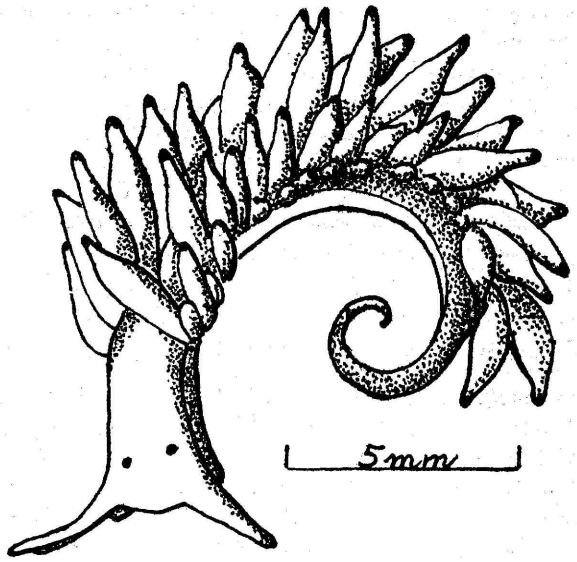
FINE DISSECTION

OF

ASCOGLOSSANS

T. Gascoigne



Hermaea variopicta

To the reader

Though this booklet refers specifically to ascoglossans, some of the remarks and more general notes are applicable to the dissection of many small molluscs and soft-bodied invertebrates.

Fine dissection is best learnt by practice. It helps if a student sometimes watches an expert at work; in this way he can pick up some useful hints. The writer was fortunate in that all his lecturers at Birkbeck College were skilled dissectors of small animals; and each had his, or her, individual style. He also recalls, with pleasure, visiting the Vrije University, Amsterdam, where Dr. J. Joosse demonstrated fine dissection. The booklet attempts in small measure to supplement, but not replace, this kind of instruction.

It is hoped that the reader will pardon the didactic style of writing; it was adopted for brevity and simplicity. The writer has confined himself to advice based on his experiences and to methods he has practised for many years. He is willing to reply to questions and to demonstrate methods described in the text. His sincere wish is that the notes will encourage students to master the art of fine dissection and that it becomes for them a valuable aid in their future researches.

Cover design. *Drawn from a colour photograph taken by M. Sordi.*

Hermaea variopicta (Costa, 1869) is a rare and brilliantly coloured Mediterranean ascoglossan. Dark and light purple, rich chestnut, and white adorn the dorsal surface. The numerous cerata are tipped with vermilion and flanked with creamy white.

In 1972 Dr. E. C. Southward found a single specimen near Plymouth. Lemche and Thompson (1974) examined it and correctly assigned the species to the genus *Hermaea*—originally Costa had placed it in a separate genus *Hermaeopsis*.

FINE DISSECTION OF ASCOGLOSSANS

Improvements and corrections

P.1, para.3, line 5. Please extend the sentence which ends "6mm" by the clause "; and there is not sufficient space between the lens and the specimen to dissect freely.'

P.2, line 13. Delete "the decade" and replace by "about"

P.7, line 4. Replace "essental" by "essential" - and line 5, "withtout" by "without"

P.8, line 2 from bottom of page. Delete "melts" and insert "cools"

P.14, line 13. Small 'a' for 'Acetocarmine'

P.16, line 16 from bottom of page. "reassemble" not "reasssmble"

Inside of back of cover. Should be a heading "ADDITIONAL DISSECTION" above Fig.11.

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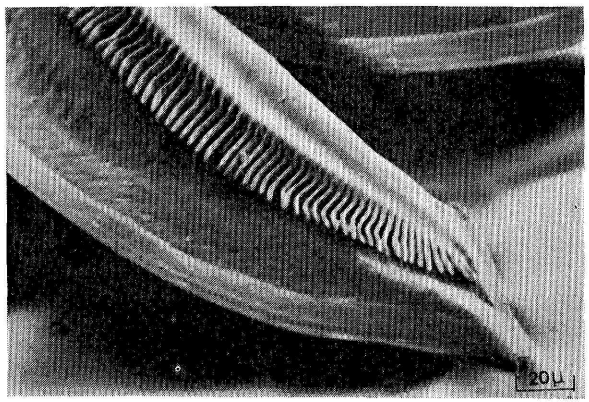
CONTENTS

(G) = note of general application



Scanning electron micrograph (reproduced by kind permission of the Quekett Microscopical Club). The cusp of a tooth of *Midorigai australis* Burn, showing a fringe of lateral processes.

The radula was prepared by the method described on p. 13, except that, after cleaning, it was dehydrated with absolute alcohol. When the alcohol had evaporated, the radula was placed on a bit of double-sided Sellotape attached to a clean metal stub. Coating with aluminium gave better results than gold and palladium.

Notes

INTRODUCTION

Fine dissection can be defined as the dissection of small animals with needles and the aid of a dissecting microscope at magnifications of x 25, x 50, and occasionally x 100. It is basic to anatomical studies of small, soft-bodied invertebrates.

The practice of fine dissection requires a reasonable amount of skill, but much depends on experience and knowhow. If a dissection turns out badly, do not doubt your skill, but question the method employed and sooner or later you will find the answer. This leads to experiment and variations in technique, which make fine dissection all the more interesting.

Another attraction is that a student, after some practice, is likely to make discoveries of his own. In the 19th century many small animals were dissected with the aid of a single lens of magnification x 20. At such a low power one simply cannot see fine anatomical detail in a specimen of length 6mm. Consequently, many published drawings by early workers are surface views of incomplete dissections; and their descriptions of the reproductive and the central nervous system are often inadequate.

Nevertheless, some specialists produced remarkable papers. For example, A. Hancock (1848) in a classic paper described the anatomy of *Limapontia capitata*, a 6mm ascoglossan. He obtained an abundant supply of specimens from the rockpools of Cullercoats Bay, Northumberland, where the species can still be found. For dissections he used a x 20 lens, fine needles, and an instrument called a compressor. He worked without stains or extra lighting. He repeated his dissections many times, and examined preparations with a microscope.

Hancock's drawings of the external features, heart, alimentary system, and reproductive system were detailed and accurate. He was aware that his description of the central nervous system was unsatisfactory. He remarked that the species was extremely minute and highly organised, and he thought the reproductive system was amazingly complicated. It is noteworthy that Hancock never again

published so detailed a description of an ascoglossan. Possibly he realised that the tools and techniques for the job had not yet been developed.

One of the first dissecting microscopes was the Greenough binocular of 1897. It became popular and was used by many zoologists. However, at a magnification of x 50, the *working distance* (distance between dissection and front lenses of objective) was far too short; and the *field of view* (area of specimen visible at a given magnification) was too small. Also, because of an optical defect, it could cause eyestrain if used for any length of time.

It seems probable that dissecting microscopes having a wide field of view and ample working distance at x 50 were not generally available until the decade 1920-1930. Modern instruments are much better than those of the '20s, and this is due to recent advances in optics and in high precision engineering.

Today, the fine dissector is fortunate; he can use a modern dissecting microscope. He has at his disposal general stains, and can prepare serial sections to check his work. He may employ a scanning electron microscope to take pictures of radulae, teeth, and penial styles.

SEA SLUGS AND ASCOGLOSSANS

Nearly all animals commonly called sea slugs belong to two orders, Nudibranchia and Ascoglossa (class Gastropoda; subclass Opisthobranchia). Of the two, the Nudibranchia has by far the greater number of species.

Nudibranchs have two pairs of tentacles, the posterior pair are called rhinophores; ascoglossans have only one pair. Nudibranchs are mostly carnivores, and ascoglossans are herbivores. The latter feed by slitting open algal filaments and sucking out the contents. The hallmark of the Ascoglossa is a small pocket, the ascus, in which discarded teeth are stored. It hangs below the buccal mass (Fig. 4A, *as*).

An unusual feature of the order is that it has two names. Some authors use Ascoglossa; others prefer Sacoglossa. A brief history of the names is as follows. In 1875 Pagenstecher established the order as the Monostichoglossa. A year later Ihering proposed Sacoglossa and at the same time Bergh used Ascoglossa. The cumbersome

name Monostichoglossa has long since fallen into disuse, though, strictly speaking, it has priority. The other two names have been used for over a century, but there is still no agreement as to which should be preferred.

There are about 200 known Ascoglossan species; some 30 are found in the Mediterranean, 10 around the British Isles, and remainder are mostly tropical or sub-tropical.

PRACTICE AND SELECTION OF SPECIES

Plenty of practice is needed to become proficient. Collect your own specimens but DO NOT OVERCOLLECT—about half a dozen of any one species should be enough. Choose common species and AVOID RARE SPECIES. For those living in the British Isles the following ascoglossans are recommended :

Limapontia capitata, easy to dissect; occurs near the top of rocky shores.

Limapontia depressa, difficult; abundant in spring on most salt marshes.

Alderia modesta, very difficult; occurs in the same habitat as *L. depressa.*

The comments about dissection refer to the central nervous system and the reproductive system. Preparations of the buccal mass and the penial style can be attempted by a beginner.

The three species are small, inconspicuous, and easily overlooked. *L. capitata* feeds on the green filamentous seaweed *Cladophora. L. depressa* and *A. modesta* feed on *Vaucheria* that occurs in green patches on the damp mud of salt marshes. When collecting it is a good plan to find the food plant and then see if the sea slugs are present. A paper by Gascoigne (1975) provides a field guide that gives more detailed information.

It is a question of "getting your eye in for the species" and then collecting is not difficult. Nevertheless, on his first attempt a beginner would be well advised to seek the help of an experienced naturalist who can recognise the species in their natural habitats. The Conchological Society has several members who would be willing to assist in this matter.

RELAXATION

If a living ascoglossan is dropped into a preservative it will contract so strongly that a complete dissection of any one system is almost impossible. Therefore it should be thoroughly relaxed before preservation, so that the foot and tail are flat, and the cerata and tentacles are extended naturally. These criteria are not easy to satisfy in practice, and a perfectly prepared specimen may not be obtained. Much depends on the selection of a suitable relaxant and the care and skill employed in its use. Pantin (1948) gave valuable advice derived from many years of experience. Steedman (1976) noted a dozen methods, the relaxants ranging from CO_2 to nembutal. Once having mastered a method that proves satisfactory, other anaesthetics should be tried, from time to time, in search of an even better one.

For small ascoglossans MS-222, a metasulphonate of benzocain, is recommended. It is a white powder and not very stable; it should be kept in a brown bottle in a cool cupboard, and a fresh supply obtained after a year. It was first used as an anaesthetic in experimental biology for the embryos and larval stages of frogs and salamanders. Before surgically operating the animal is placed in a solution of MS-222, the concentration ranging from 1 :3000 to 1 :1000, according to the species and its size (Stefanová, Puchta, and Romanovsky, 1962). If *Limapontia capitata* is placed in a low concentration of MS-222 in sea water it will stretch out and appear lifeless. If then it is transferred to fresh sea water it quickly recovers and becomes active. MS-222 probably acts as a peripheral nerve inhibitor. It is simple to use and does not damage the tissues; but see cautionary note on page 18.

Place several ascoglossans in a saucer of sea water or brackish water. Sprinkle a little MS-222 on the surface, away from the animals. Wait until they are fully extended and do not respond to the touch of a needle. For a 6mm ascoglossan this takes about 15 minutes. When they are fully relaxed, carefully transfer them to a preservative.

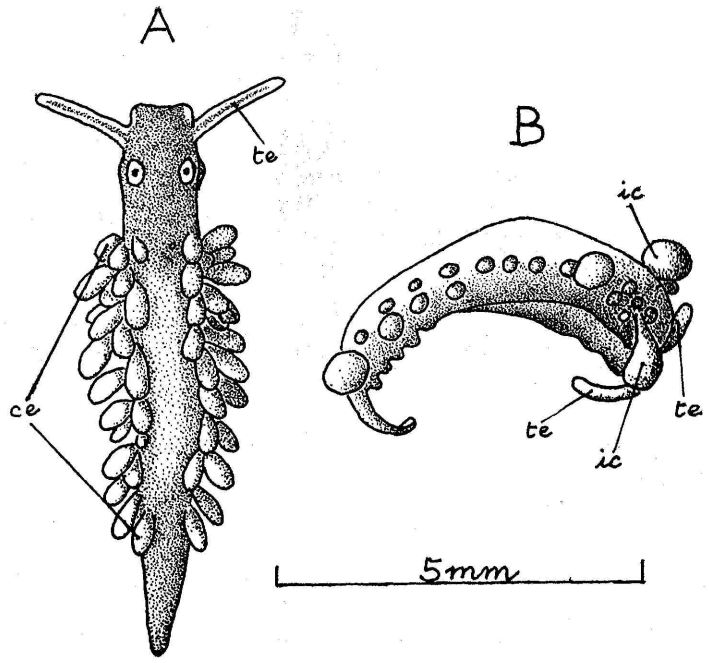


Fig. 1.

RELAXATION IS VERY IMPORTANT!

A, *Stiliger niger* Lemche, dorsal view of living animal (after Lemche, 1935). **B,** *S. niger,* preserved in 70% alcohol, without previous relaxation.

Specimen B cannot be pinned for a dorsal dissection. The anterior third of the body is strongly contracted, and this is the part where fine dissection is most needed. The buccal mass will have retracted so that the oesophagus is coiled instead of straight; and the buccal ganglia will lie behind the nerve collar, whereas they should lie in front of it. The most complicated part of the reproductive system will be clenched like a fist. For a beginner a specimen of this sort is only suitable for the radula and penial style.

Ce, cerata; ic, an inflated ceras; te, tentacle.

PRESERVATION

A GOOD DISSECTION PRESERVATIVE MAKES DISSECTION EASIER

It is customary to preserve small molluscs in 70% alcohol or Bouin's fluid. Neither of these is particularly suitable as a dissection preservative. If 70% alcohol is used, the alcohol hardens the skin, muscles, and connective tissue; whilst the water swells the mucous gland. Bouin's fluid renders nerves and ducts rather soft, so that they break easily; but it is as well to preserve 2 or 3 specimens Bouin's fluid for preparation of serial sections.

Clarke's fluid is a good dissection preservative. It is easy to prepare: 3 parts of absolute alcohol (industrial methylated spirits will do) to 1 part of acetic acid. The alcohol dehydrates the specimen and prevents the mucous gland from swelling. Acetic acid counteracts the hardening effect of alcohol and renders some tissues semi-transparent. It is very good for examining the nervous system and determining the shape of the mucous gland.

Carriker's fluid preserves some colours and makes dissection more pleasant. Its formula is: 40% formalin, 5ml; glucose, 50g; borax, 0.5g; sea water (filtered, if possible) 500ml. The formalin acts as a preservative; glucose renders the fluid hypertonic and so reduces swelling; borax prevents bacterial action and algal growth. However, the specimen should be dissected within a month, because its condition slowly deteriorates; possibly due to the small volume of formalin and the large amount of sugar in the fluid.

A concentrated solution of **picric acid** acts both as a preservative and a stain. It also renders the nerves extensible and yet tough, so that they do not break easily.

Most preservatives will keep the radula and penial style in good condition for many years. As a general rule, it is advisable to dissect specimens within a few months after their preservation. The reproductive system should be dissected within a week, if possible, for the nature of its glands changes — the prostate may become sticky; and the mucous glands harden, or crumble.

It is a good plan to employ several fixatives, placing 2 or 3 specimens in each. Often when dissection is difficult in one preservative, it is easier in another.

MICROSCOPY

The reader may be fortunate in having a modern dissecting microscope of his own, or in being able to use one at college. Buying a new one is expensive. The cost can be cut by purchasing only essental parts. For example, the microscope depicted in Fig. 2 is withtout a lower stand and mirror for transmitted light. To study part of a dissection by transmitted light it can be mounted on a slide and examined with an ordinary microscope. The microscope (Fig. 2) has no small light bulbs attached to the body by lazy-tong devices — a cheap and efficient system can easily be arranged (see next note). It is also without arm rests, and there is no optical extension for taking photographs.

Sketches of a dissection should be made at various stages and combined in a finished drawing. Making sketches will assist the memory and improve powers of observation. The drawing will illustrate important features more clearly than a photograph. As Hartley (1962) remarked, 'the facile view that photography has made drawing unnecessary must be regarded as a mischievous heresy'.

If a student proposes to buy a second-hand microscope he is strongly advised to have it examined by an expert. If he lives in, or near, London, he could seek advice from members of the Quekett Microscopical Club that holds monthly meetings at the Natural History Museum, South Kensington. Better still, he could join the Quekett Club and learn microscopy at first hand from some of its leading exponents.

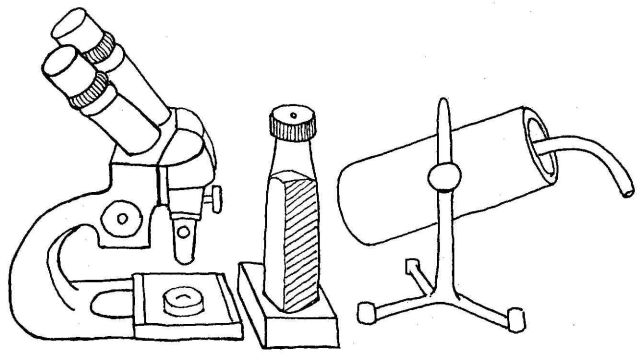


Fig. 2. Sketch of a dissecting microscope and lighting system.

LIGHTING

GOOD ILLUMINATION IS
MORE THAN HALF THE ART OF MICROSCOPY

When looking through a dissecting microscope it should be as if the observer were looking into a pleasant, brightly-lit room, and not into a dim cathedral. If the optical system is good one, tiny bits of dirt on a coin should be as clear as crystal. Avoid placing the microscope near a window and see that the room is moderately lit — sufficient to write and sketch comfortably.

A cheap and efficient lighting system is shown in Fig. 2. The lamp is one used in shops that display shirts, pullovers, and the like. It consists of a special bulb enclosed in a blackened cylinder containing a socket and a lead. The low-voltage bulb (12v; 50w) has a parabolic clear front and an Edison screw cap (E27/20). It is connected to a 12 volt transformer and from this to the mains supply. These items may be purchased inexpensively from an electrical supply store.

Between the lamp and dissection dish is a whisky flask nearly full of water. The water absorbs most of the heat from the light and so prevents the wax in the dish from melting. A hole in the stopper allows warm air to escape. The illumination is quite satisfactory when dissecting at magnifications of x 25 and x 50.

The dissection dish is about 1cm above the table, so that the forearm, from the elbow to the wrist, can rest relaxed on the table —a comfortable position during long periods of dissection.

TO MAKE A DISSECTION DISH FOR A SEA SLUG OF LENGTH 6-10mm

Obtain a sheet of pink dental wax; this does not split or crystallize like some paraffin waxes. A grape-fruit spoon that is drawn out in a spout (Fig. 3B) is useful for heating and pouring out the wax, which otherwise can be a messy and wasteful affair. Fill the spoon with bits of wax and melt them over a low flame. Pour the wax into a hollow plastic cap (Fig. 3D), about 2.5cm in diameter, until it is full. As it melts a dimple will form on the surface. Fill the dimple with melted wax so that the surface is flat

and slightly above the rim of the cap (Fig. 3D). From a local chemist buy a plastic container (approx. diameter 4.5cm and height 4.5cm). Fix the previously prepared cap with strong adhesive to the inside of the lid of the container; or to one of larger diameter, if this is preferred. The body of the container can be employed during intervals as a cover to reduce evaporation of dissection fluid (Fig. 3C).

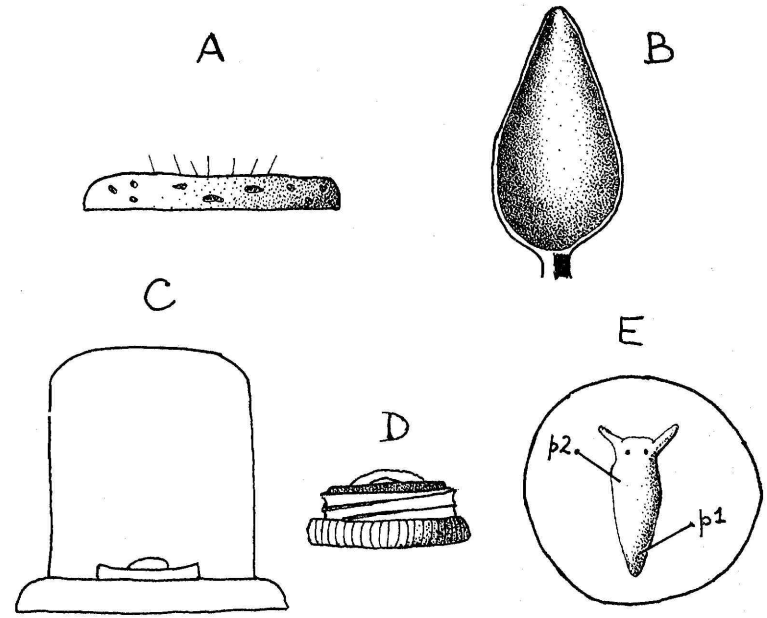


Fig. 3. A, cork pin-cushion; **B,** spout-shaped spoon; **C,** dissection dish (D) with cover; **D,** plastic cap filled with wax; **E**, position of first two pins in a dorsal lissection.

A larger dish suitable for sea slugs of length 20-30mm can be be made from a perspex box used for long coverslips.Very small specimens, 1-3mm long, can be kept reasonably steady during dissection in a drop of Berlese's fluid on a slide.

PREPARATION MOUNTANTS

There are many satisfactory mountants for serial sections, but few are suitable for preparations resulting from an ascoglossan dissection. Such a mountant should act as a clearing agent and be sufficiently viscous to hold a preparation steady when the coverslip is laid on. It must also have good optical properties.

50% Glycerol is probably the best for general purposes. **Glycerol jelly** with **fuchsin** provides good contrast, especially for radula and teeth. **Berlese's fluid** is suitable for a buccal mass. It consists of chloral hydrate, acetic acid, glucose syrup, gum arabic, and distilled water. Chloral hydrate acts as a clearing agent and clears most soft tissues leaving the radula distinct. Gum arabic and glucose syrup render the fluid viscous; acetic acid increases transparency. **Faure's fluid,** which also contains chloral hydrate, is probably a better optical medium for the only additives are gum arabic and water. Bradbury (1973) gives directions for preparing these mountants. Berlese's fluid can be obtained from a supplier (see also note p. 18).

For those interested in novelties a radula may be mounted in **Nail varnish** such as 'Cutex'. The radula should be thoroughly dehydrated and the following sequence has proved satisfactory; 70% alcohol—2 changes of absolute alcohol—transfer to a mixture of 1 part butyl alcohol and 3 parts acetone—mount radula in a drop or two of nail varnish—place the coverslip on. The varnish will set hard within a few minutes so that sealing and ringing are not necessary.

TO PIN OUT A SMALL SEA SLUG, DORSAL SURFACE UPPERMOST

Use stainless steel entomological pins of size ·0056 x 10mm. They can be obtained from a specialist supplier, such as Watkins and Doncaster Ltd., Four Throws, Hawkhurst, Kent; a hundred can be bought quite cheaply. Pin about half a dozen on a cork cushion (Fig. 3A) so that they are readily available. With your thumbnail scrape a shallow depression on the surface of the wax and in it place the sea slug. The depression should not be too deep, for this would prevent oblique light reaching the sea slug; yet of sufficient depth so that the specimen can be covered with two or three drops

of preserving fluid. Do NOT add water at this stage, because the mucous gland might swell into a shapeless blob.

One pin is insufficient to keep a sea slug stable during dissection. Three pins at the corners of a triangle would be mathematically correct. However, two pins will do to start with, and more can be added later. With fine Swiss forceps (No. 5) push the first pin (p1, Fig. 3E) through the animal's body just above the tail. Place the second pin (p2) through its left shoulder. In these positions the pins will do least damage to the internal anatomy.

Fine dissection needles can be made by firmly fixing a pin of size .0056 x 10mm to the end of a wooden cocktail stick; or the pin can be fused into the tapered end of a small glass tube. If so desired, a pin-holder chuck (No.0) with a stainless steel handle may be purchased from a watchmaker's or jeweller's supply shop.

Practise dissecting with two needles. Sooner or later one of them will bend at the tip and form a useful hook. A No. 11 Swann Morton blade can be used to cut thick transverse sections; or to divide an animal into halves along the median line, each half providing a sagittal surface.

RADULAR PREPARATIONS

Making a radular preparation is a simple and interesting exercise.

Pin out an ascoglossan, dorsal side uppermost, and open along the median line; display the buccal mass by pinning aside the skin in the head region. Sever the cerebrobuccal connectives (Fig. 4A, cb) and cut the oesophagus (oe) near the nerve collar. Remove the ligaments attaching the buccal mass to the body wall. With fine forceps hold the mass and gently pull it free from the head, taking care not to damage the ventral ascus (as). Place the unstained preparation on a slide in a drop or two of Berlese's or Faure's fluid, and display the radula in lateral view. If the ascus is not clearly visible when the coverslip is in position, gently move the coverslip with the fingers to improve the view.

In some species the smaller teeth and first elements are shed from the radula and lie heaped together in the ascus so that the first elements are often obscured. These rudiments are more easily studied in small specimens of length 2-4mm which have few discarded teeth.

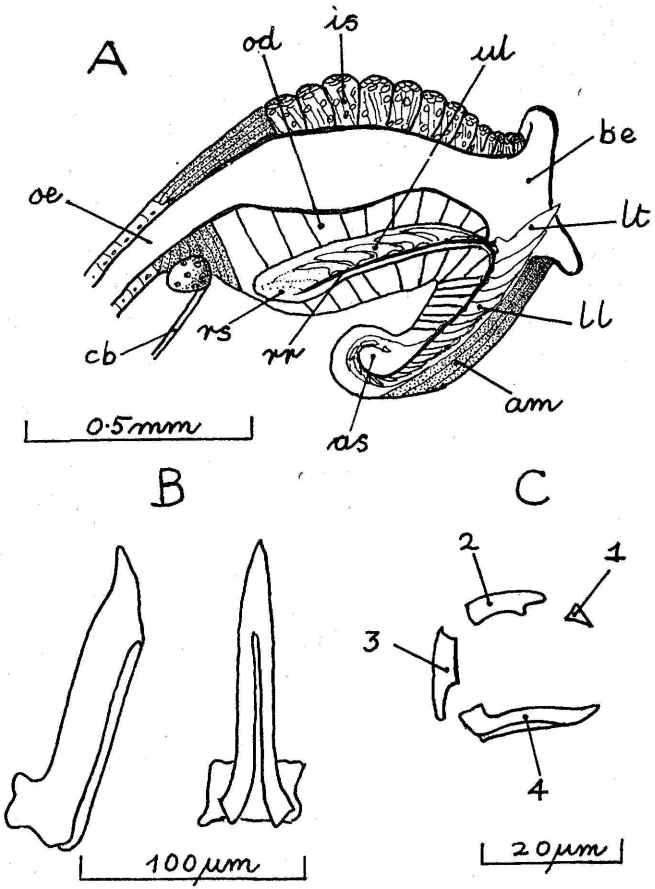


Fig. 4. Details of buccal mass, radula, and teeth

A, diagram of a buccal mass in which all elements are attached to the radular ribbon. **B,** lateral and dorsal views of a tooth of *Placida dendritica.* **C,** first elements of *Placida viridis;* 1 and 2 are rudiments; 3 is a transitonal tooth, and 4 is the first fully formed tooth.

An ascoglossan radula consists of a single longitudinal row of elements attached to the radular ribbon (rr). *The ribbon bends sharply near the buccal entrance* (be) *and ends in the ascus* (as).

Once having become proficient you will want to continue dissecting. Making a preparation of the buccal mass affords an excellent opportunity for tracing the buccal nerves; and those of cerebral ganglia, especially the anterior array. The central nervous system should be examined *in situ* — preparations of the nerve collar are seldom satisfactory.

A second method is employed to display separated teeth in different positions. Use a set-up similar to that shown in Fig. 2. Remove the flask and replace the dissection dish by a watchglass containing a 10% solution of potassium (or sodium) hydroxide, in which the buccal mass is placed. Adjust the distance of the lamp so that the solution can be maintained at a temperature of 50-60°C. It is instructive, by means of a low power objective, to observe the buccal mass as it disintegrates. Maintain the level of the solution by adding water from time to time. Add a drop of Chlorazol Black E, or Azo black, to selectively stain the radula a dark grey tone, making it easier to locate. If the tone is too dark it can be differentiated afterwards by using a 0.5% solution of sodium hypochlorite.

When the radula is free from tissues, wash it thoroughly in water to remove all traces of caustic. With a hooked needle, or fine pipette, transfer the radula to a drop of Faure's fluid on a slide. By means of needles separate the teeth and place a coverslip over them. There is no need to worry about their positions. Most of them will be lying on their sides. Slightly shift the coverslip and some will slowly rotate to reveal dorsal and ventral aspects.

STAINING

On first looking into an ascoglossan most of the viscera appear a featureless matt white with few ducts discernible. Staining is

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All elements are formed in the radular sac (rs). *They pass along as part of the upper limb* (ul) *to the lower limb* (ll). *The first elements and some teeth eventually reach the ascus where, in some species, they are shed from the ribbon and lie in a heap in the ascus.*

The ascus muscle (am) *drives the lower limb in the act of piercing an algal filament, which is performed by the leading tooth* (lt). *The interseptal and odontophoral muscles* (is *and* od) *form a pump for withdrawing cell sap and forcing it into the oesophagus* (oe).

therefore necessary to provide CONTRAST. Methyl blue is commonly used; Acetocarmine and Safranin O also give good results. Other stains could be tried, such as Alum carmine that was used by Russell (1939) who excelled at fine dissection.

Directions for preparing stains are given in Bradbury (1973). Whatever stain is used it must be CONSISTENT with the preserving fluid, otherwise the stain will be ineffective, and may form a precipitate suspended in the fluid, or deposited on the surface of the dissection.

Acctocarmine is consistent with Clarke's fluid. Dissect an ascoglossan preserved in this fluid to expose as much of the viscera as possible. Remove the fluid and cover the specimen with a few drops of Acetocarmine. Leave for about 15 minutes. Wash off excess stain with 70 per cent alcohol. If the stain has not penetrated the required region, dissect further to display it, and repeat the staining process. Water may be used to differentiate.

A few grains of table salt sprinkled over a dissection, will often lighten a stain and help to dehydrate the mucous gland.

TEMPORARY MOUNTS AND THEIR EXAMINATION

For research purposes making so-called permanent mounts is unnecessary and time-consuming. Temporary mounts in 50% glycerol, Berlese's, or Faure's fluid are preferable. The latter two fluids will set firm within a few days and require no sealing or ringing. Store the slides in a horizontal position. Cuticular structures will remain visible for 3 or 4 years, but most soft ttssues disappear in a year or less. It is inadvisable to use Canada Balsam as a mountant for an ascoglossan radula or style. The refractive index of Canada Balsam is 1.53 and this comes too near to that of ascoglossan cuticle, 1.54, so that visibility is reduced. On this point it is helpful to remember the formula: Index of visibility = the difference between the refractive indices of the object and its mountant.

It is annoying to see a well-displayed preparation move slightly as a coverslip is placed over it so that the part to be examined becomes indistinct or hidden. There is also a risk that slightly shifting the coverslip may not be successful. Try examining WITHOUT A COVERSLIP. Use an ordinary microscope with a low power objective — often sufficient to sketch most of the detail

required. Occasionally higher N.A.† is necessary. An objective designed for examining uncovered surfaces in metallurgy would be suitable; or a 'no-cover' objective may be purchased. As an expedient, stick a bit of coverglass with immersion oil to the front lens of an objective (N.A. 0.65) instead of laying it on the preparation.

After covering the preparation with a coverslip, observations can be continued with a high dry objective (N.A. 0.80). Avoid using an oil-immersion objective with a freshly-made mount. The coverslip may move when wiping off the oil—or even before this. A modern water-immersion objective (N.A. 1.00) is less open to these objections. However, high powers should be used sparingly; the depth of field is low, the area restricted, and preparations are not of uniform thickness. In this context it is a sound principle to use the *minimum* power that will reveal what is required—it is easier, quicker, and less expensive.

PREPARATION OF A PENIAL STYLE

Many ascoglossans have a penial style like that depicted in Fig. 10A. It can be displayed by a little fine dissection and a preparation made for further study.

Open the animal along the mid-dorsal line and remove the skin from the dorsal surface of the head. When housed inside the body, the penis lies in a latero-ventral position, posterior to the right eye. It also lies alongside the posterior half of the buccal mass or just beyond. The style points posteriorly. The base of the penis is attached by muscles to the body wall near the eye. Start from this region and remove the fine sheath that covers the penis with one or two posteriorly-directed strokes of a needle. At this stage it is important to *see the style,* and it can now be sketched. Take care that it remains undamaged during the rest of the operation.

Remove part of the body wall together with the penis, and the eye, which will act as a marker. Transfer to a drop of Berlese's fluid on a slide. With needles separate the penis from the body wall and remove extraneous tissues. Display the penis and style in a good position. The tip of the style is apt to stick to the underside of the coverslip, so first examine the preparation without a coverslip.

Should the penis be extruded the matter is easier. Grasp the base

† For an explanation of N.A. see footnote to Fig. 10, p. 30.

of the penis firmly with fine forceps and free it from the body with a hooked needle. Mount, display, and examine as before.

ALIMENTARY, REPRODUCTIVE, AND CENTRAL NERVOUS SYSTEMS

The best way to learn how to dissect these systems is by PRACTICE. The Appendix (p. 20) will help a student to understand technical terms used in papers. The figures illustrate, in a general way, the anatomy of those ascoglossans that do not have a shell. The text notes some morphological differences.

Endeavour to dissect a small ascoglossan in the same way as you would a dogfish or a frog. Pin it out dorsally and concentrate on one system at a time, working carefully and slowly, and observing the rules and standards of good dissection.

Do not remove parts for preparations during the course of a dissection. When examining the alimentary system it is inadvisable to begin by removing the buccal mass, for in so doing the salivary ducts will be severed and their course will be more difficult to follow. Similarly, with the reproductive system, leave the removal of the penis until the last; if the male duct is severed it will contract and valuable lead into the genital complex may be lost. Keep the number of preparations to a minimum, and avoid finishing with a "bits and pieces" dissection that may be hard to reasssmble.

The alimentary system is usually the easiest to dissect and could be attempted first. Whilst dissecting, note the level and layout of other systems.

When dissecting the nervous system make *certain* of the origin of each nerve and enter it on a general plan. In the same way, the origin, associated glands, and termination of each reproductive duct should be noted by a sketch.

In the second half of the last century, Bergh, Trinchese, and Pelseneer were among the first to describe the major systems of many ascoglossans. It is advisable to read their papers. Most students have studied French at school, so Pelseneer's famous memoir (1894) should not present much difficulty. Bergh's papers were published in German and Trinchese's in Italian. Learning both these languages to O-level standard is recommended; then, with a little help from dictionaries, translation should not be difficult.

Studying two languages may take three or four years, but it need not be an unpleasant task.

TESTS OF SKILL

There are some parts of an ascoglossan which require special manipulative skill to display. They could be regarded as test pieces, though much depends on the relaxation and preservation of the specimen, and how long it has been in preservative. So do not be discouraged, if at first you do not succeed.

1. **Heart and kidney.** Carefully remove the skin over the reno-pericardial swelling and display as shown in Fig. 5.
2. **Optic nerve**. A slender nerve (Fig. 9A, c6). Each eye is embedded in tough tissue, and here the nerve requires extra care to maintain it intact.
3. **Penial nerve** (Fig. 9B, pe). The precise origin of this nerve may vary and is, at times, difficult to display.
4. **Genital complex** (Fig. 7B). By this is meant the ducts in the region of the capsule gland and those near the bifurcation of the hermaphrodite duct. Difficult under any circumstances.

IMPROVEMENTS AND CHECKS

Try to study ascoglossans in their natural environment. KEEP THEM ALIVE for as long as possible and observe how they move, defend themselves, feed, copulate, and lay their eggs. Form and function go together and some functional anatomy will enhance a project, or a paper.

After having completed a dorsal dissection, attempt one from the ventral side; a right lateral dissection sometimes proves interesting. Transverse, horizontal, and sagittal serial sections are INVALUABLE for confirming and extending the results of dissection.

GENERAL HINTS

GO SLOW — half the pace of a normal dissection. An unfinished dissection will keep in 50% glycerol overnight, or longer. The water will evaporate, but not the glycerol. Before commencing it may be necessary to dilute the glycerol by washes of water. Pure glycerol has a refractive index of 1.47 and this may impair the resolution of an optical system.

TAKE IT EASY. Too much concentration may tense the muscles, with a loss of precise control. The trick is to keep the muscles relaxed, yet responsive, and the mind alert. Have a comfortable seat at the right height, and rest the whole of the forearm on the table.

STOP if you are bored; or if the dissection is not going well. Leave it in 50% glycerol until you are ready to continue.

When dissecting small animals it pays to keep instruments, slides, and watchglasses CLEAN. It is a good habit before dissecting to clean the upper lenses of eyepieces by breathing on them and wiping off the moisture and particles of dust with a piece of an old linen handkerchief which has been laundered many times.

Try SIMPLE remedies if the performance of a dissecting microscope is unsatisfactory. Clean the top lenses of the eyepieces and the front lenses of the objective. Readjust the light. Replace the dissection fluid. The magnification may be too high, or too low. Experience suggests that for a 6mm specimen, x 25 is suitable for general features and pinning out; x 50 for most of the dissection; and x 100 for a few details.

Special CARE should be taken when using the chemicals and mountants mentioned in the notes. If chloral hydrate gets on the fingers or hands **immediately** wash the part thoroughly in running water to prevent penetration; if the area is extensive, or deep, seek medical aid. Treat accidents with alkalis in the same way. MS-222 could cause harm if improperly used, and should be kept in a safe place out of the reach of children.

ACKNOWLEDGEMENTS

I am grateful to the Committee of the Conchological Society for encouraging me to write about fine dissection. I thank P. E. Negus, editor of the *Conchologists' Newsletter,* for permission to use excerpts and figures from the series *Ascoglossan Notes;* and A. Norris, editor of *Papers for Students,* for much constructive criticism.

I am indebted to Dr. J. Buchanan and Dr. F. Evans of the Dove Marine Laboratory, Cullercoats; W. G. Hartley, Quekett Microscopical Club; and Professor A. Graham, F.R.S., all of whom read the manuscript and suggested improvements.

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APPENDIX

DISSECTION DIAGRAMS

The diagrams are intended to give the less-specialised reader a general idea of the anatomy of many ascoglossans. The notes may help a dissector to realise what variations he may expect in some species, particularly those without shells.

HEART AND KIDNEY

In a living shell-less ascoglossan the heart can be seen beating. It lies dorsally on the median line in the anterior half of the body, just below the skin, and usually forms part of a reno-pericardial swelling. The heart consists of a thick-walled ventricle and a thin-walled auricle enclosed in a pericardium.

The aorta immediately divides into a cephalic and a visceral artery. The cephalic artery can be followed to the nerve collar, and the visceral artery descends ventrally, but their further courses are not traceable by dissection.

The structure of the heart is simple and varies little in the order Ascoglossa. Two species are described as having no heart, and there may be a few, as yet not studied, that have an atypical heart.

The kidney is a conical sac sometimes extending along the sides of the pericardium. The renal duct may follow the course of the intestine. The renal opening is minute and often difficult to locate; in many species it lies near the anus.

In bivalved gastropods the kidney is diffuse and lies near the folds of the gill.

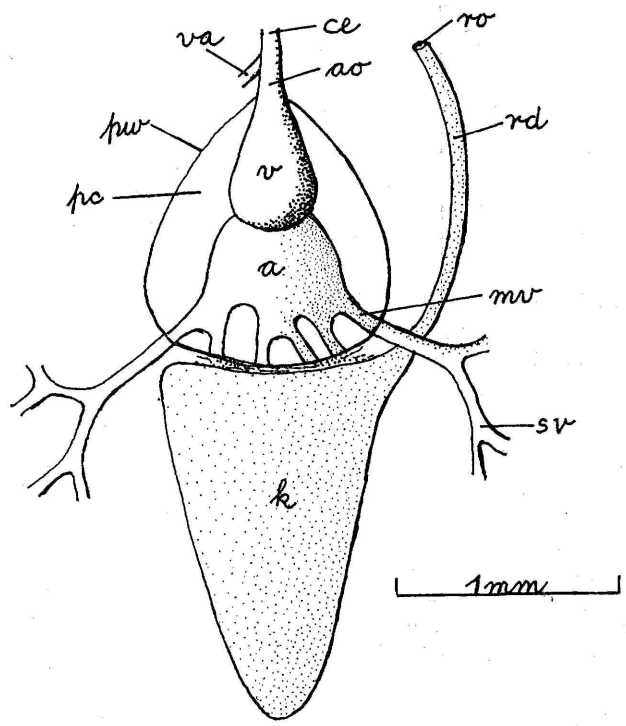

Fig. 5. Diagram of heart and kidney

a, auricle; ao, aorta; ce, cephalic artery; k, kidney; mv, main vein; pc, pericardial cavity; pw, pericardial wall; rd, renal duct; ro, renal opening; va, visceral artery; sv, surface vein; v, ventricle.

ALIMENTARY SYSTEM

Cell sap is sucked into the pharynx and passes along the oesophagus into the stomach. It is then distributed by means of lateral diverticula along both sides of the body. Branches from the diverticula enter the cerata; only one ceras is shown in Fig. 7.

Differences between species

The oral tube may be elongated into a proboscis. Buccal glands may cover the anterior half of the buccal mass. The buccal cavity may be extended posteriorly by either dorsal or ventral caeca that project externally. Some species possess a posterior muscular collar and some have a muscle pad attached to the ventral surface of the mass. The salivary glands may be simple and tubular, as in Fig. 6, or compound with foliaceous branches. In a few species the oesophageal pouch lies close to the stomach or anteriorly below the buccal mass. The intestine may be directed posteriorly. The anus can thus be placed anteriorly, or on the right side, or in the posterior part of the body, or terminally. It may be flush with the surface, or at the end of a rectal spout. Digestive diverticula can be replaced by main ducts which divide repeatedly; each final branch may supply several cerata. Species without cerata have lobed, lateral diverticula.

Shelled species (*Oxynoe, Lobiger,* and the bivalved gastropods) have a buccal mass with a typical radula. External buccal caeca may be present. The stomach is large, without diverticula, and the anus is posteriorly placed.

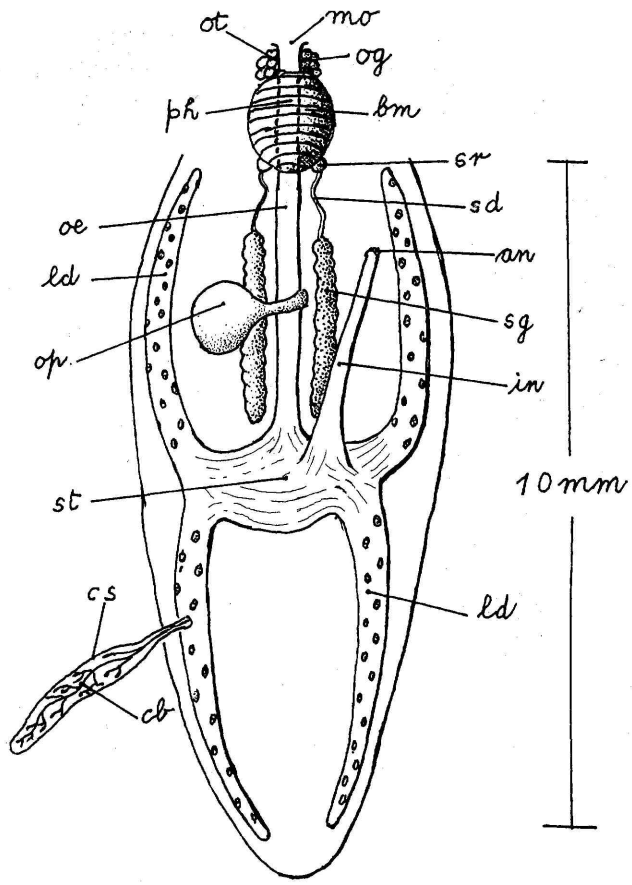

Fig. 6. Diagram of gut

an, anus; bm, buccal mass; cs, a ceras; cb, ceratal branch of gut; in, intestine; ld, lateral diverticulum; mo, mouth; oe oesophagus; og, oral glands; op, oesophageal pouch; ot, oral tube; ph, pharynx, or buccal cavity; sd, salivary duct; sg, sal. gland; sr, sal. reservoir; st, stomach.

REPRODUCTIVE SYSTEM

Fig. 7A shows the general layout. The ovotestis consists of follicles; their number varies from 10-250 according to the species. Follicular ductules lead to a median hermaphrodite duct. The duct swells to an ampulla and narrows before dividing into a male and female duct. A bursa copulatrix is present. The ill-defined fertilization region is indicated by the confluence of three ducts : female, bursal, and albumen.

There are two main types of reproductive system: diaulic and pseudo-diaulic. Fig. 7A could represent diauly. During copulation the partner's penis is thrust into the female opening and sperm pass along the female duct and are stored in the bursa copulatrix until required. The penis is usually tentaculiform and the bursa does not lie close to the body wall. Pseudo-diauly is illustrated by Fig. 7B. It is characterised by a penis with a cuticular style and a bursa that lies close to the body wall. During copulation sperm are injected by the penial style through the body wall directly into the bursa, thus bypassing the long female route. A few species are triaulic. The bursa opens to the exterior by a short duct and so there are three openings : male, oviducal, and vaginal.

Morphological variations

The prostate can be either bilobed, or compact. The bursa may be spherical, ovoid, or elongate. The extensive albumen gland and its ducts are closely applied to the branches of the gut. The albumen gland can end in tubules, lobules, or capsules; they may follow the gut into the cerata, or not. In most pseudo-diaulic species the genital complex is more compressed than in Fig. 8B. The female duct may then run along the ventral surface of the capsule gland, or be attached to its anterior border. The capsule gland can be ovoid or sausage-shaped. The spherical genital receptacle is placed anteriorly near the median line; in a few species it lies close to the left body wall. After leaving the capsule gland the female duct enters the large mucous gland, which in some species is looped and in others it is half-moon shaped.

The author has not yet made complete dissections of the system in any of the shelled species. Some of them possess flexible penial styles.

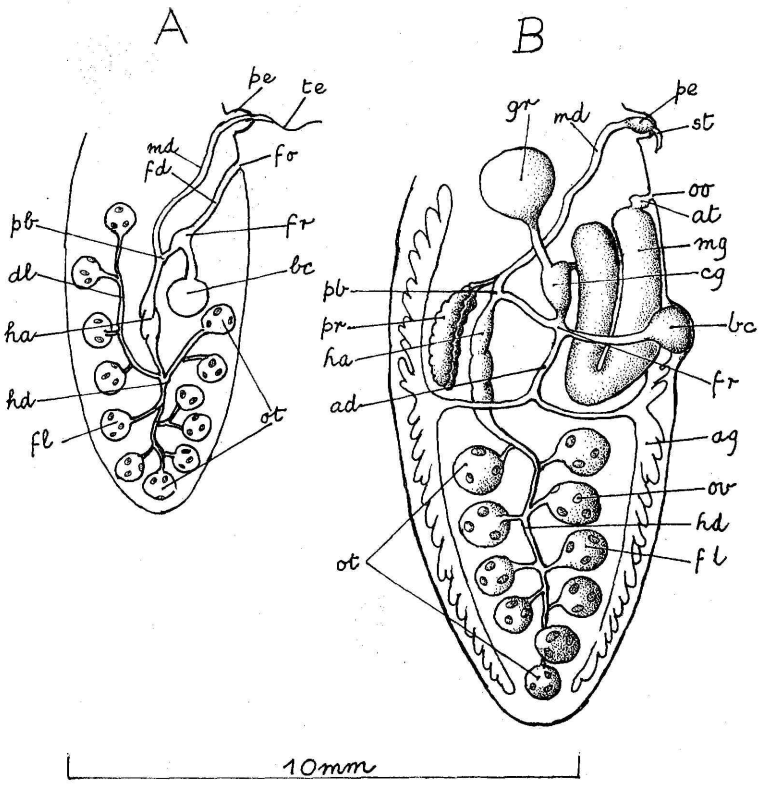

Fig. 7. Reproductive system

A, basic layout. **B**, more detailed diagram illustrative of pseudo-diauly.

ad, main albumen duct; ag, albumen gland; at, female atrium; bc, bursa copulatrix; cg, capsule gland; dl, ductule; fd, female duct; fl, follicle; fo, female opening; fr, fertilization region; gr, genital receptacle; ha, hermaphrodite ampulla; hd, hermaphrodite duct; md, male duct; mg, mucous gland; oo, oviducal opening; ot, ovotestis; ov, ovum; pb, bifurcation of hd; pe, penis, pr, prostate; st, penial style; te, tentacular extension of penis.

CENTRAL NERVOUS SYSTEM

The central nervous system consists of 8 or 9 ganglia and the nerves that arise from them.

The main ganglia are clustered around the beginning of the oesophagus. The oesophagus passes *through the nerve ring* formed by two cerebral and two pedal ganglia (Fig. 8A) together with their commissures (joining ganglia of the same name) and connectives (joining ganglia of different names). It also passes *above the visceral loop*. The loop begins on one cerebral ganglion and ends on the other (Fig. 8B). It may bear three ganglia (supra-intestinal, abdominal, subintestinal); or two, the subintestinal being absent. In many species the loop is so short that its connectives are not visible.

The buccal ganglia lie in the angle between the junction of the oesophagus with the buccal mass; each ganglion is joined by a connective to the cerebral ganglion on the same side (Fig. 8D). Before the removal of the oesophagus, only the cerebral ganglia are clearly visible (Fig. 8C). Fig. 8E shows a conventional diagram of a C.N.S., less the peripheral nerves; it is, of necessity, two-dimensional and fails to convey the fact that the cerebral ganglia sit like a saddle upon the oesophagus.

Shelled species have three ganglia on the visceral loop. In the bivalved gastropods the loop is very long and this is illustrated in Fig. 11 which shows part of the C.N.S. of *Midorigai australis*. In this species the supra-intestinal (sp) is attached to the right side of the large adductor muscle (ad) that has, as it were, pushed the loop towards the right side of the body. A short branch of sp1 passes *through* the adductor muscle and ends in an osphradial ganglion (os); ab1 swells to form a small genital ganglion (g).

The anterior array of cerebral nerves resembles that of the shell-less species.

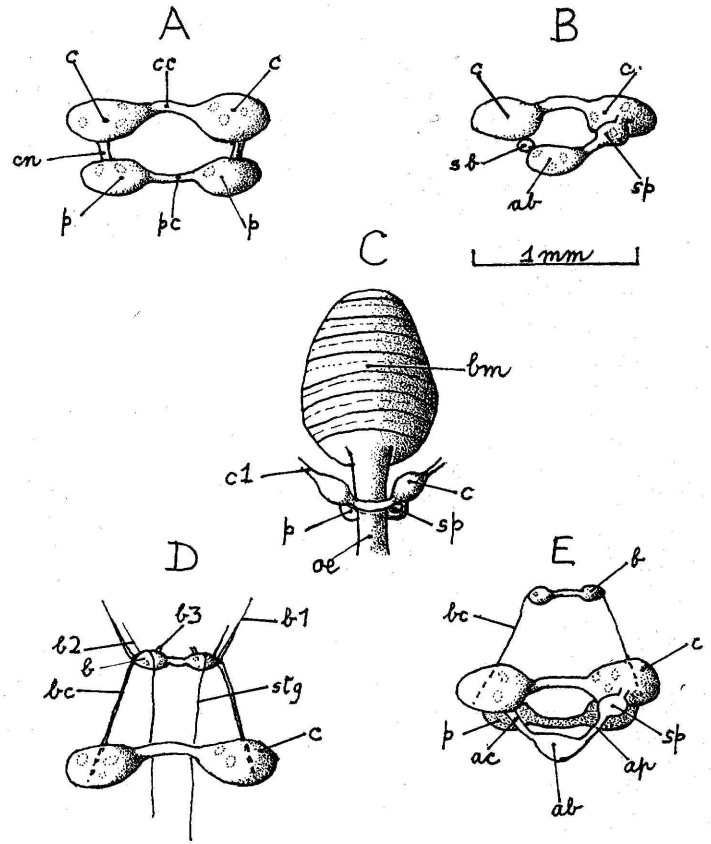

Fig. 8. Ganglia of the central nervous system

A, nerve ring; **B**, visceral loop; **C**, what can be seen of the ganglia before removal of oesophagus; **D**, buccal loop; **E**, conventional diagram of C.N.S. without peripheral nerves.

ab, b, c, p, sb, sp, abdominal, buccal, cerebral, pedal, subintestinal, supra-intestinal ganglia; b1-b3, buccal nerves; ac, cerebro-abdominal connective; ap, abdominal and supra-intestinal conn.; bc, cerebrobuccal conn.; bm, buccal mass; c1, a cerebral nerve; cc, cerebral commissure; cn, cerebropedal conn.; oe, oesophagus; bc, pedal com.; stg,stomato-gastric nerve.

PERIPHERAL NERVES

Cerebral. C1 (rhinophoral nerve) runs into the tentacle. C2 (labial nerve) has three branches : a, to the oral tube; b, to labial lobe, or anterior corner of the head; c, to the side of the head, often with a branch near the base of the tentacle. The division of c2 is variable and may occur near the origin of the nerve. C3 supplies the dorsal surface of the head. C4 is a fine nerve to the side of the neck. C6 is the slender optic nerve. A few species have an extra nerve, c7, that leads on to the surface of the buccal mass.

Some species have medio-dorsal bodies (Fig. 9A, mdb) situated each side of the cerebral commissure. Each contains two large neurons and the body is enclosed in a thin membrane that is connected by a short stalk to the cerebral ganglion on the same side. The photograph on the reverse side of the fly leaf gives an impression of what a medio-dorsal body looks like in a preparation. C1 may have a latero-dorsal body near to its base (Fig. 9A); it consists of several small neurons and one or two larger ones enclosed in a membrane. No research has been done on these bodies, but their position and structure suggest they are homologous with those found in some Pulmonates.

Pedal. There are three main pedal nerves. P1 runs to the anterior border of the foot. P2 supplies the border in the neck region. P3 is a strong nerve that runs laterally close to the margin of the foot and ends near the tail. Minor accessory nerves may be present. The penial nerve originates on the anterior border of the right pedal ganglion in most species.

Visceral loop. The supra-intestinal ganglion has only one nerve. It has been traced to the heart and kidney; sometimes it has a branch that ends midway along the right side. There are two main abdominal nerves. A1 runs medianly to the posteror region where it may have a minor ganglion with fine nerves to the follicles of the ovotestis. A2 is a genital nerve; one branch runs to the bifurcation of the hermaphrodite duct and its ampulla; the other is directed towards the capsule gland. No nerve may arise from the sub-intestinal ganglion; if one does, it leads to the body wall and may also anastomize with c5 on the left.

Buccal loop (*Fig. 8D*). B1 is a prominent nerve that leads into the buccal mass. B2 is a short suboesophageal nerve, and b3 is a fine nerve to the salivary reservoir. Two stomato-gastric nerves, when present, originate on the anterior border of the buccal ganglia and continue along the sides of the oesophagus to form a network of fine branches on the stomach.

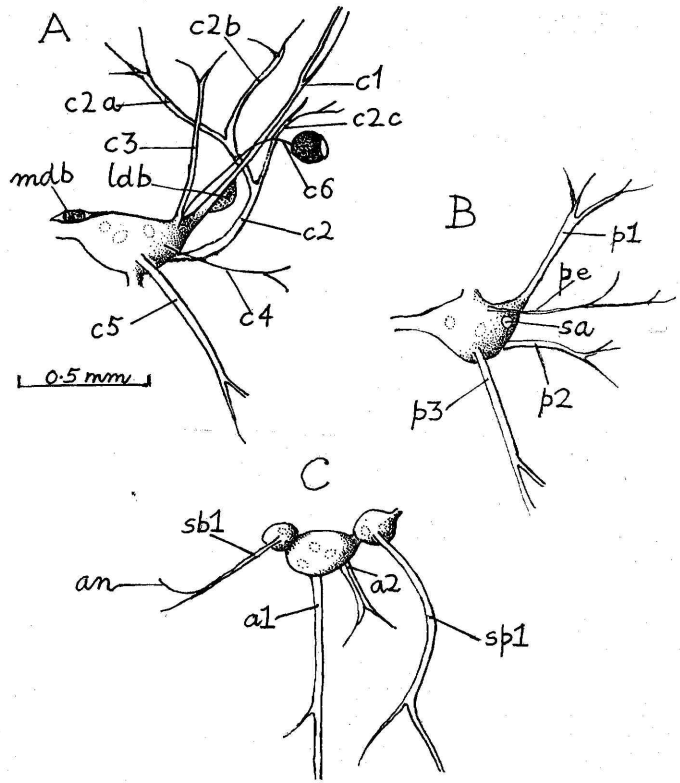

Fig. 9. Nerves arising from the main ganglia

A, cerebral nerves; **B**, pedal nerves; **C**, nerves of th visceral loop. In **A** and **B** only the nerves of the right ganglia are shown, since those of the left ganglia are similar (except a penial nerve is not present on the left).

Nerves : c1-c6, cerebral; p1-p3, pedal; pe, penial; sp1, supra-intestinal; a1, a2, abdominal; sb1, subintestinal; an, anastomosis of sb1 with c5.

ldb, latero-dorsal body; mdb, medio-dorsal body; sa, statocyst.

EXAMPLES OF PREPARATIONS

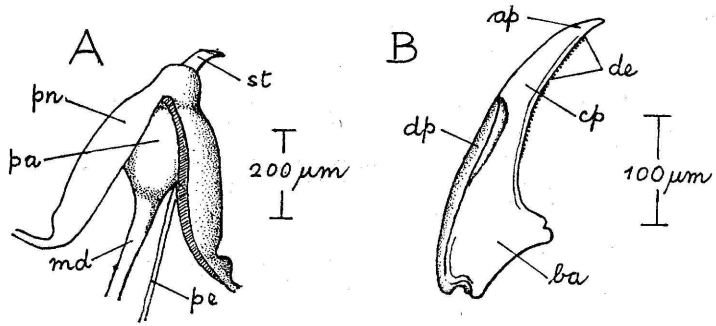

Fig. 10. Ascoglossan preparations

A, penis of *Placida viridis* displayed to show the cuticular style (st). **B**, tooth of *Elysia viridis* separated from the radula.

Ap, apex; *ba*, base; *cp*, cusp; *de*, denticles†; *dp*, depression; *md*, male duct; *pa*, penial ampulla; *pe*, penial nerve; *pn*, penis; st, cuticular style.

† For those interested in microscopy the denticles (B, de) form good test objects. They are set close together and are of average diameter 1.5µm. They should be visible as separate objects using a student's microscope with an objective of N.A. 0.65.

N.A. stands for *Numerical Aperture*. It is the measure of the power of an objective to reveal fine detail. Usually a low power objective has an N.A. of 0.1 or 0.25; medium power 0.65; and high power 0.80 (dry) or 1.25 (oil immersion).

A moment's reflection will convince the reader that the N.A. of an objective gives a better idea of the possible performance of a microscope than a figure for its magnificaion. For suppose an objective has a magnification of x 40 and the eyepiece magnifies x 10. The total magnification is then 400. Replace the eyepiece with one of x 20; the magnification is now 800. A little more detail may be revealed by the change; but in general one merely sees a more enlarged portion of the primary image that is produced by the objective. In the same way, if a photograph is enlarged the detail still depends on the original plate, or film .

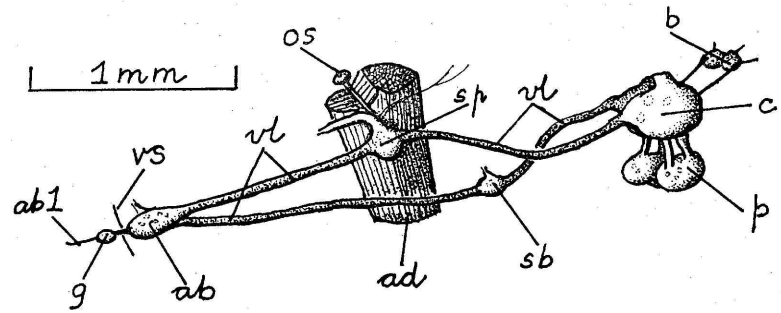

Fig. 11. Visceral loop of *Midorigai australis* **Burn**

Drawn from a complete dissection of C.N.S.

Abbreviations as for Fig. 8 with the addition of ad, adductor muscle; g, genital ganglion; os, osphradial ganglion; vl, visceral loop; vs, visceral septum.

About the author

Tom Gascoigne was educated at Birkbeck College, University of London; his lecturers in Zoology were: Professor H. G. Jackson, Professor A. Graham, Dr. Vera Fretter, T. E. Hughes, and Lilian Russell. Under their expert guidance he obtained the degrees of B.Sc. and Ph.D. and became interested in fine dissection.

At the beginning of the Second World War he enlisted in the Royal Artillery and saw active service in North Africa and Italy. After the war he was appointed Biology Master at Alleyn's School, Dulwich. During his years of teaching, over a hundred boys obtained admission to medical and dental schools.

He retired at the age of 70 and now spends most of his spare time studying ascoglossan sea slugs. So far, he has written 18 papers using the methods described in this booklet.

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The Society has a strong core of shell collectors who exhibit their shells, with well-informed comments, at our meetings. It has always been interested in taxonomy, as witness the late R. Winckworth's *British Marine Mollusca* which has remained an authoritative checklist for about 50 years. A. E. Ellis, one of our members, wrote the well-known book *British Snails*. M. P. Kerney, our non-marine recorder, recently published an *Atlas of the non-marine Mollusca of the British Isles*—the first of its kind for Britain. The Journal continues to print important papers by malacologists.

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