A STUDY OF THE REPRODUCTIVE TRACT OF *HELIX ASPERSA* MÜLLER AFTER PARTIAL GONADECTOMY

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Summary

Surgical gonadectomy was attempted in *H. aspersa*; control operations were also carried out. Mortality was high among both groups of operated animals.

The attempts at gonadectomy proved ineffective in that the surviving animals were found at dissection to have viable remnants of gonadal tissue located in pockets among the tissues of the digestive gland. It is concluded that surgical gonadectomy in this genus is not practicable since such remnants persist for at least 3 months after operation.

A total of 10 animals (6 gonadectomies, 4 controls) survived operation by periods of 7–13 weeks. The albumen glands and common ducts of these animals were subjected to histological study. No significant difference could be detected between the tissues of the gonadectomized animals and those of the controls. It is concluded that partial gonadectomy does not affect the tissues in question, and it is suggested that the genital tract of the Helicidae might not be under hormonal control of the gonad.

I. INTRODUCTION

An annual reproductive cycle has been reported in several gastropods: *Arion rufus* and *Helix aspersa* (Filhol 1938*a*, 1938*b*); *Limax maximus* (Abeloos 1943); *H. pomatia* (Ancel 1903; Baecker 1932); *Physa fontinalis* (Duncan 1958). According to Filhol (1938*a*) spermatogenesis ceases in winter in most pulmonates. Baecker (1932) has recorded cytological changes in the albumen gland of *H. pomatia* during the egg-laying season. Filhol (1938*a*) noted a decrease in size of the albumen gland of *H. aspersa* in winter. The same worker (Filhol 1938*b*) described cytological changes in the tissues of the common duct of *H. aspersa* which could be correlated with onset of winter: the epithelial cells lining the lumen of the oviducal portion of the common duct become charged with lipid, and the mucous cells outside this epithelium show regressive changes; the prostatic tissue located along the spermiducal portion of the common duct also shows regressive changes. Filhol interpreted these findings as indicating a cessation of functional activity during the winter months.

There is some evidence for gonadal control of the genital tract in gastropods. Abeloos (1943) succeeded in carrying out castration on an unspecified number of specimens of *L. maximus*. He reported that at dissection the oviducts of castrates showed no signs of glandular swelling and the albumen glands were small (". . . . l'oviducte ne montre aucun gonflement glandulaire et la glande de l'albumine est rudimentaire."), a condition contrasting with that seen in controls dissected at the same time of year (November). Abeloos suggested that the genital tract of

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Limax is under hormonal control of the gonad. Laviolette (1950) succeeded in carrying out grafting experiments on several species of the Arionidae and the Limacidae. He reported that grafts of gonad tissue taken from adult specimens of *Mesarion subfuscus* caused macroscopic changes in the genital tracts of two immature hosts of *A. rufus* within 1 month of implantation. The same worker reported that a fragment of ovospermiduct from a young specimen of *A. rufus* was implanted in the body cavity of an adult *Kobeltia hortensis* and was recovered 5 weeks later; the fragment had increased in size and showed marked histological differentiation (details not given) towards the adult condition. Laviolette stated that similar results were obtained with homografts of albumen gland material in which two specimens of *L. flavus* were involved. He also succeeded in carrying out gonadectomy on immature specimens of *A. rufus* and on an unspecified number of mature individuals of *L. maximus*. Three months after operation the latter were dissected. Macroscopic examination revealed a diminution in size of the genital tracts and particularly in the size of the albumen glands. The latter findings (except with regard to the albumen gland) do not agree with those of Abeloos mentioned above. Abeloos clearly states that 4 months after operation neither the penis nor that portion of the hermaphrodite duct left *in situ* shows any sign of change, and the oviduct merely fails to show the increase in size noted in the controls.

Studies have been carried out on the genital tracts of certain gastropods suffering from parasitic castration. This work has been reviewed by Hanström (1939). In some genera (*Paludina, Limnaea, Planorbis*) no effect has been observed, but in *Littorina* regressive changes have been described, and these changes have been taken to indicate gonadal control of the reproductive tract. However, the effects of parasitic castration must be interpreted with care, and such evidence cannot be regarded as conclusive.

The present study was undertaken to discover whether gonadectomy in *Helix* was practicable with a view to ascertaining the effect of that operation in a genus in which it has not yet been attempted.

II. Method

The snails used in this study were at least $2\frac{1}{4}$ cm in diameter, measured across the widest portion of the shell (at right angles to the longitudinal axis of the animal). In each animal the apex of the shell was removed with fine forceps so as to lay bare an area of the hump approximately 1 cm in diameter (Fig. 1). The apical whorl of the hump was then opened out centrifugally so as to reveal the greyish-coloured gonad. The integument was broken along the length of this region (parallel with the direction of curvature) and stripped away on either side, so as to lay bare the gonad.

Gonadal tissue was removed with fine forceps until the darker tissue of the digestive gland was revealed. Care was taken to avoid damaging the latter tissue. A careful search was made for the hermaphrodite duct at the base of the gonad; this duct was severed.

At conclusion of the operation the hump was replaced in position and the shell closed with "Elastoplast". Failure to do this led to high mortality, apparently

due to desiccation. In the case of control operations, the same procedure was followed but no gonadal tissue was removed and the hermaphrodite ducts were not severed. At dissection the gonadal region of the hump, the albumen gland, and the common duct of each animal were fixed in Zenker, mounted in paraffin wax, and sectioned. The stains used were Harris's haematoxylin and eosin.

III. Results

Of a large number of snails operated on between the months of September and November, 10 (i.e. 4 controls, 6 gonadectomies) survived* for periods of 7–13 weeks. They were dissected (with one exception) in January. The results given

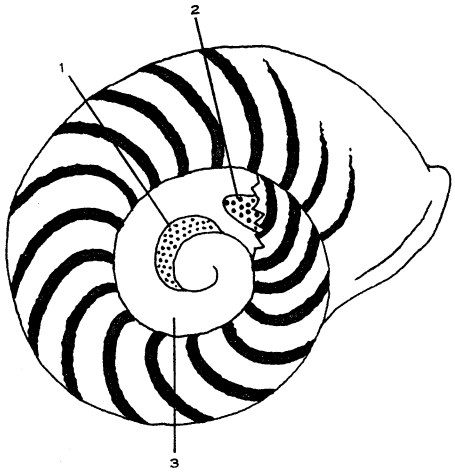

Fig. 1.—*H. aspersa*, view from above showing portion of shell removed in operation and position of the gonad relative to the surrounding tissues of the visceral hump: *1*, ovotestis; *2*, albumen gland; *3*, digestive gland.

below are derived from these animals, all of which were apparently in good condition at dissection. Removal of the "Elastoplast" revealed that in all cases the shells had been repaired by a whitish, calcareous deposit, which effectively closed the holes in the shells but did not resemble the original shell material either in colour or in texture. The remainder of the operated animals died within 7 weeks of operation; the deaths were apparently due to infection of the wounds.

(*a*) *Effectiveness of Operational Procedure*

The effectiveness of the method used was ascertained by histological examination of the gonad region. It was discovered that in no case was gonadectomy complete. It had seemed that severe damage to the ovotestis at operation might have resulted in rapid degeneration of any gonadal tissue left *in situ*; this did not prove to be the case. There was evidence of regressive changes in the gonadal

* The term "survived" is used here for convenience. It does not imply that the animals were dissected *post mortem*; they were clearly capable of surviving the operation by longer periods than those given above.

remnants of only one animal (S.8), dissected 13 weeks after operation (Plate 1, Fig. 2). In this animal some of the "follicles" left *in situ* were empty of all germinal elements except occasional oocytes, and some of the latter were evidently in process of being resorbed. The follicles contained very large granular cells with light-staining nuclei. Many of these cells showed large cytoplasmic vacuoles containing a yellowish granular material. Although their lineage is uncertain, it is at least possible that they are hypertrophied "yolk cells" (cf. Gatenby 1917) and there seems little doubt that they were responsible for resorption of the germinal elements. There is clear evidence that in some of the follicles the cells in question were being replaced by connective tissue.

There is no reason to think that the follicles described above had been damaged at the time of operation: their boundaries were intact and some were located on the periphery of the gonad, well away from the locus of operation. Apart from these follicles, the animal (S.8) possessed a number of intact gonadal remnants which contained spermatozoa and oocytes (Plate 1, Fig. 1). All the other gonadectomies (including another specimen dissected 13 weeks after operation) possessed similar remnants containing spermatozoa and oocytes and showing no evidence of regression. It therefore appears that remnants of the ovotestis of *Helix* begin to undergo regression some 3 months after partial gonadectomy, and that up to this time such remnants must be regarded as viable.

On the other hand, there was no evidence of regeneration of damaged or excised genital tissue. Laviolette (1954) reported that castrated specimens of *A. rufus* commenced regeneration of the excised gonads within 3 to 6 weeks after operation. However, Abeloos (1943) found no such regeneration in his specimens of *L. maximus*. It appears that the capacity for regenerating lost gonadal tissue is not general among pulmonates.

(*b*) *Histology*

(i) *The Albumen Gland.*—The histology of the albumen gland of *Helix* has been described: Baecker (1932), Filhol (1938*a*), Yung (1911). The gland is acinar, surrounded by a thin layer of connective tissue. In small glands the acini are round or oval in cross section, but in large glands they tend to be polygonal and closely packed. Each acinus consists of a layer of secretory epithelium, one cell thick, surrounding a central lumen. The acini are demarcated by a delicate investment of connective tissue. The glandular cells are markedly eosinophil, with basal nuclei. In small glands the cells tend to be low columnar, with relatively large, rounded nuclei (Plate 2, Fig. 3); the cytoplasm has the general appearance of a reticulum, and in some of the cells it is possible to distinguish vacuoles containing an eosinophil colloidal material.

In large glands the acini are large and the lumina are occluded (Plate 2, Fig. 4). The cell boundaries are difficult to distinguish, but when seen they show the cells to be large and polygonal, occupying the whole acinus. The nuclei are small and pyknotic, and are located against the peripheral connective tissue. The cytoplasm is filled with large globules of eosinophil colloidal material. In some cells the globules have apparently fused, forming a mass of colloid which occupies most

of the cell. In such cases the colloidal mass is surrounded by a thin layer of cytoplasm, and broken cytoplasmic threads are visible within the mass. Such glands are dense and friable and tend to be difficult to embed. The fixative caused shrinkage cracks to appear, sometimes around individual cells and sometimes around the colloidal globules. Baecker (1932) found a similar difficulty with the albumen gland of *H. pomatia* and recommended that use of fixatives containing potassium bichromate be avoided—a precaution considered unnecessary in the present work.

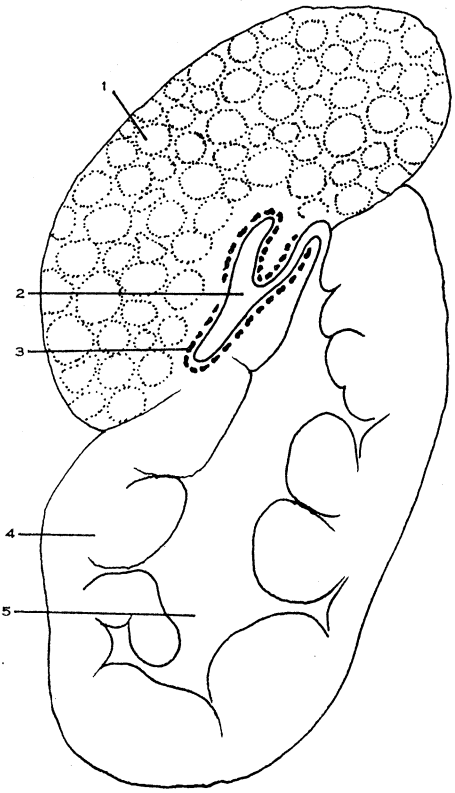

Fig. 2.—Diagrammatic cross section of common duct of *H. aspersa*: *1*, acini of prostate; *2*, spermiduct; *3*, layer of basophil cells surrounding spermiduct; *4*, mucous epithelium of oviduct; *5*, oviduct.

The controls used in the present investigation showed both of the pictures described above (cf. Plate 2, Figs. 3 and 4). Other snails (not operated on) dissected at the same time of year revealed all stages in the process of transformation from the one "type" of albumen gland to the other. It is impossible to say at this stage whether the two types of gland represent differences in physiological activity (and therefore recur in regular annual sequence) or whether the former type is characteristic of young snails and the latter of older animals. The former type is certainly characteristic of young snails, but it appears that both types may occur in older snails. This observation supports the view that the two histological types signify

differences in physiological activity rather than differences in age. This is borne out by the work of Yung (1911) and by that of Baecker (1932), both of whom report a cessation of secretory activity of the albumen gland of *H. pomatia* during the winter months.

The albumen glands of two of the gonadectomized animals are shown in Plate 3, Figures 5 and 6. Despite the evident differences between Plate 2, Figures 3 and 4 (controls), on the one hand, and Plate 3, Figures 5 and 6, on the other, there is considered to be no consistent histological difference between the albumen glands of the gonadectomized animals and those of the controls. It is therefore concluded that partial gonadectomy causes no histological change in the albumen gland of this species, at least not within 3 months of the operation.

(ii) *The Common Duct.*—The histology of the common duct of *Helix* has also been described: Yung (1911), Baecker (1932), Filhol (1938*b*). In cross section the spermiducal and oviducal portions* are clearly distinguished (Fig. 2). The oviduct is of large diameter with folded walls. The lumen is bounded by a ciliated epithelium outside of which is a high columnar epithelium which probably secretes the mucous envelopes for the eggs. The cells of the latter epithelium are faintly basophil and are sometimes vacuolated. They may be relatively small, with well-marked boundaries and rounded nuclei, or relatively large, with obscure boundaries and pyknotic nuclei. In the latter case the thickness of the epithelium is increased in proportion to the increase in size of the individual cells. Thus, the appearance of the mucous epithelium surrounding the oviduct varies considerably from animal to animal, apparently depending on the physiological state of the tissue. The mucous epithelium is bounded externally by a connective tissue sheath.

The spermiduct is of relatively small internal diameter. The lumen invariably lies at an angle to that of the oviduct from which it is partially separated by two folds or ridges of tissue. According to Filhol (1938*b*) these folds of tissue, developed in the region of the slit connecting the two lumina, can ensure almost complete separation of the two ducts.

The lumen of the spermiduct is lined with a ciliated epithelium continuous with that of the oviduct. Outside the ciliated epithelium there is a layer of markedly basophil cells, usually one cell thick (Plate 4, Fig. 7). The basophil cells are irregularly ovoid, with coarsely reticular cytoplasm and an eccentric or peripheral nucleus which it is usually difficult to detect. Each cell has a delicate cytoplasmic prolongation which passes between the ciliated cells of the surface epithelium and reaches the lumen of the spermiduct (Plate 5, Fig. 9). Similar cells were found in *H. pomatia* by Baecker (1932) who described and figured the cytoplasmic prolongations extending toward the lumen of the duct. The layer of basophil cells may completely surround the spermiduct, but more frequently is more or less restricted to one side of it. Outside the basophil cells is located the "prostatic tissue".

The prostate consists of numerous acini separated by delicate strands of connective tissue. Each acinus usually has a small central lumen surrounded by a

* Henceforward these two subdivisions of the common duct will be referred to as the "spermiduct" and "oviduct" respectively. Note that the vas deferens and oviduct *sensu stricto* are not included in this study.

columnar epithelium of eosinophil cells. The latter have rounded, basal nuclei and a coarsely granular cytoplasm. The prostatic tissue is bounded externally by a connective tissue sheath which is continuous with that of the oviduct and therefore forms an investment for the common duct.

There appears to be no significant histological difference between the common ducts of the gonadectomized animals and those of the controls except in the following respect: the layer of basophil cells surrounding the spermiduct was found to be absent from two controls (cf. Plate 4, Figs. 7 and 8). The significance of this observation is not clear. It is probably not related to the experimental procedure.

It is therefore concluded that partial gonadectomy causes no histological changes in the tissues of the common duct of *H. aspersa*, at least not within 3 months of the operation.

IV. Discussion

(*a*) *Albumen Gland*

The function of this gland in gastropod molluscs is known. In 1938 Baldwin and Bell showed that the albumen gland of *H. pomatia* secretes a complex galactose polymer which was referred to as "galactogen" since it produces galactose on hydrolysis. Duncan (1958), working on the albumen gland of *Physa fontinalis*, obtained similar results and also carried out a number of tests for other substances: "mucous, proteins, mucopolysaccharides and other sugars". The results of these tests were negative. Duncan cited the work of two other investigators in support of the view that galactogen is secreted by the albumen glands of many gastropods and is not peculiar to pulmonates.

In *Physa* each ovum is given an envelope of galactogen by the albumen gland before being invested with a double membrane and enclosed within an egg capsule. The galactogen constitutes the food supply of the embryo (Duncan 1958), and there is little doubt that it serves a similar purpose in *Helix*.

(*b*) *Common Duct*

It seems to be generally agreed that the glandular cells surrounding the oviduct in pulmonates are mucous-secreting and provide envelopes for the ova (Filhol 1938*b*; Duncan 1958). Duncan has produced experimental evidence that in *P. fontinalis* the glandular tissue of the oviduct (here separate from the vas deferens) secretes two membranes around each ovum as well as the capsular material in which a number of these "eggs" are enclosed. Details of the process of egg formation in *Helix* are not available, but it is no doubt similar to that in *Physa*, with the oviducal epithelium contributing investments for the ova.

The prostatic tissue of *Helix* is clearly defined. There is little doubt that it is analogous to, and homologous with, the prostate of *Physa* which Duncan (1958) has described as consisting of blind, finger-like follicles opening into the vas deferens. Yung (1911) has described the prostate of *H. pomatia*, the main elements of which are glandular, prismatic cells with reticular cytoplasm containing numerous calcareous granules ("granulations calcaires"). Yung put forward the view that these cells provide the eggs with the calcareous envelopes with which they are surrounded,

but this postulate was later questioned by Baecker (1932). Yung's suggestion hardly takes account of the anatomical relationships of the prostate which so evidently forms part of the male duct system.

Duncan (1958) has produced evidence that the prostate of *Physa* produces alkaline phosphatase and possibly amino acids. He took the view that the secretions of the prostate provide a fluid medium for transfer of the spermatozoa at copulation. It may be tentatively concluded that in *Helix* the prostate has a similar function.

The basophil cells which surround the spermiduct are mucous-secreting (Baecker 1932). It seems possible that they are analogous to, if not homologous with, the cells of the praeputial gland of *Physa*. Duncan (1958) has described the latter as consisting of basophil cells with long cytoplasmic necks passing through a layer of muscle tissue and reaching to the lumen of the praeputium (a sac-like structure, distal to the penis sheath, through which the penis is everted at copulation). According to Duncan the cells of the praeputial gland "produce mucins which presumably facilitate the eversion of the penis".

A praeputial gland does not occur in *Helix*. However, the basophil cells of *Helix* are strongly reminiscent of the cells of the praeputial gland of *Physa* in their structure (notably the long necks reaching to the lumen of the male duct), their mucous-secreting function, and their association with the male duct system. The fact that they occur along the spermiduct suggests that the secretion they produce is useful as a constituent of the seminal fluid and is not primarily a penial lubricant.

(c) *Conclusion*

As already mentioned, there is some evidence that the gonad exerts hormonal control over the glandular tissues of the reproductive tract in the Arionidae and the Limacidae (Abeloos 1943; Laviolette 1950). A similar situation is known in the vertebrates, where the accessory sex organs are controlled by hormones produced by the testes and ovaries. However, the results given here at least suggest caution in assuming that the same applies in the Helicidae. There is no doubt that most of the gonadal tissue was removed from the gonadectomized snails on which this study is based, and it was reasonable to expect signs of histological regression in any tissues controlled by the gonads. There was no evidence of histological regression in the albumen glands or in the glandular tissues of the common ducts, and the conclusion suggested is that these tissues are not controlled by the gonad.

However, the evidence presented is not conclusive, since it can be argued that the gonadal remnants were capable of maintaining the tissues in question in normal physiological (and histological) condition. Clearly a more effective method of gonadectomy is indicated—a method capable of complete removal or destruction of the gonadal tissue. In vertebrates the use of X-rays for the latter purpose is common practice, and the method might well be feasible in the case of *Helix*.

V. Acknowledgment

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Explanation of Plates 1–5

Plate 1

Fig. 1.—Animal S.8, dissected 13 weeks after partial gonadectomy. Intact gonadal remnant, showing follicles containing spermatozoa and oocytes. × 75.

Fig. 2.—Animal S.8 showing degeneration of a gonadal remnant. A number of the follicles contain oocytes, but spermatozoa are absent. Note very large cells occupying follicles. Arrows indicate connective tissue which is evidently in process of replacing the large cells occupying the follicles. × 75.

Plate 2

Fig. 3.—Portion of albumen gland of a control. In this animal the gland was small. Some of the acini are seen in longitudinal section. × 150.

Fig. 4.—Portion of albumen gland of a control. In this case the gland was large. Note relatively large acini occupied by cells containing large globules of colloidal material. Fixation has caused shrinkage cracks which delimit the colloidal globules as well as individual cells. × 150.

Plate 3

Fig. 5.—Portion of albumen gland of an animal dissected 11 weeks after partial gonadectomy. Acini intermediate in size between those of the controls shown in Plate 2, Figures 3 and 4. Cells show marked vacuolation. × 150.

Fig. 6.—Animal S.8, dissected 13 weeks after partial gonadectomy. Portion of albumen gland. Acini smaller than in control shown in Plate 2, Figure 4, but histology is similar. × 150

Plate 4

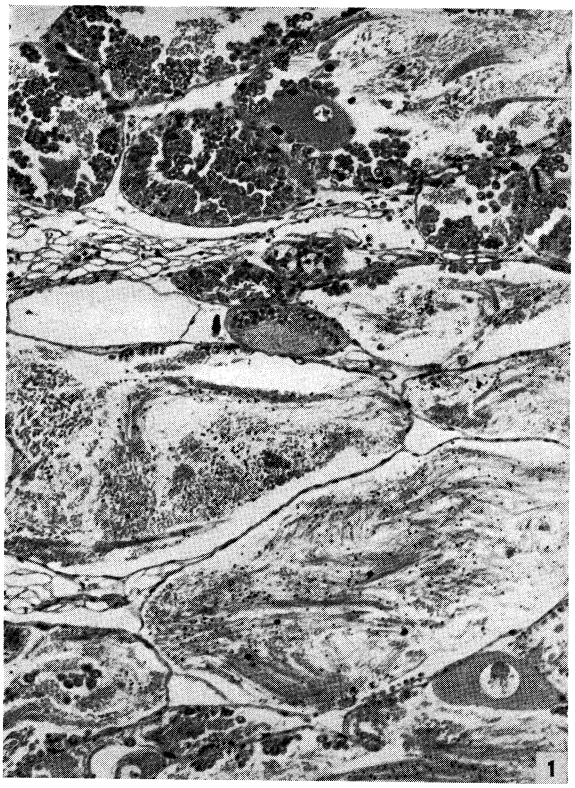
Fig. 7.—Cross section of common duct of a control in region of spermiduct. Arrows indicate the layer of basophil cells surrounding the spermiduct (*s*). Prostatic tissue occupies the upper portion of the field and mucous epithelium of the oviduct occupies the lower portion. × 150.

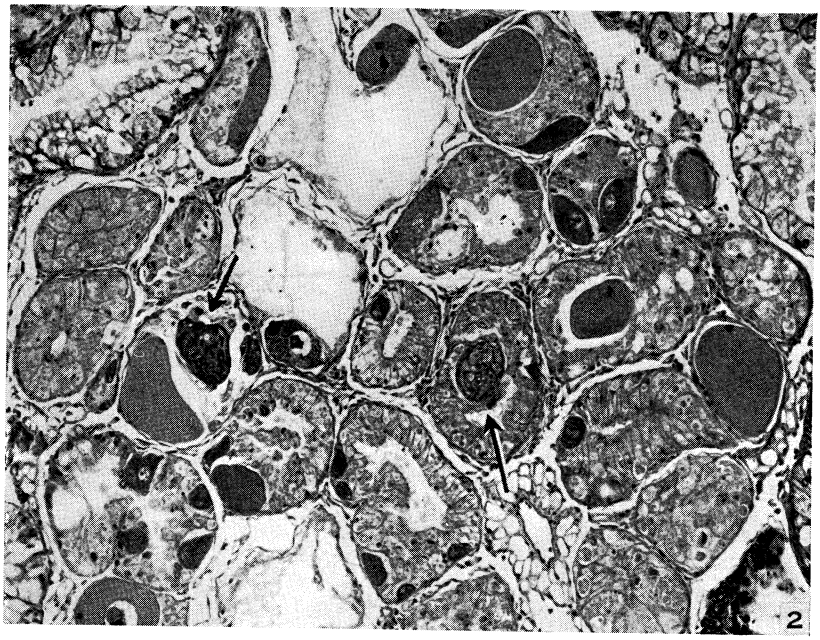
Fig. 8.—Cross section of common duct of a control; similar field to that shown in Plate 4, Figure 7. Note absence of basophil cells from region surrounding spermiduct (*s*). × 150.

Plate 5

Fig. 9.—Cross section of common duct of animal S.8, dissected 13 weeks after partial gonadectomy. Similar field to that shown in Plate 4, Figures 7 and 8. Note layer of basophil cells surrounding spermiduct (*s*). Arrow indicates neck of a basophil cell passing between ciliated epithelial cells of spermiduct to reach the lumen. × 150.

REPRODUCTIVE TRACT OF HELIX ASPERSA





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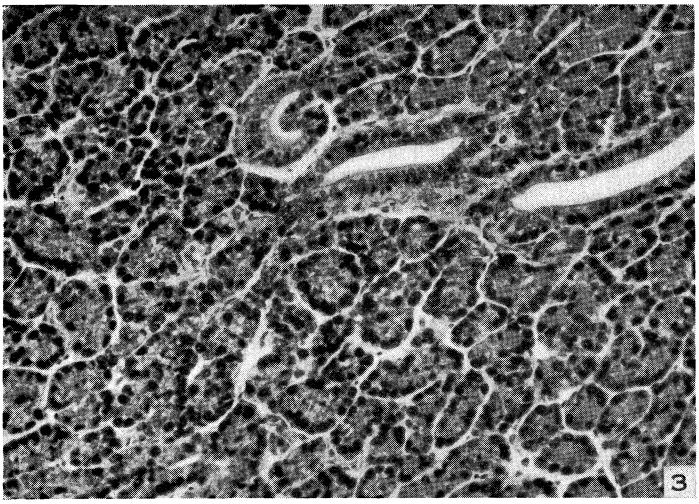

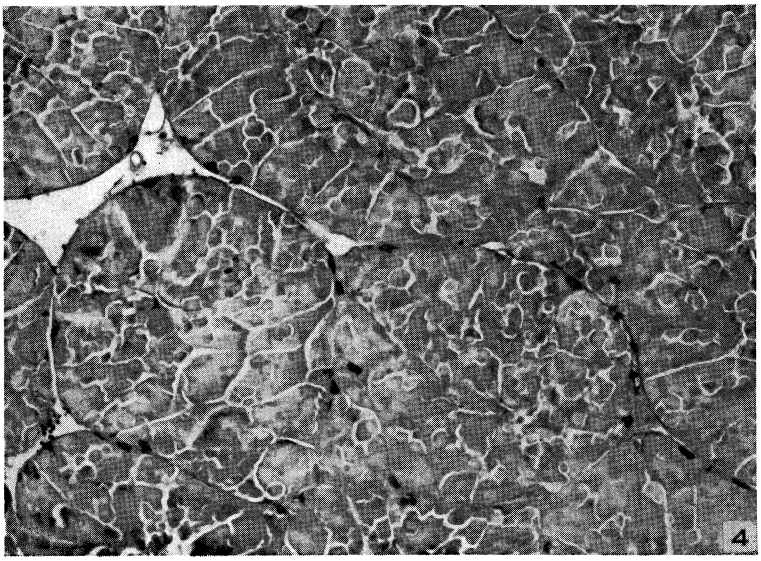

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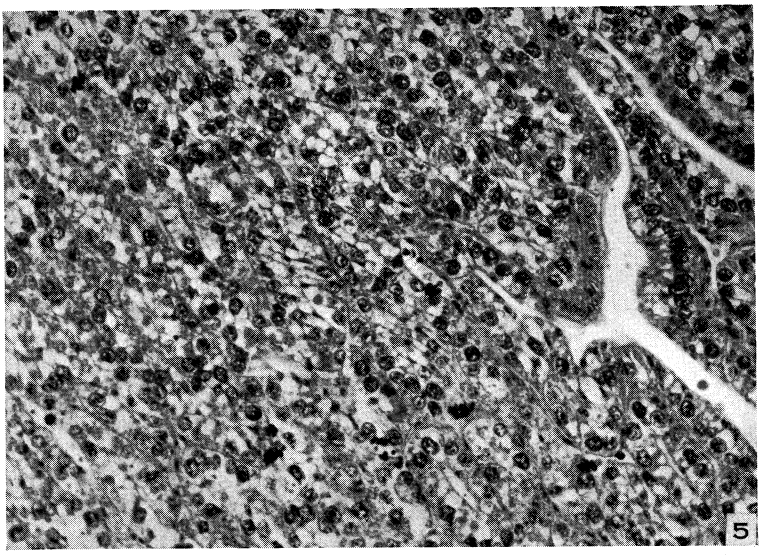

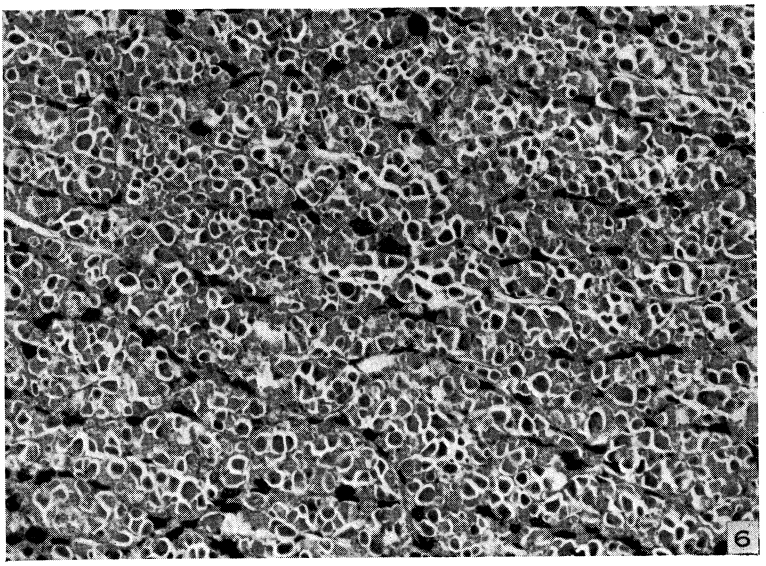

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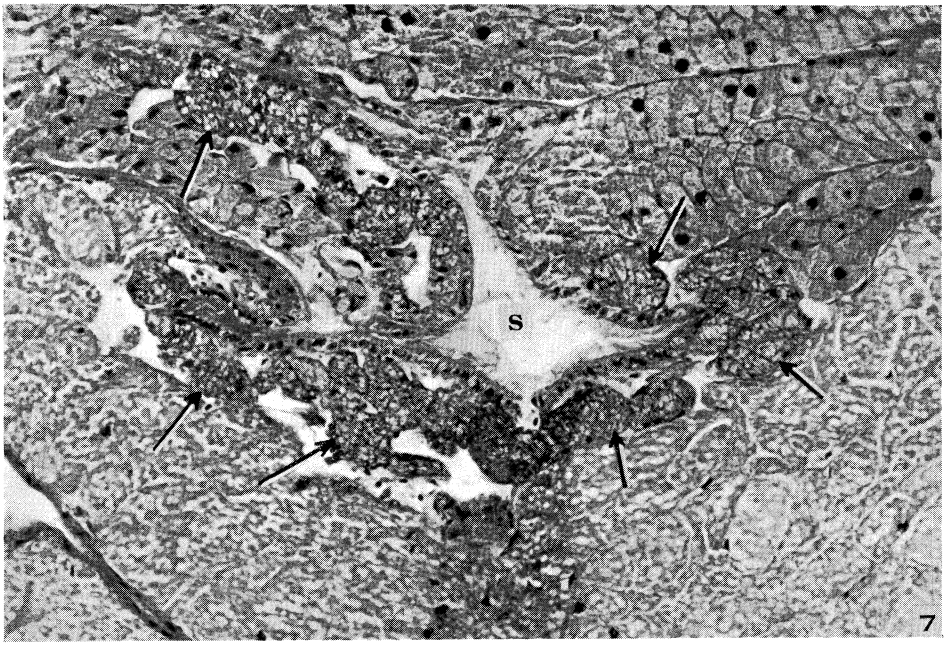

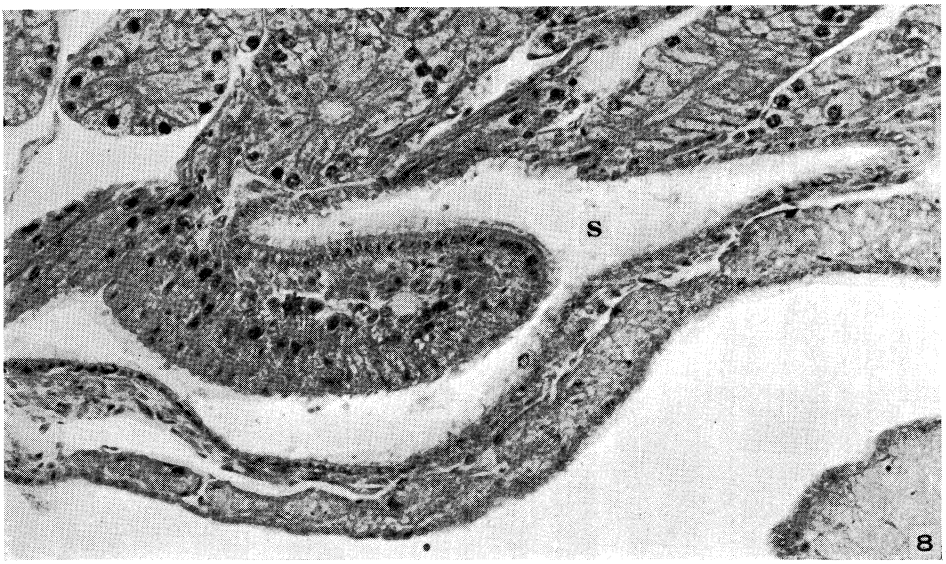

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