Studies on the Reproduction of the Dogfish

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STUDIES ON THE REPRODUCTION OF THE DOGFISH

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[Plate 20]

INTRODUCTION

In most groups of vertebrates, migration of ova from the ovary to the coelomic opening of the oviduct has been observed. This has been done by opening the abdomen of anaesthetized animals at the appropriate time. Thus, in the common fowl, Coste (1874) has found and Patterson (1910) confirmed that the infundibulum clasps the follicle before ovulation and that the pressure exerted is probably a causative factor in extrusion of the ovum. Though the process has never been observed in reptiles, the size of the ovum and proximity of the ovary to the oviducal funnel suggest that it is similar. Heape (1905) describes ovulation in the rabbit, stating that it normally occurs about 10 hr. after coition. Ovulation in the rabbit has been witnessed and described by Walton and Hammond (1928). Here, and in marsupial and placental mammals, the ovum is small and the ovary lies against the funnel, which is ciliated. It has been suggested by Corner (1921) that, in addition, peristaltic movements of the Fallopian tube may set up suction resulting in currents running into the infundibulum. There is little chance, in such cases, for an ovum to be lost in the coelom.

On the other hand, in the elasmobranch fishes and amphibians, the ova are large and the ovary far from the coelomic opening of the oviduct. Rugh (1935) has shown that despite this, the ova of the frog never become lost in the coelom during the migration. He has found that their progress is dependent entirely on ciliation. He has described the distribution of the cilia, their currents, and the path followed by the ova from the time of emergence from their follicles to their arrival in the ovisacs. Other animals studied indicate that this holds good for the Amphibia in general (Rugh 1935).

One purpose of this paper is to describe how the corresponding migration of the ova is carried out in the dogfish. It may be stated at once that despite the relatively enormous size of the dogfish's ovum, which is approximately 380 times that of the frog's ovum, and the much greater distance that the ova have to travel, the whole process can be accounted for by ciliation and closely resembles that found in amphibians.

METHOD

For this investigation, *Scylliorhynus canicula* has been used exclusively. The method of preparing dogfish for observing the action of the peritoneal cilia is to remove the head of the living fish and to open the abdomen by a median ventral incision from one end of the coelom to the other. The peritoneal wall of the decapitated fish is inclined so that a horizontally directed beam of light is reflected from its surface upward through a binocular microscope giving a magnification of about 80 diameters. It is a good plan to fold the body wall over the finger and examine the highly illuminated crest of the fold. A few transverse cuts are sometimes necessary in the body wall. In some places the ciliation is obvious, in others it is so difficult to make out that it may be nece sary to open several fish before obtaining a satisfactory observation. The best results are

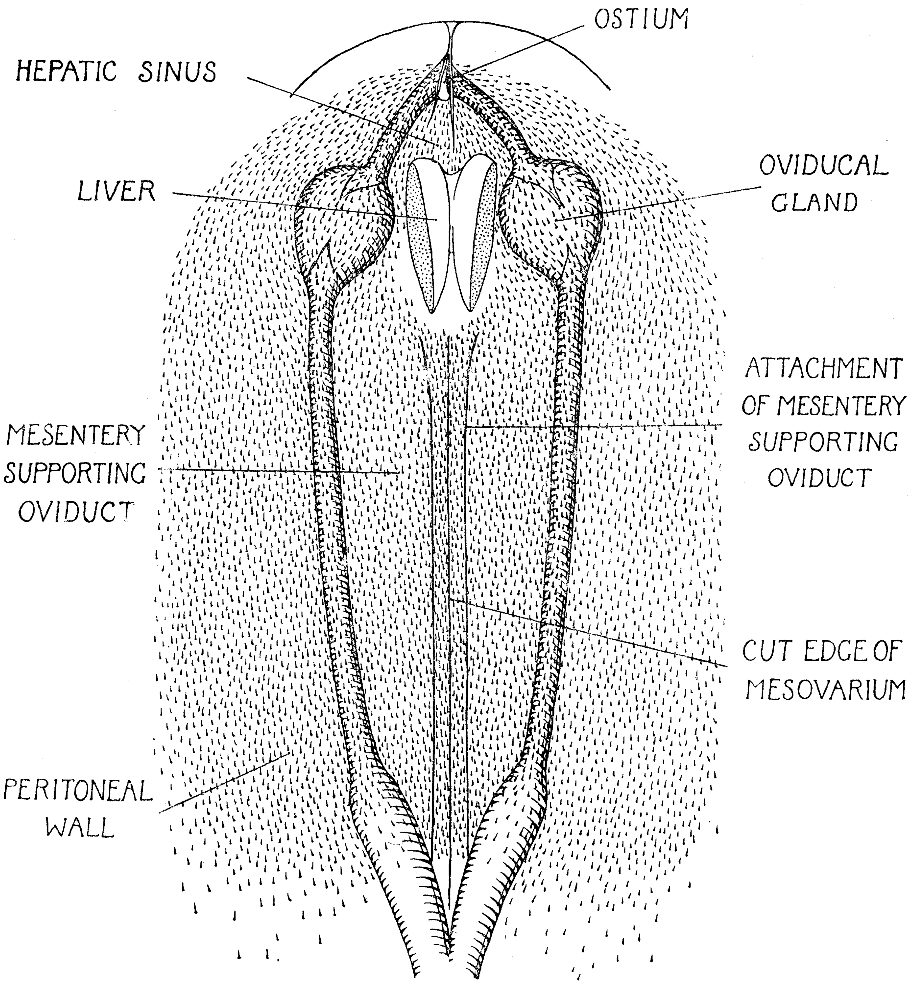

FIG. 1. Ventral view of open body cavity of adult female *Scylliorhynus canicula* showing the distribution of coelomic cilia and their convergence towards the ostium. The liver has been cut off short and all the other viscera, except the oviducts, removed. × ⅔.

obtained when the surface under observation is beginning to dry slightly and the cilia to slow down. The usual aids to tracing ciliary currents are valueless; small stationary masses are formed, round which the currents pass. There are, however, minute bubbles in the superficial mucus which are rarely difficult to find and whose movement it is easy to follow.

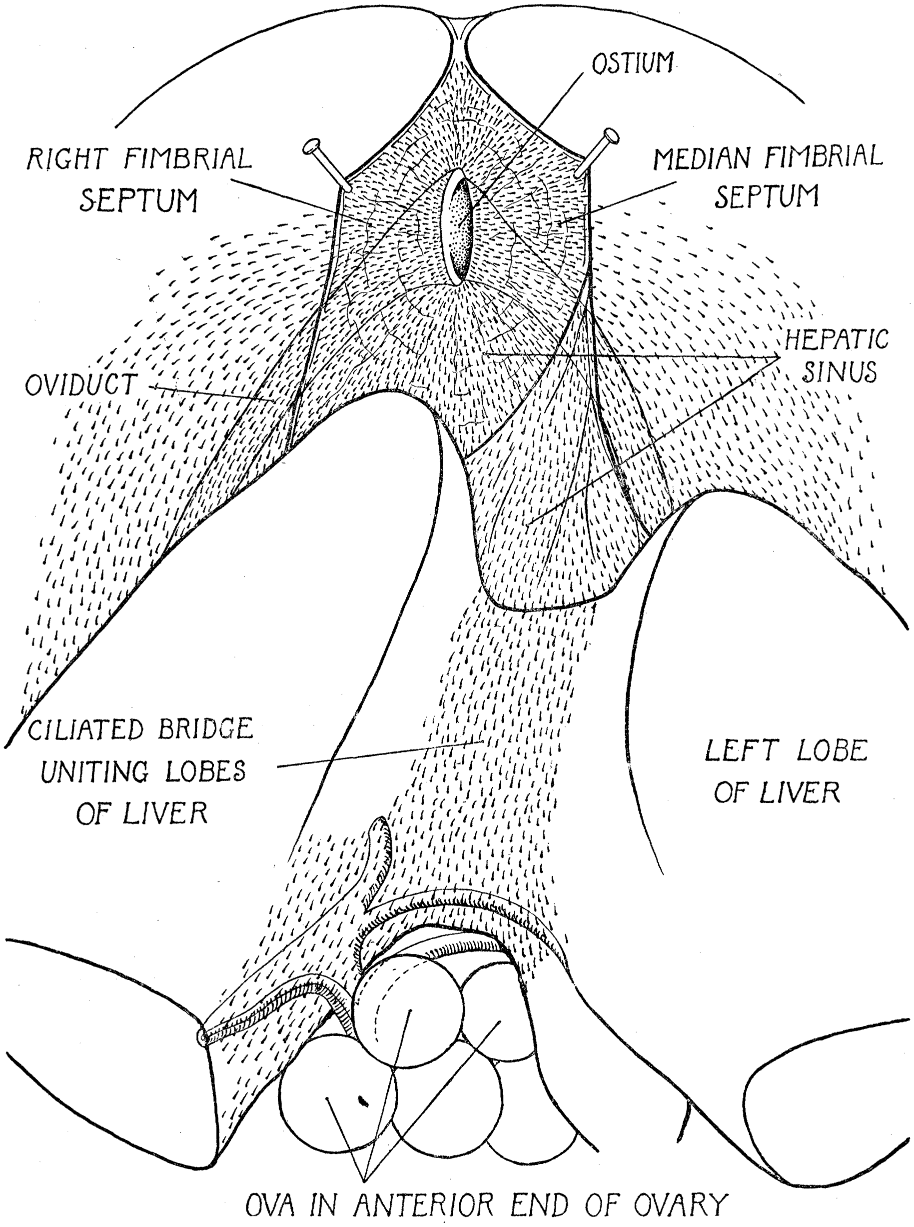

FIG. 2. Anterior end of the open body cavity of adult female *Scylliorhynus canicula* showing ostium and ciliation of surrounding organs. The fimbrial septa have been pinned apart and the liver lobes cut off short. × $1\frac{1}{3}$.

DESCRIPTIVE

The Ovary

Although the other organs of the reproductive system are paired, the ovary is single. It is also very large. In those elasmobranch fishes possessing a single ovary, the persistent ovary is believed to be that of the right side. Apart from its size, the fewer and larger ova it contains, and the absence of lobes, the ovary of the dogfish resembles a single ovary of the frog. It contains no fluid and it may be reasonably anticipated that follicular rupture and emergence of the ova resemble those of the frog. However, the placing of an ovary freshly excised from an adult female fish into 0·2% pepsin plus 0·2% hydrochloric acid in saline solution fails to cause follicular rupture and subsequent extrusion of the mature ova as it does in amphibians (Rugh 1935).

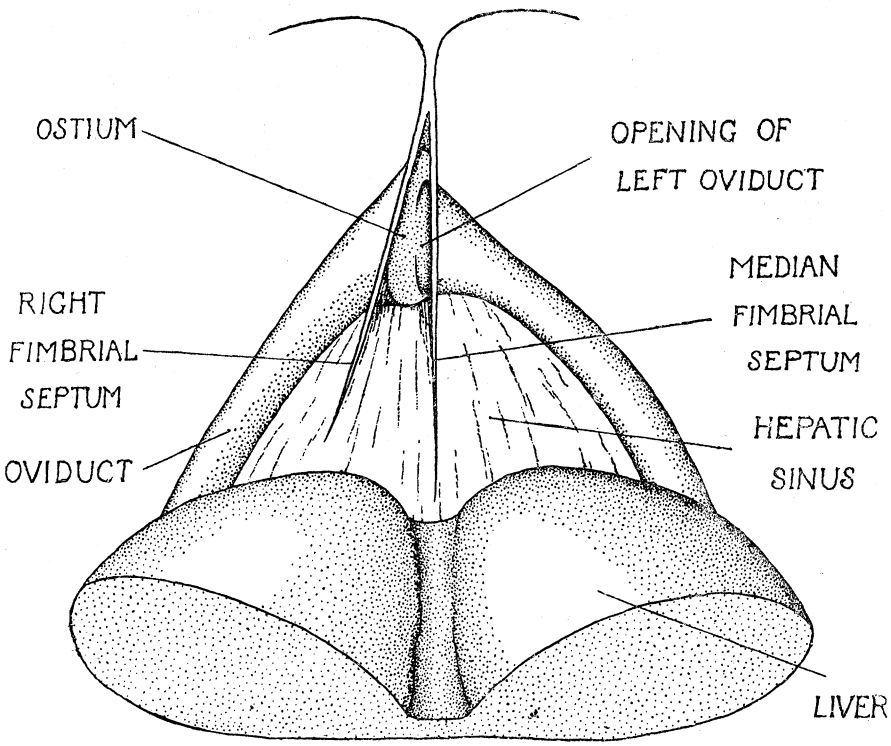

FIG. 3. (*a*) Ventral view of ostium of *Scylliorhynus canicula*. Note the asymmetry of the two fimbrial septa which have been pulled slightly apart to expose the opening of the left oviduct. The liver has been cut off short. × 1½.

The ostium

The falciform ligament supporting the ostium takes the form of two fimbrial septa, between which lies the ostium. When the ostium is closed, the two septa lie in contact with one another in a dorso-ventral plane along the mid-line, with a common attachment at one end to the anterior end of the peritoneal coelom. The two septa are joined along the mid-dorsal line above the ostium and when they are pulled apart ventrally, by a movement like the opening of a book, the ostium is revealed (figs. 3*a* and 3*b*). Posteriorly the septa merge insensibly into the wall of the hepatic sinus.

A striking feature of this region is the asymmetry. Posteriorly one septum joins the right side of the hepatic sinus; the other joins, not the left side, but the middle-line. The ostium really belongs to the right oviduct, into the side of which opens the left, just above the ostium itself (figs. 3*a* and 3*b*). In fish that have been preserved in formalin, this asymmetry is not readily noticeable, owing to shrinkage, but in fresh fish it is obvious.

In birds, the persistent ovary is believed to be that of the left side and disappearance of the right ovary has been accompanied by atrophy of the corresponding oviduct which is small and useless. In the dogfish, the asymmetry appears to represent the first stage in atrophy of the left oviduct. This atrophy has not kept pace with that of the corresponding ovary to the same extent as in birds.

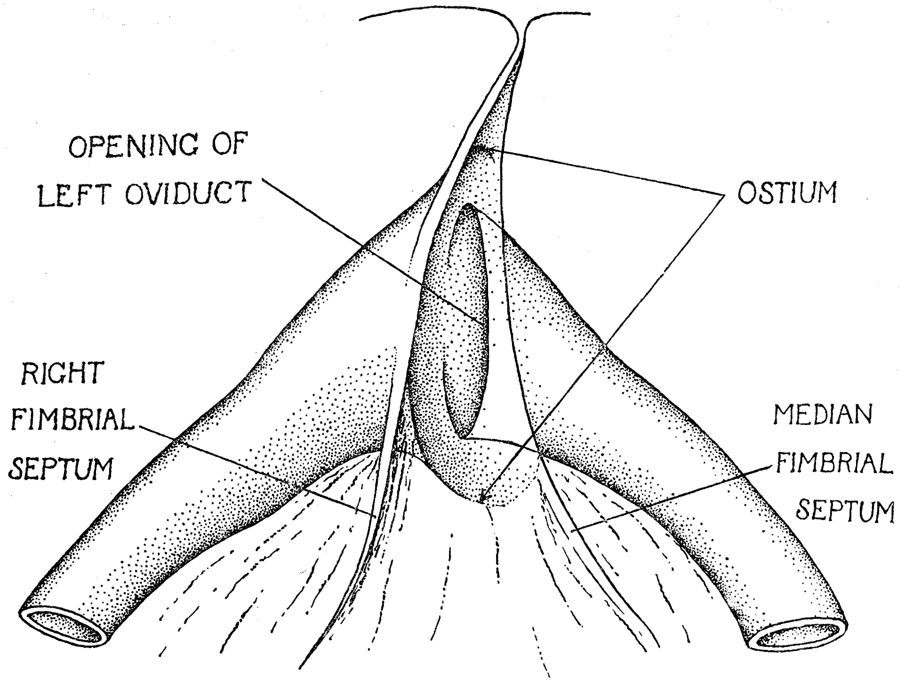

Fig. 3. (*b*) Enlarged view of ostium. The median fimbrial septum has been pulled slightly to the left to show more clearly the opening of the left oviduct into the side of the right one. × 3.

It is well known that dogfish lay their eggs containing advanced embryos in pairs, and that the developments of the two embryos, one in each oviduct, synchronize from the beginning; therefore two ova must leave the ovary together. It might be assumed, incorrectly, that the first ovum to enter the ostium would pass down the right oviduct and that, having blocked the entrance to that oviduct, the only path available to the second, would be down the left. On each of five occasions, described later, a single ovum allowed to enter the ostium without artificial aid descended the left oviduct.

The abdominal ciliation

As in the frog, the abdominal ciliation of *Scylliorhynus canicula* is confined to the adult female, where it is active at all seasons, and is absent in the male and immature female.

All currents produced by the abdominal ciliation are directed towards the ostium. The density and vigour of the peritoneal cilia are not uniform; they are at a maximum along the dorsal side and decrease ventrally and posteriorly (figs. 6 and 7).

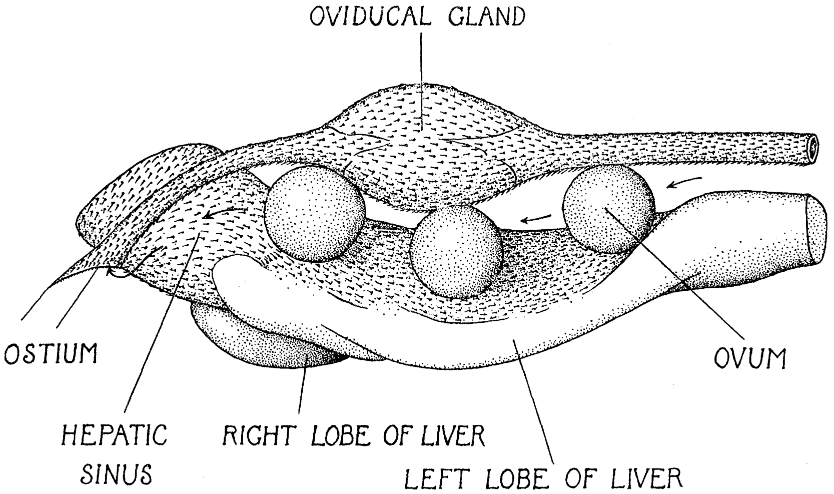

FIG. 4. Diagrammatic view of the left lobe of the liver and left oviduct of *Scylliorhynus canicula* as seen from the left side. The liver lobe has been depressed in order to show the ciliated nature of its upper (dorsal) surface. A single ovum is represented in three stages of its journey and its path is indicated by arrows. × 1.

The areas of ciliation are as follows:

(1) The whole of the peritoneal wall except the extreme anterior end. Fig. 1.

(2) The oviducts. Figs. 1, 6, 7, 8.

(3) The inner side of the mesentery supporting the oviducts. Figs. 5 and 6.

(4) Both sides of the mesovarium. Figs. 5, 6, 7.

(5) The dorsal side of the anterior portions of the liver lobes. Figs. 4 and 7.

(6) The bile duct and anterior end of the hepatic portal vein. Fig. 2.

(7) The ventral side of the bridge connecting the right and left liver lobes anteriorly. Fig. 8.

(8) The ventral surface of the hepatic sinus. Figs. 2, 4, 9.

(9) Both surfaces of each fimbrial septum.

Organs which are not ciliated are (fig. 5):

(1) The whole of the alimentary canal and its glands, except those regions of the liver stated above.

(2) The ovary.

(3) The mesentery connecting the alimentary canal to the ovary.

(4) The mesentery connecting the ascending and descending limbs of the stomach.

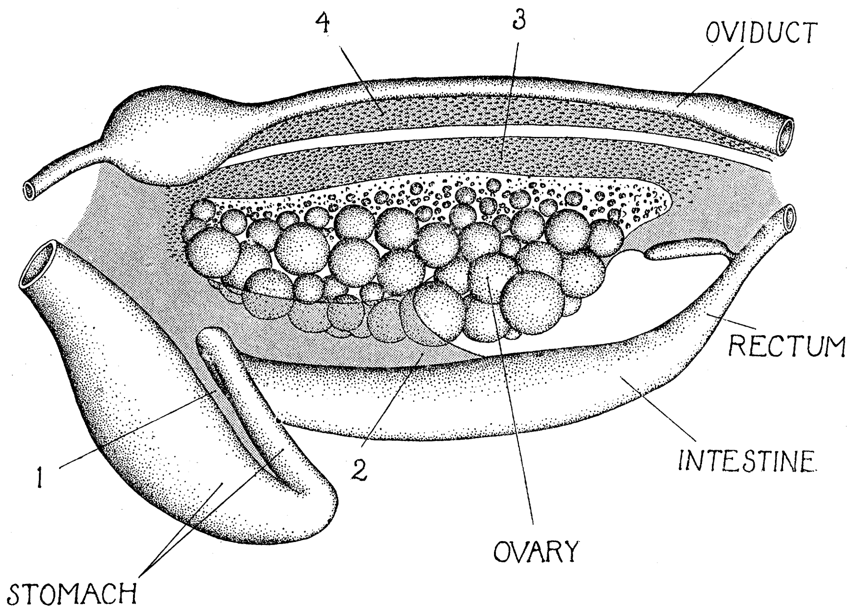

FIG. 5. Ciliation of mesenteries. Abdominal viscera of *Scylliorhynus canicula* as seen from left side with alimentary canal depressed to show the four portions of the mesentery.

1. Connecting ascending and descending limbs of stomach.
2. Connecting intestine and ovary. } Non-ciliated.
3. Supporting ovary.
4. Supporting oviduct. } Ciliated.

(The ciliation elsewhere has been omitted.) × ½.

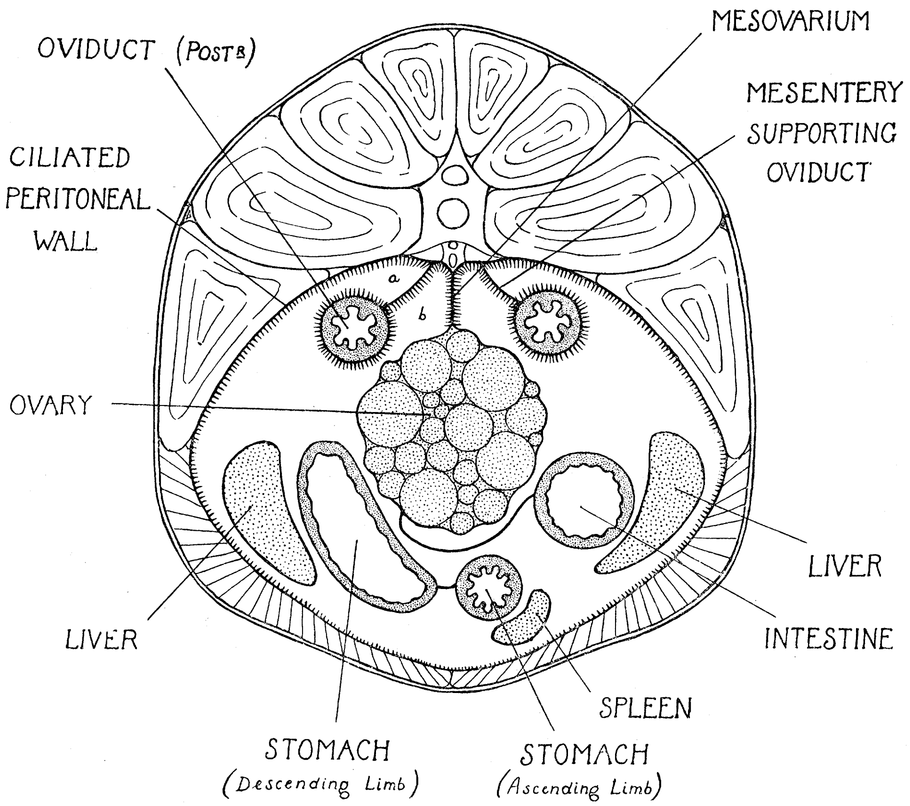

FIG. 6. Posterior view of a diagrammatic transverse section through the body of an adult female *Scylliorhynus canicula* at the level of the posterior end of the stomach, showing distribution of cilia. × 1.

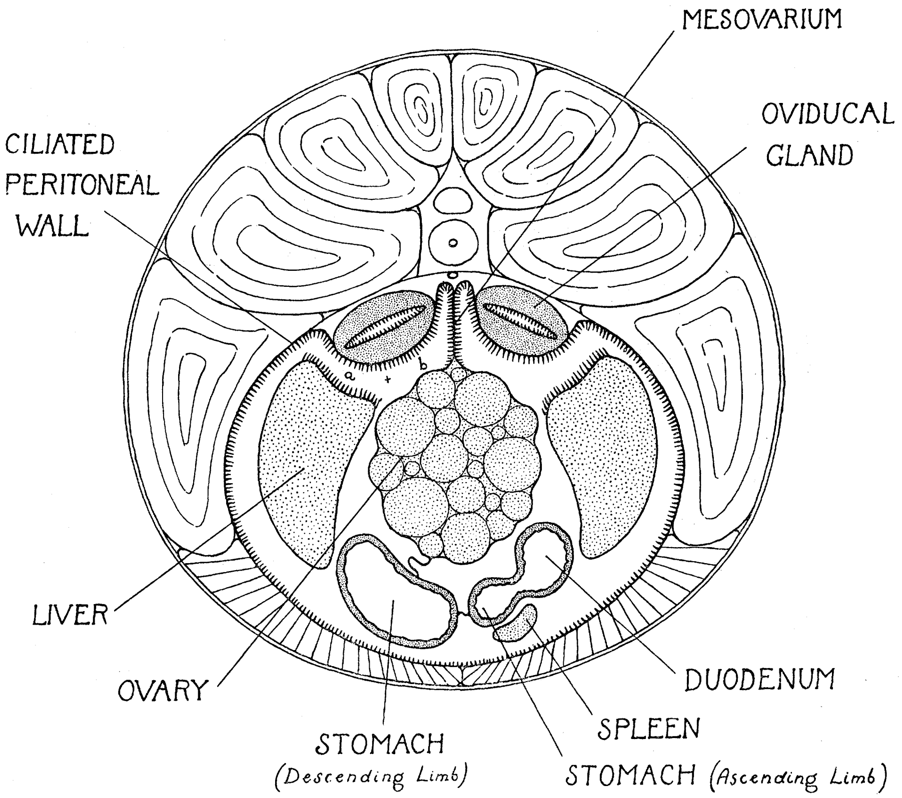

FIG. 7. Posterior view of a diagrammatic transverse section through the body of an adult female *Scylliorhynus canicula* at the level of the duodenum, showing the distribution of the cilia. × 1.

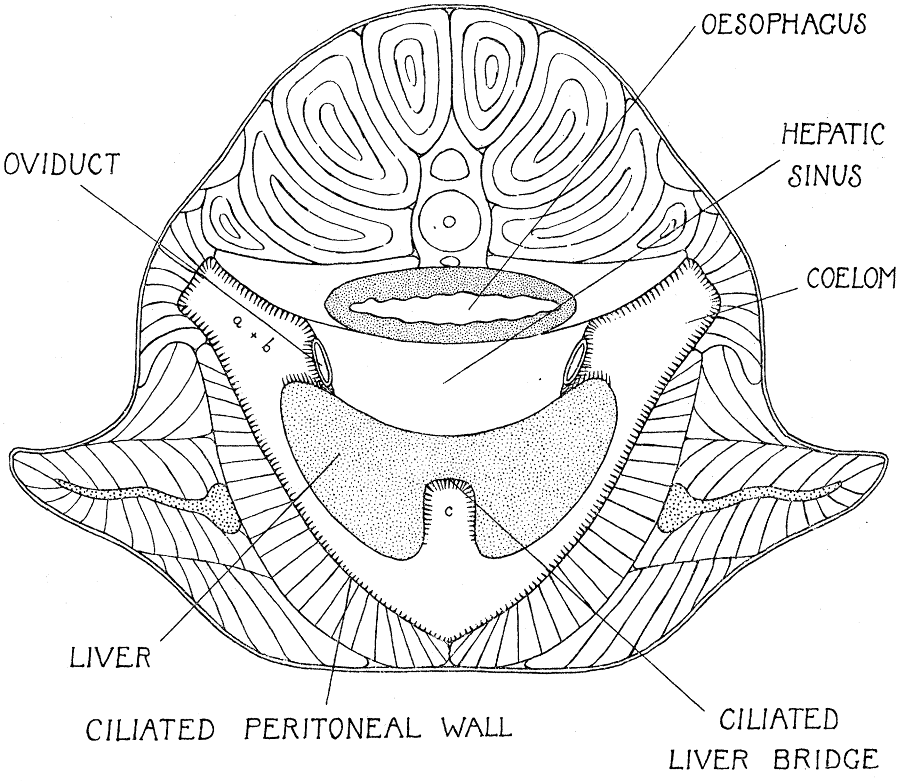

FIG. 8. Diagrammatic transverse section of an adult female *Scylliorhynus canicula* through the anterior end of the liver, showing the distribution of the cilia. × 1.

Migration of the ovum

The natural disposition of the viscera and therefore of the intervening coelomic spaces, taken in conjunction with the abdominal ciliation, must determine the path of the migrating ovum.

In order to trace the coelomic spaces, a fresh female dogfish, the body wall of which was punctured to admit the preservative, was placed in 5% formalin for 2 weeks to harden, and transverse sections were then taken through the body wall at various levels, care being taken not to displace the viscera. The results are incorporated in figs. 6–9, and point to the conclusion that the path adopted by any particular ovum depends upon the region of the ovary from which it emerges.

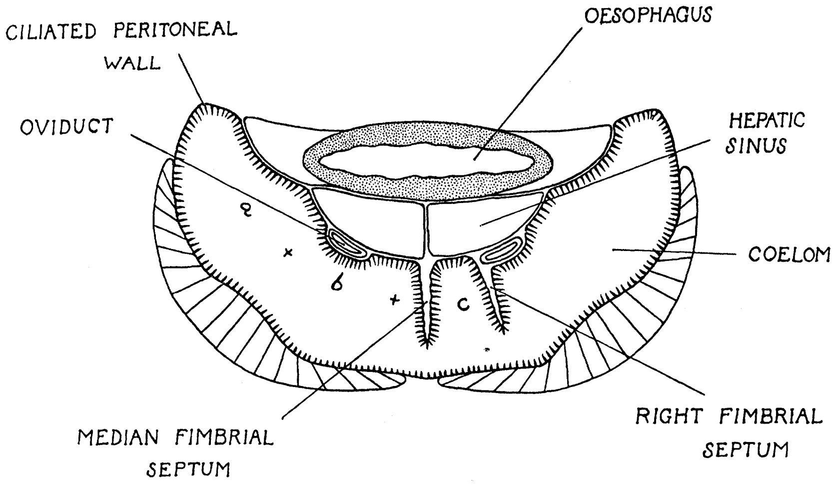

FIG. 9. Diagrammatic transverse section of an adult female *Scylliorhynus canicula* through the anterior end of the hepatic sinus, showing the distribution of the cilia. × 1.

Ova emerging from the hinder end of the ovary may move along one of two paths, indicated by the letters "*a*" or "*b*" in fig. 6. The first space (*b*) is a groove bounded laterally by the mesovarium on one side and by the oviduct on the other, dorsally by the mesentery supporting the oviduct, and ventrally by the ovary. The surfaces of all but the ovary are ciliated and therefore the major part of the surface of an ovum is in contact with ciliated epithelium. The other space (*a*) lies above the oviduct, between it and the dorsal peritoneal wall. It is less accessible to the ova and not quite so well supplied with cilia. Anteriorly the mesentery supporting the oviduct shortens and at the level of the oviducal gland, where it ceases to exist, the two paths coalesce. At this level the dorsal side of the liver lobe has become ciliated and forms part of the floor of the united spaces (fig. 7). Hereafter the ovum is propelled forwards along a narrow coelomic space bounded dorso-laterally by the body wall, laterally by the hepatic sinus, ventrally by the liver lobe and ciliated on all sides (figs. 4 and 8). Once past the liver, the ova come under the influence of the cilia on the outside of the fimbrial septum of that side and pass round its edge, to be swept into the atrium between the septa (fig. 9).

An ovum emerging from the anterior end of the ovary is propelled forward along a ventral tunnel composed dorsally and laterally of the ciliated liver bridge and ventrally of the body wall (figs. 2 and 8). Such an ovum has the major portion of its surface in contact with cilia. It is eventually swept under the hepatic sinus, between the fimbrial septa, and so into the ostium (fig. 9).

In the normal position of the fish, gravity would impede the entrance of an ovum into the ostium. The fimbrial septa, as a result of the action of their cilia, become wrapped round the ovum and bring practically the whole surface of the ovum under the influence of these cilia. The surfaces of the septa are thrown into minute folds or ripples arranged concentrically round the ostium. They may help in gripping the ovum. The nearer to the ostium, the denser and more vigorous become the cilia.

EXPERIMENTAL

Rugh (1935) finds that, in the frog, recently emerged ova are best for tracing ciliary currents. He induces ovulation in sexually inactive adult female frogs by injecting the "pars anterior" of the pituitaries of similar frogs and then opens the injected frogs after an interval. Ovulation and migration of the ova, from ovary to ostium, are quite normal and can be witnessed. Rugh also finds that the same migration can be observed when recently emerged ova from one female are introduced into the peritoneal cavity of an untreated, sexually inactive, female.

TABLE I. THE PROPORTION OF "PREGNANT" TO "NON-PREGNANT" ADULT FEMALE DOGFISH DURING DIFFERENT MONTHS OF THE YEAR

	Fish opened	"Non-pregnant"	"Pregnant"
August 1937	47	37	10
September	83	79	4
October	66	50	16
November	25	15	10
December	49	24	25
January 1938	27	16	11
February	22	10	12
March	43	21	22
April	26	7	19
May	21	12	9
June	44	22	22
July	37	13	24

Dogfish present difficulties not encountered in the frog. Attempts to induce ovulation are practically worthless because dogfish have no definite breeding season and ovulate naturally all the year round—they are merely more prolific in spring. This conclusion was the result of a record (Table I), of the condition—"pregnant" or "non-pregnant", of all adult female dogfish opened for preservation purposes at the Marine Biological Association, Plymouth, during different months of the year.

Observations on migration of the ova were made by two methods, one in which the fish were kept alive and the other in which they were freshly killed. Eleven female dogfish were available for the purpose. Subsequent autopsy revealed that three were immature and these served as controls.

Method 1

The fish is anaesthetized in a tank of sea water containing 1·5 % urethane. After 15 min. it is removed from this tank and opened by a ventral abdominal incision about $1\frac{1}{2}$ in. long. The position of the incision varies according to the place where it is intended to insert the ovum. An ovum freshly excised from the ovary of another adult female fish is introduced through the incision and placed in a selected position which is carefully recorded. The incision is sewn up so as to be water-tight by about five stitches of gut each passing through the entire thickness of the body wall and the fish is then transferred to a tank of fresh sea water. In about 2 hr. the fish has fully recovered from the effects of the anaesthetic and is swimming normally.

After an interval of time, the fish is again anaesthetized, the incision reopened and the position of the introduced ovum is recorded. At this stage the fish is either killed by pithing and the body used for Method 2, or the incision is resewn and the fish allowed to recover and the experiment to proceed for a further specified period. At the end of this period the fish is once more anaesthetized, the incision again opened and any further movement of the ovum recorded. It is then pithed and the body used as before. On one fish, recovery from anaesthesia and observation after a further interval was repeated a third time before the fish was killed. No fatalities resulted from this treatment and every fish when killed was vigorous.

Method 2

Fish which have been used for Method 1, or fresh fish, are anaesthetized as before, then killed by pithing and the abdomen opened from end to end by a ventral incision. An ovum excised from the ovary of one fish is placed in the peritoneal cavity of the same or another fish which is then immersed in a bath of sea water. The anaesthetic prevents any contortions of the body which might dislodge the ovum under observation. It does not appear to affect the ciliary action seriously as the migrations obtained are certainly not inferior to those obtained by Method 1. Sea water has almost the same osmotic pressure as the coelomic fluid and is used instead of isotonic saline to keep the cilia active. It serves the additional function of reducing the weight of the ovum to less than one-eleventh part of its weight in air. Without this immersion in saline, no migration of the ovum takes place as the cilia are crushed by it. Stitching the incision compresses the viscera and brings the normal area of ciliated epithelium in contact with the ovum. It assists migration but renders continuous observation impossible. Ciliary action continues for several hours after the fish has been pithed.

The results obtained by both methods are recorded in Table II.

TABLE II. MIGRATION OF OVA INTRODUCED INTO THE PERITONEAL COELOM OF FEMALE DOGFISH

No. of fish	Condition of fish	Size of ovum	Position of ovum. (Method 1)				Migration (+) or no migration (−)	Position of ovum. (Method 2)		Migration (+) or no migration (−)
			At insertion	1st inspection and time	2nd inspection and time	3rd inspection and time		At insertion	Ultimate (at death of cilia) and time	
1	Adolescent. Ovary immature; peritoneal cilia inactive, active in oviduct	Ripe	Between fimbrial septa, at entrance to ostium	In left oviduct $\frac{2}{3}$ way towards oviducal gland 7 hr.	At top of left oviducal gland 22 hr.	No change 47 hr.	+	...	...	...
2	Adult. Peritoneal cilia active. Mature egg-cases *in utero*	Ripe	As above	In left oviduct at top of oviducal gland 22 hr.	In opening of left oviduct (ovum regurgitated) 48 hr.	...	+	Between fimbrial septa at entrance to ostium	Half embedded in top of left oviducal gland 3 hr.	+
3	Adult in act of laying. Peritoneal cilia active	Ripe	Between left liver lobe and base of left oviducal gland	$\frac{1}{2}$ in. forward 23 hr.	No change 52 hr.	...	+ Weak	Posterior end of liver bridge	No change 5 hr.	−
4	As 3 above	Ripe	As above	No change 52 hr.	...	...	−	Anterior end of liver bridge	In left oviduct at top of oviducal gland 5 hr.	+
5	Adult. Non-pregnant. Peritoneal cilia active	Small	Dorsal to right liver lobe (posterior end)	$\frac{1}{2}$ in. forward 24 hr.	1 in. forward 52 hr.	...	+	Between right liver lobe and base of right oviducal gland	2 in. forward (within $\frac{1}{2}$ in. of ostium) 5 hr.	+
6	As 3 above	Ripe	...	...	...	...	...	As in 2 above	In left oviduct at top of oviducal gland 4 hr.	+
7	As 5 above	Small	...	...	...	...	...	As in 2 above	In top of left oviduct $2\frac{1}{2}$ hr.	+
8	As 5 above	Small	...	...	...	...	...	Between left liver lobe and top of oviducal gland	$\frac{1}{3}$ in. forward $2\frac{1}{2}$ hr.	+ Weak
					Controls					
9	Immature. Peritoneal cilia inactive	Small	As in 5 above	No change 24 hr. Ovum removed and replaced dorsal to oviduct (posterior end)	No change 52 hr.	...	−	Between right liver lobe and top of oviducal gland	No change $4\frac{1}{2}$ hr.	−
10	Immature. Only anterior peritoneal cilia active	Ripe	As 3 above, but on right side	No change 45 hr.	...	...	−	...	...	...
11	As 10 above	Ripe	As 10 above	No change 45 hr.	...	...	−	...	...	...

Ripe ovum: 1·5 cm. diam. Small ovum: 1·0 cm. diam.

Analysis of Table II

It will be noted that in all eight adult fish, where peritoneal cilia were active, some migration of the ovum took place. Such movement was always in the direction of the ostium. In the three immature fish no migration occurred.

In each of the four experiments in which an ovum was placed at the entrance to the ostium, it subsequently entered the ostium and later proceeded to descend the *left* oviduct. In three of these four cases the ovum subsequently arrived at the top of the left oviducal gland, whilst in the fourth case ciliary action had ceased before that level was reached. Twice, where Method 2 was employed, the entrance of the ovum into the ostium and its passage down the upper oviduct was actually witnessed.

Movements of the ovum are too slow to be observed continuously, and instead, observations are taken at intervals of a few minutes. The ostium stretches as the ovum sinks into it and then slowly contracts again as the ovum is engulfed. The fimbrial septa part and then come together again. There are no muscular movements of the fimbrial septa or any other organs, but as a result of their ciliary action the septa become wrapped round the ovum, thereby bringing more of it into contact with their cilia and accelerating engulfment.

Passage of the ovum through the ostium takes about 30 min. A few minutes later it appears impossible to predict into which oviduct the ovum will pass, though the asymmetry previously described seems to favour the right. Soon it becomes apparent that the ovum is entering the left oviduct which dilates considerably. Slowly it travels downwards towards the oviducal gland. There is no muscular movement of the oviduct whatever. About 2 hr. later the ovum reaches the top of the gland. These times are probably not those in the living fish during a normal ovulation; they represent the mean of only two cases (nos. 6 and 7) witnessed under conditions when the cilia were known to be slowing down. In both these cases the body cavity was widely open.

Since the entrance of the ovum into the ostium and its passage down the left oviduct was obtained when the fish was swimming dorsal side uppermost, as well as when the fish was pithed and kept ventral side uppermost, the possibility of gravity playing any part in the process is eliminated.

Only in one case (no. 4) did an ovum, inserted into the peritoneal cavity posterior to the ostium, enter the ostium. This ovum was placed in the anterior end of the liver bridge, being pushed there with the finger through an abdominal incision posterior to it. In this way the ovum was in contact ventrally with the ciliated body wall. For this reason it was not possible to witness the migration of the ovum across the hepatic sinus nor its entrance into the ostium and passage down the oviduct. As in the four previously mentioned fish, it was the left oviduct into which the ovum passed.

Although the experiment would have to be repeated on a much larger number of fish than five before a definite statement could be made, it is suggested that the first ovum of the pair liberated during a normal ovulation passes into the left oviduct, and that the second ovum, following immediately behind the first, and finding the entrance to that oviduct blocked, passes into the right.

Ova introduced into the mid-region or posterior end of the coelom show a small amount of movement, varying from one-third of an inch to two inches, over periods of 24–48 hr., always in the direction of the ostium.

From the rareness with which an adult female, on being opened, is found to contain ova in the coelom undergoing migration (there is no record that this has ever been seen), it follows that the process of migration from ovary to ostium is of comparatively short duration, probably a matter of hours and certainly not of days. As the effect of the anaesthetic on the cilia does not last for 48 hr., it is probable that during a normal ovulation the action of the peritoneal cilia is stimulated. It has been previously remarked that the vigour of the ciliary action is greatest at the ostium and falls off as the distance from the ostium increases. It follows that the rate of migration of the ovum is slowest at the posterior end of the coelom and accelerates as the ovum approaches the ostium. When a fish is not ovulating, the vigour of the ciliary action at the anterior end of the coelom is still sufficient to effect migration, though necessarily at a reduced speed, whilst at the posterior end of the coelom the ciliary action is hardly powerful enough to move an ovum under experimental conditions.

Effect of "pregnancy" on ciliary currents

The migration of ova introduced into the coelom, their entrance into the ostium, and passage down the oviduct as far as the top of the oviducal gland, is not affected by "pregnancy". Four of the fish used for this work had fully formed egg-cases *in utero*. In an adult female, action of the cilia is continuous.

The oviducal gland

In each of the five fish in which an ovum, introduced into the peritoneal coelom, subsequently entered the ostium and descended the oviduct, the ovum never succeeded in passing through the oviducal gland; usually it made no attempt even to enter it. At the most the ovum stopped when half embedded in the albumen-secreting region of the gland.

In no case was secretory activity induced in any region of the oviducal gland by the approach of the ovum or its attempt to enter the gland. The ovum is therefore not the direct stimulus inciting the oviducal gland to secrete. Borcea (1904) believes that normally the ovum incites secretion of the oviducal gland, but he adds that secretion occurs in a reflex manner and that an ovum is not the only stimulus which causes it—"other stimuli result in 'eggs' containing only albumen, if the excitement has been intense, or only filaments, if the excitement has been feeble". What these other stimuli are, Borcea does not state. On the other hand, Hobson (1930), on the evidence of a single skate, concludes that the posterior three-quarters of the egg-case is secreted before the ovum enters the oviducal gland and probably half of it before the ovum has left the ovary. In this skate ova were found in the upper oviducts, between the ostium and the oviducal gland, whilst the egg-cases were three-quarters completed.

In order to establish the truth or otherwise of Hobson's contention, a record has been made of a number of partially secreted egg-cases, with respect to presence or absence of an ovum. Several stages are represented, ranging from the earliest, where only the posterior tendrils have been secreted, to fully formed egg-cases. The results are in Table III. In every fish the condition on both sides was similar, so that each egg-case recorded represents a different fish. There was never an ovum present in a less than three-quarters completed egg-case; nor was an ovum to be found undergoing migration in the coelom or upper oviduct in any fish where the egg-cases were half secreted or less. No fish was found in the exact condition of Hobson's skate.

In one fish (no. 21), fully formed egg-cases were found containing albumen but no ovum. The egg-cases were rather small, but otherwise normal. Hobson's contention is therefore fully justified and further work will be necessary to determine the stimulus which does promote secretion of the egg-case.

For investigating the oviducal gland of the dogfish, any female with an ovary containing large ova and possessing active peritoneal cilia was assumed to be adult, though it was not possible to eliminate the chance of including an occasional adolescent fish.

The oviducal glands freshly excised from fish decapitated in the living state were fixed whole in Bouin's fluid for 3 days. Bouin's fluid was found to be a most satisfactory fixative on account of its power to penetrate relatively large pieces of tissue, without destroying the cilia. The sections were stained on the slide in Ehrlich's haematoxylin for 3 days, differentiated in acid alcohol and counterstained either with aqueous eosin (few minutes) or Van Giesen's stain (30 sec.).

For demonstrating the presence of spermatozoa in the oviducal gland, a thorough differentiation is essential before blueing with the vapour of ammonia and counterstaining. The heads of the spermatozoa then retain more haematoxylin than do the surrounding nuclei. "Van Giesen" is an excellent counterstain in that it continues the differentiation and turns the chromatin from blue to black, as well as staining the collagenous fibres of connective tissue red and other tissues yellow. Care must be taken not to overstain with "Van Giesen" as it bleaches the haematoxylin.

Historical.

Of the many authors who have investigated the oviducal gland, at least four of whom (Perravex 1884; Henneguy 1893; Widakowich 1905; Borcea 1904 and 1906) have examined longitudinal sections under the microscope, none has observed the presence of spermatozoa, although they occur in the glands of every adult female. Borcea's account is the most recent and, either the less perfect technique of 30 years ago, or poor observation, accounts for the spermatozoa having been overlooked.

In his otherwise very adequate paper (1906), Borcea has summarized separately the contributions of all authors to our knowledge of the oviducal gland of cartilaginous fish, from 1846 to 1893, before making his own contribution. His bibliography contains no reference to the work of Widakowich, and it is probable that this work was still unpublished when Borcea wrote.

In the words of Borcea..."the gland is composed of two halves, one dorsal the other ventral, the first part more developed on the right side and the other more developed on the left. In each half two zones are distinguishable, (1) a superior (anterior) zone secreting albumen and (2) an inferior (posterior) zone secreting the horny substance of the egg; and in each of these zones, an external region composed of secretory tubes and an internal lamellar system which serves to receive, conduct and dispose of the product of secretion", fig. 10. Henneguy (1893) and Widakowich (1905) have described the histology of the tubes in the albumen- and shell-secreting regions.

In preparing an oviducal gland for sectioning purposes, the ciliated peritoneal epithelium on the outside readily detaches itself, and it does not figure in the accounts of any of the authors mentioned.

Descriptive.

At rest, both sides of the oviducal gland are in contact with one another and the lamellae of one side flatten against those of the other. The tubes open in rows, one between each pair of lamellae. The lamellae of the shell-secreting zone are confined to its anterior end and are well developed, those of the albumen-secreting zone are rudimentary.

Fertilization in the dogfish is internal and it has always been assumed that spermatozoa passed upwards through the gland and that fertilization occurred in the narrow upper region of the oviduct, before the descent of the ovum into the oviducal gland. There is, however, no record of spermatozoa in this region of the oviduct. The assumption is based on the necessity for fertilization preceding envelopment of the ovum by an impenetrable egg-case, and also by analogy with the condition found in birds and reptiles where fertilization certainly precedes shell-formation. The possibility that both processes, fertilization and secretion of the egg-case, occur simultaneously, does not seem to have been considered, although this actually occurs.

The whole of the epithelium lining the oviducal gland is ciliated. Both regions of the gland, that secreting albumen and that secreting the egg-shell, contain simple tubular glands lined throughout by ciliated epithelium. Between the ciliated cells lie the secretory cells. Some of the tubes are extremely long and coiled or twisted, especially those of the shell-secreting region. The opening of each tube lies between a pair of small plates at the base of the lamellae, all the plates being parallel to one another but oblique with respect to the lamellae. The ciliated epithelium lining the tubes is continuous with that of the plates and the lamellae (fig. 15, Plate 20). The cilia on the lamellae and on the rest of the inner surface of one half of the oviducal gland can easily be seen in the living state, under a binocular microscope, by means of reflected light. By sectioning a fresh gland vertically, as in fig. 10, ciliary movement within the tubes themselves can be seen. The current is directed towards the openings of the tubes. The cilia lining the lumen of the gland beat in the direction of the cloaca.

The oviducal gland as a receptaculum seminis.

A longitudinal vertical section through an oviducal gland of an adult dogfish shows clusters of spermatozoa lying within the lumen of those tubules which secrete the egg-shell (figs. 14 and 15, Plate 20). The largest clusters are found near the openings of the glands, that is to say, beneath the bases of the lamellae, but isolated spermatozoa are found throughout the tubules of the shell-secreting zone. Shell material present in the tubules stains a reddish-brown colour with "Van Giesen", and the more deeply stained spermatozoa can be seen lying in it. No spermatozoa were ever found in that region of the oviducal gland which secretes albumen. Active spermatozoa can be seen in the shell-secreting region of the gland, if freshly excised.

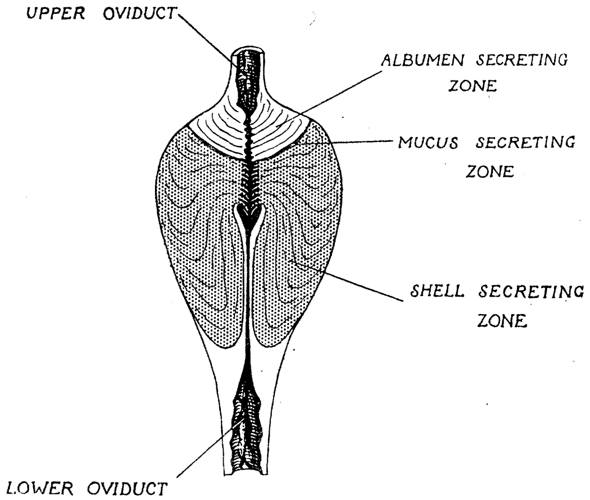

FIG. 10. Vertical section through a non-secreting oviducal gland of *Scylliorhynus canicula*. × $1\frac{1}{3}$.

Mucous glands are present both in the form of short pits in the side walls of the gland, posterior to the lamellae, and also as a single row of tubes lying between the albumen-secreting and shell-secreting zones (fig. 10). They are also ciliated throughout but no spermatozoa ever occupy them.

No spermatozoa are found anywhere in the oviducal glands of sexually immature fish. It is surprising to find, however, how early in life active cilia are to be found throughout the oviducal gland. As the spermatozoa may have a relatively long life in the oviducal gland, presumably they select the shell-secreting tubules by virtue of some nutritive material in the shell substance, which is absent from the albumen and mucus. The spermatozoa would have the same mechanical difficulty to overcome, namely, an adverse ciliary current, whichever region of the gland they entered.

The existence of a receptaculum seminis in elasmobranch fish was suggested by

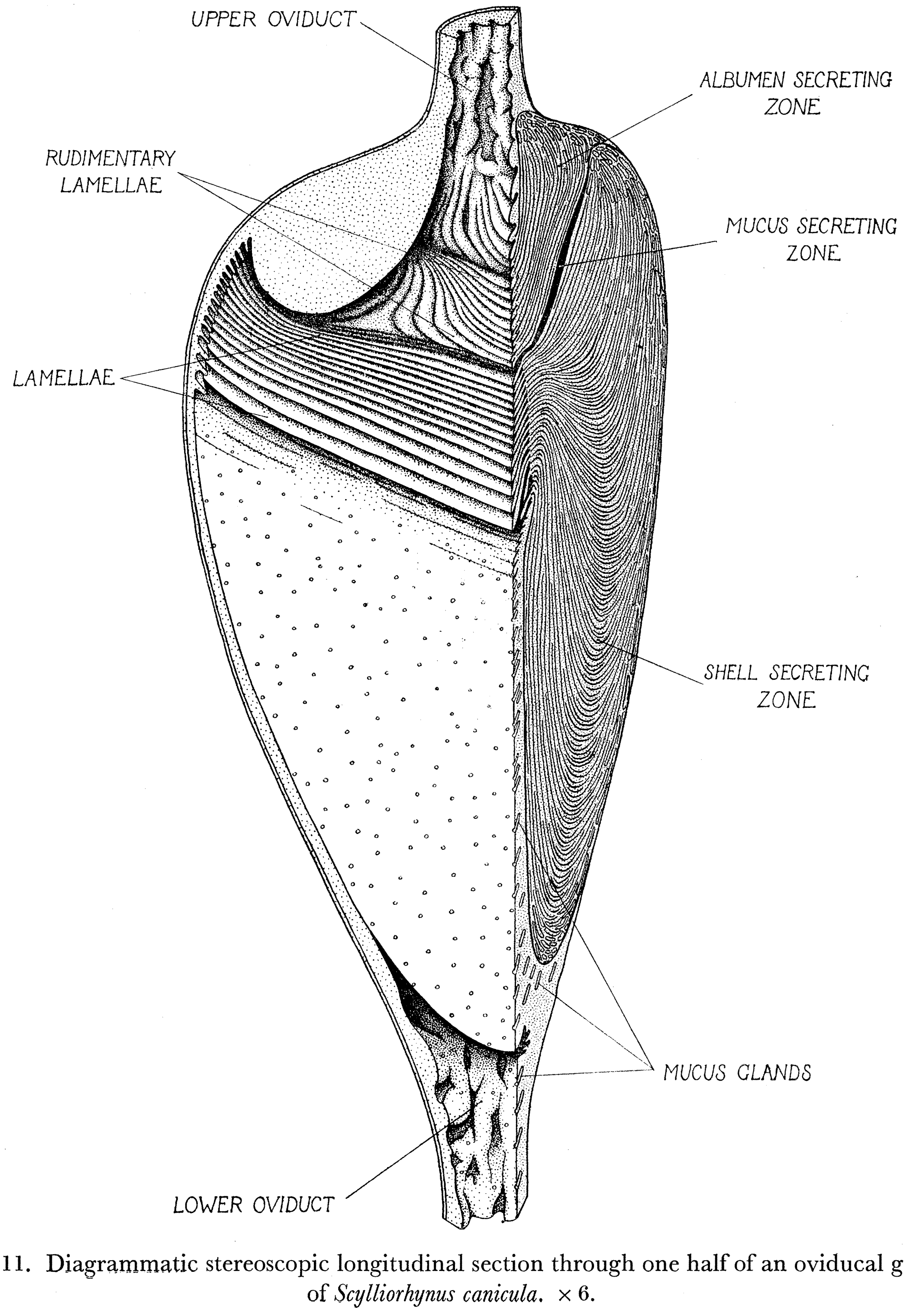

FIG. 11. Diagrammatic stereoscopic longitudinal section through one half of an oviducal gland of *Scylliorhynus canicula*. × 6.

Clark (1922). He describes a female Blonde, *Raia brachyura*, which, kept alone over a period of 5–6 weeks, laid 30 egg-cases, the ova of which were all fertile. Clark mentions that the same thing has been recorded in two other species of *Raia*. He dismisses the theory that fertilization occurs in the coelom, on developmental grounds, and concludes that "there must be a receptaculum seminis where the spermatozoa are stored...probably in the upper reaches of the oviduct". Clark omits to add that this view eliminates the possibility that copulation is the stimulus which incites ovulation.

It has already been shown that the egg-case is about three-quarters secreted at the time of arrival of the ovum in the oviducal gland. The small ciliated lamellae of the albumen-secreting zone are arranged obliquely to rotate the ovum and ensure an even distribution of albumen (fig. 11). Spermatozoa are emitted in vast numbers as the shell-secretion passes out between the lamellae. The shell-secretion hardens immediately upon leaving the lamellae and probably only a few spermatozoa escape whilst the rest become incorporated in the shell and perish. No spermatozoa can be seen in sections of the hardened egg-case, however, as the shell material, once set, is practically impervious to nuclear stains. In any case it is doubtful whether such spermatozoa would be recognizable for any length of time.

The selachian spermatozoon has been described by Retzius (1902). It is about 160μ in length and is characterized by a long, pointed, spirally twisted head (fig. 12). In stained specimens this twist is only visible under an oil-immersion lens (fig. 16, Plate 20), but in living spermatozoa it can easily be seen under a $\frac{1}{6}$ in. objective.

In the deep-seated regions of the gland, the lumen of the shell-secreting tubules is so narrow that the cilia of the surrounding cells meet in the centre. Spermatozoa in these regions lie in parallel bundles and heading inwards. No doubt a constant lashing of their tails is necessary to maintain or improve their position against the action of the cilia. In a secreting gland, a proportion of these spermatozoa are swept backwards towards the openings of the tubules. Shortly before it opens, each tubule widens, allowing the spermatozoa to turn round. As a result many of the heads of the spermatozoa in this region are U-shaped (fig. 15, Plate 20). Most of the spermatozoa, however, are swept out tail first, before they have turned.

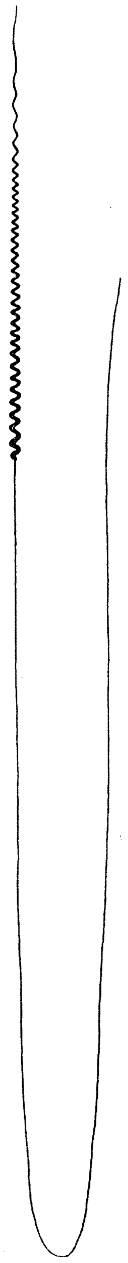

FIG. 12. A spermatozoon of *Scyliorhynus canicula*. × 2000.

Sperm content of oviducal glands.

It was decided to determine the variability of the sperm content of oviducal glands, during various phases of secretory activity and also at rest, as any variability would throw some light on the breeding habits of the dogfish.

A series of 20 secreting glands (1–20, Table III), taken from 20 different fish, covered a range from glands at the very beginning of secretion (in which only the posterior tendrils of the egg-case had been formed) to glands which had just finished secreting the anterior tendrils of the egg-case, now completed and lying in the uterus. Without exception these glands contained spermatozoa, though the content was very variable. In extreme cases a thorough search through a section of one gland revealed one or two spermatozoa only, whilst a section through another gland showed the shell-secreting tubules packed with spermatozoa. Some spermatozoa could be seen passing out with the egg-case secretion, between the lamellae. In this way, fertilization of every egg is ensured. Of these glands, some were obtained in summer (August), and some in the winter (December).

TABLE III. SPERM CONTENT OF OVIDUCAL GLANDS OF *SCYLLIORHYNUS CANICULA*. GLANDS IN VARIOUS STAGES OF SECRETION

Number of gland	Phase of secretion	Ovum present (+) or absent (−)	Details of sperm content	*
1	Posterior tendrils only secreted	−	...	++
2	Posterior tendrils only secreted	−	Few	+
3	Posterior tendrils only secreted	−	...	+
4	Posterior tendrils only secreted	−	...	++
5	$\frac{1}{8}$th of egg-case secreted	−	Few	+
6	$\frac{1}{6}$th of egg-case secreted	−	...	+
7	$\frac{1}{6}$th of egg-case secreted	−	...	+
8	$\frac{1}{6}$th of egg-case secreted	−	...	++
9	$\frac{1}{6}$th of egg-case secreted	−	Very few	+
10	$\frac{1}{3}$rd of egg-case secreted	−	Few	+
11	$\frac{1}{2}$ of egg-case secreted	−	Very few	+
12	$\frac{1}{2}$ of egg-case secreted	−	Very few	+
13	$\frac{1}{2}$ of egg-case secreted	−	...	+
14	$\frac{3}{4}$ of egg-case secreted	+	...	++
15	$\frac{9}{10}$ths of egg-case secreted	+	...	++
16	Completed egg-case in *anterior* end of oviduct	+	...	+
17	Completed egg-case in *middle* of oviduct	+	Very few	+
18	Completed egg-case in ‘uterus’	+	...	++
19	Completed egg-case in ‘uterus’	+	...	+
20	Completed egg-case in ‘uterus’	+	...	+
21	Completed egg-case in ‘uterus’	−	...	Not examined

* The symbol + denotes the presence of spermatozoa in a gland and is repeated in cases of abundance. The symbol − denotes absence of spermatozoa.

It was seen that the number of spermatozoa emitted during the manufacture of a single egg-case was a small proportion of the total. A gland would therefore pass into the resting condition, still containing spermatozoa (Table IV).

Of twelve glands in the resting condition, no two being obtained from the same fish, eleven contained spermatozoa. Again the content was very variable.

Except by segregation at the time of laying, there is no method of establishing with certainty that a "non-pregnant" female is *adult* and not merely *adolescent*. An adolescent fish would automatically give a negative result. If the accidental inclusion of a single adolescent fish be allowed, one may generalize and say that the oviducal glands of *all* adult female dogfish contain spermatozoa. It follows that copulation must recur before the gland has been exhausted of its sperm content.

TABLE IV. RESTING GLANDS

Number of fish	Details of sperm content	+ or −
1	...	+
2	...	+
3	...	+
4	? Adolescent fish	−
5	...	++
6	...	+
7	Few	+
8	...	+++
9	Very few	+
10	...	++
11	...	+
12	Few	+

SUMMARY

1. The literature concerning coelomic migration of ova, in the major groups of vertebrates, is surveyed; also that pertaining to the structure and functions of the oviducal gland in the elasmobranchs.
2. The abdominal cavity of the adult female dogfish, *Scylliorhynus canicula*, is abundantly ciliated, whilst abdominal ciliation is absent in the males and immature females.
3. The nature and relationships of the ostium are described.
4. The distribution of abdominal cilia in the adult dogfish is described and compared with that of the frog.
5. A description is given of the coelomic space and the paths of migration of ova from ovary to ostium.
6. Methods are described for obtaining coelomic migration of transplanted ova, their entrance into the ostium and passage down the upper part of an oviduct. Part of this process has been witnessed and described. It is adequately effected by ciliation and is independent of any muscular movement. It is also unaffected by "pregnancy".
7. During a normal ovulation, the ovum is not the stimulus which incites secretion of the egg-case, the egg-case is about three-quarters completed by the time the ovum arrives in the oviducal gland.
8. A transplanted ovum will descend the upper oviduct, but will not pass through an oviducal gland at rest.
9. The dogfish has no breeding season...it is always sexually active though slightly more prolific in spring.
10. A new and important function of the oviducal gland, that of a "receptaculum seminis", is described.

11. A method is given for preparing sections of the oviducal gland to show spermatozoa.

12. The oviducal glands of *all* adult female dogfish, whether secreting or at rest, contain spermatozoa. No spermatozoa are ever found in the oviducal glands of immature fish.

13. Spermatozoa are confined to that region of the gland which secretes the shell substance. Probably something in the shell secretion nourishes the spermatozoa.

14. Fertilization takes place in the oviducal gland and not in the upper oviduct as previously supposed. It is contemporaneous with secretion of the shell substance and it does not precede but follows deposition of albumen around the ovum by the anterior region of the gland.

15. A relatively small proportion of the total sperm content of an oviducal gland is emitted during the fertilization of a single ovum and the manufacture of its egg-case.

The author wishes to express his thanks to Mr H. R. Hewer, for his help in supervising this work, and to the Marine Biological Association, Plymouth, for obtaining material, and providing facilities for carrying out the experimental part of this investigation.

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Explanation of Plate 20

Fig. 13. Median vertical longitudinal section through one half of an oviducal gland of an adult dogfish (*Scylliorhynus canicula*). ×3½.

(The small rectangular area is shown enlarged in fig. 14.)

A.	Albumen-secreting zone.
I.S.	Inner surface, pitted with mucous glands.
L.	Lamellae.
Lo.O.	Lower oviduct.
M.	Intermediate mucus-secreting zone.
R.L.	Rudimentary lamellae.
S.	Shell-secreting zone.
Up.O.	Upper oviduct.

Fig. 14. Portion of the shell-secreting zone of an actively secreting oviducal gland, showing the tubules opening between the lamellae and clusters of spermatozoa in the tubules. ×60.

(The rectangular area is shown enlarged in fig. 15.)

L.	Lamellae.
O.P.	Oblique plates.
Op.	Openings of shell-secreting tubules between oblique plates.
S.	Shell-secreting tubules.
S.M.	Shell material issuing between lamellae.
Sp.	Spermatozoa, about to be ejected.
Sp.C.	Clusters of spermatozoa in deeper regions of tubules.

Fig. 15. The openings of a few shell-secreting tubules of an actively secreting oviducal gland of the dogfish, showing some spermatozoa attempting to turn round and others being ejected with the shell material. ×230.

(The small rectangular area is shown enlarged in fig. 16.)

Ci.Epi.	Ciliated epithelium.
C.T.	Connective tissue.
L.	Lamella.
O.P.	Two oblique plates with opening of tubule between them.
Sp.	Spermatozoa in shell material, being emitted between lamellae.
Sp.C.	Clusters of spermatozoa lying in shell material. (Heads only visible.)
Sp.t.	Spermatozoa in act of turning round.

Fig. 16. Portions of two lamellae of the shell-secreting zone of an actively secreting oviducal gland of the dogfish, showing the emission of spermatozoa in the shell material between the lamellae. ×1350.

Ci.	Cilia of ciliated epithelial cell of lamella.
C.T.	Connective tissue (of lamella).
N.	Nucleus of ciliated epithelial cell.
S.M.	Shell material.
Sp.H.	Entire head of a spermatozoon which is being ejected tail first. Note the spiral twist.
Sp.A.	Sharply pointed apex of head of same spermatozoon.

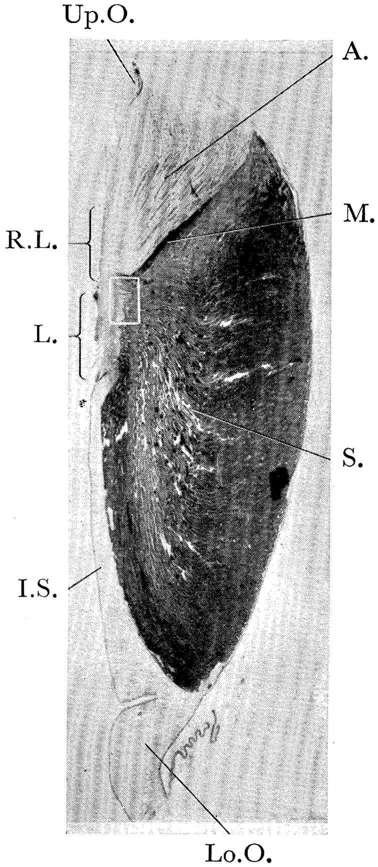


FIG. 13

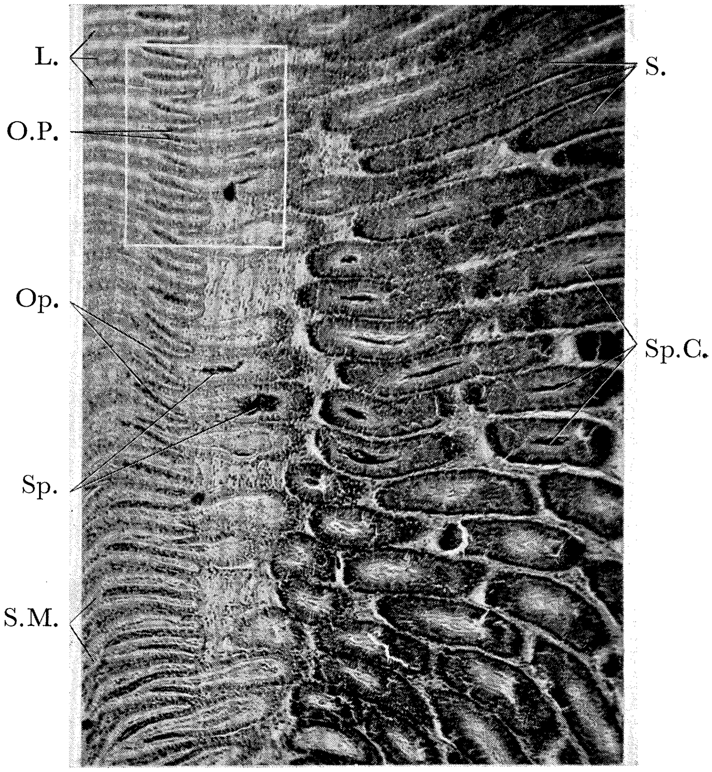
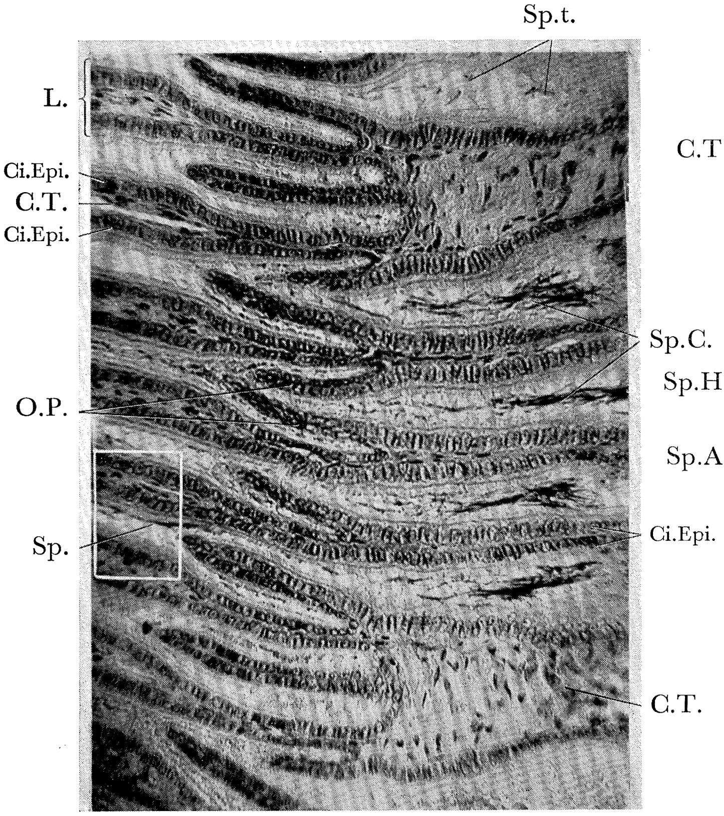


FIG. 14



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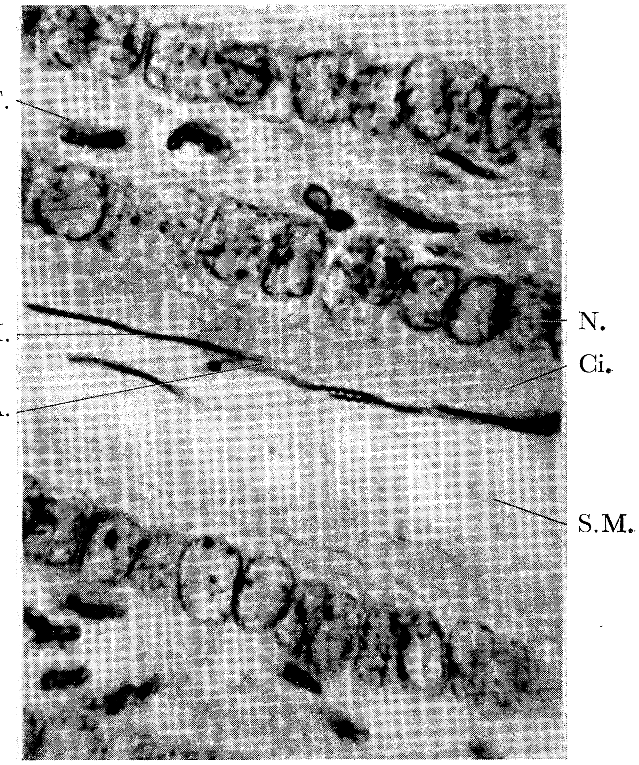

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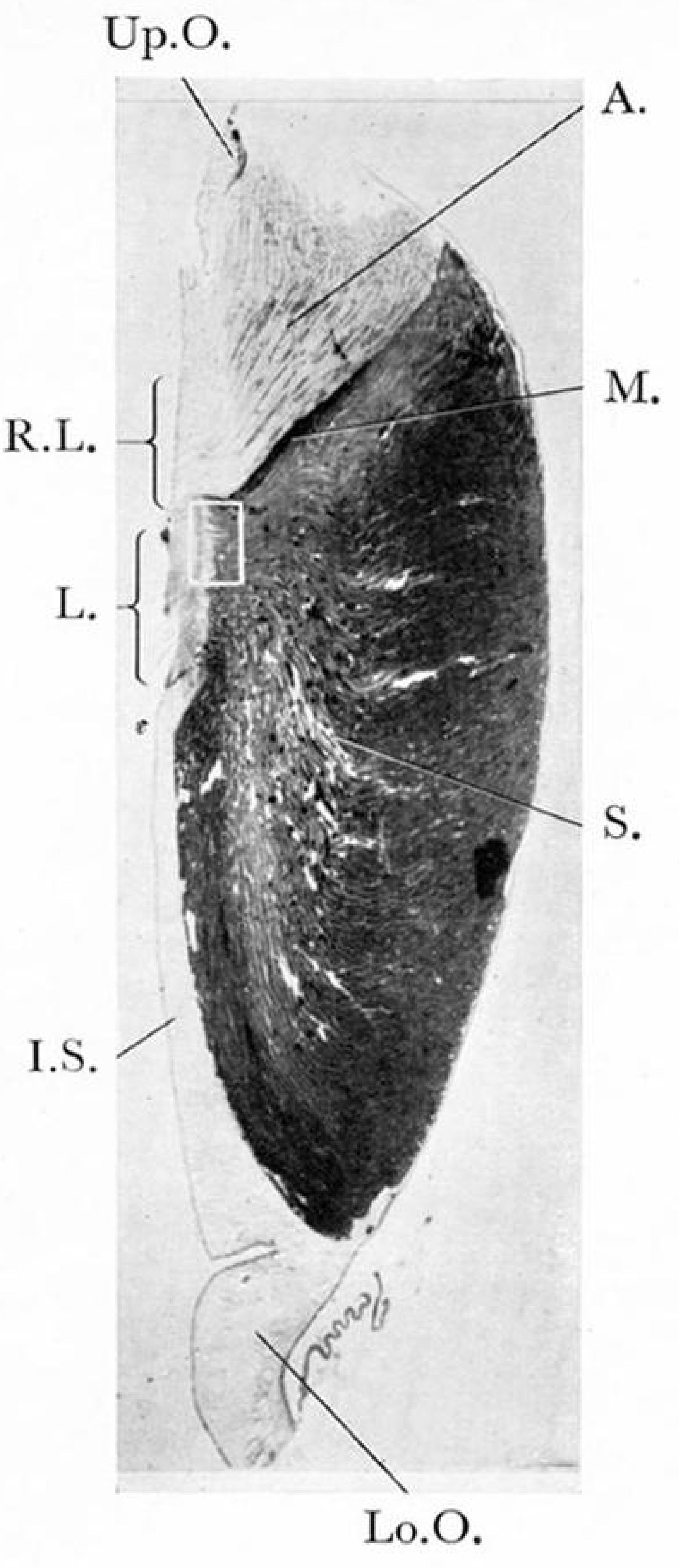

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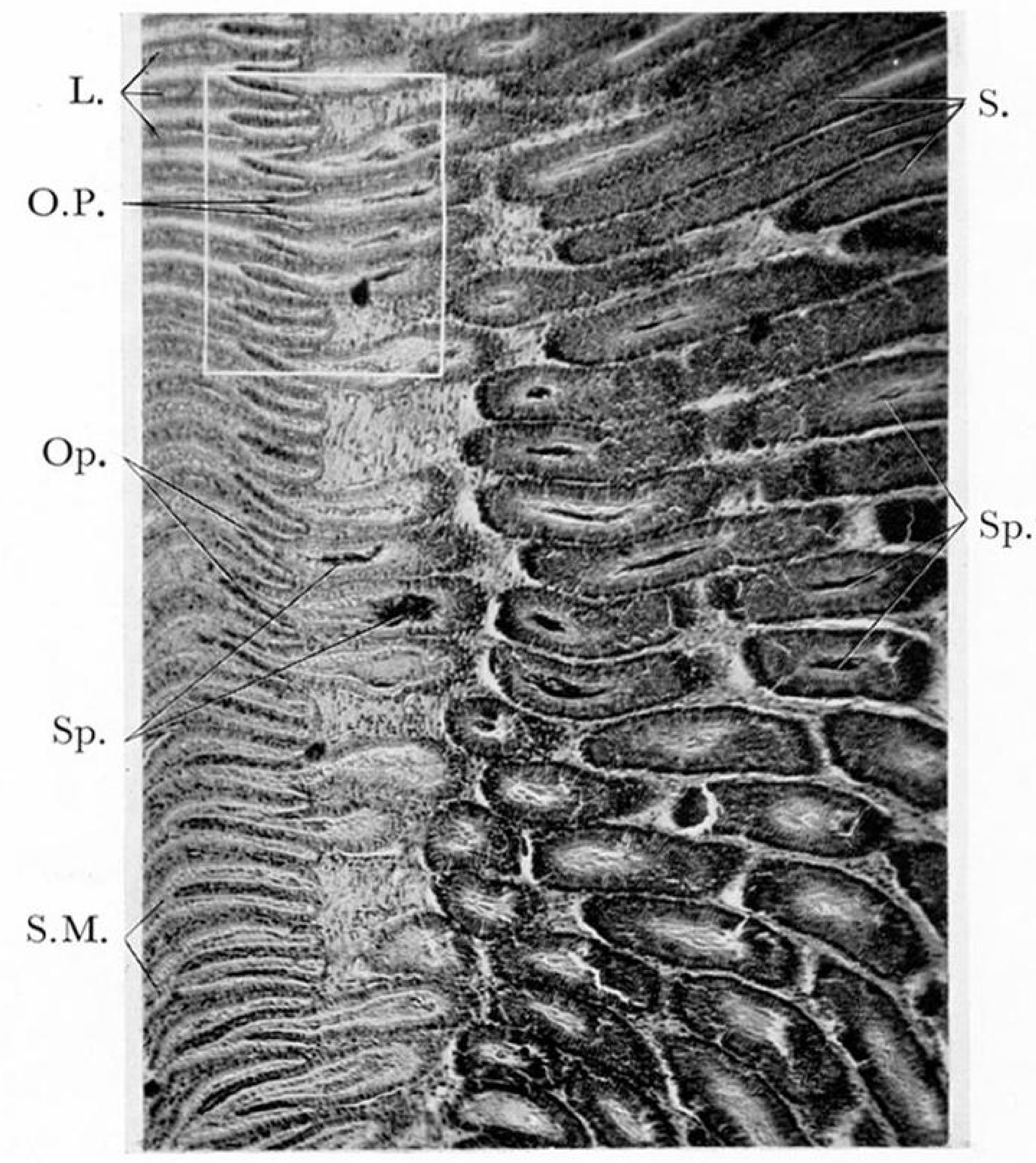

FIG. 14

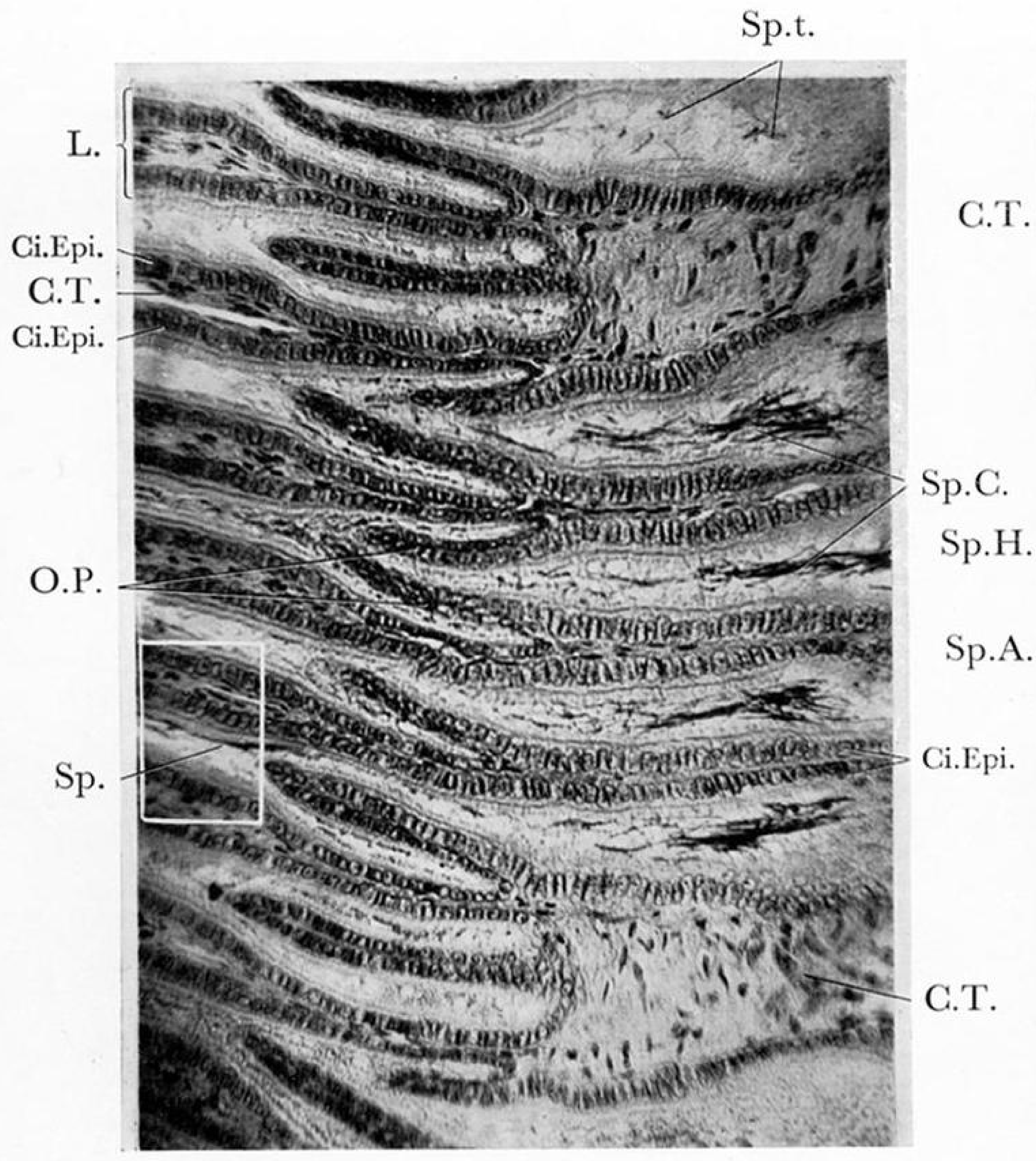

FIG. 15

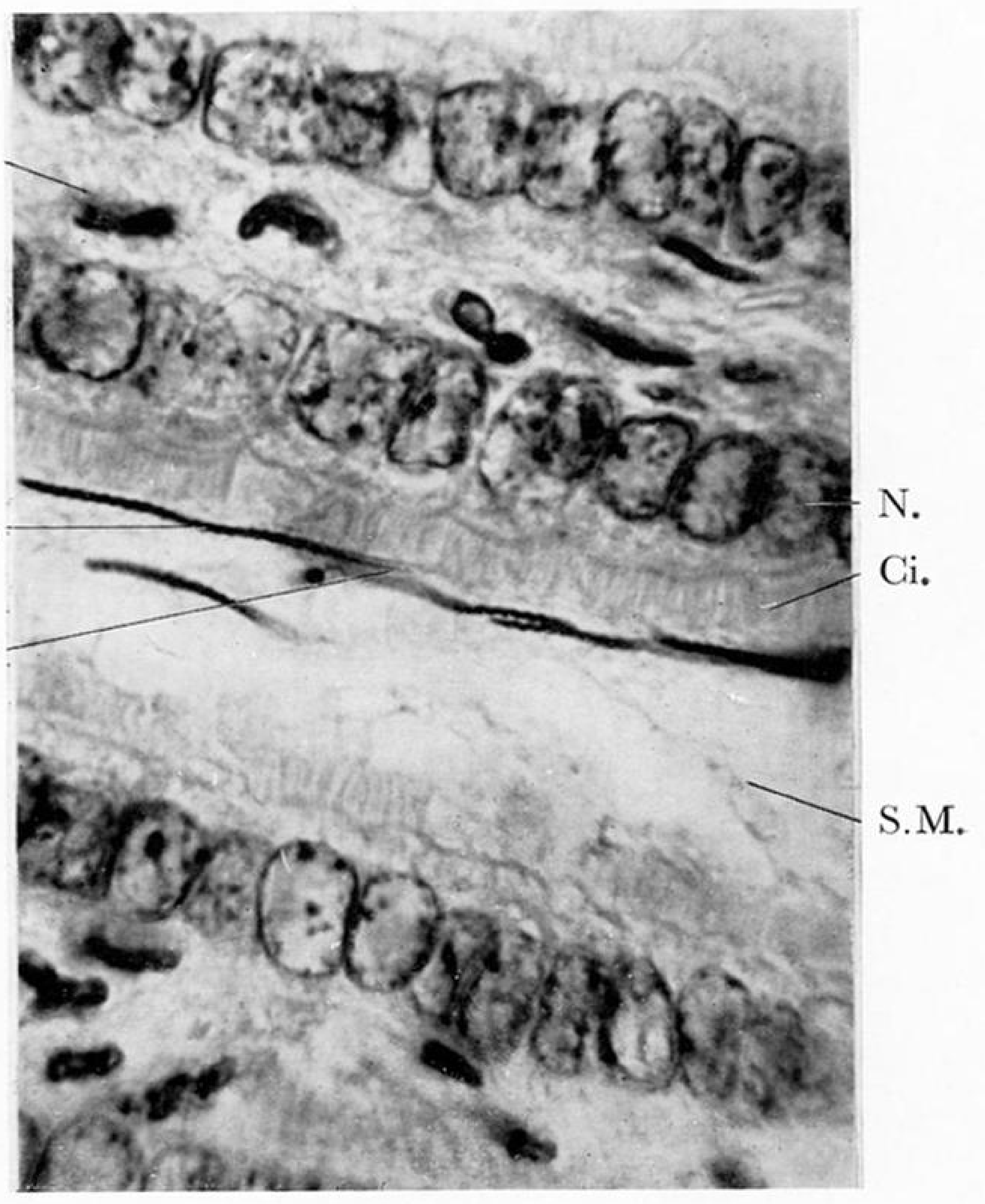

FIG. 16

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(The small rectangular area is shown enlarged in fig. 14.)

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(The rectangular area is shown enlarged in fig. 15.)

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S.M.	Shell material issuing between lamellae.
Sp.	Spermatozoa, about to be ejected.
Sp.C.	Clusters of spermatozoa in deeper regions of tubules.

FIG. 15. The openings of a few shell-secreting tubules of an actively secreting oviducal gland of the dogfish, showing some spermatozoa attempting to turn round and others being ejected with the shell material. ×230.
(The small rectangular area is shown enlarged in fig. 16.)

Ci.Epi.	Ciliated epithelium.
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