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Papers for Students No. 8

DISSECTING SNAILS

by

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Dissection is one of the standard techniques of the serious student of any animal group. It is of special importance to malacological work in the determination of similar species, working out life histories, searching for parasites etc. The purpose of this paper is to fill in some of the details which are often neglected in the few dissection manuals which include the snail. The method given below differs but little from that given by other authors, being the way of tackling any land snail, the anatomy of which may be unfamiliar to the student. It must be emphasised that there is no right or wrong way of dissecting any animal, provided that it is done in such a way as to demonstrate clearly those organs which are of interest to the investigator, so this is only a beginners' guide; once he has acquired a reasonable working knowledge of the snail's anatomy he will devise his own rapid methods of exposing those portions of the viscera which interest him, without unduly disturbing anything else.

The example chosen for description here is *Helix aspersa* Müller, being the most readily obtainable snail of a large size suited to the requirements of a beginner. *H. pomatia* L. is larger, but not materially easier to dissect. The main difference in its anatomy is that it lacks the long diverticulum of the spermathecal duct which is so prominent a feature of *H. aspersa*. As *H. pomatia* is rather local in Britain, it would be as well to practice on the commoner species. Smaller kinds, e.g. *Monacha cantiana* (Montagu), are not difficult, but it is better to begin with a big, easy species and work down to the smaller ones. As the anatomy of *Helix* is well covered by standard text books, it would be an advantage to the student to make use of these to become thoroughly familiar with the main systems in theory before starting into practice. A short bibliography is given at the end of this paper.

WARNING: UNTIL THE STUDENT IS PROFICIENT IN DISSECTING SNAILS HE SHOULD NOT ATTEMPT TO OPEN A RARE OR UNIQUE SPECIMEN, HE MAY NEVER HAVE ANOTHER CHANCE TO RECTIFY ANY MISTAKES MADE THROUGH IN-EXPERIENCE.

REQUIREMENTS. It is usual to dissect small animals under water. This is because when dissected in air the fine structures collapse into a shapeless mass, but under water the organs float free of each other and are then easily identified. The most convenient procedure is to melt soft paraffin wax into a small shallow dish, enough to provide a good thickness in which to stick pins, whilst allowing space for at least an inch of water to cover the specimen. Ordinary wax candles will do, but it is much easier to work on the black wax supplied for the purpose by most dealers in biological equipment. However, the writer finds several disadvantages in the use of wax for very small specimens; the fine entomological pins used will bend too easily, and have to be stuck into something softer, such as sheet cork or balsa wood. A piece of sheet cork is easily fixed to the bottom of the dish with any of the modern waterproof glues, e.g. "Bostik". A layer of plasticine is also good for use with cactus spines instead of pins when extremely small animals are being dissected.

Whilst all the necessary instruments can be purchased from the biological suppliers, certain of them are better if home-made, especially a seeker. The standard models are too thick and clumsy for work on snails. The best thing to do is to buy a packet of darning needles of mixed sizes, and with the aid of a robust pair of pliers, drive the eye-ends into short lengths of narrow dowel to form handles. Some of the needles can be left straight, others being bent into various angular shapes according to the requirements of the dissector. They need to be heated in a flame until blue before bending, as they are too brittle otherwise, and will snap. One indispensable tool can be made—a little hook, which is not sold anywhere to the writer's knowledge. This is done by heating the point of the needle in a flame and then pressing it vertically down on a coin, thus turning the point into a very tiny hook which can be used for a wide variety of purposes.

Other instruments which have to be bought are: a fine pair of pointed scissors (nail scissors are not suitable), and a pair of fine-pointed forceps—watchmakers' tweezers will do very well. A scalpel is useful, but needs to be pointed and kept very sharp; Swann-Morton scalpels with detachable blades are perhaps the best, the handiest blades being No. 10a or 11 (straight) and No. 12 (curved). For opening a long duct, a Borrodaile needle is good. The blade is pushed into the duct a little way, then by stroking against the edge with a seeker is can be made to shear through the duct wall with a scissor-like action.

For fixing down large snails, ordinary drapers' pins will do, but small animals such as *Oxychilus* have to be fixed with fine entomological pins or cactus spines.

For the smaller dissections some form of magnification is helpful. A watchmaker's eyeglass is cheap, but not so good because it fits one eye only, thus losing the third dimension. A fixed lens on an adjustable stand may be convenient. These tend to be expensive, but any lens (other that a "bullseye", which distorts badly) can, with a little ingenuity, be supported over the dissecting dish. A magnification of ×8 is as high as may conveniently be used, the focal length of a ×10 lens being so short as to leave insufficient working space beneath.

For magnifications above ×10 a proper binocular dissecting microscope is necessary, but this may cost anything from £40. It is impossible to dissect under an ordinary microscope, as it inverts the image. However, only the minute species need more that a ×8 lens.

KILLING. Snails are usually drowned in warm water so that they die in an expanded condition, but this takes up to 24 hours, and some of the ducts, especially the large common duct, become so bloated with water as to be unrecognisable. The writer finds that the best results are obtained by partly drowning (i.e. until the animal is fully expanded and still feebly moving), and then plunging it into boiling water to kill it. This not only loosens the shell, but fixes the tissues enough to prevent or greatly reduce bloating. Big snails like *H. aspersa* need about half a minute's boiling; smaller ones like *Cepaea nemoralis* (L.) need about a quarter minute, and very small or flat snails like *Retinella* and *Ashfordia* must not have more than a quick dip. Too much heating solidifies the animal, making it impossible to extract and virtually undissectable.

To extract the snail from its shell, insert a needle into the tail, and "unscrew" it from the shell. DO NOT PIERCE THE MIDDLE OR HEAD END AS THIS WILL DAMAGE THE INTERNAL ORGANS. If it will not come out with a gentle pull, the shell has to be cracked and removed piecemeal (as with a hard-boiled egg). KEEP THE SHELL, EVEN IF BADLY BROKEN; IT COULD BE IMPORTANT IN LATER WORK.

The extracted snail should look something like fig. 1, with some of the viscera visible through the semi-transparent mantle. The thick covering of slime can usually be wiped off with toilet tissue, or will often slide off in one lump if gently rubbed under a running tap. Methylated spirits will coagulate the slime in difficult cases. Before cutting anything, compare the cleaned snail with fig. 1 to get some idea of where a few organs lie, so that they can be avoided with the scissors.

THE DISSECTION. Now, holding the snail in the hand, cut with the scissors around the collar as indicated by the dotted lines in fig. 1. Start by pushing the scissors into the aperture of the mantle cavity and cut round to the left side of the snail first, then up the inside of the coil between the rectum and the columellar muscle to about the level of the heart, and finish with a cross-cut through the columellar muscle at right angles to the long cut. Cutting the columellar muscle allows the coil to be straightened out for about one whorl without damaging anything vital.

The collar is quite thick and awkward to cut, but usually the scissors go underneath it and sever the thin membrane which joins it to the neck, which is easier and more satisfactory.

From now on the dissection must be done under water. Fix the snail down in the dish as shown in fig. 2; turn the roof of the mantle cavity over to the animal's left side, and pin that down. EXCEPT FOR THE FOOT, MANTLE AND BODY WALL, PINS MUST NOT BE PASSED THROUGH ANY ORGANS. IT COULD DAMAGE THEM BADLY. PINS SHOULD BE PLACED SO AS TO REST AGAINST THE THINGS THEY ARE TO HOLD IN PLACE.

The cutting of the columellar muscle will have partly opened the body cavity (haemocoel) of the snail, so the next step is to complete the opening by making the cuts indicated by dotted lines in fig. 2. The whole dorsal wall of the main body can then be pinned aside in two flaps. This releases the pressure on the viscera, which spread out to look something like fig. 3. Several organs are held in place by muscles etc., which must be cut before the work can proceed. The main steps are: —

1. Cut the penial retractor muscle and pull the loop of the penis (marked "X" in fig. 3) forwards, so that it projects in front of the animal's head. Anchor it with a pin.

2. Cut the nerves and blood vessels which cross over the dart sac, so that it can be pulled out to the side. (Blood vessels are rounded and transparent as compared with the flat white opaque muscles.)

3. Cut the blood vessels at the rear end of the salivary glands as indicated in fig. 3.

4. Carefully cut away the thin membrane forming the back of the mantle cavity (dotted line in fig. 3), and with extreme care extend this cut a little way round the spire. This will expose the grey translucent albumen gland, the spermatheca half hidden below it (next to the heart), and the little hermaphodite duct (or ovotestis duct) which is a narrow, white, closely-coiled tube.

Compare with figs. 3 to 6 before each cut. AVOID PRODDING THINGS. SOME OF THE GLANDS ARE VERY BRITTLE.

The next step is to peel away the remains of the mantle and columellar muscle from the spire. This is an exceedingly delicate operation, as the digestive gland easily falls to pieces. Hold the columellar muscle with the forceps and cut around it with the scalpel or scissors; then pick off the mantle enough to release the albumen gland. Do not pull too hard, or the little common duct will snap. The albumen gland is then levered out with a seeker and laid over to the right as shown in fig. 5.

All that remains now is to unravel the remaining organs and pin them out as follows: —

1. Dig or tease out the ovotestis from the digestive gland. The two tissues look very much alike, but the ovotestis is usually paler and has a slightly different texture. Use the little hook and seeker as if they were knife and fork, holding the digestive gland down with the seeker and hooking up the ovotestis a little at a time. If this proves to be too difficult, just leave the two organs as they are.

2. Remove any pins securing the intestine and digestive gland if they are likely to be in the way during the next operation.

3. Loosen the spermatheca and follow its duct down the large common duct, to which it is attached by fibres and blood vessels, freeing it a little at a time with the scalpel-point or little hook, down to where it meets the vagina. In *H. aspersa* and some other snails, the spermathecal duct has a large tubular diverticulum arising near the base. On reaching this, detach it in the same way, but starting from the base and working up to the apex.

4. Now shift all the pins round, fixing the reproductive organs out on the right side and the digestive organs out on the left side as shown in fig. 6. As you go, trim away all superfluous pieces of mantle, blood vessel etc.: in short, MAKE THE DISSECTION LOOK AS CLEAN AND ORDERLY AS POSSIBLE.

5. Finally, make a large clear drawing of your work as accurately as posible. All scientific work should be recorded, no matter how trivial it seems to be.

If you think your drawing is sufficiently accurate, all that needs to be done about measurements is to draw a line on the side of the paper to represent a certain measurement on the specimen, and mark it accordingly in millimeters. If the line is made to represent a standard 5 or 10 mm. in every case (according to the size of the specimen), future comparisons will be made much more easily.

CONCLUSION AND FURTHER NOTES. The dissection described here is of a general nature, covering most of the snail's gross anatomy, but except for the reproductive system most snails are essentially the same internally. However, although the taxonomy of snails is based largely on their genitalia, important differences are often found between groups and species in the veins of the mantle cavity, in the nervous systems, kidneys, ureters etc. Therefore the body should be preserved, even if parts such as the radula and jaw have been removed.

If one is especially interested in the genitalia, it is convenient to remove them entirely by cutting away a small portion of the body wall around the genital opening. An interesting refinement is to split open some of the genital ducts to demonstrate folded linings, ligulae, spermatophores etc. This is standard practice with slugs.

Do not expect to find the same organs present in every snail. They may be modified or absent, or duplicated (in the case of dart sacs and mucus glands), and many oddities turn up, e.g. extra sacs, glands and diverticula, the purposes of which are often obscure. When dissecting an unfamiliar species, watch out for surprises.

The age of a specimen, and the mode of killing and preservation, have a considerable influence on the size and shape of the various parts, a fact which must be born in mind when selecting a specimen for dissection. One example of the alterations which take place during maturation is that of the spermatheca and its duct. In immature snails of different species they all look the same, but on maturation the ducts lengthen and the spermathecae take on distinctive shapes, as shown below (fig 7).

immature mature

Fig. 7. *Monacha cantiana* Mont., spermathecae.

In the above instance the radical alteration in outline may be partly due to the presence (or recent presence) of a spermatophore coiled up inside the mature spermatheca, but this can usually be seen through the semi-transparent wall.

An important point to observe is the position of the right ocular (eyed) tentacle and its muscle. In most cases they pass between the penis and the vagina (e.g. *Helix*), but in some genera they pass to the left of both (e.g. *Helicella*).

When drawing the genitalia it is preferable to draw the ventral side, as this shows some details more clearly; but each case must be judged according to its merits, and more than one drawing may be necessary.

After drawing, the specimen should be unpinned and preserved in 70% alcohol, or lesser strengths down to 50% according to how long it is to be kept. Dilute alcohol is apt to "go off" in time, but does not harden the tissues so much as a stronger one. It is well to remember, when diluting the alcohol, that the specimen itself will add much water, and to make an allowance for this. Old hard spirit material usually softens up to workable condition if it is soaked in water for some hours before dissection, whilst very hard dehydrated material is said to soften very well when soaked in a 0·8% solution of trisodium phosphate. Formalin is unsuitable for molluscan material, as it makes the tissues too brittle. The name of the specimen, together with the locality and date of capture, and any observations of interest, should be written in pencil or waterproof ink on good strong paper, and the label enclosed in a vial with the specimen. Some excellent advice on the killing and preservation of mollusca will be found in F. R. Woodward's article "Mollusca in the Museum", The Conchologists' Newsletter, Vol. 1, No. 12.

One of the chief reasons for dissecting snails is to find out something about their taxonomic position; indeed some species can only certainly be separated by an examination of their viscera. For instance, a short stout form of *Cochlicella acuta* (Müller) could easily pass for an elongated form of *C. ventrosa* (Férussac). However, the two are readily distinguished by their mucus glands (see fig. 8). In *acuta* there is a single unbranched tubular mucus gland, whereas in *ventrosa* the gland has four branches (rarely three or five).

Sometimes the genitalia of closely allied species are virtually indistinguishable, in which case one has to resort to an examination of the radula and jaw, or compare the darts. The white-lipped form of *Cepaea nemoralis* (L.) and the black-lipped form *C. hortensis* (Müller) are easily confused with the normal forms, especially in mixed colonies, but the darts are distinctive. The darts are very fragile, and often break when being removed from the dart sacs, but any fragment will do for examination. In *nemoralis* the dart has four longitudinal ridges which project at right angles, but in *hortensis* the ridges are surmounted by two more ridges, so that in cross section the dart has the shape of a "Maltese cross" (see fig. 9).

a b

Fig 9. Cross sections of darts of (a) *Cepaea hortensis* and (b) *C. nemoralis*.

The writer finds that darts are extracted in perfect condition by boiling away the whole dart sac in strong caustic soda in a test tube. As the solution is inclined to "spit" when boiled, it is safer to stand the test tube in a saucepan of boiling water to heat it. This takes very much longer, but many tubes can be boiled simultaneously, thus saving time when there are many darts to extract.

WATER SNAILS. As the anatomy of pulmonate water snails is somewhat different from that of land snails, the beginner will be wise to consult Macan (Freshwater Biological Association Scientific Publication No. 13, 1949. Wray Castle, Ambleside, Westmorland) or "A Guide to Molluscan Anatomy for Parasitologists in Africa" by C. A. Wright, published by the British Museum (Natural History) at 1s. 6d.

BIBLIOGRAPHY

Meade, A. R., 1950. *Comparative Genital Anatomy of Some African Achatinidae.* Bull. Mus. Compar. Anat. Harvard, *105*, 2, 1950. Marshall and Hurst, "Practical Zoology" (various editions up to 1924), published by Murray. Very good dissecting instructions, but difficult to follow as the figures do not cover intermediate stages.

Meisenheimer, J., "Die Weinbergschnecke *Helix pomatia* L.", Leipzig, 1912. Frequently cheaply obtained from second-hand book dealers. Many drawings covering the whole animal. Most of the German is not difficult to follow with a scientific dictionary.

Rowett, H. G. Q., 1963. "Dissection Guides. V. Invertebrates". Murray. Ten admirably clear drawings covering the general dissection of the snail and illustrating the differences between *H. aspersa* and *H. pomatia.*

SUPPLIES

Biddolph, A. Green Belt, London Road North, Merstham, Surrey.

Christie, L., 137 Gleneldon Road, Streatham, S.W.16. (Very good for forceps; stainless steel pins.)

Dutt, P. K. & Co. Ltd., 1a Howard Road, Bromley, Kent.

Flatters & Garnett Ltd., Manchester 13.

Gerrard, T. & Co. Ltd., Gerrard House, Worthing Road, East Preston, nr. Littlehampton, Sussex.

Griffin & George Ltd., Ealing Road, Alperton, Wembley, Middlesex. (Supplies of everything for scientific work, from pins to electron microscopes, at competitive prices.)

Solmedia Ltd., 35 Orford Road, London, E.17.

Southern Watch & Clock Supplies Ltd., 48-56 High Street, Orpington, Kent. (Head glasses on bands, suitable for dissection.)

Explanation of fig. 6

1. Inner side of mantle collar.
2. Oesophagus.
3. Salivary duct.
4. Vas deferens.
5. Penis.
6. Radula sac.
7. Buccal mass.
8. "Brain".
9. Right ocular tentacle.
10. Genital atrium.
11. Mucus gland.
12. Dart sac.
13. Vagina.
14. Part of columellar muscle connected to muscles of tentacles and buccal mass.
15. Spermathecal duct.
16. Diverticulum of spermathecal duct.
17. Spermatheca.
18. Intestine.
19. Prostate gland.
20. Large hemaphrodite duct.
21. Fertilization chamber.
22. Little hermaphrodite duct (or ovotestis duct).
23. Albumen gland.
24. Stomach.
25. Ducts from stomach to digestive gland or "liver".
26. Ovotestis.
27. Digestive gland or "liver".
28. Rectum.
29. Crop.
30. Ureter.
31. Kidney.
32. Salivary gland.
33. Auricle.
34. Ventricle.
35. Veins of mantle cavity or "lung".
36. Flagellum.
37. Diaphragm or body-wall.
38. "Tail".

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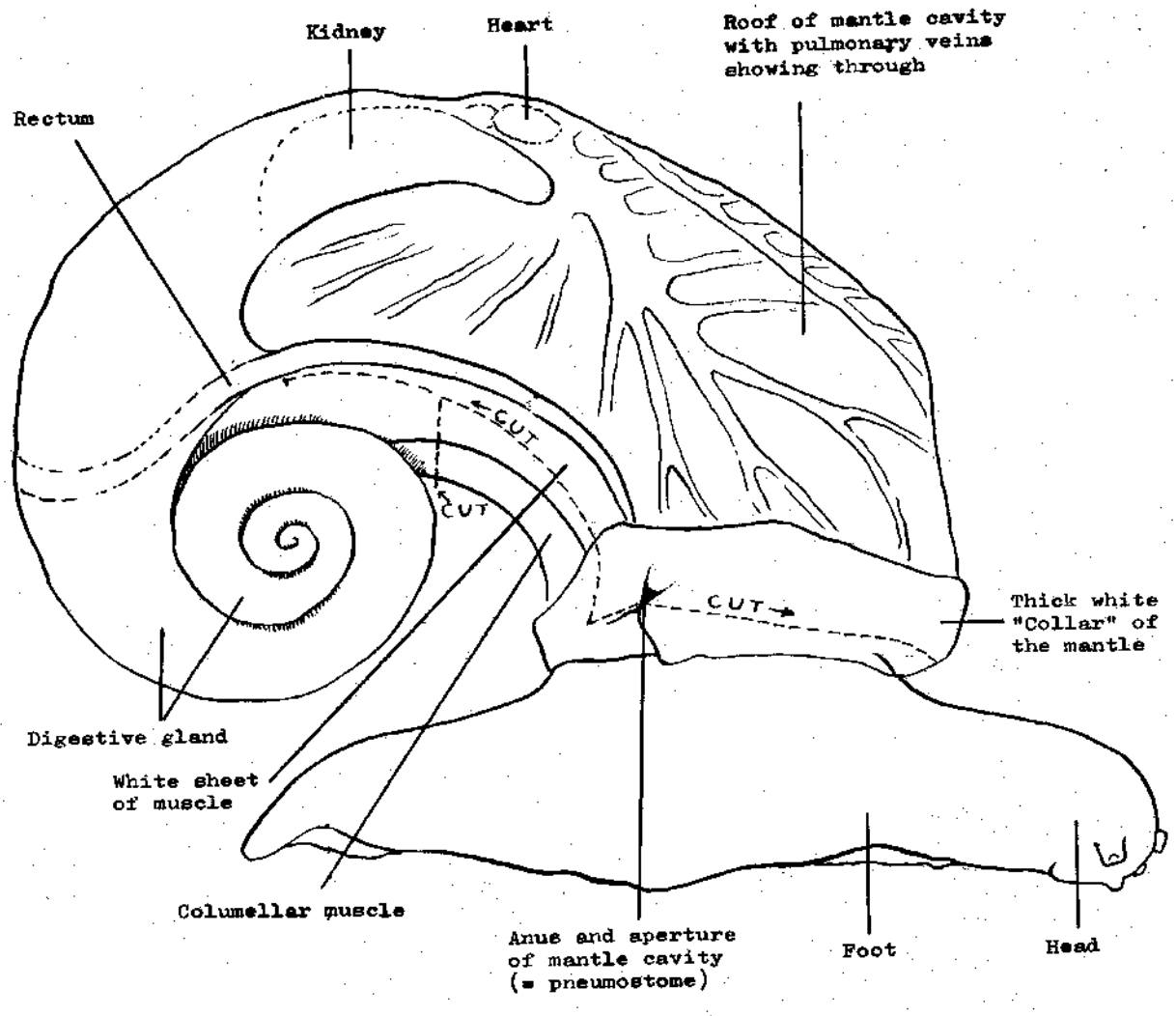
Kidney
Heart
Roof of mantle cavity with pulmonary veins showing through
Rectum
CUT
CUT
CUT
Thick white "Collar" of the mantle
Digestive gland
White sheet of muscle
Columellar muscle
Anus and aperture of mantle cavity (= pneumostome)
Foot
Head

Figure 1

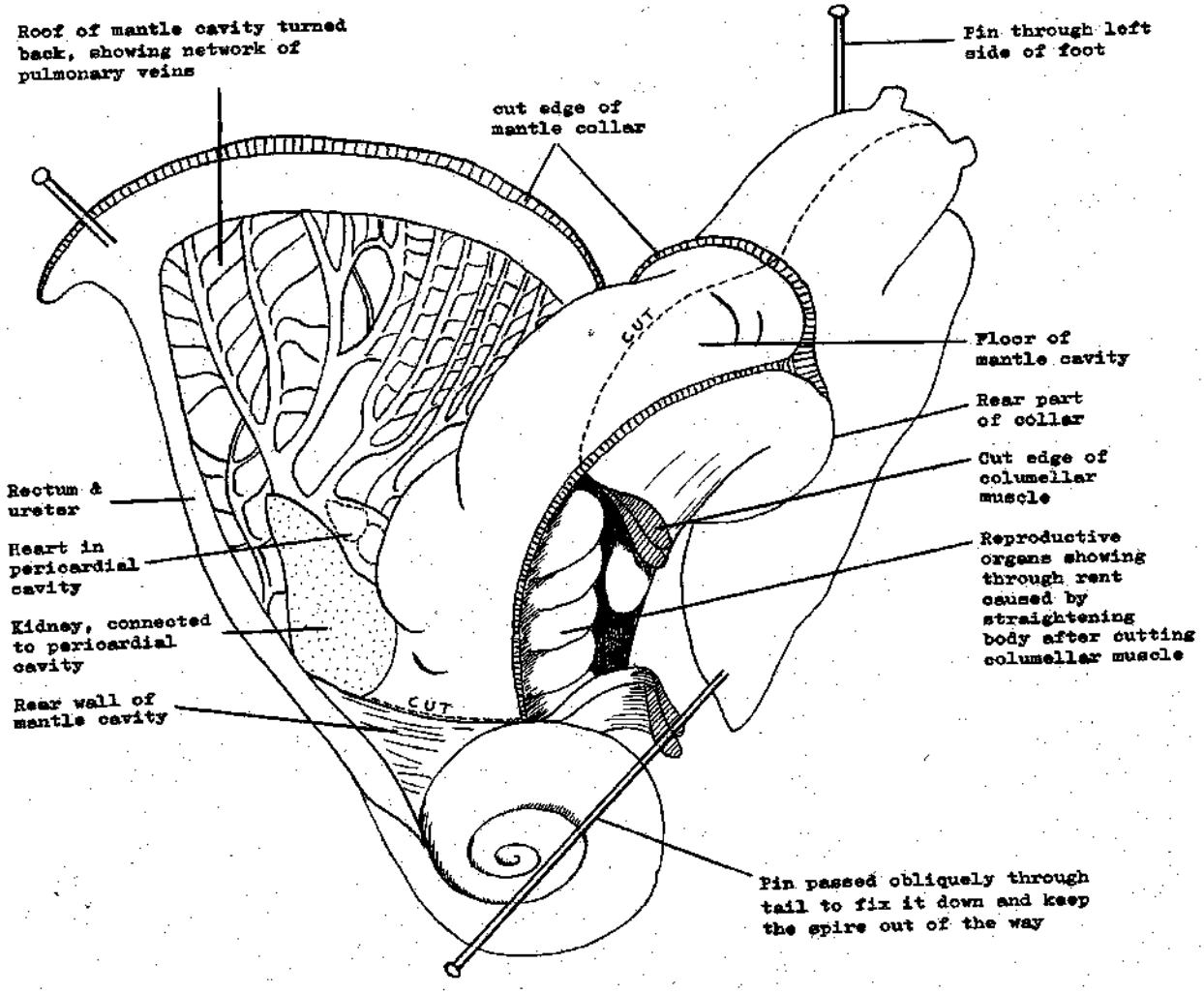
Roof of mantle cavity turned back, showing network of pulmonary veins
cut edge of mantle collar
Pin through left side of foot
CUT
Floor of mantle cavity
Rear part of collar
Cut edge of columellar muscle
Reproductive organs showing through rent caused by straightening body after cutting columellar muscle
Rectum & ureter
Heart in pericardial cavity
Kidney, connected to pericardial cavity
Rear wall of mantle cavity
CUT
Pin passed obliquely through tail to fix it down and keep the spire out of the way

Figure 2

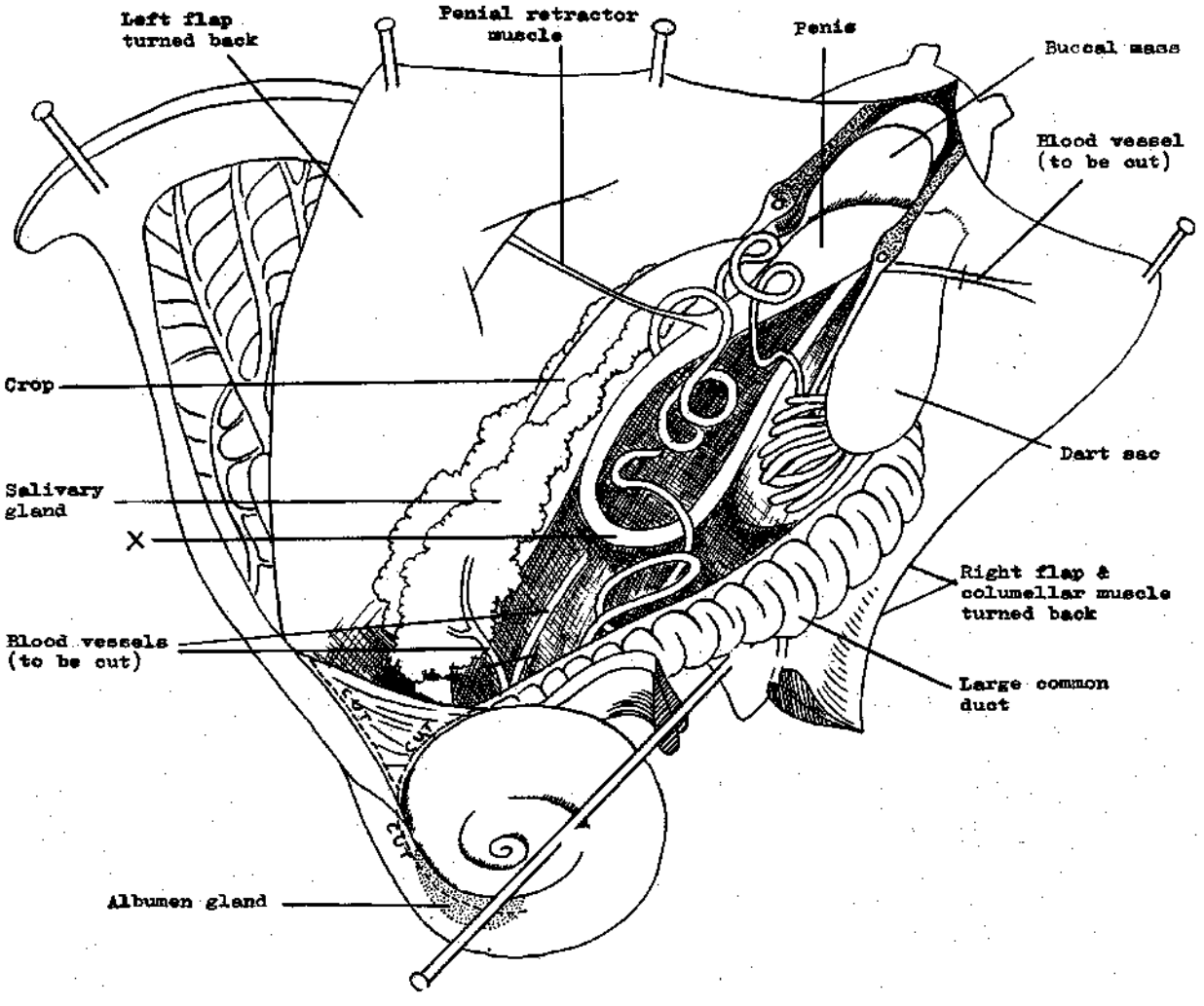
Left flap turned back
Penial retractor muscle
Penis
Buccal mass
Blood vessel (to be cut)
Crop
Dart sac
Salivary gland
X
Right flap & columellar muscle turned back
Blood vessels (to be cut)
Large common duct
CUT
CUT
CUT
Albumen gland

Figure 3

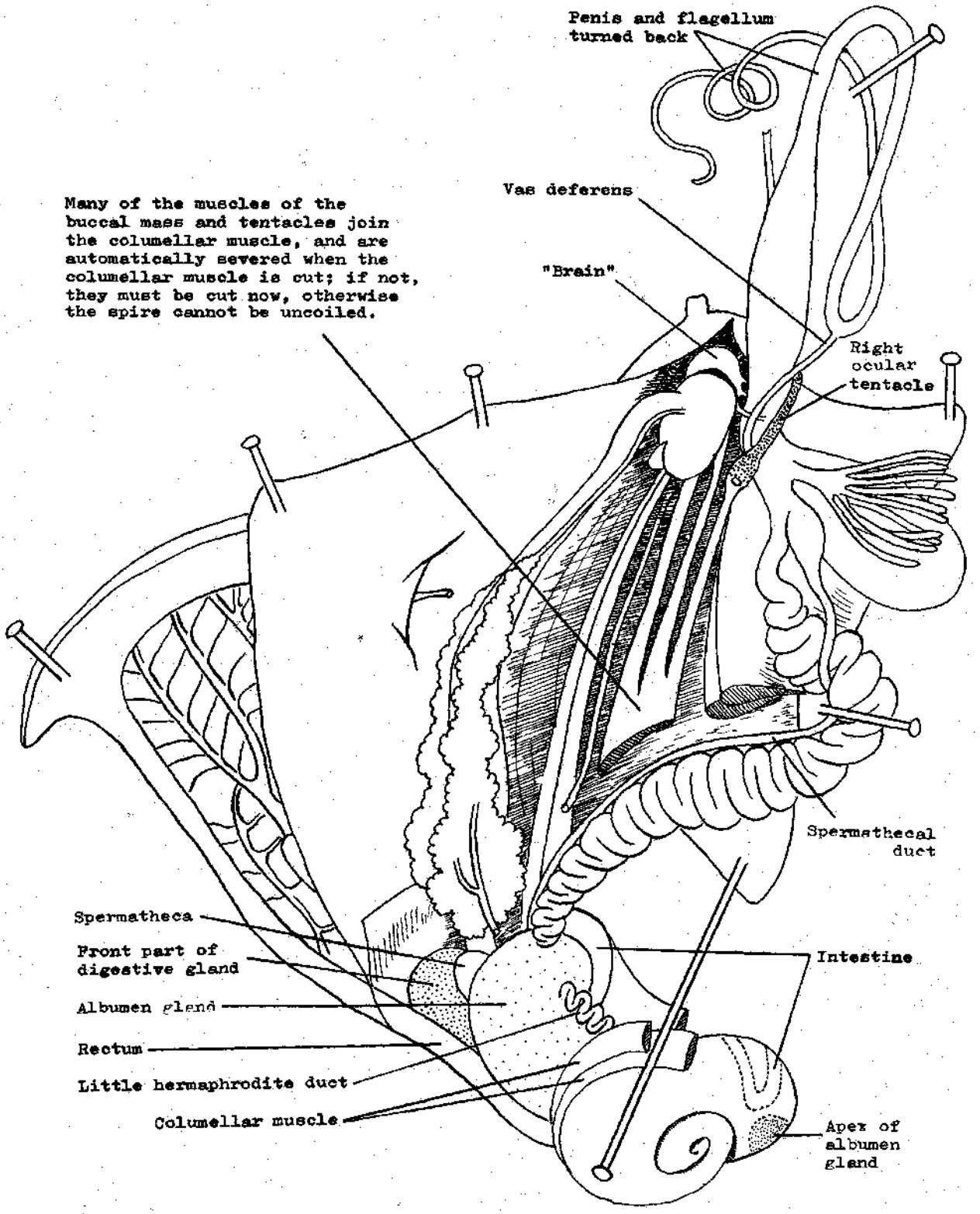
Penis and flagellum turned back
Vas deferens
Many of the muscles of the buccal mass and tentacles join the columellar muscle, and are automatically severed when the columellar muscle is cut; if not, they must be cut now, otherwise the spire cannot be uncoiled.
"Brain"
Right ocular tentacle
Spermathecal duct
Spermatheca
Front part of digestive gland
Albumen gland
Rectum
Little hermaphrodite duct
Columellar muscle
Intestine
Apex of albumen gland

Figure 4

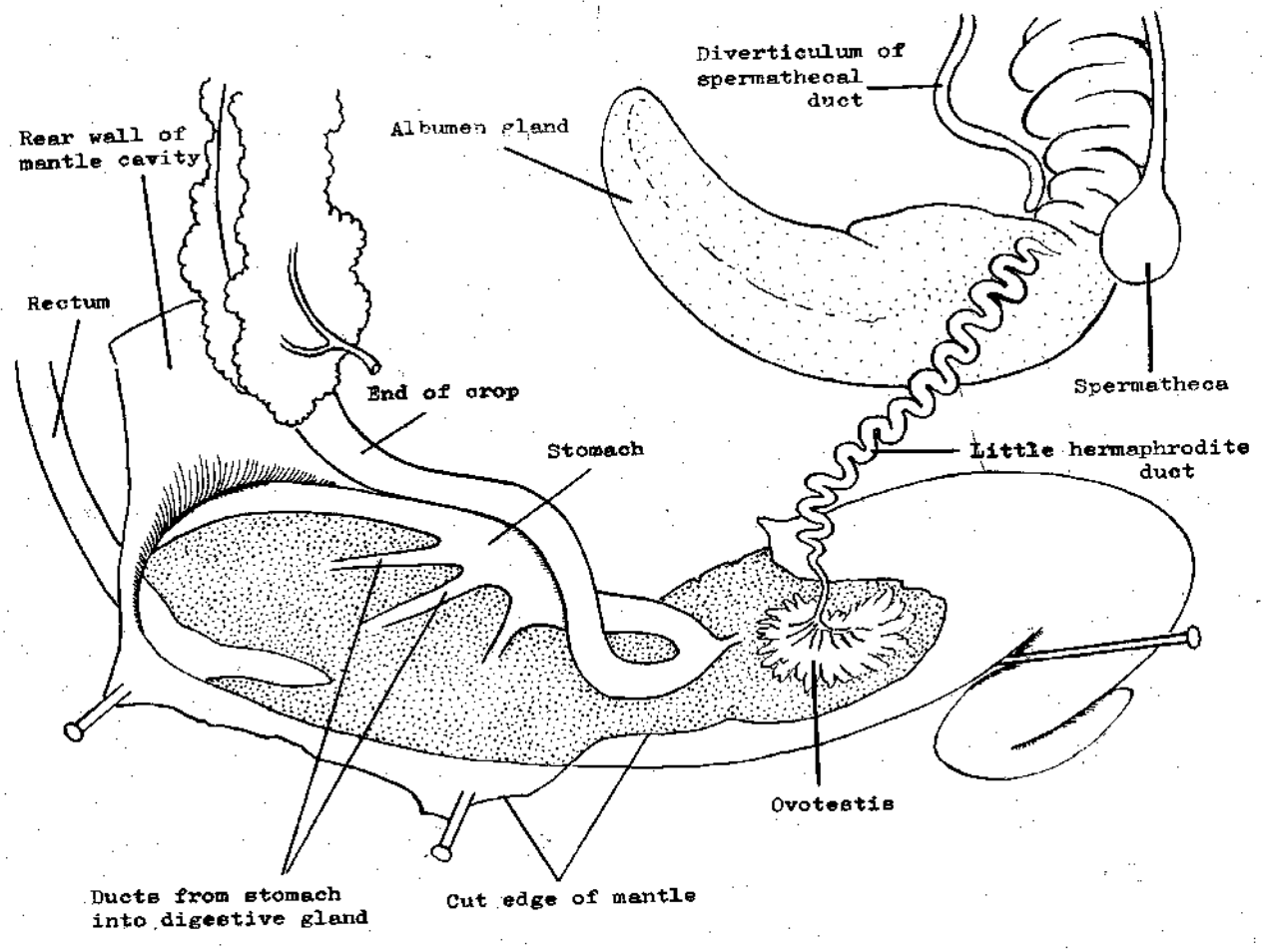
Diverticulum of spermathecal duct
Albumen gland
Rear wall of mantle cavity
Rectum
End of crop
Stomach
Spermatheca
Little hermaphrodite duct
Ovotestis
Ducts from stomach into digestive gland
Cut edge of mantle

Figure 5

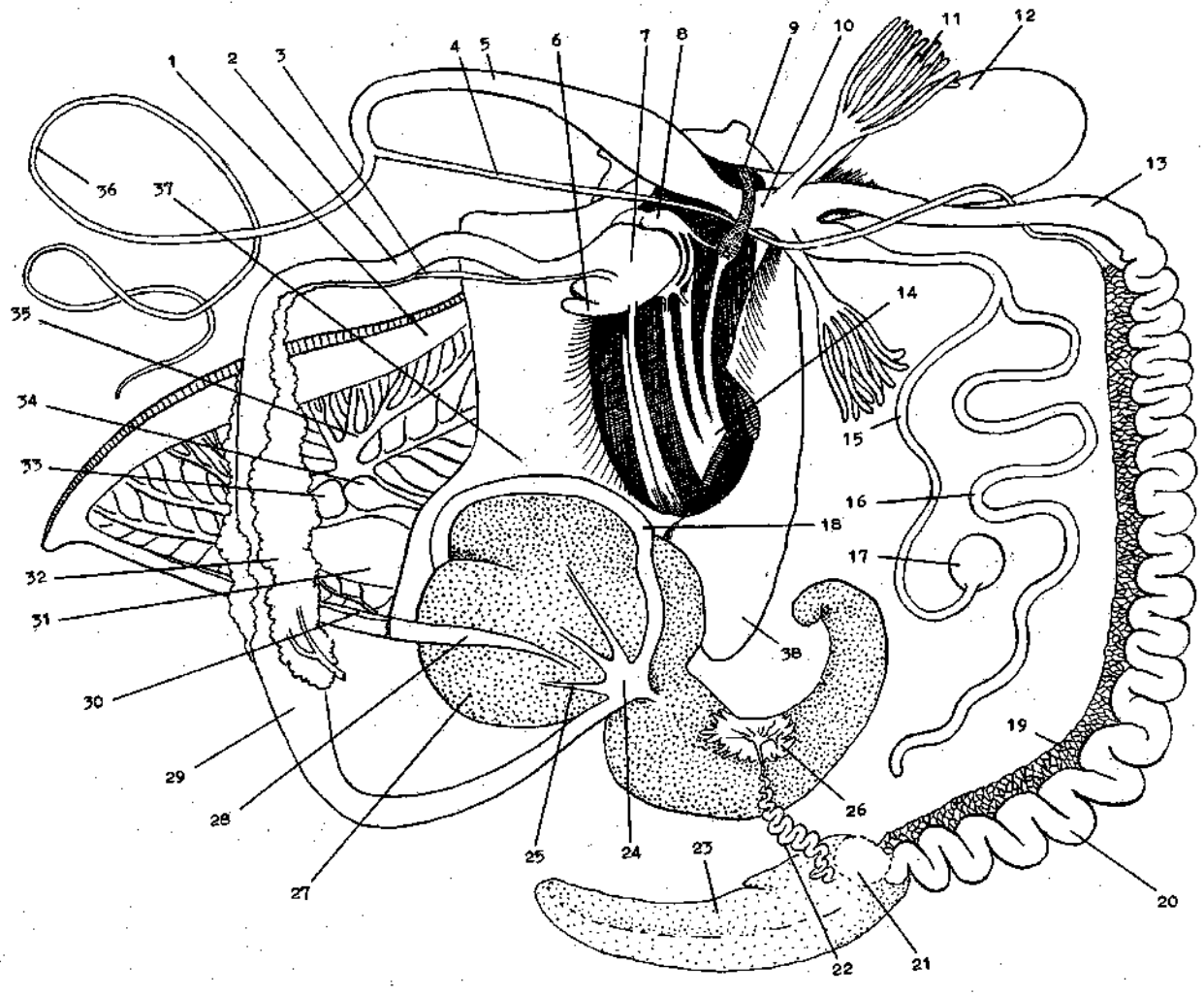
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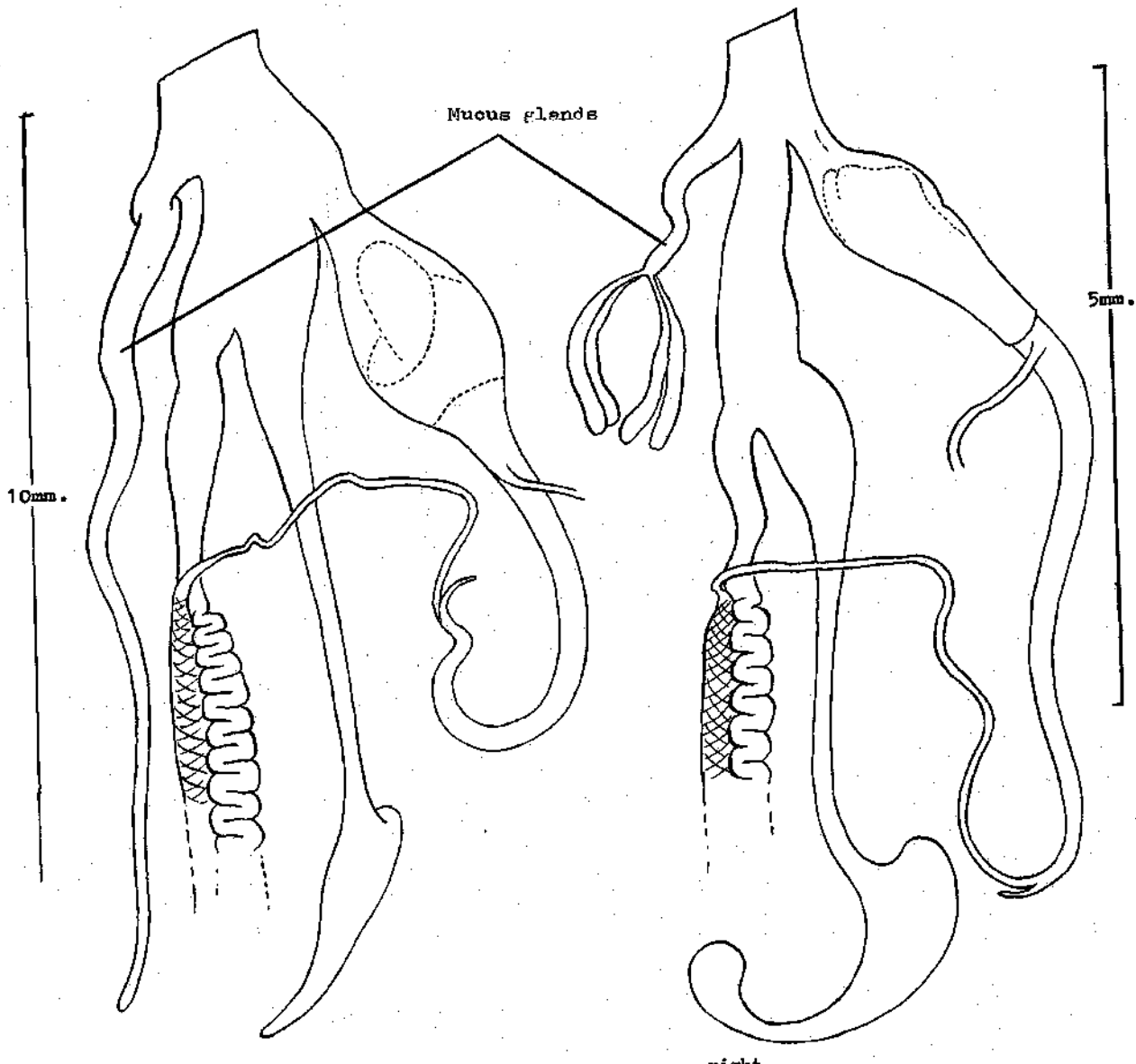
Figure 6



left
Cochlicella acuta (Müller)

right
Cochlicella ventrosa (Férussac)

Figure 8