posteriorly and joins its fellow of the other side to form the ejaculatory duct which passes to the aedeagus.

The interior of the follicle shows zones of differentiation representing the various stages of development of spermatozoa. The most peripheral region, the germarium is occupied by the zone of the spermatogonia. Each follicle of the newly hatched weevil has about sixteen such groups of spermatogonia lying external to the zones of spermatocytes and spermatids which lie internally. Developed spermatozoa are found in groups around the vas efferens, the heads of the spermatozoa being joined together, while the tails of the individuals forming the groups are free (Plate III, 1, 2). But in the region of the seminal vesicle, separate spermatozoa can be distinguished (Plate III, 3; 4).

In the follicles there is no distinct apical cell in the apex of the germarium. But each group of developing germ cells has a clear central region which is occupied by a cell-like area that does not stain with Eosin (Plate IV, 1 ; 3 ; 4). Scattered amongst the developing germ cells are small triangular cells which probably represent the cyst cells of the typical testes. These cells are found even amongst the spermatozoa (Plate IV, 1 ; 2).

The first accessory gland has an oval head, tapering into a narrow duct which turns abruptly and runs alongside the vas deferens. The second accessory gland is spherical and internally divided into unequal chambers by septa. The number of chambers in the latter ranges from 6 to 11 (Plate V, 1 ; 2).

The spermatozoa are found in the follicles even in newly emerged weevils, but the spermatozoa do not pass into the seminal vesicle until the third day after emergence. From the third day onwards the spermatozoa are stored in the seminal vesicle and progressively passed into the vas deferens. The testes enlarge steadily in the first few weeks and reduce in size as the weevil grows older. Under laboratory conditions the weevils normally start dying around the age of 90 days, but some may live even up to about 200 days. Spermatogenesis continues to take place even in the very old weevils of 180 to 200 days, and the testes of these specimens which die naturally through ageing also showed spermatogenesis (Plate VI, 1 ; 2 ; 3). Copulation too has been observed even as late as 180 days after the adult emergence.

The first accessory gland does not show any changes in the first three days after emergence, but it shows changes in appearance from the third day onwards, and enlarges subsequently. The second accessory gland, although it enlarges initially, does not show any changes throughout the adult life of the weevil.

DISCUSSION

As described above, in *S. zeamais* there are two follicles or sperm tubes in each testis, amounting to a total of four. In Coleoptera-Adephaga there is a single follicle in each testis (Chapman, 1969). But there is considerable variation in the number of follicles in Coleoptera-Polyphaga. There are 6 follicles in each of the two testes of *Phyllophaga anxia* (Le Conte) (Scarabaeidae) (Berberet and Helms, 1972), and in *Attagenus megatoma* (F.) (Dermestidae) (Dunkel and Bousch, 1968). There are 40 sperm tubes in each testis of *Agrypnus murinus* (L.) and *Agriotes obscurus* (L.), 48 in *Ctenicera aeripennis destructor* (Brown) and 50 to 60 in *Ctenicera aena* (L.) and *Ctenicera lata* (F.) (Elateridae) (Zacharuk, 1958). In *Lytta nuttalli* Say (Meloidae) there are in average 148 sperm tubes ranging from 118 to 177 (Gerber et al. 1971).

Snodgrass (1935), states that the number of sperm tubes of a male insect is less than the number of the ovarioles in the corresponding female. In *S. zeamais*, the number of ovarioles in the female is four (Ganesalingam, 1974), which is the same as the number of sperm tubes in the male.

Although the shape of the follicles in the Coleoptera in general is tubular, in *S. zeamais* they are kidney-shaped and their shape resembles those of *P. anxia* (Berberet and Helms, 1972) very much.

At the time when Khan (1949) was working on the rice weevils, they were regarded as the species *S. oryzae*. Later, Kuschel (1961), Halstead (1964) and Proctor (1971) distinguished two species, one which retained the original name *S. oryzae*, the other which was named *S. zeamais*.

Khan (1949) has described that each testis was 3 lobed in the newly emerged weevil but became divided into 5 lobes thereafter. But the present study shows that each testis contains two follicles and there is no such division as described by Khan. This has been found to be a very constant feature in all specimens dissected by the author not only of *S. zeamais* but also of *S. oryzae*. In this study in which the structure of the testis in the adult *S. zeamais* was studied from the day when the weevil emerged and in all stages subsequently, it was found that each testis, which is bilobed in structure, formed by 2 follicles at the time of emergence, remained so throughout adult life.

Only a single pair of accessory glands is described in the male *C. a. destructor* (Zacharuk 1958) and in *P. anxia* (Berberet and Helms, 1972). Gerber et al. (1971) describe 3 pairs of male accessory glands in *L. nuttalli*. But in the case of *S. zeamais* only 2 pairs of accessory glands were observed.

Khan (1949) describes the glands close to the seminal vesicle as the accessory gland and that which is farther from the seminal vesicle as the prostate gland. As no special function can be attributed to the latter, it is proposed to refer to these glands as the first accessory gland, and the second accessory gland respectively.

Khan (1949) considers that in *S. oryzae* the first accessory gland opens into the seminal vesicle, which subsequently opens into the second accessory gland ('prostate gland'). But from the present study, the seminal vesicle is distinguihsable as an enlarged reservoir which receives the vasa efferentia anteriorly, and the duct-like part which proceeds posteriorly would represent the vas deferens. In this work it was found that the vas deferens runs alongside the duct of the first accessory gland, and at the point of junction the second accessory gland opens.

Khan (1949) states that the other accessory gland which he refers to as the prostate gland, is divided into 5 equal lobes. But it was found in this study that the number of lobules of this gland varies from 6 to 11. This has been found to be so by the author in the case of *S. oryzae*. The function of these two accessory glands is no doubt the normal one of forming the medium for the transport and transfer of sperms, but the detailed structure of the gland and effects of the secreted material were not investigated.

The general pattern of spermatogenesis is similar to that described by Snodgrass (1935) and Wigglesworth (1965) for insects in general ; but unlike in the case of linearly elongated sperm tubes, in this case neither a terminal region nor an apical cell could be distinguished. Even in another Coleopteran, *Lytta nuttalli*, no apical cell could be distinguished (Gerber et al. 1971). But in this study, the cell-like area that occupies the central region of the developing germ cells may be of some significance in this connection. The appearance and the position of the triangular cells found amongst the groups of spermatocytes, spermatids and even among the spermatozoa indicate that these cells are the cyst cells or derivatives of the cyst cells, and support the conclusion that they nourish the gametes during their development, as stated by Bonhag and Wick (1953).

De Wilde (1964) presumes that separation of spermatozoa may take place by a secretion of an enzyme which may dissolve the connecting cap of the spermatozoa while they are in the seminal vesicle or while leaving the testis. This study shows that the spermatozoa are separated even before they move into the seminal vesicle. Therefore, if an enzyme is involved in the separation of spermatozoa as suggested by De Wilde, this is presumably released in the testis itself.

The movement of the spermatozoa into the seminal vesicle only on the third day after emergence of the adult *S. zeamais*, along with the changes in the accessory glands, has some significance in that the spermatozoa may be in a position to fertilize the eggs successfully only from the third day onwards.

The development of the testis of *S. zeamais* described in this study and that of the ovary of this species (Ganesalingam, 1974) show that the fertile period of the testis is greater than that of the ovary. In the female the first fully formed eggs are evident on the sixth day after emergence and the new oocytes are produced continuously only during the first fifty days of its adult life. In the male, spermatogenesis occurs even in the newly emerged adult and the spermatozoa commence to pass from the testes into the seminal vesicle from the third day onwards, and spermatogenesis continues to take place throughout its lifetime.

SUMMARY

Sitophilus zeamais Motsch. has a pair of testes, each comprising a pair of kidney-shaped follicles. The vasa efferentia of both follicles join to form a seminal vesicle from which the vas deferens proceeds. The vas deferens and the first accessory gland run alongside and open into a common duct, and at the point of junction a second accessory gland is closely attached. The common duct joins with its fellow of the other side to form the ejaculatory duct which passes into the aedeagus.

The general pattern of spermatogenesis is similar to that described in other insects in general.

There is no distinct apical cell in the apex of the germarium. Each group of developing germ cells has a clear central region which is occupied by a cell-like area. Cyst cells are found amongst the developing stages of the germ cells, even in the spermatozoa.

The spermatozoa are found to have developed even in the newly emerged weevil, but they do not pass into the seminal vesicle until the third day after emergence. The spermatozoa are stored from the third day onwards in the seminal vesicle from which they pass down into the vas deferens. The heads of the spermatozoa are joined together in the testis forming a cap, whereas the tails are free, but the spermatozoa are separated when they pass into the seminal vesicle.

Spermatogenesis continues to occur in this species throughout its adult life.

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REFERENCES

BERBERET, R. C. AND HELMS, T. J. 1972—Comparative anatomy and histology of selected systems in larval and adult *Phyllophaga anxia* (Coleoptera : Scarabaeidae). *Ann. Ent. Soc. Am.*, 65, 1026-1053.

BONHAG, P. F. AND WICK, J. R. 1953—The functional anatomy of the male and female reproductive systems of the milkweed bug, *Oncopeltus fasciatus* (Dallas) (Heteroptera : Lygaeidae). *J. Morph.*, 93, 177-284.

CHAPMAN, R. F., 1969—*The Insect: Structure and Function.* London, English University Press.

DE WILDE, J. 1964—Reproduction. In *The Physiology of Insecta.* Ed. M. Rockstein, 1, 9-58. Academic Press, New York and London.

DUNKEL, F. V. AND BOUSH, G. M., 1968—Studies on the internal anatomy of the black carpet beetle, *Attagenus megatoma Ann. Ent. Soc. Am.*, 61, 755-765.

GANESALINGAM, V. K. 1972—The development of the ovary in the adult *Sitophilus zeamais* Motsch. (Coleoptera : Curculionidae). *Proc. Cey. Ass. Advmt. Sci.* 1, 84.

GANESALINGAM, V. K. 1973—Structure and development of the testes in adult *Sitophilus zeamais* Motsch. (Coleoptera : Curculionidae). *Proc. Cey. Ass. Advmt. Sci.*, 1, 102-103.

GANESALINGAM, V. K. 1974—Morphological studies on the differentiation in the ovary of the adult *Sitophilus zeamais* Motsch. (Coleoptera, Curculionidae). *Ceylon J. Sci. (Bio. Sci.)* 11, 1-8

GERBER, G. H., CHURCH, N. S. AND REMPEL, J. G. 1971—Anatomy, histology, and physiology of the reproductive systems of *Lytta muttalli* Say (Coleoptera : Meloidea). I. The internal genitalia. *Can. J. Zool.* 49, 523-533.

HALSTEAD, D. G. H. 1964—The separation of *Sitophilus oryzae* (L) and *S. zeamais* Motschulsky (Coleoptera: Curulionidae), with a summary of their distribution. *Entomologist's mon. Mag.* 99, 72-74.

IMMS, A. D. 1964—*A General Textbook of Entomology*. London, Methuen & Co. Ltd.

KHAN, M. Q. 1949—A contribution to a further knowledge of the structure and biology of the weevils *Sitophilus oryzae* (Linn.) and *S. granarius* (Linn.) with special reference to the effects of temperature and humidity on the rate of their development. *Indian J. Ent.* 11, 143-201.

KUSCHEL, G. 1961—On problems of synonymy in the *Sitophilus oryzae* complex (30th contribution, Coleoptera : Curculionidae). *Ann. Mag. Nat. Hist.* 13, 241-244.

PROCTOR, D. L. 1971—An additional aedeagal character for distinguishing *Sitophilus zeamais* Motsch, from *Sitophilus oryzae* (L.) (Coleoptera, Curculionidae). *J. Stored Prod. Res.*, 6, 351-352.

SNODGRASS, R. E. 1935—*Principles of Insect Morphology*. McGraw-Hill Book Company, Inc. New York and London.

WIGGLESWORTH, V. B. 1965—*The Principles of Insect Physiology*. London, Methuen & Co. Ltd.

ZACHARUK, R. Y. 1958—Structures and functions of the reproductive systems of the prairie grain wireworm, *Ctenicera aeripennis destructor* (Brown) (Coleoptera : Elateridae). *Can. J. Zool.* 36, 725-751.

ABBREVIATIONS USED IN THE PLATES

AE	Aedeagus
AG 1	First accessory gland
AG 2	Second accessory gland
CA	Central area
CC	Cyst cell
DAG	Duct of the accessory gland — 1
ED	Ejaculatory duct
SC	Spermatocyte
SG	Spermatogonia
ST	Spermatid
SV	Seminal vesicle
SZ	Spermatozoa
T	Tunica
TF	Testis follicle
VD	Vas deferens
VE	Vas efferens

EXPLANATION OF PLATES

PLATE I

1. The testes of *S. zeamais* soon after emerging.

PLATE II

1. T. S. of entire testes of *S. zeamais* showing four follicles bound together by the tunica.
2. T. S. of the testis follicles showing the vasa efferentia and the seminal vesicle.
3. T. S. of the testis follicle showing the vas efferens, the seminal vesicle and the vas deferens.
4. L. S. of the second accessory gland, showing the vas deferens, the duct of the first accessory gland and the second accessory gland opening into a common duct.

PLATE III

1. T. S. of the testis follicle showing groups of spermatozoa.
2. L. S. of the testis follicle showing a single bundle of spermatozoa whose heads are joined together and the tails are free.
3. L. S. of the testis follicle showing the spermatozoa passing into the seminal vesicle.
4. L. S. of the seminal vesicle showing the spermatozoa in it.

PLATE IV

1. T. S. of a single group of developing spermatocytes showing the cyst cell at the periphery and the central area at the centre.
2. L. S. of bundles of spermatozoa showing the cyst cell.
3. T. S. of a single group of developing spermatids showing the central area at the centre.
4. T. S. of a group of developing spermatogonia showing the central area.

PLATE V

1. L. S. of the first accessory gland.
2. T. S. of the second accessory gland.

PLATE VI

1. T. S. of the testes of *S. zeamais* of 120 days old adult.
2. T. S. of the testes of *S. zeamais* of 130 days old adult.
3. T. S. of the testes of *S. zeamais*, which died on the 196th day after emergence.

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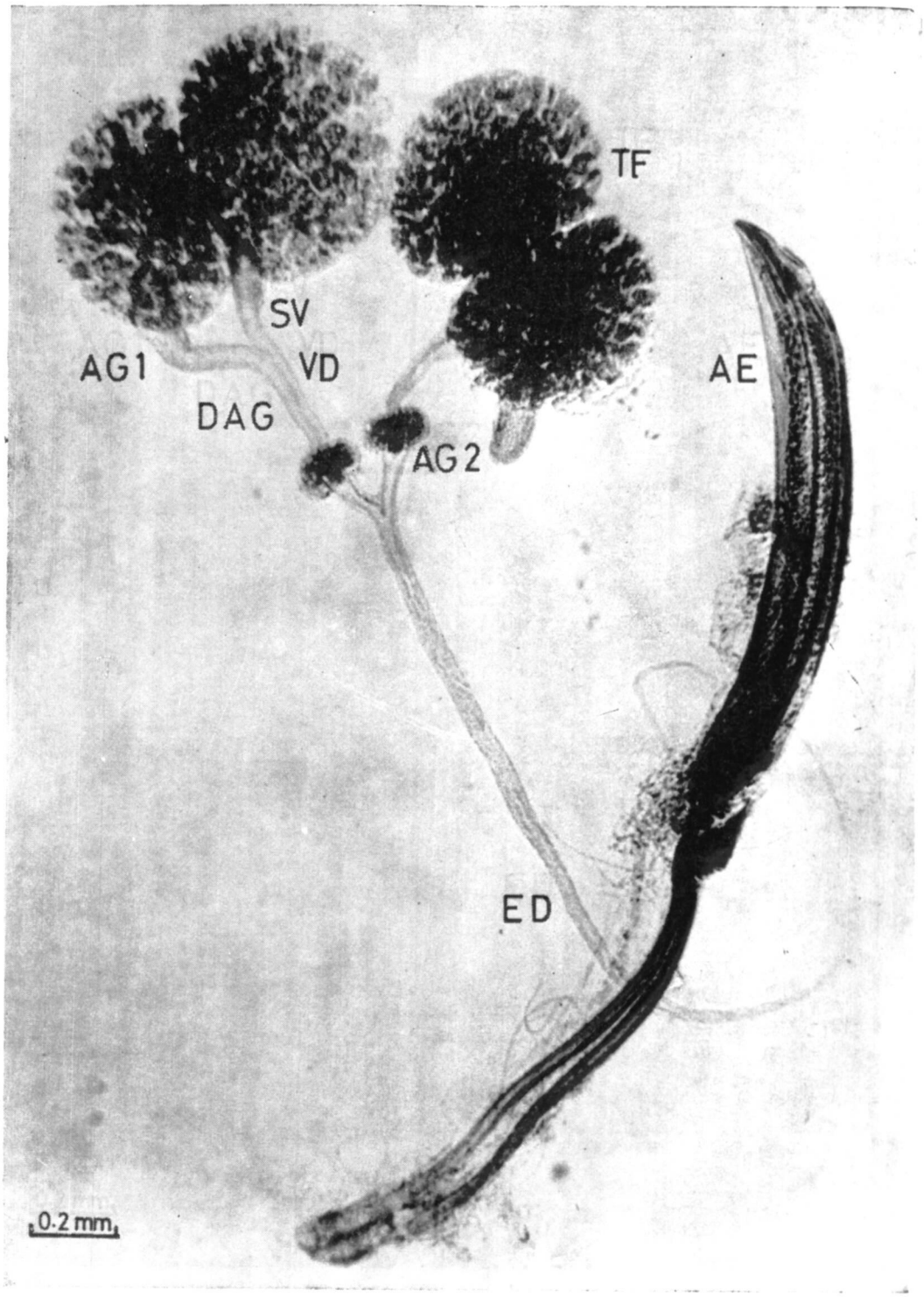
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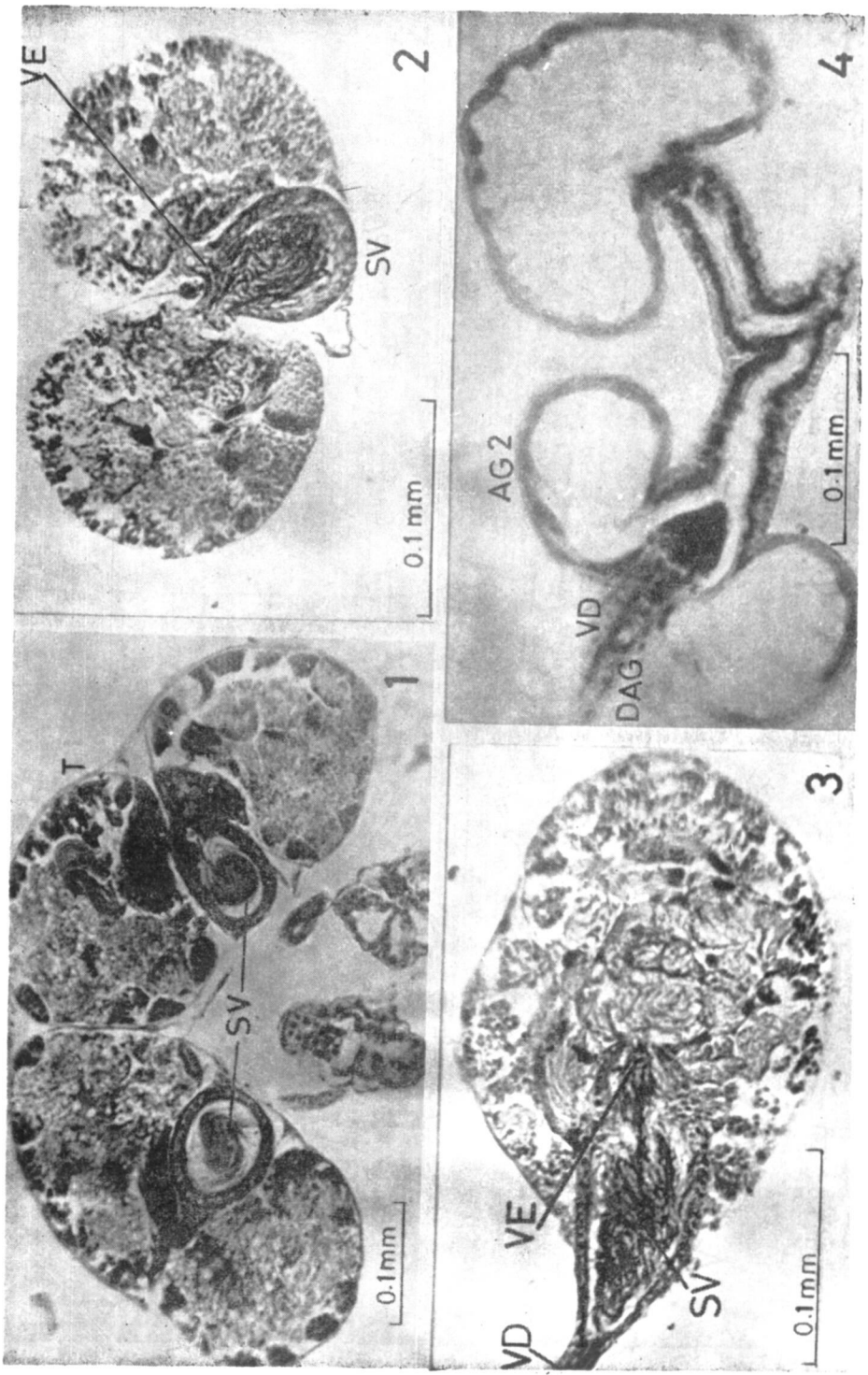
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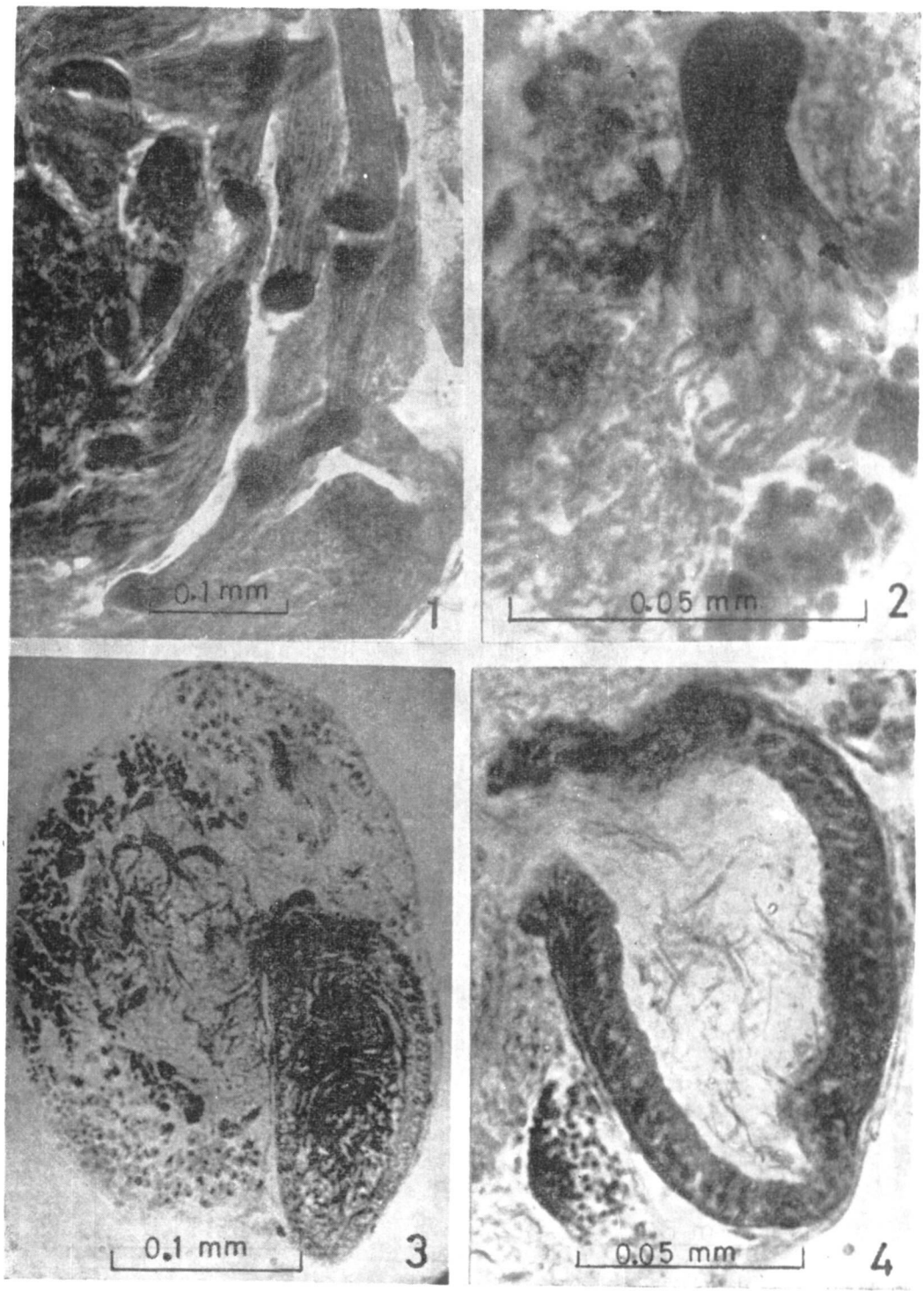
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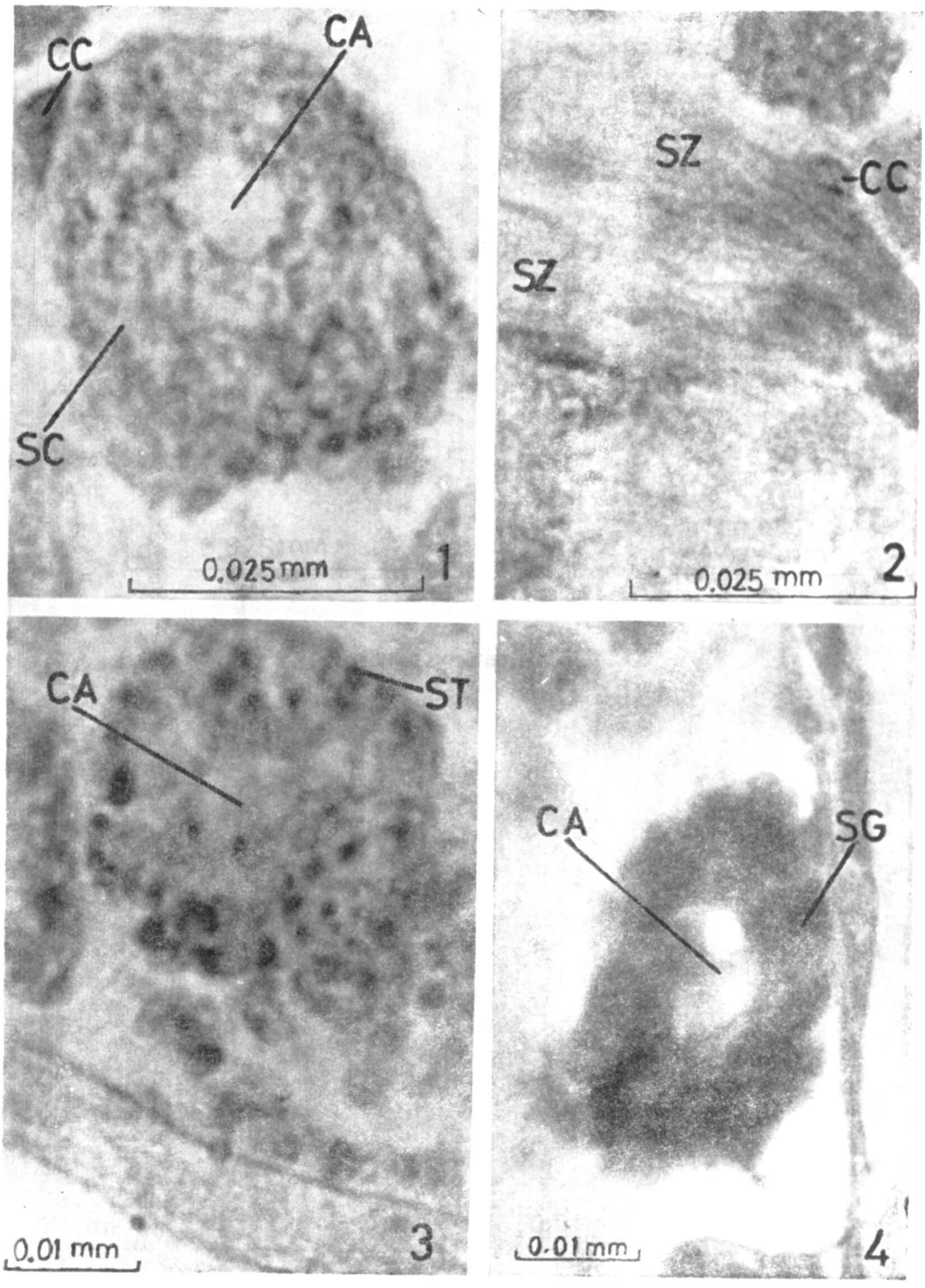
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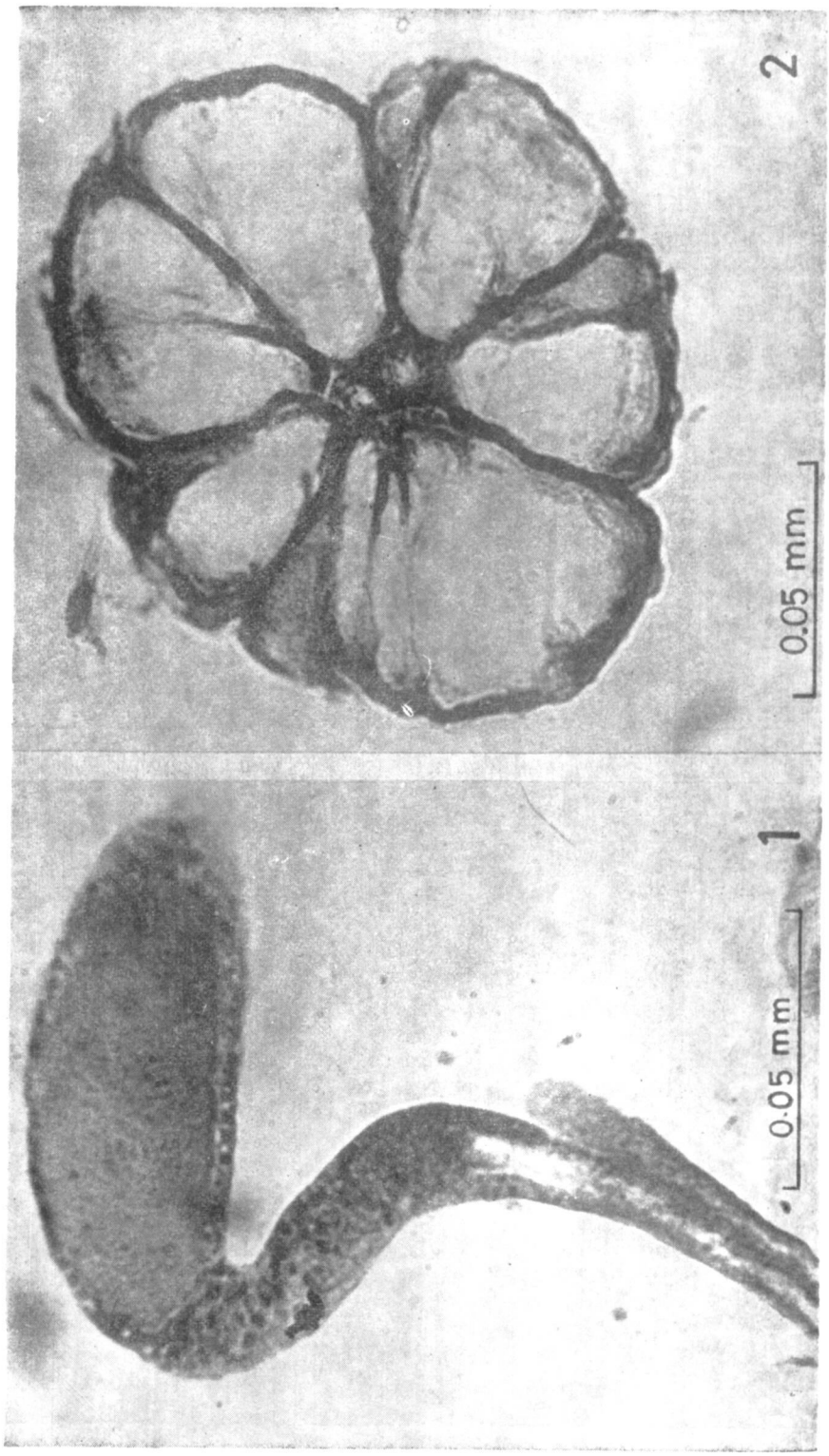
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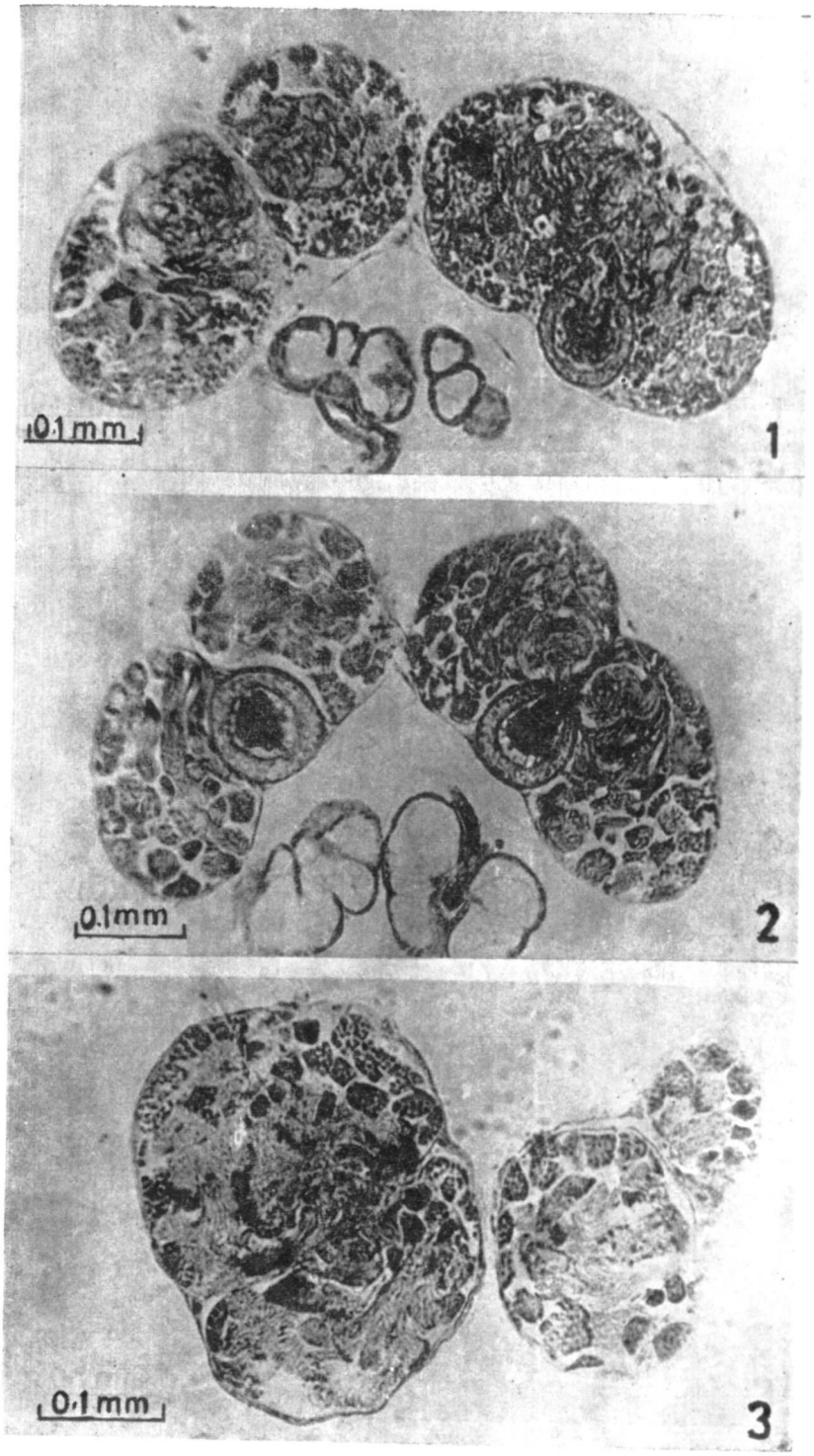
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