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Papers for Students No. 5

PREPARATION OF THE RADULA

by

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Radula is the name of the rasp like tongue which most molluscs except the lamellibranchs possess. It is situated in the head region of the snail in a structure called the buccal mass.

In Ampullaria and a few other genera many structural details of the radula can be seen with an x8 hand lens, but for the greater part of the molluscan genera a high power microscope is necessary.

Methods of preparation

Radula preparations are preferably made from spirit preserved material, but fresh material may also be used.

Fresh Material

The head of the snail is removed and placed in a small container of water with a loose fitting lid and left to rot. This process may take up to a fortnight. When seen to be free of tissue the radula is removed, rinsed in clean water and orientated on a microscope slide. Fresh material may also be prepared with sodium hydroxide as described in the section dealing with preserved material.

Orientation

A short list of instruments which will be found useful:-

2 pairs of Dumont No. 5 watchmaker's forceps. (12).
1 Windsor & Newton "oo" sable paint brush.
2 fine mounted needles.
1 pair of fine scissors.

Of the above list the most important one is the mounted needles; these must be very fine. Obtain some cactus spines from Opuntia, Echinocactus or Cereus sp. and mount with a little sealing wax or glue in the end of a

drawn out glass tube, (fig. 5). An alternative is to use the finest entomological pins mounted in the same way. The reason for the very fine needles is that the material on which you will be using them is often very small and the fine points need to be flexible. A larger point would be rigid and tend to damage the radula.

The teeth on the radula are on one side only and project towards the nascent end, at an acute angle, (fig. 2b, d). It is possible to distinguish the side on which they are from the curvature of the cleaned radula, (fig. 2c); when mounted the teeth should be on the upper surface. The correctly orientated radula is flattened out in a drop of water using the finely pointed needles. A cover slip is placed over the preparation and clipped down with a polythene clip, (fig. 3). The excess moisture is removed with blotting paper and the slide stood in a closed jar containing a half inch layer of table salt overnight or until dry, then gentle tapping of the edge of the slide on a solid object should remove the coverslip leaving the radula stuck to the slide.

Spirit preserved material

The buccal mass is removed by cutting or tearing an opening along the dorsal surface of the animal (fig. 1b) from above the mouth, as far back as the mantle. The buccal mass will be seen in the position marked in the figure. It is attached at its anterior end to the mouth and at the posterior end to the oesophagus. To remove, cut the oesophagus and firmly grasp the buccal mass with a pair of forceps and pull out. The whole of the buccal mass is placed in a 10% solution of sodium hydroxide (10) (caustic soda) and left at room temperature overnight, then a drop of chlorazol azurine (5) is added. This stains the radula blue and makes it easy to distinguish. Remove the radula with a fine paint brush and rinse in clear water, irrigating with a pipette (ear dropper) if necessary to remove any remaining adherent tissue. When clean, mount as described under the heading "Orientation".

Radulae may be examined with polarised light (9) unstained. When free of the tissue the radula is mounted in a drop of diluted glycerin (11) and a cover slip placed in position. The preparation is then examined by transmitted light through a microscope with a disk of polaroid under the substage and similar disk over the eyepiece. By rotating the eyepiece a play of colours will be seen on the radula teeth. If this method of examination gives sufficient detail for your requirements the slides may be made permanent. Remove the coverslip, replace the diluted glycerin with 50% glycerin and then pure glycerin, allowing about five minutes in each. Then finally mount in glycerin jelly (7) and ring the slide as described below.

Ringing

If radulae have been mounted in glycerin jelly, it is advisable to ring the coverslip to prevent the jelly from drying out; this is achieved by making a seal between the coverslip and the slide with a suitable compound that will cover but not affect the jelly.

The slide is clipped to a turntable (fig. 7) and a small amount of gold size (8) is applied to the edge of the coverslip with a fine paint brush and the turntable is rotated whilst the brush is held still. It may take three

or four applications of gold size to make a durable seal. The mount should be left to dry between applications.

Staining

Staining is carried out on the dried radula; the simplest way is with diluted carbol fuchsin (3). Pour a small amount of the diluted stain over the radula and gently warm over a spirit lamp. Do not allow to boil. When warmed, place on one side and allow to cool for at least ten minutes. Rinse off the excess stain with water and dip the slide into acetone (1) for about five seconds and then into carbol xylene (4) for about the same time. Wipe the excess reagent from the slide and place a small drop of canada balsam (2) in xylol over the radula and lower a coverslip into position. This process is less tedious than using glycerin jelly as it is not necessary to ring the finished preparation. It is important that the radula should not be allowed to dry between the stages of staining and mounting. If it does, it will become detached from the slide. If the radula has been treated with chlorazol azurine prior to the staining with diluted carbol fuchsin the immature teeth at the nascent end will be blue black and the mature teeth will be stained red.

Shapes and sizes

Figures 4a, 4b and 4c are drawings of radula teeth from Lymnaea, Littorina and Helix, all drawn to the same scale. The letters C.L.M. stand for central, lateral and marginal teeth. It will be noted that in Littorina the teeth are much larger than those in Helix or Lymnaea and there are fewer of them in each row, but there are more rows. The structure of the radula is closely related to the feeding habits; this should be borne in mind when making comparisons between species. The size of the animal is no guide to the length of the radula. For example, Helix has a radula of about a quarter of an inch long, but that of Littorina is about two inches.

Scanning Electron Microscopy

Since the publication of the first edition of this paper a new method of investigating surface structure has become available in the form of the scanning electron microscope (S.E.M.). This apparatus is very expensive, but as the number of universities having a S.E.M. is increasing, a word or two on preparation of material for examination.

Radulae may be prepared as described above to the stage where they are ready to be placed on the slide, instead of mounting on a microscope slide they are mounted on a metal stub, and when dry they are coated with gold and examined. The results obtained with the S.E.M. are quite different to those obtained from optical microscopy, the picture has dimension, and stereoscopic pairs can be readily obtained. Some existing publications have photographs and those interested should look at Runham (1967), Carriker (1969) and Wright (1970). Figs. 6a, b illustrate camera lucida drawings of a single central tooth from Cypraea mauritiana and Cypraea vitellus, figs. 6c, d, drawings taken from a scanning microscope picture of the same two teeth.

REAGENTS

	Reagent	Remarks
(1)	Acetone.	Very volatile and inflammable liquid obtained from local chemists.
(2)	Canada Balsam in Zylene Neutral.	G.T. Gurr in small tubes, also in 25 ml bottles.
(3)	Carbol Fuchsin (after Ziels formula)	Dissolve 10 parts of alcohol and one part fuchsin in 100 parts of 5% phenol solution. For use on radulae dilute one part of the above with 10 parts of water.
(4)	Carbol Xylene.	G.T. Gurr. 100 ml bottles.
(5)	Chlorazol Azurine.	Dry powder from G.T. Gurr in 10 gm quantities. Use as 1% solution in water.
(6)	Fuchsin (Acid).	A dry powder in 5 gm quantities for making up carbol fuchsin. G.T. Gurr.
(7)	Glycerin Jelly.	Collapsible tubes. G.T. Gurr.
(8)	Gold Size.	G.T. Gurr. 100 ml quantities.
(9)	"Polaroid" squares.	Polarizers (United Kingdom) Ltd., Optical and Scientific Equipment, 26, Stamford Street, London, S.E.1.
(10)	Solium Hydroxide.	Local chemists as 10% solution in water.
(11)	Dilute Glycerin.	Pure glycerin 30 parts, distilled water 70 parts, 2 small crystals of thymol. From local chemists.
(12)	Dumont Forceps.	C. Cooper & Co. Ltd., 93, Hatton Garden, London, E.C.1.

Address:

G.T. Gurr Division
Baird & Tatlock,
P.O. Box 1,
Romford. 1. 1HA.

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EXPLANATION OF PLATES

Plate I

Fig. 1. a. Ventral aspect of a snail showing the main anatomical features.

b. Dorsal aspect of the same snail with the shell removed and the tissue over the buccal mass dissected to show its position.

c. Profile showing the position of buccal mass.

Fig. 2. a. Spread radula showing general shape in Lymnaea sp. anterior end, A, nascent end, N.

b. Isolated radula tooth showing the baseplate dotted, and the backward projecting cusps in black.

c. Freshly removed radula showing the curling of the nascent end and the spoon shape of the anterior end.

d. Profile of a cleaned radula showing the backward projecting teeth.

Plate II

Fig. 3. a, b, c. Method of cutting polythene tube to make a simple coverslip clip.

Fig. 4. Camera lucida drawings of: a. Littorina littorea.

b. Helix aspersa.

c. Lymnaea peregra.

C = central tooth; L = lateral tooth; M = marginal tooth.

Analysis of the number of teeth per radula

	C.	L.	M.	Rows.	Total
Littorina	1	1	2	500	3,500
Helix	1	15	23	110	10,670
Lymnaea	1	11	21	83	5,395

Fig. 5. Details of mounting needle.

Plate III

Fig. 6. a, b. Camera lucida drawings of C. mauritiana and C. vitellus from a conventional microscopical preparation viewed with transmitted light.

c, d. Scanning microscope drawings taken from photographs of individual teeth as above but viewed at an angle of 45°.

Fig. 7. Ringing table.

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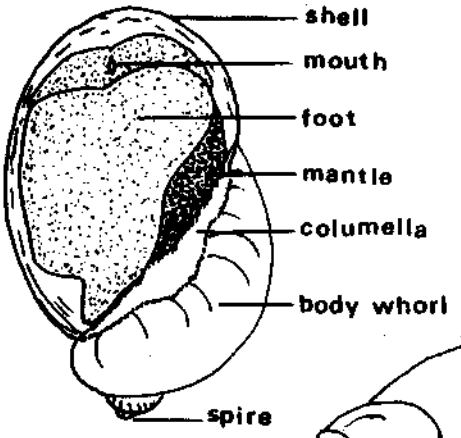
PLATE.I.



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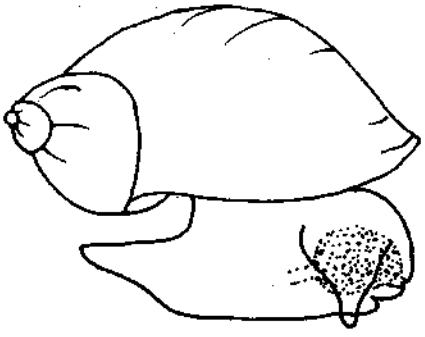

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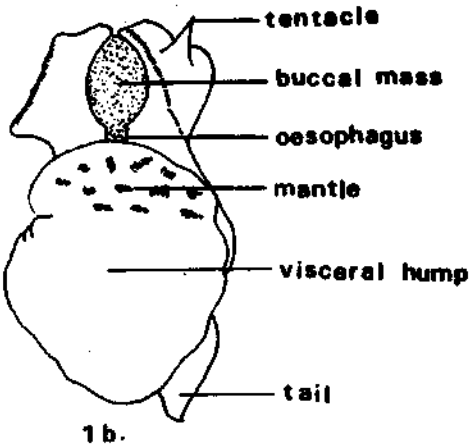
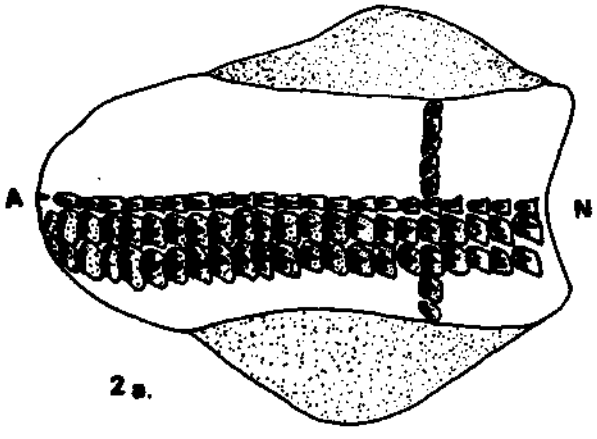


1b.

FIGURE.1.



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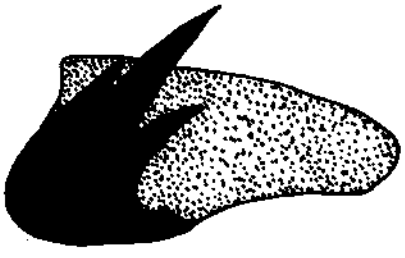


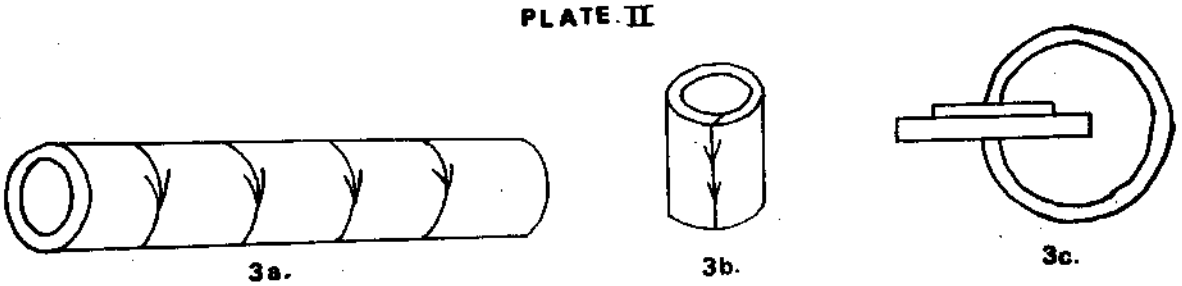
2b.

2c.

2d.

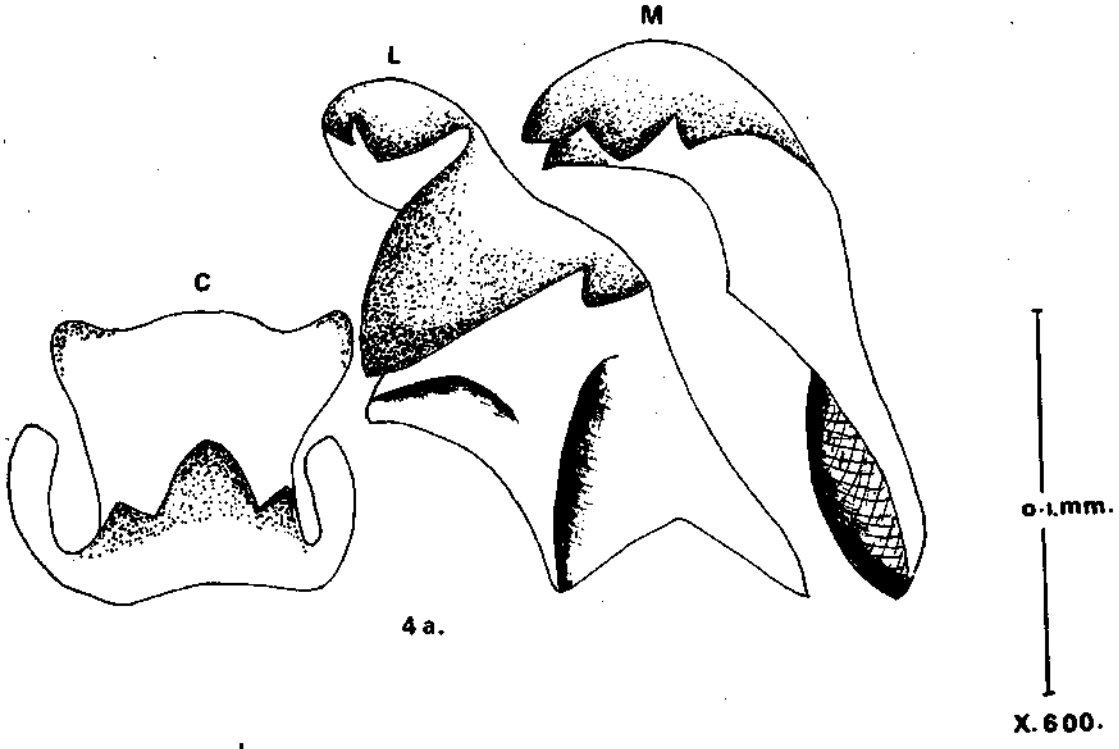
FIGURE.2.

PLATE. II

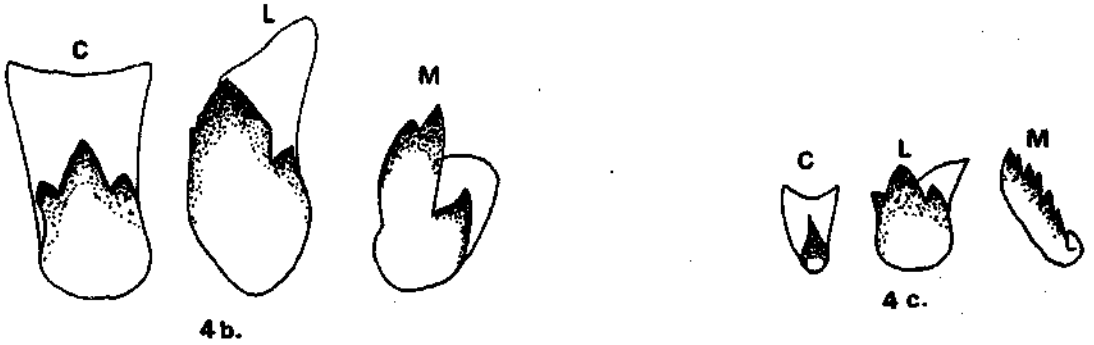


3a. 3b. 3c.

FIGURE. 3.



4a.



4b. 4c.

FIGURE. 4.

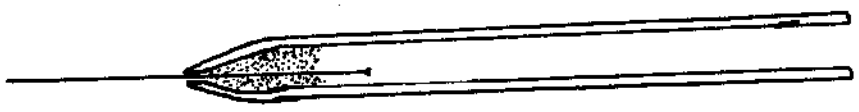
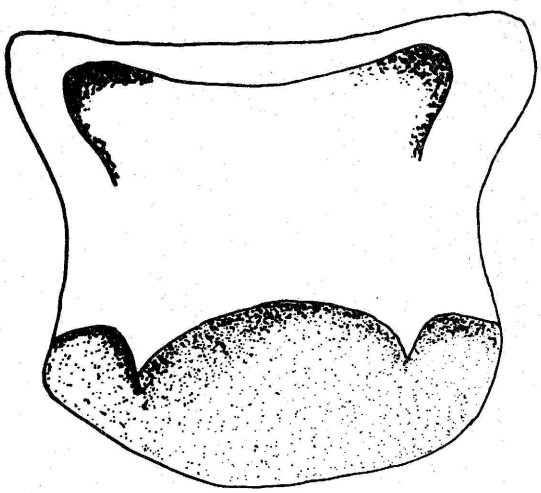


FIGURE. 5.

PLATE III



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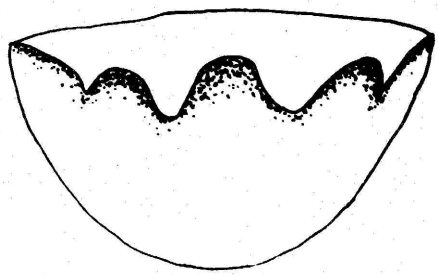
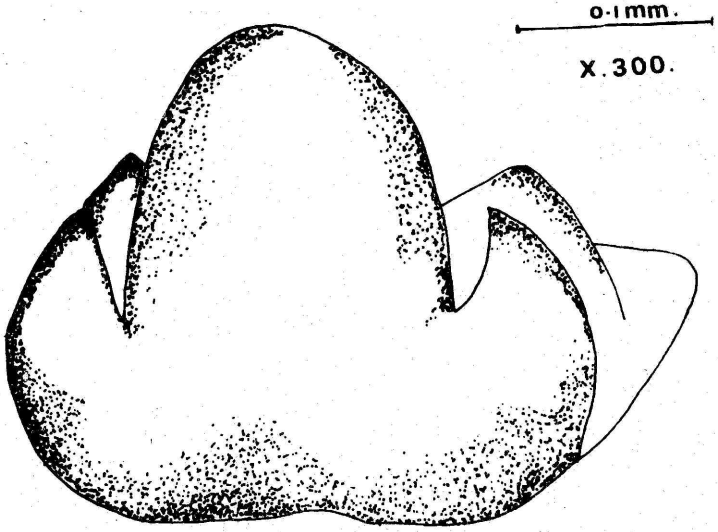


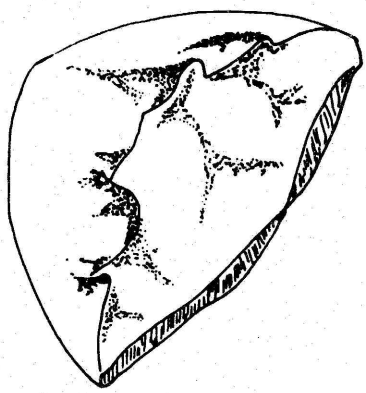
6b.



0·1mm.

X.300.

6c.



6d.

FIGURE.6.

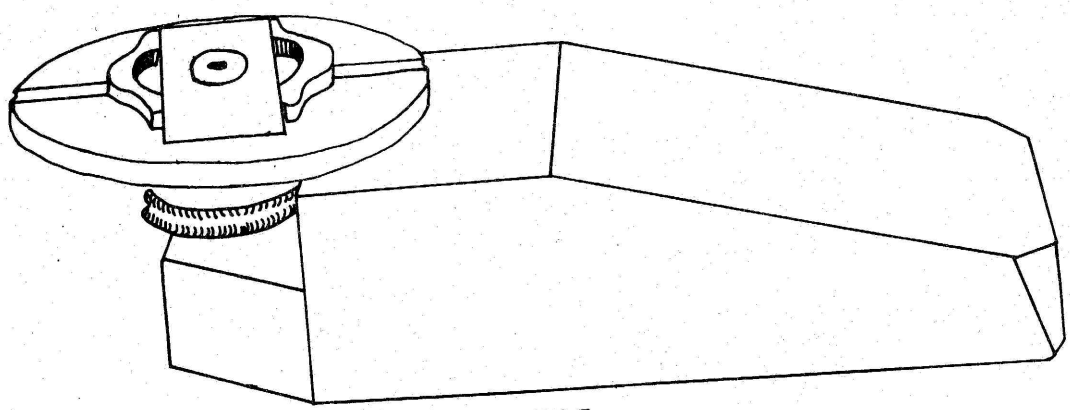


FIGURE.7.