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CONTENTS

H. ANT

- Die Bedeutung der Eiszeiten für die rezente Verbreitung
der Europäischen Landgastropoden 61

A. D. BERRIE

- Snail size in relation to infection with *Schistosoma* 23

H. H. BOER

- The effect of amphenone B on the egg production of
Lymnaea stagnalis L. 47

H. H. BOER and J. LEVER

- The effects of amphenone B on the egg production of
Lymnaea stagnalis 431

J. B. BURCH

- Chromosomes of intermediate hosts of human bilharziasis 25

J. B. BURCH

- Chromosomes of intermediate hosts of human bilharziasis 127

J. B. BURCH and J. M. HUBER

- Ploidy in mollusks 41

M. CHÉTAIL et D. BINOT

- Particularites histochimiques de la glande et de la sole
pedieuses d' *Arion rufus* (Stylommatophora: Arionidae) 269

H. COOK

- Structural details of the central nervous system of
Succinea putris (L.) 75

C. C. DAVIS

- Emergence of veliger larvae from eggs in gelatinous
masses laid by some Jamaican marine gastropods 299

G. M. DAVIS and G. K. LINDSAY

- Disc electrophoretic analysis of molluscan
individuals and populations 311

R. M. DeWITT

- Stimulation of egg production in a physid and a lymnaeid 445

P. DINAMANI	
	Variation in the stomach structure of the Bivalvia 225
T. FENCHEL	
	On the ciliated Protozoa inhabiting the mantle cavity of lamellibranchs 35
V. FRETTER	
	Some observations on neritids 79
G. GARGALLO DI CASTEL LENTINI	
	Sur quelques formes de senestrisme des <i>Helix</i> Italiennes 63
A. V. GROSSU	
	Caucasian species in central Europe: the genus <i>Lytopenia</i> (Gastropoda, Limacidae) in Rumania, and its wide variation 57
N. A. HOLME	
	Distribution of molluscs in the English Channel 53
B. HUBENDICK	
	Some aspects of vector snail control 31
J. J. LAMMENS	
	Observations on the ecology of <i>Macoma balthica</i> 81
H. LEMCHE	
	The place of Mollusca among invertebrates 7
J. LEVER	
	Modes of sorting of shell valves on a sandy beach studied with artificial valves of <i>Donax vittatus</i> 85
R. G. LUTFY and E. S. DEMIAN	
	The histology of the alimentary system of <i>Marisa cornuarietis</i> (Mesogastropoda: Ampullariidae) 375
E. H. MICHELSON	
	The specificity of hemolymph antigens in the taxonomic discrimination of medically-important snails 33
P. E. P. NORTON	
	Marine Mollusca in the Lower Pleistocene of East Anglia 55

CONTENTS (cont.)

C. M. PATTERSON

- Chromosome numbers and systematics in
streptoneuran snails 37

C. M. PATTERSON

- Chromosome numbers and systematics in
streptoneuran snails 111

M. PETITJEAN

- Structure microscopique et nature mineralogique de la
coquille des principaux Muricidae 69

B. PRESCOTT and C. P. LI

- Antimicrobial agents from sea food 45

M. RAINER

- Chromosomenuntersuchungen an gastropoden
(Stylommatophora) 341

O. RAVERA

- The effect of X-rays on demographic characteristics of
a freshwater gastropod, *Physa acuta* Drap. 51

O. RAVERA

- The effect of X-rays on the demographic characteristics
of *Physa acuta* (Gastropoda: Basommatophora) 95

C. S. RICHARDS

- Genetic studies on *Biomphalaria glabrata* (Basommatophora:
Planorbidae), a third pigmentation allele 335

N. W. RUNHAM and K. ISARANKURA

- Studies on radula replacement 73

G. P. SELLMER

- Functional morphology and ecological life history of the gem
clam, *Gemma gemma* (Eulamellibranchia: Veneridae) 137

B. J. SMITH

- Correlation between neurosecretory changes and maturation
of the reproductive tract of *Arion ater* (Stylommatophora:
Arionidae) 285

C. R. STASEK

- Views on the comparative anatomy of the bivalved Mollusca 67

CONTENTS (cont.)

T. E. THOMPSON	
Development and life history of <i>Archidoris pseudoargus</i>	83
T. E. THOMPSON	
Adaptive significance of gastropod torsion	423
J. W. TIEZE-DAGEVOS	
The effect of an experimental molluscicide on the eggs of <i>Australorbis glabratus</i>	29
H. VAN DER SCHALIE	
The role of snail intermediate hosts in culturing <i>Schistosoma japonicum</i>	17
H. VAN DER SCHALIE	
Hermaphroditism among North American freshwater mussels	77
W. J. VAN DER STEEN	
Atmospheric air pressure and egg production of <i>Lymnaea stagnalis</i> , under laboratory conditions	49
J. A. VAN EEDEN	
Distributional trends of four species of freshwater snails in the Republic of South Africa with special reference to the intermediate hosts of <i>Bilharzia</i>	21
C. A. WRIGHT	
Intermediate host-parasite relationships in African schistosomiasis	15

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of the

Symposium on MALACOLOGY AND PARASITOLOGY

and the

SECOND EUROPEAN MALACOLOGICAL CONGRESS

(Copenhagen, 10-14 August, 1965)

Edited by G. HØPNER PETERSEN and J. KNUDSEN

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PREFACE

When the preparations for the Second European Malacological Congress were taken up for general consideration it was realized that the contact between the pure malacologists, be they amateurs or professional scientists, and those working in applied malacology was not as close as it could advantageously be. It was felt that it would have a scientific value if a problem of general interest to both groups could be taken up for more extensive analysis, and so, the idea sprang up to arrange a symposium on malacology and parasitology. It was hoped that if they met and listened to each other, several pure malacologists would learn more about the problems and undertakings of those in the applied field, and vice versa, and thus contribute to bridge the gap. The financial support necessary to carry out this idea was generously granted by the IUBS, and the Symposium was then arranged so that it immediately preceded the Congress, enabling those who wished to attend both arrangements to do so by merely arriving two days earlier.

For practical reasons, this double arrangement will not be treated as two separate parts in the present volume as, for easier orientation of the reader, it was found more suitable not to have separate indices, etc.

It is a pleasure to express the gratitude of the organizers for the generous and most valuable help obtained from many sides, and to thank Dr. C. A. Wright for accepting the invitation to give a lecture at the Symposium.

First of all, we are indebted to the members of the Honary Committee for the important support they gave to the meeting.

Secondly, we wish to express our gratitude to the Director of the Zoological Museum, Dr. H. Volsøe, for permission to use the rooms and facilities of the museum. We also extend our best thanks to the leaders of the institutions visited during the excursions, Dr. G. Mandahl-Barth (Dansk Bilharziosis Laboratorium, Charlottenlund), Dr. A. Schiøtz (Danmarks Akvarium, Charlottenlund), Dr. G. Thorson (Marinbiologisk Laboratorium, Helsingør), and to Dr. E. Rasmussen (Vellerup Vig Laboratorium at the Isefjord), as well as to the two excursion leaders, Dr. T. Wolff and Dr. B. Muus. Also, we are grateful to the conveners of the sessions for the interest they each took in running their part of the meetings.

It is impossible here to acknowledge all the assistance we received from many sides, but it may be justified to extend our most deepfelt thanks to Mrs. A. Volsøe, Mrs. Lisbeth Wolff, Mr. Hans Madsen and DIS Congress Service.

We are especially indebted to Dr. J. B. Burch, the editors of MALACOLOGIA, and the Institute of Malacology, who relieved us of a difficult problem when they generously offered to publish this report in MALACOLOGIA.

HENNING LEMCHE
(President)

CONTENTS

	Page
Preface	iii
Introduction	1
Program	3
Excursions	5
Presidential address	7
Report on the General Assembly of Unitas Malacologica	
Europaea	11
Symposium on Malacology and Parasitology	
WRIGHT, C. A.: Intermediate host-parasite relationships in African schistosomiasis	15
VAN DER SCHALIE, H.: The role of snail intermediate hosts in culturing <i>Schistosoma japonicum</i>	17
VAN EEDEN, J. A.: Distributional trends of four species of freshwater snails in the Republic of South Africa with special reference to the intermediate hosts of <i>Bilharzia</i>	21
BERRIE, A. D.: Snail size in relation to infection with <i>Schistosoma</i>	23
BURCH, J. B.: Chromosomes of intermediate hosts of human Bilharziasis	25
TIEZE-DAGEVOS, J. W.: The effect of an experimental molluscicide on the eggs of <i>Australorbis glabratus</i>	29
HUBENDICK, BENG T.: Some aspects of vector snail control	31
MICHELSON, E. H.: The specificity of hemolymph antigens in the taxonomic discrimination of medically-important snails	33
FENCHEL, TOM: On the ciliated protozoa inhabiting the mantle cavity of Lamellibranchs	35
Second European Malacological Congress	
PATTERSON, C. M.: Chromosome numbers and systematics in streptoneuran snails	37
BURCH, J. B. & HUBER, J. M.: Polyploidy in mollusks	41
PRESCOTT, B. & LI, C. P.: Antimicrobial agents from sea food	45
BOER, H. H.: The effect of Amphenone "B" on the egg production of <i>Lymnaea stagnalis</i> L.	47
VAN DER STEEN, W. J.: Atmospheric air pressure and egg production of <i>Lymnaea stagnalis</i> , under laboratory conditions	49
RAVERA, O.: The effect of x-rays on demographic characteristics of a freshwater Gastropod, <i>Physa acuta</i> Drap.	51
HOLME, N. A.: Distribution of molluscs in the English Channel	53
NORTON, P. E. P.: Marine mollusca in the Lower Pleistocene of East Anglia	55
GROSSU, A. V.: Caucasian species in central Europe: the genus <i>Lytopenella</i> (Gastropoda, Limacidae) in Rumania, and its wide variation	57
ANT, H.: Die Bedeutung der Eiszeiten für die rezente Verbreitung der Europäischen Landgastropoden	61
GARGALLO DI CASTEL LENTINI, G.: Sur quelques formes de senestrisme des <i>Helix</i> Italiennes	63

CONTENTS (Continued)

STASEK, C. R.: Views on the comparative anatomy of the bivalved Mollusca67
PETITJEAN, M.: Structure microscopique et nature mineralogique de la coquille des principaux Muricidae69
RUNHAM, N. W. & ISARANKURA, K.: Studies on radula replacement73
COOK, H.: Structural details of the central nervous system of <i>Succinea putris</i> (L.)75
VAN DER SCHALIE, H.: Hermaphroditism among North American freshwater mussels77
FRETTER, V.: Some observations on neritids79
LAMMENS, J. J.: Observations on the ecology of <i>Macoma balthica</i>81
THOMPSON, T. E.: Development and life history of <i>Archidoris pseudoargus</i>83
LEVER, J.: Modes of sorting of shell valves on a sandy beach studied with artificial valves of <i>Donax vittatus</i>85
List of participants87
Index91

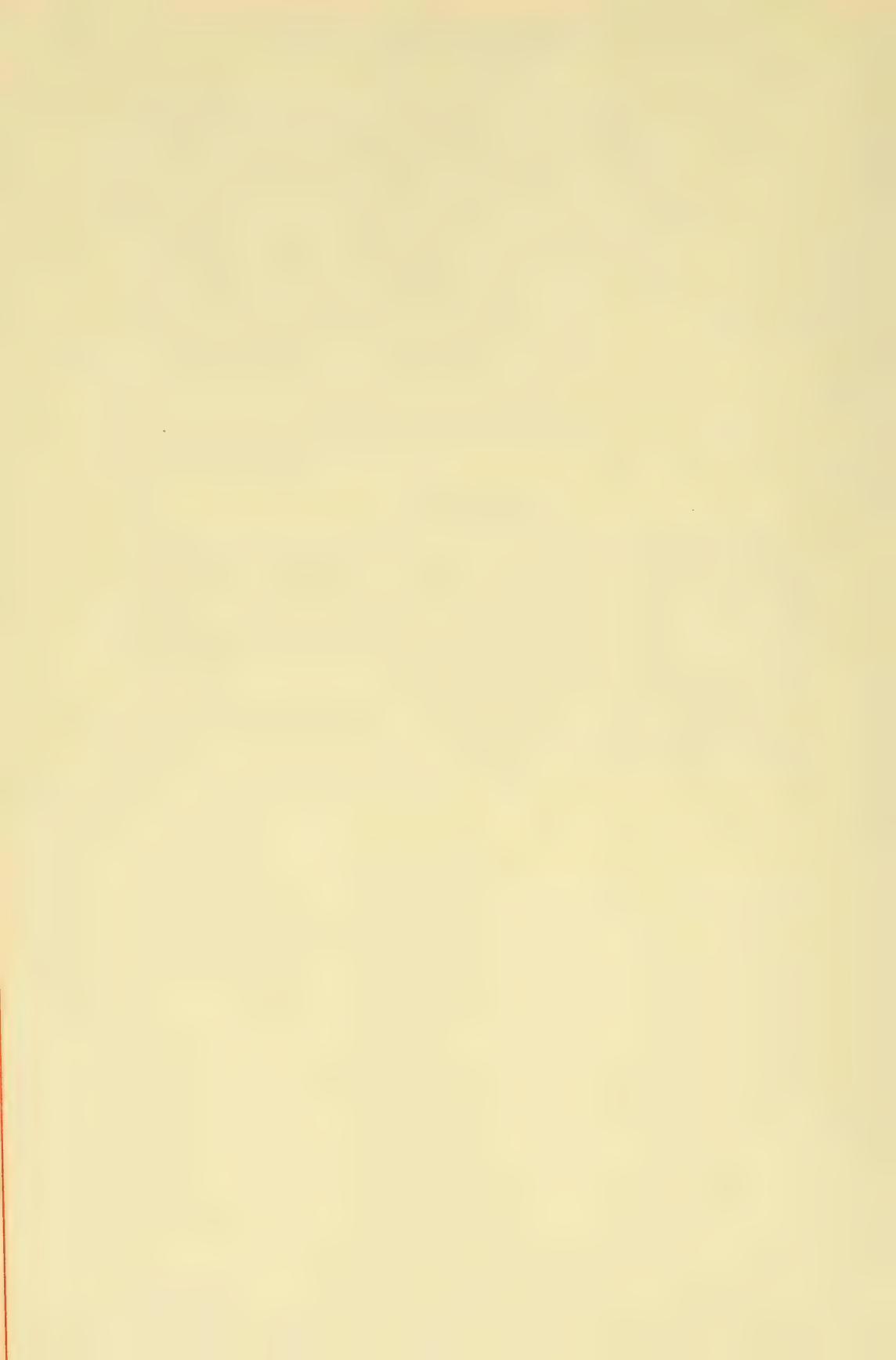
INTRODUCTION

The Second European Malacological Congress was held in Copenhagen, at the Zoological Museum of the University of Copenhagen, from August 12th to 14th, 1965. Prior to the Congress, on August 10th and 11th, a Symposium on Malacology and Parasitology was held, and after the Congress, on August 15th, an excursion to Møns Klint was undertaken.

In connexion with the Congress, the General Assembly of the *Unitas Malacologica Europea* was held on August 14th.

The Symposium was welcomed by Dr. H. Lemche, President of the U. M. E. and the Congress was welcomed by Dr. Helge Volsøe, who also demonstrated the new museum building.

On August 14th a dinner was held at the University.



PROGRAM

SYMPOSIUM ON MALACOLOGY AND PARASITOLOGY

August 10th. Convener: H. Lemche

- C. A. Wright: Intermediate host-parasite relationships in African schistosomiasis.
H. v. d. Schalie: Bionomic studies of the snail intermediate host of oriental schistosomiasis.
J. A. v. Eeden: Trends in the distribution of the intermediate hosts of *Bilharzia* in South Africa.

August 11th. Morning Session. Convener: B. Hubendick

- A. D. Berrie: Snail size in relation to infection with *Schistosoma*.
J. B. Burch: Chromosomes of intermediate hosts of human bilharziasis.
J. W. Tieze-Dagevos: The effect of an experimental molluscicide on the eggs of *Australorbis glabratus*.

August 11th. Afternoon session. Convener: O. E. Paget

- B. Hubendick: Aspects on vector snail control.
E. H. Michelson: The specificity of hemolymph antigens in the taxonomic discrimination of medically-important snails.
T. Fenchel: On the fauna of ciliated protozoans in the mantle cavity of molluscs.

SECOND EUROPEAN MALACOLOGICAL CONGRESS

Section on Ecological Physiology (August 13th). Convener: J. Lever

- C. M. Patterson: Chromosome numbers and systematics of streptoneuron snails.
J. B. Burch & J. M. Huber: Polyploidy in molluscs.
B. Prescott: Antimicrobial agents in molluscs.
H. H. Boer: The effect of Amphenone B upon the egg production of *Lymnaea stagnalis* L.
W. J. v. d. Steen: Atmospheric pressure and egg production of *Lymnaea stagnalis* under laboratory conditions.
O. Ravera: Effect of X-radiation in fresh water gastropods.

Section on Systematics and Zoogeography (August 13th). Convener:

- Z. Filatova
R. Turner: Consequences of a systematic revision of the Teredinidae. (Not published).
N. Holme: Distribution of Molluscs in the English Channel.
P. E. P. Norton: Marine Mollusca in the Lower Pleistocene of East Anglia.
H. Ant: Der Einfluss der Eiszeiten auf die recente Verbreitung der Landgastropoden Europas.

Section on Structural Physiology (August 14th). Convener: J. B. Burch

- C. R. Stasek: Form and symmetry in the bivalved Mollusca.
M. Petitjean: Structure microscopique et nature mineralogique de la coquille des principaux Muricidae europeens actuels et fossiles.

- N. Runham: The radula with reference particularly to electron microscope studies.
- H. Cook: Structural details of the central nervous system of *Succinea putris*.
- H. v. d. Schalie: Sexual differentiation in North American Naiades.

Section on Ecology (August 14th). Convener: A. D. Berrie

V. Fretter: Some observations on neritids.

- J. J. Lammens: Observations on the ecology of *Macoma balthica*.
- T. E. Thomson: Development and life history of *Archidoris pseudoargus*.
- J. Lever: Modes of sorting shell valves on a sandy beach studied with artificial valves of *Donax vittatus*.

EXCURSIONS

Danish Bilharziosis Laboratory and Danmarks Akvarium (August 10th)

Dr. A. Schiøtz, director of Danmarks Akvarium and Dr. G. Mandahl-Barth, Director of the recently established Danish Bilharziosis Laboratory showed their institutions to the approximately 75 participants in the excursion.

Frederiksdal (August 12th and 13th)

Departure by bus from the Zoological Museum to Lyngby and from there by motorboat across the lake Lyngby Sø to Frederiksdal, the classical working area of O. F. Müller, who was a private tutor to the Schulin family from 1753 to 1767. By special permission from the present owner of the mansion Frederiksdal, Count S. Schulin, the participants of the excursions were allowed to visit the park. After this visit the excursion proceeded to the surrounding forest, visiting the lakes Bagsværd sø, Hulsø and Furesø. Finally a motorboat took the excursion across Furesø to Holte. The total number of participants amounted to approximately 70.

Marinbiologisk Laboratorium, Helsingør (August 12th and 13th)

The participants of the excursions were welcomed by Professor G. Thorson. The laboratory and the research vessel "Ophelia" was demonstrated by Professor Thorson, Dr. K. Ockelmann and Dr. C. Nielsen. The staff of the laboratory had arranged an exhibition of about 40 species of living molluscs from the Øresund and of photographs of marine animals of the region. In addition, the collection of prosobranch egg capsules from many parts of the world as well as samples of the local marine meiofauna containing many juvenile molluscs was demonstrated. The total number of participants was approximately 70.

Isefjordslaboratoriet, Vellerup Vig (August 13th)

Dr. E. Rasmussen, the leader of the laboratory, gave an introductory lecture on the environmental conditions and the ecology of the marine invertebrates of the fjord area. An exhibition of about 100 species of invertebrates, including some 50 living species of molluscs, had been arranged. The research work of the laboratory was discussed. This excursion had 36 participants.

Møn (August 15th)

The excursion, which had 48 participants, left the Zoological Museum at 8 a.m. going directly to the east coast of the island of Møn, a cliff over 100 m high of Senonian chalk, which has been dislocated and folded during the glacial period. Some of the participants went to the beach (with good possibilities for collecting fossils), while others remained in the forest on top of the cliff collecting pulmonates. On returning to Copenhagen a short visit was paid to the church of Elmelunde (on Møn) richly decorated with medieval wall paintings (15th century).

PRESIDENTIAL ADDRESS

Ladies and Gentlemen!

To many of you, it may be of little surprise that I have chosen for today's speech the topic:

The Place of Mollusca among Invertebrates

Since *Neopilina* was recognized nine years ago, this animal has usually been taken as a support for the view that molluscs derive from metameric animals. However, I myself have gradually been driven towards the opposite conclusion.

It struck me first that the nephridial openings in molluscs are always placed at the bases of the ctenidia, in the same manner as the nephridia in arthropods open at the bases of some legs. Conditions in annelids are such that they fit into this same scheme. Hence it seemed that ctenidia and arthropod limbs had something to do with each other, and I began an attempt to find out what should be considered the most primitive structure of a ctenidium.

Yonge has made excellent comparisons of the functional relationships of the gills or ctenidia in the several classes of molluscs, but his prototype seems too elaborate to have been the ancestral one. Ctenidia fixed along the one side of their stem, e.g. the efferent one, cannot be closely related to those fixed along the opposite side. An intermediate stage with short ligaments seems necessary during the change-over, in order that the water current can flow and the ctenidia function during many generations, providing time enough for the change to take place. Hence, the Bivalvia and the Cephalopoda cannot derive their ctenidia along exactly the same lines as can the Gastropoda. However, the Polyplacophora and the Tryblidiacea possess ctenidia of an intermediary type with no supporting rods or other specializations, but still there are inhalant and exhalant water chambers, the later being placed between the perforated wall of the ctenidia and the foot. The shape and arrangement are so easily derived from each other in these two groups that I infer their water currents to be similar as well, even though these have never been observed in *Neopilina*. If, in *Neopilina*, the foot is reduced sufficiently to expose the ventralmost side of the pallial cavity, the basalmost and longest lamella in each ctenidium will become exposed to the surface of the substratum, and the movements possible from the ctenidial musculature will be able to effect locomotion of the whole animal. The said musculature is elaborate, consisting of two pairs of muscles from the dorsal shell to the interior of the gill, dividing up into each of the lamellae found there. Hence, as soon as they become exposed, the basalmost lamellae may be able to produce wavy movements of value for locomotion. We have no proof that this is the explanation of the formation of a telopodite, but it is at least a possible one.

It is a fact, however, that the leg muscles in trilobites arose from the dorsum in a place - marked by strong apodemes - which corresponds in position exactly with that of the insertions of the gill muscles in molluscs. Also, a comb-shaped gill arose from the lateral side of each limb base in trilobites, and its shape and position are such that by imagining an elongation of its stem and the addition of quite a number

of lamellae, it can easily be derived from ctenidia of the shape still seen in *Neopilina*. However, the respiratory currents in molluscs are brought about by ciliary action, whereas the trilobites - being arthropods - may be supposed to have had no cilia if we extrapolate from the lack of cilia in modern arthropods. But the gill lamellae in trilobites are known to have been somewhat flattened, with the broader sides facing each other, almost as in a common comb. These broader areas seem to have been covered by soft epithelium, and so the possibility is open that they were covered by dense cilia whose movements produced the necessary water currents, in a manner very much like that found in chitons today.

If arthropod limbs are to be derived from molluscan ctenidia, the fossil record would be expected to show a gradual decrease in importance of the gill part of the limb relative to the telopodite, and so it is in arthropods in general. Some Middle Cambrian (Burgess Shale) fossils described by **Walcott** have gill-limbs with no telepodite at all (*Leanchoilea*, *Opabinia*) and a short or almost non-existent head. Other fossils from these same strata have similar gill-limbs alone on the opisthosoma, but both telopodites and gills on the prosoma. (Compare the development with gradual displacement posteriorly of the different limb functions in recent Crustacea.) *Limulus* represents almost a similar stage even though the gills on the true walking legs have disappeared and slight rudiments of telopodites are present on the opisthosoma.

The blood circulation is identical in principle in *Neopilina* and in a scorpion, the venous blood in both cases collecting ventrally and thence flowing to the respiratory organs (ctenidia/book lungs), and from there dorsally and medially to the ventricle and aorta that carry the blood anteriorly. From the head, the blood runs more ventrally backwards to course the same route once more. Annelids have a similar arrangement which, however, does not seem to be intermediate between the two other groups.

A paired ventral nervous system is present in all three groups, and a rudiment of a visceral cord is found in some primitive polychaetes - and in *Limulus*. The position of the visceral cord in the latter is precisely the same relative to the dorso-ventral muscles as in *Neopilina*, but the source of innervation differs. The mouth, terminal in annelids, is ventral in both molluscs and in more primitive arthropods, and the two later groups have also the same antero-dorsally directed curvature of the oesophagus and crop, with a branched liver attached to the short stomach. These same conditions are known to have prevailed in the Middle Cambrian arthropods as well.

All these facts indicate an origin of arthropods directly from a molluscan ancestor, and hence we need an explanation of how metamerism came into existence. For orientation, let us remember that the gill muscles and the dorsoventral ones insert very close together on the interior side of the integument of the dorsum, as can easily be seen in *Neopilina*. This same animal also possesses a medial extension from the ring of insertions of the continuous pallial muscles for each of the other groups of dorsally inserting muscles. This arrangement is suggestively like that found in coelenterates if the dorsum is taken to be homologous to the aboral side in the latter group. Then, the extensions indicate obsolete septa. The Cambrian fossil *Cambridium* has a similar arrangement but with interesting differences. One of the species has a few strong elongate scars indicating septa-

like protrusions on the inside of the shell. In another species these septa are lower but more numerous, and many more secondary or tertiary ones are inserted between the primary ones and are especially distinct marginally. If only the primary scars survive, conditions will be almost as in the tryblidians.

Further, *Cambridium* shows a region at the one end (supposedly the anterior end) as having less scars. In Gotlandian Rugosa or Tetra-coralia, a similar pattern of septa is found, with two areas of growth. If only the hindmost of these is retained, conditions will be closely similar to those in the Ceriantharia, and in the arthropods and annelids. Of course, the Rugosa, being all too recent in appearance, cannot be the true ancestors of the arthropods, but they may show how metamerism can have arisen.

The Rugosa were high and horn-shaped. Most early molluscs had similarly high and slightly curved shells. Septal subdivision was not very evident on the outside of rugosan shells even though there may have been appendages, etc. developed around the oral area in a manner which revealed the septal arrangement. Similarly, molluscs may have ventrally placed appendages which, together with the scars on the inside of the shell, demonstrate septal arrangements. Even in trilobites, this same situation is found in the very youngest stages of some of the earliest representatives where no subdivisions of the larval dorsum are visible though segmentation appears gradually with increasing age. Hence, trilobite larval head development does not indicate any fusion of once separate segments.

Neopilina also provides us with a clue to the problem of how many primary segments have been involved. The eight dorsal insertions of the dorso-ventral muscles form two groups, the foremost three being inserted higher up in the shell than the remaining five. Chitons have that same number, but in other molluscs there are only the first three pairs and a single or paired fourth one instead of the five posterior ones. In their early larvae, *Haliotis* and other primitive prosobranchs show six muscle cells placed obliquely over the alimentary canal. With their first contraction (at about 30 hours larval age), they twist the ventral part of the animal relative to the visceral part so that a torsion of 90° is produced. Then, the six cells form three pairs which develop into the three larval pairs of retractor muscles, one preoral and two perioral in their ventral insertions. A stronger, unpaired postoral muscle representing the fourth pair develops later. In prosobranchs the larval pairs disappear, thereby permitting torsion to continue around the fourth one up to 180° . In opisthobranchs, all four pairs survive as long as the shell does, preventing further torsion. An apparent detorsion is produced by differential growth of the different parts. In bivalves, conditions appear primitive in the prosobranchs (*Nucula*) where four retractor pairs are present, and in the fossil *Babinka* from the Ordovician. Apparently scaphopods fall into a similar pattern. In cephalopods, indications of the three larval pairs are extremely scarce, but I have found them in one Jurassic genus.

Turning to the crustacean larva, the nauplius, and remembering that the dorso-ventral muscles and the limb/gill muscle insert together, we find muscles to the distinctly preoral first antennae, and two perioral pairs - to the second antennae and the mandibles - at a stage where

only a posterior bud indicates the place of the body proper with all its postoral muscles, etc. The bryozoan cyphonautes larva has the same four pairs of muscles, exactly at the stage preceding that where it settles on the substratum to form a short-lived molluscan stage before the interior tissues disintegrate prior to the growth of a stolonical bud which develops into the ancestrula.

This all shows us that there was at first a primitive tetracyclomeric type of organism. Of the four segments around the central axis, the one called the D-quadrant enlarged enormously, and it is the subdivision of this quadrant that constitutes true metamerism. These ideas take us back to the Coelenterata, which means that the molluscs must be derived almost directly from something like that group, and that true metamerism came into existence only in a very few members of the molluscan phylum. From molluscan-like ancestors, arthropods and annelids have evolved independently. That molluscs do not originate from annelids is demonstrated very well in the larva of *Lopadorhynchus*, which at a stage where it has three pairs of appendages has also a clear cut, longitudinally cleft foot and even a foot gland. Moreover, a hump on the dorsum indicates the place where the shell should be expected to be present.

Hence, I propose molluscs as rather a primitive group of invertebrates representing a stage in evolution rather than a branch, to be placed immediately above the coelenterate level. The consistency of the number four in the cyclomeric arrangement is to be expected if mechanical laws have governed the first cleavages of the first metazoans, but it excludes the derivation of the Metazoa from the Acoela.

On the other hand, molluscs do not seem to form a very close group. The Cephalopoda seem to have originated from coelenterates almost on their own, as indicated both in their embryology and in the evolution of their shell, and both the Mono- and the Polyplacophora have evolved towards metamerism much more than the remainder. Bivalves, scaphopods, and gastropods have retained original tetra-cyclomeric symmetry with an enormous growth of the D-quadrant - as in cephalopods. But it does not seem possible to derive bivalves from gastropods or vice versa.

HENNING LEMCHE

REPORT ON THE GENERAL ASSEMBLY OF
UNITAS MALACOLOGICA EUROPAEA

by the Secretary, Dr. A. ZILCH

The 1965 meeting of the General Assembly of Unitas Malacologica Europaea took place on Saturday, August 14, at 1:00 p.m. in the Zoological Museum of Copenhagen University. Dr. Lemche, the President of Unitas, welcomed the members and asked Mr. Crawford to preside over the assembly again since he had proved to be an excellent chairman at the two sessions of the Congress in London, during which the formation of Unitas was discussed.

The assembly followed the order of the agenda which had been mailed to all members on May 7, 1965, in accordance with paragraph 8 of the Rules of the Unitas.

1. Confirmation of new members.

The new members of Unitas as listed in appendix 1 and 2 of the agenda were confirmed.

2. Report by the President of Unitas' work.

Dr. Lemche gave a short review on the development of Unitas during the first three years of its existence. Until August 1965, 120 members had joined Unitas, as listed below:

Ordinary members (personal 92, collective 9)101
Corresponding members (all personal) 19

At the final meeting of the First European Malacological Congress on September 21, 1962, 52 persons had signed the membership list. One of them, Dr. Herbert Kaltenbach, died in the meantime. Altogether 83 members of the First European Malacological Congress joined Unitas.

Only 37 persons later joined Unitas as a result of our invitation form which was inserted in all European malacological periodicals and sent to private addresses.

The 120 members came from 27 countries:

a) Ordinary members in 19 countries:

Austria (2), Belgium (1), Denmark (7), Egypt (2), France (11), Germany (13), Great Britain (26), Hungary (2), Israel (1), Italy (8), Netherlands (8), Norway (2), Poland (1), Portugal (1), Rumania (2), Sweden (4), Switzerland (7), Turkey (1), Yugoslavia (2).

b) Corresponding members in 8 countries:

South Africa (1), South Australia (1), Canada (1), Curaçao (1), Ghana (1), Hawaii (1), New Zealand (1), U. S. A. (12).

3. Presentation of statement of accounts by the Treasurer.

The Treasurer, Dr. Forcart, presented the following statement of accounts (in Swiss Francs) for the period from October 1, 1962, to June 30, 1965. The statement had been approved by the auditors Mr. Kuiper and Dr. Paget.

	S. Fr.
Income	3,279.30
Expenditure	1,052.65
Account Schweizerischer Bankverein	2,226.65

4. Postal ballot on new councillors.

As laid down in paragraph 11 of the Rules of Unitas as approved on September 21, 1962, any ordinary personal member may be nominated for the Council, either by the Council or by any group of five ordinary personal members, at any time up to three months before a General Assembly. The Secretary had received only one proposal, signed by seven ordinary members on May 1, 1965, for the election of the Council for the period from 1965 to 1968. According to paragraphs 10 and 11 of the Rules this proposal had been mailed as a ballot to all 92 ordinary members on June 3, 1965. 52 ordinary members voted with the following result:

President

- a) Professor, Dr. M. de Larambergue, France33
- b) Dr. O. Paget, Austria19

Vice President

- a) Dr. O. Paget, Austria20
- b) Dr. C. O. van Regteren Altena, Netherlands32

Secretary

- Dr. A. Zilch, Germany52

Treasurer

- Dr. L. Forcart, Switzerland52

Member of Council

- a) J. F. Peake, B.Sc., England21
- b) Dr. C. O. van Regteren Altena, Netherlands11
- c) Dr. B. Hubendick, Sweden31

In a letter of July 27, 1965, addressed to the President and the Secretary, Professor Larambergue had declared that he would be unable to accept the position of President even if he received a majority of votes. Thus the following office holders were elected members of Council:

- President: Dr. Oliver Paget (Vienna, Austria)
- Vice President: Dr. C. O. van Regteren Altena (Leiden, Netherlands)
- Secretary: Dr. Adolf Zilch (Frankfurt, Germany)
- Treasurer: Dr. Lothar Forcart (Basle, Switzerland)
- Member of Council: Dr. Bengt Hubendick (Gothenburg, Sweden)

5. Election of auditors.

The following members were appointed auditors for the period from 1965 to 1968: S. P. Dance (London), J. G. J. Kuiper (Paris).

6. Subscription fees for the next period.

The annual subscription rates of 10.00 Swiss Francs for ordinary members and 5.00 Swiss Francs for corresponding members were not altered.

7. Fixing of year and place of the next Congress.

The President elect, Dr. Paget, informed the members of an invitation from the Austrian Ministry of Education to hold the Third European Malacological Congress in Vienna in 1968. The invitation was accepted.

8. Revision of rules.

All the proposals for alteration of the rules as listed in appendix 3 of

the agenda were approved by the General Assembly except for the first sentence of number 8: "As in the past, the annual subscriptions cover the period from October 1 each year to September 30 of the following year".

9. Report from the Congress committee on common research projects.

At the opening session of the Congress on August 12, Dr. Lemche had asked Dr. Paget to form a committee (which he did, with Dr. Ant and Mr. Waldén) to discuss common research projects and make proposals to the General Assembly. This committee was not able to give any definite proposals because the limited time did not allow them to decide on such a difficult complex of questions. Then various suggestions by members were discussed but without achieving a result. The question of common research projects has been the subject of further considerations since. At present Dr. Paget is preparing a circular which soon will be distributed to all members of Unitas.

10. Any other business.

Mainly one question was intensively discussed: The printing of the Proceedings of this Second European Malacological Congress.

A. ZILCH
Secretary

INTERMEDIATE HOST-PARASITE RELATIONSHIPS IN
AFRICAN SCHISTOSOMIASIS

C. A. WRIGHT

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(Read at the morning session, 10 August, 1965)

ABSTRACT

Medical malacologists must attempt to take a worm's-eye view of the Mollusca. This contribution set out a few recent ideas and the results of some current work in the field of intermediate host-parasite relationships of the schistosome parasites of man in Africa.

The snail hosts of *Schistosoma mansoni* in both the Ethiopian and Neotropical regions belong to the planorbid genus *Biomphalaria* whose closest affinities are probably with the Palaearctic *Planorbis*. The hosts of *S. haematobium* are species of *Bulinus*, anatomically related to *Indoplanorbis* but anatomically and serologically distinct from the Australasian *Isidorella* which, despite its superficial similarity to *Bulinus*, is probably closer to *Physastra*.

The occurrence of fossil shells of the *Bulinus africanus* species-group in deposits of Pleistocene or late Pliocene age in Katanga (Leriche, 1925) suggests that the present-day species groups were well differentiated when the early hominids appeared during the Pleistocene era in East Africa. The fossil evidence suggests that the early Australopithecines were often closely associated with water (Cole, 1964) and thus these rapidly-evolving primates presented excellent potential host-species for the various mammalian schistosome parasites which were almost certainly already in existence. Subsequent parallel evolution of the hominids with their schistosomes has resulted in the exceptionally host-specific parasites which we know to-day.

Throughout the Digenea the larval stages are tissues parasites of their molluscan hosts and the relationship between host and parasite is very close (Wright, 1965). Characteristic behaviour patterns of the miracidia help to ensure contact between the larvae and their hosts (Wright, 1965b). The selectively "specific" stage in the relationship follows penetration of the miracidia into the snails. If the host is not a suitable one, a rapid tissue reaction destroys the invading larva. A form of hypersensitivity may also occur in which snails which are normally susceptible to a certain strain of parasite may be killed by larvae of another strain. Both of these forms of immunity may be incomplete, and in these cases it is possible that selection will result in the establishment of a new strain of parasite compatible with that particular strain of host. Susceptibility to infection is not, therefore, a simple matter determined solely by the characteristics of the snails; it can only be defined in terms of a parasite strain, and the over-all relationship must be thought of as *compatibility* between populations of both snails and parasites.

The present distribution of the bulinid snails and their associated urinary

schistosomes was illustrated and discussed, particular attention being given to the *B. forskalii* complex because the higher level of specific differentiation in this group (probably due to its greater antiquity) provides a background for interpreting the situation in the *B. africanus* and *B. truncatus* complexes. Finally some results of chromatographic analysis of body surface mucus (Wright, 1964) and electrophoretic separation of egg-proteins of snails (Wright & Ross, 1965) were shown and their uses in discriminating between morphologically similar populations and in grouping species complexes were discussed.

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THE ROLE OF SNAIL INTERMEDIATE HOSTS IN CULTURING
*SCHISTOSOMA JAPONICUM*¹

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(Read at the morning session, 10 August, 1965)

ABSTRACT

Few laboratories maintain the cycle of *Schistosoma japonicum*. As is generally known, most of the basic research involving human schistosome cycles has been undertaken with pulmonates such as *Australorbis*, now called *Biomphalaria*, which serve as hosts for *S. mansoni*. Consequently, studies of Oriental schistosomiasis are relatively uncommon in part because it has been difficult and painstaking to culture the amphibious *Oncomelania*. Anyone who has worked with the *S. japonicum* cycles realizes that there is no comparison between the relative ease with which the planorbid snails can be cultured and maintained and the problems one faces with *Oncomelania* needed for studies of Oriental blood fluke. Just the differences in susceptibility of the two groups, the greater time needed for development of the parasite in *Oncomelania*, the difficulty getting a good shed of cercariae, the need for picking the cercariae from the surface rather than using intra-peritoneal methods with cercariae as is often done with *Biomphalaria*, etc.—all are factors that tend to make the *S. mansoni* cycle the one most commonly used; the need for studies of *Oncomelania* is obvious.

To solve some of the problems relating to the biology of *Oncomelania* and its maintenance in laboratory culture it was possible to work intensively with two species of *Pomatiopsis* living in the vicinity of Ann Arbor, Michigan. It was established that *Pomatiopsis* and *Oncomelania* are similar in the following ways: (1) *ecologically* all are amphibious snails living in nature under essentially similar conditions; (2) in *life history* both groups produce single eggs, laid on wet mud or loam (or coconut husks) and covered with a jacket of slime and mud; (3) in many ways they are similar in *gross morphology*; (4) both groups are "*microphagous herbivores*" and have a style-feeding mechanism with a pellet compressor producing similar fecal pellets; (5) their *growth and development* in laboratory culture are in many ways the same; and (6) in both groups the *mode of progression* is a looping gait. These similarities among the snails in the genera *Pomatiopsis* and *Oncomelania* and their bearing in a series of studies designed to understand better their relationships are shown in the following investigations.

In distribution and natural history the field studies (van der Schalie & Dundee, 1955; Dundee, 1957; van der Schalie & Getz, 1962a) undertaken all tended to emphasize that the conditions observed for *Pomatiopsis* were not very different from those observed in the field where *Oncomelania* are

¹This work was carried out under the sponsorship of the Commission on Parasitic Diseases, Armed Forces Epidemiological Board, and was supported by the Office of The Surgeon General, Department of the Army.

found. Pictures taken both in the region of Totoni (Yamanashi Prefecture), Japan, and in Palo, Leyte, Philippines, indicate that the general habitat conditions are very similar for both groups. The basic pattern as it relates to these animals in nature indicates that the conditions established for them in the laboratory can also be similar in many respects for both groups.

Several studies have been undertaken on the morphology (van der Schalie & Dundee, 1956; Dundee, 1957; van der Schalie & Getz, 1962b; and Davis, 1966) of both the *Pomatiopsis* and *Oncomelania*. While the systems are basically similar, problems remain since the names of organs cannot, as yet, relate some organs to their function. For example, the term "pallial oviduct" or "accessory gland" has been used interchangeably. While some attempt has been made to relate genital structures to their function, it is still not definite how each of the organs in the female tract relates to the egg-laying process. Also, the functional relations of the digestive tract needs more careful study.

Both in the field and in the laboratory these groups are conditioned to terrestrial existence while actually they are restricted to the conditions that tend to limit gill-breathing operculates. Since moisture is very vital, studies (van der Schalie & Getz, 1961) have been made that indicate that in *Pomatiopsis cincinnatiensis* the young require more moisture than adults in such a way that in a moisture gradient newly hatched young selected 90 to 100 percent saturation; the adults selected 30 to 60 percent. When temperature and moisture responses were studied (van der Schalie & Getz, 1963) there were several basic responses which these groups shared in common. In one case, a temperature response in the field clearly relates to the survival of *P. cincinnatiensis* since if it could not tolerate a lower range of temperature in the fall of the year it would not reach the top of the bank where it spends the winter.

It was not possible to study the life history of the *Oncomelania* in the field, but studies of the life history of *Pomatiopsis cincinnatiensis* (van der Schalie & Walter, 1957) clearly indicated that this animal is an annual with the eggs appearing in certain well delineated zones along the bank of the river; they usually hatch early in August. In their restricted range this species could serve well in field tests with molluscicides. In *P. lapidaria* (van der Schalie & Dundee, 1959) the life cycle was found to be more like *Oncomelania* in that it is biennial as determined by 869 soil samples in two transects in a typical habitat throughout the active seasons of the year. The peak of egg production was mid-June; tests of several substrates to determine which were best for egg-laying were inconclusive. While both *P. cincinnatiensis* and *P. lapidaria* show basic similarities ecologically to *Oncomelania*, these species remain ecologically isolated (van der Schalie & Getz, 1962) even in the occasional sites where their ranges and habitats overlap.

Information obtained from the field and laboratory studies of *Pomatiopsis* together with the laboratory investigations of *Oncomelania*, made possible the development of laboratory culture techniques which enable both a highly predictable rate of production of young, as well as a rapid rate of growth and development of all four *Oncomelania* (van der Schalie & Davis, 1966); it was also possible to show that overcrowding not only produced stunting among these amphibious snails but also suppressed their sexual development (van der Schalie & Davis, 1965). The possible causes for this stunting are at present under investigation.

In addition to these studies which deal with the basic biology of this Hydrobiid group, the availability of these snails made possible several additional investigations. Basch (1959) determined that both *Pomatiopsis lapidaria* and *P. cincinnatiensis*, as well as *Oncomelania nosophora*, can serve as hosts for *Paragonimus kellicotti*. Getz (1962) indicated that the habitats of the *Pomatiopsis* snails in relation to the mammals and rodents that might conceivably serve as hosts in nature are such as to preclude any danger that cercariae would reach suitable hosts. Davis (1962) showed that *P. cincinnatiensis* can serve as a theoretical model for measuring secondary productivity; Davis (1963; 1964) also studied regeneration and shell formation in *O. formosana*. Burch (1964) studied the cytotaxonomy of *Oncomelania* and concluded that the four nominal species were four ecological races or subspecies, at best. With respect to speciation, Davis and Lindsay are making an intensive study of species relations among *Oncomelania* and related genera, using disc electrophoresis and immunobiological techniques.

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DISTRIBUTIONAL TRENDS OF FOUR SPECIES OF FRESHWATER
SNAILS IN THE REPUBLIC OF SOUTH AFRICA WITH SPECIAL
REFERENCE TO THE INTERMEDIATE HOSTS OF *BILHARZIA*

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(Read at the morning session, 10 August, 1965)

ABSTRACT

In this country, as elsewhere on the continent, the geographic distribution of the snail intermediate hosts of *Bilharzia* exceeds that of the disease. It can neither be established, however, whether these snails have already colonised all the areas potentially capable of supporting them nor can the suitability for colonisation of any new area at present be assessed by the application of any known criteria.

The known geographic distribution of *Biomphalaria*, *Bulinus* (*Physopsis*), *Lymnaea natalensis* and *Bulinus* (*Bulinus*) *tropicus* suggests that *B. tropicus* might be the first to colonise any new area, followed successively by *L. natalensis*, *Physopsis* and *Biomphalaria*. This conclusion seems to be supported by the differential tolerance of these species to certain unknown factors associated with increasing altitude and water pollution. The association between *Physopsis* and *L. natalensis* is statistically so significant that these two snail forms may be regarded as having very similar biological requirements, and for this reason the relative abundance or otherwise of the latter species in a given area may be taken as an index of the suitability of that area for colonisation by *Physopsis*.

SNAIL SIZE IN RELATION TO INFECTION WITH *SCHISTOSOMA*

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(Read at the morning session, 11 August, 1965)

ABSTRACT

In the Mtwara Region of Tanzania three potential intermediate host snails of *Schistosoma haematobium* have been found. Two of these, *Bulinus (Physopsis) globosus* (Morelet) and *Bulinus (Physopsis) nasutus nasutus* (Martens), occur together in certain seasonal pools and form interbreeding populations exhibiting a wide range of intermediate characters. Regular observations were made on seven such hybrid populations during which 5170 snails were examined and 404 of them were found to have mature infections of *S. haematobium*. Few snails under 7 mm shell height had mature infections and the percentage infection was highest among the largest size groups.

The study extended over two years and the data obtained from each habitat in each year were compared. This showed that more infections were always found in the year in which the mean size of all specimens examined was the greater. Further analysis showed that there was a strong positive correlation between the infection rate found in each habitat in each year and the proportion of the snails over 10 mm shell height which were examined from that habitat in that year.

CHROMOSOMES OF INTERMEDIATE HOSTS OF HUMAN BILHARZIASIS^{1,2}

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(Read at the morning session, 11 August, 1965)

ABSTRACT

The basic haploid chromosome number of the genus *Bulinus* is 18 (Burch, 1960b, 1964a; Natarajan, Burch & Gismann, 1965). Eighteen pairs of chromosomes have been found in *B. beccarii*, *B. forskalii*, *B. globosus*, *B. guernei*, *B. jousseamei*, *B. reticulatus*, *B. senegalensis*, *B. tropicus tropicus*, *B. t. angolensis* and *B. t. zanzebaricus*. Chromosome numbers resulting from aneuploidy were found in *B. forskalii* (n=19) from Angola, and *B. natalensis* from Rhodesia (meiotic cells had 18, 19, 20 and 21 chromosomal elements). Tetraploidy (n=36) occurs in the "Truncatus species group" of the subgenus *Bulinus* s.s.: *B. coulboisi*, *B. truncatus truncatus*, *B. t. rohlfisii* and *B. t. sericinus*. Hexaploidy (n=54) and octaploidy (n=72) occur in various *Bulinus* populations in Ethiopia, a region where tetraploidy and normal diploidy also occur.

The occurrence of populations with differing polyploid chromosome numbers in the Ethiopian highlands, where supposedly only *Bulinus truncatus sericinus* occurs (Mandahl-Barth, 1965), raises a question concerning both their nomenclature and the systematics of the genus in that and similar geographical areas. Individuals with differing polyploid numbers are undoubtedly reproductively isolated from each other. Therefore, from a biological point of view, they are distinct species. Most likely they also exhibit morphological differences.

Information currently at hand suggests that the "Truncatus" and the "Tropicus species groups" can be distinguished by their chromosome numbers. The former group is apparently infective, the latter refractive, to infection with *Schistosoma haematobium*. In critical geographical areas where the distribution of these two groups overlap, chromosome number determinations should prove helpful to workers trying to identify the species. Chromosome number determinations may also help in discerning which field populations are capable of transmitting bilharziasis.

All species in the genus *Biomphalaria* that have been studied to date, *B.*

¹Contribution No. 15, Intermediate Hosts of Schistosomiasis Program, Institute of Malacology.

²Grateful acknowledgement is made to the U. S. National Science Foundation for funds (grant GB-3786) to attend the Second European Malacological Congress for the presentation of this paper. The researches on which much of this paper is based were supported (in part) by a Public Health Service research career program award (number 1-K3-AI-19, 451) and by research grants from the National Institute of Allergy and Infectious Diseases, U. S. Public Health Service (grant 2 T1 AI 41) and the U. S. National Science Foundation (grant GB-787).

alexandrina alexandrina, *B. glabrata*, *B. pfeifferi pfeifferi*, *B. p. gaudi*, *B. p. madagascariensis* and *B. sudanica tanganyicensis*, have 18 pairs of chromosomes. Chromosome numbers, therefore, have not yet been helpful in systematics, although they do indicate a strong conservativeness in chromosome numbers that is characteristic of gastropods in general.

The group of snails transmitting schistosomiasis japonica was divided into three genera and nineteen species by Bartsch (1936, 1939, 1946). Abbott (1948) and Kuo & Mao (1957) considered the group to contain only one genus with four species: *Oncomelania formosana* (Taiwan), *O. hupensis* (China mainland), *O. nosophora* (Japan), and *O. quadrasi* (Philippines). Burch (1960a, 1964b) found that the latter four species had 17 pairs of chromosomes, and that the pairing behavior of their chromosomes at meiosis in F_1 hybrids was normal. Only normal bivalents with one, two or three chiasmata were observed, and no univalents, trivalents, or quadrivalents were found. In addition, all segments of each chromosome seemed to pair completely. When this cytological information is coupled with the great morphological similarity found between the four species, the ease of their hybridization, and the nonreduced viability of the hybrids, then the four so-called "species" of *Oncomelania* should be interpreted as no more than geographical populations or races of the same species. On shell shape, *O. nosophora*, *O. formosana* and *O. quadrasi* form a north to south stepcline, the Japanese species being relatively long and slender, the Philippine species being relatively shorter and broader, and the Taiwan species falling between the two extremes. If the populations of the four main geographical regions of their distribution are treated as races, then the trinomial nomenclature would be: *Oncomelania hupensis hupensis* Gredler 1881, *O. hupensis quadrasi* Möellendorff 1895, *O. hupensis formosana* Pilsbry & Hirase 1906, and *O. hupensis nosophora* Robson 1915.

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A more detailed account of these studies will be published in a later issue of *MALACOLOGIA*.

THE EFFECT OF AN EXPERIMENTAL MOLLUSCICIDE ON
THE EGGS OF *AUSTRALORBIS GLABRATUS*

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(Read at the morning session, 11 August, 1965)

ABSTRACT

An attempt has been made to determine the reason(s) for the lack of ovicidal activity of N-trityl-morpholine (N. T. M.) which is lethal to the intermediate host snails of schistosomiasis in minute dosages.

An indication that lack of penetration is the governing factor was obtained by means of micropuncture.

In a series of experiments a successive removal of the various barriers around the egg (capsule membrane, jelly mass, egg membranes) was attempted.

Experiments with completely sealed egg masses of various stages exposed to N. T. M. of 5-50 p.p.m. for 24 hours, indicated that at least older eggs are not penetrated by this chemical until time of hatching. From these experiments it can also be concluded that the soft jelly mass, the immediate cover of the eggs, acts as the most important accumulator of molluscicide so that long after the batches have been removed from the poisoned medium the young snails die as soon as they hatch, when they start feeding on the jelly. This effect is the same whether the capsule membrane is removed after exposure or not.

Isolated eggs without jelly which were exposed to 5 or 10 p.p.m. of N. T. M. for 24 hours gave unaffected young snails which hatched and behaved normally.

The effect of N. T. M. on embryos exposed by micropuncture is rather peculiar when exposure takes place at the "trochopore" stage. A high percentage (80% at exposure to 5 p.p.m. for 24 hours) stays behind in growth, eventually developing into "dwarf" snails which never hatch and die inside the egg after normal hatching-time.

Isolation of these eggs and culturing under axenic conditions has been attempted and is in progress. Nothing can be said with certainty about the reasons for the difference in dimension between these "dwarf" snails and normal, fully mature embryos.

SOME ASPECTS OF VECTOR SNAIL CONTROL

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ABSTRACT

At present, the only practical way of counteracting the spread of snail-transmitted diseases is by suppressing the vector snail populations. Efforts to this effect have, so far, been focused mainly on mollusciciding. However, it should be remembered that mollusciciding represents only one of three main possibilities in snail-control work, the other two being ecological and biological control, respectively. In ecological control, the environment is changed and made unsuitable for the snails; in biological control, natural competition or predation of any kind is utilized. Mollusciciding is still expensive, has only a temporary effect and may have unfavourable side effects. Ecological control may be permanent but requires high initial costs. Biological control, if the right means are found, may be inexpensive and permanent.

In Puerto Rico, the ampullariid snail *Marisa cornuarietis* (L.) has completely suppressed many populations of the schistosome vector generally called *Australorbis glabratus* (Say). A research project¹ carrying out basic research on *Marisa*, comprising basic and functional morphology, population dynamics, competition activities, etc., is now being carried out in Gothenburg and Cairo. Dr. Emile S. Demian of Ain Shams University in Cairo, the co-principal investigator of the project, is responsible for running the competition and predation experiments. Together with Dr. Ramsis G. Lutfy he has studied the predation of *Marisa* on *Bulinus truncatus* (Audouin), a transmitter of urinary bilharziasis; *Biomphalaria alexandrina* (Ehrenberg), a transmitter of intestinal bilharziasis; and *Lymnaea caillaudi* Bourg., a transmitter of *Fasciola gigantica* (Cobbold).

Laboratory studies clearly show that *Marisa* is an efficient predator on *Bulinus*. The methods of predation are threefold: adult *Marisa* were observed to attack and devour adult *Bulinus* of various sizes; adult and young *Marisa* devoured newly-hatched *Bulinus*; and newly-hatched and young *Marisa* destroyed egg-masses of *Bulinus* and consumed the eggs.

Adult and juvenile *Marisa* devour newly-hatched *Biomphalaria*, newly-hatched and juvenile *Marisa* destroy and consume egg-masses of *Biomphalaria*. However, large and small-sized *Marisa* fail to kill and devour adult *Biomphalaria*. This failure is not due to any specific distaste but to mechanical obstacles provided by the *Biomphalaria* shell with its long whorl and narrow aperture.

The relation between *Marisa* and *Lymnaea* is similar to that between

¹The project is supported by a research grant (AI 04906) from the National Institute of Allergy and Infectious Diseases, U. S. Public Health Service.

Marisa and *Bulinus*.

Demian and Ramsis have also studied the ecology of the above-mentioned predation. They found that the rate at which adult individuals of *Marisa* predated on adult and juvenile *Bulinus* increased with temperature, reached its highest value between 26° and 30°C, and decreased again at still higher temperature.

The predatory activity normally decreases in presence of abundant vegetable material edible to the predator. However, there is an indication that adult *Marisa*, while practising predation on *Bulinus*, develops a certain progressive predatory habit, and that with development of such habitual predation its attack on *Bulinus* becomes less affected by the presence of abundant vegetable diet.

The rate of predation is more or less directly proportional to the ratio of predators to prey, and is far less affected by variations in the water volume, i.e., by differences in the population densities of the two competing snails relative to the water volume.

There is ample proof that the predation of *Marisa* of all ages on the egg-masses and newly-hatched individuals of *Bulinus* does not take place only accidentally while the predators are browsing and feeding on aquatic plants, but also deliberately. Nor is this kind of predation so much influenced by the water volume as it is by the absolute numbers of predators and prey.

The results concerning the relations between *Marisa* on the one hand and *Biomphalaria* and *Lymnaea* on the other are similar to those reported here regarding the ecology of predation on *Bulinus*.

As a result of the *Marisa* project the following papers have been published. Others are in press or under preparation.

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THE SPECIFICITY OF HEMOLYMPH ANTIGENS IN THE TAXONOMIC
DISCRIMINATION OF MEDICALLY-IMPORTANT SNAILS

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(Read at the afternoon session, 11 August, 1965)

ABSTRACT

The study was initiated to determine the immunologic specificity of snail hemolymph antigens and to evaluate their use in serotaxonomic studies. Hemolymph antigens were obtained from snails of the families Planorbidae and Lymnaeidae. The interfacial ring test (IRT) and a gel-diffusion system were employed to compare the capacity of the various antigens in producing precipitins against specific snail antisera. Antigenic differences between genera were demonstrable in the IRT, but the specificity of the precipitin reactions was dependent upon absorption from the antisera of familial cross-reacting antibodies. Congeneric species or strains of a species could not be differentiated by the IRT. Gel-diffusion studies confirmed the results of the IRT with respect to generic differences; in addition, with some antisera this technique proved sensitive enough to demonstrate antigenic differences between congeneric species. However, strains of a species could not be distinguished in this manner.

Snail hemolymphs could be impregnated into filter paper discs, eluted in 0.85% saline up to 9 days later, and still retain their antigenicity. In one experiment, hemolymph-impregnated discs were prepared from African snails in Tanzania, air mailed to Boston, and successfully identified 6 days later by means of immunologic techniques.

The results obtained from this study indicate that, in spite of certain limitations, serologic techniques may serve as valuable adjuncts to the methods now employed by the molluscan taxonomist. For example, serologic evidence suggests that species assigned to the anominal genera *Australorbis*, *Tropicorbis*, and *Biomphalaria* are congeneric—a concept recently set forth by several malacologists on the basis of anatomical studies. Furthermore, there is serologic evidence which suggests that snails now assigned to the genus *Lymnaea* may, in fact, belong to more than one genus.

A detailed account of this study will be published in "The Journal of Parasitology".

ON THE CILIATED PROTOZOA INHABITING THE MANTLE
CAVITY OF LAMELLIBRANCHS

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(Read at the afternoon session, 11 August, 1965)

ABSTRACT

It has long been known that the mantle cavity of bivalves harbours a rich fauna of ciliates (see References and papers cited therein). In the work reviewed here (see Fenchel (1965) for a full report), 57 species of Scandinavian bivalves were investigated and found to harbour a total of 42 morphologically defined species of ciliates, of which 14 were new to science.

Systematically, these ciliates (with two exceptions) belong to the holotrich order Thigmotrichida and the peritrich suborder Mobilina. The thigmotrichs are all parasites or commensals; the greatest number of species live in molluscs, and the order can be shown to have evolved mainly within this host group. The Peritrichida Mobilina, on the other hand, are found in or on a great variety of aquatic hosts.

All ciliates from bivalves can be classified as belonging to one of three types adapted to live in the mantle cavity of the host: 1) Filter-feeders utilizing the water currents produced by the host, 2) Particle-feeders feeding on particles and/or mucus lying on the gills and mantle wall and 3) Parasites feeding on the epithelial cells of the gills. The peritrich representatives all belong to the first group, whereas the thigmotrichs are represented in all three groups.

One species of lamellibranch may harbour several species of ciliates and even several representatives of the same genus. In such cases statistical analysis shows that the infection frequencies and population sizes of each species are independent of one another. This aspect was studied in detail in *Mytilus edulis*. This mussel harbours two filter-feeding ciliates, viz. *Ancistrum mytili* and *Peniculistoma mytili*. It could be shown that the two species occupy two separate ecological niches in the host. The former species feeds on smaller particles than the latter and is exclusively found on the gills whereas the latter predominantly is found on the labial palps of the host.

The infection of the host lamellibranchs takes place after metamorphosis by ciliates which for some reason have left their original host, and which are passively imbibed by the new host. It was shown for several species of ciliates that they may survive in filtered sea-water for 100-200 hours. The infection frequency of a ciliate species in a given host population is dependent on the density of the latter.

The population sizes of filter-feeding ciliates are dependent on the amounts of nutrients contained in the water pumped by the host and on the amount of water pumped. Thus, it was shown that the population sizes of *Ancistrum* and *Peniculistoma* are much smaller in mussels exposed to air at certain times and, therefore, unable to pump water continually.

Of the 42 ciliate species studied, 21 were strictly host-specific. Thus, of two *Abra* species, living side by side in some localities in the Gullmarfjord, each harbours a characteristic ciliate fauna with no species in common. *Cardium lamarcki* and *C. edule* each harbours a different species of *Trichodina*. Experimental cross-infections were either negative or else the ciliates died after a very short time. Ciliates which were not host-specific occurred only in a few--usually systematically or ecologically related--host species.

In several cases it was found that systematically related bivalves harbour systematically related ciliates. For example, the mytilids and the lucinid-thyasirid group both have a ciliate fauna characterized by special genera and subgenera indicating that the commensals have evolved in parallel with the evolution of the host families.

The ciliate species are not evenly distributed within the host group. Bivalves which normally form the densest populations in nature contain the greatest number of species (*Mytilus edulis* and *Macoma balthica*, for example, harbour respectively 7 and 6 species of ciliates) while on the other hand, lamellibranchs which are rare or occur sporadically on the bottom contain either few or no ciliate species. Other factors, such as morphology of the mantle cavity, may also explain some cases of distribution. The pectinids, for example, never contain ciliates, probably due to the open mantle cavity and the strong water currents produced by these bivalves, which render it difficult for commensals to keep attached to the host.

Too few data are available to allow one to draw far-reaching conclusions concerning the geographic distribution of the ciliates living in bivalves; but, with very few exceptions, nothing refutes the assumption that the ciliates occur wherever their hosts are found. Thus, the ciliates of *Mytilus edulis*, *Macoma balthica*, *Cardium lamarcki* and *Mya arenaria* (with one exception) follow their hosts into the brackish Baltic Sea. The ciliates of *Mytilus* (from any locality) are more tolerant to low salinities than are related ciliates from less euryhaline hosts.

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CHROMOSOME NUMBERS AND SYSTEMATICS IN
STREPTONEURAN SNAILS¹

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(Read at the morning session, 13 August, 1965)

ABSTRACT

An increasing number of studies of molluscan chromosomes and cytogenetics are now being published. Burch (1965) summarized current information on chromosome numbers in regard to systematics in the Euthyneura. The present report summarizes information known for the Streptoneura.

Reliable reports of chromosome numbers exist for only 26 of the 121 recent families of the Streptoneura. Of the estimated 2,800 recent genera and subgenera in this subclass, there is information available on only 64 genera (or 1 out of every 45).

In the Archaeogastropoda, 25 species representing 7 of the 21 recent families have chromosome information available. Haploid chromosome numbers in this order range from $n=9$ in the superfamily Patellacea to $n=18$ in the superfamily Trochacea. The superfamily Pleurotomariacea has information for one family, Haliotidae, where $n=17$. In the Fissurellacea, chromosome numbers are known only for 3 species of the Fissurellidae, which have haploid numbers of $n=16$ and $n=17$. In the Patellacea, all members of the 2 families investigated, Acmaeidae and Patellidae, have the haploid chromosome number 9. The Trochacea has cytological information for the Trochidae and Turbinidae; all species investigated had a haploid number of $n=18$. In the Neritacea, 3 species of the Neritidae have haploid chromosome numbers ranging from $n=11$ to $n=14$.

Sixty-six species representing 13 of the 81 recent families in the Mesogastropoda have been investigated cytologically. Chromosome numbers in this order range from 7 haploid in the Viviparacea to 20 haploid in the Pleuroceridae. Both recent families of the Viviparacea, Viviparidae and Pilidae, have cytological information available. Chromosome numbers range from $n=7$ to $n=14$. One species of the Valvatidae (Valvatacea) has been studied. It had a haploid chromosome number of 10. In the Littorinidae (Littorinacea) chromosome numbers range from $n=15$ to $n=17$. In the Rissoacea, chromosome numbers are known for 3 families: Hydrobiidae ($n=16$ and $n=17$), Bithyniidae ($n=17$) and Assimineidae ($n=12$ and $n=15$). Four families of the superfamily Cerithiacea have information on chromosome numbers. The haploid chromosome numbers for these families are $n=16$ to $n=19$ (excluding possible polyploidy) in the Thiaridae; $n=9$ to $n=20$ in the Pleuroceridae; and $n=18$ in both the Potamididae and Cerithiidae. In the Hipponicacea $n=17$ occurs in the Hipponicidae, and in the Naticacea

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$n=16$ occurs in the family Naticidae.

There are 19 recent families in the Neogastropoda, but chromosome numbers are available for only 6 of them (23 species). The range in haploid chromosome numbers is from a low of 28 to a high of 36. Both of these extremes occur in the Buccinacea. The one reported exception occurs in the Muricacea, i.e., a species with races of 13 and 18 pairs of chromosomes. Other chromosome numbers in the Muricacea range from $n=30$ to $n=35$ (family Muricidae). The Buccinacea has information on 4 families; Pyrenidae ($n=28$ to $n=35$), Buccinidae ($n=35$ and $n=36$), Nassariidae ($n=34$) and Fasciolaridae ($n=35$). One family, Mitridae, of the Mitracea, has 1 species studied, which showed a haploid chromosome number of $n=30$.

The Streptoneura, like the Euthyneura, exhibits a conservativeness in regard to chromosome numbers, with variation in chromosome numbers seldom more than ± 2 bivalents in the lower taxa. With the fragmentary information now available, there appears to be no general clear-cut correlation between low chromosome numbers and "primitiveness" among the various groups, as apparently can be shown for the Euthyneura (Burch, 1965). However, within the Viviparacea such a correlation may exist.

Reliable cytogenetic studies on hybridity and sex determination in the Streptoneura are very few. Staiger (1954) found that hybrids between $n=13$ and $n=18$ forms of *Thais* (*Purpura*) *lapillus* had 5 metacentric chromosomes paired with 10 acrocentrics. Burch (1964) studied F_1 hybrids among 4 nominal species of *Oncomelania* ($n=17$) and found them all to have 17 normal bivalents during meiosis and concluded that the 4 species were in reality only races of one species.

Studies on sex chromosomes in mollusks include those of Jacob (1959a, b) who reported an X-O sex determining mechanism for *Melania crenulata* and an X-Y mechanism for *Paludomus tanschaurica*. Burch (1960) and Patterson (1963) showed *Pomatiopsis lapidaria* to have an X-O sex determining mechanism and *P. cincinnatiensis* to have an X-Y mechanism. *Tulotoma angulata* shows a similar sex chromosome dimorphism, with X-X in the females and X-Y in the males (Patterson, 1965).

Much work is still needed on cytology of the Streptoneura, since such an extremely small number of species have been studied. It is hoped that our work, and that of our colleagues, will fill much of the existing void in the next few years.

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A more detailed account of these studies will be published in a later issue of *MALACOLOGIA*.

POLYPLOIDY IN MOLLUSKS¹J. B. BURCH² and J. M. HUBER*Museum of Zoology, University of Michigan, Ann Arbor, Michigan, U. S. A.*

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ABSTRACT

Polyploidy is the multiplication of the normal chromosome number of an organism. Because of the inability of a newly derived polyploid to breed with its sibling diploids it is usually chromosomally sterile. Accordingly, polyploidy is a method, and the only clearly established one, of instantaneous speciation. Its importance and predominance in producing new species of plants is well-known. Its significance in animal speciation is generally agreed by cytologists to be slight. Nearly all proven cases occur in permanently hermaphroditic species that are capable of self-fertilization or asexual reproduction, or in those species that have abandoned sexual reproduction in favor of parthenogenesis. Reasons given for the rarity of polyploidy among animals are the disturbance of the sex chromosome-autosome balance, the improbability of a newly arisen polyploid individual finding a polyploid mate, or in developmental difficulties in cellular differentiation.

In hermaphroditic animals, such as euthyneuran snails, there are no sex chromosomes (White, 1945; Inaba, 1953; Burch, 1960b). A newly arisen polyploid need not find a mate; but through self-fertilization it can produce its own strain, if it is not chromosomally sterile. It would seem, then, that the only barriers to polyploidy would be the infrequency of accidents of chromosomal doubling, the infrequency of self-fertilization in many groups, the infrequency of the production of viable interspecies hybrids, and possible developmental difficulties. That polyploidy has been found in less than 3% of euthyneuran species examined (Burch, 1965) indicates that one, some, or all of these factors are important in reducing its occurrence. One such factor is likely lack of self-fertilization, which probably does not occur in many of the hermaphroditic euthyneuran groups. In the Planorbidae, where self-fertilization is known to exist and many specimens of some populations are aphyllid (*e.g.*, see Larambergue, 1939) only about 7% of those species examined to date are polyploid. But on the other hand, in the Lymnaeidae, where self-fertilization is also known to

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occur, none of the 40 species studied are polyploids. So one or more of the other factors limiting polyploidy must be operating in lymnaeid snails.

Seemingly reliable reports of polyploidy in mollusks are those of Sanderson (1940) for *Potamopyrgus jenkinsi*; Jacob (1959) for *Melanooides lineatus*, *M. scabra* and *M. tuberculatus*; Burch (1960a) for *Gyraulus parvus*; Burch, Basch & Bush (1960) for *Ferrissia parallela*, *F. tarda* and *Ancylus fluviatilis*; Burch (1960c, 1964, 1965) for *Bulinus coulboisi* and *B. truncatus*.

In Ethiopia we recently found various populations (mostly unnamed species) with chromosome numbers of $n=18$, $n=36$, $n=54$ or $n=72$. Cytologically, the various polyploids behave as diploids, i.e., all chromosomes of the first meiotic divisions are bivalents. Therefore, we conclude that most likely these polyploids are allopolyploids. If this is indeed the case, the minimum number of species involved would be eight. Currently, only one species is reported to occur in that region (Mandahl-Barth, 1965).

Steusloff (1942) suggested that polyploidy might occur in the land snail order Stylommatophora. So far, we have found no evidence of polyploidy in this group (Burch, 1965), except possibly in the Succineidae, which has a chromosome number range of $n=5-22$. However, a comparison of morphological groupings with chromosome numbers does not indicate the occurrence of polyploidy in succineid snails.

Very few species of pelecypods have been studied, but our preliminary observations on the Sphaeriidae, an hermaphroditic group capable of self-fertilization, suggests that polyploidy occurs, perhaps commonly, in this family.

Most changes in chromosome numbers in mollusks have obviously been brought about through aneuploidy. When polyploidy occurs, it usually does so at the species level and probably has been of little significance in the derivation of higher taxa.

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A more detailed account of these studies will be published in a later issue of *MALACOLOGIA*.

PROC. SECOND EUROP. MALAC. CONGR.

ANTIMICROBIAL AGENTS FROM SEA FOOD

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(Read at the morning session, 13 August, 1965)

ABSTRACT

The ocean and its inhabitants have become a steadily important source of biologically active substances in recent years. A number of antimicrobials have already been isolated from sea material. In our laboratory we have studied the abalone (*Haliotis rufescens*), oyster (*Ostrea virginica*), clam (*Mercenaria mercenaria*), sea snail (*Tegula gallina*), queen conch (*Strombus gigos*) and squid (*Loligo pealii*), successfully isolating both an antibacterial and an antiviral substance that we have termed paolin 1 and 2 respectively. The term "paolin" simply means abalone extract in Chinese.

Paolin 1 was prepared from the above molluscs by cellulose ion-exchange chromatography or by acetic acid extraction. The product was usually a white powder, readily soluble in water, non-dialyzable, stable at a temperature of 95°C for 45 minutes and resistant to trypsin and pepsin digestion. It was precipitated by all protein precipitants tested. On hydrolysis, it yielded 18 amino acids and glucose. Chromatographic fractions derived from abalone or oyster material containing paolin 1 appeared to have a molecular weight of 5000 to 10,000 and an isoelectric point of pH 4.4. Acute toxicity studies of oyster extract containing paolin 1 were carried out in white Swiss mice. Doses of 1, 2 and 4 gm/kg of extract were tolerated by all animals tested. No gross pathology was found upon sacrifice of all survivors at the end of 7 days. In one anesthetized cat, an intravenous injection of 25 mg/kg showed no pharmacodynamic effects; and a dose of 50 mg/kg caused slight transient decrease of blood pressure. Subacute toxicity tests were made on 3 dogs subjected to oral administration of 20 mg/kg daily for 4 weeks. There was no indication of clinical toxicity attributable to the oyster extract, nor was any gross pathology of the important organs noted. Thus, the paolins seem to be relatively nontoxic in the limited number of animal experiments.

Antibacterial activity was tested in Difco nutrient broth medium containing various concentrations of paolin 1 against *Staphylococcus aureus*, *Streptococcus pyogenes*, beta hemolytic and *Salmonella typhi*. Gram positive as well as gram negative organisms were inhibited by 20 ug/ml of paolin 1. Purification of paolin 1 from oysters by paper chromatography produced a product which inhibited the growth of *S. aureus* at a concentration of 1 PPM.

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The antiviral paolin 2 inhibited the early phase of multiplication of poliovirus, influenza B virus or herpes simplex virus in tissue culture, reducing the virus yield by more than 90 percent. Antiviral activity against poliovirus and influenza B virus infection was observed in mice fed natural shellfish materials or their acetic acid extracts. The acetic acid extract from oysters was further purified by alcohol fractionation. A single intraperitoneal dose of 2.5 mg/kg or 5 mg/kg of this purified material given a few hours before or after intracerebral infection with Type I poliovirus (mouse-adapted) or intranasal infection with influenza B virus reduced the death rate by 25 to 50 percent as compared to the untreated controls.

The paolins may thus constitute another class of substances concerned with defensive mechanisms of animals against invading microorganisms, including viruses.

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THE EFFECT OF AMPHENONE "B" ON THE EGG PRODUCTION OF
LYMNAEA STAGNALIS L.

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(Read at the morning session, 13 August, 1965)

ABSTRACT

Amphenone "B" (3,3¹-bis (p-aminophenyl)-butanone-2-dihydrochloride) has frequently been used to study endocrine organs in vertebrates. When administered to *L. stagnalis* in rather low concentrations (0.001%), by dissolving it into the medium, the egg production of the snails showed major abnormalities. When compared to the controls, not only was the number of egg capsules deposited by the experimental animals lower, but these capsules also contained fewer eggs. In addition, the eggs were usually quite abnormal, showing several degrees of damage. They often contained many more egg cells than the single one normally found. Moreover, the egg membranes were frequently broken or even lacking; and the viability of the eggs was low. After treating the animals for only one week with Amphenone "B", during which time the medium was renewed 3 times, the phenomenon lasted for several months. Many of the snails never again produced normal egg capsules.

¹A more extensive paper on the subject will be published in *Malacologia*.

PROC. SECOND EUROP. MALAC. CONGR.

ATMOSPHERIC AIR PRESSURE AND EGG PRODUCTION
OF *LYMNAEA STAGNALIS*, UNDER LABORATORY CONDITIONS

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(Read at the morning session, 13 August, 1965)

ABSTRACT

Specimens of *Lymnaea stagnalis*, kept individually in what is usually regarded as constant conditions, were shown to exhibit large day-to-day fluctuations in egg production. Three independent experiments, each with 50-100 individuals, revealed the daily number of egg masses (= egg capsules) to be correlated with the total daily change in atmospheric air pressure. In the course of one experiment, a sudden and persisting decrease in the mean size of the egg capsules (= eggs) was noticed; at the same time the sign of the correlation between number of egg masses and air pressure changed from positive to negative. During the other two experiments the correlation was positive.

As in creeping through the water the snails normally undergo changes in pressure in the order of magnitude of 1 mm Hg in a few seconds, it seems highly improbable that the relatively minute changes in atmospheric air pressure can be of influence. Thus, another pressure-correlated external factor seems to be effective. Details will be discussed in a later paper.

The study was made possible by a grant of the Netherlands Organization for the Advancement of Pure Research (Z. W. O.).

THE EFFECT OF X-RAYS ON DEMOGRAPHIC CHARACTERISTICS OF A
FRESHWATER GASTROPOD, *PHYSA ACUTA* DRAP¹

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(Read at the morning session, 13 August, 1965)

ABSTRACT

The effects of X-radiation on the demographic characteristics of *Physa acuta* Draparnaud were determined by measuring the rates of adult mortality, fecundity and fertility and the percentage of egg viability. The adults were irradiated in an unfiltered beam (300 KV, 10 mA) with total dosages ranging between 2000r and 220,000r. The dose rate ranged between 1000r and 2000r per minute. The mortality of the animals irradiated with 10,000r was very similar to that of the controls; with 54,000r, the mortality did not become significantly different from that of the controls until 20 days after irradiation; with 100,000r, the mortality on the 18th day had increased to 95%; and with 220,000r, all the animals were killed in one day. Up to a dose of 10,000r, the fecundity of the irradiated animals was reduced but not strongly influenced.

The effects of radiation on the number of eggs per capsule and on the production of capsules were also studied. A dose of 2000r reduced both viability and fertility. An evident recovery of the germ tissues of individuals irradiated with 2000r and 10,000r was observed. The role played by the interaction of temperature and radiation had more effect on the reproductive processes than on the survival of the adults.

¹A more detailed paper on these experiments will be published in *Malacologia*, under the title "Effects of X-rays on *Physa acuta* (Gastropoda: Basommatophora)".

PROC. SECOND EUROP. MALAC. CONGR.

DISTRIBUTION OF MOLLUSCS IN THE ENGLISH CHANNEL

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(Read at the morning session, 13 August, 1965)

ABSTRACT

A survey of the bottom fauna of the English Channel has been made by means of over 300 samples taken with a special anchor-dredge. Along the length of the Channel there is a gradation in conditions from the western Channel, which is relatively deep and has a thermocline in summer, to the eastern channel, where stronger tidal streams and shallow water result in complete vertical mixing throughout the year. The distribution within the Channel of lamellibranch molluscs (also echinoderms) has been studied in some detail and a number of distribution patterns distinguished. The northern boundaries of many warm-water species lie in the region of the English Channel, and the possible effects of changing sea temperatures on their distribution was briefly considered.

Part of this survey has already been published (*J. Mar. Biol. Ass. U. K.*, 41, 1961, 397-461), and a second paper is to appear in vol. 46, no. 2 of the same Journal during 1966.

MARINE MOLLUSCA IN THE LOWER PLEISTOCENE OF EAST ANGLIA

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(Read at the morning session, 13 August, 1965)

ABSTRACT

Recent micropaleontological work (on pollen and Foraminifera; West, 1961; Funnell, 1961; Funnell & West, 1962) has queried earlier, stratigraphic, findings on Late Pliocene and Early Pleistocene marine deposits with Mollusca ("The Craggs") of East Anglia (*e.g.* by the malacologist Harmer, 1898, 1900, 1902, 1914-1925). Lack of quantitative data in Harmer's work and that of other malacological biostratigraphers made a re-study of already published work pointless. A new quantitative method was developed to record details of Mollusca and use them for interpretation as well as leaving them available for re-appraisal later if need be. The paper took, as example of the techniques, the work on one (important) site, the Royal Society Borehole at Ludham.

All molluscs larger than 135 microns across were counted. In this, one gastropod apex, two bivalve hinges, or eight plates of a polyphacophoran, were reckoned one individual. The frequency of each species in each sample was given as a percentage (% total number of all molluscan individuals in that sample). Granulometric analyses of sediments were made. Ecological groups (groups of species with definite present-day ecological significance) were recognised amongst the fossil populations. The change in importance (vertically) of these groups was studied by histograms showing the frequency of each group (*i.e.* the frequencies of the constituent species, compounded) at each level. This permitted attempts to assess the extent of seabed from which the "Post-mortem" faunal assemblage in each sample had been collected during deposition. Comparison of present-day habits of well-known infauna species with the sediments in which they occurred allowed assessment of their provenance, *i.e.* possibly *in situ*, or not.

Attempts were also made to assess water depth by study at each level of the proportions of the fauna which nowadays inhabit certain definite ranges on the seabed. It was thought possible that deposition began in less than 50m of water, the sea becoming progressively shallower until only a few metres deep, when Mollusca finally disappeared from the sequence. A sequence of six Assemblage Zones was established on a basis of faunal and sedimentary change.

An empirical method was tried for recognising "semiglacial", "Semi-interglacial" and "interglacial" hydro-climates on the basis of groups of Mollusca from the succession of Late- and Postglacial raised beaches in Oslofjord and Bohuslän (molluscs recorded by Brøgger, 1901 and I. Hessland 1946; the raised beach successions can be correlated by careful study of the foraminiferal and pollen analytic work of Feyling-Hanssen, 1957, Brotzen, 1951, and Hafsten, 1960). The method failed when applied to the Ludham deposits. It is thought that the North Sea of the time may have been

"Boreal" as regards average temperatures, but that a great annual temperature range existed. Temperature-ecotypes of the species, now extinct, may have been present. It is a future problem to try to understand the temperature regime in the North Sea at the time the Ludham mollusc Zones were accumulating (*i. e.*, most of Harmer's "Icenian Stage" and a short period prior to it which is not similar to his "Butleyan Stage"). Oxygen-isotope paleotemperature methods may be very useful here.

A paper "Marine Mollusca in the Early Pleistocene of Sidestrand, Bramerton and the Royal Society Borehole, Ludham, Norfolk" (Norton, unpublished) has been submitted for publication; it covers these topics fully.

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CAUCASIAN SPECIES IN CENTRAL EUROPE: THE GENUS *LYTOPELTE*
(GASTROPODA, LIMACIDAE) IN RUMANIA, AND ITS WIDE VARIATION

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(Read at the morning session 13, August, 1965)

ABSTRACT

The main characteristic of a faunistic province is its large number of endemic species, the presence and origin of which may be explained by historical factors and by the geomorphological peculiarities of the province. The geographical situation of Rumania has conditioned a special malacological fauna, consisting of *species relictæ* and of *endemic* ones; these show the historical relations to other neighbouring provinces and to the fossil fauna, which, after an invasion of new elements, formed the recent population of this province. The definition and limitation of these provinces was made by P. FISCHER (1887) and W. KOBELT (1904); and, much later, A. GROSSU (1943, 1962) characterized Rumania as a peculiar malacological province from the faunistical standpoint.

Our attention was especially attracted by the discovery of certain species existing both in Rumania and in the Caucasus, and we were interested in attempting to establish historical relations between the two remote provinces. These common species are: *Serrulina serrulata* C. PFEIFFER, *Deroceras melanocephalus* SIMRTH, *D. transcaucasicus* SIMRTH, *D. subagrestis* SIMRTH, etc., some of which were only recently identified in the Rumanian fauna (GROSSU & LUPU 1957, 1959). Among these species, the discovery in Central Europe (Poland, Germany) of *Serrulina serrulata* and *Boetgerilla vermiformis* WIKTOR brought about recent discussions on their presence and their dispersion (WIKTOR 1959; SCHMID GUNTER 1963; GROSSU 1964).

In addition to these species common to both provinces, Rumania and the Caucasus, some new ones were recently described, e.g., *Daudebardia dobrogica*, *Limax dobrogicus*, *L. zilchi*, *Deroceras schleschi*, *D. forcarti*, etc. These are much related to the Caucasian elements and at the present are only known from Rumania; they are considered to be endemic (GROSSU & LUPU 1959, 1960). The recent identification of the genus *Lytopelte* in the Carpathian mountains has been of particular interest. Species of this genus have previously been known only in the Caucasus, and by following their distribution and variation in the Rumanian fauna we arrived at some surprising results. From the mountainous Carpathian region, five new species of the genus *Lytopelte* have thus far been described, namely, *L. moldavica*, *L. occidentalis*, *L. suboccidentalis*, *L. herculana*, *L. olteniana* (GROSSU & LUPU 1961; GROSSU 1964, 1965). All these belong to the subgenus *Liolytopelte* and are much smaller in size than the Caucasian species of the same subgenus. Each of the described species is distinct in colour and in morphology of the reproductive organs; each of them lives isolated in mountainous regions which are far from one another and separated by deep valleys. The special Rumanian climatic and geomorphological conditions have caused changes in their morphology, separating them both from one another and from the Caucasian species from which they became

isolated during a previous geological period. As to the morphology of the genitals, there is great variation in the penisform. In *Lytopelte moldavica*, which we think to be the most primitive species, it is simple and without any appendix, like a sandbag; but in the other species it becomes more complicated as a result of the appearance of the distal part of an appendix, which acquires a more and more varying aspect in form and size. Its most complicated forms are found in the individuals collected in the most southern regions, i.e., the Retezat Mountains and Băile Herculane (*L. suboccidentalis* and *L. herculana*). In such cases not only is its external form altered, but the stimulating body inside the penis also shows different aspects, which, related to other features, allow us to differentiate between the species mentioned above.

The presence and variation of the Carpathian *Lytopelte* species, as well as of those in the genus *Deroceras*, may demonstrate that we are dealing with a genetic centre whose past and present factors differ from those of the Caucasus; these have brought about profound modifications which have been emphasized by selection and have led to the appearance of new species. At the same time, in different regions inside Rumania, some new species have appeared; these are the result of certain complex influences (isolation, climate, relief, etc.) which may explain their changes and their scanty distribution. These new species of *Lytopelte* are nearly always found in association with other gastropods endemic in the region or, frequently, with endemic plants, therefore demonstrating some long standing influences of an historical process. We think, accordingly, that this cannot be a question of Caucasian relicts but of endemic species developed independently in this part of Europe.

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DIE BEDEUTUNG DER EISZEITEN FÜR DIE REZENTE VERBREITUNG
DER EUROPÄISCHEN LANDGASTROPODEN

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ABSTRACT

Zu Beginn des Tertiärs setzte eine langsam fortschreitende Abkühlung ein, die von 21°C im Eozän bis etwa 14°C mittlerer Temperatur im Pliozän reichte. Bis zum Ende des Tertiärs herrschte in großen Teilen Europas eine sehr wärmeliebende Flora und Fauna. Etwa 60% der rezenten mitteleuropäischen Landgastropoden lebten bereits im Tertiär. Bei Einbeziehung aller europäischen, vor allem der mediterranen Arten ist dieser Prozentsatz sicherlich noch höher. Parallel den wechselnden Glazialen, Interstadialen und Interglazialen traten große Faunenschiebungen auf, die wir heute kaum noch rekonstruieren können. Gleichzeitig kam es durch die Änderungen der Auslesebedingungen zur Artneubildung: doch ist die Zahl der neu entstandenen Arten wahrscheinlich gering. Für die Beurteilung des rezenten Faunenbildes ist die letzte (Würm)-Eiszeit am wichtigsten. Infolge der großen Südausdehnung des Eisblocks zogen sich alle wärmeliebenden Arten nach Süden zurück. Etwa 30% harrten im mitteleuropäischen Periglazialraum aus. Die übrigen lebten in Glazialrefugien. Es lassen sich folgende Refugialräume unterscheiden: 1. Ostküste Spaniens, Westküste Italiens; 2. Raum Oberitalien bis Kleinasien (in viele kleine Einzelrefugien aufgelöst); 3. asiatisches Waldgebiet westlich des Ural; 4. Südengland. Als weitere Refugialräume, von denen aber postglazial keine größeren Ausdehnungen mehr stattfanden, kommen hinzu: Nunatakr in den Alpen (und evtl. Skandinavien), die Nordwestküste Skandinaviens und lokalklimatisch begünstigte Stellen im mitteleuropäischen Periglazialraum. Im Zuge der postglazialen Klimaänderungen (Atlantikum) wurde das frühe postglaziale Verbreitungsbild ebenfalls wieder abgewandelt. So wurden einerseits die postglazialen Waldarten disjungiert, was zur Entstehung des boreoalpinen Verbreitungstypus führte, während andererseits die mediterranen Arten ihr Areal weiter nordwärts vorschoben. Beispielsweise erreichte *Pomatias elegans* England noch vor Abtrennung vom Festland. Daher können sich heute die Verbreitungsgebiete von sibirop-asiatischen und mediterranen Arten überlagern, je nach den lokal- bzw. ökoklimatischen Verhältnissen. Ein Teil der stenotopen sibirop-asiatischen Arten ist daher heute auf Mittelgebirgsspitzen beschränkt. Diese Arten sind nicht, wie häufig angenommen wird, Glazialrelikte. Als echte Glazialrelikte können nur Arten gelten, die im Würmperiglazial weiter verbreitet waren und heute hier Reliktpopulationen besitzen, während sich ihr Hauptverbreitungsgebiet heute im hohen Norden bzw. in den Alpen findet. Derartige Beispiele gibt es unter den Landgastropoden Mitteleuropas nicht. Die wärmeliebenden Arten, die ihr Areal im Atlantikum bis Süddänemark ausgedehnt hatten, wurden in der nachfolgenden Klimaverschlechterung ebenfalls disjungiert. Arten dieses Ausbreitungstypus

haben am Nordrand ihres Verbreitungsgebietes heute Reliktpopulationen inne, vornehmlich an warmen Südwesthängen.

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SUR QUELQUES FORMES DE SENESTRISME DES *HELIX* ITALIENNES

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ABSTRACT

Nous jugeons convenable de signaler quelques phénomènes de senestrisme, observés dans la faune malacologique italienne chez certaines espèces du genre *Helix*.

Les *Helix* sont des animaux très intéressants, du point de vue écologique: nous trouverons donc des *Helix* caractéristiques qui vivent sur la montagne et dans la plaine, aux caractères fixés et bien déterminés, par rapport aux lieux d'origine.

Naturellement, comme chez tout organisme vivant, les *Helix* aussi peuvent subir des variations: parmi lesquelles, la plus évidente est celle qui sort du phénomène du senestrisme.

En 1880 Bourguignat avança la supposition que le phénomène était causé par un trouble soudain pendant la fécondation, par exemple une chute de l'animal. De nos jours, chez les *Helix* on n'a pas encore réussi à interpréter le fait, du point de vue génétique. Par les études de Crampton sur la *Limnaea peregra*, on a observé que, en cas d'une *Limnaea sinistrorsum* et d'une *Limnaea dextrorsum*, les générations se transmettent à travers l'action de gènes maternels qui impriment le caractère au cytoplasme de l'oeuf, et que à la deuxième génération, en accouplant les hybrides entre eux, nous avons une progéniture suivant le rapport trois à un, selon la loi de Mendel.

Ce serait très suggestif de pouvoir avancer une hypothèse fondée sur une base strictement embriologique, au cas où on arriverait à démontrer que les oeufs de *Helix* sont typiquement régulatifs. On sait, en effet, que chez les jumeaux monovulaires de *Triton* de l'ordre des salamandres, le phénomène du *situs inversus* est fréquent, et que toujours dans les organes régulatifs, la duplication des structures suit la loi de la symétrie spéculaire de Batteson. On sait que la segmentation de l'oeuf de *Helix* se passe de façon que chaque nouveau quatuor de blastomères tourne alternativement de 90° par rapport au précédent; selon Morgan, l'orientation des futures coquilles est déterminée par l'orientation du fuseau mitotique, à la première segmentation de l'oeuf. Si l'oeuf de *Helix* est régulatif et, au cas où on sépare, pour des causes accidentelles, les deux blastomères initiaux, leur morphogenèse pourrait suivre la loi spéculaire de Batteson, de sorte que la position des deux coquilles des deux animaux d'origine monovulaire, après cette rotation, serait spéculaire.

Cela signifierait que tout exemplaire *sinistrorsum* de *Helix* de l'espèce normalement *dextrorsum*, serait nécessairement et exclusivement un jumeau monovulaire d'un autre animal tourné d'une façon normale. Chez certains types de *Helix* exotiques, tels les groupes des *Ariophantacea*, *Bertiae*, *Camena*e et de *Nanine*, etc, le phénomène du senestrisme semble

presque physiologique. Mr. Francesco Settepassi, pendant sa longue vie de malacologue, a remarqué que le phénomène du senestrisme n'est presque jamais présent aux endroits où la température est basse; Mr. Sacchi, dell' Aquarium de Naples, affirme n'avoir jamais trouvé un exemplaire avec cette anomalie, après avoir examiné des animaux par milliers à des endroits différents, et aux grandes hauteurs au-dessus du niveau de la mer. L'absence, au-dessus de la haute altitude et dans les régions circumpolaires, de phénomènes de senestrisme, pourrait s'expliquer par la basse température qui aurait une action, chez les *Helix*, inhibitive à l'égard de la production de jumeaux. Cette hypothèse se trouverait revalidée par le fait que la distribution des espèces normalement senestrorsum, mais avec un fort pourcentage d'exemplaires dextrorsum (et donc toujours suivant l'hypothèse monovulaire) est tropicale, donc mégathermique.

Morgan, ensuite, à reproduit dans son laboratoire le senestrisme chez la *Cumingia* à une température donnée, en comprimant les oeufs qui sont régulatifs. Aussi le Prof. Piersanti suppose que la sinistrorsité des *Helix* doit dériver de un inversion dans la dislocation des blastomeres embryonales. Après avoir avancé cette hypothèse, qui n'est qu'une hypothèse de travail, dont le control impliquerait l'accouplement, en laboratoire, des différentes espèces de *Helix*, à des différentes températures, durée d'insolation, pression atmosphérique, etc., nous venons à présenter les cas de senestrisme des *Helix* italiennes, que j'ai trouvées, et dont j'établis les zones et surtout les hauteurs où elles ont été découvertes:

Helix (Helix) pomatia Lin.

Deux exemplaires adultes, l'un: 43mm. de haut, 41mm de large. L'autre: 40mm. de haut, 39mm de large. Trouvés dans la plaine en Lombardie. (Coll. Settepassi).

Helix (Cryptomphalus) aspersa Mull.

Deux exemplaires, l'un: 33 mm de haut, 32mm de large. L'autre 34mm de haut, 35mm de large, trouvés près Palerme, par Monterosato, en 1883.

Helix (Arianta) arbustorum Lin.

M. le Prof. C. Piersanti a trouvé dans le 1930, dans le Valle Venosta, un exemplaire de 12,5mm de hauteur, et 20mm de large.

(Riv. Studi Tridentini di Scienze Naturali, 1936, fasc. 3, tav. II, fig. 9).

Helix (Cepaea) nemoralis-etrusca Ziegl.

21mm de haut, 28mm de large. Trouvée à Monte Gabberi (800m au-dessus du niveau de la mer) en 1932 par Settepassi.

Helix (Cepaea) nemoralis-etrusca Ziegl.

17mm de haut, 22mm de large. Trouvée à Camaiore (150 m au-dessus du niveau de la mer), Alpes Apuane, par M. le Docteur Del Prete, en 1876.

Helix (Eobania) vermiculata Mull.

Deux exemplaires, l'un: 23mm de haut, 30mm de large, trouvée à Palerme dans la plaine, au mois d'octobre 1911, par Monterosato. L'autre de 19mm de haut, 26mm de large, trouvée à Monte Cuccio, le 1902.

Helix (Cantareus) aperta Born

20mm de haut, 23mm de large. Trouvée à Monte Pellegrino (Palerme), à 200m au-dessus du niveau de la mer, par M. le Marquis de Monterosato (collect. Settepassi).

Helix (Xerolauta) peninsularis Monts.

Exemplaire adulte, la seule connue: 18mm de haut, 24mm de large. Trouvée à Cerveteri à 70m au-dessus du niveau de la mer, en 1937, par Settepassi.

Helix (Opica) surrentina Schm.

La seule connue. 9mm de haut, 17mm de large. Trouvée à Capri sur la route de Anacapri, au mois d'avril 1963 par Settepassi.

Helix (Xerocincta) profuga-etrusca Issel

Exemplaire la seule connue: 5mm de haut, 7mm de large. Trouvée à Carsoli (Lazio) à 400m au-dessus du niveau de la mer, en 1943 par Settepassi.

Helix (Monaca) carthusiana Mull.

9mm de haut, 15mm de large. Trouvée à Rome, dans la zone Sedia del Diavolo (Chaise du Diable) en 1940 par Settepassi.

Helix (Theba) consona (Z.) Rossm.

Exemplaire la seule connue: 7mm de haut, 12mm de large. Trouvée à Castel del Monte (Bari), à 350m au-dessus du niveau de la mer, oct. 1958 par Settepassi.

VIEWS ON THE COMPARATIVE ANATOMY OF
THE BIVALVED MOLLUSCA

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ABSTRACT

Comparative anatomical studies of the Bivalvia have commonly resorted to uncritical use of superficial conchological characters and reliance upon overly emphatic generalizations. These tendencies have inhibited evolution of comparative anatomical concepts of the class.

Often put forth is the point of view referred to by Allen (1963) that the shell and mantle of the bivalves, and of all other Mollusca as well, comprise an entity evolutionarily separable from the rest of the body; albeit these entities are said to profoundly influence one another. Yet, so profoundly do the presumed entities interact that no separation, in the sense usually expressed, can be shown to exist; for the anatomical bases of the concept of independent entities are invalid in precisely all those genera hitherto used to prove the view. The most famous in this regard is *Tridacna*, in which the mantle, and hence the shell, has been said to have literally rotated 180° about the body.

The "demarcation line" of the shell, now necessarily used in support of the concept, is unreliable, as demonstrated by the cardiid series *Clinocardium*, *Dinocardium*, *Fragum*, and *Corculum*. In this series of genera all of the same family, and also in the related Tridacnidae, the demarcation line is variously located and does not correspond to anatomically comparable regions of the body, nor even of the shell alone.

Cartesian, or deformed coordinates, such as used by D'Arcy Thompson, can be successfully employed in the Bivalvia, both ontogenetically (illustrated by *Isognomon*) and in comparisons between groups (illustrated by *Clinocardium*, *Corculum*, *Solen*, and *Chlamys*). Pallial innervation, which was not used in construction of the coordinates, follows the "deformations" in every instance where it is known and thus provides a proof of the applicability of this graphic technique to bivalves. That this technique is applicable disproves the concept of independent entities since the "deformations" affect as a unit the mantle and the remainder of the body.

Such "deformations" not only aid in visualizing comparative differences and similarities, but they may lead to lines of approach for other kinds of study. For example, their application to a size series of *Isognomon* (Pteriacea) illustrates differential enlargement of the posterior portions of the shell and body. Relative and absolute phylogenetic enlargement of these portions seems to have occurred in the Paleozoic pteriacean genus *Myalina*, judging from the diagrams by Newell (1942, p. 47). [It may be noted that while Newell regarded the Myalinidae to be Mytilacea, it was Nicol (1958) who held the family to be pteriacean in character.] By comparisons with dimyarians, similar enlargements of the posterior regions are to be

found in the Tridacnidae and in the Pectinacea (Stasek, 1962, 1963).

The ostensible significance of this relative and often absolute enlargement, which involves the water-circulating and primary feeding structures, is hypothesized to be that all such suspension-feeding monomyarians evolved but have not necessarily remained fixed organisms in geographical regions or in environmental situations where the ambient water contained a relative paucity of useable food material. It is suggested that because food was relatively scarce, natural selection led to increase in the size of the water-circulating and feeding equipment, with all adjacent structures, such as the posterior adductor muscle, being similarly affected by the "deformation". The foot remained relatively small and became ineffective in locomotion, for increase in the size of the anterior and locomotory regions of the body would have negated the adaptive significance incurred through differential augmentation of the quantity of water circulated and potential food collected. Hence, with few exceptions, these organisms are inactive and attached, one implication being that the less energy expended in moving about the better. Clear, tropical waters may exemplify one of the general environmental situations in which this evolutionary process could have occurred, although few details are definitely known of the specific ecology of the bivalves under consideration.

Behavioral modifications utilizing the expanded siphonal regions often followed the resultant exposure of these bivalves to predators. Examples are the swimming ability of *Pecten* and the aiming-spurting behavior of *Tridacna* (Stasek, 1965).

The view here briefly outlined seems to offer frontiers for comparative studies, perhaps throughout the Mollusca, that would not have been conceivable had the concept of independent entities gone unchallenged. Other applications along these lines are now in progress.

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STRUCTURE MICROSCOPIQUE ET NATURE MINÉRALOGIQUE DE
LA COQUILLE DES PRINCIPAUX MURICIDAE EUROPEENS,
ACTUELS ET FOSSILES

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ABSTRACT

La structure microscopique des coquilles s'étudie en y pratiquant des lames minces, comme on le fait en Géologie pour les roches. Le Microscope polarisant y révèle les détails de l'architecture ainsi que la nature minéralogique des différentes couches. Dans les coquilles de Mollusques, il peut s'agir de calcite ou d'aragonite. Les couches de calcite de l'aragonite se différencient bien de l'anagouite à l'observation des lames minces. Dans les coquilles la calcite est en effet formée de cristaux assez gros pour que leurs limites se voient nettement. On observe donc alors une couche formée de cristallites distincts, blancs, gris ou noirs, s'éteignant à tour de rôle de façon franche. L'aragonite au contraire est microcristalline. Elle montre de brillantes couleurs de polarisation, et donne des extinctions mouvantes. La présence de l'un ou l'autre de ces minéraux est vérifiée sur de petits fragments des couches, au moyen des Rayons-X, par la méthode des poudres de Debye & Scherrer, qui donne des diagrammes spécifiques.

Chez les Prosobranches et, plus particulièrement, chez les Meso- et Neo-Gastropodes, deux types de structure s'observent en général:

—ou bien les coquilles sont formées d'une couche externe de calcite (le cortex), et d'une couche interne d'aragonite (l'ostracum);

—ou bien elles sont constituées seulement d'un ostracum aragonitique.

Dans les deux cas, l'ostracum a sensiblement la même structure: il est formé de "couches principales" superposées. Chacune est due à l'empilement de feuillets cristallins perpendiculaires aux surfaces externe et interne de la coquille. Dans deux couches successives, les feuillets sont dans des directions perpendiculaires. Si, dans la couche la plus externe, ils sont perpendiculaires aux lignes de croissance, dans la seconde couche, ils leur sont parallèles, et vice-versa.

Nous avons montré dans un travail précédent que certains caractères structuraux et minéralogiques ont, dans la famille des Muricidae, une valeur systématique au niveau soit du genre, soit du sous-genre. C'est en particulier le cas pour la présence ou l'absence du cortex calcitique, qui semble bien être de valeur générique. Deux autres caractères paraissent propres aux sous-genres:

1) la forme de la zone de jonction entre le cortex et l'ostracum; elle peut être soit parallèle à la surface interne de la coquille (type "droit"), soit ondulée ou même, dans les cas extrêmes, plissée en doigts de gant (type "villeux");

2) l'orientation des feuillets de la couche externe de l'ostracum par rapport aux lignes de croissance.

Dans les mers européennes actuelles, les Muricidae ne sont représentés que par un nombre assez restreint de genres et d'espèces. Il n'en était pas de même au Tertiaire où la plupart des genres de la famille étaient représentés par d'assez nombreuses espèces.

Les Rapaninae comprennent une espèce actuelle de *Rapana* en Mer Noire. Comme tous les membres de la tribu, cette espèce possède un cortex calcitique qui occupe environ la moitié de l'épaisseur de la coquille. Sa zone de jonction avec l'ostracum est ondulée. Les feuillettes de la couche externe de l'ostracum sont perpendiculaires aux lignes de croissance.

Les Muricinae typiques sont presque tous entièrement aragonitiques et, dans cette sous-famille, les feuillettes de la couche externe de l'ostracum sont perpendiculaires aux lignes de croissance. La structure des espèces fossiles étudiées n'a subi aucune transformation secondaire et a pu être considérée comme originelle. Dans chacun des genres étudiés, la structure de la coquille et sa nature minéralogique sont en tous points semblables dans les espèces tertiaires et dans celles des mers actuelles. La structure décrite (un ostracum seulement) a donc été observée dans les espèces suivantes:

- *Murex (sensu stricto) carbonnieri* Jousseau (actuel, méditerranéen, immigrant de la Mer Rouge);
- *Murex (Tubicauda) spinicosta* Bronn et *Murex (Haustellum) haudmuticus* Cossman & Payraudeau (tertiaire d'Aquitaine);
- *Bolinus brandaris* L. et *Trunculariopsis trunculus* L. (actuels, méditerranéens);
- *Chicoreus dujardini* Tournouer; *Phyllonotus capgrandi* Tournouer; *Homalocantha (heptagonatus) pauli* Tournouer; *Poirieria elatospira* Cossman & Payraudeau (tous tertiaires d'Aquitaine);
- *Pterynotus tricarinatus* Lmk. (tertiaire parisien);
- *Muricopsis blainvillei* (actuel, méditerranéen) et *M. corniculatus* (tertiaire d'Aquitaine);
- *Typhis sowerbyi* Broderip (actuel, méditerranéen) et *T. intermedius* Bellardi (tertiaire d'Aquitaine).

Les Drupinae (ou Purpurinae, ou Thaisinae, suivant les auteurs) montrent une plus grande variété des structures.

Des caractères semblables à ceux des Muricinae s'observent chez *Vitularia linguabovis* Basterot (tertiaire d'Europe, espèce ubiquiste), ainsi que dans les espèces du genre *Ocenebrina*: *O. aciculata* Lmk. (actuel, Atlantique, Méditerranée) ou *O. excoelata* Cossman & Payraudeau (tertiaire d'Aquitaine).

Par opposition avec ces espèces, le genre *Ocenebra* possède un cortex calcitique, représentant environ la moitié de l'épaisseur coquille, de la coquille on le trouve aussi bien dans les espèces actuelles *O. erinacea* L. (Atlantique, Méditerranée) et *O. torosa* Lmk. (Adriatique), que tertiaires (*O. dufresnoyi* Grateloup). Dans l'ostracum de ces espèces, les feuillettes de la couche externe sont perpendiculaires aux lignes de croissance.

En ce qui concerne les pufres proprement dites, deux espèces sont actuellement assez communes: *Nucella lapillus* L. possède un cortex extrêmement épais, qui forme la presque totalité de la coquille. L'ostracum est réduit à un mince glaçage à la surface interne de la coquille. La jonction entre cortex et ostracum est de type "droit". Dans les derniers tours de la spire, on n'observe que deux couches dans l'ostracum, et les feuillettes de la couche externe sont *parallèles* aux lignes de croissance.

Thais (*Stramonita*) *haemastoma* Chemn. possède aussi un cortex calcitique, mais il est très mince, et c'est l'ostracum qui occupe le plus de place dans la coquille. Les deux couches sont séparées par une zone de jonction plissée, de type "villeux". L'ostracum est formé de trois couches de feuillets, dont les plus externes sont perpendiculaires aux lignes de croissance.

Enfin, on trouve dans les mers actuelles des représentants de la sous-famille des Trophoninae. Ils se répartissent entre les deux genres *Trophonopsis* et *Boreotrophon*. Ces deux genres se distinguent nettement, comme s'était déjà le cas pour *Ocenebra* et *Ocinebrina*.

Les espèces du genre *Trophonopsis* (*T. muricatus* Wood, *T. barvicensis* Johnson) sont entièrement aragonitiques.

Celles du genre *Boreotrophon* (*B. clathratus* L., *B. truncatus* Ström) possèdent un important cortex calcitique, d'aspect souvent granuleux et parfois de coloration vert-jaunâtre. Il constitue environ la moitié de l'épaisseur totale de la coquille.

Dans les deux genres cependant, l'ostracum n'a le plus souvent que deux couches, et les feuillets de la plus externe sont, dans les deux cas, *parallèles* aux lignes de croissance.

Par conséquent l'étude de la structure microscopique et de la nature minéralogique de la coquille peut être une aide pour le systématique. Elle nous donne en effet des critères supplémentaires de distinction générique ou sous-générique. Ceci peut permettre, par exemple, de donner sa place systématique réelle à une espèce *incertae sedis* ou controversée, si les autres critères morphologiques ne donnent pas de certitude. D'autre part, certains caractères structuraux fourniront peut-être, après une étude encore plus fine, et plus approfondie, la possibilité d'arriver à des déterminations spécifiques, surtout sur des fragments, peu identifiables autrement.

PROC. SECOND EUROP. MALAC. CONGR.

STUDIES ON RADULA REPLACEMENT

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ABSTRACT

The process of radula replacement has been described by Runham (1963) for *Lymnaea stagnalis*. Recent work in this laboratory has been concerned with a comparative study of replacement and electron microscopy. In *Lymnaea stagnalis* the radula is replaced at a rate of 2.98 rows a day; and, in the other 12 species investigated, it varied from 1.22 in *Pomatias elegans* to 5.02 in *Littorina littorea* and *Agriolimax reticulatus*. In *Helix aspersa* these rates were found to vary with age (at 20°C it was 7.0 rows at one week old and decreased to 3.6 in adults) and temperature (from 0 at 0°C to 3.6 at 20°C). In spite of the great differences in rate of replacement at different temperatures, the lengths were not affected. Starvation and aestivation did not apparently affect the rate of replacement, but in hibernation it appeared to depend on the depth of sleep. In long term aestivation experiments the radula of *H. aspersa* was found to be up to 3 times longer than normal because of 1. elongation of the radular gland and 2. detachment of the old radula from the underlying epithelium, but owing to lack of feeding it did not get broken up.

The most interesting findings in the electron microscope study have been concerned with the structure of the odontoblasts. In adult *Patella vulgata* these can be as large as $400\mu \times 2\mu$. From their upper surfaces very thin fibres have frequently been described in the older literature; these were found to be of a very variable diameter and there were many from each cell. They were found to extend into the radula for at least two teeth rows, probably further. Within these cells innumerable microtubules of 180-200 Å diameter and of very great length, were found in addition to the usual cytoplasmic inclusions.

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STRUCTURAL DETAILS OF THE CENTRAL NERVOUS SYSTEM OF
SUCCINEA PUTRIS (L.)

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ABSTRACT

Previous investigations have shown the Succineidae to be a family which takes a unique place among the Stylommatophora because it has some features in common with basommatophoran snails. For this reason a study of the morphology of the central nervous system of *Succinea putris* was undertaken. From the literature it is apparent that such characteristics as the presence or absence of medio-dorsal bodies, the nature of the derivatives of the embryonic cerebral tubes, and the location of groups of cells reacting positively with stains which demonstrate neurosecretory materials, differ markedly between Stylommatophora and Basommatophora. One of the reasons for examining these characteristics in *S. putris* was to further clarify the systematic position of the Succineidae.

That *S. putris* possessed medio-dorsal bodies has been noted previously. The structure of these dorsal bodies differs from that of other Stylommatophora found thus far.

Derivatives of the embryonic cerebral tube of *S. putris* form the pro-cerebrum. In this part of the cerebral ganglion and in the remnant of the cerebral tube, a glandular structure, the cerebral gland, is often found. This situation is similar to that found in other Stylommatophora. However, in *S. putris* the tube also forms a lateral lobe which is attached to the cerebral ganglion via two connectives. The presence of a lateral lobe has not been reported for any other stylommatophoran species but is widely distributed among basommatophoran snails. However, unlike the Basommatophora, the lateral lobe of *S. putris*, does not contain a cerebral gland.

The nature of some groups of neurons in *S. putris*, particularly in the cerebral ganglion, is similar to that of the Basommatophora in some respects. Other cell types closely resemble the situation in other Stylommatophora.

These phenomena are discussed in greater detail in *Archives Néerlandaises de Zoologie*, 1966, 17: 1-72.

HERMAPHRODITISM AMONG NORTH AMERICAN
FRESHWATER MUSSELS

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ABSTRACT

Coe (1943) published at length on the sexual differentiation among mollusks. However, little has been contributed on the numerous species of freshwater mussels in the United States. Intensive work with this naiad group during the past several years indicates that most of the species have separate sexes (dioecious). Only three appear to be usually hermaphroditic; several have occasional hermaphrodites. Altogether 1,733 freshwater mussels were relaxed in sodium nembutal, killed and fixed (usually in Bouin's fluid), sectioned using the paraffin technique, and then stained with haematoxylin and eosin. An additional 2,000 specimens were sectioned using a cryostat.

The following four species were found to be hermaphroditic: *Anodonta imbecillus* Say, *Lasmigona compressa* (Lea) and a near relative *Lasmigona subviridis* (Conrad), and *Carunculina parva* (Barnes). Considered on a family basis, there were no Margaritanidae that were usually monoecious or hermaphroditic. Two species, *Margaritana margaritifera* (L.) and *Cumberlandia monodonta* (Say), each had an occasional hermaphrodite. Additional study is needed in this group. Among the widespread family Unionidae, representatives of all three subfamilies were studied. The results can be summarized, as follows:

Unioninae - Some 561 specimens in this subfamily were sectioned, and altogether 29 species were represented. None were found to be usually monoecious; six were occasionally hermaphroditic. Since specimens in this group often show striking visceral coloration running to orange, pink or bright crimson, some have questioned whether this feature was sex determined. Sections of both orange and white *Fusconaia flava* (Raf.) indicated that the color was present in both males and females. This observation agrees with Ortmann (1912), who indicated there is no relation of these colors to sex.

Anodontinae - It was possible to section 19 species representing 414 specimens in this group. As already indicated, two (*Anodonta imbecillus* and *Lasmigona compressa*) are usually monoecious; the remaining 7 were only occasionally so. It was surprising to find that *Anodonta imbecillus* had representatives in Florida which were dioecious, indicating that such a wide ranging species may not be consistently hermaphroditic. In the case of *Lasmigona compressa*, its habitat is usually very small creeks often too small for other naiades. In this case there is a question as to whether hermaphroditism may be related to difficult ecological conditions and hence may be an adaptive mechanism.

Lampsilinae - Of the 734 specimens sectioned, representing 46 species,

7 had occasional hermaphroditism; one is usually monoecious, *i.e.* *Carunculina parva* (Barnes). While several southern species of *Carunculina* were studied, none were hermaphroditic. Tepe (1943) observed that an occasional male follicle of *C. parva* contained eggs. This same condition was observed (H. & A. van der Schalie, 1963) in one specimen of some 200 of *Actiononaias ellipsiformis* (Conrad) sectioned. This specimen represented one of the most bizarre hermaphrodites found in that eggs and sperm were scattered in a most haphazard way. A report on this species and one other in another genus will be published elsewhere.

Purchon (1951), Fretter & Graham (1964), and others, have indicated that hermaphroditism appears more commonly among freshwater than among marine mollusks. Some authors have also emphasized that the monoecious condition is apt to appear under conditions in which the animal is confronted with difficulties in its normal reproductive activity. While the relatively few (only three) naiades, among some hundred species examined, were usually hermaphroditic, the causes are still quite obscure. A substantial amount of data to indicate hermaphroditism as conditioned by ecology is wanting in the three species cited. Further investigations are warranted.

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A more detailed account of these studies will be published in a later issue of *Malacologia*.

SOME OBSERVATIONS ON NERITIDS

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ABSTRACT

The Neritacea comprises a group of over 580 living species of gastropods and are recorded from the mid-Devonian (Knight *et al.*, 1960). They stand apart from other prosobranchs in displaying an assemblage of both primitive and advanced characters: the former indicate their aspidobranch ancestry; the latter indicate a long period of independent evolution and warrant the isolation of these gastropods in a separate order of the subclass Prosobranchia. A number of advanced characters are admirably described by Bourne (1908, 1911); but further investigation of members of the most primitive of the six families of the subclass, the Neritidae, reveals more of these and a new interpretation of some already known (Fretter, 1965).

An important feature in the evolution of prosobranchs is the loss of the right kidney coincident with the elaboration of a genital duct which allows for internal fertilization and the production of egg capsules. There is evidence that in the Neritacea the glandular moiety of the kidney as well as the renal duct are taken over by the genital system, the former becoming that part of the glandular genital duct posterior to the mantle cavity. The genital duct grows forwards through the mantle skirt in the anterior pallial vein, separating the right and left hypobranchial glands; and the rectum follows the same course. The position of the glandular duct is unique for prosobranchs; and its complexities arise without the suppression of the right gill, which remains vestigial, and the right hypobranchial gland. A minute duct leaves the genital duct and opens to the mantle cavity at the base of the vestigial gill. It bears the same relationship to this gill as the duct of the left kidney to the functional gill.

The mantle cavity of *Nerita* is used as a lung when the snail is out of water. The large aspidobranch gill, its tip free from membrane attachments and its filaments without skeletal supports, is then folded back in the mantle cavity; and the parabranchial vein allows the ctenidial circulation to be reduced. This vein runs parallel with the efferent branchial vein and joins it near the auricle. It collects blood from the mantle skirt and also a more elaborate respiratory surface, the dorsal body wall. The body wall is thick and numerous blood spaces give it a spongy texture. These spaces receive blood from the anterior aorta, and they open into the general haemocoel.

The rhipidoglossan radula works in conjunction with the buccal fold which functionally replaces jaws in depressing the anterior tip of the odontophore as it is protruded and spreading the radular teeth. The development of the fold would account for the loss of salivary glands which are replaced by buccal and sublingual glands. The oesophageal glands extend forward to the posterior limit of the buccal cavity where they open to the oesophagus

anterior to the area of torsion. This is a modification of the primitive disposition of the glands displayed in Pleurotomarians (Fretter, 1964).

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OBSERVATIONS ON THE ECOLOGY OF *MACOMA BALTHICA*

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(Read at the morning session, 14 August, 1965)

ABSTRACT

Special attention is paid to reproduction and growth in a tidal-flat population of *Macoma balthica*, near Den Helder, in the north-west part of Holland. The shells show concentric areas, called sectors, separated by rings caused by slower growth in summer and autumn. By counting these rings, up to six year classes can be distinguished.

A connection between year class, spawning time and growth has been found: animals of the first year class complete spawning first and also begin to grow earlier, while the animals of the other year classes follow in succession, in both respects. This may be explained by their digging into different depths in the flat. The animals which live more peripherally probably attain the critical temperature earlier. The spawning period is limited rather sharply (2 to 3 months) and differs from one year to the next, depending on temperature. Normal growth cannot begin before spawning has ended and takes place mainly from the end of the spatfall to the middle of June.

Three parameters are taken for shell growth: 1. height of the shell and its sectors, measured from umbo to the middle of the shell edge and to the middle of the rings, 2. the projected surface on the median plane of the shell and its sectors, and 3. the thickness of the shell, measured by the weight, as compared to the projected surfaces.

A "catching-up phenomenon" in terms of the height of the sectors is found, particularly between the first and the second sectors of several year classes, if specimens are placed in a series with increasing first sector height. This phenomenon disappears if the projected surfaces of these sectors are considered in the same way, but then it is found to occur between the first and the third sectors. This can be explained by the large growth in thickness in the third year.

The animals have the largest growth in their second year, both in terms of height and of projected surface. But if the increase in thickness is considered, the growth in the third year is the largest. The growth in terms of height in the first year is usually larger than that in the third and subsequent years, but generally smaller if the growth in terms of projected surface is considered.

Finally, it was found that in the spring of some years the growth of all sectors was large, while in other years it was small. From observations in the population during 6 successive years, it is concluded that a cold spring is favourable for growth in each year class. Hardly any fluctuations are found in the growth of the first year class.

These phenomena will be discussed in greater detail in a future publication.

DEVELOPMENT AND LIFE HISTORY OF *ARCHIDORIS PSEUDOARGUS*

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ABSTRACT

Strong evidence has accumulated that many small British dorid nudibranchs have annual life-cycles, spawning in the spring before dying off. The maximal life-span is one year (Thompson 1958, 1961, 1964). A number of gaps remained. The histology of the reproductive organs at various seasons and the pathology of post-sexual adults were untouched areas of study. Attention is now concentrated on the common intertidal dorid, *Archidoris pseudoargus* (Rapp).

Copulation is usually reciprocal; seminal fluid is pumped into the bursa copulatrix of the partner, from whence spermatozoa pass to the receptaculum seminis where they are nourished and stored until required. The breakdown of excess sperm in the bursa and the relationship between the receptacular endothelium and the stored allosperms (*i.e.*, foreign sperms received during copulation) were investigated with the electron microscope. As in insects (Hinton 1964), it seems unlikely that the intrinsic motility of the spermatozoa plays a part in their translocation either within the male tract or on their way to the receptaculum of the partner.

During oviposition, ripe oocytes are conducted along a longitudinal ciliated tract through dense masses of autosperms (the individual's own spermatozoa) in the vesicula seminalis. They meet active receptacular allosperms in the capsule-gland, and fertilization occurs. Many valve-mechanisms were discovered which guide the gametes during various reproductive activities. Female gametes are transported solely by ciliary means, whereas male gametes are translocated almost entirely by peristaltic muscular activities of the ducts. The electron microscope shows the presence on the spermatozoon of a keel which takes the form of a ridge commencing behind the head and spiralling to the tip of the tail. Ciné-photomicrographic observations on swimming allosperms showed that thrust is provided by a series of propagated waves originating at the neck and progressing rearwards along the flagellum; the waves are uniplanar and approximately symmetrical on the two sides. As normal allosperms progress forwards, they spin in a clockwise direction when viewed from the front. This spinning may exceed 8 rev./sec. It seems likely that spinning is brought about by the spiral keel, a spiral filament on the head, and the helical form of the head itself through their differential alteration of the moving spermatozoon's resistance to torque. Experiments with glass scale-models towed in fluids of various viscosities furnished support for this theory. The functional significance of spinning progression is as yet uncertain.

Observations on a population of *A. pseudoargus* in the Isle of Man proved the maximal life-span to be approximately one year (contrary to Miller's (1962) opinion). Young ones with undifferentiated gonads came into the

samples in early autumn. They grew rapidly, and ripening of the ovotestis began. Within a month some oocytes had ripened to apparent maturity, and the production of tailed spermatozoa was under way. During the winter months, more and more oocytes were brought to maturity. Ripe autosperms passed to the vesicula seminalis. The adults reached maximal size in the spring and spawning commenced. Copulation and oviposition took place time and again, each mating providing ample allosperms to fertilize several egg-masses. Feeding declined and the adults began to subsist solely on the reserves of the digestive gland. New waves of oocytes replenished the gonad so long as food-reserves remained. Finally, death occurred when the digestive gland had shrunken past a level beyond which the digestive cells rounded off and drifted away from the basement membrane of the lobules. Most other organ-systems still appeared normal.

In other British localities slight differences occur in that it appears that there may be a second autumnal breeding period, but there is no evidence that anywhere the normal life-span greatly exceeds one year.

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MODES OF SORTING OF SHELL VALVES ON A SANDY BEACH
STUDIED WITH ARTIFICIAL VALVES OF *DONAX VITTATUS*¹

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ABSTRACT

It is well-known that sand is selectively transported by water currents, dependent on size, shape and specific gravity of the particles. Comparable sorting of shell valves has only been incidentally reported but has never been the subject of extensive experiments. In the present study, 24.000 brightly coloured artificial valves of *Donax vittatus* were used. They were copies of the left and right valves of 3 complete shells of different sizes (19.7, 24.2, and 28.7mm length respectively). In a fraction of the valves, holes of 1, 2, or 3mm diameter were drilled; these holes had the exact shape of holes frequently bored in bivalves by prosobranch snails of the genus *Natica*. Of each of these 24 categories, 1000 valves were used. At low tide the valves were laid out (in a circle with a radius of 1m) at the lower part of the off-shore slope of a sand bar. During the next ebb-tide the distribution of the transported valves was studied. The results showed that clear selections had occurred according to size, symmetry (left and right valves), presence or absence of a hole, and diameter of the hole. Some implications of this type of work for the study of marine deposits were discussed.

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INDEX

- Abra*, 36
Actiononaias ellipsiformis, 78
Agriolimax reticulatus, 73
Ancistrum mytili, 35
Ancylus fluviatilis, 42
Anodonta imbecillus, 77
Archidoris pseudoargus, 83
Australorbis, 17, 33
Australorbis glabratus, 29, 31
Babinka, 9
Bilharzia, 21
Biomphalaria, 15, 17, 21, 26, 31, 32, 33
Biomphalaria alexandrina, 31
Biomphalaria alexandrina alexandrina, 26
Biomphalaria glabrata, 26
Biomphalaria pfeifferi gaudi, 26
Biomphalaria pfeifferi madagascariensis, 26
Biomphalaria pfeifferi pfeifferi, 26
Biomphalaria sudanica tanganyicensis, 26
Boetgerilla vermiformis, 57
Bolinus brandaris, 70
Boreotrophon, 71
Boreotrophon clathratus, 71
Boreotrophon truncatus, 71
Bulinus, 15, 21, 25, 32
Bulinus africanus, 15, 16
Bulinus beccarii, 25
Bulinus coulboisi, 25, 42
Bulinus forskalii, 16, 25
Bulinus globosus, 23, 25
Bulinus guernei, 25
Bulinus jousseaumei, 25
Bulinus nasutus nasutus, 23
Bulinus natalensis, 25
Bulinus reticulatus, 25
Bulinus senegalensis, 25
Bulinus tropicus, 21
Bulinus tropicus angolensis, 25
Bulinus tropicus tropicus, 25
Bulinus tropicus zanzibaricus, 25
Bulinus truncatus, 16, 31, 42
Bulinus truncatus rohlfsii, 25
Bulinus truncatus sericinus, 25
Bulinus truncatus truncatus, 25
Cambridium, 8, 9
Cardium edule, 36
Cardium lamarcki, 36
Carunculina parva, 77, 78
Chicoreus dujardini, 70
Chitons, 8
Chlamys, 67
Clinocardium, 67
Corculum, 67
Cumberlandia monodonta, 77
Cumingia, 64
Daudebardia dobrogica, 57
Deroceras, 58
Deroceras forcarti, 57
Deroceras melanocephalus, 57
Deroceras schleschi, 57
Deroceras subagrestis, 57
Deroceras transcaucasicus, 57
Dinocardium, 67
Donax vittatus, 85
D-quadrant, 10
Fasciola gigantica, 31
Ferrissia parallela, 42
Ferrissia tarda, 42
Fragum, 67
Fusconaia flava, 77
Gyraulus parvus, 42
Haliotis, 9
Haliotis rufescens, 45
Helix, 63
Helix arbustorum, 64
Helix aperta, 65
Helix aspersa, 64, 73
Helix carthusiana, 65
Helix consona, 65
Helix nemoralis-etrusca, 64
Helix peninsularis, 65
Helix pomatia, 64
Helix profuga-etrusca, 65
Helix surrentina, 65
Helix vermiculata, 64
Homalocantha, 70
Indoplanorbis, 15
Isidorella, 15
Isognomon, 67
Lasmigona compressa, 77
Lasmigona subviridis, 77
Leanchoilea, 8
Limax dobrogicus, 57
Limax zilchi, 57
Limulus, 8
Liolytopelte, 57
Littorina littorea, 73

<i>Loligo pealii</i> , 45	<i>Oncomelania hupensis nosophora</i> , 26
<i>Lopadorhynchus</i> , 10	<i>Oncomelania hupensis quadrasi</i> , 26
<i>Lymnaea</i> , 31, 32, 33	<i>Oncomelania nosophora</i> , 19, 26
<i>Lymnaea caillaudi</i> , 31	<i>Oncomelania quadrasi</i> , 26
<i>Lymnaea dextrorsum</i> , 63	<i>Opabinia</i> , 8
<i>Lymnaea natalensis</i> , 21	<i>Ostrea virginaca</i> , 45
<i>Lymnaea peregra</i> , 63	<i>Paludomus transchaurica</i> , 38
<i>Lymnaea sinistrorsum</i> , 63	<i>Paragonimus kellicotti</i> , 19
<i>Lymnaea stagnalis</i> , 47, 49, 73	<i>Patella vulgata</i> , 73
<i>Lytopenete</i> , 57, 58	<i>Pecten</i> , 68
<i>Lytopenete herculana</i> , 57	<i>Peniculistoma mytili</i> , 35
<i>Lytopenete moldavica</i> , 57	<i>Phyllonotus capgrandi</i> , 70
<i>Lytopenete occidentalis</i> , 57	<i>Physa acuta</i> , 51
<i>Lytopenete olteniana</i> , 57	<i>Physastra</i> , 15
<i>Lytopenete suboccidentalis</i> , 57	<i>Physopsis</i> , 21
<i>Macoma balthica</i> , 36, 81	<i>Planorbis</i> , 15
<i>Margaritana margaritifera</i> , 77	<i>Poirieria elatospira</i> , 70
<i>Marisa</i> , 31, 32	<i>Polyplacophora</i> , 7
<i>Marisa cornuarietis</i> , 31	<i>Pomatias elegans</i> , 61, 73
<i>Melania crenulata</i> , 38	<i>Pomatiopsis</i> , 17, 18, 19
<i>Melanoides lineatus</i> , 42	<i>Pomatiopsis cincinnatiensis</i> , 18, 19, 38
<i>Melanoides scabra</i> , 42	<i>Pomatiopsis lapidaria</i> , 18, 19, 38
<i>Melanoides tuberculatus</i> , 42	<i>Potamopyrgus jenkinsi</i> , 42
<i>Mercenaria mercenaria</i> , 45	<i>Pterynotus tricarinatus</i> , 70
<i>Murex carbonnieri</i> , 70	<i>Rapana</i> , 70
<i>Murex haudmuticus</i> , 70	<i>Rugosa</i> , 9
<i>Murex spinicosta</i> , 70	<i>Saimonella typhi</i> , 45
<i>Muricopsis blanvillei</i> , 70	<i>Schistosoma haematobium</i> , 15, 23, 26
<i>Muricopsis corniculatus</i> , 70	<i>Schistosoma mansoni</i> , 15, 17
<i>Mya arenaria</i> , 36	<i>Schistosoma japonica</i> , 17, 26
<i>Myalina</i> , 67	<i>Serrulina serrulata</i> , 57
<i>Mytilus</i> , 36	<i>Solen</i> , 67
<i>Mytilus edulis</i> , 35, 36	<i>Straphylococcus aureus</i> , 45
<i>Natica</i> , 85	<i>Streptococcus pyogenes</i> , 45
<i>Neopilina</i> , 7, 8, 9	<i>Strombus gigas</i> , 45
<i>Nerita</i> , 79	<i>Succinea putris</i> , 75
<i>Nucella lapillus</i> , 70	<i>Tegula gallina</i> , 45
<i>Nucula</i> , 9	<i>Tetracorallia</i> , 9
<i>Ocenebra</i> , 70, 71	<i>Thais haemastoma</i> , 70
<i>Ocenebra dufresnovi</i> , 70	<i>Thais lapillus</i> , 38
<i>Ocenebra erinacea</i> , 70	<i>Trichodina</i> , 36
<i>Ocenebra torosa</i> , 70	<i>Tridacna</i> , 67, 68
<i>Ocinebrina</i> , 70, 71	<i>Triton</i> , 63
<i>Ocinebrina aciculata</i> , 70	<i>Trophonopsis</i> , 71
<i>Ocinebrina excoelata</i> , 70	<i>Trophonopsis barvicensis</i> , 71
<i>Oncomelania</i> , 17, 18, 19, 26, 38	<i>Trophonopsis muricatus</i> , 71
<i>Oncomelania formosana</i> , 19, 26	<i>Tropicorbis</i> , 33
<i>Oncomelania hupensis</i> , 26	<i>Trunculariopsis trunculus</i> , 70
<i>Oncomelania hupensis formosana</i> , 26	<i>Tryblidiacea</i> , 7
<i>Oncomelania hupensis hupensis</i> , 26	

Tulotoma angulata, 38
Typhis intermedius, 70

Typhis sowerbyi, 70
Vitularia linguabovis, 70

Mj - Malacologia

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THE EFFECT OF X-RAYS ON THE DEMOGRAPHIC
CHARACTERISTICS OF
PHYSA ACUTA (GASTROPODA: BASOMMATOPHORA)¹

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EURATOM
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ABSTRACT

The effects of x-radiation on the demographic characteristics of *Physa acuta* Draparnaud were determined by measuring the mortality, fecundity and fertility rates of the adult and viability of its eggs. The adults were exposed to an unfiltered beam (300 KV, 10 mA)² at doses ranging between 2 and 220 KR. The dose rate ranged between 1 and 2 KR · min⁻¹. Comparing with the controls: the mortality of animals irradiated at 10 KR was quite similar; at 54 KR, it did not significantly differ until 20 days after irradiation; at 100 KR it rose to 95% on the 18th day; all snails were killed in 1 day by 220 KR. The production of egg-capsules and the number of eggs they contained were reduced by irradiation, but not greatly, up to 10 KR. A dose of 2 KR did reduce viability and fertility. An evident recovery of the germ tissues was noted in individuals irradiated with 2 and 10 KR. Maintenance at higher temperature increased the effect of irradiation on the reproductive processes of adults to a greater extent than it did on survival.

INTRODUCTION

To study the effects of ionizing radiations on the demographic characteristics of freshwater Gastropods, it is first necessary to acquire a fundamental knowledge of their effects on those processes which determine an organism's status within a complex group of populations.

These processes are: fecundity, fertility and viability of the eggs. The radiosensitivity of the adult form is also exceedingly important.

Relatively little work has been done on the radiosensitivity of Gastropods: Richards (1914), Sonehara (1933), Bonham (1949, 1955) and Cather (1959) carried out research on embryonic development; Bonham & Palumbo (1951),

Azevedo & Carvao Gomez (1956) on the sensitivity of the adult; Laviolette & Cuir (1959) on the gonads; Hug (1958) and Born (1960) on the tentacle reflexes and Perlowagora-Szumlewicz (1964a, b, c, d) on the embryo development and demography.

This paper is the first report of an intensive study on the effects of x-irradiation on *Physa acuta* Draparnaud, a very common freshwater snail in Europe.

MATERIALS AND METHODS

Physa acuta were collected at or near Angera, Lake Maggiore, Italy. Two series of investigations were made on the mortality and reproductive capacity

¹From a paper presented at the Second European Malacological Congress held in Copenhagen, Denmark, August, 1965.

²KV = kilovolt (10³ volts); mA = milliampère (10⁻³ ampères); KR = kiloroentgen (10³ roentgens).

of this snail, when irradiated at different doses. Specimens were collected in September 1963 (Experiment A) and at the beginning of August 1964 (Experiment B). Interaction of temperature and irradiation was studied in *Physa* collected in July 1964 (Experiment C).

The snails used in this study, were exposed to laboratory conditions for at least 2 weeks before irradiation. They were kept in filtered lake water (about 50 ml per individual). Air was continuously bubbled into the aquaria and the water renewed every 3 days. The water temperature for experiments A and B was maintained at $20^{\circ} \pm 1^{\circ}$ C. The molluscs fed on a standard food, consisting of a mixture of powdered lettuce (91%), silt (2%), CaCO_3 powder (2%) and agar-agar (5%), which had been stirred at 100° C for 30 minutes and cooled; about 1 gm (dry weight) of this food was the individual ration per day. For Experiment C, on the effect of temperature (10° C and 30° C) on radiation effects, pH and oxygen content of the water were determined periodically. The water used for refilling the beakers used in that experiment had a mean oxygen content of 8.8 mg/l at 10° C and 7.4 at 30° C. The pH was 7.7 at 10° C and 7.9 at 30° C. After irradiation, the number of dead animals was determined daily and the egg capsules were collected at regular time intervals. In order to follow the embryonic development of the broods of irradiated individuals, the egg capsules were kept in glass containers filled with filtered lake water and periodically observed with a stereoscopic microscope until hatching occurred.

The material was exposed to an unfiltered beam of 300 KV x-rays produced by a Seifert-Isovolt x-ray machine operating at 10 mA. For Experiment A the dose rate was $1 \text{ KR} \cdot \text{min}^{-1}$, for Experiments B and C it was $2 \text{ KR} \cdot \text{min}^{-1}$. In order to avoid any diurnal variation in radiosensitivity of the animals (Pizzardello et al., 1963) they were irradiated at the same time

every day, i.e. between 13:00 and 14:00 hrs.

RESULTS

The growth curve of an isolated population, i.e. one without immigration or emigration, is the resultant of its death and birth rates. Therefore, measuring these rates is the first step towards determining the effects of radiation on population dynamics.

Adult mortality

In order to measure the influence of x-irradiation on the mortality of *Physa* Experiment A was initiated with 80 adults. One group of 20 snails was retained as the control, and the other 3 were exposed respectively to 2 KR, 10 KR and 100 KR. The mortality of snails which received a dose of 2 KR and 10 KR was similar to that of unirradiated animals, whereas it was much higher in the animals irradiated with 100 KR. For the first 17 days after irradiation, the cumulative mortality of the groups irradiated with 100 KR was 15%, on the 18th day it increased to 95% and by the 22nd day all animals had died.

The purpose of Experiment B was to study the effects produced by other doses using a larger number of individuals: 752. Groups of snails were irradiated with 28 KR, 54 KR, 110 KR and 220 KR. It was observed that irradiation with 110 KR caused a flaccid paralysis during the first 24 hours: from the 48th hour until death the molluscs moved very slowly, secreted abundant mucus, ate only a small amount of food and, consequently, produced a very little amount of excrement. This behavior was also observed in Experiment A at the dose of 100 KR.

From the comparison of the survival curves for the first 50 days after irradiation, calculated for each dose (Fig. 1) we may observe the following: (1) the differences between controls and animals irradiated with 28 KR and 54 KR did not become significant until 20 days

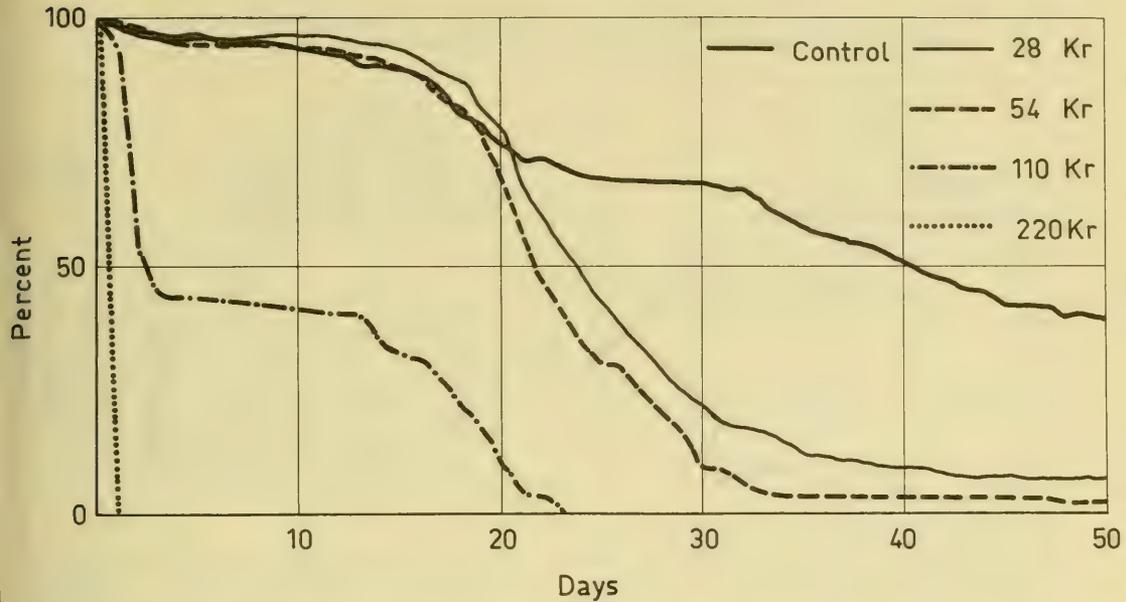


FIG. 1. Percentage of survival in a group of 752 *Physa acuta* kept at 20° C and irradiated at different doses (Experiment B).

TABLE 1. Reproductive characteristics of 80 *Physa acuta* (Experiment A) during a period of 136 days after exposure to x-rays at 20° C

Dose KR	Nos. used	Fecundity	Viability	Fertility	Total eggs
0	20	6762	94.7	6402	7384
2	20	4989	51.7	2577	5782
10	20	5965	44.5	2655	8011
100	20	2189	0.0	0	788

Fecundity = number of eggs that would be produced by 1,000 adults in one day.

Viability = percentage of hatchlings per 100 eggs.

Fertility = number of hatchlings produced by 1,000 adults in one day.

after irradiation, after which time they were pronounced; (2) mortality at 110 KR, very similar to that calculated for 100 KR in Experiment A, was very high for the first 2-3 days after irradiation, then, until the 13th day it was negligible, as was that of the controls. From the 13th-24th day mortality showed a very similar pattern to that observed from the 15th-30th day in animals irradiated with 28 KR and 54 KR. From this pattern it seems that the irradiated ani-

mals died from different types of damage; (3) the individuals irradiated with the highest dose (220 KR) lived only for 2-3 days.

Fecundity³

Since these snails are hermaphrodites

³Following Allee et al. (1955) *fertility* is used to designate the number of young produced, and *fecundity* for the egg production.

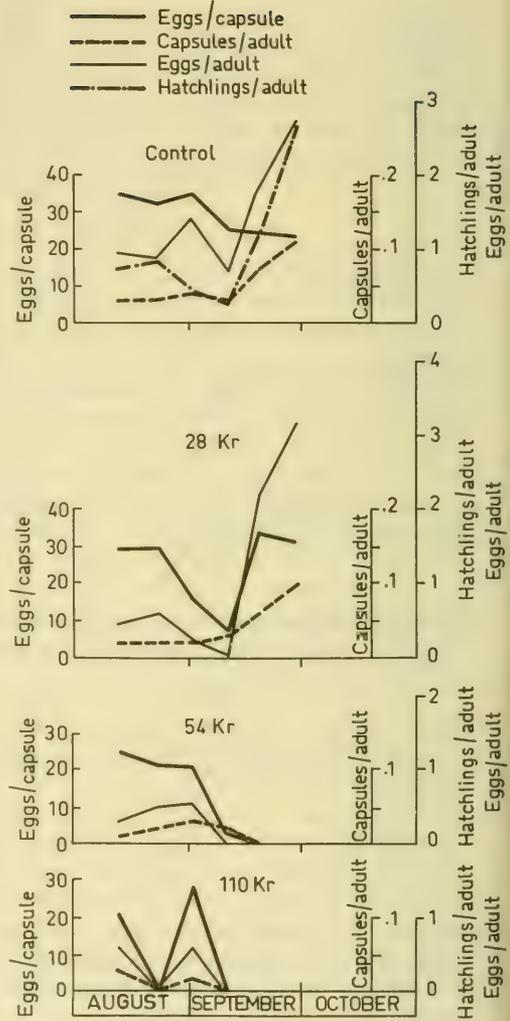
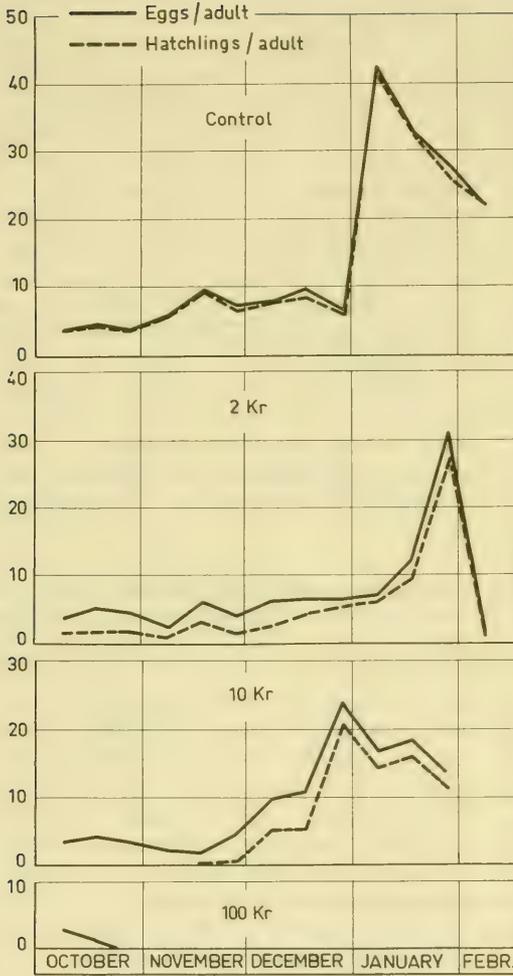


FIG. 2. Mean number of eggs and hatchlings per adult, after irradiation at different doses, at 20° C (Experiment A). Mean values calculated for periods of 10 days.

FIG. 3. Mean number of eggs, capsules, hatchlings per adult and mean number of eggs per capsule, at 20° C, after irradiation at different doses (Experiment B). Mean values calculated for periods of 10 days.

TABLE 2. Egg-production (fecundity*) of *Physsa acuta* irradiated at different doses and temperatures as compared to their controls, for the first 50 days after irradiation

Dose KR	20° C (Expt. B)				30° C (Except C)			
	Fecundity*		Total number of eggs produced		Fecundity*		Total number of eggs produced	
	N	%	N	%	N	%	N	%
0	1273	100.0	6327	100.0	1261	100.0	1667	100.0
28	495	38.9	1782	28.0	156	12.3	158	9.5
54	400	31.4	1197	18.9	145	11.5	111	6.6
110	432	33.9	451	7.1	0	0.0	0	0.0

N = absolute values.

% = percentage in relation to the controls.

* = number of eggs from 1,000 adults per day.

and all individuals were fecund, the ratio between the number of eggs and the number of living adults is a measure of the specific fecundity of the population. This index was expressed as the number of eggs from 1,000 adults per day.

In Experiment A the egg capsules were usually collected daily until the death of the last adult (136 days after irradiation). The fecundity values for Experiment A are given in Fig. 2. For the controls the number of eggs per adult rapidly increased at the beginning of January and maintained a rather high value until the end of the experiment on February 14. A very similar pattern was found for the group irradiated with 2 KR and 10 KR. This increase was independent of environmental conditions and was not affected by irradiation (at least up to 10 KR). For the group irradiated with 100 KR, oviposition ceased within a month and 11 days before the death of the last survivor. From the data listed in Table 1, we may conclude that fecundity was not strongly influenced by doses of x-radiation up to 10 KR.

In Experiment B, egg capsules were collected every 3-4 days for about 50 days. The results of this experiment are illustrated in Fig. 3. Soon after treatment, all individuals (except those irradiated with 220 KR, which survived

only 1 day) produced eggs, but the duration of the fecundity period decreased with increasing dose. For the controls, although the laboratory conditions (e.g. food, temperature, light) were constant, the number of eggs per adult rapidly rose during the experiment (i.e. from August through September). The data for the first 50 days after irradiation, listed in Table 2, indicate a clear reduction in fecundity of the irradiated animals.

Production of egg-capsules and number of eggs per capsule⁴

For Experiment A, the data on the number of eggs per capsule and capsules per adult are given in Fig. 4, which shows clearly the reduction in the number of capsules per adult, and of eggs per capsule, caused by exposure to x-rays.

To study the effect of radiation on the frequency distribution of eggs per capsule, the data were pooled for successive 20-day periods. In Fig. 5 it may be noted that, in irradiated animals the modal number of eggs per capsule shifted to the left and then to the right; i.e. the frequency of the egg capsules containing

⁴See Bondesen (1950) for the egg-capsules morphology of *Physsa*.

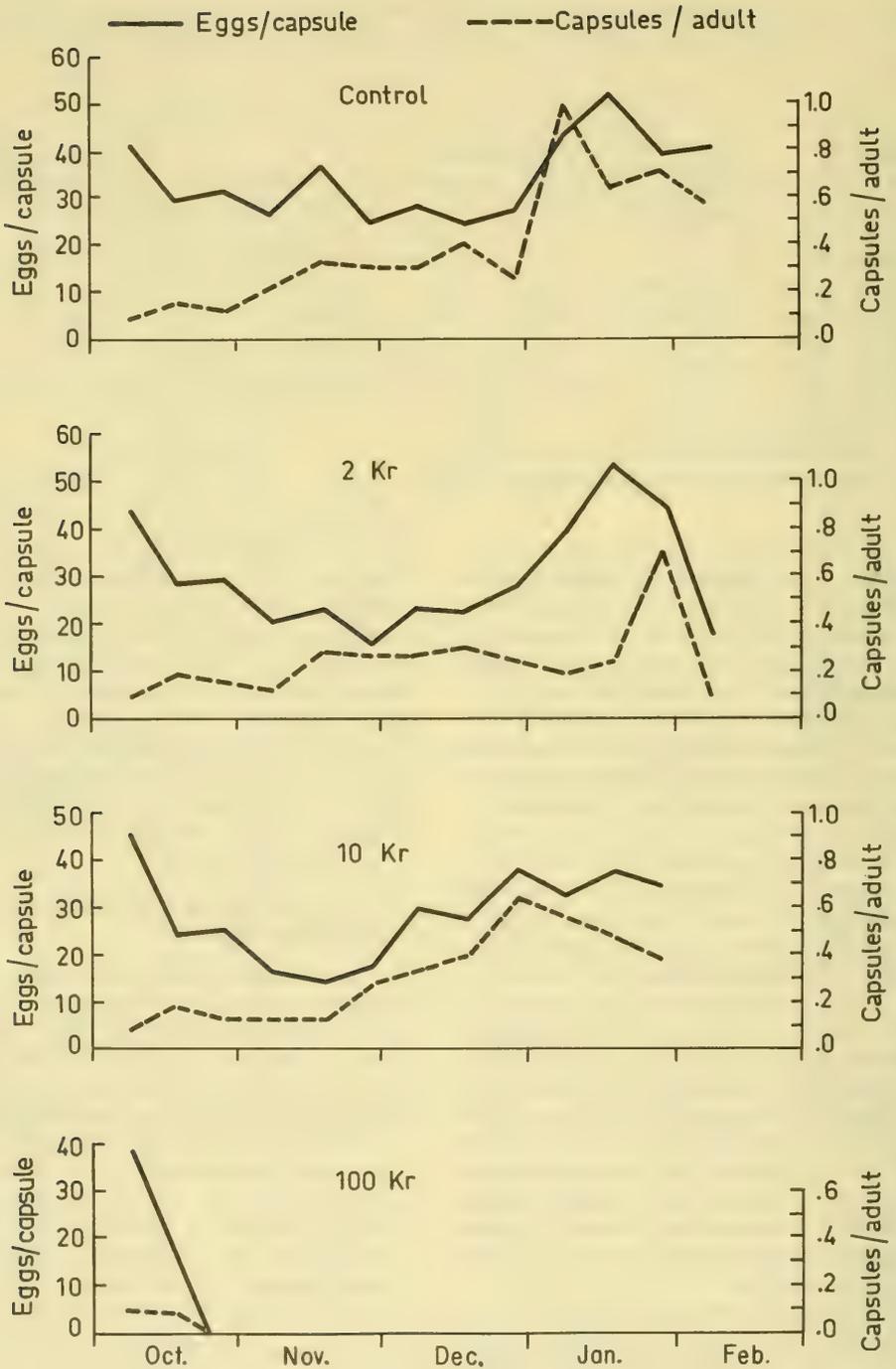


FIG. 4. Mean number of eggs per capsule and number of capsules per adult, at 20° C, after irradiation at different doses (Experiment A). Mean values calculated for periods of 10 days.

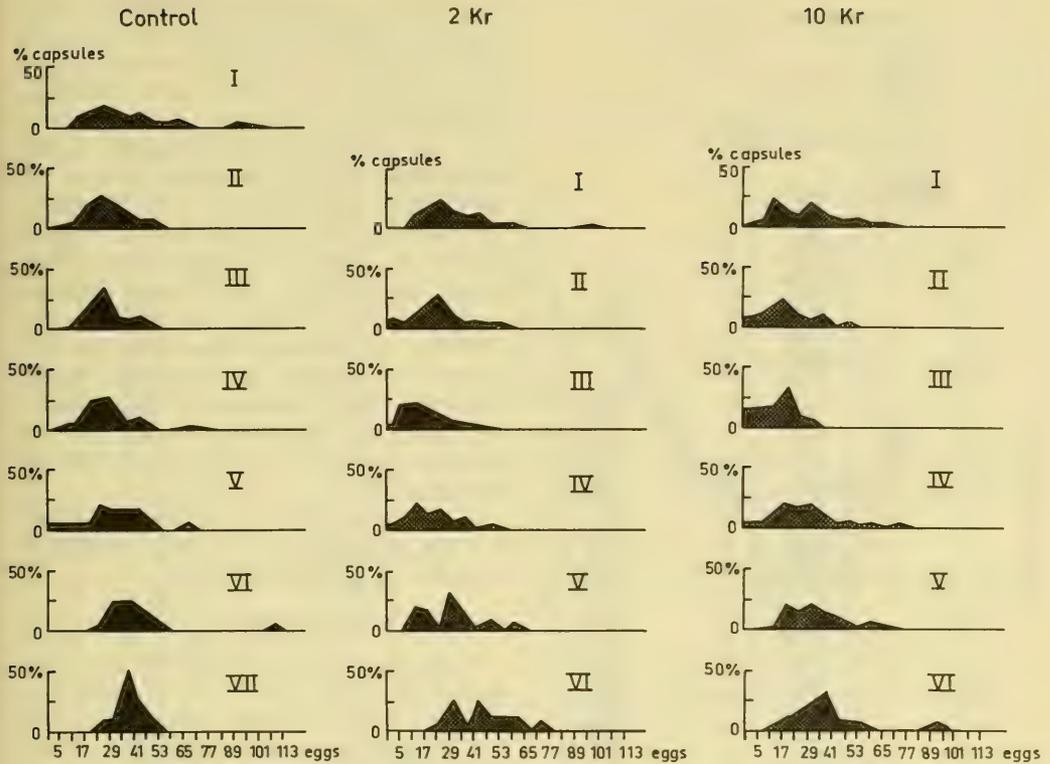


FIG. 5. Mean frequency of eggs per capsule, expressed as percentage, and calculated for successive 20-day periods (I, II, III, etc.). Experiment A.

a higher number of eggs showed a decrease, which was then followed by an increase, whereas the control showed only a shift toward the right, i.e. the frequency of capsules with a high number of eggs increased from autumn to winter.

To clarify this pattern, the mean number of eggs per capsule per 20-day period was plotted (Fig. 6). The values initially fell with increasing dose but then tended to exceed the control values, i.e. there was an overshoot, which resembled that noted by Whitfield in various organisms after radiation induced mitotic delay (personal communication).

The variation of the number of eggs per capsule and that of capsules per adult in Experiment B is illustrated in Fig. 3. This figure shows that, in the control, the number of capsules per adult increased during the experiment

(i.e. August and September). Since the mean number of eggs per capsule slightly decreased toward the end of the experiment, the observed increase of the number of eggs per adult was due only to a greater production of capsules. The mean number of capsules per adult decreased with increasing dose.

For the groups irradiated with 100 and 110 KR, the egg-capsule, which usually is kidney-shaped, was frequently more curved, so that its posterior and anterior ends would touch. This ring-like shape was not due to a larger number of eggs contained in the capsule, as has been observed in unirradiated eggs by Bondesen (1950), but probably to the manner in which this spawn was attached to the wall of the aquarium. For mechanical reasons the eggs contained in these capsules were ellipsoidal,

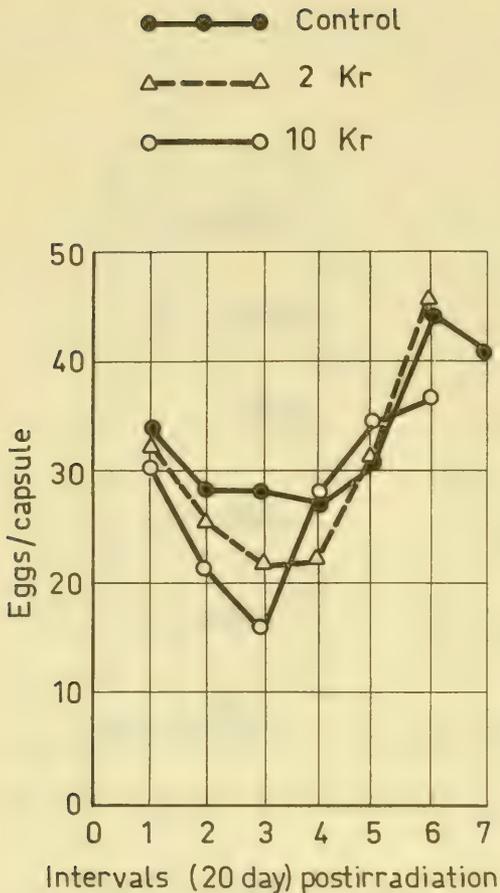


FIG. 6. Mean number of eggs per capsule for successive 20-day periods (Experiment A).

with their major axis oriented along the radius of the ring.

Viability

Viability was expressed as the number of hatchlings per 100 eggs. The ratios calculated for Experiment A are reported in Table 1.

Irradiation with 2 KR and 10 KR considerably reduced the viability of the eggs. No viable egg was produced during the first 50 days after irradiation with 10 KR. However, after this time, viable eggs began to be produced again and after 3.3 months, the percentage of viable eggs had risen to 88% (Fig. 7).

In order to study the development of

eggs from irradiated individuals, egg-capsules containing a total of 706 eggs were isolated soon after spawning and observed every 2 days until hatching. Observations were begun on November 4 (i.e. 22 days after irradiation); they were repeated on December 10 (i.e. 62 days after irradiation) with 459 eggs. The results, summarized in Fig. 8, confirmed an evident recovery of the germ tissue cells of the individuals exposed to 2 KR and 10 KR. In addition, the earlier, November, observations revealed a slower development in the embryos from irradiated individuals, whereas, in December, their rate of development was very similar to that of the controls.

In Experiment B, viability was 64.9% for the controls and zero for irradiated animals (at 28 KR and above).

Fertility or birth-rate

Fertility (number of hatchlings produced by 1,000 adults in 1 day) displayed a wide and daily variation in both the control and irradiated groups. There was no noticeable difference between the controls and the irradiated groups with respect to the amplitude of the variations.

For Experiment A, the variations of fertility are illustrated in Fig. 2, and for Experiment B in Fig. 3. These figures show that, in the control, the number of hatchlings increased during the experiment. The data for Experiment A, listed in Table 1 show that fertility was reduced by relatively low doses (2 KR and 10 KR). Fertility was clearly impaired by irradiation in Experiment B (28 KR and above), the value for the controls being 826, whereas no egg produced by irradiated snails was fertile.

Interaction of temperature and irradiation (Experiment C)

Several authors have studied the effect of temperature during or after x-irradiation on other poikilothermic animals (i.e. Gros & Bloch, 1957; Pendlebury & Banham, 1962; Tantawy, 1963 and

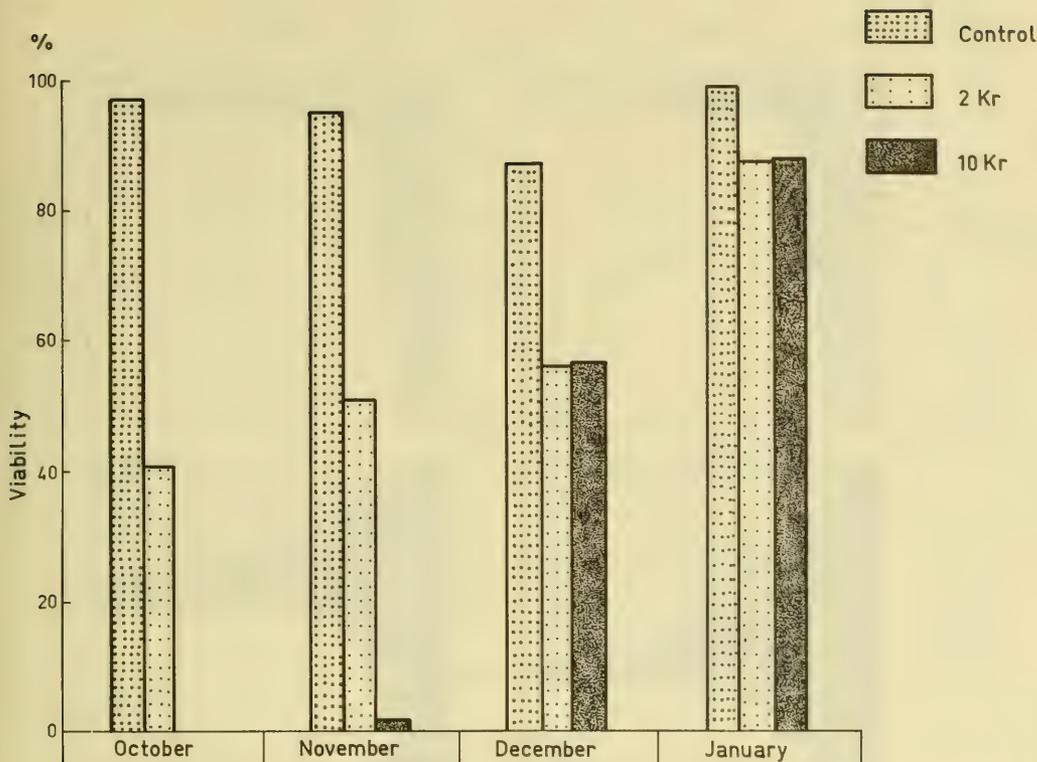


FIG. 7. Viability of *Physa* eggs, expressed as percentage of hatchlings (Experiment A) after irradiation at different doses.

Giavelli & Parazzi, 1963). Because no studies have been made with molluscs, an attempt was made to test the effect of temperature on *Physa acuta*, which is able to live in a relatively wide range of temperature.

Soon after collection (July, 1964) the adults were separated at random into 2 groups of 200 individuals each. The first group was kept at $10^{\circ} \pm 1^{\circ} \text{C}$, the second one at $30^{\circ} \pm 1^{\circ} \text{C}$. The aquaria were kept in the dark for 12 hours and in the light (7,000 lux) for 12 hours. Each sample was divided at random into 20 groups of 10 individuals each. An attempt was made to maintain the environmental conditions as constant as possible. Each group was kept in a beaker filled with 800 ml of filtered

lake water which was renewed daily.

After 20 days, 50 snails from each temperature treatment were irradiated at each of the following doses: 0, 28,

TABLE 3. Survival time;* from 50 *Physa* each for the different doses and temperatures

Dose KR	Temperature	
	10°C	30°C
0	23	21
28	24	22
54	15	17
110	12	4

*Time in days when 50% mortality is reached.

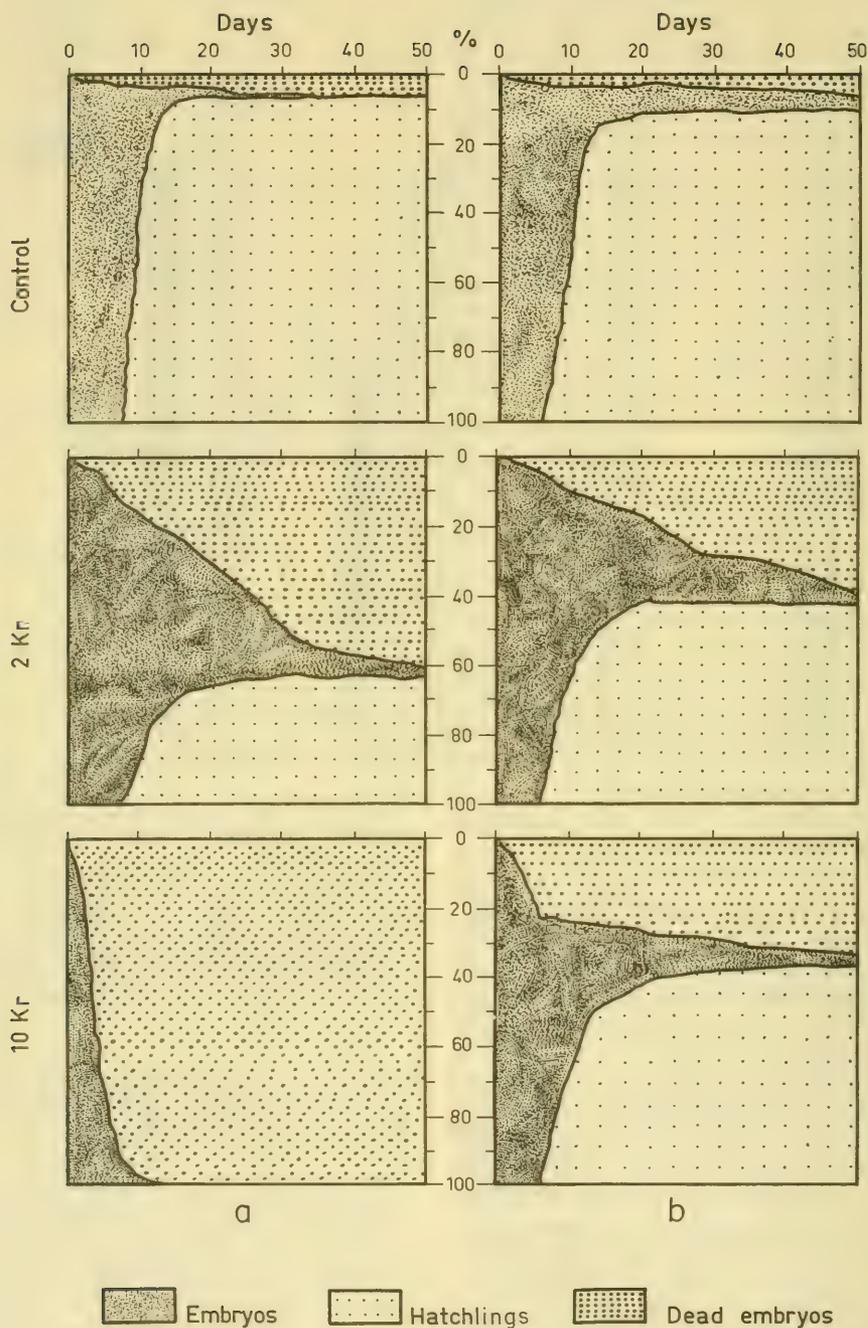


FIG. 8. Percentage of live and dead embryos and hatchlings from eggs laid at two different times (a and b), that is, 32 and 68 days after irradiation of the parents (Experiment A).

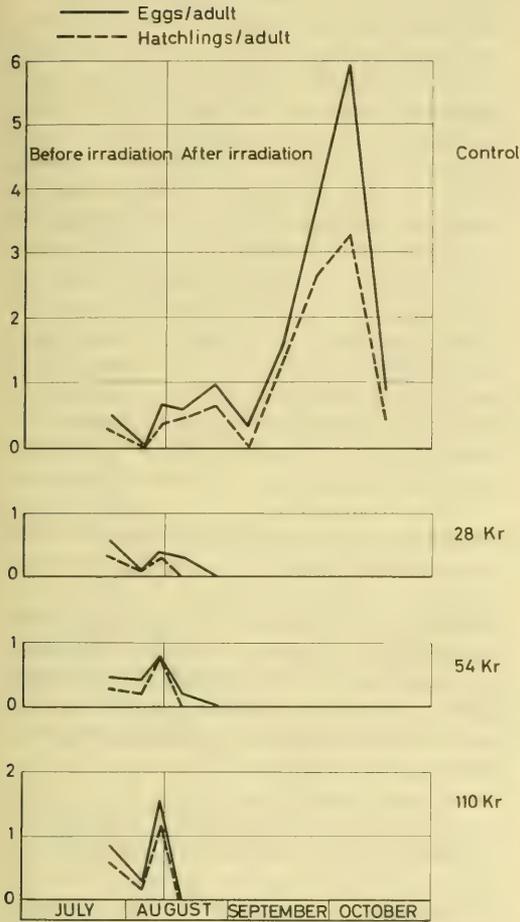


FIG. 9. Mean number of eggs and hatchlings per adult before and after irradiation at different doses, at a breeding temperature of 30°C (Experiment C). Values calculated for 10-day periods.

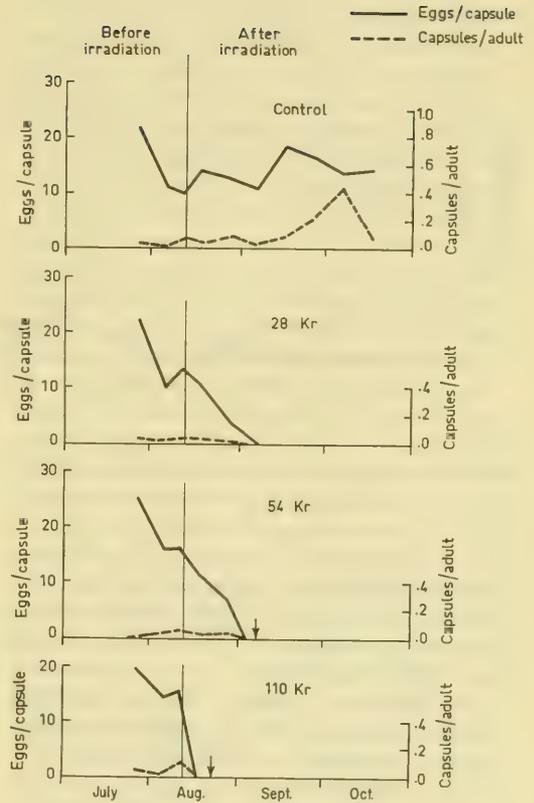


FIG. 10. Mean number of eggs per capsule and of capsules per adult before and after irradiation at different doses, at a breeding temperature of 30°C (Experiment C). Mean values calculated for 10-day periods. Arrows indicate death.

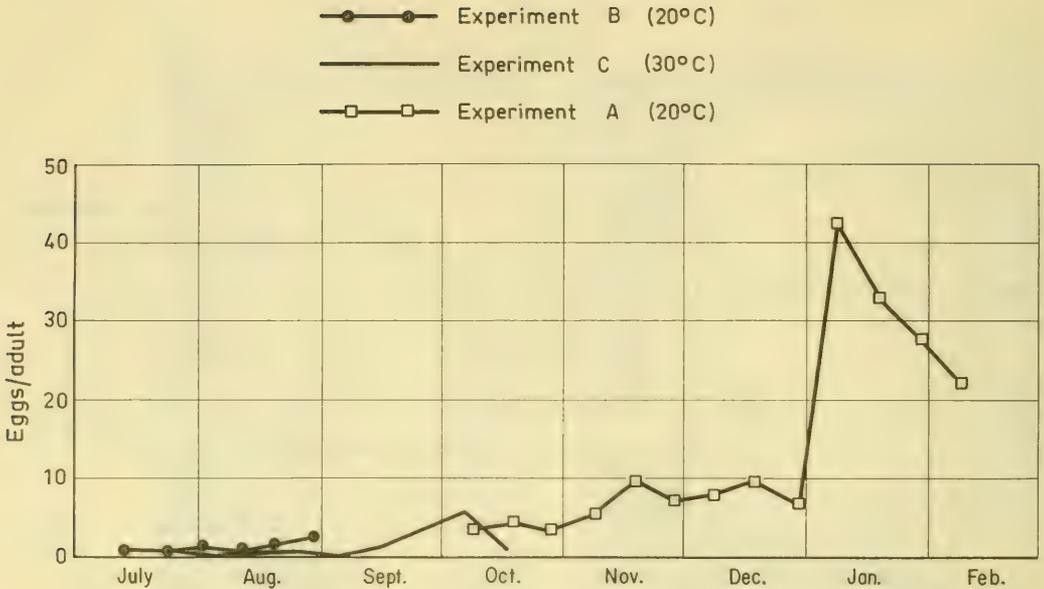


FIG. 11. Mean egg-production of controls (calculated for 10-day periods) from experiments carried out at different periods of the year.

54 and 110 KR (at $2 \text{ KR} \cdot \text{min}^{-1}$). From the data listed in Table 3 it is evident that the mortality of adults was not much affected by temperature when irradiated with 28 KR and 54 KR.

At 10°C no animal, whether control or irradiated, produced eggs. For this reason, the author compared the fecundity data obtained from the experiment at 30°C with those from Experiment B, which had been carried out at 20°C (Table 2). From this table it is evident that radiation-induced change in egg production and fecundity are markedly affected by temperature.

The deleterious effect of x-rays on the reproductive characteristics of snails kept at 30°C are also clearly shown in Figs. 9 and 10. For instance, the period of egg-production of the irradiated snails was shortened as compared to that of controls and of snails exposed to the same dose and kept at 20°C (Fig. 3). In addition, the number

of eggs per capsule and of capsules per adult was markedly reduced. No egg produced by the irradiated animals was fertile. For the controls at 30°C the mean value of fertility was 856 and that of viability 67.9%.

DISCUSSION AND CONCLUSIONS

The effects of irradiation on the reproductive capacity of *Physa acuta* should be discussed at the outset since it is obviously the most radiosensitive of the processes which ultimately determine the organism's persistence within a given ecosystem. Irradiation reduced the fecundity of the adult and the viability and fertility of the eggs. Particularly interesting were the effects of radiation on egg viability. During the first 50 days after irradiation with 10 KR, the animals were totally sterile, that is, they produced eggs but these were not fertile.

Eggs produced after that time became increasingly fertile and soon hatchable, i.e. the viability of the embryo was restored. A similar but less striking, progression of events was observed when snails were irradiated with only 2 KR, whereas 100 KR totally destroyed the reproductive capacity of this species. The ultimate cause of the recovery observed at the lower doses (and of the other reproductive changes) probably lies in vast shifts of the cell populations which constitute the reproductive tissues. In very general terms it may be proposed that irradiation destroys cells in certain radiosensitive stages of development, such as ova and spermatozoa. These cells are eventually replaced by less radiosensitive ones which regain their mitotic capacity and eventually produce functional germ cells.

Perlowagora-Szumlewicz (1964d) also observed a recovery of fertility in *Australorbis glabratus* irradiated with 4980 and 7105 R.

The effects of radiation on the reproductive capacity (or, at least, the fecundity) of *Physa* are amplified by increasing the temperature from 20° C to 30° C. Therefore, any prediction of the final results of radiation on the demographic picture must include in its formulation the environmental temperature.

The fecundity (i.e. index of egg production) of untreated *Physa* was very different for different experiments. The value for Experiment A (6762) was about 5x that of Experiment B (1273) and of Experiment C (1261). For the latter experiment, carried out at 30° C, the values of fecundity for the control were calculated for the period before and after irradiation. The value for the former period (mid-July to mid-August) was 420 and that for the latter (mid-August to mid-October) was 1261. In summary, the values of fecundity for the controls of our experiments were: 420 (July-August), 1261 (August-October), 1273 (August-September) and 6762 (October-February). Besides, as regards the observations carried from October to

February, the values calculated for October-November were lower, those from December-February again higher. It would thus appear that the fecundity of *Physa* increases from summer to winter (Fig. 11). This view is supported by several other observations on *Physa acuta* kept in the laboratory under constant conditions.

Fecundity, viability and fertility data for the controls at 30° C were very similar to the data noted for the controls kept at 20° C and observed during the same summer months, but very different from those calculated for the controls kept at 20° C during autumn and winter. From this comparison it seems that the internal cycle may influence the rate of increase more strongly than temperature. It is noteworthy that the ratio (5.3) between the fecundity values of the controls in the experiment (A) carried during October-February and that (B) during August-September was very similar to the ratio (5.1) between the fecundity of the groups exposed to the highest dose in these same 2 experiments: 100 KR in the former and 110 KR in the latter. Since in both experiments the highest dose reduced the fecundity to 1/3 that of the control (see Tables 1 and 2), the effect of irradiation on fecundity seems independent from the absolute value of the fecundity and, consequently, from its seasonal cycle.

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RESUMEN

EFECTOS DE LOS RAYOS-X EN LAS CARACTERISTICAS DEMOGRAFICAS DE *PHYSA ACUTA* (GASTROPODA: BASOMMATOPHORA)

O. Ravera

Los efectos fueron determinados por las medidas de mortalidad, fecundidad y fertilidad del adulto y la viabilidad de sus huevos. Los adultos se expusieron a rayo sin filtro (300 KV, 10 mA) en dosis variando entre 2 y 220 KR. La frecuencia de las dosis fue entre 1 y 2 KR min.⁻¹. Comparados con los controles: la mortalidad de individuos irradiados a 10 KR fue casi similar; a 54 KR no hubo diferencia significativa hasta los 20 días de irradiación; a 100 KR se elevó a 95% en el 18^o día; todos los caracoles murieron en un día a 220 KR. Producción de cápsulas ovígeras y el número de huevos contenidos fue reducido por irradiación; pero no mucho hasta 10KR. Una dosis de 2 KR redujo viabilidad y fertilidad. Recuperación de los tejidos germinales se notó en individuos irradiados con 2 y 10 KR. Mantenimiento de altas temperaturas aumentaron el efecto de irradiación sobre el proceso reproductivo de los adultos en proporción mayor a la supervivencia.

АБСТРАКТ

ВЛИЯНИЕ РЕНТГЕНОВСКИХ ЛУЧЕЙ НА ХАРАКТЕРИСТИКУ НАСЕЛЕНИЯ *PHYSA ACUTA* (GASTROPODA: BASOMMATOPHORA)

O. Равера

Определялось влияние рентгеновских лучей на характеристику и изменения населения *Physa acuta* Draparnaud, путем измерения степени отмирания, плодовитости, скорости прироста взрослых форм и выживаемости яиц этих моллюсков. Взрослые моллюски подвергались воздействию пучка прямых (незаэкранированных) рентгеновских лучей (300 KV, 10 mA) при дозировке от 2 до 220 KR. Сила облучения изменялась от 1 до 2 KR/мин. Отмирание животных, облученных при 10 KR было сходно с контрольным; при 54 KR оно отличалось от контрольных лишь незначительно в течение периода 20 дней после облучения; при 100 KR отмирание на 18ый день достигло 95%; при 220 KR все моллюски были убиты в течение одного дня. После облучения, силой в 10 KR продукция яйцевых капсул и количество яиц в них уменьшалось незначительно; доза в 2 KR уменьшила выживаемость и плодовитость. У моллюсков, облученных при 2 и 10 KR наблюдалось ясное восстановление зародышевой тканм. При повышении температуры содержания моллюсков влияние облучения на процессы размножения взрослых форм сказывались сильнее, чем на их выживаемости.

CHROMOSOME NUMBERS AND SYSTEMATICS IN STREPTONEURAN SNAILS^{1,2}

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ABSTRACT

Information has been compiled of present knowledge on chromosomes of streptoneuran snails. Haploid chromosome numbers range from 7 to 36. Within the Streptoneura, the Archaeogastropoda ($n=9-18$) and Mesogastropoda ($n=7-20$) have relatively low numbers, whereas the more advanced order Neogastropoda ($n=13-36$) has higher numbers, i. e., with the exception of 1 species, $n=28$ or more.

The Streptoneura, like the Euthyneura, are conservative in regard to chromosome numbers, the variation in chromosome numbers seldom exceeding ± 2 bivalents in the lower taxa. There appears to be no general clear-cut correlation between low chromosome numbers and "primitiveness" among the various groups, except perhaps in the Viviparacea.

Polyploidy, suspected in the Hydrobiidae (Sanderson, 1940) and reported in the Thiaridae (Jacob, 1959a), needs to be confirmed in both.

Two studies on chromosomes of hybrids in the genera *Oncomelania* and *Purpura*, the only such studies in the Mollusca, have given results promoting a clearer understanding of inter- and intraspecific morphological variation in their immediate taxa.

Sex chromosomes have been reported in several species; and, in at least 2 species, *Pomatiopsis cincinnatiensis* and *P. lapidaria*, they exhibit differential specific characters.

Since the chromosomes of less than 0.3% of currently recognized streptoneuran species have been studied critically, cytological information is extremely fragmentary. The current review is presented as a background for further cytotaxonomic studies in the Streptoneura.

Chromosome studies and cytotaxonomy have been of considerable interest to molluscan systematists in the past few years. Current information available on chromosome numbers and systematics in euthyneuran snails has recently been presented by Burch (1965). Although the Streptoneura are a group as large as the Euthyneura, if not larger, there is less information on its

chromosome numbers. Only 105 species have been investigated in the Streptoneura, in contrast to about 275 species for the Euthyneura. Streptoneuran mollusks are especially interesting for chromosome investigations because most species have separate sexes and are therefore useful for studies of sex determining mechanisms in addition to cytotaxonomy.

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²This paper is dedicated to Dr. Berwind P. Kaufmann on the occasion of his seventieth birthday.

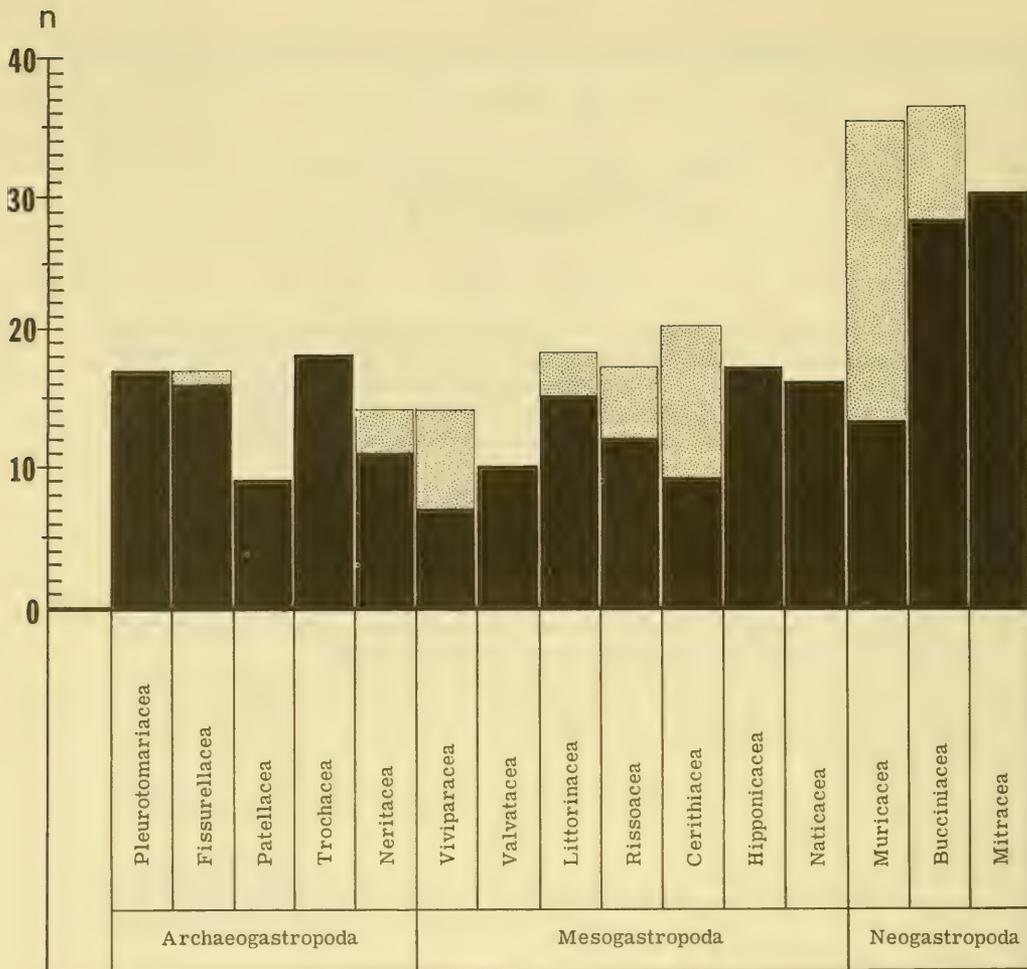


FIG. 1. Haploid chromosome numbers (n) in the streptoneuran superfamilies. The stippled areas indicate the range between the lowest and highest numbers known. The polyploid chromosome numbers (in the Cerithiacea) are not shown.

The earliest published reports of chromosome studies of streptoneuran mollusks date back to the early 1900's. However, due to the limitations of optical equipment and inferior cytological techniques, many of these early reports are inaccurate and unreliable. These early reports were based upon material prepared by paraffin section technique which compresses the chromosomes to as much as $1/3 - 1/2$ of their size, as seen in acetic-orcein squash preparations, the technique used in our labora-

tory. Because of this compressed condition, accurate chromosome counts are often difficult; nor can the morphological characteristics of the chromosomes be easily discerned, which makes karyotype analyses almost impossible. Thus, information from older reports which were based upon such inadequate techniques are not included in this paper. They first need to be verified with the use of more modern techniques and optical equipment.

The reliable and pertinent papers upon

which this discussion is based include the following: Franz (1932), Pollister & Pollister (1940, 1943), Staiger (1950, 1954), Inaba (1958, 1965), Ramamoorthy (1958), Jacob (1959a, b), Burch (1960), Nishikawa (1962), Rainer (1963), Patterson (1963, 1965, 1966) and Lutfy & Demian (1964). Some additional information included in this paper is from recent unpublished work of Dr. Burch and myself.

REVIEW OF CHROMOSOME NUMBERS

Taylor & Sohl's (1962) "Outline of Gastropod Classification" lists 121 recent families for the gastropod subclass Streptoneura. Reliable information on chromosome numbers is available for only 26 of them, or 1 out of every 5. If one considers genera and subgenera, the dearth of information is even more striking: of an estimated 2800 recent genera and subgenera of the Streptoneura, information on chromosome numbers exists only for 64. This is a ratio of information of 1:45 only. In the Archaeogastropoda there are 21 families, for 7 of which we have chromosome information. The Mesogastropoda contains 81 families, with chromosome numbers available for only 13; and in the Neogastropoda there are 19 families, with chromosome numbers known in 6 of them.

Although the information for such a large and diverse group of gastropods is but fragmentary, the existing information nevertheless needs to be reviewed and summarized.

Haploid chromosome numbers of the order Archaeogastropoda range from a low of 9 in the superfamily Patellacea to a high of 18 in the superfamily Trochacea (Fig. 1). In the order Mesogastropoda, they range from 7 haploid in the Viviparidae, to 20 haploid in the Pleuroceridae (Cerithiacea), excluding possible polyploidy in the Thiaridae. However, in the advanced Neogastropoda, the haploid chromosome numbers all range from

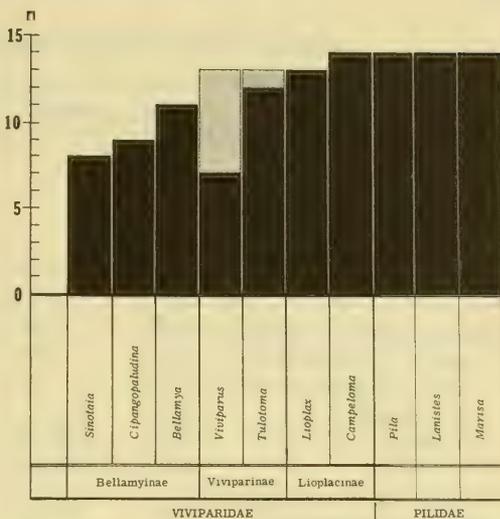


FIG. 2. Haploid chromosome numbers (n) in the Viviparacea. The stippled areas indicate the range within the genera.

28 - 36, except for the muricid *Purpura lapillus*, which is reported to have 13 and 18 pairs of chromosomes (Staiger, 1954).

Reliable information concerning chromosome numbers is available for 25 species of the order Archaeogastropoda (Table 1). Of the 3 recent families of the superfamily Pleurotomariacea, only 1 species, *Haliotis japonicus*, representing the family Haliotidae, has been investigated cytologically: it has a chromosome number of $n=17$. In the superfamily Fissurellacea there is only 1 family, the Fissurellidae, of which 3 species have been investigated: *Macroschisma sinensis*, $n=16$; *M. dilatata*, $n=16$; and *Clypina picta* with $n=17$. In the Patellacea there are 3 recent families, with chromosome information available for 2 of them, the Acmaeidae and Patellidae. All members of these 2 families have been found to have the haploid chromosome number of $n=9$. Of the 6 recent families in the superfamily Trochacea, chromosome data are available for only 2: the Trochidae and Turbinidae. In both of these families all species have been found to have the

TABLE 1. Haploid chromosome numbers in the Archaeogastropoda

Species	Haploid No.	Source	Reference
Order Archaeogastropoda			
Superfamily			
Pleurotomariacea			
Family Haliotidae			
<i>Haliotis japonica</i>	17	Japan	Nishikawa, 1962
Superfamily Fissurellacea			
Family Fissurellidae			
<i>Macroschisma sinensis</i>	16	Japan	Nishikawa, 1962
<i>M. dilatata</i>	16	Japan	Nishikawa, 1962
<i>Clypina picta</i>	17	Japan	Nishikawa, 1962
Superfamily Patellacea			
Family Acmaeidae			
<i>Patelloida saccharina lanx</i>	9	Japan	Nishikawa, 1962
<i>P. pygmaea</i>	9	Japan	Nishikawa, 1962
<i>P. lampanicola</i>	9	Japan	Nishikawa, 1962
<i>Notacmea schrenckii</i>	9	Japan	Nishikawa, 1962
<i>N. concinna</i>	9	Japan	Nishikawa, 1962
<i>N. fuscoviridis</i>	9	Japan	Nishikawa, 1962
Family Patellidae			
<i>Cellana toreuma</i>	9	Japan	Nishikawa, 1962
<i>C. eucosmia</i>	9	Japan	Nishikawa, 1962
<i>C. nigrolineata</i>	9	Japan	Nishikawa, 1962
Superfamily Trochacea			
Family Trochidae			
<i>Cantharidus collichroa</i>	18	Japan	Nishikawa, 1962
<i>Thalotia japonica</i>	18	Japan	Nishikawa, 1962
<i>Monodonta labio</i>	18	Japan	Nishikawa, 1962
<i>M. neritoides</i>	18	Japan	Nishikawa, 1962
<i>Tegula lischkei</i>	18	Japan	Nishikawa, 1962
<i>T. nigerrima</i>	18	Japan	Nishikawa, 1962
<i>T. rustica</i>	18	Japan	Nishikawa, 1962
<i>T. pfeifferi carpenteri</i>	18	Japan	Nishikawa, 1962
Family Turbinidae			
<i>Turbo cornutus</i>	18	Japan	Nishikawa, 1962
<i>Lunella coronata coreensis</i>	18	Japan	Nishikawa, 1962
<i>Astralium haematragum</i>	18	Japan	Nishikawa, 1962
Superfamily Neritacea			
Family Neritidae			
<i>Puperita japonica</i>	11	Japan	Nishikawa, 1962
<i>Clithon retropictus</i>	12	Japan	Patterson, 1967
<i>Dostia violacea</i>	14	Japan	Patterson, 1967

TABLE 2. Haploid chromosome numbers in the Mesogastropoda

Species	Haploid No.	Source	Reference
Order Mesogastropoda			
Superfamily Viviparacea			
Family Viviparidae			
Subfamily Bellamyinae			
<i>Sinotaia histrica</i>	8	Japan	Inaba, 1965
<i>Cipangopaludina malleata</i>	9	U. S. A.	Pollister & Pollister, 1940, 1943
<i>C. malleata</i>	9	Japan	Inaba & Tanaka, 1953
<i>Bellamyia dissimilis</i>	11	India	Ramamoorthy, 1958
<i>B. bengalensis</i>	11	India	Ramamoorthy, 1958
Subfamily Viviparinae			
<i>Viviparus contectus</i>	7	Europe	Franz, 1932
<i>V. contectus</i>	7	Europe	Rainer, 1963
<i>V. ater</i>	9	Europe	Franz, 1932
<i>V. ater</i>	9	Europe	Rainer, 1963
<i>V. viviparus</i>	10	Europe	Franz, 1932
<i>V. viviparus</i>	10	Europe	Rainer, 1963
<i>V. georgianus</i>	12	U. S. A.	Pollister & Pollister, 1940, 1943
<i>V. contectoides</i>	13	U. S. A.	Pollister & Pollister, 1940, 1943
<i>V. contectoides</i>	13	U. S. A.	Patterson, (unpublished)
<i>V. intertextus</i>	13	U. S. A.	Pollister & Pollister, 1940
<i>Tulotoma magnifica</i>	12	U. S. A.	Pollister & Pollister, 1940, 1943
<i>T. angulata</i>	13	U. S. A.	Patterson, 1965
Subfamily Lioplacinae			
<i>Lioplax subcarinata</i>	13	U. S. A.	Pollister & Pollister, 1940, 1943
<i>Campeloma decisum</i>	13 or 14	U. S. A.	Pollister & Pollister, 1940
<i>C. ponderosum</i>	14	U. S. A.	Pollister & Pollister, 1940, 1943
<i>C. p. coarctatum</i>	14	U. S. A.	Patterson (unpublished)
<i>C. subsolidum</i>	13 or 14	U. S. A.	Pollister & Pollister, 1940
Family Pilidae			
<i>Pila ovata</i>	14	Egypt	Lutfy & Demian, 1964
<i>Lanistes bolteni</i>	14	Egypt	Lutfy & Demian, 1964
<i>Marisa cornuarietis</i>	14	Egypt	Lutfy & Demian, 1964
Superfamily Valvatacea			
Family Valvatidae			
<i>Valvata tricarinata</i>	10	U. S. A.	Burch, (pers. comm.)
Superfamily Littorinacea			
Family Littorinidae			
<i>Nodilittorina picta</i>	15	Japan	Nishikawa, 1962
<i>N. granularis</i>	18	Japan	Nishikawa, 1962
<i>Littorina brevicula</i>	17	Japan	Nishikawa, 1962
<i>Littoraria strigata</i>	17	Japan	Nishikawa, 1962
Superfamily Rissoacea			
Family Hydrobiidae			
<i>Pomatiopsis</i>			
<i>cincinnatiensis</i>	16	U. S. A.	Burch, 1960
<i>P. cincinnatiensis</i>	16	U. S. A.	Patterson, 1963

TABLE 2. (continued)

Species	Haploid No.	Source	Reference
<i>P. lapidaria</i>	17	U. S. A.	Burch, 1960
<i>P. lapidaria</i>	17	U. S. A.	Patterson, 1963
<i>P. californica</i>	17	U. S. A.	Burch, (pers. comm.)
<i>Oncomelania formosana</i>	17	Formosa	Burch, 1960
<i>O. formosana</i>	17	Formosa	Patterson, 1963
<i>O. nosophora</i>	17	Japan	Burch, 1960
<i>O. hupensis</i>	17	China	Burch, 1960
<i>O. quadrasi</i>	17	Philippines	Burch, 1960
Family Bithyniidae			
<i>Bithynia usseriensis</i>	17	Japan	Patterson, 1967
<i>Mysorella costigera</i>	17	India	Patterson, (unpublished)
Family Assimineidae			
<i>Assimineea japonica</i>	12	Japan	Patterson, 1967
<i>A. parasitologica</i>	12	Japan	Patterson, 1967
<i>A. castanea</i>	15	Japan	Patterson, 1967
<i>A. yoshidayukioi</i>	15	Japan	Patterson, 1967
Superfamily Cerithiacea			
Family Thiaridae			
<i>Melanoides tuberculatus</i>	16	India	Jacob, 1959a
<i>M. tuberculatus</i> (polyploid)	45-47	India	Jacob, 1959a
<i>M. (Tarebia) lineatus</i>	35-36	India	Jacob, 1959a
<i>Thiara scabra</i>	38-39	India	Jacob, 1959a
<i>Melania (Radina) crenulata</i>	18	India	Jacob, 1959a
<i>Paludomus tanschaurica</i>	19	India	Jacob, 1959a
Family Pleuroceridae			
<i>Semisulcospira habei</i>	8	Japan	Burch & Davis (pers. comm.)
<i>S. decipiens</i>	12	Japan	Burch & Davis (pers. comm.)
<i>S. niponica</i>	12	Japan	Burch & Davis (pers. comm.)
<i>S. reticulata</i>	12	Japan	Burch & Davis (pers. comm.)
<i>S. nakasekoe</i>	13	Japan	Burch & Davis (pers. comm.)
<i>S. multigranosa</i>	14	Japan	Burch & Davis (pers. comm.)
<i>S. kurodai</i>	18	Japan	Burch & Davis (pers. comm.)
<i>S. libertina</i>	18	Japan	Patterson, 1967
<i>S. libertina</i>	18	Japan	Burch & Davis (pers. comm.)
<i>S. ornata</i>	18	Japan	Patterson, 1967
<i>S. trachea</i>	18	Japan	Patterson, 1967
<i>S. reiniana</i>	20	Japan	Burch & Davis (pers. comm.)
Family Potamididae			
<i>Cerithidea rhizophorarum</i>	18	Japan	Nishikawa, 1962
<i>C. cingulata</i>	18	Japan	Nishikawa, 1962
<i>C. djadjariensis</i>	18	Japan	Nishikawa, 1962
<i>Batillaria zonalis</i>	18	Japan	Nishikawa, 1962
<i>B. multiformis</i>	18	Japan	Nishikawa, 1962

TABLE 2 (continued)

Species	Haploid No.	Source	Reference
Family Cerithiidae			
<i>Proclava kochi</i>	18	Japan	Nishikawa, 1962
<i>Contumax kobelti</i>	18	Japan	Nishikawa, 1962
Superfamily Hipponicacea			
Family Hipponicidae			
<i>Amalthea conica</i>	17	Japan	Inaba, 1958
Superfamily Naticaacea			
Family Naticidae			
<i>Neverita didyma</i>	16	Japan	Nishikawa, 1962

chromosome number $n=18$. In the superfamily Neritacea with 6 recent families, only 3 species representing 1 family, the Neritidae, have been investigated. These species and their haploid chromosome numbers are *Puperita (Heminerita) japonica* ($n=11$), *Clithon retropictus* ($n=12$) and *Dostia violacea* ($n=14$).

A total of 66 species of the Mesogastropoda have been investigated cytologically (Table 2). There are 2 recent families in the Viviparacea, the Viviparidae and Pilidae. In the family Viviparidae, the most primitive subfamily, Bellamyinae, has a range of haploid chromosome numbers from 8 - 11 (Fig. 2). In the subfamily Viviparinae the haploid numbers range from 7 - 13, while in the most advanced subfamily of this group, the Lioplacinae, the range is from 13 - 14 haploid. The members of the closely related and somewhat more advanced family Pilidae that have been examined have haploid chromosome numbers of $n=14$.

The superfamily Valvatacea has one recent family, the Valvatidae, with a haploid chromosome number of 10 reported for 1 species.

The Littorinacea contains 4 recent families, with cytological information available in only 1, the Littorinidae. The chromosome numbers in this family range from 15 in *Nodilittorina picta* to $n=18$ in *N. granularis*.

The superfamily Rissoacea is a large and diverse one, with 18 recent families. Chromosome numbers are known in only 3 of these, the Hydrobiidae, Bithyniidae and Assimineidae. In the Hydrobiidae the chromosome numbers for the 7 species investigated are $n=16$ and $n=17$. Rhein (1935) reported the diploid chromosome number of *Potamopyrgus jenkinsi* from Europe to be 20-22. Sanderson (1940) found British specimens to have a diploid number of 36-44 and suggested that the British specimens may be a tetraploid race derived from the Continental diploid race. More exact cytological studies are needed to confirm the existence of polyploidy in *P. jenkinsi*. Only 2 species of the Bithyniidae have recently been studied cytologically: *Mysorella costigera* from India and *Bithynia usseriensis* from Japan. Both have a haploid chromosome number of $n=17$.

Four Japanese species in the family Assimineidae have been studied cytologically. The haploid chromosome number of *Assimineia japonica* and *A. parasitologica* is $n=12$ while *A. castanea* and *A. yoshidayukioi* have $n=15$.

Of the 17 recent families in the Cerithiacea, only 3 have representatives with known chromosome numbers. In the Thiaridae, the range in haploid chromosome numbers is from 16 to 45-47. Jacob (1959a) reports *Melanoides*

TABLE 3. Haploid chromosome numbers in the Neogastropoda

Species	Haploid No.	Source	Reference
Order Neogastropoda			
Superfamily Muriceae			
Family Muricidae			
<i>Bedevina birileffi</i>	30	Japan	Nishikawa, 1962
<i>Purpura lapillus</i>	13, 18	France	Staiger, 1950, 1954
<i>P. bronni</i>	30	Japan	Nishikawa, 1962
<i>P. clavigera</i>	30	Japan	Nishikawa, 1962
<i>P. luteostoma</i>	30	Japan	Nishikawa, 1962
<i>Chicoreus asianus</i>	34	Japan	Nishikawa, 1962
<i>Hexaplex trunculus</i>	35	France	Staiger, 1950
<i>Tritonalia erinaceus</i>	35	France	Staiger, 1950
Superfamily Buccinaceae			
Family Pyrenidae			
<i>Columbella versicolor</i>	28	Japan	Nishikawa, 1962
<i>C. rustica</i>	34	France	Staiger, 1950
<i>Anachis misera</i>	32	Japan	Nishikawa, 1962
<i>Pyrene bicineta</i>	34	Japan	Nishikawa, 1962
<i>P. testudinaria tylerae</i>	35	Japan	Nishikawa, 1962
Family Buccinidae			
<i>Pisania ferrea</i>	35	Japan	Nishikawa, 1962
<i>P. maculosa</i>	35	France	Staiger, 1950
<i>Euthria cornea</i>	35	France	Staiger, 1950
<i>Buccinum undatum</i>	35	France	Staiger, 1950
<i>Cantharus subrubiginosus</i>	35	Japan	Nishikawa, 1962
<i>Babylonia japonica</i>	36	Japan	Nishikawa, 1962
Family Nassariidae			
<i>Nassarius livescens</i>	34	Japan	Nishikawa, 1962
<i>Tritia festiva</i>	34	Japan	Nishikawa, 1962
Family Fascioliariidae			
<i>Fasciolaria lignaria</i>	35	France	Staiger, 1950
Superfamily Mitraceae			
Family Mitridae			
<i>Pusia hizenensis</i>	30	Japan	Nishikawa, 1962

tuberculatus to have a haploid number of $n=16$ and, what he calls the polyploid race of this species, to have $n=45-47$. Jacob reported *Melania (Radina) crenulata* to have $n=18$ and *Pahdomus tanschaurica* to have $n=19$. Because of the uncertainty of Jacob's results for the polyploid race, more exacting studies are desirable. Similarly, it would be beneficial to study more thoroughly the chromosomes of *Melanoides (Tarebia) lineatus* and *Thiara scabra*, which were reported to have

chromosome numbers of $n=35-36$ and $n=38-39$ respectively.

In the family Pleuroceridae, 11 species of *Semisulcospira* have chromosome numbers that range from 8-20 haploid. *S. libertina* was reported by Inaba & Tanaka (1953) to have a haploid chromosome number of $n=8$. However, during recent studies in Japan, I examined many populations of this species, including specimens from the type locality, and all had a haploid chromosome number

of $n=18$. Furthermore, Burch & Davis (personal communication) also found *S. libertina* to have 18 bivalents. They have extended chromosome studies that provide data on 8 additional species of *Semisulcospira*.

All 7 members of the families Potamididae and Cerithiidae so far studied have a haploid chromosome number of $n=18$.

There are 3 recent families in the superfamily Hipponicacea, with chromosome information available for 1 family, the Hipponicidae. *Amalthea conica* has a haploid chromosome number of $n=17$.

The superfamily Naticacea contains only 1 family, the Naticidae, in which *Neverita (Glossoulax) didyma* has a chromosome number of $n=16$.

The species of the order Neogastropoda (Table 3) investigated cytologically number 23. There are 2 recent families in the superfamily Muricacea, with information on chromosome numbers in 1 of these families, Muricidae. Four species have haploid chromosome numbers of 30, 1 species a haploid number of 34 and 2 species with the haploid number 35. In addition, the haploid numbers 13 and 18 are found in different populations of one species, *Purpura lapillus*. These latter numbers are by far the lowest yet found in the Neogastropoda.

There are chromosome numbers for members of 4 of the 7 recent families of the Buccinacea. In the family Pyrenidae the haploid numbers range from $n=28$ for *Columbella (Euplica) versicolor* to $n=35$ for *Pyrene testudinaria tylerae*.

Five members of the family Buccinidae have haploid numbers of 35 while *Babylonia japonica* has a chromosome number of $n=36$, the highest number known in the Neogastropoda.

In the family Nassariidae, *Nassarius (Niota) livescens* and *Tritia (Hiria) festiva* both have the haploid chromosome number of $n=34$.

In the Fascioliariidae, *Fasciolaria lignaria* has the haploid number 35.

There is 1 recent family in the superfamily Mitracea. *Pusia hizenensis*, of the family Mitridae, has a haploid chromosome number of 30.

The haploid chromosome numbers reliably reported in the various superfamilies of the Streptoneura are shown in Fig. 1. The stippled areas represent ranges of variation in chromosome numbers found in the superfamilies of the respective suborders.

CHROMOSOME CYTOLOGY OF HYBRIDS

As far as the author knows there have been only 2 studies on the chromosomes of hybrids of known origin in the Streptoneura. The first study is that of Staiger (1954) on various populations of the muricid *Thais (Purpura) lapillus*. In hybrid populations between an $n=13$ and an $n=18$ form, individuals were found in which, during meiosis, 5 metacentric chromosomes are paired with 10 acrocentrics. Presumably the 10 acrocentrics arose by splitting of the centromeres of 5 metacentric chromosomes. The arms of the 10 acrocentric chromosomes remain homologous to the 10 arms of the 5 metacentric chromosomes of the 13 chromosome race.

Burch (1964) studied F_1 hybrids of the 4 nominal species of the Oriental hydrobiid genus *Oncomelania*, at Michigan, several years ago. All specimens studied of each of the populations had 17 pairs of chromosomes, and an examination of late prophase or diakinesis chromosomes of the various F_1 hybrids revealed no apparent anomalies that did not also occur as prevalently in the normal parents. Only normal bivalents with 1, 2 or 3 chiasmata were observed, and no univalents, trivalents or quadrivalents were found. In addition, all segments of each chromosome seemed to pair completely. Considering this information in conjunction with the great ease of hybridizing the various nominal species of *Oncomelania*, the non-reduced viability of the hybrids, and the morpho-

logical and biochemical similarities of the parent populations, it was concluded that these 4 nominal species, although isolated and widely separated geographically, were in reality only races of 1 species. Recent attempts to cross species of *Oncomelania* with species of the closely related North American *Pomatiopsis* failed.

SEX CHROMOSOMES

Sex chromosomes have been reported in 9 species of marine gastropods, 3 freshwater species, *Melania crenulata*, *Paludomus tanschaurica* and *Tulotoma angulata*, and 2 amphibious species, *Pomatiopsis lapidaria* and *P. cincinnatiensis*. All of the investigations on sex chromosomes in marine gastropods were published before 1931 and were based upon material prepared by the paraffin section technique. Concerning these reports Nishikawa (1962) states: "According to the author's view, the X-element designated by some authors is no other than the chromosome which is mechanically displaced unusually by the influence of technical procedures." In his chromosome study of 53 species of marine gastropods, Nishikawa found no evidence of sex chromosomes. The inability of Nishikawa and others to identify sex chromosomes may be due partly to inadequacies of the paraffin section technique.

One of the recent reports of sex chromosomes in mollusks is that of Jacob (1959a,b). He reports *Melania crenulata* to have a chromosome number of $2n=35$, with an X-O sex determining mechanism. These data need to be verified since the material was prepared by the paraffin section technique and the morphology of the chromosomes is so obscure that an accurate karyotype analysis is extremely difficult. Jacob further reports *Paludomus tanschaurica* to have a chromosome number of $2n=38$, with X and Y sex chromosomes present in the males. Although these observations were from squash preparations,

only male meiotic cells were examined, hence the evidence is not yet conclusive.

Burch (1960) and Patterson (1963) have shown the sex determining mechanisms in 2 species of *Pomatiopsis*. *P. lapidaria* has a male diploid chromosome number of 33 with an X-O sex determining mechanism. The X chromosome is the largest of the complement and in the male it has no corresponding homologue. In *P. cincinnatiensis* the diploid number is 32 with a σ X-Y sex determining mechanism. In σ *P. cincinnatiensis* the X and Y elements are the 2 largest chromosomes of the complement, with one medianly constricted and the other subterminally constricted. Neither of these has a homologue and hence they presumably are dimorphic sex chromosomes. Female members of the species must be investigated before one can designate which of these dimorphic sex chromosomes is the X and which is the Y.

Sex chromosomes in both male and female individuals of the viviparid species, *Tulotoma angulata*, have been studied (Patterson, 1965). The somatic karyotype of *T. angulata* consists of 3 pairs of metacentric chromosomes, 7 pairs of sub-metacentrics and 2 pairs of almost acrocentric chromosomes. Two relatively large chromosomes in spermatogonial metaphase cells cannot be matched with morphologically similar homologues, as was the case in *P. cincinnatiensis*. One is medianly constricted, the other is distinctly submedianly constricted. These are presumably the sex chromosomes, one being an "X", the other a "Y". In oögonial metaphase cells, the corresponding submedianly constricted chromosome is duplicated and the metacentric chromosome is lacking. This indicates that the sub-metacentric chromosomes are the X's and that the metacentric is a Y chromosome. Thus, in *T. angulata*, the sex-determining mechanism is XX in the female and XY in the male.

Therefore, we conclude that morphologically distinguishable sex chromo-

some do exist in some species of the Streptoneura and probably can be demonstrated in a great number of other species if proper techniques are used and the pertinent division stages are found.

CHROMOSOME NUMBERS AND SYSTEMATICS

Changes in chromosome numbers in animals, other than those changes produced by polyploidy, may occur in several ways: 1) by chromosomal fusion; 2) by fragmentation; 3) by mitotic or meiotic non-disjunction; and 4) by translocations involving a supernumerary fragment serving as a donor of a centromere and 2 telomeres. In the first case, the result is a decrease in chromosome number, and in the other 3 cases, an increase in number. It has been shown by Burch (1965) that, in the Euthyneura, chromosome numbers of the various taxa are, in general, extremely conservative, which indicates that mechanisms responsible for addition or deletion of chromosomes in that subclass must operate at a very low frequency or efficiency. Since the lower numbers almost invariably go with lower groups, chromosomal fusion is probably a very rare occurrence in the Euthyneura.

In the Streptoneura, the case does not seem so clear-cut; or at least in many instances it is impossible to correlate lower chromosome numbers with species considered "primitive" by the taxonomists and morphologists. Nevertheless, there are some correlations which are worth mentioning. First, however, it should be pointed out that in the Streptoneura there is also evidence of a considerable amount of conservativeness in chromosome numbers. For example, all 9 species studied of the 2 Patellacea families have the haploid chromosome number 9. All 11 species studied of the 2 Trochacea families have the haploid number 18. All 3 genera of the Pilidae have the haploid number 14. And in

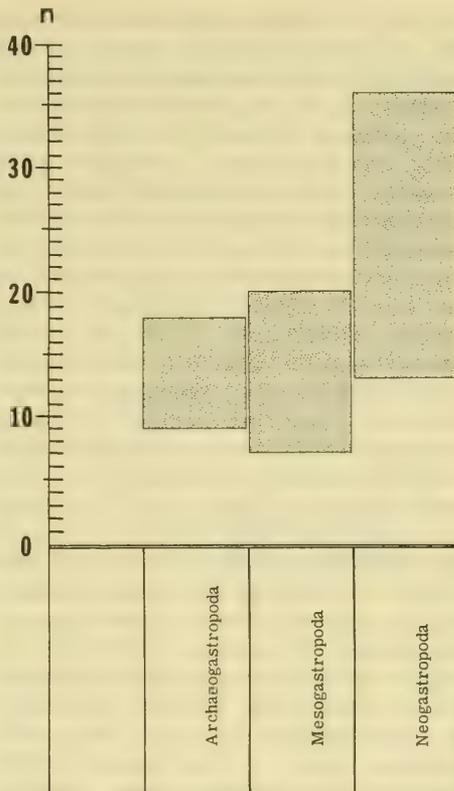


FIG. 3. Range of haploid chromosome numbers (n) in the streptoneuran orders. Polyploid chromosome numbers (in the Mesogastropoda) are not shown.

those families or genera where there is chromosomal number variation, this variation is usually not more than ± 2 bivalents. Sometimes, however, the variation in a lower taxon was found to be great: 5 different chromosome numbers with a maximum variation of 12 was found in the genus *Semisulcospira*, and a variation of 6 in a single species of *Purpura* (see below). Such variation, however, is not a common occurrence.

The range of haploid chromosome numbers in the Archaeogastropoda is from 9-18 (Fig. 3); in the Mesogastropoda it is from 7-20; and in the Neogastropoda the range is from 13 - 36. In the latter case, the lower limit of 13 is due to a single species, *Purpura*

lapillus. All other species have at least 28, or usually 30, or more, pairs of chromosomes. The high numbers of the Neogastropoda, the most advanced order, are perhaps the most striking feature about this histogram. It might further be mentioned that Nishikawa (1962) found difficulty in explaining the great differences in the chromosome numbers reported for *P. lapillus* ($n=13$; $n=18$), and the other 3 species of *Purpura* that he studied, which had the haploid number 30. He suggested that the basic number of the order is one of the higher numbers because of the great frequency of higher numbers found in the Neogastropoda.

Because of their wide range of chromosome numbers (Table 2; Fig. 2), it is especially interesting to compare the taxa within the Viviparacea. The more primitive viviparid subfamily Bellamyinae is characterized by having generally lower chromosome numbers ($n=8-11$). In the Viviparinae the range is $n=7-13$, with the greater number of species having haploid chromosome numbers above 11, the highest number reported in the Bellamyinae. Furthermore, the most advanced subfamily, the Lioplacinae, is characterized by haploid chromosome numbers of 13 and 14. The most advanced family of the Viviparacea, the Pilidae, have only the haploid number 14, the highest number found in the Viviparidae.

The above is a compilation of present knowledge on chromosomes of streptoneuran mollusks. Because only relatively few species have been studied, the gaps in our knowledge are large. But it did seem worthwhile to review the literature and to compile the references as a base for further studies. With the increased attention being given to the chromosomes of mollusks in recent years, it is hoped that rapid advances will soon be made in our knowledge of the chromosomes and their behavior in the large and diverse molluscan class Streptoneura.

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ADDENDUM

Butot and Kiauta have recently determined the chromosome number of 8 species belonging to the Rissoacea [Butot, L. J. M. & Kiauta, B., 1966, Notes on the cytology of Rissoacea. I. Cytotaxonomical conditions in some Hydrobiidae and Assimineidae (Gastropoda Streptoneura). Basteria, 30(2/3): 21-35]. Members of the family Hydrobiidae, *Hydrobia neglecta*, *H. stagnorum* and *H. ulvae* all had the haploid chromosome number $n=18$. In the Bithyniidae,

Bithynia leachi and *B. tentaculata* had 17 bivalents present during meiosis. *Assimineea grayana* (Assimineidae) had a haploid chromosome number of $n=12$.

The haploid chromosome numbers found in the 8 species studied by Butot & Kiauta have all been previously reported for other species of these families (see Table 2); thus, their information further illustrates the conservativeness in regard to chromosomal complement change in the Mollusca.

RESUMEN

NUMERO CROMOSAMATICO Y SISTEMATICA DE
CARACOLES ESTREPTONEUROS

C. M. Patterson

Se compila la información sobre el conocimiento actual de cromosomas de estreptoneuros. El número de cromosomas haploidos varía entre 7 y 36. Entre los Streptoneura, los Archaeogastropoda ($n=9-18$) y Mesogastropoda ($n=7-20$) tienen números relativamente bajos, mientras en el orden más avanzado Neogastropoda ($n=13-36$) es alto, con excepción de una especie, $n=28$ o más.

Los Streptoneura, igual que los Euthyneura, son conservativos en lo que concierne al número cromosomático, y la variación raramente excede + 2 bivalentes en los taxa inferiores. Aparentemente no hay correlación bien definida entre números bajos de cromosomas y la "primitividad" entre los varios grupos, excepto quizá en Viviparacea. Poliploidia, sospechada en los Hydrobiidae (Sanderson, 1940) y señalada para los Thiaridae (Jacobs, 1959a), necesita confirmación.

Dos estudios sobre cromosomas de híbridos, únicos en Mollusca, en *Oncomelania* y *Purpura*, han promovido una interpretación más clara de las variaciones morfológicas interespecíficas e intraespecíficas en sus grupos inmediatos. Cromosomas sexuales se han registrado en varias especies y, al menos una de ellas, *Pomatiopsis cincin-natiensis* y *P. lapidaria*, muestran caracteres específicos diferenciales.

Mientras que los cromosomas de menos del 0,3% de las especies de Streptoneura corrientemente reconocidas han sido estudiadas críticamente, la información citológica es fragmentaria en extremo. La revisión es presentada como un antecedente para futuros estudios citotaxonómicos en los Streptoneura.

АБСТРАКТ

ЧИСЛО ХРОМОСОМ И СИСТЕМАТИКА БРЮХОНОГИХ
МОЛЛЮСКОВ ИЗ STREPTONEURA

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В работе приводятся данные о современном состоянии наших знаний о хромосомах у брюхоногих моллюсков из подкласса Streptoneura. Число гаплоидных хромосом у них колеблется от 7 до 36. Относительно низкое число хромосом из группы Streptoneura имеют Archaeogastropoda ($n=9-18$) и Mesogastropoda ($n=7-20$), в то время, как более высоко организованные моллюски из Neogastropoda обладают большим числом хромосом ($n=13-36$), за исключением одного вида, имеющего 28 хромосом или даже больше. Моллюски из Streptoneura, как и из Euthyneura очень консервативны с точки зрения числа хромосом и изменения их количества, которое у более низких систематических категорий редко превышает ± 2 бивалента. Ясная корреляция между малым количеством хромосом и "примитивностью" моллюсков, видимо, отсутствует, за исключением, может быть Viviparacea. Полиплоидия, предполагаемая у Hydrobiidae (Sanderson, 1940) и указанная для

Thiaridae (Jacob, 1959a), нуждается в подтверждении.

Имеются два (единственные для моллюсков) исследования по хромосомам у гибридов из родов *Oncomelania* и *Purpura*, которые дали ясное представление о меж- и внутривидовых морфологических изменениях у близких таксонов из этих групп.

Половые хромосомы, которые были исследованы у нескольких видов, по крайней мере у двух (*Pomatiopsis cincinnatiensis*, и *P. lapidaria*) показывают видовую дифференциацию.

Поскольку лишь у 0,3% (или даже меньше) видов моллюсков из **Streptoneura** были просмотрены с точки зрения хромосом, цитологические данные по ним очень фрагментарны. Настоящий обзор представляет собой основу для дальнейшего цитотаксономического исследования моллюсков из **Streptoneura**.

CHROMOSOMES OF INTERMEDIATE HOSTS OF HUMAN BILHARZIASIS^{1, 2}

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ABSTRACT

The chromosomes have been studied of many species of the snail taxa which include intermediate hosts implicated in the transmission of human bilharziasis. Some of the results indicate that present taxonomy of these snails is unsatisfactory.

The occurrence of populations with differing polyploid chromosome numbers in the Ethiopian highlands, where supposedly only *Bulinus truncatus sericinus* occurs, raises a question concerning both their nomenclature and the systematics of the genus in that and similar geographical areas. Individuals with differing polyploid numbers are undoubtedly reproductively isolated from each other. Therefore, biologically they are distinct species, and most likely they will be found to exhibit morphological differences.

Until recently those snails involved in the transmission of schistosomiasis japonica were grouped into 3 genera and 19 species. The chromosomes have been studied of laboratory produced F₁ hybrids of the 4 key species of the 4 main geographical areas where these snails are found. The pairing behavior of their chromosomes at meiosis appeared to be normal. Only normal bivalents with 1, 2 or 3 chiasmata were observed, with no univalents, trivalents or quadrivalents being found. In addition, all segments of each chromosome seemed to pair completely. When this cytological information is coupled with the great morphological similarity found between the 4 species, the ease of their hybridization, and the non-reduced viability of the hybrids, then these 4 nominal species of *Oncomelania* should be interpreted as no more than geographical populations or races of the same species.

For several years we have been studying at the University of Michigan the chromosomes of various snails, some species of which belong to taxa that contain species implicated in the transmission of human bilharziasis (schistosomiasis). Some of the data that we have obtained have a bearing on the

systematics of these medically important snails, and it is on this information that the present paper is based.

The genus *Bulinus*, intermediate hosts of *Schistosoma haematobium*

The basic chromosome number (x) of this genus is the same as that of its

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TABLE 1. Chromosome numbers of *Bulinus*

Species	Haploid chromosome number	Source	Reference
Africanus group			
<i>B. africanus ovoideus</i>	18	Tanzania	Burch, unpubl.
<i>B. globosus</i>	18	Liberia, Ghana, Zambia, Rhodesia, S. Africa	Natarajan et al., 1965
<i>B. nasutus nasutus</i>	18	Tanzania	Burch, unpubl.
<i>B. nasutus productus</i>	18	Tanzania	Burch, unpubl.
<i>B. jousseaumei</i>	18	Senegal	Natarajan et al., 1965
<i>B. ugandae</i>	18	Tanzania	Burch, unpubl.
Forskaliï group			
<i>B. beccarii</i>	18	W. Aden	Natarajan et al., 1965
<i>B. forskaliï</i>	18	W. Aden, Ghana, Tanzania, S. Africa	Natarajan et al., 1965
	19	Angola	Natarajan et al., 1965
<i>B. reticulatus</i>	18	W. Aden	Natarajan et al., 1965
<i>B. senegalensis</i>	18	Senegal	Natarajan et al., 1965
Tropicus group			
<i>B. guernei</i>	18	Senegal	Natarajan et al., 1965
<i>B. natalensis</i>	18-21	Rhodesia, S. Africa	Burch, 1964a
<i>B. tropicus tropicus</i>	18	Kenya, Rhodesia, S. Africa	Burch, 1964a
<i>B. t. angolensis</i>	18	Zambia	Burch, 1964a
<i>B. t. zanzebaricus</i>	18	Tanzania	Burch, 1964a
<i>B. sp.</i>	18	Ethiopia	Burch, 1964a
Truncatus group			
<i>B. coulboisi</i>	36	Tanzania	Burch, 1964a
<i>B. schackoi</i>	36	Ethiopia	Burch, unpubl.
<i>B. truncatus truncatus</i>	36	Sardinia, Iran, Iraq, Egypt, Sudan	Burch, 1964a
<i>B. truncatus rohlfsi</i>	36	Ghana	Burch, 1964a
<i>B. sp.</i>	36	Ethiopia, W. Aden	Burch, 1964a, unpubl.
<i>B. sp.</i>	54	Ethiopia	Burch, unpubl.
<i>B. sp.</i>	72	Ethiopia, W. Aden	Burch, 1964a, unpubl.

family, the Planorbidae, i. e., $x=18$ (Table 1) (Burch, 1960b, 1963, 1964a, 1965, 1966; Natarajan et al., 1965). However, since polyploidy occurs in this genus, a comparison of chromosome numbers of the various species brings to light some of the still unresolved problems of systematics of the planor-

bid subfamily Bulininae.

The 5 species and 1 additional subspecies studied in the bulinine "africanus species group" all had 18 pairs of chromosomes, as did all populations except 1 of the 4 species studied of the "forskaliï species group", and nearly all of the "tropicus species group". The 1

specimen of *Bulinus forskalii* (Ehrenberg) that we studied from Angola had 19 elements present at diakinesis and Metaphase I (Natarajan et al., 1965). The meiotic elements of this specimen all appeared to be normal bivalents, but accurate counts could not be made on mitotic cells. This specimen may have come from an aneuploid race; it may have been an aneuploid individual of a normal 36 ($n=18$) chromosome race, or it may have been a specimen with supernumerary or B chromosomes.

In specimens of *Bulinus* "*?natalensis*" from Rhodesia most of the Metaphase I cells had 18 chromosomal elements, but some cells had 19. On the other hand, in *Bulinus natalensis* (Küster) from the same country, most Metaphase I cells had 19 chromosomal elements, but some cells had 20 and 21. These varying chromosome numbers may be the result of hybrid origins of the 2 populations.

In the "*truncatus* species group", we have studied *Bulinus truncatus truncatus* (Audouin) from Sardinia, Iran, Iraq, Egypt and the Sudan (Burch, 1964a; Natarajan et al., 1965). All of our specimens had 36 pairs of chromosomes, as did *B. truncatus rohlfsi* (Clessin) from Ghana. This species, with a wide geographic distribution, is obviously a tetraploid. *B. coulboisi* (Bourguignat) from Tanzania also had 36 pairs of chromosomes, as did topotype specimens of *B. schackoi* (Jickeli) from the Toquor River at Mekerka, Ethiopia. Other populations of *Bulinus* with 36 pairs of chromosomes occur in the Moggio River at Moggio, Ethiopia (Burch, unpublished) and at Belas, Western Aden Protectorate. The species or subspecies to which these should be relegated is doubtful at present.

At least 1 population in Ethiopia, located in a small stream about 15 miles north of Addis Ababa on the Debra Marcos Road has specimens with 54 pairs of chromosomes (Burch, unpublished). These are obviously hexaploids. Another population, about 1 mile north of this one, consists of octaploid

individuals with 72 pairs of chromosomes. At least 1 population with 72 pairs of chromosomes occurs also at Tarbak, Western Aden Protectorate (Burch, 1964a).

Finding such polyploid populations in nature raises a question concerning both their nomenclature and the systematics of the genus *Bulinus* in the geographic area of the polyploids. Mandahl-Barth (1965) considers only 1 species of *Bulinus* s.s. to occur in Ethiopia. The polyploid populations are undoubtedly reproductively isolated from individuals of other populations with different chromosome numbers in this polyploid series (Burch & Huber, 1966). From a biological point of view, these polyploid populations represent various distinct species. Most likely they also exhibit morphological differences. The differences in habitats of the Ethiopian populations are shown in Table 2.

Brown (1965) found considerable variation in shell shape in the specimens he collected in Ethiopia. Some of his samples contained a wide range in shell shapes, other samples were uniform. The local variation he found calls to mind my recent experiences (unpublished) in collecting *Bulinus* s. s. specimens in Tanzania. In several populations 2 size groups were present, large specimens that were obviously adults, and smaller specimens at first thought to be young of the larger specimens. However, cytological observations showed that while the larger specimens had 18 pairs of chromosomes, the smaller specimens had 36 pairs of chromosomes. These were obviously mixed populations of 2 separate and distinct species, presumably *B. tropicus* (Krauss) and *B. coulboisi*, since both occur in the area.

Information currently at hand suggests that the "*truncatus*" and "*tropicus*" species groups can be distinguished by their chromosome numbers. If such is the case, then chromosome numbers may be helpful in placing snail populations into their species group category, which is

TABLE 2. Habitats and chromosome numbers of *Bulinus* s. s. in Ethiopia

Locality	Habitat at time of collection	Haploid chromosome number
Lake Awasa	Large lake; very warm surface water; snails in very shallow water on aquatic vegetation.	18
Lake Bishoftu	Crater lake; surface water not as warm as in L. Awasa; snails under rocks at margins of lake (none on aquatic vegetation).	18
Lake Tana	Very large lake; snails under and on rocks at margins of lake.	18
Moggio River	Slow flowing stream of medium size; relatively warm water, snails on aquatic vegetation, which is especially abundant near shore.	36
Toquor River	Small, cold-water stream flowing over rocks and boulders; many areas with relatively steep gradient; snails on vegetation in quieter areas.	36
Small stream 15 mi. north of Addis Ababa	Very small, cold-water stream, hardly more than a seepage area; snails on aquatic vegetation.	54
Small stream 16 mi. north of Addis Ababa	Small, cold-water stream, with relatively slight gradient; mud banks; aquatic vegetation sparse; snails on banks, rocks and vegetation.	72

important since species of the "truncatus group" can be infected with *Schistosoma haematobium*, but species of the "tropicus group" have not yet been implicated in the transmission of bilharziasis on the African continent (unless an as yet unstudied species from Madagascar that serves as host and is distinct from *B. livatus* (Tristram) will be found to belong to the "tropicus" group). However, if it is subsequently found that tetraploidy arose independently on 1 or more occasions in both species groups, then chromosome numbers *per se* will be of little value in separating the 2 groups. Nevertheless, knowledge of chromosome numbers will remain important in separating and understanding various species.

The genus *Biomphalaria*, intermediate hosts of *Schistosoma mansoni*

In the genus *Biomphalaria*, 5 species and 2 additional subspecies have been

studied from a number of localities in 9 countries (Table 3) (Natarajan et al., 1965; Burch, unpublished). All species had 18 pairs of chromosomes. Chromosome numbers, therefore, have not been helpful in systematics in this genus, but do indicate a strong conservativeness in chromosome numbers that is characteristic of gastropods in general (see also Burch, 1965).

The genus *Oncomelania*, intermediate hosts of *Schistosoma japonica*

Before discussing available cytological data on *Oncomelania*, it is pertinent to review recent concepts of systematics of the genus, on which there have been several different and quite diverse opinions, especially on the number of taxa that comprise the group. The most extreme view on the side of the "splitters" is that of Bartsch (1936, 1939, 1946), where the genus *Oncomelania* of other authors was divided into 3 genera.

TABLE 3. Chromosome numbers of *Biomphalaria*

Species	Haploid chromosome number	Source	Reference
<i>B. alexandrina alexandrina</i>	18	Egypt	Natarajan et al. , 1965
<i>B. choanomphala choanomphala</i>	18	Tanzania	Burch, unpubl.
<i>B. glabrata*</i>	18	Puerto Rico	Burch, 1960b
<i>B. pfeifferi pfeifferi</i>	18	Tanzania, Rhodesia, S. Africa	Natarajan et al. , 1965
<i>B. p. gaudi</i>	18	Liberia, Ghana	Natarajan et al. , 1965
<i>B. p. madagascariensis</i>	18	Madagascar	Natarajan et al. , 1965
<i>B. sudanica tanganyicensis</i>	18	Tanzania	Natarajan et al. , 1965

*This species is usually placed in the nominal genus *Australorbis* or *Planorbina*, but Opinion 735 of the International Commission on Zoological Nomenclature has ruled that *Biomphalaria* has precedence over these 2 generic names.

Oncomelania was retained for *O. hupensis* Gredler, and included *O. moellendorffi* (Schmacker & Boettger), *O. longiscata* (Heude), *O. elongata* Bartsch, *O. schmackeri* Moellendorff, *O. multicosta* Bartsch, *O. costulata* (Heude), *O. crassa* (Heude) and *O. yaoi* Bartsch; the genus *Katayama* was retained for *Katayama nosophora* Robson, including "*Katayama*" *nosophora yoshidae* Bartsch, "*K.*" *formosana* (Pilsbry & Hirase), "*K.*" *lii* Bartsch, "*K.*" *fausti* Bartsch, "*K.*" *cantonii* Bartsch and "*K.*" *tangi* Bartsch; the genus *Schistosomophora* was described as a new taxon to include *Prosothenia quadrasi* Moellendorff and "*S.*" *minima* Bartsch, "*S.*" *robertsoni* Bartsch and "*S.*" *slatteri* Bartsch. In this scheme, the vectors of Oriental bilharziasis and allied species consisted of 3 genera, 19 species and 2 additional subspecies.

Abbott (1948) considered the group to consist of only 1 genus, *Oncomelania*, and this genus to contain only 4 species, 1 of which had an additional variety, *O. nosophora slatteri*. *O. nosophora* was believed to occur in Japan and on the China mainland; *O. hupensis* inhabited

the China mainland; *O. formosana* was found on Taiwan (Formosa); and *O. quadrasi* was found in the Philippines. Kuo & Mao (1957) went even further and presented the opinion that ". . . the specific term *Oncomelania hupensis* Gredler should be used for all *Oncomelania* snails involved in the transmission of schistosomiasis japonica in China." Also they rejected the validity of the variety *slatteri*.

Burch (1960a) determined the chromosome numbers of these 4 nominal species as understood by Abbott and Kuo & Mao. All 4 have the same chromosome number, $n=17$, $2n=34$, which is interesting, but not much help from a standpoint of taxonomy (except in a negative way when contrasted to the fact that the 2 Michigan species of the closely related *Pomatiopsis* each has a different chromosome number). Burch, Moose, Williams & Patterson (unpublished) studied the chromosomes of hybrids of the 4 nominal species (see Burch, 1964b). One might expect that such a study of hybrids between different species would give extremely valuable clues as to the relation-

TABLE 4. Revised nomenclature in the genus *Oncomelania*

Species	Distribution
<i>O. hupensis hupensis</i> Gredler, 1881	China mainland
<i>O. hupensis quadrasi</i> (Moellendorff, 1895)	Philippines
<i>O. hupensis formosana</i> (Pilsbry & Hirase, 1906)	Taiwan (Formosa)
<i>O. hupensis nosophora</i> (Robson, 1915)	Japan

ships of these species. Such an assumption is based on the fact that homologous chromosomes pair at meiosis, and non-homologous chromosomes, or non-homologous segments of chromosomes, do not normally pair. Therefore, the more distantly related species might be expected to have more non-pairing chromosomes and more closely related species to have fewer non-pairing chromosomes. A study of the chromosomes of snails of presumably known hybrid origin has been done previously only by Staiger (1954) on the marine snail *Purpura*.

A careful examination of late prophase or diakinesis chromosomes of the various F₁ hybrids of the 4 nominal species of *Oncomelania* revealed no apparent anomalies that did not also occur as prevalently in the normal parents. Only normal bivalents with 1, 2 or 3 chiasmata were observed, and no univalents, trivalents or quadrivalents were found. In addition, all segments of each chromosome seemed to pair completely. When this information is coupled with the great ease of hybridizing the various nominal species, and the nonreduced viability of the hybrids, then grounds for a different interpretation of systematics in *Oncomelania* are available. This interpretation is that the 4 so-called "species" of *Oncomelania* are no more than geographical populations or races of the same species. Such an interpretation is compatible with what is known about the morphology of the geographic populations, since distinguishing differences between the 4 are either

slight or intergradations exist (e.g., the ribbing of *O. hupensis*). In fact, on shell shape, *O. nosophora*, *O. formosana* and *O. quadrasi* form a north to south step-cline, the Japanese specimens being relatively long and slender, the Philippine specimens being relatively shorter and broader, and the Taiwan specimens falling between the 2 extremes.

Therefore, if one uses reproductive isolation (including chromosomal homology) as a main criterion for determining which populations are distinct species and which are not, then in the case of *Oncomelania* what was formerly considered 3 genera with 19 species is in reality no more than 1 species with several geographical races. This case reveals some of the excesses of classical taxonomy. The new data would change nomenclature within the genus to that shown in Table 4.

Acrylamide gel electrophoresis of foot muscle proteins of *Oncomelania* supports the classification derived from hybridization and cytological studies (G. M. Davis, personal communication). Although certain population differences were found within the *O. hupensis formosana* complex on Taiwan, the 4 geographic races were quite similar in their protein patterns. Although electrophoretically similar, the races showed slight differences, which were of a greater magnitude than those differences of the Taiwan populations of *O. h. formosana*. The electrophoretic similarities of the geographic races were especially notable when compared to the

relatively gross differences exhibited between the 4 species of *Pomatiopsis* that Davis studied (unpublished).

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RESUMEN

CROMOSOMAS DE HUESPEDES INTERMEDIARIOS
DE BILHARZIOSIS HUMANA

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Los cromosomas han sido estudiados en muchas especies de caracoles de los grupos taxonómicos que incluyen huéspedes intermediarios implicados en la transmisión de bilharziosis. Algunos de los resultados indican que la presente taxonomía de esos caracoles no es satisfactoria.

La presencia de poblaciones con números diferentes de cromosomas poliploides en tierras altas etiópicas, donde se supone que habita solamente *Bulinus truncatus sericinus*, plantea una cuestión de nomenclatura y sistemática en el género en áreas geográficas similares. Individuos con número poliploideo diferente están sin duda aislados reproductivamente unos de otros. Así, biológicamente son especies distintas y probablemente podrán encontrarse en ellas diferencias morfológicas.

Hasta ahora, estos caracoles implicados en la transmisión de la esquistosomiasis japonesa, se agrupaban en tres géneros con 19 especies. Los cromosomas han sido estudiados en híbridos F_1 producidos en laboratorio de las cuatro especies clave, de las cuatro áreas geográficas principales donde habitan. Durante el apareamiento de los cromosomas en meiosis, el comportamiento pareció ser normal. Solo se observaron bivalente normales con 1, 2 o 3 fusiones cruzadas (chiasmata), sin ningún univalente, trivalente o cuadrivalente. Además, todos los segmentos de cada cromosoma parecieron acoplarse completamente. Cuando esta información citológica se combina con la gran similitud morfológica que existe entre las cuatro especies, el caso de sus hibridaciones y la capacidad no reducida de los híbridos, entonces esas cuatro especies nominales de *Oncomelania* deben interpretarse únicamente como poblaciones geográficas o razas de la misma especie.

АБСТРАКТ

О ХРОМОСОМАХ У ПРОМЕЖУТОЧНЫХ
ХОЗЯЕВ ЧЕЛОВЕЧЕСКОГО БИЛХАРЦИАЗИСА

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Были изучены хромосомы у многих видов улиток, среди которых встречались промежуточные хозяева и передатчики человеческого билхарциазиса. Судя по некоторым результатам исследования, систематика этой группы моллюсков находится в настоящее время в неудовлетворительном состоянии.

Нахождение популяций моллюсков с различным числом полиплоидных хромосом в горных районах Эфиопии, где предположительно встречается только *Bulinus truncatus sericinus*, подымает вопрос относительно их видового состава и систематического положения рода, как в исследованном, так и в других близких географических районах.

Как известно, индивидуумы с различным числом полиплоидных хромосом, несомненно должны быть репродуктивно изолированы между собой. Поэтому биологически они являются различными

видами и, вероятнее всего будут обладать и морфологическими различиями. До сих пор указанные выше виды моллюсков, являющиеся передатчиками *schistosomiasis japonica*, разделялись на 3 рода и 19 видов. Нами были исследованы хромосомы первого поколения (F_1) гибридов, полученных в лаборатории путем скрещивания четырех основных видов моллюсков из четырех главных районов, где эти моллюски были найдены. Оказалось, что парное поведение их хромосом при мейозисе, повидимому является для них нормальным. Для них также нормально наличие только бивалентов с одним, двумя или тремя перекрестами; одно-, трех- или четырехваленты ни разу найдены не были. Кроме того, все сегменты каждой хромосомы, видимо сливаются полностью с другой. Когда имеются такие цитологические сведения, к которым присоединяются - большое морфологическое сходство, найденное между четырьмя видами, легкость их гибридизации и неумещающаяся жизнеспособность полученных гибридов, то тогда эти 4 вида *Oncomelania* должны рассматриваться не более, как географические популяции или расы одного и того же вида.

FUNCTIONAL MORPHOLOGY AND ECOLOGICAL LIFE HISTORY
OF THE GEM CLAM, *GEMMA GEMMA* (EULAMELLIBRANCHIA: VENERIDAE)^{1,2}

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ABSTRACT

A comprehensive study of the gem clam, *Gemma gemma*, was made at Union Beach, New Jersey during the period 1955-1958. It embraced a general study of functional morphology as well as growth, reproduction, population density, mortality, parasites and predators.

The gem clam is ovoviviparous, utilizing the demibranchs as brood pouches. It has a maximum length of 5 mm and is found in sandy situations in intertidal and subtidal estuarine areas.

Gemma occurs from Labrador to Texas. It has been introduced to the west coast where it now ranges from Puget Sound to San Diego.

Gemma has no actively swimming pelagic stage, and dispersal, particularly of the juveniles, is probably accomplished through currents and wave action.

The anatomy is described largely from serial sections. Special attention was paid to the inhalant and exhalant siphonal membranes, which are situated inside and at the base of each siphon. The former is an arched curtain that may be lowered to deflect silt-laden water away from the gills. The latter has not previously been described in detail in other clams. It possesses a vertical ovoid slot and can perhaps be completely closed. This membrane might (1) assist in withdrawal of the valvular membrane of the exhalant siphon, (2) slow or stop water movement through the clam thus aiding cleansing actions, (3) prevent loss of gametes during ovulation and (4) aid in liberation of juveniles.

Growth was studied by sampling clams from the same population at monthly or semi-monthly intervals. A method was developed for separating juveniles from sand, using a concentrated solution of $ZnCl_2$.

Juveniles liberated in summer average approximately 375μ in length; they attain about 2 mm in the fall, and reach adult size (about 4 mm) the following summer. The growing period begins in April and ends, at the latest, in November.

Gem clam females retain their young within the inner and outer demibranchs, where they may be found at all times of the year. A few females attain sexual maturity in the fall of their 1st year of life at the age of about 4 months and carry their young through the winter. The majority mature during the spring and liberate 100-200 juveniles during the summer with greatest numbers being released in July.

In vitro experiments indicate that embryos of *Gemma* can be cultured outside the mother during at least the latter half (i. e. last week) of their develop-

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mental period, and raised to maturity.

A table of over 100 species of brood-protecting clams is given with notes on various aspects of their reproduction.

Population densities of 5 year classes were studied. During the summer, density may reach 200,000 or more per square meter. A steady decline in numbers was observed at Union Beach during the period of study. Biomass (wet weight including shell) was calculated for this period. Seasonal fluctuations were regular and of considerable magnitude. Minimal and maximal values varied from about 2-200 gms per square meter.

The maximum life span of *Gemma* at Union Beach is 2 years, the mean life span only 1.13 months. The mortality rate averages approximately 40% per month.

Predators of the gem clam include a sea anemone, a gastropod, 5 species of crabs, the horseshoe "crab", an elasmobranch fish and 18 species of shore birds, half of them ducks.

Larval stages of 3 trematodes are parasitic on *Gemma*. A metacercaria (*Parvatrema borealis*) is found between the shell and mantle of most gem clams at Union Beach but do little damage. Sporocysts situated in the gonads and containing furcocercous cercariae are probably of the same species. Although destroying the gonads, they infrequently infect *Gemma* (about 1%) and thus have a negligible effect. A sporocyst with microcercous cercariae (*Cercaria adranocerca*), also found only occasionally in the gonads, is similar in its effect. An unidentified metacercaria, briefly described herein, was also found.

Gemma gemma appears to have both positive and negative value to man. It is a part of the diet of game ducks. It probably acts as a buffer species against predators of commercial species of clams, but, on the other hand, may serve to maintain populations of predatory gastropods during a dearth of economically important species of clams. It also acts as first and second intermediate host for trematodes which may infect, as adults, animals important to man.

CONTENTS

	Pages		
I	INTRODUCTION	139	IX REPRODUCTION 177
II	SYSTEMATICS	139	1. Method of Reproduction . . . 177
III	DISTRIBUTION	139	2. Factors Influencing Develop- ment of Brood Protection . . 178
VI	PHYSICAL ENVIRONMENT . . .	141	3. Relationship of Embryo to Maternal Organism 184
V	BIOTIC ENVIRONMENT	143	4. Sex Ratio, Sexual Maturity, Reproductive Periods and Fe- cundity 188
IV	FUNCTIONAL MORPHOLOGY . . .	144	5. Development Within the Brood Chamber 190
	1. Methods	144	6. Size and Time of Release of Young 192
	2. Shell	144	X POPULATION DENSITY 194
	3. Mantle	147	XI MORTALITY 200
	4. Foot	152	XII PREDATORS 204
	5. Muscular System	154	XIII PARASITES 206
	6. Digestive System	156	XIV RELATIONS TO MAN 208
	7. Circulatory System	158	ACKNOWLEDGEMENTS 209
	8. Ctenidial System	159	LITERATURE CITED 209
	9. Nervous System	161	APPENDIX 215
	10. Reproductive System	163	
	11. Excretory System	169	
VII	FOOD AND FEEDING	170	
VIII	GROWTH	170	

I. INTRODUCTION

The gem clam, *Gemma gemma*, a member of the Family Veneridae, is a very common ovoviviparous estuarine clam, but has received only the most casual study in spite of its ubiquity and abundance in favorable environments. Except for the usual brief descriptive material found in books on shells, *Gemma* has but scant representation in the literature. It generally appears only in check lists drawn up from ecological surveys or in studies of food habits as a dietary component of animals feeding in the intertidal zone. The reasons for the scarcity of substantial research on this clam are probably its small size - according to Abbott (1954) the smallest marine clam known - and its lack of commercial value. It is of some importance to man in that it is a portion of the diet of economically important game ducks. It may also provide an alternative food supply to predators of commercial species of bivalves, i.e. act as a buffer species, and it may also maintain these predator populations during a dearth of commercially important bivalves. Its presence may deter the development of populations of such clams. In addition, it acts as the first and second intermediate host for trematodes which perhaps infect, as adults, animals important to man. In the present study, practical considerations were subordinate to an interest in gaining fundamental knowledge of this form.

The aim of this inquiry was to study as many aspects of the functional morphology and ecological life history of *Gemma gemma* as was practicable, on the basis of monthly samples collected over a 3-year period. Observations were made on distribution, reproduction and population density, natality, growth and mortality.

II. SYSTEMATICS

1. Synonymy

According to Römer (1865) and Morse

(1919), *Gemma gemma* (Totten, 1834) Dall, 1902, has been regarded as the juvenile of the hard clam or quahog, *Mercenaria mercenaria* for many years. This misconception has not yet entirely disappeared from the shellfish industry. There is no mistaking the difference between these 2 species, as recently-set quahogs possess only a few concentric ridges deeply etched in the shell, while *Gemma* possesses many fine ridges or sometimes none (Fig. 2).

Gemma gemma was first described by Totten in 1834 and initially placed in the genus *Venus*. It has subsequently undergone seven nomenclatural changes, its present status having been established by Dall (1902). Reference may be made to Palmer (1927-29) for details.

2. Common Names

The name given to this clam in shell books is usually the gem shell or amethystine gem shell because of the color of the more highly pigmented individuals. It has also been referred to as the duck clam although it shares this name with other small clams, such as the smooth and channeled duck clams (*Laviosa* spp.) which form a part of the diet of ducks. It is understandable that conchologists, in naming a mollusk, are prone to refer to a structure which holds their greatest interest and for students of food habits to incorporate an important predator in the name. In the light of its appearance and status as a living organism rather than a mere shell, it seems more appropriate simply to call it the gem clam.

3. Subspecies

According to Abbott (1954), a number of forms or subspecies have been described: *G. purpurea* Lea, *manhattensis* Prime and *fretensis* Rehder.

III. DISTRIBUTION

1. Geographic Range

a. East Coast

All authorities consulted give Labrador or Nova Scotia as the northern limit of the gem clam, but there is less agree-

ment as to its southern extreme. Earlier publications limit it to Massachusetts or New York Bay (Dall, 1902; Palmer, 1927-29) while later authors extend it to North Carolina (Johnson, 1934; Pratt, 1935; Rogers, 1936; Smith, 1937; Miner, 1950), and even the Bahamas and Texas (Abbott, 1954). This lack of accord may be due to confusing *Gemma gemma* with *Gemma purpurea*, since the range of the latter is given from Cape Cod to the Bahamas and Texas (Palmer, 1927-29; Johnson, 1934).

b. West Coast

Gemma purpurea is found in areas along the West Coast from Puget Sound to San Diego where plantings of East Coast oysters, *Crassostrea virginica*, were made in the latter part of the 19th century; it was introduced unintentionally at the time (Townsend, 1896; Hanna, 1939; H. Orcutt, personal communication).

In the records of duck stomach examinations placed at my disposal by the Patuxent Research Refuge at Laurel, Maryland, it is noted that a bufflehead (*Bucephala albeola*) collected on November 21, 1919, at Keku Pass, Alaska, contained the shell of a clam identified as *Gemma gemma*. This location is about 750 miles NNW of Puget Sound. Dr. Myra Keen of the Department of Geology, Stanford University, Stanford, California, (personal communication), in commenting on this entry, believes *Gemma* could easily be mistaken for *Transennella tantilla*, which is found in that area.

Adams (1862) cites *Gemma* under the mollusks of Japan and postulates a migration route from the American west coast. Römer (1865) points out that *Gemma* was not known at that time from the west coast and considers its movement from the east coast through the Arctic Sea hardly possible. Kuroda & Habe (1952) also list *Gemma* among the marine mollusks of Japan, but, curiously, references which they cite to support its inclusion in their list make no allusion

to its distribution outside the North American continent.

2. Habitat Range

Bradley & Cooke (1959) report that in Sagadahoc Bay, Maine, the gem clam is restricted to the intertidal zone, but that in Chesapeake Bay it also lives below the low tide line. The latter distribution is true for Raritan Bay, New Jersey, the site of this study. Gem clams were taken with a Peterson dredge 1600 yards offshore where depth is about 7 feet at mean low water.

3. Means of Dispersal

Following release from the female, juveniles of *Gemma* take up an infaunal existence immediately, since they lack a swimming organ. This poses the question of how *Gemma* has become distributed as extensively as it has. Nelson (1928) reports behavior of post-veliger *Mytilus edulis* juveniles which, he believes, is an important factor in the wide distribution of this species. Included is the formation of a flotation gas bubble and attachment to the surface film by foot, siphon and byssus. None of these activities has been observed in *Gemma*. It seems probable, however, that wave action and currents may act to remove the young from the bottom and transport them passively to other areas. Accordingly, on June 25, 1956, at Union Beach, New Jersey, the site of this study, 20 liters of water passing over a sand bar were collected by dipping the wave crests to avoid disturbing the bottom sediments and strained through a No. 18 plankton net. Water depth at the time varied from 1-4 inches due to wave action. Fifteen juvenile *Gemma*, lengths ranging from 330-510 μ , were found in the sample. Unfortunately, concomitant sampling of the sand bar was not of a nature which would yield quantitative results, therefore, no comparison can be made regarding the proportion of juveniles in the plankton and those in the sediments.

Carriker (1955, unpublished data)

found juvenile *Gemma* in a salt pond on Gardiners Island, New York, in hard clam planktonic larval traps which were 10 inches to 5 feet off the bottom and placed in currents ranging from 8-80 cm/sec. Gem clam juveniles were found in all of the 12 traps which were set out for 1-2 weeks, the openings of which were covered with screening having an aperture size ranging from 360-670 μ . The "catch" of the 3 most successful traps totalled 113 *Gemma*. There was a positive correlation between number of juveniles trapped and current velocity although nothing was known about local concentrations of gem clams in the trap vicinities.

Sullivan (1948) found recently liberated *Gemma* juveniles in plankton tows, but stated that this was only on rare occasions, such as after particularly heavy storms. She did not state how far from the bottom her collections were made.

Bradley & Cooke (1959) believe wave action to be important in the transport of both juvenile and adult gem clams, movement of the latter being enhanced by possession of algal tufts on the shells of many.

Baggerman (1953) found that young *Cardium* up to 2 mm in size were transported over sand flats by water currents.

In experiments designed to study effect of currents on movements of juveniles of *Mya arenaria*, Baptist (1955) found that small clams placed on an artificial sand flat were moved according to their size and to the velocity of the current. Of the 2-4 mm size, 100% were rolled along the surface, being unable to burrow when the velocity was 25 cm/sec.

Dow & Wallace (1955) have reported involuntary translocation by wave action of quahog populations, the members of which averaged 100 times the size of juvenile gem clams and 10 times that of adults. It is not difficult to conceive the effect of such forces on these minute clams.

No study has been made of the behavior or tactic responses of juvenile gem clams to factors which might enhance

their removal from the bottom sediments, but it appears likely that under suitable conditions of current velocity, sufficient numbers enter the plankton for short periods to provide means for the dispersion of this species.

IV. PHYSICAL ENVIRONMENT

Information gathered during this study is based largely on collections made on the south shore of Raritan Bay at Union Beach, New Jersey, from April 1955 through August 1958.

The general area is characterized by a series of low bars and sloughs which run parallel to the shoreline, but which constantly and moderately shift position and direction. The collecting area is located at the juncture of the beachfront and a sod bank of a salt marsh promontory known as Conaskonk Point at latitude 40° 27' 18" N., longitude 74° 10' 29" W. Collecting was confined to a sand bar having an area of approximately 1000 square meters.

1. Methods

At each visit to the collecting site, air and water temperatures were taken to the nearest 0.5°C, generally within 2 hours before low water (See Table 1 and Appendix). Water temperature was taken on the bottom at a water depth of one foot. Sand temperatures were taken beginning in May 1957, one-half inch below the surface of the sand exposed at low tide.

Surface salinities were determined beginning in March, 1956 using a set of "Gemware" sea water hydrometers, Model No. 110, accurate to within 0.0002° Sp. Gr. Water densities were converted to salinities using U. S. Coast and Geodetic Survey tables (1953b).

2. Temperature

Records of the U. S. Coast and Geodetic Survey (1951) covering the period 1945-1950 give the mean high surface water temperature as 24°C in July, at Sandy Hook (9 miles to the east of Union Beach),

TABLE 1. Representative hydrographic data for the collection site at Conaskonk Point, Union Beach, New Jersey (1957). For data of other years, see Appendix.

Date	Time Relative To Low Water	Temperature (°C)			Salinity (‰)
		Air	Water	Sand	
Jan. 17	-1.5 hr.	-7.0	0.0	-	23.3
Feb. 1	-1.5	1.0	1.5	-	22.4
Mar. 16	-1.0	12.0	9.5	-	22.4
Apr. 11	-1.0	8.5	9.5	-	14.5
May 11	0.0	14.0	15.0	15.0	24.1
May 28	-	18.0	21.0	23.0	22.5
June 11	0.0	26.0	23.0	25.5	24.2
June 26	-2.0	24.0	25.5	26.0	25.2
July 10	-3.0	24.5	24.5	25.0	27.6
July 26	-0.75	25.0	27.5	26.5	26.8
Aug. 8	-1.0	26.0	26.5	27.0	27.1
Aug. 28	-1.0	23.0	23.0	24.0	26.1
Sept. 9	-0.5	-	19.5	-	26.5
Sept. 22	-1.5	26.0	22.5	24.0	26.7
Oct. 7	-0.5	16.0	15.0	16.5	26.3
Nov. 5	-0.75	12.0	12.0	13.5	24.4
Dec. 19	-1.0	14.0	7.0	11.0	24.1

and 1.5°C as the low in February.

Extremes in water temperature at Union Beach ranged from 0.0-30.0°C although, on one occasion at low tide, the shallow water of a tide pool reached 35°C and, on another, the interstitial water of a sand bar was found to be frozen. Since these determinations were made only at monthly intervals during the colder half of the year and at semi-monthly intervals during the warmer half, precise ecological inferences cannot be drawn from them. Following high temperature conditions during July, 1955, windrows of dead gem clams covered the sand flats in some places so densely as to give the impression that snow had fallen. During the 7-day period preceding this observation (July 21), maximal air temperatures at Newark Airport exceeded 32°C for 6 out of the 7 days. In addition, low water of spring tides came during the mid-day period.

3. Salinity

Salinity extremes ranged from 27.6-13.1‰, the high and low periods being mid-summer to early fall and April,

respectively. According to U.S.C.G.S. records (1953a) the surface salinity at Sandy Hook has a mean high of about 25.5‰ in October and a mean low of about 20.4‰ in April. The collecting area was on the south side of the bay where salinity was generally lower than in other parts and it was closer to the mouth of the Raritan River than is Sandy Hook. Therefore, it is expected that salinities there were consistently lower than those at Sandy Hook.

4. Substratum

The bottom consisted of a silt-sand mixture overlying a layer of clay which, in the sloughs, was as little as 3 inches from the surface. The sand layer consisted of 2 strata, an upper, containing *Gemma* and other bottom-dwelling animals, and a lower layer blackened by the presence of sulfides. The upper surface of this reducing stratum often began at a depth of about 2 inches or less.

The sand was rather fine; about 75% of it passed through a screen having an aperture size of 295 μ . The receding

tide often deposited a fine layer of silt on the surface of the sand.

5. Exposure

The mean tidal amplitude at this location was 5 feet, with a 6 foot range during spring tides. *Gemma* was found both below and above mean low water level and some were exposed upwards of 4 hours during low water of spring tides.

6. Wave Action and Turbidity

In general, on calm days, the water was fairly clear, the bottom being easily visible at depths of 3-4 feet. Since at least a light breeze was almost always blowing, fine surface sediments were stirred at late ebb and early flood resulting in high turbidity in water covering the collecting site. It is generally accepted that large quantities of suspended material have a tendency to foul filtering and respiratory organs (Nelson, 1938; Yonge, 1960). In combination with periods of exposure, this no doubt appreciably reduces the feeding time of the intertidal clams compared with those situated below mean low water level.

V. BIOTIC ENVIRONMENT

1. Animal Associates

The Union Beach sand flat was a typical bivalve-annelid community, *Nereis virens*, *N. limbata*, *Glycera dibranchiata* and a capitellid polychaete being the dominant worms with *Cistenides gouldii* found occasionally. Of the bivalves, *Mya arenaria*, although not abundant, was present and frequently associated with it was the interesting "sand swallow" *Macoma balthica*. At and below the low water level, *Mercenaria mercenaria* and *Crassostrea virginica* were found. Occasionally shells of *Ensis directus* and *Mytilus edulis* washed up. Thick clusters of *Modiolus demissus* covered the sod bank. Of the gastropods, *Nassarius obsoletus*

abounded during the summer and *Polinices heros* was seen at times of exceptionally low water. The small salt marsh pulmonate, *Melampus lineatus* was abundant in the marsh grasses.

Arthropods, such as *Carcinides maenas*, *Panopeus herbstii* and, less frequently, *Callinectes sapidus* wandered over the flats. *Ovalipes ocellatus* moved into the area on one occasion during a period of high salinity. *Uca pugnax* was numerous in the escarpment formed where the salt marsh met the sand flat. The sand shrimp, *Crago septemspinosa*, was plentiful with lesser numbers of *Palaemonetes vulgaris* associated with it. *Limulus polyphemus* spawned in great numbers in late spring and immature individuals were present throughout the summer. A burrowing sea anemone, *Paractis rapiformis*, was found at the mean low water level and below. The nomenclature adopted for mollusks is that of Abbott (1954), and for the other animals listed above, that of Miner (1950).

Animals, approximating the size of *Gemma*, and found in or on the sand, were a tiny tube-building spioniformian polychaete, an isopod (*Edotea* sp.), gammarid amphipods, ostracods and nematodes.

During warm periods of the year, clusters of a sessile ciliate were found attached to the dorsal shell region of almost all gem clams. They were peritrichs of the Tribe Loricata, Family Vaginicolidae.

The larval stages of at least 3 species of digenetic trematodes found in gem clams are discussed later under "Parasites."

2. Plant Associates

A cordgrass, *Spartina alterniflora*, covered the salt marsh to the water's edge. Its roots form a matrix with byssi of ribbed mussels to build a sod which resists for a time the pounding of the surf at high water. It ultimately fails to hold out against the attrition of the sea and, at intervals, fragments

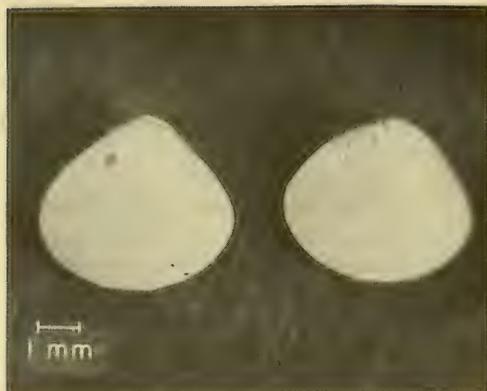


FIG. 1. Two adult individuals of the 1955 year class, collected Aug. 8, 1956, showing annual growth arrest ring (dark line). Anterior end at right.

break loose and scatter on the flats. Further wave action reduces the fragments to silt and detritus which form a layer over the sand and collect in the beach wrack. Above the high water mark was a large stand of reed, *Phragmites communis*.

The dominant macroscopic alga was a species of *Enteromorpha* which often collected inches deep where the sloping sandy beach met the more level sand flat.

During the summer and fall, *Enteromorpha compressa* was occasionally attached to the posterior region of the shell of *Gemma*. Bradley & Cooke (1959) note that gem clams in Maine were similarly garnished.

VI. FUNCTIONAL MORPHOLOGY

1. Methods

a. Gross dissection

The small size of gem clams (max. length = 5 mm) makes anatomical study by dissection difficult and this method was employed to a lesser degree than that of histological sectioning. Clams preserved in 5% neutral formalin were utilized because of the hardened state of their tissues. They were opened by



FIG. 2. Adult clam of the 1954 year class, collected Dec. 27, 1955, showing pigmentation of first year portion of shell and lack of it in second year. This was typical of most members of that year class.

cracking the shell and picking away the pieces of broken shell. A variety of tools suitable for this work were fashioned from insect pins, fine forceps, hairs mounted on needles, dissecting needles sharpened to form a knife edge and glass micropipettes drawn out in a flame. All work was done at a magnification of 10-60X.

b. Histological

Serial sections of a total of 25 clams were prepared. The majority of clams were cut in the transverse plane, but a few were cut in sagittal and frontal planes. They were fixed in Bouin's solution which also dissolves the shell. Orientation in paraffin was accomplished by use of a dissecting microscope, lamps to keep the paraffin in a melted state and heated needles for manipulation. Harris' hematoxylin and eosin were used for staining. Sections were cut at 10 μ .

2. Shell

a. General description

The shell is rounded and subtriangular

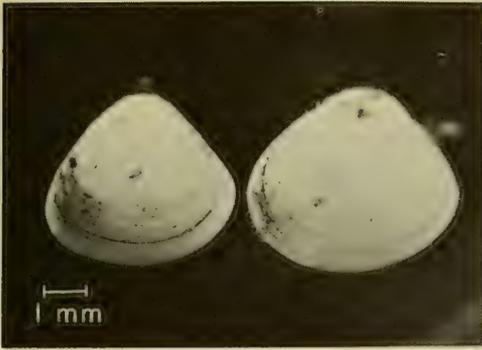


FIG. 3. Two adult individuals of the 1955 year class, collected Aug. 8, 1957, showing 2 annual growth-arrest rings. These are the only 2, out of approximately 6,000 clams examined, which were found to have attained 2 years of age. Length of individual on right is 5.2 mm.

in shape with a large, faintly impressed lunule but no escutcheon. The exterior is glossy with irregular concentric sculpturing; the spaces between sulci varying in width. The slightly anterior beaks are small, incurved and generally eroded (Figs. 1, 2). Dentition is teleodont, the cardinal teeth being divergent with 2 large teeth in the left valve and 3 in the right. The teeth of the right valve are dissimilar in that the median member is large, the posterior large and narrow and the anterior slight and feeble. A long, thin, not easily distinguishable ridge forms a lateral tooth posteriorly in the left valve and anteriorly in the right. A small but distinct pallial sinus is present which is triangular and vertically directed. Crenulations are found along the inner ventral shell margins.

Some shells are devoid of pigmentation, but when color is present, the anterior portion and basal margin, inside and out, may be a pale reddish-violet, the remainder being a reddish-purple, darker at and near the superior and posterior margins. Pigmentation appears to vary with time and area.

The first year's growth of the 1954 year class was strongly tinted but color was lacking in shell laid down during its second year's growth (Fig. 2). Specimens received from Maine and Massachusetts in 1956 were strongly colored as were clams collected at Gardiners Island, New York, in 1954 and 1955. This strong pigmentation is reflected in the observation of Morse (1919) on New England *Gemma*, who states that "some beaches are tinged purple by their number."

A growth arrestment "ring" is apparent following resumption of growth during late spring and early summer of the second growth period. This indication of the gem clam's second year of life is sometimes made more prominent by differences in pigmentation of shell laid down one year as contrasted with that of the next. Where no such contrast is afforded, accumulation of a dark-colored material along the indented edge of the ring clearly marks its position (Figs. 1 and 2). Bradley & Cooke (1959) confirm the presence of this ring and its usefulness in age determination. Evidence that this ring represents the period of cessation of growth during the first winter of life is presented under "Growth." A second year ring, indicating longer life, has been observed in only 3 clams out of slightly more than 6,500 examined (Fig. 3).

b. Ligament

The ligament of *Gemma* is external and situated posterior to the beaks (opisthodetic). It is of the paravincular type being hemicylindrical in shape (L, Figs. 4, 18, 22). This is more nearly true at the ends of the ligament than in the central portion where it is thicker and has the aspect of a half-rod. In addition to the periostracum, it is seen, in section, to consist of 2 layers which, according to Owen et al. (1953) correspond to the 2 shell layers. When the valves are closed, the outer layer is under tension while the inner is com-

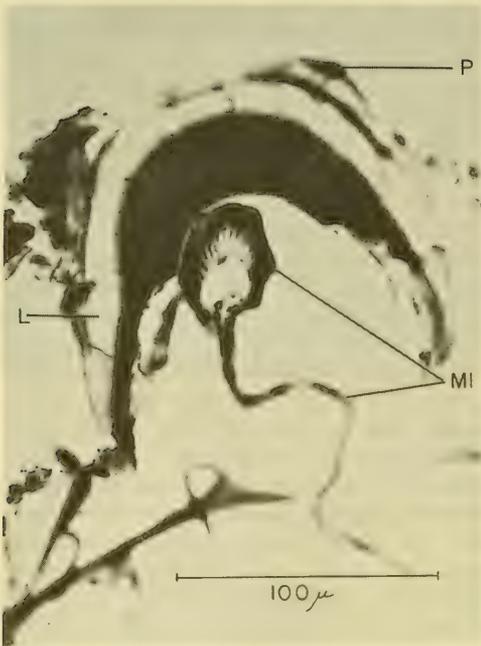


FIG. 4. Transverse section of dorsal shell region showing ligament and mantle isthmus.

LIST OF ABBREVIATIONS

A	atrium (so-called auricle)
AAM	anterior adductor muscle
An	anus
ARM	anterior retractor muscle
BG	byssal gland (so-called)
C	concretion
CC	cloacal chamber
CG	cerebral ganglion
CGC	cerebral ganglia commissure
CPC	cerebro-pedal connective
CVC	cerebro-visceral connective
DD	digestive diverticula
E	embryo(s)
ELP	external labial palp
Es	esophagus
ES	exhalant siphon
ESM	exhalant siphonal membrane
F	foot
GF	gill filament
GFD	gill filament, direct lamella
GFR	gill filament, reflected lamella
GS	gastric shield
I	intestine

ID	inner demibranch
ILJ	interlamellar junction
ILP	internal labial palp
IRT	inner rejection trough
IS	inhalant siphon
ISM	inhalant siphonal membrane
K	kidney
L	ligament
LP	labial palp
M	mouth
Ma	mantle
Mu	mucus
MC	mantle cavity
Me	metacercaria
MI	mantle isthmus
OD	outer demibranch
OF	ovarian follicle
OO	oviducal orifice
P	periostracum
PA	posterior aorta
PAM	posterior adductor muscle
PaS	pallial sinus
PC	pericardial cavity
PG	pedal ganglion (ia)
PRM	posterior retractor muscle
PS	pedal sinus
RD	renopericardial duct
RO	renal orifice
RPO	renopericardial orifice
S	stomach
SD	sperm duct
Si	siphon(s)
Sp	spermatozoa
SRM	siphonal retractor muscle
SS	style sac
St	statolith
T	testis
V	ventricle
VG	visceral ganglion (ia)
VM	valvular membrane

pressed. In clams fixed during their second growing season, the inner layer is subdivided into 2 laminations which react differently to staining. The innermost, which is shorter than the outer one along the longitudinal axis, is thickest in its central region, thinning out as it passes toward the ends of the ligament and terminating before reaching the ends. Figure 4 shows only the outer lamination of the inner layer since the section is from the region beyond the posterior termination of the inner lami-

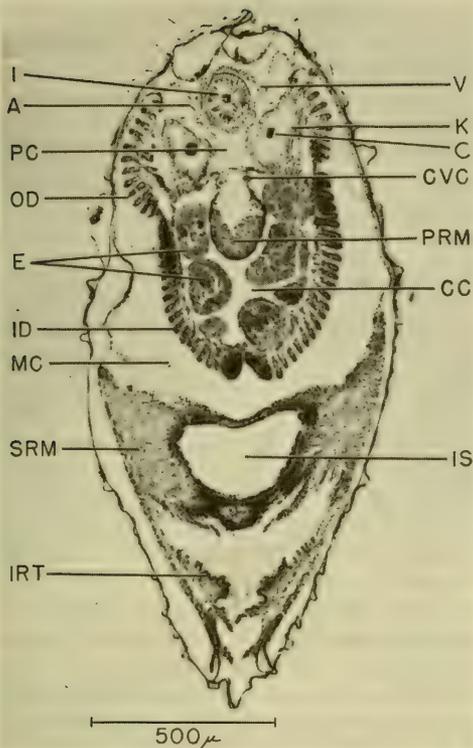


FIG. 5. Transverse section through adult in posterior region.

nation. The juncture of these laminations might be interpreted as a continuum from the shell of a "bunched" group of translucent laminae (Shuster, 1956) laid down during a period of slow growth, i.e., winter, and, when traced to the external shell surface, correspond to the growth-arrest line. Insufficient material was obtained to identify with certainty the layers and laminae. Formation of the ligament is discussed under "Mantle."

c. Periostracum

The periostracum is secreted by a row of glands situated on the inner surface

of the outer mantle lobe. Kellogg (1892) mentions secretory cells in folds of the mantle as producing the "horny cuticle" which covers the shell. Drew (1906) figured this gland in the giant scallop with a strand of periostracum issuing from it and called it the cuticular gland. Nelson (1938) draws attention to this gland in the oyster terming it the pallial gland. It would seem most appropriate to designate it the periostracal gland.

In carefully prepared transverse sections of the gem clam, the periostracum may be seen as a thin layer completely covering what was the outer shell surface to such a degree of fidelity that each concentric ridge is clearly evident in the undulations of the periostracum (P, Fig. 11). Its continuity with the secretion of the periostracal gland may be distinctly seen in these sections.

3. Mantle

a. General

The mantle (Ma) is thin and uniform (Fig. 22) except (i) in the region of the isthmus where the 2 mantle lobes join dorsally (MI, Fig. 4), (ii) along its margins where it forms longitudinal folds (Fig. 11) and (iii) posteriorly where it creates the siphons (Figs. 5, 6, 12). The lateral portion possesses 2 layers. The outer secretory layer consists of a single row of low columnar cells having irregular, rounded to slightly elongated, lightly-staining nuclei. The cytoplasm of these cells is greatly vacuolated. The inner layer, which is 2 cells thick, is composed of flattened cells with spherical, darkly-staining nuclei (Ma, Fig. 23, 35). Slight separations between the 2 cell layers are presumed to be blood spaces. In prepared sections, cilia are not apparent on the medial surface except in the region just dorsal to the inner fold as later described. Observations on the living clam, however, demonstrate their presence.

The dorsal connection of the mantle lobes, called the isthmus, consists of a rather high ridge expanded at the top

where it passes beneath the ligament (Fig. 4). In transverse section, the structure of this ridge can be seen by following the thin mantle lobes as they proceed dorsally and medially. As they approach the median sagittal plane, they descend a short distance, approach one another, then ascend to meet at the midline, there to pass dorsally in apposition until they terminate in the expansion mentioned earlier. This structure is composed of tall columnar cells which are applied to the under surface of the ligament. This region of the isthmus, according to Owen et al. (1953), secretes the inner ligament layer while the ends of the isthmus, which represent the outer mantle folds, secrete the outer layer. Details of ligament structure and formation may be found in Yonge (1957) and Ansell (1961a).

The mantle border is unfused along its entire length except at the isthmus and where it forms the siphons. In the living animal, this border is a very delicate shade of light green. Three lobes or folds are present along the edge (Ma, Fig. 11). These are the inner, middle and outer folds. The inner fold (infolded ridge, Drew, 1906; velum, Dakin, 1909; pallial curtain, Nelson, 1938; muscular lobe, Quayle, 1952) is the largest and may be raised to meet its counterpart at the midline to effect a functional fusion except for a region just anterior to the siphons. On the basis of laboratory observations, this opening appears to serve 2 functions: first, as an incurrent aperture supplementing the inhalant siphon - indeed, the volume of water entering here appears to exceed that of the siphon - and, second, as the site for the elimination of rejecta from the pallial cavity. This opening, called the 4th pallial aperture, has been described in *Spisula solidissima* (Kellogg, 1915) and *S. subtruncata* (Yonge, 1948). Yonge also describes an aperture in *Lutraria lutraria*. The mantle borders in this case are united by fusion of the cuticle (Atkins, 1937). Yonge believes that this opening is primarily associated with removal of rejecta. Plankton and

starch suspensions taken into either incurrent opening of the gem clam appear, within a few seconds, as rejecta, forming an aggregate at the posterior end of this mantle aperture where the mantle edges converge and fuse at the base of the incurrent siphon.

A study of transverse sections discloses a series of small ciliated ridges and grooves on the inner mantle surface which apparently aid in moving rejecta to this aperture. They extend from the middle of the clam to the anterior end of the aperture running between and parallel to the insertion of the mantle at the pallial line and the inner edge of the base of the inner fold. Anteriorly there are one or 2 grooves which increase to 5 or 6 at the aperture. They bear a strong resemblance to those Nelson (1938) found in the inner rejection trough of *Crassostrea virginica* and have been so designated (IRT, Fig. 5). The epithelial cells of the grooves possess long cilia and take up more stain than adjacent cells. Accumulated rejecta are carried away by water currents or slight contractions of the adductors. This mantle aperture closes more readily than does the inhalant siphon when the clam is subjected to heavy concentrations of suspended material.

The middle or sensory fold of the mantle (ophthalmic pallial fold, Dakin; tentacular fold, Nelson) has 2 secondary folds in *Gemma*; the medial division bears no tentacles and the lateral appears to act as a guide for the sheet of periostracum which is closely applied to it. The outer fold (shell fold, Dakin; pallial border,³ Nelson; secretory lobe, Quayle) is relatively small. The middle fold is subdivided in *Gemma*, which possesses the 4 folds that Ansell (1961a) describes as being typical of Venerids, but the proportions and functions of the folds

³Pallial border and pallial curtain are terms coined by Nelson (1938) for the outer and inner folds, respectively, of the mantle border. His figure labels for these structures, however, are switched.

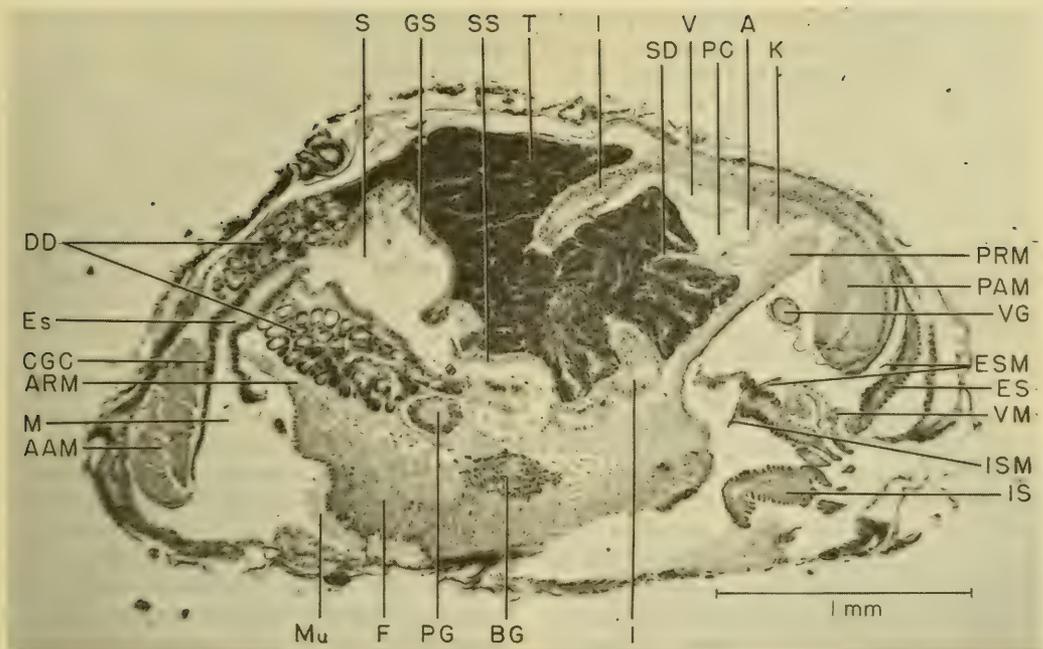


FIG. 6. Median sagittal section of adult.

differ somewhat.

In addition to the juncture of the mantle at the isthmus, there are 3 fusions posteriorly to form the 2 siphons.

The siphons are united over their entire length. The exhalant siphon possesses a thin, semi-transparent sleeve, the valvular membrane (Yonge, 1957), which is an extension of this siphon. The inhalant siphon bears 8-12 tentacles which are loosely interdigitated over the opening when the animal is pumping water. These tissues possess some photosensitivity, since they are often withdrawn when subjected to suddenly increased or decreased illumination. The foot, mantle border and siphons may be withdrawn in a flash when the animal is disturbed. When this occurs, the valvular membrane (VM, Fig. 6; Fig. 7) is drawn into the interior of the base of the siphon and is everted when pumping resumes.

b. Siphonal membranes

Two structures are associated with the siphons, the inhalant and exhalant siphonal membranes (ISM, ESM, Fig. 6). It is thought that the latter has not been previously described in any structural or functional detail. They are membranes, each having a central opening, and situated in the cloacal and pallial chambers at the bases of the siphons, partially covering the internal siphonal apertures (Figs. 6, 7, 8). Although muscle fibers are seen in the membranes, their orientation could not be definitely ascertained.

The description of these membranes is based upon sections prepared from gemma clams which were fixed without relaxation. It is not known, therefore, how closely their dimensions approximate the natural state or to what degree their muscle fibers are contracted.

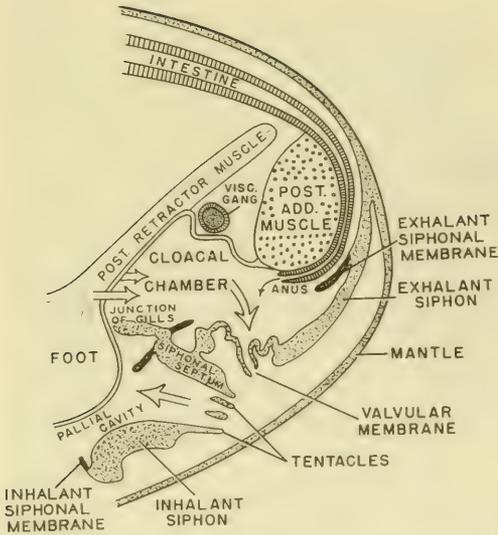


FIG. 7. Semi-diagrammatic representation of parasagittal section of posterior region slightly to one side of the median plane to show direction of water flow and siphonal membranes (solid black). Siphons contracted.

Following the method of His for preparing geometrical reconstructions of serial sections (Guyer, 1917), sagittal sections were projected on ruled paper so that outlines of important anatomical boundaries could be made.

The membrane associated with the inhalant siphon surrounds the opening like an arched curtain dorsally and laterally but not ventrally and it therefore appears that this orifice cannot be completely closed. Pelseneer (1911) refers to a "valvule du siphon branchial" at the interior origin of the inhalant siphon projecting into the pallial cavity which is found in *Cardium*, *Venus*, *Meretrix* and *Poromya*. He links the presence of the valve with the condition of short siphons. His description and figures are too inadequate to provide comparison with the membrane under discussion and their correspondence is uncertain.

Kellogg (1892) found a structure similar to the one in *Gemma* in *Mercenaria mercenaria*, *Mytilus edulis*, *Mya arenaria* and *Spisula solidissima*

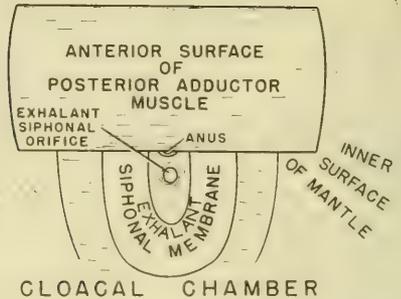


FIG. 8. Semi-diagrammatic view of exhalant siphonal membrane as seen from interior of cloacal chamber. Demibranchs not shown. Length of posterior adductor muscle somewhat exaggerated.

and called it the branchial membrane but could offer no function for it. Dodgson (1928), as well, notes this membrane in *M. edulis* and termed it the inhalant or pallial velum. Ansell (1961a) reports siphonal membranes in *Venus ovata*, *V. casina* and *Gafrarium minimum* and suggests that the membranes control water currents passing through the mantle cavity. Kellogg (1915) later reported it in *Schizothorus nuttalli*, *Tivela stultorum*, *Mytilus californianus*, *Saxidomus gigantea* and *Spisula polynyma* in addition to those mentioned in his earlier report, but dropped *Mercenaria mercenaria* and *Mya arenaria* from the list of the clams possessing it. In the latter paper, he calls it the siphon membrane and suggests its function as that of an organ which helps to prevent fouling of the gills. He states that the membrane "may be raised to admit the incurrent stream freely, or may be drawn downward so as to throw the stream toward the mantle edges. It seems probable that its function is to throw the current downward . . . and away from the gills when much sediment is present." He found another structure usually associated with this membrane which presumably prevents the downward-directed

current from washing the mantle collections forward in the mantle chamber. It is a canal formed by ridges of erectile tissue at the posterior ventral margins of the mantle which may be elevated and bent toward each other to form a waste canal. Collections of rejecta from the mantle enter the canal at its anterior end, pass backwards to accumulate posteriorly for subsequent expulsion. No indication of this canal as such can be found in *Gemma* although the inner rejection trough described earlier apparently performs the function of transporting rejecta posteriorly along the same path as the waste canal, but not acting as a covering against the incoming water current. Dodgson (1928) and Yonge (1948) both concur with Kellogg on the function of the (inhalant) siphonal membrane as being an adaptation to a silty environment.

The exhalant siphon of the gem clam has a similar but more complete membrane guarding the inner aperture at the point of water egress. This membrane (ESM, Fig. 6, 12; Figs. 7, 8) possesses a medially-located, long, ovoid slot about 5 times longer than its greatest width. Laboratory observations indicate that this orifice can be completely closed, thus effectively stopping water movement through the clam. The previously mentioned valvular membrane or extension on the exhalant siphon has been observed to contract, particularly at the distal end, decreasing its diameter so that the external opening of the valvular membrane is closed. From the relatively small amount of muscle tissue in such a thin structure, it seems unlikely that closure of this opening against the exhalant stream could be effected without the assistance of the exhalant siphonal membrane. This reasoning is admittedly meager evidence for deducing the closure of the siphon membrane, but another action of the valvular membrane supports this contention. When the siphons are withdrawn between the margins of the valves, the valvular membrane is drawn, by inversion, into the lumen of the siphon.

Rather than postulate a complex arrangement of muscles capable of performing this movement, it is simpler to infer that the exhalant siphonal membrane is so attached that its contraction serves to move it more internally, so that, like the mammalian diaphragm, it would create a reduced pressure within the siphonal lumen. External pressure then forces the valvular membrane inward. The success of this action is, of course, based on closure of the siphonal membrane. Carriker (1961) describes the collapse and withdrawal of the valvular membrane but holds the *inhalant* siphonal membrane responsible.

The only other references encountered in the literature to a somewhat similar structure have been in connection with rock borers and mussels. Yonge (1958) describes such a membrane in *Petricola carditoides*. This structure is similar to that in *Gemma*, but possesses an annular opening. Cilia, which completely cover the inner surface of the membrane, are said to aid in the outward movement of rejecta. This does not appear to completely answer the question of function, since cilia, if necessary at all in the strong excurrent stream, would act similarly if they just lined the siphon. The interposition of a membrane partially blocking the excurrent opening requires further functional explanation. Like membranes were found in *Pholadidea loscombiana* and *Petricola pholadiformis* by Purchon (1955a, b).

Field (1922) and Dodgson (1928) reported 2 curtains, one above and one below the exhalant siphon opening, which can be brought together to close off the current. Field calls the upper element the anal membrane while Dodgson refers to both as exhalant vela. To have employed the word velum in naming these structures has only further compounded the usage of an overworked term. It has been used to refer to a structure in hydrozoan medusae, rotifers, certain ciliates, lancelets, crocodiles, scallops and veliger larvae. It is proposed, therefore, that, following Yonge's usage,

these membranes be called siphonal membranes, inhalant and exhalant, as the case may be.

Assuming that the exhalant siphonal membrane in *Gemma* can be closed, an attempt should be made to determine or at least speculate as to how it serves the animal. A minimum of 4 possibilities exist.

The first has already been discussed in connection with observations on the closure and retraction of the valvular membrane. The siphonal membrane may thus assist in the withdrawal of the valvular membrane. Carriker (1961) describes the valvular membrane in the juveniles of *Mercenaria mercenaria* which is lost as the animal matures. Quayle (1952) also reports a similar structure in the spat of *Venerupis pullastra* and Ansell (personal communication) has found it in 3 other venerid genera, but does not think that the exhalant siphonal membrane can be closed in any of the species he has studied.

The discovery of an exhalant siphonal membrane closely resembling that of *Gemma* in species bearing such a valvular membrane would tend to support the first proposition.

The second possibility lies in the ability of the membrane to slow or stop the general movement of water through the clam. Such a function might be related to a silty environment; reduction of water flow may enhance the performance of cleansing mechanisms, since normal water movement produces a counter-current to the movement by cilia of rejected particles across the inner mantle surface. A reduction in water flow would also permit the continuance of aerobic respiration with less fouling of the respiratory surface. The membrane may also be of value in reinforcing cleansing movements. Closure of the membrane would tend to prevent passage of water out of the exhalant siphon during adductor contractions, thus aiding in the expulsion of silt and rejecta through the separation between the mantle borders. If this be an adaptation

to turbid waters, the membrane should be sought for in clams inhabiting this type of environment.

The third and fourth possible functions may be related to the entrance and exit of the gem clam's embryos into and out of the brood chambers in the ctenidia. As the female gametes leave the oviducal orifices on their way into the brood chambers, they presumably stand in danger of being swept into the cloacal chamber and out of the exhalant siphon. It is at this time that the exhalant siphonal membrane could eliminate this possibility by closing, thus suspending the outgoing current during the critical transfer. It is also conceivable that rapid, alternate opening and closing of the membrane orifice in conjunction with a similar action of the valves could create pressure differentials in the brood chambers sufficient to dislodge and carry out the young at the end of their period of development. Narrowing of the ex-current stream at the siphonal membrane would possibly increase its velocity sufficiently to propel them out through the exhalant siphon. Liberation of juveniles has been observed in the laboratory and the act is accompanied by slight contractions of the mantle and adductors prior to their arrival into the cloacal chamber. When expelled, they emerge with considerable velocity and settle several millimeters from the female. Other brood-protecting species of lamellibranchs should therefore be examined for a similar exhalant siphonal membrane.

4. Foot

The foot of the gem clam is a well-developed, active organ occupying the whole of the ventral surface of the visceral mass (F, Figs. 6, 9, 10, 22). It has a median longitudinal furrow along the posterior 2/3 of its ventral edge (Fig. 22).

The foot can be extended to a distance which approximates the clam's length. The tip shows considerable mobility, moving readily and quickly in any

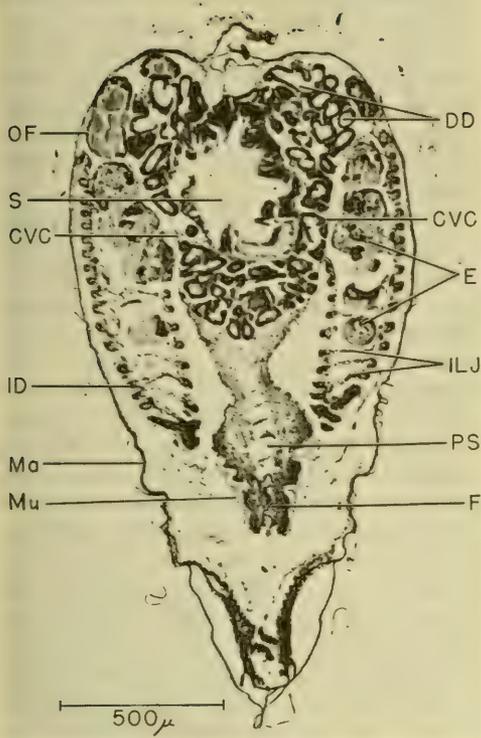


FIG. 9. Transverse section through adult at level of stomach.

direction. Adult clams, placed on the surface of the sand flat, disappear in less than a minute.

The epithelium of the foot is thrown into parallel ridges and consists of a single layer of low columnar, almost cuboidal, cells. No cilia can be seen in sections, but they have been observed on the foot of living juveniles. In adults, particles can readily be seen to pass over the surface of the foot posteriorly and then dorsally toward the base, although Ansell (1961a) states that in the "adult [Venerid] there are no ciliary movements on the surface of the foot."

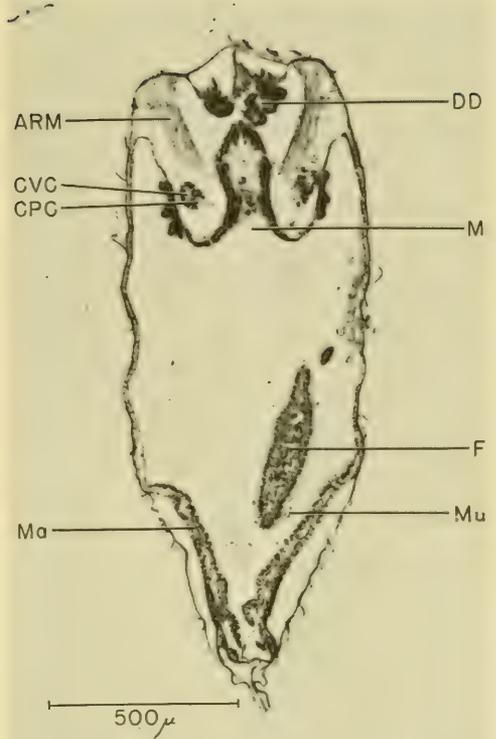


FIG. 10. Transverse section through adult at level of mouth.

A large blood sinus, expanding anteriorly, extends throughout the length of the foot and is traversed by muscle bundles uniting the 2 lateral faces of the foot. Other muscle fibers are seen to run in various directions. The muscles governing major movements of the foot will be discussed subsequently under "Muscular System."

Beneath the epithelial layer of the foot, several conspicuous glands are present which open laterally at the surface and secrete a lightly-staining homogeneous material, apparently a type of mucus, which spreads out over the

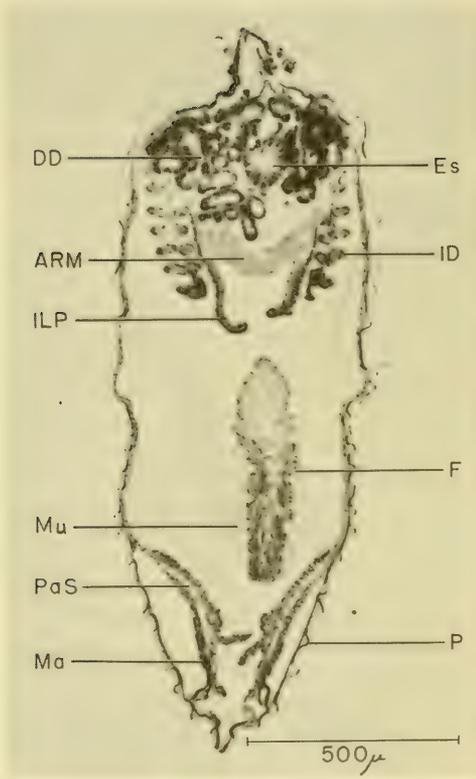


FIG. 11. Transverse section through adult at the level of the internal palps, showing the lobes of the mantle.

surface of the foot. This secretion (Mu) may be seen adhering loosely to the foot epithelium in Figs. 6, 9, 10, 11 and 16.

Although Dall (1902) states that the foot is not byssiferous, and the writer has never observed a byssus in either the juvenile or adult, a prominent gland is present in the foot which appears actively to secrete some substance, if not a byssus (BG, Fig. 6). This gland has been designated the byssal gland in *Gemma*, because of its doubtless homology with byssus-forming glands in other lamellibranchs. The major portion

of the gland is centrally located and, from it, a duct passes tortuously in an antero-ventrad direction to open at the anterior end of the median groove. Secretory cells surround the duct along its entire length and present an appearance difficult to interpret. Their cytoplasm takes up so much stain that nuclei can only occasionally be distinguished and the cells are arranged in groups with indistinct cell membranes.

The substance issuing from the duct orifice is similar in appearance, presumably mixes with that secreted by the subepithelial foot glands mentioned earlier and spreads out over the surface of the foot (Figs. 6, 9, 10, 11, 16). This material may aid in keeping the foot clean and lubricating it in its passage through the sand, but observations on the locomotion of juveniles and adults over glass surfaces indicate that this substance possesses considerable adhesive qualities. Juveniles can readily climb steeply-inclined surfaces and the adult foot has sufficient anchorage to aid in pulling the clam forward. The highly adhesive characteristics of the foot of early *Mytilus* dissoconchs have been noted by Nelson (1928). In byssus-forming species, the functioning of the gland is associated with a sessile existence, whereas in *Gemma*, the gland enhances mobility by the adhesive quality of its secretion, enabling the gem clam to gain a better purchase on the substrate. Burrowing ability must have high survival value in forms such as *Gemma* which are easily dislodged from the bottom by wave action.

5. Muscular System

a. Pedal musculature

According to Pelseneer (1906), lamellibranchs normally possess 4 pairs of foot muscles. Two pairs, the protractor pedis and the elevators, are not present in *Gemma*.

The anterior retractor muscles have their origin on the valves just posterior to the dorsal portion of the anterior

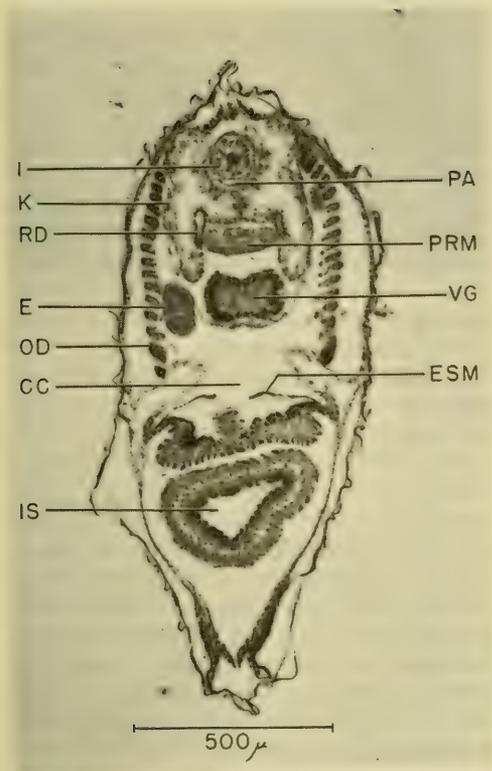


FIG. 12. Transverse section through adult at the level of the visceral ganglia.

adductor muscle (ARM, Figs. 6, 10). Passing posteriorly and ventrally, they converge and join one another ventral to the oral end of the esophagus, there to form a dorso-ventrally flattened bundle. As the bundle proceeds toward the foot, the sides rise to form a "V" at its entry into the base of the foot (ARM, Fig. 11).

The posterior retractors originate just anterior to the dorsal portion of the posterior adductor. They pass anteriorly and ventrally, converging, and join dorsal to the visceral ganglia to form a bundle similar to that formed

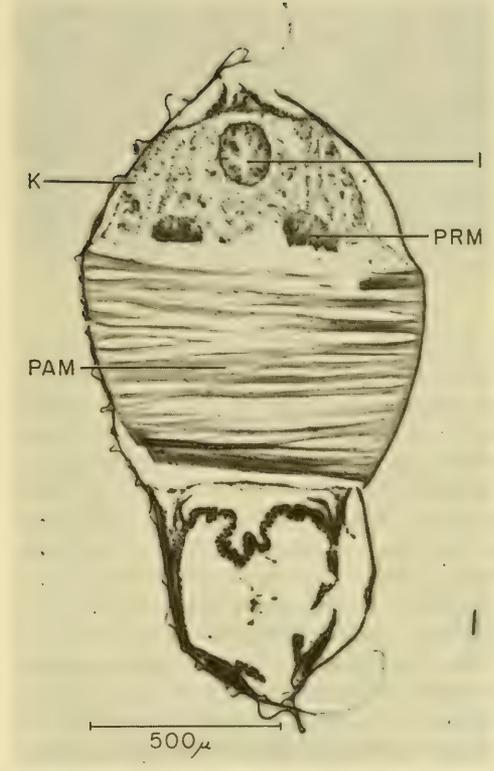


FIG. 13. Transverse section through adult at the level of the posterior adductor muscle.

by the anterior retractors (PRM, Figs. 5, 6, 12, 13, 19). In both instances, fibers forming the arms of the "V" separate and pass down the lateral regions of the foot.

b. Pallial musculature

Pelseneer (1906) groups the adductor muscles with muscles of the mantle, terming the adductors transverse pallial muscles (AAM, PAM, Figs. 6, 13). There is nothing unusual in *Gemma* about these frequently-described muscles; thus they will not be treated in detail. Each adductor possesses both "striated" and

"smooth" fibers; in cross-section, the striated fibers are smaller and more compactly arranged. In the anterior adductor muscle, the striated element is the more posterior of the 2 types of fibers, while in the posterior adductor, it is situated anteriorly.

Rather prominent siphonal retractor muscles (SRM, Fig. 5) have their origin in the mantle above the pallial line at the level of the posterior end of the foot. They pass medially for only a short distance to enter the siphons.

c. Ctenidial musculature

A small pair of muscles associated with the gills are considered worthy of mention because of infrequent reference to them in the literature. Their points of origin are on the inner dorsal aspects of the valves about 1/3 the distance from the posterior end from where they descend ventrally, laterally and slightly posteriorly to insert at the juncture of the inner and outer demi-branches. Monk (1928) found this muscle, which he called the levator branchiarum, in *Sphaerium notatum*, and gave what he believed to be the first description of this structure. Earlier, Pelseneer (1911) referred to a "retracteur de la branchie" in *Anomia*, *Aenigma*, *Hemipecten*, some *Ostrea* and certain Solenidae (*Tagelus*), but his description is so meager that it is difficult to determine whether his "retracteur" is homologous with the levator of *Sphaerium* and *Gemma*.

6. Digestive System

a. Alimentary canal

The mouth opens ventrally and is situated behind the anterior adductor muscle with the anterior lip bordering the ventral aspect of this muscle (M, Figs. 6, 10). The borders are simple and are continued on either side by the labial palps which are discussed later under "Ctenidia." The esophagus, which is round in cross-section and is surrounded by digestive diverticula, rises dorsally for about half its length and

then turns somewhat posteriorly to enter the stomach (Es, Figs. 6, 11).

The stomach lies in the median plane; its inner walls folded into numerous ridges and grooves, all heavily ciliated (S, Figs. 6, 9). Presumably these aid in the movement of materials to and from the head of the crystalline style and into ducts of the digestive gland. Nelson (1918) first showed in *Modiolus* the roles of ridges and ciliated tracts. At least 4 openings lead into ducts of the digestive gland. A translucent, cuticular layer, the gastric shield, may be seen on the dorsal and posterior aspects of the stomach wall (GS, Fig. 6). Cilia are found covering the surface of all epithelia of the alimentary canal except that underlying the gastric shield.

The anterodorsal end of the stomach opens into a diverticulum which turns laterally to the left and ends blindly among the acini of the digestive gland. This is assumed to be the food-sorting caecum. At its opening into the stomach it has 5-6 grooves which unite and diminish in number distally. A portion of the gastric shield enters this caecum and lines part of its ventral and posterior walls.

The relationship of the crystalline style to the intestine varies considerably in lamellibranchs. The range includes those forms where the style lies in the intestine itself, through those where longitudinal ridges or typhlosoles partially separate the style region from the intestine, to those where the 2 are completely separate (Nelson, 1918). *Gemma* is in the second category. The intestine and style sac communicate with one another, but with typhlosoles effecting a partial separation.

The intestine is lined on its dorsal, left and ventral faces by epithelium typical of a style sac, while the right side has typical intestinal epithelium (Fig. 14). The epithelium of the style sac continues anteriorly into the stomach along its floor to a point about half way to the esophagus.

The style is rather short and stout,

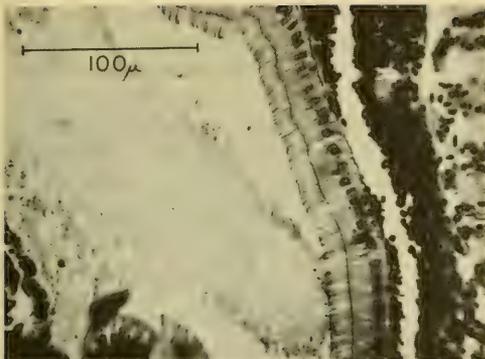


FIG. 14. Section through the crystalline style and style sac showing the style substance in center and ciliated columnar epithelium lining the style sac at right.

its length being only about 5 times its diameter. On one occasion the styles were removed from 5 clams. The clams were kept covered at all times by sea water in finger bowls and were dissected under water less than 30 minutes after their removal from a sand flat which itself was covered by water. In each case the crystal-clear style underwent dissolution and disappeared less than 45 seconds after its removal from the style sac. The water temperature was 21.5°C. The pH of the water was not determined. The rapidity with which the styles dissolve is a function of their surface-volume relations and the pH of the surrounding medium. The minute size of the gem clam style presents more surface area relative to volume than most styles. Yonge (1926) found that the style of *Ostrea edulis* dissolved more rapidly on the alkaline side of the isoelectric point of its globulin than on the acid side.

The intestine arises from the ventral side of the tube it shares with the style slightly anterior to the end of the tube. It passes anteriorly for a very short distance, turns to the right and continues its turn until it is directed posteriorly. After passing beyond the end of the style

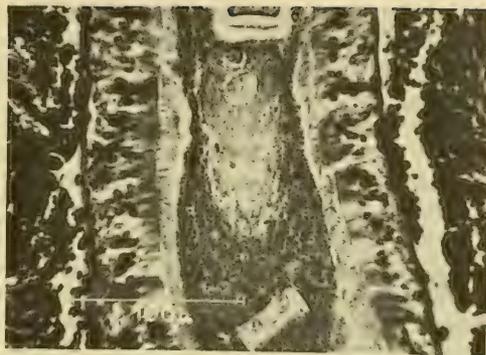


FIG. 15. Longitudinal section through intestine showing contents consisting of organic detritus and the frustules of 3 diatoms. The nature of the cilia and distribution of the nuclei are in strong contrast to that of the style sac epithelium (see Fig. 14).

sac, it ascends dorsally and anteriorly through the gonad. As it rises, it again is directed somewhat posteriorly until it enters the pericardial cavity where it penetrates the ventricle. It continues posteriorly, passing over, down and around the posterior adductor muscle ending at the anus on its ventral aspect. The diameter of the intestine is quite uniform throughout its length until it reaches the adductor muscle where it gradually becomes constricted to about 1/4 of its former size (I, Figs. 5, 6, 12, 15).

b. Digestive diverticula

Digestive diverticula surround the anterior part of the stomach and esophagus and almost entirely fill the visceral mass at this level. They extend anteriorly to end above the mouth opening and, except for the dorsal aspect of the stomach, posteriorly almost to the end of that organ. In this region, the diverticula are surrounded by the gonad (DD, Figs. 6, 9, 10, 11).

The crypts or acini lead into small canals joining larger canals which in turn open into the stomach. The cytological

details of the epithelium lining these elements, their ciliation and the number of the several primary ducts which enter the stomach are not clear from the sections prepared for this study. Although the crypts appear to be devoid of cilia, squash preparations of fresh digestive diverticula made following the feeding of a suspension of *Chlorella*, revealed active movement of algal cells within the crypts. This is due, of course, to ciliary activity somewhere within the system of canals, if not in the crypts. Muscle tissue is present in the crypts or their ducts since the whole organ was in slow constant movement at all times.

7. Circulatory System

a. Heart

The heart lies in the pericardial cavity (PC, Fig. 5) and surrounds the intestine. It consists of a muscular ventricle (V) and 2 thin-walled, conical atria (A). The epithelium of the pericardial glands may be clearly seen investing the walls of the atria in the sections under appropriate magnification. The cells of the glands often have spaces between them, the distal ends of the cells being rounded, but no flagella are seen. The brownish color so often reported as being characteristic of these cells cannot be seen.

No attempt was made to determine the heart rate of adult clams, but the heart of a 0.5 mm juvenile was observed to average 6 beats per minute at 26.0°C.

b. Arteries

Two aortae lead blood away from the ventricle: an anterior vessel situated dorsal to the intestine and a posterior one ventral to it (PA, Fig. 12). Although a dilation of the posterior aorta known as the aortic bulb (or *bulbus arteriosus*) is commonly found in venerids (Pelseneer, 1906), none is apparent in *Gemma*. Possibly because of the nature of the preparations, valves at the aortic roots could not be distinguished, but a

sphincter at the root of the posterior aorta is evident.

The small size of the gem clam makes difficult the task of tracing branches of the aortae. Since other excellent treatments of circulatory systems of similar forms (Field, 1922; Dakin, 1909) are available, their description as well as that of the venous system is not attempted.

c. Sinuses and Blood

The most prominent blood cavity is the pedal sinus in the foot (PS, Fig. 9). It is rather capacious anteriorly, diminishing in size from the middle of the foot to its posterior end. It is continuous with sinuses which pass dorsal and parallel to the anterior and posterior retractor muscle bundles (ARM, PRM, Figs. 5, 11).

Another conspicuous sinus is the pallial sinus which extends along the mantle lobe above and below its attachment to the pallial line (PaS, Fig. 11). It occupies approximately the ventral third of the lobe until it reaches the siphonal region where it branches to continue over and below the mass of mantle tissue forming the siphons. Other smaller sinuses and lacunae may be seen surrounding various organs, but their extent and limits are difficult to discern.

Amoebocytes may be seen in the heart, vessels and sinuses as well as extravascularly in intercellular spaces. Their role in intracellular digestion has been stressed by Yonge (1926) and others, and that in defense reactions by the work of Stauber (1950), who studied phagocytosis and elimination of non-metabolizable india ink particles by these cells in the oyster. Tripp (1958) has shown that intravascularly-injected bacteria, erythrocytes and yeast cells are digested by amoebocytes of the oyster, whereas bacterial spores and starch, which resist enzymatic destruction, are transported ultimately through the epithelial layers to the exterior by the same means.

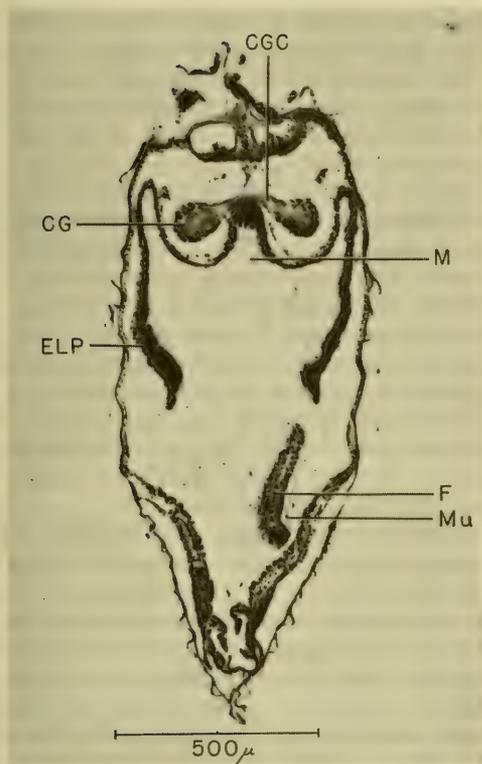


FIG. 16. Transverse section through the adult at the level of the external labial palps.

In fixed and stained preparations, the amoebocytes are irregular in shape, often with one or more pseudopodia extended. Some even assume a stellate form. Their nuclei are large and darkly stained. They are usually widely scattered, but occasionally appear in small clumps.

8. Ctenidial System

a. Labial palps

The palps consist of 2 pairs of appendages approximately triangular in

shape, smooth on the unapposed surfaces and ridged on the apposed surfaces of each pair. They hang suspended in the pallial chamber. The junction between the external palps runs dorsal to the mouth to form the upper lip (ELP, Fig. 16). The connection between the internal palps forms the lower lip. In fixed material, the external palps are generally in an extended state whereas the internal pair have a tendency to curl inward, sometimes almost a full turn, so that the smooth sides facing the median plane are markedly concave (ILP, Fig. 11). Six ridges are present on the apposed surfaces of the gem clam's palps. The anterior ends of the inner demibranchs project between the 2 palps on each side.

The epithelium of the smooth surfaces consists of a single layer of cuboidal cells while that of the ridged surfaces is a layer of columnar cells which bear cilia. No cilia can be demonstrated on the smooth surfaces, although certain workers (Matthews, 1928, and others) have noted sparse clumps on these surfaces.

Internally, each palp consists of spongy connective tissue, blood spaces and muscular bands.

b. Ctenidia

The chief functions of the ctenidia or "gills" of lamellibranchs are respiration and food-getting. Pomeroy (1951) has shown that calcium ions are absorbed from sea water by these organs in the oyster. Some ovoviviparous species, such as *Gemma*, utilize the demibranchs as pouches in which the embryos develop (ID, Figs. 9, 22). The ctenidia have elicited considerable interest in the past, which has found expression particularly in the exhaustive studies of Ridewood (1903) and Atkins (1936, 1937). Only certain aspects of the ctenidia will be discussed here with emphasis on their function in brood protection.

According to the terminology of Lan-

kester⁴ and of Ridewood (1903), the ctenidia are synaptorhabdic, since the interfilamentar junctions are cellular in nature. No plication occurs and the filaments are therefore homorhabdic. The inner demibranchs have about 50 filaments; the outer, about 30.

In the majority of lamellibranchs, a ctenidium has an outer and inner demibranch. Each demibranch is usually reflected back on itself so that there is a descending element, the lamella (directed ventrally), and an ascending lamella (directed dorsally). Due to the orientation of the outer demibranchs of *Gemma*, it will be convenient to adopt the terminology of Lacaze-Duthiers (1856) and substitute direct for descending and reflected for ascending. The inner demibranch is typical in that it possesses both direct and reflected lamellae and is oriented in the usual fashion (ID, Figs. 9, 22). The outer demibranch, however, appears to have only one lamella, the direct, which passes dorsally from its proximal attachment to the visceral mass at the gill axis to join the visceral mass distally at its juncture with the mantle (OD, Fig. 5). This condition is found in certain other bivalves, and Ridewood (1903), in commenting on this, says: "It might be suggested here that in the upturned lamella ... the whole of the outer demibranch is present and that the position of the bend in the filaments has gradually approached the axis, or retreated from it and finally disappeared."

On the other hand, Pelseener (1911) considered that dorsal orientation of the outer demibranch in the Anatinacea is not due to upward bending, but that what remains of this demibranch is nothing but the supra-axial extension, which is a continuation, above the gill axis, of the reflected lamella found on the outer demibranch of most eulamelli-

branches.

The outer demibranchs of the gem clam are quite small and are located posteriorly (OD, Figs. 5, 12). They run with the inner demibranchs for only about the last half of the length of the latter and extend somewhat posteriorly to them. The length of the filaments of the outer demibranchs is about 1/4 that of the inner.

The picture presented by the ctenidia of *Gemma* is representative of certain other brood-protecting species in that it has the characteristic inner demibranch, but an atypical outer demibranch. In the Anatinacea,⁵ many of which are brood-protecting, the outer demibranch is similar to that of *Gemma*, whereas *Lasaea* has a direct lamella ventrally disposed. In *Teredo*, it is a mere vestige and in *Lucina*, *Scioberetia* and *Montacuta*, it is entirely lacking.

Although many eulamellibranchs have 2 sets of orad directed longitudinal food currents, one at the ventral margin of each demibranch and the other along the gill axes between the bases of the 2 demibranchs on each side, *Gemma* has only the former. This type of gill is designated as Type E in the classification of Atkins (1937), which includes most of the Tellinidae, the Semelidae and the Anatinacea. This current was demonstrated in *Gemma* using a suspension of No. 600 carborundum powder and observing the movement of particles placed on the upturned direct lamella of the outer demibranch. There was no interruption of the passage of particles across the gill axis so that they all proceeded to the marginal furrow of the inner demibranch.

The inner demibranchs (ID, Fig. 18) are prominent structures which extend from palps to siphons. According to Pelseener (1906), the upper edges of

⁴In a series of unpublished lectures given at the Royal Institution in his capacity as Fullerman Professor.

⁵In the classification scheme of Pelseener (1906), the Anatinacea are a suborder corresponding to the suborder Anomalodesmata of Thiele (1935).

the reflected lamellae are joined to the visceral mass in all eulamellibranchs. This connection is seen in prepared sections of *Gemma* as a tenuous but definite tissue junction which is easily broken when preparing sections (Fig. 22), or when dissecting live or fixed specimens. The fragility of this tissue may account for occasional references in the literature to the lack of such a connection. (Hansen, 1953, for *Transennella tantilla*;⁶ Matteson, 1948, for *Elliptio complanatus*).

Beyond the posterior end of the visceral mass, the distal edges of the reflected lamellae of the inner demi-branches lose their connection with the mass and converge across the median plane joining by a ciliary junction so as to form a part of the floor of the cloacal chamber (CC, Fig. 5). The gradual transition from the typical 2 lamellae at the anterior and middle region of the demibranch to one lamella at the posterior end is seen as one examines the sections in sequence. Although both lamellae progressively decrease in height, the reflected lamella does so earlier than the direct, so that the bend in the filaments approaches and finally meets the distal end of the reflected lamella as the ciliary junction is formed. The marginal food furrow disappears at this point as well. The floor thus formed extends to the intersiphonal septum and completes the separation between the 2 opposing sets of water currents (Fig. 7).

The direct lamellae of both inner and outer demibranchs lose their proximal attachment to the visceral mass posteriorly at the level of the siphons. The brood chambers formed by each thus become continuous and allow free passage of juveniles from the outer demi-

branches to the inner ones. Thence, the embryos (E) may pass into the cloacal chamber, through the aperture of the exhalant siphonal membrane (ESM) and finally out of the exhalant siphon (ES, Figs. 5, 6, 12).

9. Nervous System

Description of this system is based solely on a study of serial sections which were not stained for nervous tissue. Therefore, only the major structures and nerves were observed. A summary of the identifiable parts arranged according to their origins follows.

- (1) cerebral ganglia:
 - anterior pallial nerves
 - cerebro-pedal connectives
 - cerebro-visceral connectives:
 - renal nerves
- (2) pedal ganglia:
 - pedal nerves
- (3) visceral ganglia:
 - posterior adductor nerves
 - branchial nerves
 - posterior pallial nerves:
 - siphonal nerves

a. Ganglia and their branches

In primitive clams, such as *Nucula* and *Solenomya*, the cerebral and pleural ganglia are distinct and separate, but in the more advanced species, such as *Gemma*, they are intimately fused (Pelseneer, 1906). Following common usage, the cerebro-pleural ganglia will be referred to as the cerebral ganglia.

These ganglia are found above the mouth opening high up on the posterior face of the anterior adductor muscle. They are well separated, but are connected by a stout commissure (Figs. 6, 16). Three paired branches leave these ganglia.

The anterior pallial nerves pass anteriorly to the junction of the upper lip and external labial palps, then ventrally to pass under the anterior adductor muscle and enter the mantle where they are lost.

The cerebro-pedal connectives, which

⁶Hansen (personal communication) states that re-examination of his sections has revealed the presence of this connection in some of his presumably more carefully prepared sections.

link the cerebral and pedal ganglia, leave the former and, buried in fibers of the anterior retractor muscles, pass down into the foot to join the pedal ganglia. It is generally agreed (Pelse-ner, 1906; Dakin, 1909; Field, 1922) that, in spite of their proximity to the pedal ganglia, the statocysts are not innervated by those centers. The statocyst fibers arise in the cerebral ganglia, pass down the cerebro-pedal connectives part way to emerge from them as the statocyst nerves. These nerves were not seen in *Gemma*.

The cerebro-visceral connectives (CVC) leave the ganglia with the cerebro-pedal connectives but soon diverge from the latter to pass posteriorly to the visceral ganglia. They follow a straight, somewhat superficial course through the visceral mass which takes them through the digestive diverticula on either side of the stomach (Fig. 9). They continue through the gonad (Figs. 18, 22) and pass parallel to and just beneath the sperm ducts (Figs. 19, 20) or oviduct (Fig. 23). They proceed between the kidneys (Figs. 5, 25) where they give off a renal nerve on each side. Continuing further, they become associated with the posterior retractor muscles (PRM), at first running above them (Fig. 5), then lateral to and finally below them as the muscles begin their ascent to their origins. At this point, the connectives enter the visceral ganglia. Fibers from the osphradial ganglia, which are adjacent to the osphradia, are reported to pass to the cerebro-visceral connectives, but the ultimate innervation of these organs is not settled. Pelse-ner (1906) states that, where they can be demonstrated, the fibers originate in the cerebral ganglia. On the other hand, Dakin (1909) states that they arise in the visceral ganglia. The course of these fibers could not be ascertained in the gem clam.

The pedal ganglia are intimately fused, in contrast to the cerebral ganglia. They are found below the posterior end of the stomach well up in the visceral mass and thus are not actually in the foot

(PG, Figs. 6, 17). Three large pairs of pedal nerves are given off in anterior, ventral and posterior directions.

The visceral ganglia are also in juxtaposition and, as in *Thracia*, a member of the Anatinacea, are located on the anterior face of the posterior adductor muscle (VG, Figs. 6, 12). In most lamellibranchs, they are found on the ventral surface. Three paired branches leave these ganglia.

Anteriorly, the branchial nerves leave the ganglia below the cerebro-visceral connectives and pass ventrally to enter the inner demibranchs near the posterior ends of the latter, where the upper edges of the reflected lamellae lose connection with the visceral mass.

A small pair of posterior adductor nerves leave the posterior ends of the ganglia to supply these muscles.

A large pair of nerves, the posterior pallials, leave the posterior ends of the ganglia. They soon give off a small branch, the siphonal, and shortly thereafter become lost in the mantle.

b. Special sense organs

The osphradia, believed to be organs of chemoreception, are situated in the roof of the cloacal chamber ventral to the visceral ganglia. From their similarity to osphradia of gastropods and their position in the chamber, earlier workers readily concluded that they test the respiratory fluid (Pelse-ner, 1906) and initiate reflexes to regulate water flowing past them (Vlès, 1909). From experiments Allen (1923) conducted on osphradia of freshwater mussels, he concluded they are sensitive to a number of substances in solution and that the clam responds in such a way as to expell noxious materials and prevent their subsequent entry.

At first thought, the position of osphradia in the excurrent stream seems remote and futile since the character of the water would not be known until just before leaving the animal. If, however, particulate matter were to interfere with the function of the

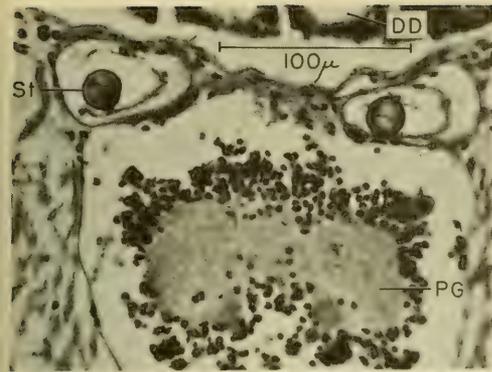


FIG. 17. Section showing pedal ganglia and statocysts.

osphradia, their location in the excurrent stream becomes logical, since the water has been freed of such matter by the gills and other means, but still contains solutes which could be detected by the osphradia.

The osphradia of *Gemma* are seen as paired thickenings of epithelium of the cloacal chamber just ventral and slightly anterior to the visceral ganglia. Cells of this organ bear a superficial resemblance to those of the pericardial gland in that they are long, sometimes separated from adjacent cells and are rounded on their distal ends. Some are large and pear-shaped. The epithelium of the osphradia is 3-4 times thicker than the adjacent areas.

Paired statocysts are present in the gem clam. They are located dorsally and laterally to the pedal ganglia. An ovoid capsule encloses a smaller one of similar shape and within the latter is a single statolith (St, Fig. 17). Connective tissue fibers or loosely packed cells are seen in the space between the outer and inner capsules which serve to support the inner capsule. The statolith is a greenish, somewhat transparent, almost spherical body. A slight constriction girdles it and fine striae appear to radiate from a common center. In one specimen 2 statoliths were found

in each of a pair of statocysts.

Although presence of cilia or tactile hairs is generally associated with the inner lining of the statocyst, none was demonstrated in *Gemma*. This has been a common experience among investigators of this organ and some have voiced the suspicion that their apparent absence may be due to the method of histological preparation. In the general work on mollusks by Pelseener (1906) may be found a figure of the statocyst of *Cyclas* which exhibits cilia although the author allows that they "may be absent in some." Field (1922) dissected out the statocysts of living *Mytilus edulis* and found them to contain "vibratile cilia." Drew (1906), as well, found cilia in histological preparations of the statocysts of *Pecten tenuicostatus*. On the other hand, Dakin (1909) failed to find them in *P. opercularis* and *P. maximus*, but found the statocysts bounded by a layer of "sensory cells." Earlier, Drew (1895) was unable to demonstrate the presence of cilia in *Sphaerium sulcatum* but acknowledged that the fault may have lain in the preparation. Monk (1928) was also unable to find them in *S. notatum*, but, using as his criterion the presence of cilia elsewhere in the preparation, he concluded that the histological method employed was not responsible for their absence. Whether cilia or hair cells are truly lacking in some forms or whether special methods are sometimes required to demonstrate them, can only be answered by further work.

10. Reproductive System

Reproductive and excretory systems of mollusks are closely associated anatomically. During their evolutionary history, however, there has been a tendency toward separation of these systems until, in eulamellibranchs, they are completely distinct. Their past relationship is best shown in the manner in which their ducts open to the exterior, often in close association with one another.

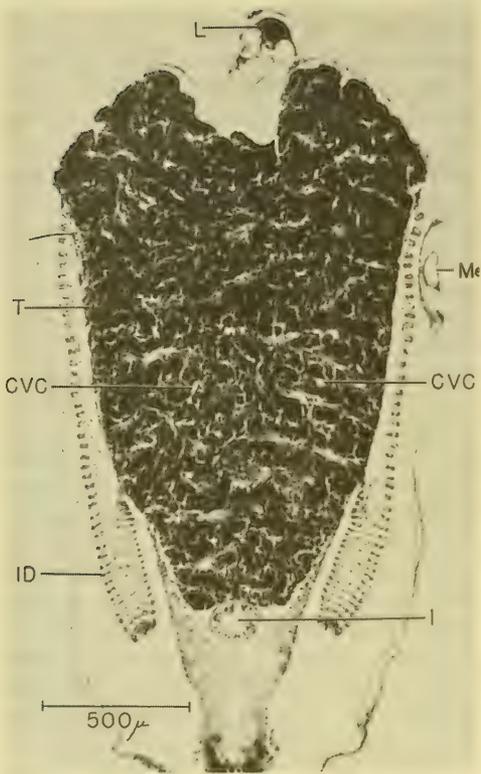


FIG. 18. Transverse section through middle of adult male clam. Compare with female in Fig. 21.

a. Male

According to Pelseneer (1906), the molluscan gonad is a paired structure although this is not always readily apparent, as in the gem clam, because the testicular follicles are packed together (T, Fig. 18).

Two lobes of the testes extend anteriorly on each side as far as the esophagus in the dorsal region of the visceral mass and lateral to the digestive diverticula. Although posteriorly, the gonads take up more space than the

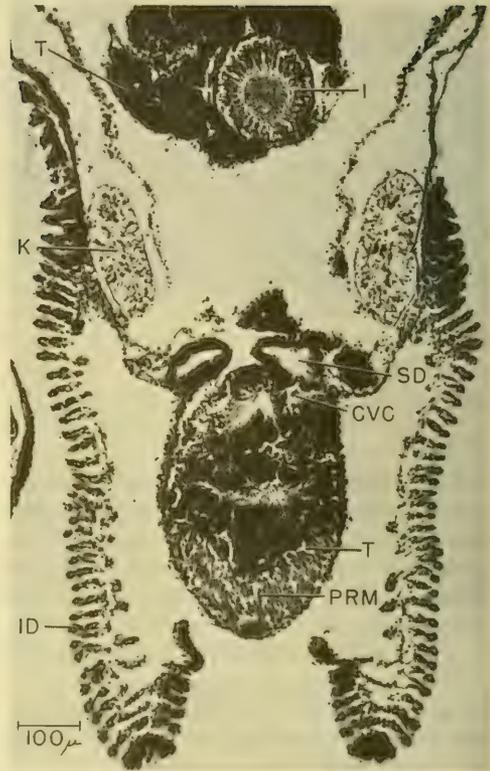


FIG. 19. Transverse section of male through the sperm ducts.

diverticula, the allocation is about equal at the mid-region of the stomach. At the level of the pedal ganglia and posterior end of the stomach, the entire visceral mass is occupied by the testes except for a small portion taken up by the alimentary tract (Figs. 6, 18). Posteriorly, at the level of the heart and kidney, they end as 2 median lobes one above the other (Fig. 19).

Loosanoff (1937) found that the hard clam (*Mercenaria mercenaria*), a close relative of the gem clam, develops, at a few months of age, a primary gonad which is bisexual and contains the ante-

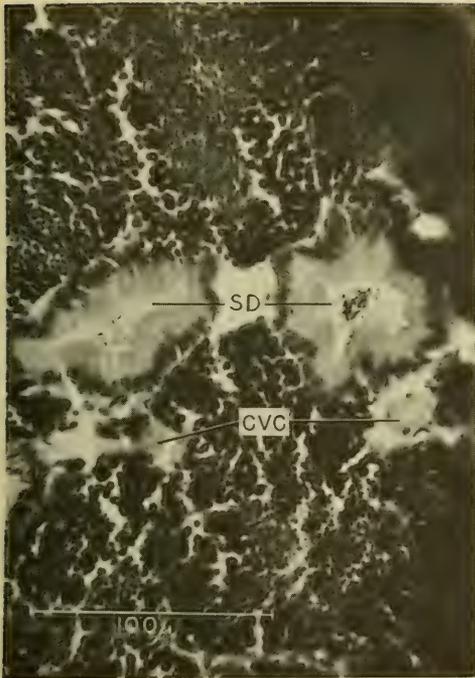


FIG. 20. Section through sperm ducts showing cells undergoing spermatogenesis in testicular follicles as well as mature spermatozoa in lumina of sperm ducts.

cedent cells of both sexes. Shortly thereafter, it is given over to spermatogenesis in almost all individuals. Some clams function as males by the end of the first summer, others in their second summer. About half the individuals, however, begin developing and functioning as females in the spring of their first year and the sex ratio remains essentially equal thereafter. A negligible number of true hermaphrodites was found in his study.

No gem clams less than one year in age were sectioned, and it is therefore not known whether they pass through a protandric phase. Of 25 examined, all were found to possess reproductive cells of only one or the other sex. Fretter & Graham (1964), however, state that *Gemma* is a simultaneous hermaph-

rodite.

The follicles in early summer present the typical picture of spermatogenesis with larger cells (spermatogonia and primary spermatocytes) at the periphery, smaller ones (secondary spermatocytes and spermatids) in intermediate positions and spermatozoa in the lumen (Fig. 20).

The sperm duct, which begins near the posterior end of the testes, is a dorso-ventrally flattened, thick-walled, ciliated tube. It soon bifurcates and passes posteriorly as paired ducts (SD, Figs. 19, 20) which open into the epibranchial chambers just anterior to the renocloacal orifices. The walls of the ducts are composed of a single layer of ciliated columnar cells, the nuclei of which are at the ends farthest from the lumen. Spermatozoa, possessing the typical crescent-shaped head of venerid spermatozoa, are frequently seen in the lumina (Fig. 20).

b. Female

The main bulk of the ovaries lies posterior to the stomach (Fig. 21). Follicles (OF) extend anteriorly in the dorsal part of the visceral mass as 2 lateral lobes and posteriorly as a single median lobe. The right lobe extends as far as the anterior half of the stomach (OF, Fig. 9), whereas the left lobe extends to a point not quite as far anteriorly, presumably to accommodate the food-sorting caecum on that side of the stomach. Distribution of ovarian material is about equal at the level of the posterior end of the stomach and pedal ganglia. Here, except for space occupied by the alimentary tract, the dorsal region is taken up by the ovaries, the ventral, by the digestive diverticula. The visceral mass is given over entirely to ovaries at the level of the pedal ganglia and for a short distance posterior to them (Fig. 22). The gonads end as a single lobe at the level of the anterior end of the heart.

It can hardly be said that the gem clam possesses an oviduct. The most

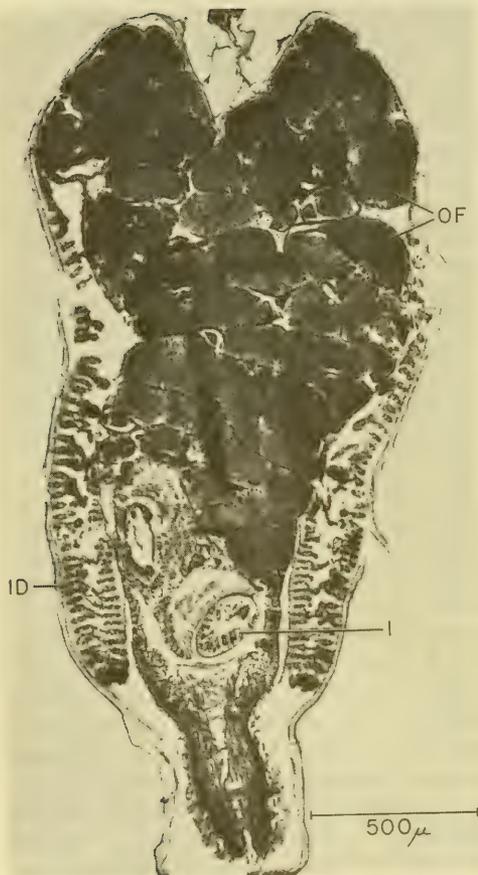


FIG. 21. Transverse section through middle of adult female clam to show the extensive nature of the gonad at this level. Germ cells at late stage of development. (Compare with male in Fig. 18). Clam collected June 26, 1957.

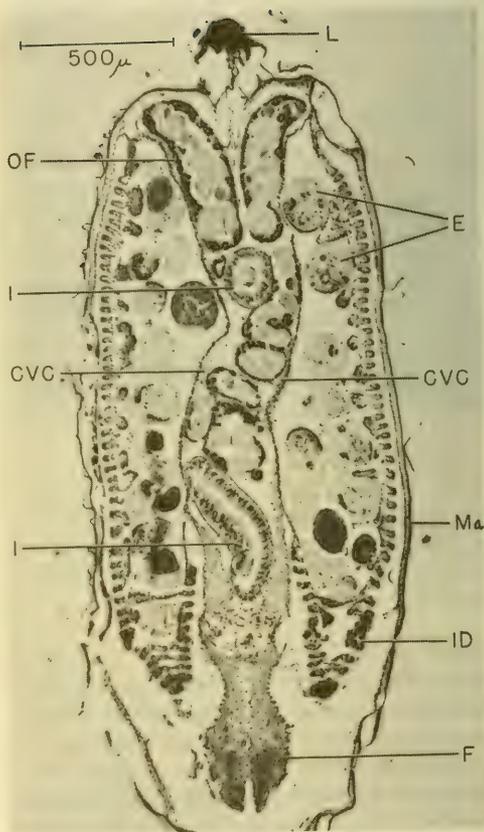


FIG. 22. Transverse section through adult female clam somewhat posterior to the level shown in Fig. 21 showing the gonad and embryos in inner demibranchs. Germ cells at earlier stage of development than those seen in Fig. 21. Clam collected June 26, 1957.

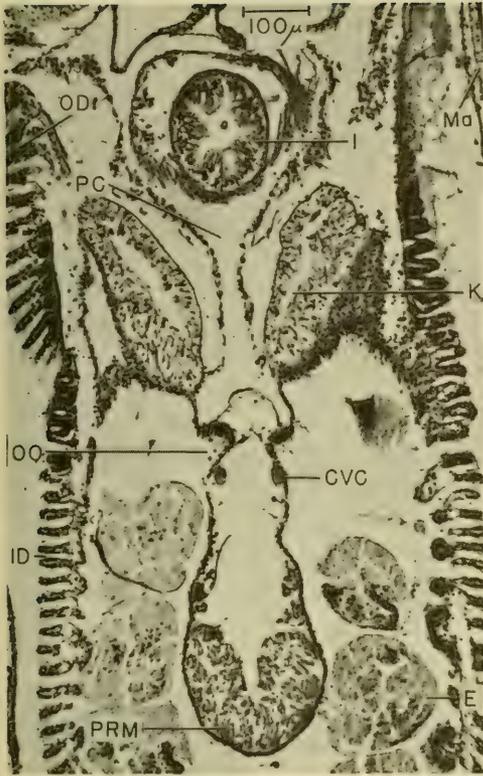


FIG. 23. Transverse section through female at level of oviducal orifices. Clam collected June 26, 1957.

posteriorly located follicle in each gonad narrows almost to a point and then opens into the epibranchial chambers on both sides very close to the midline by 2 very short ciliated orifices (OO, Fig. 23) which are anterior to the excretory openings. These openings constitute points of egress of germ cells from the gonads to the brood pouches. The openings and the brood pouches communicate with the cloacal chamber.

In the female, follicles are larger, less numerous and more variable in size than in the male. The wall of the follicle consists of 1 or 2 layers

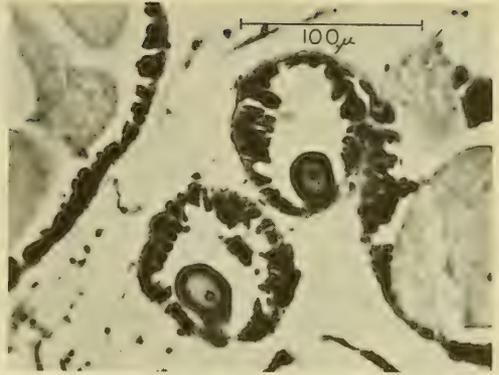
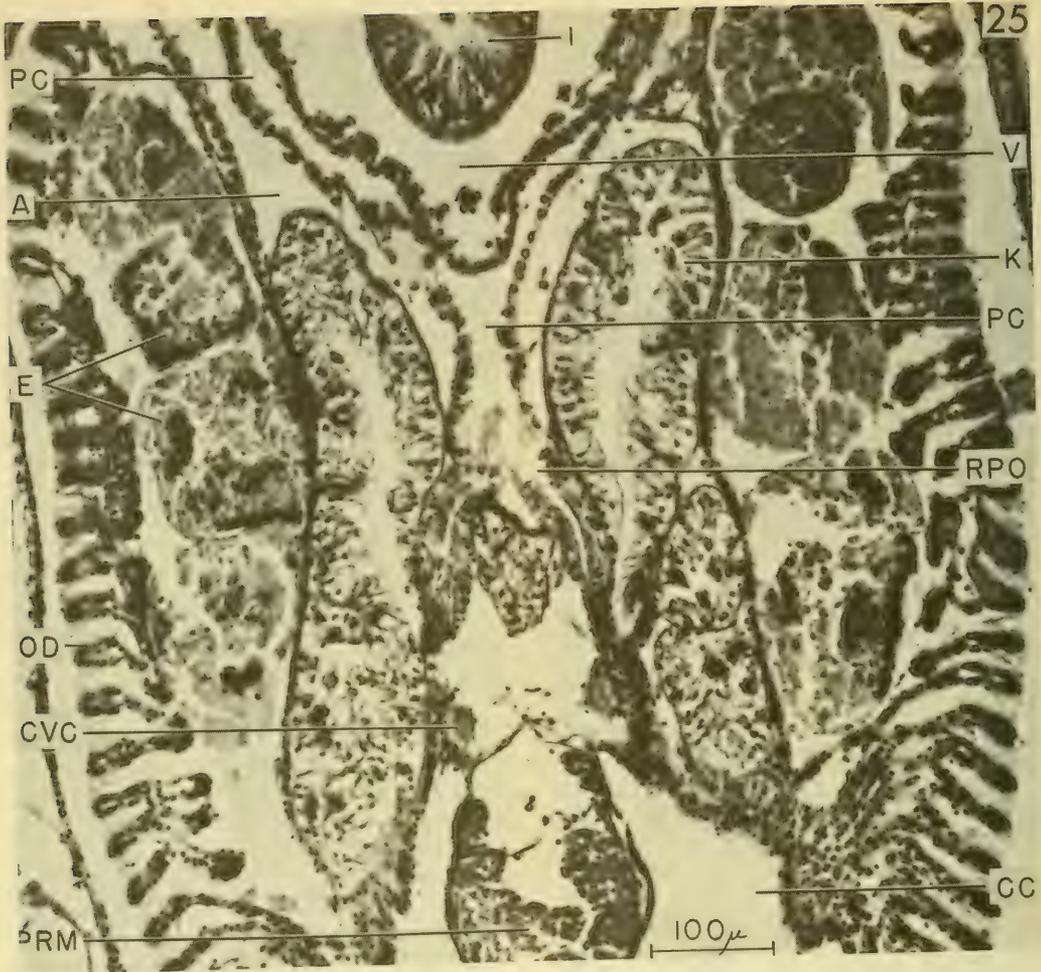


FIG. 24. Section through ovarian follicles showing germ cells in various stages of development. Clam collected June 26, 1957.

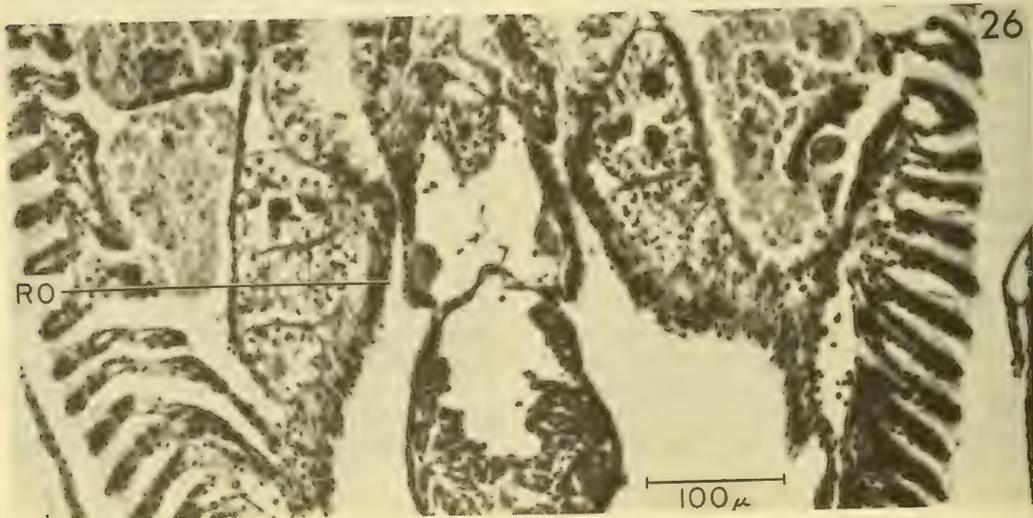
of flattened cells serving as a base for support of the germinal epithelium, cells of which project into the lumen (Fig. 24).

In sections made in early summer, a relatively large number of germ cell precursors are present in the epithelium with considerably fewer later stages still attached or in the lumen. Figure 24 shows 2 small follicles with precursors and rather undeveloped germ cells attached to the follicle wall with vacant space in the lumina. Adjacent follicles contain larger cells in a later stage of development, which are occupying the entire lumen.

It has been the practice of many to employ a rather loose terminology in designating fertilizable germ cells of mollusks. The literature of early embryology points out that, in many, if not in most mollusks, female germ cells are released and fertilized either in the germinal vesicle stage or metaphase of the first maturation division. They are thus primary oocytes about to become secondary oocytes, yet they are almost invariably referred to as ova. The term gamete is usually given to a germ cell capable of union with a germ cell from the opposite sex. It would seem appropriate, therefore, to



25



26

refer to the female germ cell at this stage as a gamete rather than an ovum.

11. Excretory System

The organs of excretion in lamelli-branches have been variously called nephridia, renal tubes, organ of Bojanus and kidneys. They are open, tubular structures consisting of a pair of modified coelomoducts leading from the pericardial cavity to the exterior. The lumina of these ducts, those of the genital organs and the pericardial cavity represent the coelom in mollusks. In the formation of urine, the processes of filtration, secretion and reabsorption play a part. The maintenance of a homeostatic balance of water, inorganic ions and nitrogenous wastes involves, in addition to the kidney, the pericardium, gills, mantle and digestive gland.

The kidneys of *Gemma* are U-shaped tubes which extend beneath the pericardium from a point below the anterior end of the ventricle to a point above the middle of the posterior adductor muscle (K, Figs. 6, 12, 13, 25, 26). Openings are found at both ends of the tubes. Blood is filtered through the wall of the ventricle into the pericardial chamber (Picken, 1937), the kidneys receiving the filtrate through 2 internal openings, the renopericardial orifices (RPO, Fig. 25), which open into the pericardial cavity below the mid-region of the ventricle. The wall of an afferent renopericardial duct (RD, Fig. 12) consists of a single layer of ciliated cuboidal epithelium, the cilia of which are very long and fill the lumen of the tube at its anterior end. The ciliary beat is said to be in the direction of the external openings (Monk, 1928; Scheer, 1957). The tube passes posteriorly under the main bulk of the kidney (Fig.

12), where, as it approaches the bend of the "U", its histological character changes to that of the anteriorly-directed limb, the so-called glandular portion.

The glandular part of the kidney appears as an elongated sac which connects transversely with its partner at the posterior level of the ventricle. The lumen of the glandular portion is at times capacious (K, Fig. 12) and at other times almost occluded by the variable nature and thickness of its wall (K, Fig. 13). The wall is made up of long, loosely-arranged cells which are considerably vacuolated, with nucleus and protoplasmic material situated at the base of each cell. The free ends are often decidedly convex. The wall is thrown into folds giving an irregular appearance to the cavity. In addition, numerous diverticula, some of major proportions, lead off from the main cavity.

It is difficult to say if cilia are present in the glandular portion of the kidney of the gem clam. In the literature there is generally neither a positive nor negative statement made in this respect. Monk (1928) does not find cilia here in *Sphaerium notatum*. On the other hand, Duwe (1958) states that cilia are present in *Unio*, while Dakin (1909), although noting "delicate processes" in *Pecten tenuicostatus*, is inclined to believe they are due to an excretion which forms in a fibrous manner. These processes are observable in *Gemma* and give the distal ends of the cells a frayed appearance suggestive of apocrine secretion.

The lumen of the glandular part of the kidney often contains irregularly-shaped, bluish-gray concretions (C, Fig. 5). They are also seen within the cells lining the lumen. It is the glandular

FIG. 25. Transverse section through adult female gem clam showing (internal) renopericardial orifices. The 2 regions labelled "pericardial cavity" do not appear to be continuous with one another. This is probably due to a slight displacement of tissue during preparation of the section.

FIG. 26. Same as Fig. 25 except slightly posterior.

part of the kidney which is believed to play a part in the secretory aspect of renal function in mollusks. Here nitrogenous wastes are extracted from the blood and inorganic ions returned.

The kidneys end as short efferent tubules which open into the cloacal chamber as external renal orifices (RO, Fig. 26) below and behind the renopericardial openings (RPO). The excretory openings are close to and slightly posterior to the genital orifices.

VII. FOOD AND FEEDING

Gemma is a filter feeder which strains suspended material from the water. It is presumed that, as in other lamellibranchs, latero-frontal cilia of the demibranchs accomplish the filtering, and food particles so captured are transported to the mouth in mucus strings. Food material, which can only be identified as diatoms and organic detritus, is commonly seen in the digestive tract when sections are examined (Figs. 6, 15). Presence of food in the tract neither signifies that it will be digested and utilized nor that it is necessarily indigestible. Studies on the nutrition of lamellibranchs have thus far yielded only limited information. Loosanoff and his associates, however, have done considerable work in this field, particularly with larval clams. Studies have recently been made by Dean (1957) on the nutrition of 1- and 2-year old oysters. No attempt was made to concentrate on this aspect in the gem clam except for certain studies on the movement of particles on the gills and movement of particles within the digestive diverticula.

Bradley & Cooke (1959) have observed that the feeding of *Gemma* is largely confined to night time or periods of subdued light. Turbidity of the water may thus influence feeding activity due to its shading effect.

VIII. GROWTH

1. Methods

A high mortality rate averaging about

40% each month (Fig. 47) ruled out the marking and measuring of individual gem clams at periodic intervals in the work area. When a species has a fairly limited and known breeding period, like *Gemma*, (see Fig. 42), the best or only method available for measuring growth is that of taking successive samples of approximately known mean age from a population. This was the method used.

If the mean values for size are to be truly representative of individual growth, it must be assumed that clams of a particular size range within a year class are not moving into or out of the sampling area as this would tend to alter the mean size. Since this possibility has not been ruled out, the values given must be looked upon with that reservation.

Another assumption must be made that may not necessarily be warranted. That is, that mortality rates of all sizes of clams within a year class are the same. This, also, has not been demonstrated and there is some evidence that differential mortality does occur.

Sampling was, for the most part, restricted to a particular sand bar of an area of about 1000 m², which was above the mean low water level. The choice of this level reduced the possibility of losses from the sampler which might occur had the samples been taken under water.

The samplers were a series of glass jars with a diameter across the mouth of 2.1, 6.0 and 10.7 centimeters. The size of the sampler used was governed by the relative density of clams at the time of sampling. Collections were made at monthly or semi-monthly intervals from April, 1955, through August, 1958. The number of samples of each year class at each collection varied from 1-50, although the minimum was generally 10, with an overall average of about 18. (See Table 6 and Appendix for number of samples at each collection.) Since the area covered by any given sampling averaged only about 1/15,000 of the total sampling area, it was felt that the effect of sampling

on reducing the population was negligible.

The sand bar which ran parallel to the shore was divided along its long axis into 3 belt transects; one along the land-facing slope, one on the sea-facing slope and one along the crown. One-third of the total sample was taken randomly in each of the transects.

The sampler was pressed into the bottom to a depth of about 2 inches, as previous studies had established that no living clams were present below a depth of 1 inch. Unfortunately these studies were made during the warm season, and no attempt was made to determine the depth at which the clams might be found during the winter. A winter observation by Bradley & Cooke (1959) in Maine revealed that some gem clams were to be found at a depth of as much as 3 inches. At least half of the samples in the present study, however, were taken down to or into the black reducing layer where no living gem clams are found. Samples at Union Beach are probably comparable to those taken by Bradley & Cooke, since their sampler went to a depth of 1 and 3/8 inches. The samples were transferred to a series of 12-inch diameter nested screens of such mesh size that the bottom screen would hold the smallest clam. (A 65 mesh per inch screen was used during the summer when juveniles were present in the sediments. This screen has a pore size of 210 μ . Screens of larger aperture sizes were employed as growth proceeded.) As much sand as possible was removed. Prior to measurement the sample was preserved in 10% formalin with 2.5% hexamine (hexamethylenetetramine) as a neutralizing and buffering agent. Samples of newly-liberated juvenile clams could not be screened, as, at this stage, they approximate the size of sand particles. These particular samples were placed in an aqueous solution of $ZnCl_2$ having a specific gravity of about 1.95, which separated the sand from the clams by causing the clams to rise to the surface due to their differences in density

(Sellmer, 1956). It was necessary to treat the screened samples in a similar fashion to remove shell trash and pebbles.

For growth studies, only the maximum antero-posterior dimension (length) was determined. An attempt to measure 100 clams of each year class in all samples was, for the most part, successful. This goal was not always attained, especially at the beginning and end of the life span when densities were low. (See Table 2 and Appendix for numbers measured on each collection date.) All clams were measured with an ocular micrometer in a stereoscopic microscope; those less than 1 mm were measured at 60X to the nearest 10 μ while those greater than 1 mm were measured at 20X to the nearest 39 μ . A total of 6,524 clams were measured.

Since many dead clams, even those devoid of soft parts, did not gape, it was necessary to distinguish with certainty between those which were alive and those which were dead at the time of collection. Appearance of the shell exteriors of living and dead gem clams differs considerably; the former exhibit a sheen, particularly in the region of new shell, whereas dead clams are dull and lusterless. The most important criterion for distinguishing between them, however, is the ability of fresh adductor muscle to transmit light through the shell more readily than does other tissue. Adductors of living clams and those preserved when alive, appear as bright spots when light is passed through the clam. This phenomenon is assumed to be due to an internal scattering effect (a property of Lucite) which, by repetitive reflection within the cylindrical muscle, serves to channelize light in its passage through the animal. It might be expected that judgment by this criterion would be less accurate at times of near freezing temperatures when muscle tissue of dead clams is better and longer preserved. A study using 250 clams was made to test the observer's ability to judge from external appearances as

TABLE 2. Representative growth data for *Gemma gemma* (1956 year class). For data of other years, see Appendix.

Collection Date	Number Measured	Mean Length (μ)	Range (μ)
1956:			
June 11	20	557	470-660
June 25	50	398	340-520
July 11	100	581	350-950*
July 23	100	752	400-1380**
Aug. 8	100	1157	600-1980
Aug. 23	100	1343	850-2418
Sept. 5	100	1744	1140-2340
Sept. 20	100	1805	1287-2613
Oct. 19	100	1813	1326-2730
Nov. 18	100	1850	1248-2574
Dec. 20	100	1923	897-3549
1957:			
Jan. 17	100	1907	1248-2964
Feb. 1	100	1928	975-3237
Mar. 16	100	1883	1209-3588
Apr. 11	100	1969	1248-3081
May 11	100	2213	1365-3432
June 11	100	3058	2184-3705
June 26	100	3690	2301-4641
July 10	100	4018	3042-5148
July 26	100	4238	3198-5226
Aug. 8	100	4220	3510-4875
Aug. 26	13	4142	3588-4875

* one individual measured 1870 μ

** one individual measured 2340 μ

to whether or not a clam was alive when collected. An error of less than 0.5% was noted.

2. Distinguishability of Year Classes

Study of the growth of the gem clam was focused on individual year classes. Where size variations are great enough, age groups may often be distinguished from one another when plotted as size-frequency polygons. Since variation in growth rates eventually causes age groups to merge due to the faster growing members of one group overtaking the slower members of the next older group, it is necessary that individual clams be identified with a specific age group. This problem was simplified due to relatively rapid growth of a given year class which formed a size-frequency polygon well along the

size scale when the next year class appeared in the samples. The maximum life span is, at most, 2 years, so that there are never more than 2 year classes present at any one time. When, during fall and winter, polygons of 2 successive year classes approach and slightly overlap, shell characteristics, particularly the growth-arrest ring earlier described and possessed by members of the older year class, allow separation of equal-sized individuals into their respective year classes. Inspection of clams at monthly (and bi-weekly intervals during the warm season) forestalled the possibility of mistaking a growth-arrest ring due to water disturbances or reproductive activity with the growth-arrest ring due to cessation of growth during the winter.

Length-frequency distributions of 3

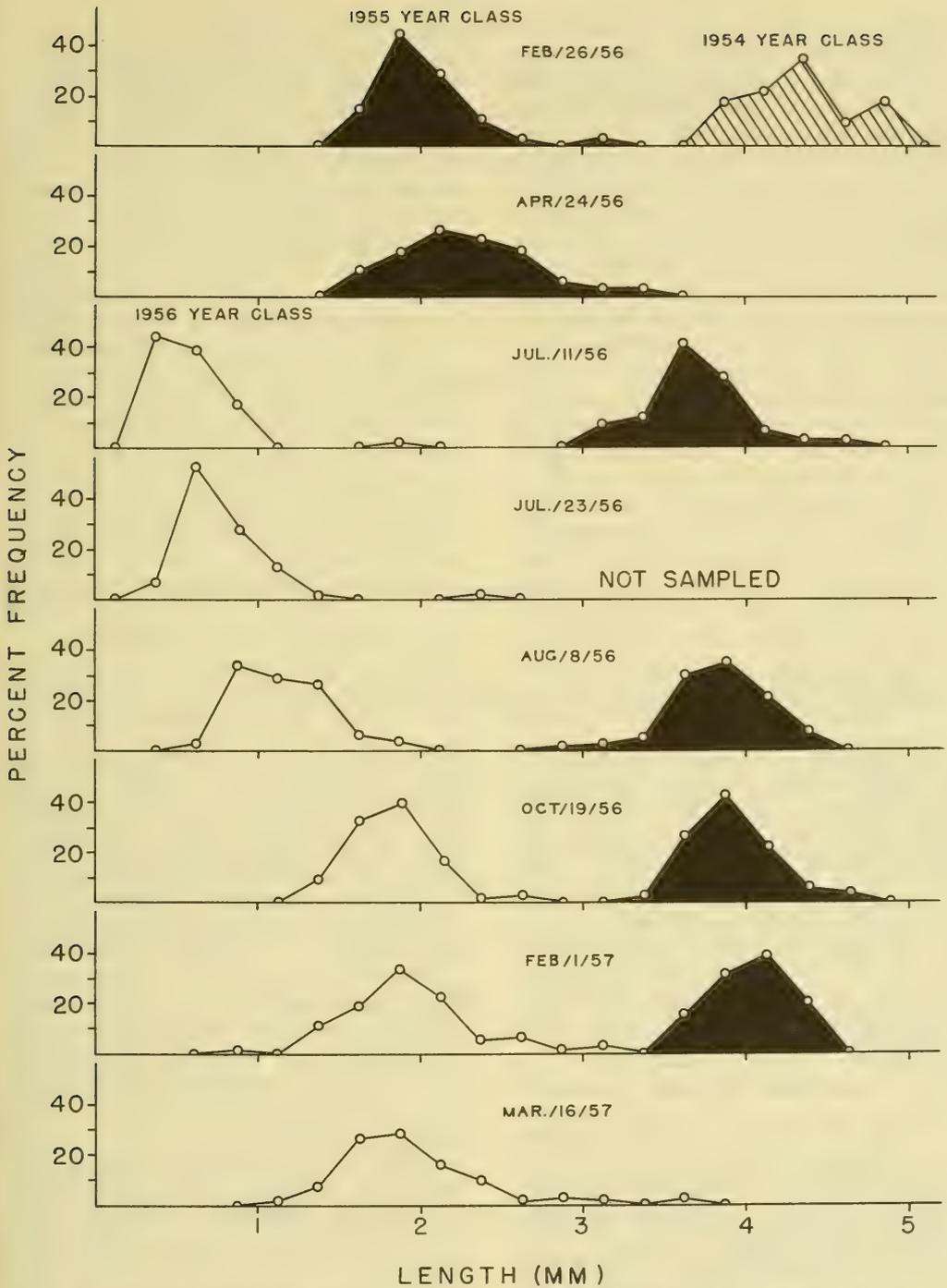


FIG. 27. Selected length-frequency polygons of 3 year classes of *Gemma gemma* to show discreteness of age groups. (The 1954 year class is cross hatched; the 1955 class solid black; the 1956 class white).

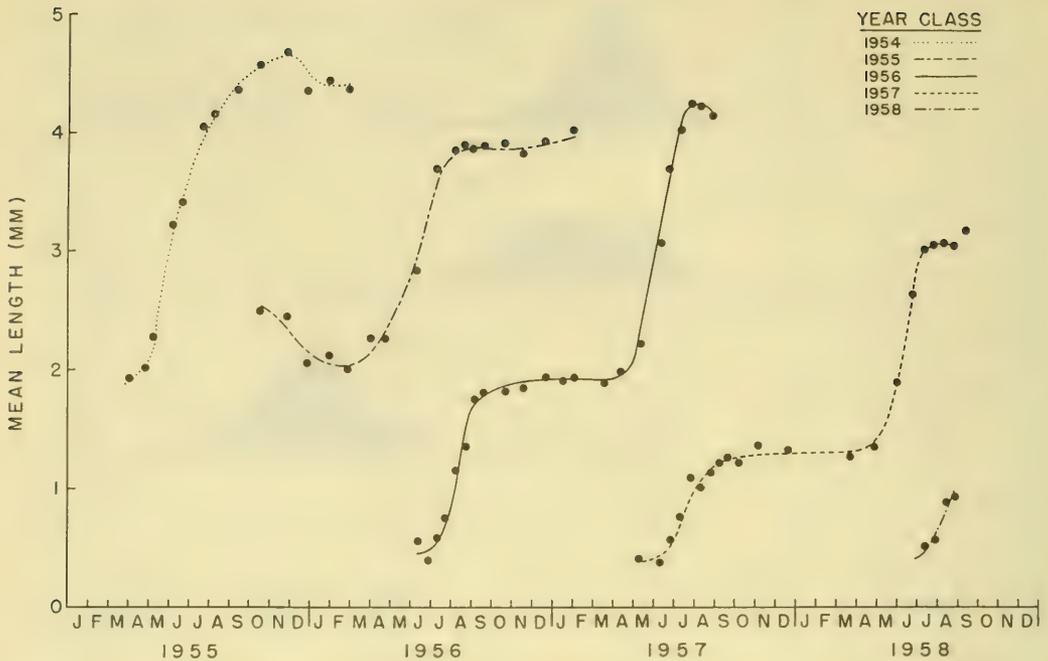


FIG. 28. Growth curves of 5 year classes of *Gemma gemma*. In most cases, each datum point represents the mean length of 100 clams. See Table 2 and Appendix for further information.

year classes are shown in Fig. 27. The discreteness of these age groups is easily seen. The older, larger year class (1954) dropped out during the winter leaving the younger year class (1955) to be joined by a new year class (1956) the following summer. That this sequence is repeated each year is seen by the coincident distributions in February of each year as well as in the comparable months of April, 1956 and March, 1957.

3. Growth of 1954-1958 Year Classes

The growth curves of 5 year classes are shown in Fig. 28. None can be said to represent the entire life span of any year class. The 1954, 1957 and 1958 classes are incomplete due to the present study beginning and ending during their life spans. The 1955 class is not represented by the younger stages due to the lack, at that time, of a technique for collecting them. Study of the 1956

class was terminated prematurely by heavy mortality as a result of crab predation. Since, however, the second (and last) growth period had been completed by the 1956 and 1957 year classes, their curves are essentially complete.

These curves show a number of things, some only in a very general way. Included are initial and terminal sizes with intermediate values, year to year variations, seasonal changes in growth rate, number of year classes present at a given time, spanning time and life span. The latter 2 will be dealt with later in more detail.

Considerable variation in growth can be seen from year to year. This may be observed especially when the 1956 and 1957 year classes are compared. Temperature is the factor most often thought of in connection with growth of poikilothermous animals because of its effect on their metabolic rate, feeding activity and production of their food.

Since water temperatures were taken only at the time when the population was sampled, no extensive data have been gathered with respect to this factor. Mean monthly air temperatures at the U. S. Weather Bureau's station at Newark, New Jersey, for the period covered by this study are suggestive of a correlation between the characteristics of these growth curves and temperature, but no serious attempt has been made to develop possible relationships.

The curves show, as might be expected, a rapid increase in size beginning in late spring with a general leveling off as temperature decreases in the fall. The data indicate that growth begins in April, but they are not complete enough to signify very precisely the terminal month. It can be said that it falls somewhere between August and November.

A marked difference between the 1956 and 1957 year classes is seen in mean length, which each attained at the end of their growth period in 1957. The seemingly poor growth of the 1957 year class is even more striking when compared with the excellent concomitant growth of the 1956 year class. The cause of the early demise of the 1956 year class as a population may also be responsible for the apparent poor growth of the 1957 year class. It is postulated that large numbers of lady crabs (*Ovalipes ocellatus*) which almost certainly eliminated the 1956 year class during August, 1957 (see "Predators"), may have extended their predatory activities into the ranks of larger members of the 1957 year class. Some evidence of this is seen in a comparison of size-frequency polygons (not shown) of this class. A small proportion of the larger members of the year class present in late July were not present in late August. Attention is directed to growth data of the 1957 year class in the Appendix, where a decrease in maximum size during August may be seen.

In Fig. 28, decrease in mean size

is evident in both the 1954 and the 1955 year classes during the winter of 1955-1956, but not during subsequent winters. Several factors could account for this phenomenon. The sizes of the samples might be too small to truly represent the population or there might be an actual shift in the size structure of the population. Movement of smaller-sized clams into or larger sizes out of the sampling area would decrease the mean size as would also a higher mortality rate among the larger clams.

Both Weymouth (1923), who studied the Pismo clam, and Smith et al. (1955), who worked with the soft-shell clam, noted decreases in mean size at particular times and suggested that their samples may have been too small.

The number of gem clams in the 1954 year class measured during December, January and February averaged only 12 per month. The small size of these samples are easily subject to sampling error. On the other hand, the number of 1955 year class clams measured during the same period averaged over 200 per month. The *t* test was applied to the 1955 year class to determine whether sampling variation could account for the difference in mean size value of this year class before and after the size decrease. A value for *t* of 9.98 was obtained indicating that the probability was less than 0.01 that the decrease observed was due to sampling variation.

The most likely explanation may be found in changes in the size composition of the gem clam population within the sampling area due to migrations and/or mortality of particular size groups within a year class.

Lindner & Anderson (1956) and Haven (1957), in studying the shrimp *Penaeus setiferous* and the marine teleost *Micropogon undulatus*, respectively, noted a leftward shift of size-frequency polygons during the winter. As a possible explanation, they proposed emigration and mortality of the larger animals and entry of the smaller sizes into the

population.

Small gem clams are no doubt moved about by waves more readily than large ones. The possibility exists, though it was not investigated, that this action resulted in an accumulation of smaller sizes in the sampling area, therefore reducing the mean size of sampled clams.

The generally high mortality rate (Fig. 47 and Table 7) and the difficulty in finding a suitable substance for marking large numbers of tiny clams ruled out any experiment designed to determine active, unidirectional migratory behavior of the gem clam. Therefore, it is not certain whether migration could contribute to the size decrease.

Hatton (1938) discovered a decrease in size in 3 groups of barnacles situated at different water levels. He was unable to explain it. Deevey (1947), however, in citing the work of Hatton, suggests that size decrease of a population may be due to differential mor-

tality.

This suggestion most probably offers an explanation for the case in *Gemma*. Examination of Fig. 29 shows that a considerable proportion of the larger members of the 1955 year class in the population in October of that year were not present 4 months later.

Causes for differential mortality have been discussed in the literature. In his study of the bay scallop, Belding (1910) noted that older and faster growing members of a year class were the first to die when environmental stresses were placed upon them. Pearl & Miner (1935) cite a number of investigations which demonstrate an inverse correlation between rate of living and duration of life and state that that rate is a major factor in determining longevity. Comfort (1956) also suggests that a process of morphogenetic aging occurs at different rates depending on rate of growth. He cites findings of a number of investigators which show that the

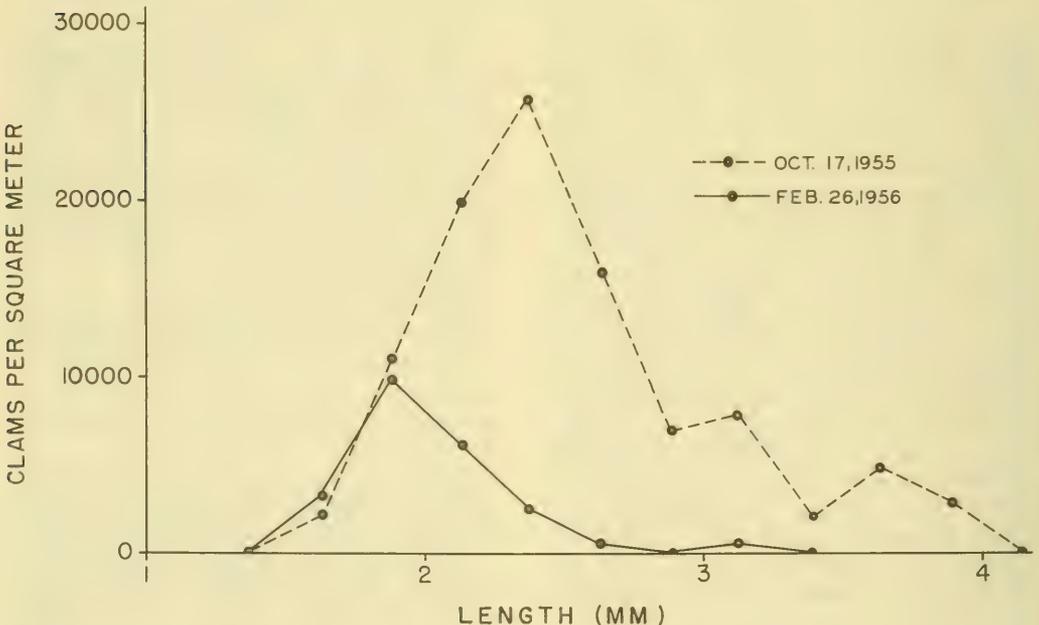


FIG. 29. Length-frequency distributions of the 1955 year class on 2 different dates to show evidence of the disappearance of larger members of the year class during the interim period.

shortest-lived individuals were those with the highest growth rates.

It would appear that temporally or physiologically older individuals are less likely to survive environmental buffetings, and the below-normal temperatures which prevailed during the period of size decrease of *Gemma* could be a factor in the possibly higher mortality of the larger members of the 2 year classes. Selective predation could play a part here, but, at present, the possible factors are not known.

IX. REPRODUCTION

1. Method of Reproduction

The reproduction of *Gemma* has been variously described as viviparous, ovoviviparous, larviparous, incubatory and brood-protecting. Search for definitions of these conditions reveals that the multiplicity of terms results from a variety of criteria employed to describe this method of reproduction. Most of them involve the following situations: (1) nutritional relationship to the maternal organism, (2) stage of development at birth, (3) location of embryos within the female, (4) presence or absence of membranous covering at birth, (5) degree of activity at birth, (6) degree of protection against environmental vicissitudes and (7) site of fertilization. Some of the foregoing are obviously interrelated.

Some can be more properly applied to the gem clam than others and the elimination of those less suitable is in order.

Larviparity is the condition where the mother releases the young as larvae. Since the transition of larva into adult is frequently gradual, the problem of distinguishing between the 2 often involves an arbitrary decision. When the young of *Gemma* leave the mother, they are fully-shelled miniatures of the adult and might be called juveniles rather than larvae. To be sure, they undergo further growth and development but, in

general, the adult structures are laid down prior to release. This is quite in contrast to other genera, such as *Ostrea* and *Teredo*, where the young are released as veligers and bear little resemblance to the adult. It is felt, therefore, that the term larviparous is inappropriate when applied to *Gemma*.

The term brood-protecting may be employed here but, in its broadest sense, this concept embraces all forms of maternal solicitude and a more restrictive term is sought.

Ovoviviparity is used to describe the condition where the embryo is retained within the mother, during which time considerable development ensues. Prior to birth, the yolk of the egg is the only food supply. According to the generally accepted sense of the work, *Gemma* can be classified as being ovoviviparous since the young are retained within the mother, but do not receive nourishment. That which constitutes being within the mother, however, has been open to various interpretations. In mollusks location of the embryos may range from a site within the ovaries or their tubules, through a position within the water tubes of the demibranchs, to being situated in the mantle cavity. Pelseneer (1935) reserves the first of these sites for the ovoviviparous condition, but when the young have left the genital orifices, although still in contact with the mother, he terms it incubation. Moreover, he divides incubation into those instances where the young are in contact with tissue and those in which they are not (e.g., shell) and designates these conditions as internal and external incubation, respectively.

Incubation is usually defined as a period during which an organism develops under conditions favorable to its development; at the end of this period, the organism becomes manifest by birth or hatching. Pelseneer has changed the connotation of incubation from that of a time period to that of a condition based on location. Further, he has restricted the general sense of

the word to exclude ovoviviparity which would be included under incubation as originally defined.

In making a distinction between a site in the ovary or oviducts and a site elsewhere in the body (demibranchs or pallial cavity), Pelseneer is distinguishing between contact with different types of tissue. The difference, however, between contact with mesodermally or ectodermally-derived tissue is slight in contrast to the difference between tissue contact itself or none at all as in the case of embryos attached externally to the mother's shell or in egg capsules adhering to the substratum. The degree of protection afforded embryos in contact with maternal tissue is usually much greater, and the step toward viviparity much easier, than with those embryos outside the external openings of the mother.

Viviparity is generally used to refer to the condition where organisms nourish their young while they are held within the mother, and, since this is not believed to occur in *Gemma*, it may be discarded. Pelseneer (1906) flatly asserts that "viviparous lamellibranchs are unknown." It seems apparent that viviparous animals have passed through an evolutionary series from oviparity through ovoviviparity to their present condition. Contact with maternal tissue by the young places them in a position to develop a nutritional relationship with the mother. Non-nutritional relationship with the maternal tissue is a convenient criterion with which to delimit the condition of ovoviviparity. In view of the location of gem clam embryos within the mother and the nature of their relationship to her, ovoviviparity is the most appropriate term to be used to describe this aspect of reproduction in *Gemma*.

2. Factors Influencing Development of Brood-protection

Although there exist a number of scattered records on the occurrence of brood-protection and ovoviviparity in

lamellibranch mollusks, there are relatively few publications that deal with the subject in anything but a cursory way. Consequently, an attempt was made to tabulate such information as was available in the hope of arriving at a better understanding of the occurrence and significance of this phenomenon.

This information may be found in Table 3 in which marine and brackish-water clams are arranged by family together with information on size, type of brood protection and details that were available on other aspects of reproduction. Species whose young lack a pelagic phase are included since it is inferred that the mother, in these instances, protects her young from the dangers of planktonic life in some way if only by attaching her eggs to the substratum.

It is seen by the large number of families represented, that some form of brood protection has arisen repeatedly and independently among lamellibranchs. Ovoviviparity is found in all orders except the Protobranchia and in almost half of the eulamellibranch families.

Widespread occurrence of protection of young makes it tempting to speculate on factors which might bear a relationship to this condition. An interesting fact emerges from a study of Table 3. Except for members of 2 families, the Ostreidae and the Teredinidae, all known ovoviviparous clams, for which size is recorded in the literature, are less than 1/2 inch long. It is granted that the absence of pelagic larvae in some larger species does not necessarily imply ovoviviparity, since eggs could be fastened to the substratum. However, no clam less than this size, the reproductive behavior of which is known, is oviparous. Since the size of the eggs cannot fall below a certain limit, because of the physiological demands of embryonic development, small clams are limited in the number of eggs which they can produce. A gravid gem clam may contain a maximum of only 300 young (Table 5), whereas the oviparous American oyster, *Crassostrea virginica*, in contrast, may

TABLE 3. List of marine and brackish water bivalves possessing some form of brood protection. In general, the terminology from the reference is used except where "viviparous" has been employed. Taxa used are from Pelseneer (1906).

Taxon	Aver. or max. length (mm)	Form of Brood Protection and Other Notes on Reproduction	Reference
PROTOBRANCHIA			
Nuculidae			
<i>Nucula nucleus</i>	23.0	Taken for granted that pelagic life is very short	Jørgensen, 1946
<i>N. tenuis</i>	4.75	Undoubtedly non-pelagic or very short pelagic life	Thorson, 1936
<i>N. t. expansa</i>	17.4	Pelagic stage presumably very short or absent	Ockelmann, 1958
<i>N. delphinodonta</i>	5.0	20-70 eggs in gelatinous sac attached to posterior end of valves, non-pelagic development 3-4 weeks, liberated in adult state	Drew, 1901
Nuculanidae			
<i>Leda pernula</i>	32.0	Larval development non-pelagic or very short	Ockelmann, 1958
<i>L. minuta</i>	15.7	Very short pelagic stage	Ockelmann, 1958
<i>Yoldia hyperborea</i>	44.7	Pelagic stage presumably very short or absent	Ockelmann, 1958
<i>Y. thraciaeformis</i>	35.5	Pelagic stage presumably very short	Ockelmann, 1958
<i>Portlandia arctica</i>	28.5	Pelagic stage of short duration	Ockelmann, 1958
<i>P. lenticula</i>	8.3	Pelagic stage very short or non-existent	Ockelmann, 1958
<i>P. fraterna</i>	3.7	Pelagic stage very short or quite suppressed	Ockelmann, 1958
FILIBRANCHIA			
Arcidae			
<i>Arca vivipara</i>	-	Incubatory	Pelseneer, 1906
<i>A. pectuncutoides grandis</i>	16.9	Pelagic stage, if any, short	Ockelmann, 1958
<i>A. glacialis</i>	25.6	Probably non-pelagic, protandric hermaphrodite (?)	Ockelmann, 1958
<i>A. frieli</i>	16.9	Probably pelagic stage, if any, very short	Ockelmann, 1958
<i>Adacnarca nitens</i>	-	Incubatory	Burne, 1920
Limopsidae			
<i>Limopsis</i> spp.	6.4-12.7	Incubatory	Pelseneer, 1906
<i>Cyrella minuta</i>	-	Incubatory, rel. size of young to adult 1 : 3.5	Howard, 1953
<i>Hochstettaria</i>	-	Incubatory	Bernard, 1897
Philobryidae			
<i>Philobrya setosa</i>	2.75	Incubatory, 12 embryos, 1/9 diameter of adult	Howard, 1953
<i>P. costata</i>	-	Incubatory, 9-10 embryos	Bernard, 1897
<i>P. aviculoides</i>	-	Incubatory, 12 embryos	Bernard, 1897

Table 3 (continued)

Taxon	Aver. or max. length (mm)	Form of Brood Protection and Other Notes on Reproduction	Reference
Mytilidae			
<i>Dacrydium vitreum</i>	6.2	Pelagic stage very short or quite suppressed	Ockelmann, 1958
<i>Modiolaria nigra</i>	47.0	Egg strings attached to byssus of parent	Ockelmann, 1958
<i>M. lateralis</i>	25.0	Spins fibrous nest	Miner, 1950
<i>M. discors</i>	25.0	Egg string around mother in a "nidus"	Thorson, 1946a
<i>Crenella glandula</i>	6.4	Builds nest	Miner, 1950
<i>C. decussata</i>	4.6	Pelagic stage rather short or entirely lacking	Ockelmann, 1958
Propeamussidae			
<i>Propeamussium imbriferum</i>	20.8	Assumed pelagic stage short or almost lacking	Ockelmann, 1958
<i>P. groenlandicum</i>	29.5	Assumed pelagic stage short or almost lacking	Ockelmann, 1958
Ostreidae			
<i>Ostrea edulis</i>	80.0	Embryos held in pallial & branchial chambers	Abbott, 1954
<i>O. lurida</i>	75.0	Embryos held in pallial & branchial chambers	Abbott, 1954
<i>O. permollis</i>	75.0	Embryos held in pallial & branchial chambers	Abbott, 1954
<i>O. frons</i>	50.0	Embryos held in pallial & branchial chambers	Abbott, 1954
<i>O. equestris</i>	50.0	Embryos held in pallial & branchial chambers	Abbott, 1954
<i>O. angasi</i>	-	Embryos held in pallial & branchial chambers	Pelseneer, 1906
EULAMELLIBRANCHIA			
Astartidae			
<i>Astarte borealis</i>	55.0	Eggs in gelatinous envelopes attached to substratum	Thorson, 1946a
<i>A. elliptica</i>	40.0	Eggs in gelatinous envelopes attached to substratum	Thorson, 1946a
<i>A. montagui</i>	26.0	Eggs in gelatinous envelopes attached to substratum	Thorson, 1946a
<i>A. crenata</i>	32.0	Very short or no pelagic stage assumed, adhesive eggs	Ockelmann, 1958
<i>A. sulcata</i>	22.9	Very short or no pelagic stage assumed, adhesive eggs	Ockelmann, 1958
Carditidae			
<i>Milneria kelseyi</i>	6.4	50 young in external pouch in indented ventral shell margins covered with sheet of periostracum	Abbott, 1954
<i>Thecalia</i> spp.	-	Young held in exterior cavity in fold of the shell	Bernard, 1898

Table 3 (continued)

Taxon	Aver. or max. length (mm)	Form of Brood Protection and Other Notes on Reproduction	Reference
Carditidae (cont.)			
<i>Cardita concamerata</i>	-	Incubatory	Bernard, 1898
<i>Venericardia purpurata</i>	-	Incubatory	Burne, 1920
Condylocardiidae			
<i>Condylocardia</i> spp.	1.2	Incubatory, 1 embryo, 300 μ , released in adult state	Pelseneer, 1935
Modiolarcidae			
<i>Modiolarca trapezina</i>	-	Young incubated in gills, 20 embryos	Pelseneer, 1935
<i>M. magellanica</i>	11.0	Incubatory, young liberated in adult state, 330 μ	Pelseneer, 1903
<i>Phaseolicama</i>	-	Brood-protecting	Thorson, 1946b
<i>Kidderia</i>	-	Brood-protecting	Thorson, 1946b
Cyamiidae			
<i>Cyamium minutum</i>	3.0	2-6 eggs within capsule attached to substratum	Matveeva, 1953
<i>Turtonia minuta</i>	3.0	Eggs attached to substratum or byssus	Ockelmann, 1958
Diplodontidae			
<i>Thysaria gouldi</i>	9.0	Pelagic stage presumably short or absent; earliest stages possibly developing in gills	Ockelmann, 1958
<i>T. equalis</i>	8.8	Pelagic stage very short or quite suppressed	Ockelmann, 1958
<i>Axinopsis orbiculata</i>	4.6	Pelagic stage presumably very short or absent	Ockelmann, 1958
Lucinidae			
<i>Lucina</i> spp.	Mostly < 6.4	Gills often used as brood chambers	Miner, 1950
<i>L. lactea</i>	12.0	Incubatory but swimming larvae, embryo length 250 μ duration of embryonic life > 15 days	Pelseneer, 1926
Montacutidae			
<i>Montacuta ferruginosa</i>	7.0	Young in demibranchs, possesses only internal ones, 140 μ when liberated	Pelseneer, 1935
<i>M. clarkiae</i>	2.5	Incubatory, 20-50, 400 μ embryos, pelagic life	Pelseneer, 1935
<i>M. phascolionis</i>	-	Incubatory, pelagic life, 415 μ when liberated	Pérès, 1937
<i>Isoconcha sibogai</i>	5.5	Incubatory, liberated in adult state	Pelseneer, 1911
<i>Synapticola</i>	-	Incubatory	Pelseneer, 1911
<i>Jousseamiella</i>	-	Incubatory	Pelseneer, 1911
Leptonidae			
<i>Lepton parasiticum</i>	small	Young brooded inside mantle cavity, about 12 embryos liberated in adult state	Pelseneer, 1935
<i>Kellia suborbicularis</i>	3.75	Large numbers of young held in inner demibranchs, young 64-80 μ when liberated, pelagic life	Howard, 1953

Table 3 (continued)

Taxon	Aver. or max. length (mm)	Form of Brood Protection and Other Notes on Reproduction	Reference
Leptonidae (cont.)			
<i>Lasaea rubra</i>	2.5	12-22 300 μ long embryos in inner demibranchs, released in adult state	Howard, 1953
<i>Aligena elevata</i>	5.1	Brood-protecting but some pelagic life, 150 μ when liberated	Thorson, 1946a
<i>Bornia longipes</i>	8.5	Incubatory	Pelseneer, 1906
<i>B. corbuloides</i>	-	Incubatory	Bernard, 1898
<i>Mysella bidentata</i>	-	Incubatory	Howard, 1953
<i>M. japonica</i>	-	Incubatory, young 124 μ when liberated	Miyazaki, 1935
<i>Cyamium minutum</i>	-	Incubatory, 7-14 embryos	Morse, 1919
Galeommidae			
<i>Galeomma turtoni</i>	5.0	Incubatory	Lebour, 1938
<i>Entovalva mirabilis</i>	-	Young in pallial cavity, pelagic life	Voeltzkow, 1891
<i>Scioberetia</i>	-	Incubatory	Pelseneer, 1911
Cardiidae			
<i>Cardium elegantulum</i>	13.4	Eggs develop in brood pouches in folds of ventral parts of mantle	Ockelmann, 1958
<i>C. exigium</i>	-	Eggs in gelatinous envelopes attached to substratum	Thorson, 1946a
<i>Pseudokellya</i>			
<i>cardiformis</i>	-	Young in inner demibranchs	Pelseneer, 1906
Veneridae			
<i>Psephidia lordi</i>	6.4	Young inside adult	Abbott, 1954
<i>P. ovalis</i>	4.0	Young inside adult, embryo length 1 mm	Howard, 1953
<i>P. brunnea</i>	4.5	Young inside adult, embryo length 1.5 mm	Howard, 1953
<i>Parastarte triquetra</i>	3.2	Incubatory	Dall, 1893
<i>Transennella tantilla</i>	6.0	Young in inner demibranchs	Hansen, 1953
<i>Gemma gemma</i>	5.0	Young in inner and outer demibranchs	Perkins, 1869
<i>Gomphina fluctuosa</i>	23.3	Non-pelagic development assumed	Ockelmann, 1958
Tellinidae			
<i>Macoma moësta</i>	30.1	Pelagic stage assumed to be very short or lacking	Ockelmann, 1958
<i>M. loveni</i>	16.7	Pelagic stage assumed to be very short or lacking	Ockelmann, 1958
<i>M. torelli</i>	13.6	Pelagic stage assumed to be very short or lacking	Ockelmann, 1958
Teredinidae			
<i>Teredo navalis</i>	51.0 (400.0)*	500,000-1,000,000 embryos in gill brood pouch	Hill & Kofoid, 1927
<i>T. diegensis</i>	50.0	490 embryos in brood pouch	Hill & Kofoid, 1927
<i>T. parksi</i>	-	Embryos in brood pouch	Hill & Kofoid, 1927

*Total length including soft parts

Table 3 (continued)

Taxon	Aver. or max. length (mm)	Form of Brood Protection and Other Notes on Reproduction	Reference
Lyonsiidae <i>Lyonsia arenosa</i>	24.6	Pelagic stage presumably very short or absent	Ockelmann, 1958
Anatinidae <i>Anatina elliptica</i>	-	Incubatory	Burne, 1920
Clavagellidae <i>Aspergillum javanicum</i>	-	Incubatory	Lacaze-Duthiers, 1870
Pandoridae <i>Pandora rostrata</i>	-	Incubatory	Pelseneer, 1911
<i>P. elongata</i>	-	Incubatory	Pelseneer, 1911
<i>P. glacialis</i>	27.3	Pelagic stage probably very short or lacking	Ockelmann, 1958
Thraciidae <i>Thracia distorta</i>	-	Incubatory	Pelseneer, 1935
<i>T. septentrionalis</i>	25.8	Pelagic stage presumably very short or lacking	Ockelmann, 1958
<i>T. myopsis</i>	33.0	Pelagic stage presumably very short or lacking	Ockelmann, 1958
<i>T. devexa</i>	40.0	Pelagic stage presumably very short or lacking	Ockelmann, 1958
SEPTIBRANCHIA			
Cetoconchidae <i>Cetoconcha</i>	-	Incubatory	Pelseneer, 1911
Verticordiidae <i>Lyonsiella abyssicola</i>	5.3	Pelagic stage presumably very short or lacking	Ockelmann, 1958
Poromyidae <i>Poromya granulata</i>	12.0	Pelagic stage presumably very short or lacking	Ockelmann, 1958
Cuspidariidae <i>Cuspidaria obesa</i>	11.0	Pelagic stage probably very short or lacking	Ockelmann, 1958
<i>C. subtorta</i>	8.4	Pelagic stage presumably very short or lacking	Ockelmann, 1958
<i>C. glacialis</i>	28.4	Pelagic stage presumably very short or lacking	Ockelmann, 1958

discharge over 100,000,000 eggs in one spawning (Galtsoff, 1964). It appears then that ovoviviparity is an evolutionary adaptation to a reduced number of young.

Although it may be argued that ovoviviparous Teredinids and Ostreids are several times larger than the rest of

the ovoviviparous forms listed in Table 3, they are, nevertheless, small when compared to the oviparous members of their families.

All fresh-water lamellibranchs are ovoviviparous except one species, *Mytilopsis leucophaeta*, a Dreissenid. It is

only about 12 mm long, but is oviparous. The small Sphaeriids are probably limited by body size in the number of eggs they can produce, but the remaining fresh-water clams, although also ovoviviparous, are much larger. Other grounds than size need therefore be postulated for their adopting this mode of reproduction.

The fresh-water environment appears to discourage most free-swimming larvae and promotes ovoviviparity. The direction of river flow and low density of fresh water may be cited as 2 important factors in support of this idea. The seaward direction of water flow in rivers tends to prevent entry of weakly-swimming marine larvae into fresh-water regions. Needham (1950) states that "The existence of minute free-swimming larvae in the plankton is a positive bar to the colonization of fluviatile fresh water." The relative absence of salts makes fresh water less buoyant than sea water, probably raising energy expenditures of swimming larvae beyond their metabolic means. Accordingly, Pennak (1953) notes the paucity of fresh-water invertebrates which have planktonic young. This may help to explain why fresh-water mussels are an exception to the rule of size proposed earlier. The distribution of many marine invertebrates is dependent upon a planktonic stage during the life cycle. Since this seems more or less denied to the freshwater mussels, they have resorted to temporary parasitism for their dispersal, and time spent within the brood pouch permits the development of structural adaptations, i.e., hooks and byssi, for this transient mode of life. Although ovoviviparity is usually associated with relatively few young, the large numbers of glochidia produced by freshwater mussels are doubtlessly linked to the hazards of parasitism.

Brood protection may have developed in some animals as a means of restricting them to environmental regions most suitable to their physiological requirements since brood protection acts as a

deterrent to dispersal. Thorson (1946b) states that, in an area north of Point Conception, California, where the continental shelf is narrow and the deep sea much closer than usual, there is a fairly large number of brood-protecting species (actininians, asterids, holothurians). Thorson notes, too, that all Antarctic lamellibranchs investigated are brood-protecting and speculates that food conditions are such as to be able to support little planktonotrophic life.

3. Relationship of Embryo to Maternal Organism

a. Introduction

In forms which retain their young, the female functions to a greater or lesser degree in enhancing development and survival of the embryos. The major requirements of the embryo are a supply of organic and inorganic substances including water and oxygen, disposition of wastes and protection from environmental factors inimical to survival. Viviparous animals assume a greater responsibility for these embryonic needs than do ovoviviparous animals. It becomes necessary to resort to experimental means to evaluate the role of the mother and to learn what her contributions are. The crucial experiment involves removal of embryos from the mother to determine whether they can still continue normal development. By subjecting them to a variety of conditions, something can be learned about factors the mother may or may not supply.

According to Pelseneer (1935), embryos of *Anodonta*, *Ostrea edulis* and *Montacuta ferruginosa* can develop following removal from the mother, but those of *Teredo navalis* only when prematurely expelled. He also states that embryos of ovoviviparous clams may sometimes be nourished by maternal cells. There is evidence that the embryos of *Anodonta* absorb amoebocytes which traverse the gill epithelium and that those of *Cyclas* absorb epithelial

TABLE 4. Data relative to experiments conducted to determine survival of *Gemma* embryos *in vitro*

Exp. No.	Begin. Date (1957)	Embryonic Stage	No. Embryos Used	Mean Length from 10 Embryos (μ)	Salinity (‰)	Temperature (°C)
1	Aug. 4	1/8 Shell	40	210	27.1-27.6*	22.0-25.0*
2	Aug. 28	1/4 Shell	60	255	26.1-27.1	19.5-22.5
3	Sept. 11	No Shell	20	170	26.5	22.0-24.5

*First 7 days only

cells and glandular secretions of the brood pouch.

It is not known whether *Gemma* provides this form of nourishment for its embryos and, if it did, how critical the need would be, as evidenced by the nature of development following removal from the mother. To determine the relative importance of certain factors of environment, a series of experiments was performed. Since the mother provides her embryos with circulating water which is probably relatively free of microscopic life, but contains some of her metabolites, an attempt was made to produce experimentally some of these conditions.

b. Methods

Embryos, removed by dissection from gravid females, were used to test various combinations of circulating, non-circulating, filtered and unfiltered sea water, with and without adults present. Two methods of filtering employed are described below. Ten embryos for each environmental condition tested were placed in 4-inch diameter glass specimen dishes which contained 200 ml of sea water. In all, 8 out of 12 possible combinations were tested, although not all 8 were tested in any one experiment. Three experiments were run and, in each, embryos in different stages of development were utilized: in one experiment, 20 embryos prior to shell development; in a second, 40 having a shell covering about 1/8 of the body surface; and in a third, 60 with a 1/4-

developed shell. Further details are given in Table 4. Water was changed daily at which time the embryos were inspected at a magnification of 60X to count survivors and to remove dead embryos. Observations were continued as long as any embryos remained alive. Development of the shell took 6-7 days.

The sea water used for the experiments was obtained every 2 weeks at Union Beach, New Jersey. It was kept at 6°C until 24 hours before its use, when it was allowed to come to room temperature. It was filtered by 2 methods. In one, it was passed through a Millipore filter, type HA (Hydrosol Assay) of a calculated pore size of 0.45 μ , using a faucet aspirator. In the other it was gravity-filtered twice through E & D No. 615 (rapid) filter paper. The unfiltered water was mixed well and allowed to settle for 2 minutes and then drawn off from the surface with a bulb pipette. Water in the dishes containing embryos was changed once a day and at that time temperature was taken.

Circulation and aeration was provided by an air lift system using a No. 25 Marco air pump delivering cotton-filtered air into 3-inch rigid butyrate plastic tubing air lifts which drew up water from the specimen dishes and discharged it directly into the same dishes. It was calculated that a quantity of water 70 times that of the volume contained in a dish passed through the air lift in 24 hours.

The conditions to which the embryos were subjected are presented in tabular

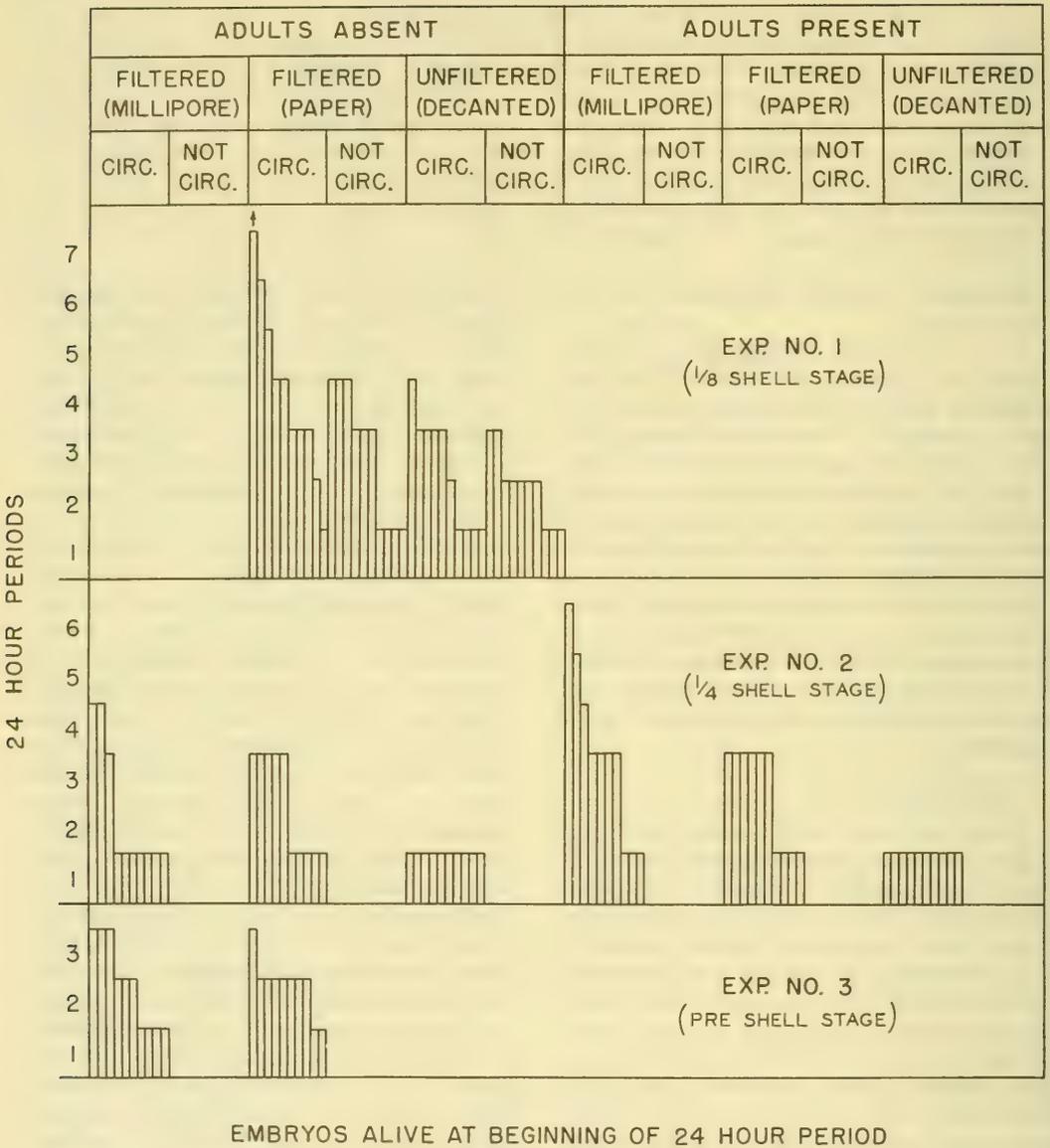


FIG. 30. Survival of *Gemma* embryos at different stages of development *in vitro*. Ten embryos each (represented by maximum width at base of bar) were subjected to the various conditions indicated. Middle and lower ranks of heading refer to state of water (circ. = circulated). Progressive narrowing of bar upwards represents mortality. Survival of embryos was not observed in second 24-hour period of experiment No. 2. See Fig. 31 for other details.

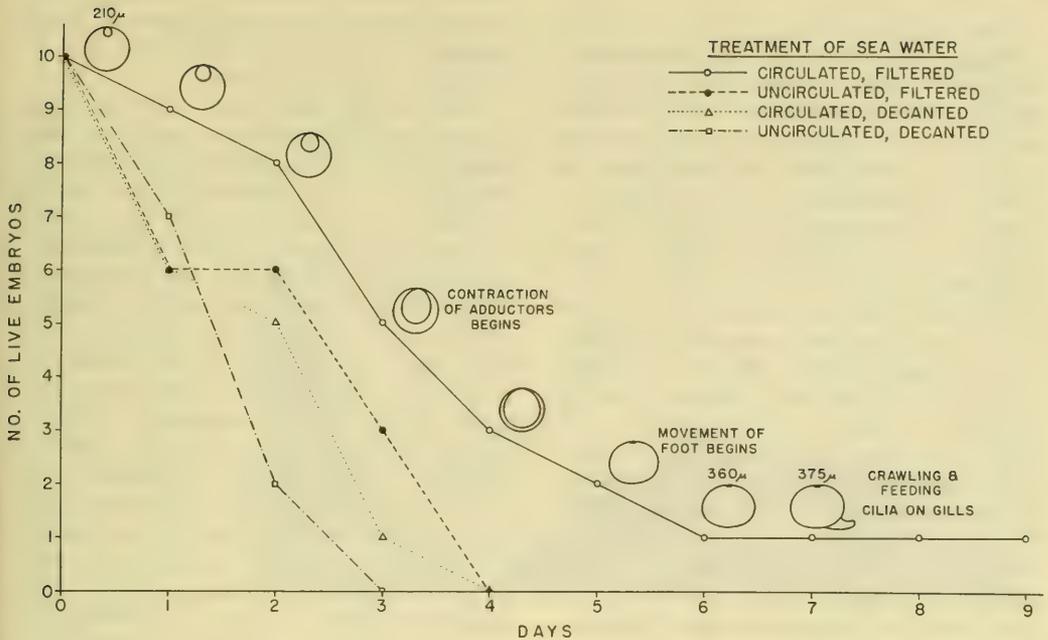


FIG. 31. Development and survival of *Gemma* embryos *in vitro* (experiment No. 1, initial stage: 1/8 shell approx.). The small inner circles in the embryos represent the progressive development of the shell, which completely enclosed the soft parts by the 5th day. Above 3 of the embryos, the length is given in μ . No adult clams were present in the specimen dishes containing the embryos in this experiment.

form in Fig. 30.

c. Results

Of the 120 embryos used in all experiments, 14 survived at least 3 days, 3 for 5 days, and one grew and developed for 98 days at the end of which time the experiment was accidentally terminated. (During a change in its culture water, the baby was thrown out with the bath!) The most favorable environment appeared to be circulating, filtered sea water. A beneficial or detrimental effect through the presence of adults was not demonstrated.

In Fig. 31, the results of experiment No. 1 are also presented, but in a manner different from Fig. 30 along with additional information relating to development.

The single clam, which survived for

98 days in experiment No. 1, attained a length of 895μ . After the 7th day of that experiment, when the juvenile began crawling about, the water in the dish was changed daily until the experiment ended. Unfiltered decanted water was used to provide nourishment. The clam was measured at each water change up to the 42nd day, after which time it was measured every 3rd day. During this period, the temperature and salinity ranges were $16.5\text{--}24.5^{\circ}\text{C}$ and $24.4\text{--}27.1$ ‰, respectively.

d. Discussion

As the experiments were of a preliminary nature, no definite conclusions can be drawn. The experiments, however, are worth repeating on a larger scale since they show the feasibility of maintaining prematurely removed em-

bryos alive *in vitro*.

The relatively poor results obtained in Experiment 3 may be due to the early (pre-shell) stage of the embryos at the beginning of the experiment. Only further work can demonstrate whether there is a critical point prior to which time the embryo is dependent on the mother or whether refinement of experimental techniques can further extend the non-dependent period.

In many mollusks shell development begins shortly after fertilization: *Venerupis semidecussata*: 17 hours (Cahn, 1951); *Mercenaria mercenaria*: 24 hours (Carriker, 1956); *Crassostrea virginica*: 30 hours (Stafford, 1913); *Mytilus edulis*: 48 hours (Field, 1922); *Ostrea lurida*: 96 hours (Hopkins, 1937). It is interesting to note that the last named, an ovoviviparous species, takes the longest time. Since, in *Gemma*, the time from the beginning of shell development to the stage ready for liberation is less than a week, it appears that the total developmental period may occupy less than 2 weeks during the warm season. This being true, it may be concluded that at least half of the development of *Gemma* embryos may be successfully completed outside of the mother.

In the case of the embryo which survived over 3 months, it is highly probable that it would have attained adulthood had the experiment continued.

4. Sex Ratio, Sexual Maturity, Reproductive Periods and Fecundity

A total of 881 clams was examined for sex and presence of embryos. Members of the 1954, 1955 and 1956 year classes were used for this purpose. Although the entire life span was covered, temporal subdivisions of this period were not represented by equal numbers of clams. Special attention was devoted to the April-September period, within which time, year-old clams were liberating young. Since environmental conditions vary during a specific period in successive years and because rela-

tively small numbers of clams were examined during certain periods, some aspects of reproduction could not be determined with certainty.

Most of the data were obtained from dissection of preserved material. Sex was determined by examining gonads. In sexually mature specimens, the female gonad is characterized by large, irregularly-shaped, white, opaque eggs, whereas the testes are translucent and composed of small, branching follicles. Embryos, when found in the brood pouches, were counted and classified largely by absence or presence of shell and its relative development. Observation of the shell was facilitated by staining the embryos for a minute or 2 with gentian violet. Normally white embryonic tissue stained a dark purple except for the regions beneath the transparent shells which stood out in contrasting white.

In all, the sex of 602 clams was determined; the ratio over the entire period being 53.6 males per 100 clams.

Of 881 clams examined, 285 contained embryos in their brood chambers. Those devoid of embryos were either males or immature, unfertilized, sterile or spawned-out females. Results of this examination are given in Fig. 32.

Some young-of-the-year females contain early embryos in the fall which develop into the fully-shelled stage during the winter, causing a decrease in the number of females with early embryos and an increase in the number of those having fully-shelled embryos. During spring there is another increase in incidence of early embryos which replace fully-shelled juveniles liberated during the summer as seen in Fig. 32 by the change in proportions of the different stages. During the fall, the number of females bearing fully-shelled stages increases and probably all go into their second winter bearing fully-shelled embryos.

Because the reproductive behavior of the gem clam has been deduced from an analysis of limited data from Union

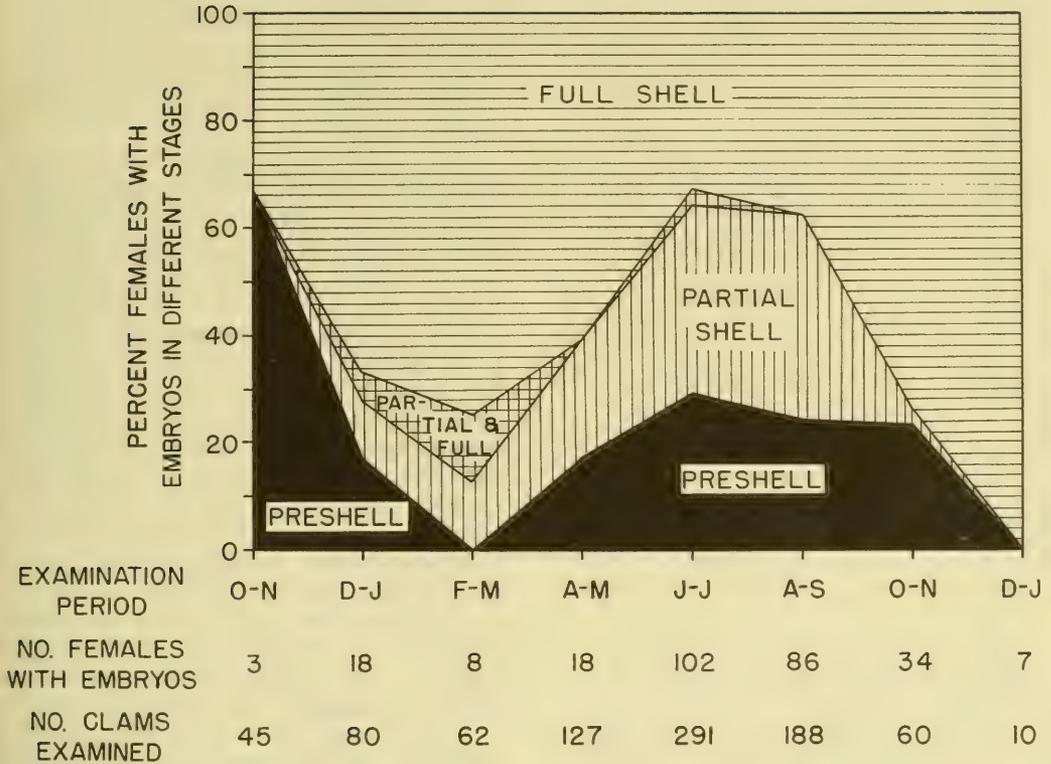


FIG. 32. Relative proportions of 3 embryonic stages found in females throughout the life span of the gem clam. The data have been drawn from the 1954, 1955 and 1956 year classes to represent the average condition during the examination periods. Data are grouped into 2-month periods, so as to include meaningful numbers of clams. Hyphenated letters are initial letters of 2 successive months. Duration of study covered the period October, 1955 - January, 1957.

Beach, New Jersey, the following description must serve, at least for the present, as a tentative statement of this process.

Sexual maturity is attained by a few clams in the fall of their first year when they reach a length of approximately 2 mm and are about 4-5 months old. At this time, they produce embryos which soon develop to the fully-shelled stage and are carried through winter to be released the following summer. Embryo production ceases for the winter but begins again in spring and continues through the summer. During summer, the shelled embryos, which had been

held in the brood chambers through the winter, are liberated along with those initiated during late spring. A second brood of embryos is produced by those females which have liberated their first brood. In a few clams, early embryonic stages are found along with a few fully-shelled embryos. Whether some of the females liberate the second brood during the same summer is not known, but judging by the apparent rapidity of development noted in the *in vitro* experiments, it may be possible. In all events, most of the year-old females enter their second autumn carrying embryos, which are ready for release. Liberation of

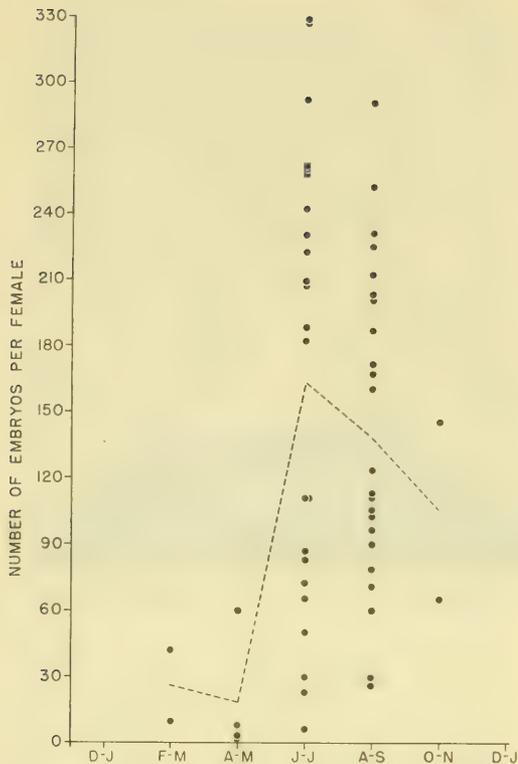


FIG. 33. Number of early (pre-shell) embryos found in sexually mature females. Data from 1954, 1955 and 1956 year classes. Letters on abscissa refer to initial letters of months indicating 2-month periods during which clams were examined. Broken line indicates mean. See also Table 5.

these embryos apparently did not take place during the period of observation since the females (as well as the males) were observed to die during the second winter of life.

In an attempt to determine the brood size of *Gemma*, a total of 20,593 embryos from 276 clams was counted and graded as regards development of the shell, with the results summarized in Table 5. Hansen (1953) found embryos in all stages of development in the gills of female *Tranennella tantilla*, a close relative of *Gemma*. In *Gemma* itself, however, all embryos, in a given female,

were, with few exceptions, in only one of the 3 stages of development named in Table 5. Figure 33 shows considerable range in number of early embryos carried by females and lack of a tendency to form a mode. This is also true of later stages.

The present study did not yield enough information to give a very reliable estimate of brood size. First, the interval most intensively sampled was that of the "spawning" period when, in many cases, the number of fully-developed embryos ready for release was so low as to indicate that an unknown number had already been liberated. A better value could be obtained from samples during the period just prior to spawning; but, for several reasons, relatively few clams were examined at that time. Secondly, the number of early or partially-developed embryos should provide a measure of the entire brood to be liberated, but a decline in the mean number of embryos during the developmental period indicated either that some prenatal mortality occurred, or that a part of the brood was prematurely aborted. Since certain facts necessary for the accurate determination of brood size still remain unknown, only an approximation can be made.

Although a potential of about 300 young is apparent in Table 5, a more conservative estimate of 100-200 juveniles released per female per season is given pending further study. The rate of development seen in the *in vitro* experiments as well as the presence of fully-shelled embryos held by females over the winter shown in Fig. 32, hint at the possibility of the production and liberation of 2 broods during the summer.

5. Development Within the Brood Chamber

In oviparous clams, the period during which fertilization takes place is coincident with the spawning period. In the gem clam, the fertilization period may be much longer than the time during which the female actually liberates her

TABLE 5. Number of embryos in different stages of development per female through major portion of life span (1954, 1955, 1956 year classes). See also Fig. 33.

Examination Period	Number of Embryos per Female								
	Pre-shell stage			Partial shell stage			Full shell stage		
	No. Fem.	Mean	Range	No. Fem.	Mean	Range	No. Fem.	Mean	Range
Dec. Jan.	0	-	-	4	39	29-56	13	18.2	2-62
Feb. Mar.	2	26	10-42	2	27	11-43	5	12.2	2-50
Apr. May	4	18.3	2-60	3	11.3	10-13	13	14.1	1-97
June July	24	163	7-328	49	80.8	3-214	34	37.6	1-125
Aug. Sept.	23	138	26-290	29	106	2-315	30	27.7	1-185
Oct. Nov.	2	105	65-145	1	9	-	25	35.4	1-192
Dec. Jan.	0	-	-	0	-	-	7	50.7	4-146

young. Since the majority of early embryos were found in females in the period between April-May and October-November (see Fig. 32), it appears that this is the time when males release their sperm.

It is presumed that the method of fertilization follows that of other incubatory species where spermatozoa released into the water by the male are drawn into the incurrent siphon of the female, pass through the gill ostia (Figs. 37, 39) and fertilize the female gametes either in the demibranchs, epi-branchial chambers or oviduct.

Very early embryos (2-cell stage) have been observed in the brood chambers in sectioned material. On one occasion when adult clams in the laboratory were subjected to a temperature of 34°C, for 3 hours, zygotes and very early cleavage stages were aborted and continued their development to the 16-blastomere stage. The few zygotes

measured ranged from 170-180 μ .

Embryos develop within the brood chambers for an undetermined period of time. Differentiation is undoubtedly suspended during the winter but, from observations made while raising embryos *in vitro*, it appears that development proceeds at a rapid rate during warm periods and may be accomplished in as little as 2 weeks.

Early and intermediate stages are shown in Figs. 22 and 34, while the fully-developed embryo is presented in Figs. 35-40. These figures show selected serial transverse sections through the same embryo from anterior to posterior end. Since differentiation of structures and organs was not followed through progressive stages, accuracy in identification may be doubtful in some cases. Identification of structures was made by comparison with adult anatomy of the gem clam as well as reference to other works on lamellibranch develop-

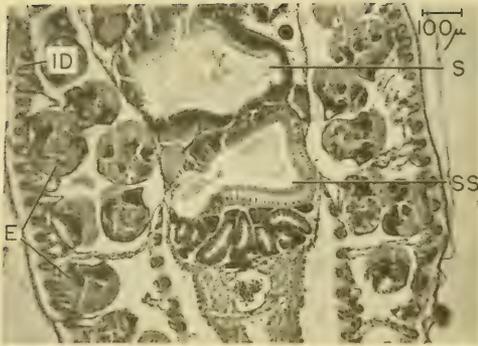


FIG. 34. Transverse section through adult female collected on June 11, 1957 and containing embryos in an intermediate stage of development.

ment, particularly that of Quayle (1952).

Although an empty egg membrane was found clinging to the shell of many juveniles liberated in the laboratory, this structure was demonstrable only in sections of very early embryos. What appears to be this membrane in Figs. 35-40 is actually the periostracum, since a careful study of many sectioned embryos shows it to originate invariably from the secretory lobe of the mantle (Fig. 37).

6. Size of Young and Time of Release

The only reference to the size of *Gemma* juveniles is that of Sullivan (1948) who approximated their size from plankton samples where she occasionally found them. They averaged 410μ in length, the smallest being 295μ .

In the present study, a mean value somewhat less than that of Sullivan's was found. The length-frequency distribution of 87 juvenile clams from 10 samples collected on May 11, 1957 had a mean of 373.3μ with a standard deviation of 21.56. The limited range and close approximation to a normal curve of their length-frequency distribution is interpreted to mean that liberation had occurred very shortly before col-

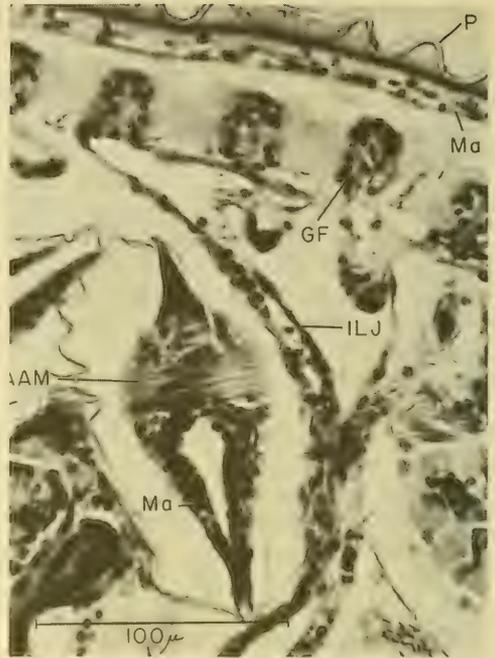
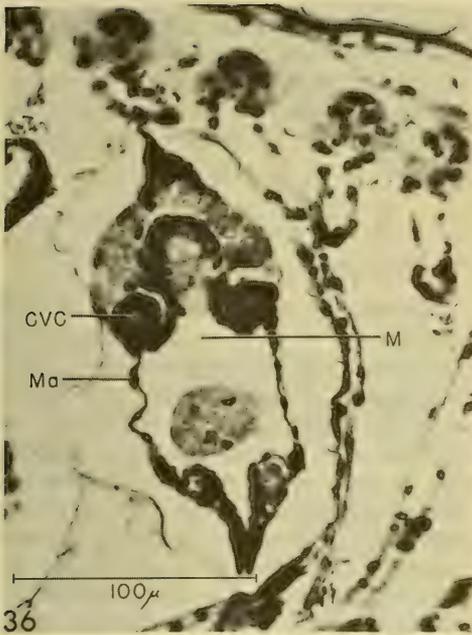


FIG. 35. Transverse section of fully-developed embryo at level of anterior adductor muscle in inner demibranch of adult female collected on June 11, 1957. Figs. 35-40 are selected sections of the same individual from anterior to posterior end. In this figure only, labels for embryo are at left, adult at right.

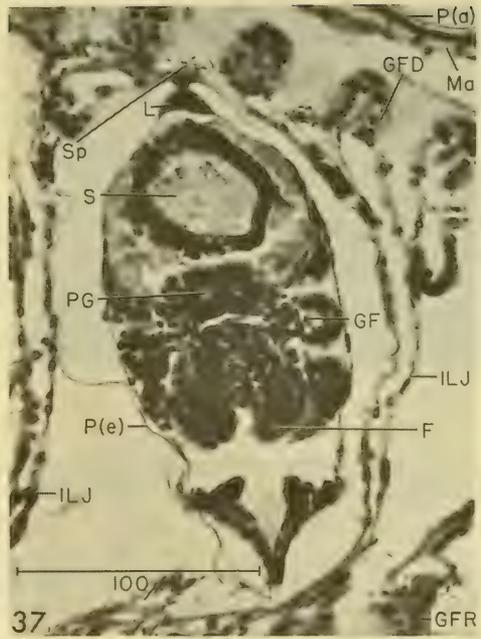
lection.

The largest clam among the presumably just-released juveniles collected in the field had a length of 430μ . Knowing the area covered by the samples, the density per square meter of clams this size and smaller was calculated for each collection date during the summers of 1956-1958. The results are graphically presented in Fig. 42.

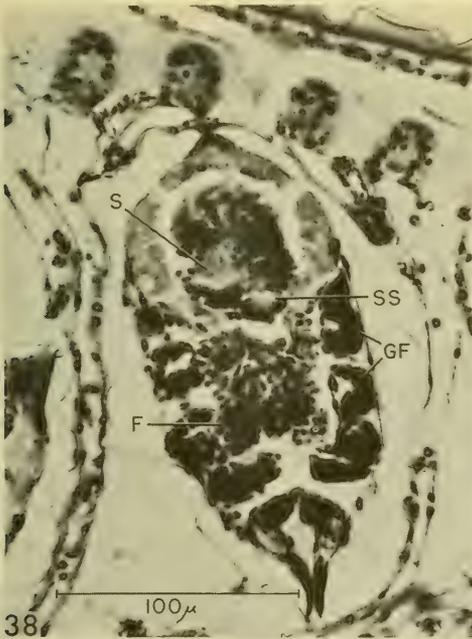
It is seen that "spawning" occurs chiefly within a 2-month span, although the period of highest intensity may last for only about 3 weeks. There is, of course, some variation in the initiation and completion of spawning related probably to temperature conditions. When the results of the 3 years are



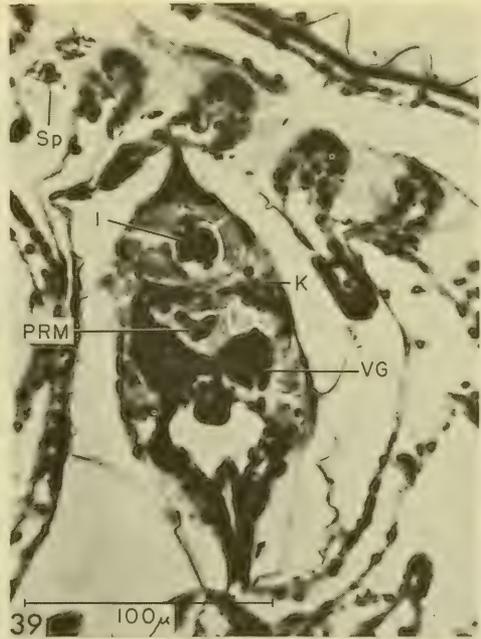
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39

- FIG. 36. Transverse section of embryo at level of mouth. Compare with Fig. 10.
 FIG. 37. Transverse section of embryo in region of stomach (the periostracum of the embryo and adult are differentiated by (e) and (a) respectively, surrounding tissues of parent also labelled).
 FIG. 38. Transverse section through mid-region of embryo.
 FIG. 39. Transverse section of embryo at level of visceral ganglia. Compare with Fig. 12.

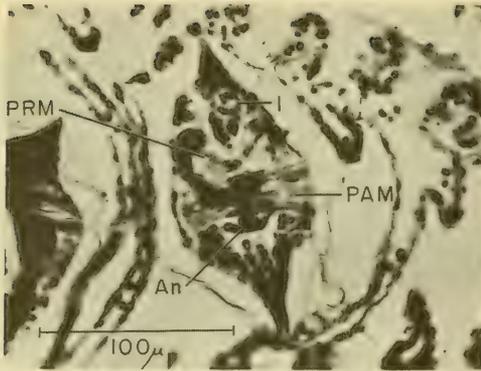


FIG. 40. Transverse section of embryo at level of posterior adductor muscle. Compare with Fig. 13.

combined, it is found that approximately 30% of the young were liberated in June, 50% in July and 10% in August.

A decrease in numbers released in successive years is noted. This is in accord with a similar decrease in density of adults during the same period. A group of young juveniles, including some just released, is seen with an adult in Fig. 44.

X. POPULATION DENSITY

1. Introduction and Methods

Knowledge of several aspects of the life history of an organism can be gained from a study of fluctuations in population size. These include reproductive activity, life span, mortality and direction of density trends. Magnitude of a population is often expressed in terms of numbers per unit of area or volume. Where size of individuals varies considerably, as in *Gemma*, it is more satisfactory to use some sort of biomass measure such as weight, volume

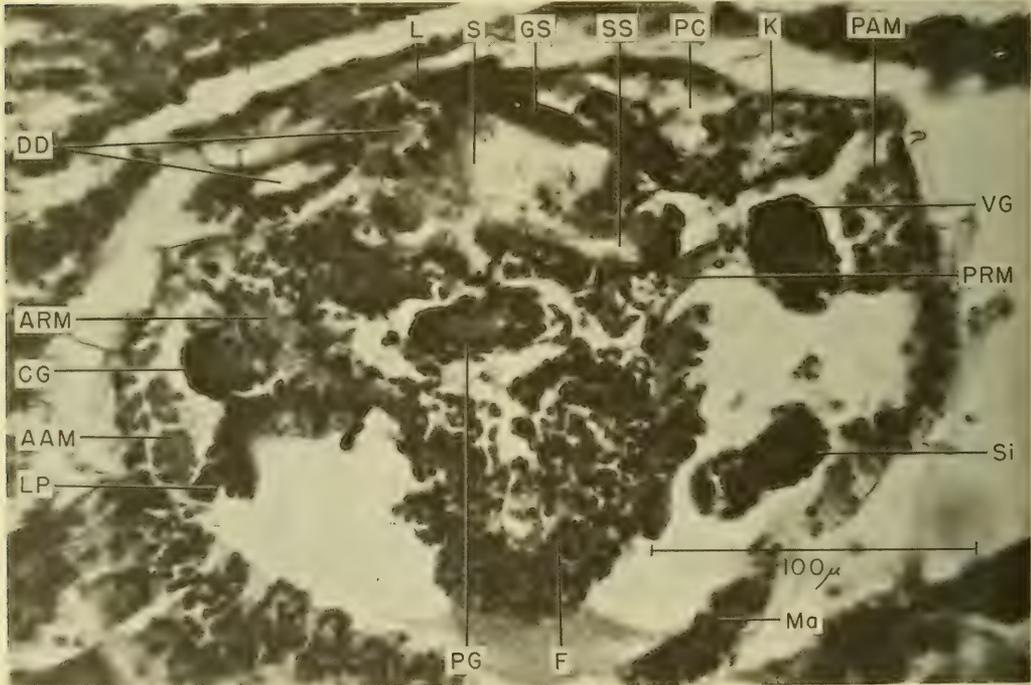


FIG. 41. Sagittal section of embryo in inner demibranch of adult female collected on August 26, 1957. Compare with Fig. 6.

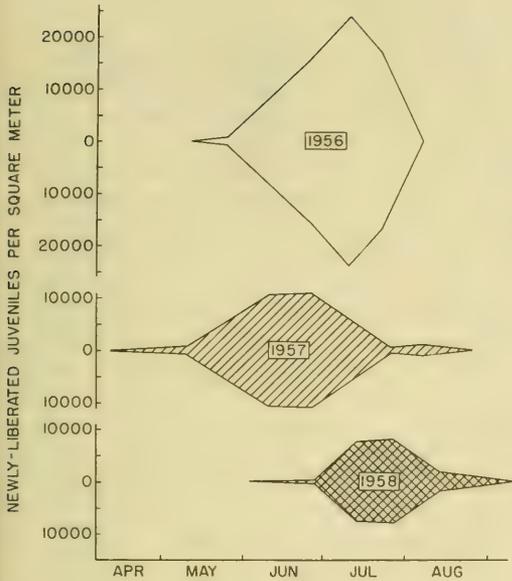


FIG. 42. Densities of newly-liberated juveniles of 3 year classes of *Gemma*.

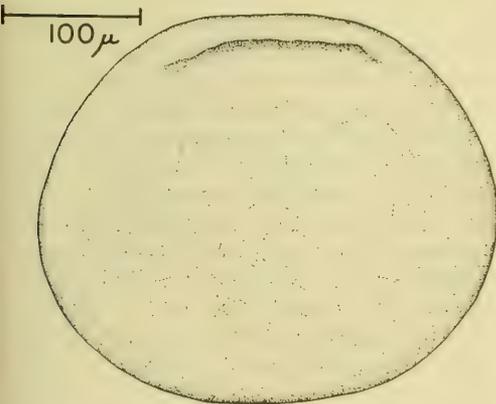


FIG. 43. Recently liberated juvenile gem clam. (Drawn by M. R. Carriker).

or chemical determination to indicate protoplasmic content. Both numbers and weight have been used in the present study.

Relatively high concentrations of



FIG. 44. Recently released juveniles of the 1957 year class shown with a 4 mm adult of the 1956 year class. All clams collected on June 11, 1957. Smallest individuals seen here are size of those just released.

different species of clams in one square meter of bottom have been reported in the literature. Moulton & Coffin (1954) found 270,000 recently-set *Mercenaria mercenaria* in Maine, where they had probably been concentrated by the action of waves and currents. A density of 100,000 *Cardium edule* is described by Orton (1937). Coe (1955), who has studied highly variable fluctuations of *Donax gouldii*, reports concentrations up to 20,000. Weymouth et al. (1925) found "an unusually good set" of *Siliqua patula* (length 2.5 mm) with a density of 15,600. As many as 10,000 *Nucula proxima* were found by Sanders (1958). An account of 8,250 *Spisula subtruncata* is given by Davis (1923), and Stephen (1928) reports an average of 3,500 *Tellina tenuis*, 2 mm in length, at favorable locations.

Densities of *Gemma* are such as to have caused investigators to describe this clam as occurring in "enormous" or "immense" numbers (Verrill & Smith, 1874; Sumner et al., 1911; Morse, 1919; Turner, 1951; Stickney & Stringer, 1957). These densities have not been given quantitative expression until recently (Bradley & Cooke, 1959) and it is

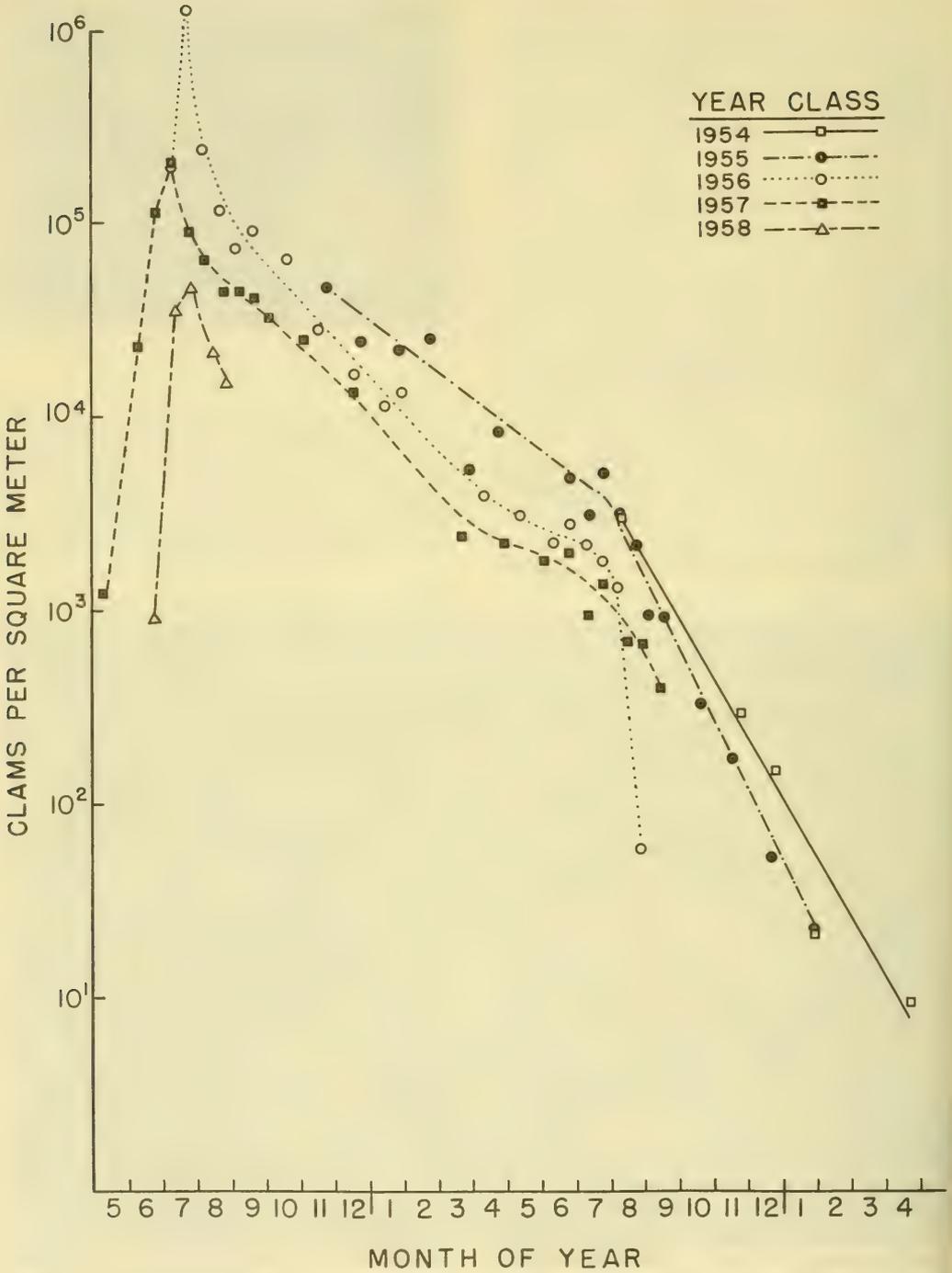


FIG. 45. Densities per m^2 of *Gemma gemma* for part or all of life spans of 5 year classes plotted semilogarithmically.

TABLE 6. Representative population density data for *Gemma* (1956 year class). For data of other years, see Appendix.

Date	No. of samples	Total area of samples (cm ²)	No. of clams in total sample	No. of clams /m ²
1956:				
July 11	1	28.2	499	176,450
July 23	1	28.2	3221	1,138,967
Aug. 8	10	34.6	758	218,822
Aug. 23	12	41.6	442	106,327
Sept. 5	15	52.0	348	66,978
Sept. 20	15	52.0	430	82,852
Oct. 19	20	69.2	400	57,804
Nov. 18	20	69.2	179	25,867
Dec. 20	9	254.5	379	14,892
1957:				
Jan. 17	15	424.2	437	10,302
Feb. 1	15	424.2	524	12,117
Mar. 16	35	989.8	274	2,768
Apr. 11	12	1078.9	377	3,494
May 11	10	899.1	257	2,858
June 11	12	1078.9	226	2,095
June 26	15	1348.7	346	2,565
July 10	18	1618.4	324	2,002
July 26	18	1618.4	265	1,637
Aug. 8	18	1618.4	200	1,236
Aug. 26	21	1888.1	11	58

the intent in the present study to describe these densities and their fluctuations somewhat in detail. That densities reported for other clams do not generally attain those given for *Gemma* may simply be a reflection of the size of the individuals studied. In almost all investigations concerned with densities of post-larval bivalves, clams smaller than 2 mm have not been collected (*Tivela stultorum*, Weymouth, 1923; *Siliqua patula*, Weymouth et al., 1925; *Tellina tenuis*, Stephen, 1928; *Kellia suborbicularis*, Lebour, 1938; *Tivela stultorum*, Coe, 1947; *Donax gouldii*, Coe, 1955). At this length, the gem clam is already half grown. Densities given here begin at the time of liberation when juveniles are less than 0.4 mm. It is, therefore, quite possible that densities comparable to those of the gem clam might be attained by other species early in life.

The sampling method has already been described. Table 6 and the Appendix present data on number of samples and clams taken on each collection date. A total of 18,486 clams was collected for this phase of the study.

2. 1954-1958 Year Classes

a. Numbers

Densities throughout part or all of the life spans of 5 year classes of *Gemma* are shown superimposed on one another for purposes of comparison in Fig. 45. Rise and fall in numbers is dependent upon the interplay of natality and mortality. Curves presented here are resultants of these opposing tendencies. The highest value shown of over a million in a square meter must be looked upon with some reservation as this value was derived from a single sample. The majority of values were,

however, based on 10 or more samples and are probably close to actual densities.

Bradley & Cooke (1959) report densities of gem clams in Maine of 8-20 times those found in this study. Considerable variation in density no doubt exists between localities, but the disparity in values between those of Maine and Union Beach is due, in part, to a difference in method of reporting. Bradley & Cooke combine clams of 2 year classes and part of a third (the youngest). Densities reported in the present study are by individual year classes. When density data for Union Beach are combined in the manner of the Maine study, densities are comparable.

It is clearly evident from Fig. 45 that the *Gemma* population at Union Beach during the period of study was in a state of decline. If year classes are compared at the same stage of their life span, namely when they have attained an age of one year, it is seen that the 1956 year class was about 50% less numerically than the 1955 year class and that of 1957 about 30% less than that of 1956.

Bradley & Cooke, who found a similar decline from 1950 to 1956, have offered suggestions for possible causes: (1) high frequency of summer storms transporting gem clams, especially juveniles, to less favorable environments, (2) salinity decreases killing juveniles and (3) decrease in life span. Neither their study nor this one has attempted to relate the first 2 possibilities to the decline. The third suggestion has considerable merit. The number of offspring produced by a stable population is adjusted to life span. Should life span decrease, insufficient young are produced to fully replace adults which die. It was noted earlier that adult females were gravid as they entered their second winter, but did not survive at Union Beach to liberate young the following summer.

Intraspecific competition attending initially high densities may have started

the decline. A decrease in life span may also be causally related to high densities, as was observed in *Drosophila* populations by Pearl et al. (1927).

Sufficient information is not available to say whether lowered natality, heightened mortality, or both, may be responsible for the observed decline at Union Beach.

There is no reason to think that the gem clam is necessarily headed toward local extinction. The current decrease may be only the declining phase of a secular cycle. Coe (1957) records the resurgence of a number of intertidal populations following periods of low density.

Bradley & Cooke report finding fewer young of the year than adult clams. This was never true at Union Beach (see Fig. 45). In late summer there were always 30-50 times as many young clams as adults and, in July, many times more than that. The reason for the difference in findings between Maine and Union Beach may lie in sampling methods. Bradley & Cooke used a 20 mesh screen after having tested for the proportion of clams which would pass through this screen, but would be held by a 65 mesh screen. Of the total number of clams, 13% passed through the 20 mesh screen. These were less than 1 mm in length. The date of their test was not given.

Calculations from growth and density data at Union Beach for the years 1956-1958 indicate that, of the total number of clams of all year classes in collections made in the middle of June, July, August and September of those years, an average of 35, 83, 38 and 11% respectively, would have been lost through a 20 mesh screen. During the other part of the annual cycle, 100% recovery would have been made.

One also wonders whether all clams were retrieved from their test sample. These clams are not much larger than the sand grains in which they are mixed and have a similar appearance. Using data of Bradley & Cooke on sample volume and grain size, it is estimated

that about 8.7 in^3 of sand passed through the 20 mesh screen and contained approximately 14.8×10^6 sand grains. The 72 clams which they found are thus in the ratio of 1 clam to about 200,000 sand grains. It is believed that, if the test were made in mid-summer, many could have been overlooked and that the sieve and search method is not adequate when newly-liberated juveniles are in the sediments.

Bradley & Cooke found that, following the density decline of 1950 to 1956, density increased in 1957. This increase may be only illusory. At Union Beach, liberation of juveniles occurred earlier in 1957 than in any of the 3 years during which liberation was studied (Fig. 42). If the situation were similar in Maine, then the apparent increase could have been due to collecting a greater proportion of the new year class than in other years since these clams, in 1957, had had a longer period during which to grow and were less likely to be missed. Since the numerical size of the new year class is many times that of the previous generation, the number of larger, and detected, members might be considerable and would add appreciably to the total collection.

b. Biomass

Reference was made earlier to the greater value of a biomass measure over numerical densities in determining the importance of species in a community. The value of an organism as a producer as well as a consumer can be better assessed when weight is substituted for numbers. This approach has been utilized to great practical advantage in computing optimum yields for such economically important species as fish (Ricker & Forester, 1948) and oysters (McHugh & Andrews, 1954).

With an animal as small as the gem clam, it would be impractical to weigh each collection made. A newly-liberated juvenile is only about 30 micrograms in weight, an adult, about 30 milligrams. To make possible translation of length

into weight, an equation relating these 2 values was developed.

Clams of various sizes, which had been preserved in 10% neutral formalin and whose shells were tightly closed, were used for this determination. Eleven groups of 100 each, whose mean length was known and which were spaced fairly evenly along the size range of *Gemma*, were chosen. Each group was air-dried to a constant weight and weighed to the nearest milligram. From these data, the following equation was derived:

$$Y = 0.006851 X^{2.79}$$

where X is length in micra and Y is weight in micrograms. Thus, the weight of a clam of known length could be calculated or read from a curve constructed from the equation. The term weight, as used here, is wet weight, because the tissues still contained moisture, although the external shell surfaces of the clams were dried. Monthly or semi-monthly collections of usually 100 clams, whose mean length had been determined (Table 2), were used to calculate biomass. The weight of clams/m² was obtained by multiplying the calculated weight of a clam, whose length was the mean of a given collection, by the number of clams per square meter on that date. These points are plotted in Fig. 46. A smoothed curve for these points was obtained from values read off the smoothed growth and density curves found in Figs. 28 and 45.

Curves in Fig. 46 show that a *Gemma* population exhibits a seasonal fluctuation in biomass. Mortality is occurring in the population at all times although its rate varies. During the summer, the combined effects of natality and growth offset the downward trend of mortality so that biomass increases temporarily. Biomass of a given year class increases during the first summer of life due both to natality and growth, whereas only growth is responsible for increase during its second summer. Biomass reached a maximum of 200 g/m² in 1956 and dropped to a minimum of 2 g/m² in

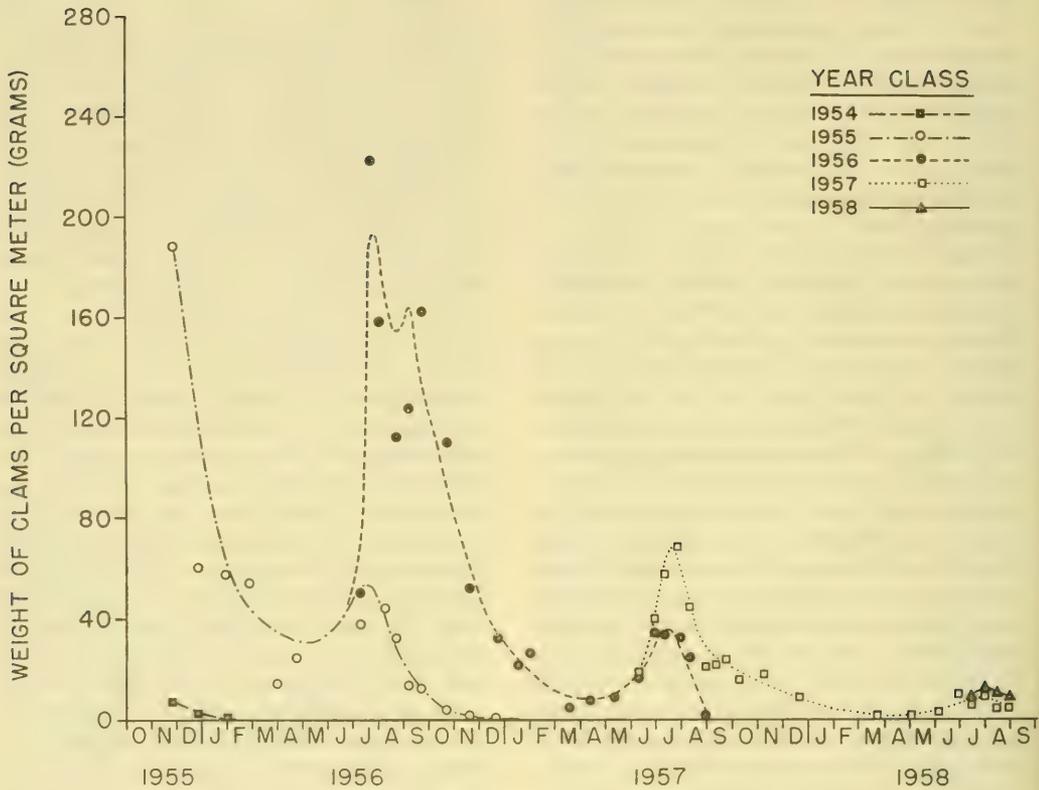


FIG. 46. Wet weight (including shell) of *Gemma gemma* for part or all of life spans of 5 year classes. Datum points are derived from density datum points as given in Fig. 45 and weight at mean length datum points, as given in Fig. 28. Smoothed curve obtained from points on smoothed curves of the same figures.

1958.

A general decline in biomass took place over the period of study. This corresponds to the similar decline in density during the same period.

XI. MORTALITY

1. Life Table

Mortality rates are derived from relative numbers of surviving members of an original group during succeeding age intervals. This and corollary vital statistics may be concisely summarized

in a life table. Beginning with a cohort, real or imaginary, whose members start life together, the life table states for every interval of age the number of deaths, survivors remaining, rate of mortality and expectation of further life. In his review of this subject, Deevey (1947) summarizes information up to that time and gives the method for computation of life tables.

The collection of data for the preparation of life tables in animals already studied (man, rotifers, mountain sheep, insects, barnacles, rabbits, brachiopods,

TABLE 7. Life table for the gem clam, *Gemma gemma*. Age reckoned from time of liberation. Data based on 1954-1957 year classes at Union Beach, New Jersey. Mean length of life = 1.13 months.

x	x^1	d_x	l_x	$1000q_x$	e_x
Age (Months)	Age as % Deviation From Mean Length of Life	Number Dying in Age Interval out of 100,000 Liberated	Number Surviving at Beginning of Age Interval out of 100,000 Liberated	Mortality Rate per 1000 Alive at Beginning of Age Interval	Expectation of Life, or Mean Life- time Remain- ing to those Attaining Age Interval (Months)
0- 1	- 100.0	79,000	100,000	790.0	1.09
1- 2	- 11.5	8,800	21,000	419.1	2.32
2- 3	+ 77.0	3,700	12,200	303.3	2.63
3- 4	+ 165.5	2,850	8,500	335.3	2.56
4- 5	+ 254.0	1,850	5,650	327.4	2.59
5- 6	+ 342.5	1,240	3,800	326.3	2.61
6- 7	+ 431.0	850	2,560	332.0	2.55
7- 8	+ 519.5	550	1,710	321.6	2.70
8- 9	+ 608.0	355	1,160	306.0	2.75
9-10	+ 696.5	191	805	237.3	2.74
10-11	+ 785.0	116	614	188.9	2.43
11-12	+ 873.5	137	498	275.1	1.88
12-13	+ 962.0	188	361	520.1	1.40
13-14	+1050.5	92	173	537.6	1.38
14-15	+1140.0	43	81	530.1	1.39
15-16	+1227.5	20	38	526.3	1.40
16-17	+1316.0	9	18	500.0	1.39
17-18	+1404.5	5	9	555.6	1.28
18-19	+1493.0	2	4	500.0	1.25
19-20	+1581.5	1	2	500.0	1.00
20-21	+1679.0	1	1	1000.0	0.50

fish and birds) is hindered by obstacles presented in the nature of their life history and behavior. Age at death or age of survivors when collected, is sometimes difficult to determine. Migratory behavior may make continuous observation of a given population difficult. Considerable size difference between juvenile and adult and the existence of a pelagic stage in some species make sampling sometimes impossible for all age groups. The longevity of some animals may require protracted study. A prolonged spawning period may render identification of year classes difficult.

It is believed that the gem clam is

well suited for a study of this nature. *Gemma* is non-migratory and it is assumed, although possibly incorrectly, that active or passive immigration and emigration in a sampling area cancel each other out, thus a given population can be kept under continuous observation. Since *Gemma* young are liberated during a period of relatively short duration, have no pelagic existence and become evenly distributed among the adults, all age groups are presumed to be sampled equally. Well-defined growth rings allow accurate determination of age. Finally, a short life span permits collection of data in a short

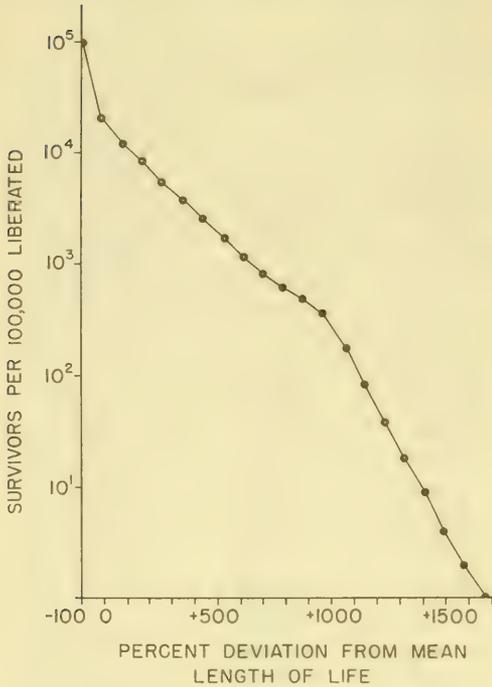


FIG. 47. Survivorship curve (1_x) for *Gemma gemma* plotted semi-logarithmically, age being expressed as percentage deviation from mean length of life. The curve gives the number surviving at the beginning of each age interval starting with an imaginary group of 100,000 clams (compare with Table 7).

period.

Because all life table functions can be calculated from each other, information used in preparing them can be obtained from several sources. A life table for the gem clam (Table 7) was prepared by combining data from densities of the 1954-1957 year classes (see Fig. 45). Survivorship (Fig. 47) and mortality rate curves (Fig. 48) have been plotted from this table (see discussion below).

2. Life Span

The maximum observed life span of gem clams at Union Beach was 2 years (Fig. 3). Extremely few individuals attained this age, however, and the

average life span was considerably less than this since the great preponderance of individuals died in the first few months of life (Table 7). In order to calculate mean life span, life spans of groups of individuals dying during succeeding age intervals were used and these values were weighted according to the number dying during each interval. Values found in the life table in columns headed x and d_x were used. A mean life span of 1.13 months was obtained. This may appear at first to be very short, but is easily understood when reference to the table reveals that almost 80% of the cohort die within the first month of life.

The conclusions of Bradley & Cooke on average life span of *Gemma* of 1.8-2.6 years are at great variance with the value obtained in this study. The maximum life span they found was demonstrably greater than ours, but their method of age determination and the fact that many young of the year were probably not included in their estimation, tend artificially to advance their calculated average age.

They have defined an age group as follows: that a clam was a given age if it had lived into that year even though it may have died early in that year, e.g., "the animal was considered to be 2 years old if it had lived into its second year." To Bradley & Cooke, the year begins with growth beyond the previous growth-arrest ring. This growth begins late in spring prior to attainment of one year of age. Thus their "2-year old" could be actually less than one year and, at most 1 and 3/4 years of age. Their study, which included both modern and ancient gem clams, revealed, that, among recent populations (since 1949), no clams actually attained 4 years of age. Some, however, which lived a few hundred years ago, apparently had a life span of over 6 years.

3. Survival and Mortality Rates

Pearl & Miner (1935) describe 3 survivorship curves. In Type I, the

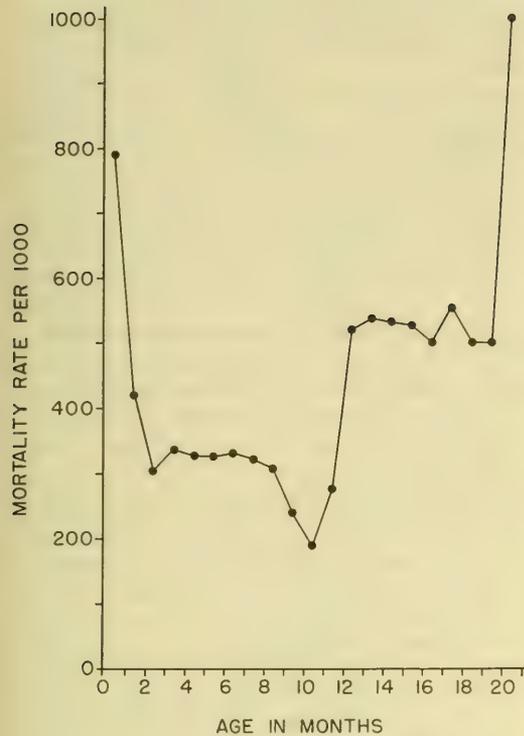


FIG. 48. Mortality rate curve ($1000q_x$) for *Gemma gemma*. It gives the number dying during successive intervals divided by the number of survivors at beginning of interval ($d_x/1_x \times 1000$) (see Table 7).

negatively skew rectangular, members of a cohort die more or less simultaneously at the end of their life span. Type II is diagonal, implying a constant mortality rate at any age. Type III, the positively skew rectangular, shows very heavy mortality early in life, survivors continuing with little mortality to an advanced age.

The survivorship curve for *Gemma* presented in Fig. 47 is a modified Type II, showing a Type III characteristic early in life and a Type I characteristic in later life. Two breaks are seen where mortality rate changes.

The mortality rate curve given in Fig.

48 describes in reverse the survivorship curve. It has the value of a more accentuated ordinate scale, and changes in rate and level of mortality are more readily seen. A high early mortality soon levels off and is maintained until the following spring when it decreases even more, but then takes a sudden turn upward to a higher level which is maintained to the end of life. Causes for varying levels and rate changes are not readily apparent. The summer period is obviously the time during which greatest fluctuations occur. It is at this time that predators are presumably most active. Many of the predators of *Gemma* are known (Table 8), but the time and extent of their activities, and which age groups of *Gemma* are affected, has not been investigated. It is possible, nevertheless, to make certain other observations concerning characteristics of the life history of *Gemma* which may influence mortality.

Reference has already been made to the probably greater susceptibility of juveniles to wave action and their removal to less favorable environments.

Intraspecific competition accompanying high densities which obtain early in life cannot be ignored. Since high numbers at this time are associated with much smaller size, it is more meaningful to seek information from biomass (Fig. 46). It is precisely during the period of greatest biomass that mortality rate is high (for juveniles) or rises sharply (for 1-year olds). The maintenance of this high level for 1-year olds when biomass falls again, requires another explanation.

The rise in mortality rate for 1-year olds coincides with the period of liberation of juveniles and production of another brood. It is possible that a drain on physiological resources of the individual may render it more susceptible to hostile environmental factors. Paine (1963) suggests the same possibility for brachiopods which suffer a similar heightened mortality in summer.

TABLE 8. A list of predators of *Gemma gemma* compiled from the literature, and laboratory and field investigation.

Scientific Name	Common Name	Reference
COELENTERATA		
<i>Paractis rapiformis</i>	Burrowing sea anemone	Sellmer
ARTHROPODA		
<i>Callinectes sapidus</i>	Blue crab*	Sellmer
<i>Ovalipes ocellatus</i>	Lady crab	Sellmer
<i>Carcinides maenas</i>	Green crab	Glude, 1955
<i>Panopeus herbstii</i>	Mud crab*	Sellmer
<i>Neopanopeus texana sayii</i>	Mud crab*	Sellmer
<i>Limulus polyphemus</i>	Horseshoe "crab"	Packard, 1880; Shuster, 1950
MOLLUSCA		
<i>Polinices</i> spp.	Sand-collar snail	Turner, 1951; Hanks, 1953; Sawyer, 1950
CHORDATA		
Pisces		
<i>Rhinoptera bonasus</i>	Cow-nosed ray	Mc Dermott**
Aves		
<i>Nycticorax nycticorax</i>	Night heron	(Greely)***
<i>Branta canadensis</i>	Canada goose	(Cottam)
<i>B. bernicla</i>	Brant	(Cottam)
<i>Anas platyrhynchos</i>	Mallard	(McAtee)
<i>A. rubripes</i>	Black duck	Forbush, 1925
<i>A. acuta</i>	Pintail	(McAtee)
<i>Aythya americana</i>	Redhead	Cottam, 1939
<i>A. valisineria</i>	Canvasback	Cottam, 1939
<i>A. marila</i>	Greater scaup	Cottam, 1939
<i>A. affinis</i>	Lesser scaup	Cottam, 1939
<i>A. bucephala clangula</i>	Goldeneye	(Cottam)
<i>Clangula hyemalis</i>	Oldsquaw	Cottam, 1939
<i>Squaterola squaterola</i>	Black-bellied plover	(Sperry)
<i>Capella gallinago</i>	Common snipe	Sperry, 1940
<i>Calidras camutus</i>	Knot	Sperry, 1940
<i>Erolia minutilla</i>	Least sandpiper	(Sperry)
<i>Limnodromus griseus</i>	Dowitcher	Sperry, 1940
<i>Ereunetes pusillus</i>	Semipalmated sandpiper	(Sperry)

*Laboratory feeding.

**Personal communication.

***References in parentheses are unpublished data from stomach examination forms supplied through the kindness of Fred H. Dale, Assistant Director, Patuxent Research Refuge, Laurel, Maryland. Name is that of examiner.

XII. PREDATORS

The gem clam serves as food for a carnivorous gastropod, certain arthropods and chordates and a coelenterate. These predators are summarized in Table 8.

Predation on *Gemma* by boring snails of the genus *Polinices* has been reported by Sawyer (1950), Turner (1951) and Hanks (1953) who believe it to be the chief food source of these snails when they are young. Although *Polinices* is present at Union Beach, no drilled shells

were ever found there. In a collection of *Gemma* made in the Navesink River, New Jersey, in 1955, however, almost 10% of the shells were found to be drilled.

Since the holes were abraded, the characteristics defining the species which had bored them had become indistinct and they were shown to 2 investigators acquainted with snail borings (Drs. M. R. Carriker and J. E. Hanks). Both believed that the borings were probably the work of *Polinices*.

Results of laboratory and field studies indicate that crabs are important predators on the gem clam. Two species of mud crabs, the green crab and the blue crab, readily accepted gem clams when offered to them. In one instance, a green crab, having a carapace width of 4 cm, consumed 50 adult gem clams in 30 minutes. Shells have also been found in the stomachs of the above crabs which were collected in the field. At low tide in a salt pond at Gardiners Island, New York, many small piles of broken *Gemma* shells were seen on the exposed bottom. Although their formation was never observed, they resembled the shell piles left by laboratory-fed crabs. Bradley & Cooke (1959) have also observed chipped and broken *Gemma* shells in Maine which they believe may be due to predation by the green crab.

In late August, 1957, the 1956 year class of *Gemma*, which had a density of about 1000/m² 2 weeks earlier, had all but disappeared. The flat was strewn with broken gem clam shells and the presence of hundreds of shed carapaces of the lady crab (*Ovalipes ocellatus*) attested to an invasion by this species during the previous 2 weeks. Five were caught and *Gemma* shells were found in the stomachs of all. Measurement of 45 carapaces indicated that the crabs were half grown. They had never been seen in the area at any other time during the period of study. It is believed that high salinities resulting from below normal precipitation

allowed the crabs to exploit this newly-available food source.

Gem clams were early noted by Packard (1880) and later by Shuster (1950) as food for the horseshoe "crab". A young *Limulus* with a prosomal width of 6 cm was collected at Union Beach and observed for the next few days. During this time, it passed fecal aggregations of broken *Gemma* shells containing 268 umbones which presumably represented 134 clams.

An interesting predator of *Gemma* is a burrowing sea anemone, *Paractis rapiformis*, which is found in great numbers at Union Beach. It is 4-6 inches long when relaxed and secures its holdfast to the packed clay or black reducing stratum. The sand-colored tentacles are even with the level of the sand. These animals were found to contain 50-100 *Gemma* in their gastrovascular cavities.

A large number of shore birds feed on the gem clam. The relative importance of *Gemma* in the diet of these birds is not known in most cases. Martin & Uhler (1939) found that in over 12,000 duck stomachs examined, all clams contributed only about 5% of the total contents. Of the ducks listed in Cottam's study (1939), *Gemma* contributes 6.5% to the diet of one and less than 2% to the others. Moreover, molluscan contribution to the diet of ducks may actually be less than was measured volumetrically since mollusks are probably triturated and digested more slowly than soft-bodied forms taken with them, thus tending to be held longer and even accumulated. They are, therefore, probably counted for more than their actual contribution.

Certain bottom-feeding fishes have been described as feeding on "small bivalve mollusks." McDermott (personal communication) found *Gemma* shells in the stomach of the cow-nosed ray. No fish stomachs were examined during this study, but the gem clam may well be a part of the diet of some fishes feeding in the intertidal zone.

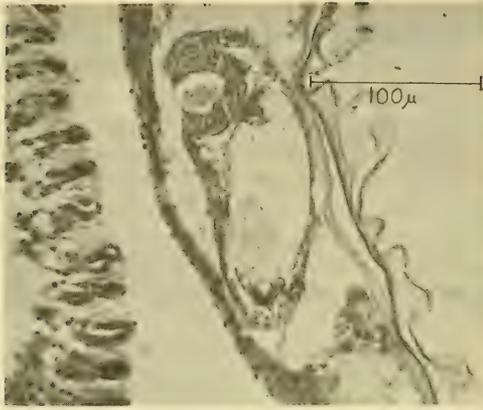


FIG. 49. Section of metacercaria of *Parvatrema borealis* through oral sucker and intestinal cecum. Mantle and inner demi-branch to left, shell to right.

XIII. PARASITES

Two new species of trematodes from *Gemma* have been described (Stunkard & Uzmann, 1958, 1959). These parasites were observed early during this study and information supplementing their published description is given here.

One species is represented by a metacercaria, *Parvatrema borealis*, found between the shell and mantle. It is believed by Stunkard & Uzmann that sporocysts containing furcocercous cercariae, which were located in the gonad of *Gemma*, may belong to the same species. A related sporocyst, *P. borinquense*, infects *Gemma purpurea* in Puerto Rico, but the metacercaria is found in a snail, *Cerithidia costata* (Cable, 1953).

The other species, *Cercaria adrano-cerca*, represented by sporocysts and microcercous cercariae, is also situated in the gonads. In addition, a second, possibly new species of metacercaria was found in gem clams. A brief description is given below.



FIG. 50. Left valves of 2 half-grown gem clams showing cavity on inner surface of valve dorsal to pallial sinus. Cavity marks former location of metacercaria and is probably produced through interference with shell deposition and/or chemical erosion.

1. Metacercariae

A total of 1359 clams was dissected during the period July, 1954-August, 1957. Incidence of infection of clams longer than 1.5 mm showed greatest fluctuation during the warmer period of the year and an average of 37 clams was inspected each month during the warmer half of the year. Since incidence of infection proved to be relatively constant during the colder period, clams were checked during only one cold season.

The metacercariae of *Parvatrema* are generally found in a cluster slightly anterior to the posterior adductor muscle. They are individually enclosed by a thin transparent membrane. Stunkard & Uzmann (1958) refer to the metacercaria as being unencysted, as the senior author (oral communication) holds that a proper cyst should be thicker than the one present and consist of some host contribution. Cysts are not evident in section (Fig. 49), but are readily demonstrable when the clam is dissected.

There is a definite tissue response to the worm on the part of the host. In the region adjacent to the metacercaria, the mantle loses its histological organization and becomes somewhat thickened (Fig. 49). In addition, there is often a concavity in the shell (Fig. 50) which may be the result of an interference with shell deposition by the altered mantle and the interposition of the body of the worm which separates it from the shell. Acid metabolites from the worm may also act on the shell, eroding it chemically. No evidence was found of other harmful effects on the host.

Overall incidence of infection varied between 14 and 100%. It remained over 90% during most of the year, but fell each year early in the summer to 50% or less. It soon returns later in the summer to its former high level. During the fall of 1958, however, casual inspection revealed only a 10-20% incidence of infection. Stunkard & Uzmann report 10% infection in clams at Boothbay Harbor, Maine in 1957, with 1-10 metacercariae per clam.

Numbers of larval worms per host varied with incidence of infection. Only 1-2 metacercariae were found in each clam when incidence was low in the summer, but 10-150 were present in winter, when most clams were infected.

The number of metacercariae increases as size of clam increases, possibly indicating continued infection. If the period of infection is of short duration, an alternative explanation may be that size of the clam places a limit on the number of worms it can harbor.

The seasonal decline observed is difficult to understand, but may be brought about by selective mortality of infected clams.

Attempts to experimentally infect 9-day and 25-day old white Pekin ducklings failed. Stunkard & Uzmann (1958) infected eider ducks (*Somateria mollissima*) recovering adults from the intestine. No effort was made during the present study to find *Parvatrema borealis* adults in predators of *Gemma*.

The list of gem clam predators in Table 8 offers a number of possibilities for further study.

Another species of metacercaria was observed on one occasion during the summer of 1957. While inducing gem clams to shed cercariae using elevated temperature and light, several clams shed clusters of metacercariae, some clusters containing as many as 50 of them. They differed in several ways from *Parvatrema borealis* metacercariae. The cyst of *Parvatrema* is ovoid and not much larger than its body which lies in an extended position. The cysts of this species is conical and 5-6 times as long as the larval worm, which is curled upon itself and situated near the base of the cyst. They also differ in color, *Parvatrema* being a grayish to olive-green, the other olive to bluish-green. The ratio between diameters of oral and ventral suckers of *Parvatrema* is about 2:1 whereas that of the other is about 1:1. A single excysted and extended, living worm measured $175 \times 71 \mu$. No further studies have been made on this species.

2. Other Trematode Stages

Sporocysts containing furcocercous cercariae, and believed by Stunkard & Uzmann (1958) to belong to the species *Parvatrema borealis*, are found in gonads of the gem clam. These authors thought that sporocysts of this species were situated in the interlobular spaces of digestive diverticula, but my own studies, which included the opportunity to examine Stunkard & Uzmann's material, show them to be only in the gonads.

The possibility of parasitic castration or other interference with reproduction is indicated by the fact that of the 13 infected clams found, the sex of which was indeterminable, none contained embryos. Evidence for gonadal damage in the male is seen in Fig. 51. Coe (1955) found that the sporocyst of *Postmonorchis donacis* rendered the bean clam *Donax gouldii* sterile and Uzmann (1953) found that sporocysts of *Cer-*

caria milfordensis had the same effect on *Mytilus edulis*.

Of 1624 gem clams checked, 1359 by dissection, 265 by induced liberation of cercariae, 17 (1.05%) cases of furcocercous cercarial infection were found. Stunkard & Uzmann found an incidence of 0.37%. It is believed that this low incidence of infection has relatively little effect on the reproductive potential of the gem clam as a population.

Sporocysts containing microcercous cercariae of the species *Cercaria adranocerca* are also found in gem clam gonads. Parasitic castration is indicated since no embryos were found in 15 infected clams. In 1624 clams, the incidence of infection was 0.92%. Stunkard & Uzmann (1959) found an incidence of somewhat less than 1% in clams from Maine.

XIV. RELATIONS TO MAN

1. Positive Value

It has already been indicated that *Gemma* is found in the diet of at least 18 shore birds (Table 8), many of which are game birds. The gem clam, however, actually contributes probably very little to the total diet and thus is of no great importance as food for these animals.

Gemma may act to some degree as a buffer for commercially important clams. Glude (1955) reported that from an examination of 1448 stomachs of green crabs collected in the field, *Gemma* was 4th in order of preference based on frequency. In laboratory studies, the order of preference of the green crab was *Mya*, *Macoma*, *Gemma* and *Mercenaria*, indicating *Gemma* as a buffer species for *Mercenaria* when the latter is young.

2. Negative value

Gemma has been shown to act as the first and second intermediate host for 3 species of trematodes which, as adults, may infect game birds important to man.

Gemma may act as a secondary food

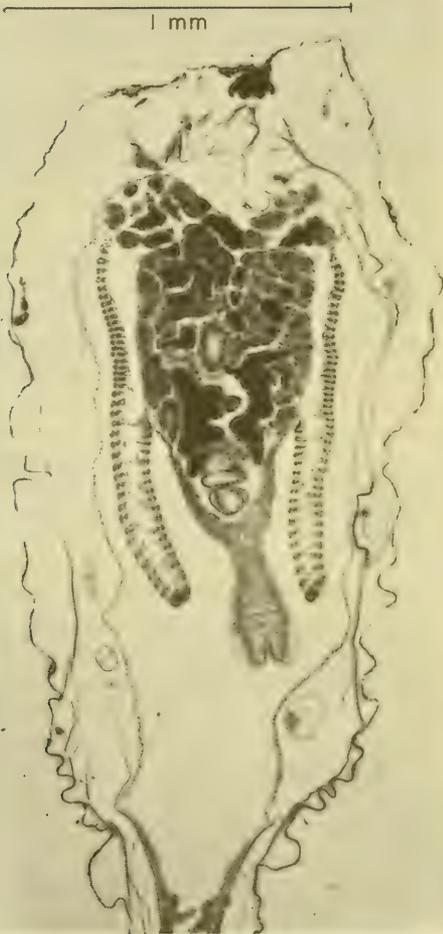


FIG. 51. Cross-section through adult male gem clam at level of gonad. Light areas in visceral mass are sporocysts of the species *Parvatrema borealis* and/or *Cercaria adranocerca*. Deeply stained areas are persisting testicular follicles. Compare with Fig. 18. Note metacercariae (*P. borealis*) between shell and mantle.

supply for predators of commercially important bivalves, thus maintaining a predator population during a dearth of their primary food supply. This thesis has been advanced by Sawyer (1950) and Turner (1951) for the boring snail *Polinices duplicatus* in Massachusetts. Although Hanks (1953) believed that the bulk of the diet of young *Polinices* snails consisted of gem clams, he did not think that these latter were the sole factor in the preservation of a starving snail population, and pointed out that other gastropods, *Littorina littorea* and *Nassarius obsoletus*, also act as substitutes.

Gemma has recently come under suspicion as being, in some way, inimical to the welfare of the soft shell clam, *Mya arenaria*. Bradley & Cooke (1959) have suggested that, in Maine, *Mya* sets are more successful when the density of gem clams is low, and that few *Mya* are found when it is high. Similar observations have been made at Union Beach. During the period of this study, there was no set of *Mya* until 1958, when gem clam density was lowest. A good set of 0.5-2.0 mm long *Mya* (8,888/m²) appeared that summer.

That this antagonism works both ways was demonstrated by Bradley & Cooke, who showed that in a limited number of samples, significantly fewer gem clams were found in the proximity of adult soft shell clams where the gem clams might be deprived of food and be subjected to metabolites excreted by the soft shell clams. The tentative hypothesis that these 2 species are mutually antagonistic can only be proven through further study.

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APPENDIX

TABLE 1a. Hydrographic data for Conaskonk Point, New Jersey (1955)

Date	Time Relative to Low Water hr.	Temperature (°C)	
		Air	Water
Apr. 2	+0.5	-	18.5
Apr. 25	-1.0	-	10.5
May 7	-0.5	-	20.0
June 7	-0.5	-	23.0
June 20	-0.75	-	28.0
July 21	-0.5	33.0	30.0
Aug. 8	-1.0	25.0	26.0
Aug. 10	0.0	22.0	22.0
Aug. 14	0.0	29.0	27.5
Sept. 12	0.0	20.0	22.0
Oct. 17	0.0	14.5	17.5
Nov. 26	0.0	3.0	4.5
Dec. 27	0.0	- 4.0	0.0

TABLE 1b. Hydrographic data for Conaskonk Point, New Jersey (1956)

Date	Time Relative To Low Water hr.	Temperature (°C)		Salinity (‰)
		Air	Water	
Jan. 30	0.0	12.0	2.0	-
Feb. 26	-1.0	6.0	5.0	-
Mar. 31	-1.0	7.0	6.0	16.6
Apr. 24	-1.0	11.0	9.0	16.9
May 26	-1.5	20.5	16.0	21.6
June 11	-1.0	25.0	23.5	20.8
June 25	-1.0	28.0	25.0	23.3
July 11	-1.0	28.0	25.0	24.3
July 23	-1.0	30.5	24.5	23.5
Aug. 8	-1.0	27.0	25.5	24.1
Aug. 23	-1.0	21.0	25.0	25.1
Sept. 5	-1.0	25.5	28.5	25.5
Sept. 20	-1.0	16.0	19.0	23.9
Oct. 2	-1.0	14.0	18.0	24.7
Oct. 19	-1.0	11.0	14.0	22.9
Nov. 18	-1.0	7.0	9.5	20.6
Dec. 20	-2.0	10.5	7.0	20.4

TABLE 1c. Hydrographic data for Conaskonk Point, New Jersey (1958).

Date	Time Relative To Low Water hr.	Temperature (°C)			Salinity (‰)
		Air	Water	Sand	
Mar. 4	-	-	-	-	16.6
Mar. 22	-0.75	5.5	5.0	7.5	14.7
Apr. 29	-	-	-	-	13.1
June 2	-0.25	21.0	20.5	22.0	21.2
June 27	-1.0	22.0	21.5	24.0	23.4
July 13	-0.5	24.0	26.0	29.0	23.4
July 27	-	-	-	-	25.2
Aug. 13	-1.0	25.0	25.0	25.5	26.4
Aug. 28	-1.0	20.5	21.5	21.5	24.8

TABLE 2a. Growth data for *Gemma gemma* (1954 year class)

Collection Date	Number Measured	Mean Length (μ)	Range (μ)
1955:			
Apr. 2	100	1925	1365-2964
Apr. 25	100	2014	1482-3237
May 7	100	2270	1560-3354
June 7	100	3248	2106-4056
June 20	100	3413	1989-4212
July 21	45	4047	3198-4680
Aug. 8	50	4155	3627-4797
Sept. 12	12	4356	4017-4758
Oct. 17	26	4750	4251-5343
Nov. 26	23	4668	4056-5304
Dec. 27	8	4339	3783-4953
1956:			
Jan. 30	6	4433	3939-4719
Feb. 26	23	4364	3900-4914

TABLE 2b. Growth data for *Gemma gemma* (1955 year class)

Collection Date	Number Measured	Mean Length (μ)	Range (μ)
1955:			
Oct. 17	100	2494	1716-3939
Nov. 26	100	2440	1677-3978
Dec. 27	311	2055	1521-4329
1956:			
Jan. 30	200	2114	1521-4212
Feb. 26	100	1996	1521-3227
Mar. 31	100	2261	1716-3471
Apr. 24	100	2260	1560-3432
June 11	100	2835	2174-4017
July 11	48	3688	3081-4680
Aug. 8	100	3838	2886-4290
Aug. 23	100	3887	3315-5070
Sept. 5	72	3858	3510-4758
Sept. 20	100	3882	3120-4407
Oct. 19	61	3904	3471-4602
Nov. 18	44	3818	3393-4719
Dec. 20	26	3918	3315-4407
1957:			
Feb. 1	13	3999	3549-4368

TABLE 2c. Growth data for *Gemma gemma* (1957 year class)

Collection Date	Number Measured	Mean Length (μ)	Range (μ)
1957:			
May 11	4	405	380- 430
June 11	87	373	320- 430
June 26	100	562	370- 820
July 10	100	751	400-1443
July 26	100	1082	410-2340
Aug. 8	200	999	400-2379
Aug. 26	100	1123	440-1989
Sept. 9	100	1195	700-2028
Sept. 22	100	1260	790-2145
Oct. 7	100	1199	800-1989
Nov. 5	100	1347	1053-1950
Dec. 19	100	1309	880-1872
1958:			
Mar. 22	100	1265	900-1911
Apr. 29	100	1333	940-2028
June 2	100	1882	1599-2730
June 27	100	2614	1677-3315
July 13	50	2955	2106-3822
July 27	50	3035	2301-3510
Aug. 13	100	3058	2301-3861
Aug. 28	117	3024	2496-3900

TABLE 2d. Growth data for *Gemma gemma* (1958 year class)

Collection Date	Number Measured	Mean Length (μ)	Range (μ)
1958:			
July 13	50	495	360- 740
July 27	50	563	360- 870
Aug. 13	79	873	340-1794
Aug. 28	86	926	350-1794

TABLE 6a. Population density data for *Gemma gemma* (1954 year class)

Date	No. of Samples	Total Area of Samples (cm ²)	No. of Clams in Total Sample	No. of Clams /m ²
1955:				
Aug. 10	7	88.2	24	2,736
Nov. 26	5	141.4	4	284
Dec. 27	5	141.4	2	142
1956:				
Jan. 30	15	424.2	1	24

TABLE 6b. Population density data for *Gemma gemma* (1955 year class)

Date	No. of Samples	Total Area of Samples (cm ²)	No. of Clams in Total Sample	No. of Clams /m ²
1955:				
Nov. 26	5	141.4	588	41,746
Dec. 27	5	141.4	311	22,081
1956:				
Jan. 30	15	424.2	845	19,919
Feb. 26	5	141.4	325	22,971
Mar. 31	10	282.8	139	4,912
Apr. 24	55	1080.0	814	7,546
June 25	10	282.8	125	4,431
July 11	6	169.8	48	2,827
July 23	25	705.0	325	4,610
Aug. 8	20	565.6	166	2,935
Aug. 23	22	622.2	125	2,009
Sept. 5	28	791.8	71	897
Sept. 20	30	2697.3	233	864
Oct. 19	20	1798.2	56	312
Nov. 18	30	2697.3	44	163
Dec. 20	50	4495.5	23	51
1957:				
Feb. 1	50	4495.5	10	22

TABLE 6c. Population density data for *Gemma gemma* (1957 year class)

Date	No of Samples	Total Area of Samples (cm ²)	No. of Clams in Total Sample	No. of Clams /m ²
1957:				
May 11	10	34.6	4	1,157
May 28	12	41.5	0	0
June 11	12	41.5	87	20,929
June 26	6	20.8	215	103,565
July 10	3	10.4	186	179,383
July 26	12	41.5	340	81,888
Aug. 8	18	62.3	363	58,125
Aug. 26	12	41.5	172	41,185
Sept. 9	15	51.9	206	39,692
Sept. 22	18	62.3	232	37,412
Oct. 7	18	62.3	182	29,210
Nov. 5	21	72.7	165	22,709
Dec. 19	12	208.0	250	12,019
1958:				
Mar. 22	30	848.4	190	2,240
Apr. 29	30	1086.8	226	2,080
June 2	30	1086.8	182	1,675
June 27	18	1618.4	305	1,885
July 13	15	1348.7	120	889
July 27	18	1618.4	206	1,273
Aug. 13	18	1618.4	106	655
Aug. 28	21	1888.1	120	636

TABLE 6d. Population density data for *Gemma gemma* (1958 year class)

Date	No. of Samples	Total area of Samples (cm ²)	No. of Clams in Total Sample	No. of Clams /m ²
1958:				
June 27	10	34.6	3	868
July 13	10	34.6	109	31,503
July 27	12	41.5	176	42,389
Aug. 13	12	41.5	80	19,750
Aug. 28	18	62.3	85	13,648

RESUMEN

MORFOLOGIA FUNCIONAL E HISTORIA ECOLOGICA DE LA ALMEJA
GEMMA GEMMA (EULAMELLIBRANCHIA, VENERIDAE)

G. P. Sellmer

Un estudio comprensivo de la pequeña almeja *Gemma gemma* se realizó en Union Beach, Nueva Jersey, durante el período 1955-1958. Incluye la morfología general funcional, así como crecimiento, reproducción, densidad de población, mortalidad, parásitos y predadores. La almeja es ovovivípara, utilizando las branquias medias como sacos marsupiales. Tiene una longitud máxima de 5 mm y se encuentra en lugares arenosos de las zonas de marea de áreas estuáricas. *Gemma* habita desde Labrador hasta Texas; ha sido introducida en la costa oeste donde vive desde Puget Sound hasta San Diego.

Gemma no tiene estado pelágico natatorio activo y la dispersión, particularmente de los jóvenes, se cumple probablemente por la acción de las corrientes y oleaje.

Se describe la anatomía, revelada en su mayoría por cortes seccionales en serie. Especial atención se prestó a las membranas de los sifones inhalantes y exhalantes que están situados dentro, y en la base, de cada sifón. La primera membrana es una cortina arqueada que puede descender para desviar de las branquias el agua cargada de limo. La segunda no había sido hasta ahora descrita en detalles para otras almejas; posee una ranura oval vertical y posiblemente puede cerrarse por completo. Esta membrana también puede, (1) ayudar a la retracción de la membrana valvular en el sifón exhalante, (2) retardar o interrumpir el movimiento del agua a través del cuerpo, contribuyendo a la acción de limpieza, (3) prevenir pérdidas de gametas durante la ovulación, y (4) asistir a la expulsión de las almejititas que nacen.

El crecimiento fue estudiado en muestras de una misma población a períodos mensuales y quincenales. Se elaboró un método para separar los individuos jóvenes de la arena, usando una solución concentrada de $ZnCl_2$.

Los jóvenes nacidos en verano tienen un tamaño medio de 373μ de largo; llegan hasta 2 mm en otoño, alcanzando el tamaño adulto de 4 mm en el verano siguiente. El crecimiento empieza en abril y termina, lo más tarde, en noviembre.

Las hembras retienen las crías dentro de las secciones interna y externa de la branquia media, encontrándose en toda época del año. Unas pocas hembras alcanzaron madurez sexual en el otoño del primer año, aproximadamente a los 4 meses de edad y llevaron sus crías todo el invierno. La mayoría maduran durante la primavera siguiente y dan nacimiento a 100-200 jóvenes durante el verano, con el mayor número nacidos en julio. Experimentos *in vitro* indicaron que los embriones pueden cultivarse fuera del cuerpo materno, por los menos durante la última mitad del desarrollo, y criados hasta madurez. Se da una tabla de más de 100 especies de almejas incubadoras sobre varios aspectos de la reproducción.

Se estudió la densidad de la población en clases de 5 años. Durante el verano la densidad alcanza 200.000 por m^2 . Una constante declinación en número se observó en Union Beach durante el estudio. Biomasa (peso húmedo incluyendo la concha) se calculó para el período. Fluctuaciones sesionales fueron regulares y de magnitud considerable. Valores mínimos y máximos variaron entre 2-200 gramos por m^2 .

Los predadores incluyen una actinia, un gastrópodo, 5 especies de cangrejos, la "cacerola" (*Limulus*), un pez elasmobranquio, y 15 especies de aves acuáticas la mitad de ellas patos.

La duración máxima de la vida de *Gemma* en Union Beach es de 2 años, con un promedio de 1,13 meses. Mortalidad mensual es alrededor de 40%.

Estados larvales de tres tremotodes parasitan en *Gemma*. Una metacercaria (*Parvametra borealis*) se encuentra entre la concha y el manto de la mayoría de las almejas, pero causan poco daño. Esporocistos situados en las gonadas y conteniendo cercarias furrocercas son probablemente de la misma especie. Aunque destruyen las gonadas estos infecta *Gemma* con muy poca frecuencia (alrededor de 1%) y el efecto es negligible. Un esporocisto con cercarias microcercas (*Cercaria adranocerca*) también se encontró ocasionalmente en las gonadas con similar efecto. Además se encontró una metacercaria no identificada que aquí se describe.

Gemma gemma parece tener valores positivos y negativos para el hombre. Es parte de la dieta de los patos de caza y problememente actúa como especie amortiguadora contra los predadores de otras especies comerciales. Pero, por otra parte, puede servir para alimentar poblaciones de gastrópodos predadores durante la carestía de otras almejas economicamente importantes. También como primer y segundo huéspedes intermediario de trematodes que pueden infectar, como adultos, animales útiles para el hombre.

АБСТРАКТ

ФУНКЦИОНАЛЬНАЯ МОРФОЛОГИЯ И ЭКОЛОГИЧЕСКАЯ ХАРАКТЕРИСТИКА
ДВУСТВОЧАТОГО МОЛЛЮСКА
GEMMA GEMMA (EULAMELLIBRANCHIA, VENERIDAE)

Г. П. СЕЛЛМЕР

В 1955-58 г.г. в районе Юнион Биу, Нью Джерси, было проведено всестороннее исследование моллюска *Gemma gemma*, включавшее как общее изучение функциональной морфологии, так и рост, размножение, плотность поселений, смертность, паразитов и врагов (хищников).

Gemma - живородящий моллюск, у которого полужабы играют роль выводковых камер. Наибольшая длина гемм 5 мм; обитает на песчаных пляжах литорали и в верхней сублиторали, в эстуариях. Встречается в районе Атлантического побережья США от Лабрадора до Техаса; была интродуцирована также на тихоокеанское побережье, где теперь распространена в районе от Пэджет - Саунда до Сан-Диего, Калифорния.

Геммы не имеют активно-плавающей личиночной стадии и их распространению (особенно молодежи), вероятно способствуют течения и действие волн.

Анатомия этих моллюсков описывается, главным образом, на основании серий срезов. Особое внимание было обращено на строение дыхательной и выдыхательной сифональных мембран, расположенных внутри, у основания каждого сифона. Клапан дыхательного сифона имеет вид дугообразно-изогнутой пленки-которая может опускаться, чтобы отклонить от жабр ток воды, содержащей илстые частицы; клапан выдыхательного сифона у других гемм никогда раньше детально не описывался. Он имеет вертикальную овальную щель и, видимо, может полностью закрываться. Этот клапан может: 1) способствовать отодвиганию клапана выдыхательного сифона, 2) замедлять или останавливать движение воды через внутреннюю полость моллюска, помогая таким образом её очищению, 3) предотвращать потерю гамет во время овуляции и 4) помогать выходу молодежи.

Рост гемм изучался путём взятия проб моллюсков из одной и той же популяции через месячный или двухнедельный интервалы. При этом отделение молодых форм от песка производилось при помощи концентрированного раствора $ZnCl_2$.

Молодь, появляющаяся летом, имеет, в среднем, длину около 2 мм, и достигает 4 мм (взрослые) на следующее лето. Рост гемм начинается в апреле и заканчивается, самое позднее, в ноябре.

Геммы-самки вынашивают свою молодь на внутренней и внешней полужабрах, где их можно найти в любое время года.

Лишь немногие самки достигают половозрелости на первом году жизни, в возрасте около 4-х месяцев и тогда вынашивают свою молодь всю зиму. Большинство же самок созревает весной и дадут в течение лета 100-200 экземпляров молоди, наибольшее количество которых выходит в июле.

Эксперименты *in vitro* показывают, что эмбрионов гемм можно успешно выращивать вне материнского организма, по крайней мере в течение последней недели периода их развития, доводя их до половозрелого состояния.

В работе приводится список более 100 видов моллюсков, проявляющих заботу о потомстве, а также замечания о различных способах их размножения.

Изучалась плотность популяций данного вида (при классовом промежутке в 5 лет). В течение лета эта плотность поселений может достигать 200 тыс. экз. и более на 1м^2 . В течение периода наблюдений на Юнион Бич происходило устойчивое уменьшение количества экземпляров. За этот период была подсчитана биомасса (сырой вес вместе с раковиной); сезонные её колебания имели правильный характер и достигали значительной величины. Минимальные и максимальные её значения колебались от 2 до 200 $\text{г}/\text{м}^2$.

Наибольшая продолжительность жизни гемм в районе Юнион Бич составляла 2 года, а средняя - всего 1,13 месяца. Темп отмирания составлял, в среднем около 40% в месяц.

Врагами гемм являются различные хищные формы: актинии, брюхоногие моллюски, подковообразные "крабы", рыбы из *Elastobranchia* и 18 видов береговых птиц (половину из них составляют утки).

Личиночные стадии 3 видов трематод паразитируют на геммах. Метацеркарии (*Parvatrema borealis*) были найдены между раковиной и мантией большинства гемм, обитающих на Юнион Бич, но особенно вреда они не приносят. Спороцисты, найденные в гонадах и содержавшие фуркоцеркальных церкарий, возможно относились к тому же виду. Хотя они и разрушают гонады, они редко заражают гемм (около 1%) и поэтому не приносят большого вреда. Такую же роль играют и спороцисты с микроцеркальными церкариями (*Cercaria adranocerca*), также иногда находимые в гонадах гемм. Отмечены также поea неопределенные метацеркарии, кратко описанные в работе.

Gemma gemma видимо имеет и положительное и отрицательное значение для человека. Они составляют частично пищу для диких уток. Возможно они являются формами, промежуточными между хищниками, потребляющими промысловые виды моллюсков и тем самым в какой то степени предохраняют их. С другой стороны, они же могут служить для прокорма популяций различных хищных форм при уменьшении количества важных промысловых видов моллюсков. Геммы также служат первым и вторым промежуточными хозяевами для трематод, которые могут заражать животных, важных для человека.

VARIATION IN THE STOMACH STRUCTURE OF THE BIVALVIA

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ABSTRACT

The stomach structure of 36 species of bivalves has been described, using a uniform terminology. The details of structure of the stomach of various groups of bivalves have been compared with earlier studies on the subject by Graham (1949) and Purchon (1956a, 1957, 1958, 1960).

The most significant aspects of structural variation appear to be those resulting from the interaction between the walls of the stomach and the ducts of the digestive diverticula. These open either individually or in groups into the stomach walls, or join to open by large common ducts. In the Eulamellibranchia mainly, the stomach wall adjusts itself to accommodate the ducts into definite areas, embayments or pockets of the wall. These modifications of the stomach wall at the sites where ducts open may also be followed by other changes, of which the most noteworthy is that shown by the major typhlosole of the stomach, which invades the regions where ducts of the digestive diverticula have become aggregated. Thus in the extreme line of modification, as revealed in some of the Eulamellibranchia, the typhlosole expands greatly and becomes intimately associated with the openings of the ducts in special pockets (the caeca) of the stomach wall.

The degree of relationship between the stomach and the ducts of the digestive diverticula is believed to be significant in assessing the evolution of stomach structure. An estimate of this relationship is diagrammatically represented in Fig. 22, which represents the main patterns of variation in stomach structure.

Three main patterns of variation, which represent the stomachs of 3 main groups of bivalves, have been initially recognized as follows: (1) Arcacea + part of Anisomyaria (Fig. 22C), (2) remainder of Anisomyaria (Fig. 22D) and (3) Eulamellibranchia (Fig. 22E). Within the Eulamellibranchia, 3 subsidiary lines of modification (Fig. 22E¹ - E³) have been indicated, which present a sequential or phased relationship between the stomach and the digestive diverticula. The protobranch stomach reveals a structural pattern which incorporates the basic features of the major groups above, but in that group the duct system of the digestive diverticula has been modified without basic changes in the stomach wall (Fig. 22A); a similar line of modification is revealed in one line of the Eulamellibranchia (Fig. 22E²). The Septibranchia represent an extremely simplified plan, both of the stomach and the digestive diverticula (Fig. 22B).

To base structural analyses on more functional lines, there is need to know more about the functions of the digestive tubules and ducts, the crystalline style and the stomach epithelia.

INTRODUCTION

A comparative study by Graham (1949) of the structure of the stomach in 16

species of bivalves has formed the basis for later studies on the subject (Allen, 1954, 1958; Dinamani, 1957; Owen, 1953; Purchon, 1954, 1955, 1956a, 1957,

1958, 1959, 1960), and stimulated interest in the functional interpretation of its structure. Purchon (1956a *et seq.*) in fact extended this study to cover the stomachs of 60 genera of bivalves, with the object of analysing structural variation, and describing stomach function for recognition of phylogenies within the class. He also proposed a scheme of classification, taking the structure of the stomach as his criterion, and divided the class into 5 orders, as many as there were stomach types. In the studies on the stomach presented in this paper, I have been able to distinguish 4 main types of stomachs (I consider the Septibranchia as possessing a fifth type of stomach, but have not personally examined the stomach of any member of this order). My types do not conform to Purchon's, primarily because we have selected different morphological features of the stomach as bases for distinction. Moreover Purchon has confined his attention to the stomach, and has not included details of structure of the tubules and ducts of the digestive diverticula in his exhaustive survey. However, the structure and arrangement of the ducts of the digestive diverticula have been revealed by Owen (1955, 1956, 1959) to be important in discussing the functions of the bivalve digestive system. I have indicated in the present study on the stomach of about 36 species of bivalves belonging to 20 families, that the main variations in stomach structure are due to the interaction of the stomach walls with the duct system of the digestive diverticula. The variation revealed in the structure of the stomach forms the subject matter of this paper, and the corresponding changes in the duct system of the digestive diverticula will be discussed in a later paper.

MATERIALS AND METHODS

The structure of the stomach has been examined in 36 species of bivalves, listed under 4 main groups: Protobranchia, Anisomyaria I, Anisomyaria II and Eu-

lamellibranchia. The arrangement has been made on the basis of similar stomach structure, and does not wholly conform to any major scheme of classification of the Bivalvia in use at present. Thus, 2 major innovations in the classification below are the splitting up of the Anisomyaria into 2 groups and the shifting of the Gastrochaenidae next to the Unionidae. The Arcacea have been included with a number of filibranchs and placed in the first group of the Anisomyaria, while the Anomiidae, Pectinidae (and the Limidae¹) have been separated as the second group of Anisomyaria. The adapedont family Gastrochaenidae possesses a type of stomach similar to the schizodont families of Unionidae and Trigonidae, and on that account has been placed close to them.

Family	Species
PROTOBRANCHIA	
Nuculidae	<i>Nucula layardi</i> A. Adams
ANISOMYARIA	
Arcidae	<i>Arca concamera</i> Bruguère <i>Arca inaequalvalvia</i> Bruguère <i>Arca rhombea</i> Born
Limopsidae	<i>Limopsis belcheri</i> (Adams & Reeve)
Mytilidae	<i>Modiolus undulatus</i> (Dunker) <i>Modiolus striatulus</i> (Hanley) <i>Arcuatula</i> sp. <i>Perna viridis</i> (Linnaeus) <i>Lithophaga gracilis</i> (Philippi) <i>Botula cinnamomea</i> (Lamarck)
Isognomonidae	<i>Isognomon nucleus</i> (Lamarck)
Pteriidae	<i>Pinctada anomoides</i> (Reeve)

¹The placement of the Limidae is based on published information (Graham, 1949; Purchon, 1957).

Family	Species
	<i>Pinctada vulgaris</i> (Schumacher)
Pinnidae	<i>Pinna bicolor</i> (Gmelin) <i>Pinna atropurpurea</i> Sowerby
Ostreidae	<i>Ostrea (Crassostrea)</i> <i>madrasensis</i> Preston <i>Ostrea forskalii</i> Gmelin
ANYSOMYARIA II	
Pectinidae	<i>Pecten crassicosatus</i> Sowerby <i>Amussium</i> <i>pleuronectes</i> (Linnaeus)
Anomiidae	<i>Placenta placenta</i> (Linnaeus)
EULAMELLIBRANCHIA	
Unionidae	<i>Lamellidens corrianus</i> Lea
Gastrochaenidae	<i>Gastrochaena</i> <i>impressa</i> Deshayes
Carditidae	<i>Begonia variegata</i> (Bruguère)
Erycinidae	<i>Galeomma</i> <i>paucistriata</i> Deshayes
Veneridae	<i>Meretrix casta</i> (Chemnitz) <i>Cataleysia opima</i> (Gmelin) <i>Chione tiara</i> (Dillwyn) <i>Venerupis</i> <i>macrophylla</i> Deshayes <i>Sunetta effosa</i> Hanley
Mactridae	<i>Standella pellucida</i> (Gmelin)
Psammobiidae	<i>Sanguinolaria</i> <i>(Soletellina) diphos</i> (Gmelin)
Tellinidae	<i>Tellina ala</i> Hanley
Solenidae	<i>Solen annandalei</i> Preston
Pholadidae	<i>Martesia striata</i> (Linnaeus) <i>Jouannetia cumingii</i> (Sowerby)

and preserved in 70% alcohol), before dissection of live material. Most of the specimens, however, were examined in the live condition, and dissected after removal of the right valve under a stereoscopic binocular microscope for the study of ciliary currents in the stomach. Ciliary currents, were first studied using powdered carmine or indian ink, but both of these clump quickly with mucus in the stomach and proved not quite as workable as indian-ink sticks (water dispersible) ground up in the same medium as that in which the animal was being examined. But the most useful materials for studies of ciliary currents were those 'available' within the animal itself, like the intestinal contents and sexual products: for example, the gut of forms such as *Tellina*, *Sanguinolaria* and *Standella* are usually crammed with superfine mud, and the coils of the gut in these forms lie in a mass behind the stomach; a little of this mud, taken at the tip of a blunt needle and applied on the ciliary surfaces, proved a very satisfactory medium. In other species such as *Ostrea*, *Isognomon* and *Lithophaga*, specimens of which were sexually mature at the time of collection, the milky gonadal discharges could be drawn into a fine pipette and strewn on the ciliary areas of the stomach. This may also have the advantage that particles not 'foreign' to the animal are being employed, and the action of the ciliary areas on these particles may approximate to their natural or normal mode.

In naming the areas, folds and pouches of the stomach, the terminology introduced by Graham (1949) has been followed, with modification suggested by Owen (1953) and Purchon (1954, 1955). The system followed by Purchon (1956a *et seq.*) in his subsequent studies of the stomach, whereby numerous sorting areas are recognized and designated by numbers, has not been made use of in this study. Whenever a species, or an allied species, has already been described in the literature, description of stomach structure has been limited to

The structure of the stomach was studied first in preserved specimens (fixed whole in 1% chromic acid, washed

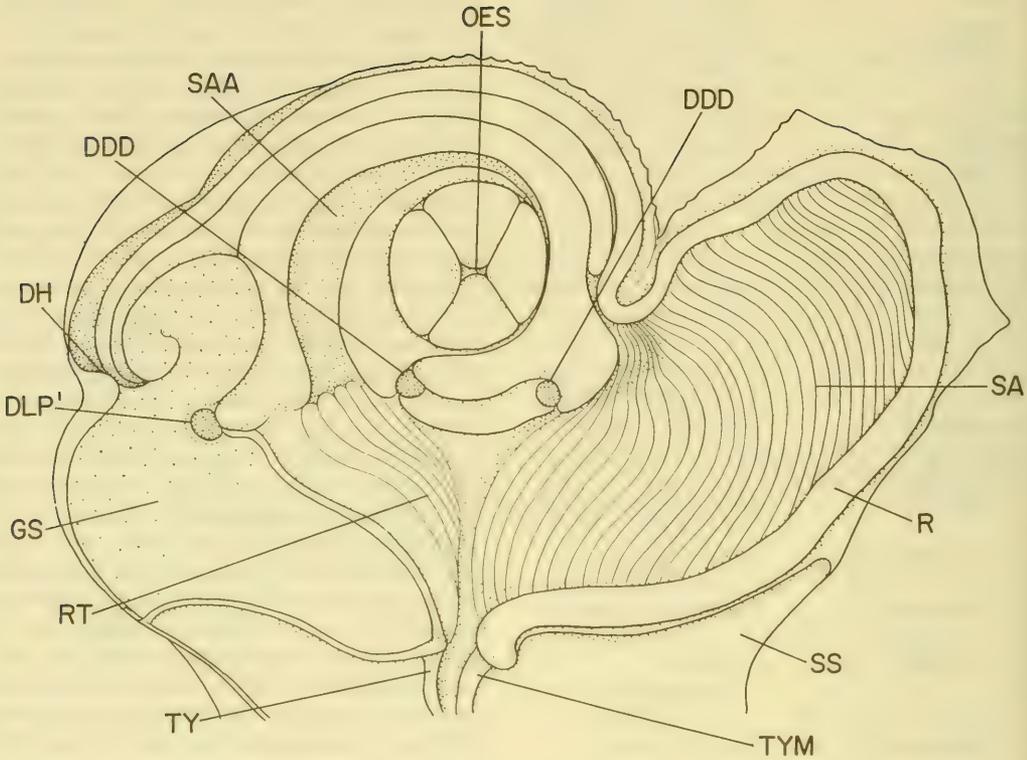


FIG. 1. Interior of the stomach of *Nucula layardi* viewed posteriorly. (For lettering, see p 229)

essentials only, but using the terminology applied in the present paper.

PROTOBRANCHIA

Nuculidae

Nucula layardi A. Adams

Fig. 1

Graham (1949) and Purchon (1956a) have described the stomach of *Nucula hanleyi*, and Owen (1956) that of *N. sulcata*, and the descriptions given by them are more or less identical, except for the nature of the major typhlosole. According to Purchon, the major typhlosole does not enter the stomach, but Owen and Graham state that it extends over the left wall of the stomach to the left

opening of the digestive diverticula. Graham considers that it extends into the duct, while Owen regards it as terminating at the opening of the duct. All the authors describe the great extension of the minor typhlosole into the stomach in the form of a wide 'U', surrounding a grooved area and terminating on the right side of the stomach wall near the oesophageal opening. However, the study of the present material seems to suggest that the disposition of both the typhlosoles inside the stomach needs reconsideration.

The opening of the oesophagus into the stomach in *Nucula layardi* (OES) is situated in a median circular depression in the anterior wall. The opening itself is in the form of a X-shaped slit, formed

KEY TO THE LETTERING OF THE FIGURES

AF	anterior fold		digestive diverticula are concentrated
AT	acceptance tract		
AXF	axial fold	LF	lateral fold
AXF'	terminal portion of the axial fold	LP	left pouch or homologous area
BP	blind pouch	LP'	ridge above left pouch
BP'	entrance to blind pouch	LR	lateral ridge
C	channel-like area on posterior right wall	MG	opening of midgut
CL	crenate lobes	OD	oval depressions along posterior right wall
DDD	openings of the ducts of the digestive diverticula (not specified)	OES	oesophagus
DDL	ducts opening on the left wall of the stomach	PDC	posterior dorsal caecum
DDR	ducts opening on the right wall of the stomach	PDC'	opening to the posterior dorsal caecum
DG	dorsal groove	R	ridges on the wall of the dorsal hood or the right wall
DH	dorsal hood	RC	right caecum
DLP	ducts opening into the left pouch	RDC	region of the right wall where openings of the ducts of the digestive diverticula are concentrated
DLP'	ducts opening at or close to the site of the left pouch	RG	rejection groove
FSC	food sorting caecum	RT	rejection tract
FSC'	extension of food sorting caecum	SA	sorting area
GA	grooved area in left wall or floor of stomach	SAA	anterior sorting area
GI	main intestinal groove	SAP	posterior sorting area
GIA	beginning of main intestinal groove	SS	style sac
GP	posterior groove	TY	major typhlosole
GS	gastric shield	TYA	portion of the major typhlosole between the right and left caeca
GSD	spur of gastric shield in dorsal hood	TYT	terminal portion of the major typhlosole
GSL	spur of gastric shield in left pouch	TYM	minor typhlosole
LC	left caecum	TR	trough-like area between the openings of the midgut and the style sac
LDC	region of the left wall of the stomach where openings of the ducts of the		

Note: Figures showing the interior of the stomach have been made after opening it by a dorsal and posterior longitudinal incision running from the opening of the oesophagus to that of the midgut. The terms left and right refer to this median line. Arrows in figures show direction of ciliary currents.

by the inward projection of 4 longitudinal ridges from the circumference. Graham's figure of the stomach of *N. hanleyi* shows 4 folds at the opening of the oesophagus into the stomach. Immediately below the oesophageal aperture are the openings of 2 ducts, (DDD). These are placed at opposite ends of a shallow oval depression in the anterior wall. To the left of the oesophagus is a narrow outpocketing of the stomach wall, which Owen calls the 'caecum' and which Purchon refers to as a hemispheric

pocket containing a sorting area (SAA). At the mouth of the sorting area, and to its left, is placed the left opening of the digestive diverticula (DLP').

Running from near the border of the anterior sorting area (SAA) towards the intestinal opening are a few parallel ridges and grooves, which constitute the 'rejection tract' (RT). The disposition of the typhlosoles inside the stomach is as follows: arising from the style sac (SS) and encircling the sorting area on the right wall (SA) in the form of a U-

ANISOMYARIA - I

shaped loop is a ridge (R), which seems to end on the right side, near the oesophageal aperture. This ridge has been described as the minor typhlosole by Graham, Owen and Purchon. However, careful examination shows that in *Nucula layardi* this ridge does not end near the oesophageal aperture, but runs through a shallow depression in the stomach there, which has been described as another sorting area by Purchon (1956a: Fig. 2, SA'). The ridge emerges from this depression and curves upwards and to the left, to run above the oesophageal opening into the dorsal hood (DH). Thus the ridge described as the minor typhlosole and that running into the dorsal hood (termed the 'right fold' by Owen (1956) and the 'longitudinal ridge' by Purchon (1956a) are continuous and one in *N. layardi*. Dr. Owen (personal communication) has confirmed that this is also true for *N. sulcata*. Thus, if the whole ridge is regarded as the minor typhlosole it would represent a unique modification in the Nucleidae. However, at the junction of the stomach proper with the style sac, the 'minor typhlosole' is interrupted by a ridge which divides these 2 regions of the stomach. It is therefore possible that the minor typhlosole (TYM) terminates within the style sac itself, and that the ridge extending into the stomach is part of the fold arising from the dorsal hood.

Similarly, it is possible that the major typhlosole (TY) does not extend into the stomach. The ridge which has been identified as an extension of the major typhlosole (Graham, 1949; Owen, 1956) into the stomach appears to be part of another fold, the 'left fold' of Owen (1956), which arises above the left duct opening, a counterpart of which has also been indicated by Graham (1949). However, the fold does not enter the dorsal hood, but arches over the roof of the stomach and ends near the oesophageal opening. It is possible that this fold is homologous with another fold which forms a dominant structural feature in the filibranch stomach (see below, *axial fold*).

The stomachs of 15 species belonging to 7 families of Anisomyaria, which share certain distinctive features, have been considered together in this section. One of the most characteristic of these is the presence of a fold which forms an important structural element in the stomach. The identity of this fold has not been fully recognised, nor has it been adequately described, in earlier descriptions of the stomach (Graham, 1949; Purchon, 1957). The fold, having a broad or narrow base, arises from the floor of the stomach, between the major typhlosole and the left pouch. It forms a major portion of the left wall of the stomach, and extends in the form of a ridge across the roof to terminate anteriorly on the right wall, near the oesophageal opening. The major typhlosole accompanies the fold along a greater or lesser part of its course, and marks the fold's morphologically anterior limit. The left pouch and the dorsal hood mark the posterior limits of the fold. This fold forms such a constant feature of the stomach in these families that the degree of its development and its relation to the major typhlosole constitute important factors influencing stomach form and structure in the different species within the order. This fold has been described as a portion of the left wall of the 'caecum' by Graham (1949), and as the 'fold forming the posterior wall of the caecum' by Purchon (1957). In view of the importance of the fold and its position in the stomach, it is here designated as the *axial fold*. The axial fold is present in all the Anisomyaria, though it is much reduced in the pseudolamellibranch families, Pectinidae, Anomiidae and Limidae. It also seems to be partly represented in the Protobranchia. Along the base of the axial fold, ciliary currents are powerful, and carry particles towards the dorsal hood and the left pouch. This portion of the axial fold has been described as a sorting area by Purchon (1957: SA²), but there is only one type

of current in this area.

ARCIDAE

Arca concamera Bruguière, Fig. 2

A. inaequalvia Bruguière

A. rhombea Born

The structure of the stomach in these 3 species generally resembles that of *Anadara granosa* described by Purchon (1957). They were examined in detail to estimate the limits of structural variation within a genus. The stomach of *A. concamera* is figured (Fig. 2), and the areas and folds labelled according to the terminology followed in the present study. The distal portion of the major typhlosole marks the posterior boundary of a grooved area extending over the ventral and left wall of the stomach. This area, described as the food sorting caecum or its extension by Purchon (1957) is termed the sorting area (SA) here, though the portion of the sorting area anterior to the axial fold may be regarded as an extension of the food sorting caecum (FSC') (see remarks on 'food sorting caecum' under Mytilidae). The axial fold (AXF) is very prominent and arises near the left wall of the stomach. The major typhlosole (TY) lies in front of this fold and accompanies it over the roof of the stomach to terminate (TYT) in a triangular pocket on the anterior right wall, close to the oesophageal opening. The ducts of the digestive diverticula open individually but in 4 groups, below the oesophageal aperture, at the junction of the sorting area with a smooth area of the anterior wall (DDR, DDD). Each group consists of 4 main ducts, and thick ridges of the stomach wall run into the ducts.

The left pouch (LP) is placed immediately above the common opening of the midgut (MG) and style sac (SS), and a faint groove with a ridge alongside runs from the pouch across the base of the axial fold and joins the intestinal groove (GIA) accompanying the major

typhlosole. The left pouch contains 2 recesses, into each of which open 2 main ducts that penetrate the mass of the digestive diverticula on the left side. The gastric shield (GS) lying above the left pouch, skirts the dorsal and posterior boundary of the left pouch. The dorsal hood (DH) lies above the gastric shield in the form of a cup-shaped depression, and its left wall bears a few ridges (R) running into the depths of the hood. The shield has 2 thickened projections which invade the posterior and ventral sides of the hood.

In *Anadara inaequalvia*, the area around the common opening of the midgut and the style sac shows a few folds which converge on the small lobe of the minor typhlosole and lead into the midgut. The left pouch is a deeper recess than it is in *A. concamera*, and the axial fold is better developed. The axial fold, accompanied by the major typhlosole, is thrust into a triangular pocket on the anterior right wall. The stomach of *A. rhombea* resembles that of *A. inaequalvia* more closely than that of *A. concamera*. The area around the left pouch is slightly modified: the ventral wall of the pouch bears a series of grooves, which join and run in the form of a channel to join the intestinal groove. The sorting area is not crimped at its junction with the smooth area of the wall below the oesophageal aperture, and the ducts, 24 in number, open in a linear series along the sorting area.

The course of ciliary currents is similar to that of *Anadara granosa* (Purchon, 1957).

Limopsidae

Limopsis belcheri (Adams & Reeve)

Fig. 3

Purchon (1957) has indicated the main features of the stomach of *Limopsis vaginatus* without giving a figure. The single specimen of the present species, obtained from a dredge collection, had not been ideally preserved and was rather brittle when dissected. Yet it

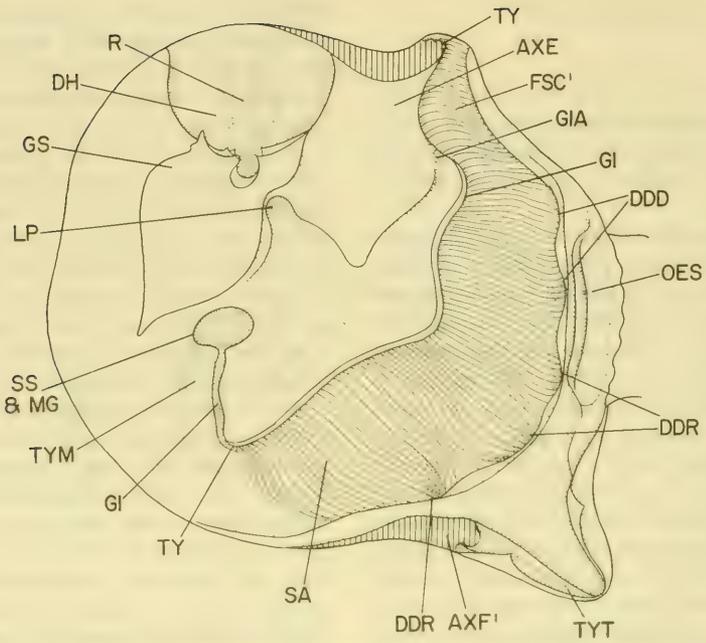


FIG. 2. Interior of the stomach of *Arca concamera*. (For lettering, see p 229)

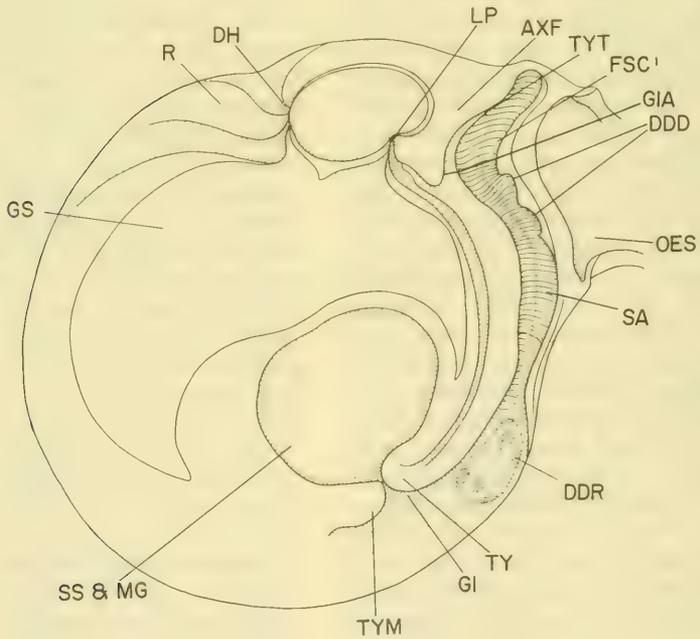


FIG. 3. Interior of the stomach of *Limopsis belcheri*. (For lettering, see p 229)

was possible to make out the general disposition of the parts of the stomach, and since these observations agree broadly with Purchon's description, a figure is also given (Fig. 3).

The anterior chamber of the stomach into which the oesophagus (OES) opens is not as spacious as has been described in *Limopsis vaginatus*, since the posterior chamber of the stomach appears to be pushed forwards, and forms the most conspicuous region of the stomach. The sorting area (SA) extends transversely in the anterior part in the form of a narrow strip. The common opening of the midgut (MG) and the style sac (SS) is large and is placed almost in the middle of the floor of the stomach. The minor typhlosole (TYM) projects as a small lobe from the common opening towards the right. The major typhlosole (TY), after veering to the right from the midgut, bends back sharply and runs along the anterior left wall. Since the wall of the stomach in the neighbourhood of the left pouch was not intact, it was not possible to determine how the typhlosole is related to the grooves and ridges of the pouch. However, the disposition of the typhlosole seems to be as shown in Fig. 3, and since this arrangement resembles the condition in *Glycymeris violacescens* (Purchon, 1957), it is of interest.

The dorsal hood (DH) is fairly deep, and is bounded by 2 broad ridges (R), one anteriorly and the other posteriorly, the anterior ridge being part of the axial fold (AXF) which is not well developed in this species. The gastric shield (GS) is large and the 2 prongs of the shield are thrust deep into the dorsal hood and the left pouch (LP), and embrace a thick ridge between these structures. The openings of the ducts of the digestive diverticula are, as far as could be ascertained, distributed as shown in the figure: the depression on the right wall, placed immediately right of the sharp bend of the typhlosole, is particularly large and seems to accommodate the openings of 4 main ducts (DDR). The 2

groups of ducts on the left side mentioned by Purchon (1957) in *Limopsis vaginatus* seem to open in this species just below the oesophageal aperture (DDD). Another peculiar feature of the stomach is that the walls around the gastric shield as well as the lining of the main ducts are coated with a dark brown pigment.

Mytilidae

The terms 'caecum' and 'food sorting caecum' have been used to denote the same structure in the Bivalvia (Graham, 1949), though it seems desirable to restrict the term 'food sorting caecum' to denote the outpocketing on the ventral and left side of the stomach, placed morphologically anterior and ventral to the axial fold, into which the major typhlosole sends a U-shaped limb. It is homologous with the extension of the sorting area anterior to the axial fold, as described in forms such as *Arca*, but in the Mytilidae particularly, the food sorting caecum is vastly modified, as shown in the examples described below. It is possible that the food sorting caecum acts more as a temporary reservoir of food than as a sorting caecum since, in specimens of *Perna viridis* starved for a day or two, the normal accumulation of food particles seen in the food sorting caecum disappears. Within the food sorting caecum, the cilia on the major typhlosole and the grooves work more or less in the manner described for *Modiolus modiolus* by Nelson (1918), the general trend being to direct particles into the main intestinal groove.

Perna viridis (Linnaeus)

Figs. 4a, b

There is broad agreement in general features with that of the stomach of *Mytilus edulis* described by Graham (1949); but the food sorting caecum, the arrangement and distribution of the ducts of the digestive diverticula and the configuration of the walls appear to be different in the present species.

The major typhlosole (Fig. 4a, TY)

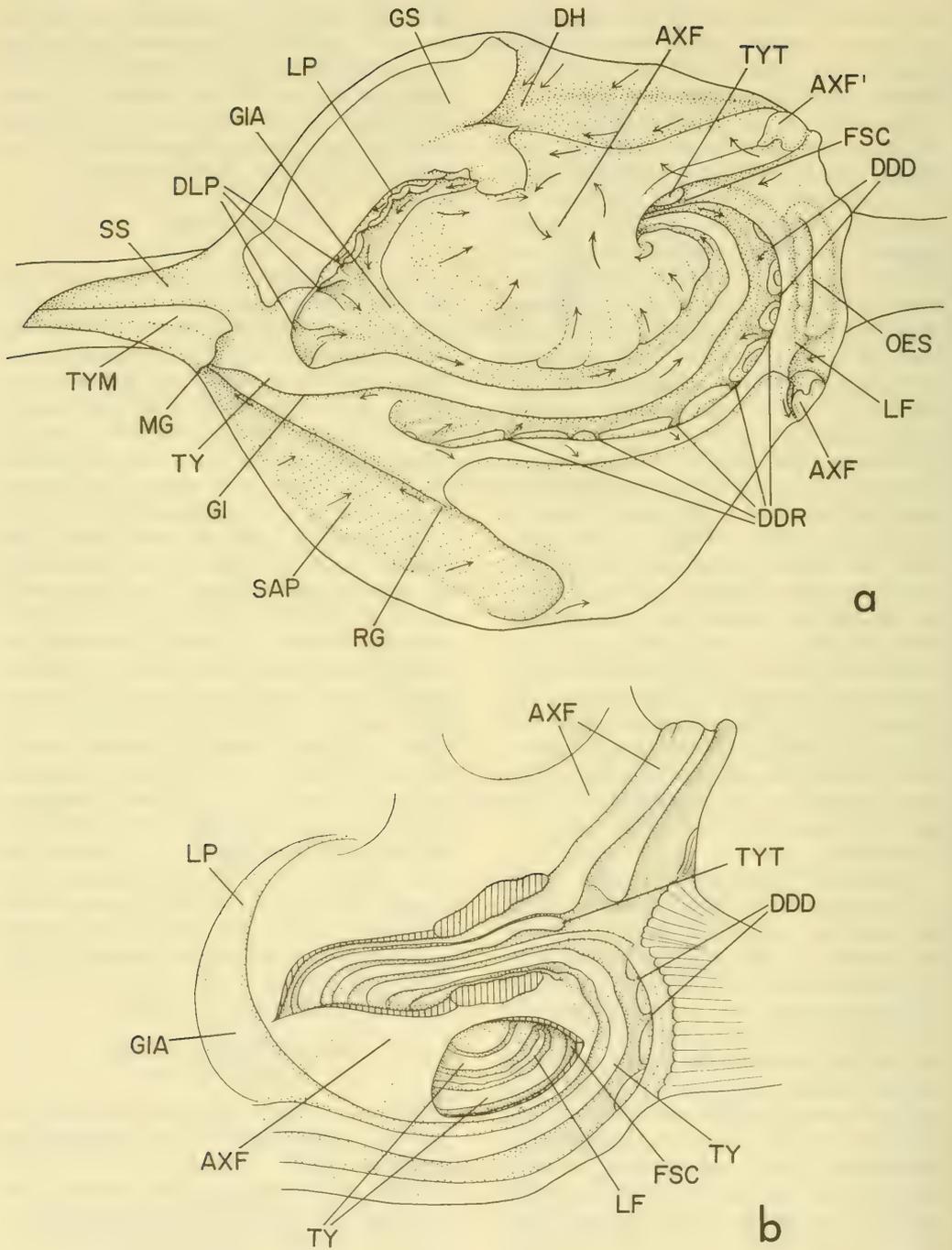


FIG. 4. Stomach of *Perna viridis*. a, interior view; b, stomach opened behind the region of dorsal hood and viewed posteriorly to show opening of oesophagus. (For lettering, see p 229)

emerges from the midgut (MG) and runs anteriorly over the floor of the stomach; it then turns left to run into the food sorting caecum (FSC). The food sorting caecum is a blind outpushing of the floor of the stomach in the form of a closely wound planispiral consisting of nearly 2 turns (see Fig. 4b). The major typhlosole runs through the spirals of the food sorting caecum up to its tip (the descending limb), and then doubles back and retraces its course (ascending limb) to the stomach proper. It emerges from the food sorting caecum above the descending limb and terminates (TYT) on the left wall close to the side of the oesophageal opening. Graham's figure of the caecum of *Mytilus edulis* is different in that the typhlosole is shown as ending within the caecum.

The main intestinal groove (GI) has its origin (GIA) near the left pouch (LP); it follows the major typhlosole into and out of the food sorting caecum, and eventually runs into the midgut. A flat striated fold commences at the tip of the food sorting caecum, bounded on either side by the 2 limbs of the typhlosole. It runs through the spirals of the food sorting caecum and, after emerging, turns right and runs as a broad shelf immediately below the oesophageal opening; it then turns backwards and runs parallel to the major typhlosole over the floor of the stomach. This fold, termed here the lateral fold (LF), appears to be similar to the 'fold' described by Graham (1949) in *Mytilus edulis*, though in *P. viridis* the 10 ducts of the digestive diverticula of the right side open (DDD, DDR) along the side of the fold facing the major typhlosole.

To the right of the lateral fold is the posterior sorting area (SAP), which occupies a pocket-like vestibule on the right dorsal wall, but does not extend into the dorsal hood. The posterior sorting area is limited anteriorly by a groove (RG), which seems to correspond to the rejection groove described in some Eulamellibranchia (Owen, 1953; Dinamani, 1957) since it drains the sorting

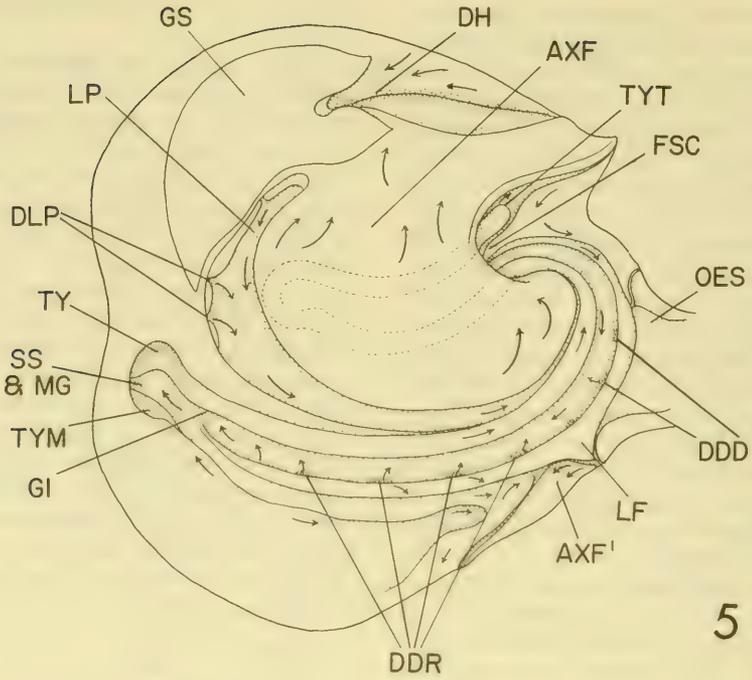
area and conveys particles into the midgut.

The ducts of the digestive diverticula of the left side (DLP) open in a shallow area below the gastric shield (GS). Small grooves lead from the openings of the ducts and join to form the beginnings of the intestinal groove (GIA). This area corresponds to the left pouch (LP); Purchon (1957) regards a similar region in *Lithophaga nasuta* as possibly constituting the left pouch. The axial fold (AXF) arises from the floor of the stomach above the food sorting caecum. Its basic structure and relationship to the food sorting caecum is shown in the stereogram (Fig. 4b). The axial fold ends (AXF') on the right wall and since the major typhlosole is folded into the food sorting caecum, the typhlosole does not accompany the axial fold all the way as in *Arca*.

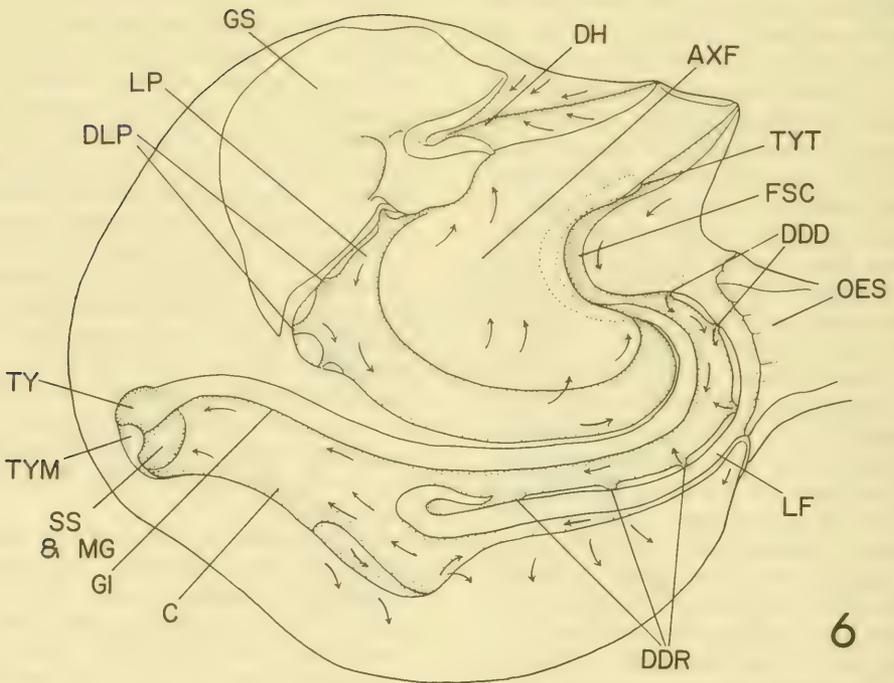
Ciliary Currents:

All particles entering the stomach through the wide oesophagus first come into contact with the portion of the axial fold that arches across the roof of the stomach opposite to the opening of the oesophagus. Along the whole surface of the axial fold strong ciliary currents move upwards and towards the left. At the point where the axial fold terminates on the right wall, a groove runs round from the tip of the fold as a well-defined channel along the posterior boundary of the axial fold into the dorsal hood. Most of the particles which come into contact with the anterior part of the axial fold are fed into this channel, and are thus passed on to the dorsal hood; those particles that are deflected towards the left wall escape into the food sorting caecum.

Along the broad base of the axial fold currents are stronger and converge towards the dorsal hood and the left pouch. These currents meet with those carrying particles via the groove accompanying the axial fold mentioned above, and where the 2 currents meet a small eddy-like effect is seen. The



5



6

FIG. 5 & 6. Stomach in the Mytilidae. Interior of the stomach of: 5. *Modiolus undulatus*; 6. *Modiolus striatulus*. (For lettering, see p 229)

crystalline style normally projects into the stomach up to the point where these currents meet and where particles entering the stomach accumulate. As the style rotates, these particles are wound in the form of a string around its tip.

In the region of the left pouch 2 types of currents are present: above the openings of the ducts currents are directed backwards, while in the shallow basin of the left pouch, particles move towards the intestinal groove to the left of the major typhlosole. These particles enter the food sorting caecum. Particles accumulating thus in the food sorting caecum pass out of it along the walls of the food sorting caecum as well as along the limbs of the major typhlosole (via the intestinal groove) and the lateral fold. The walls of the food sorting caecum, as shown in the stereogram, are actually formed by the basal walls of the axial fold and the same pattern of currents exists here; particles from the food sorting caecum are brought up by these currents along the axial fold to the region of the dorsal hood and tip of the style. The region of the food sorting caecum between the ascending and descending limbs of the typhlosole, as well as the area of the stomach floor where 10 ducts open, is lined by faint grooves and ridges. In this whole area, the action of the cilia keeps the particles out of the ducts and moving towards the intestinal groove. Along the fold of the major typhlosole cilia beat forwards or laterally so that particles are carried either along the typhlosole or into the intestinal groove on either side.

Modiolus undulatus (Dunker), Fig. 5

Modiolus striatulus (Hanley), Fig. 6

Arcuatula sp., Fig. 7

Lithophaga gracilis (Philippi), Fig. 8

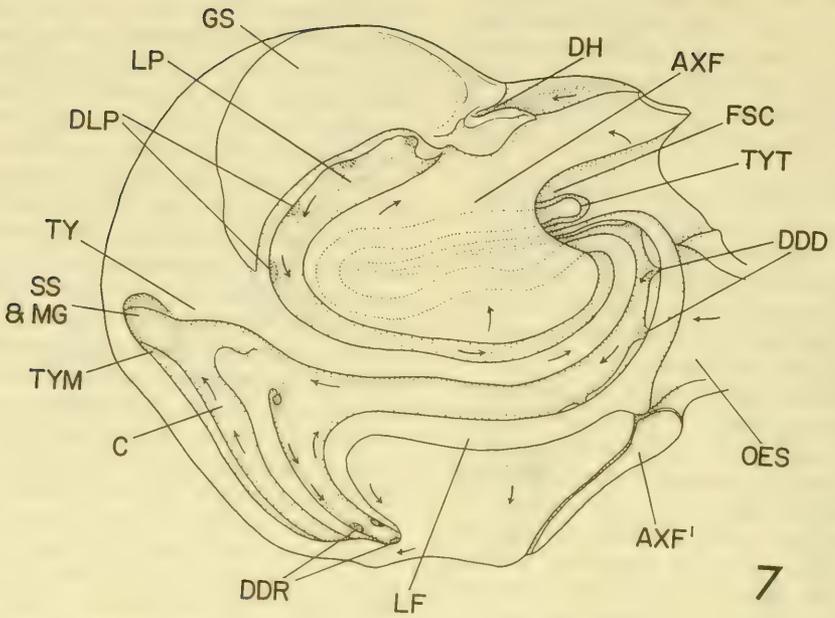
Botula cinnamomea (Lamarck)

Stomach structure in other Mytilidae remains remarkably constant in the different genera and species, and broadly resembles that of *Perna viridis*; the various examples figured and described

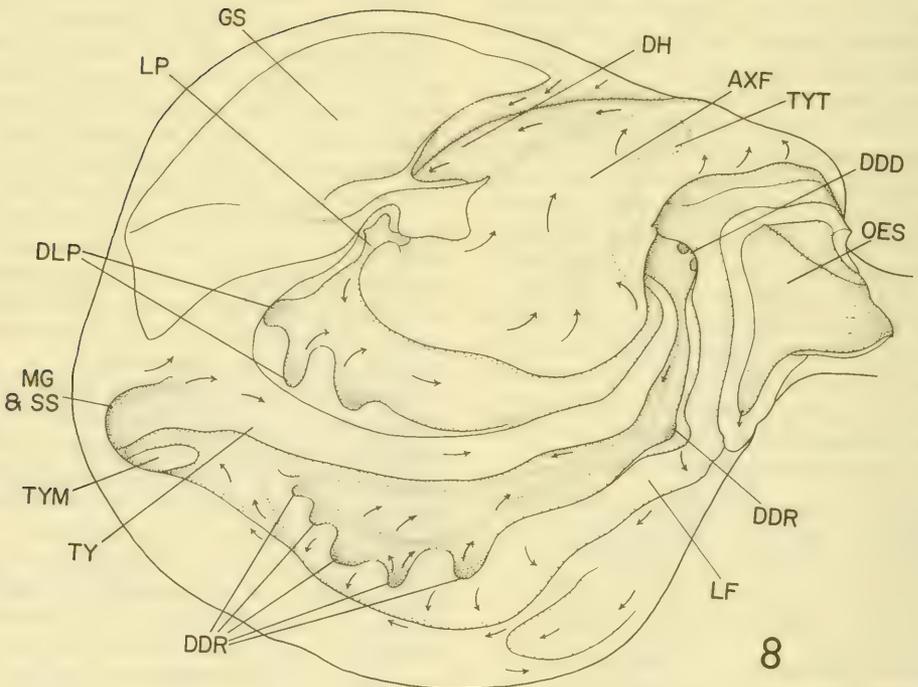
in the present study serve to illustrate the nature of variation in the form of the axial fold and the food sorting caecum. Regarding other Mytilidae, Nelson (1918) has figured the stomach of *Modiolus modiolus*, and Purchon (1957) has described that of *Lithophaga nasuta*.

In both species of *Modiolus* examined in the present study, the axial fold is prominent and the major typhlosole (TY) is intimately associated with it. In *M. undulatus* (Fig. 5) the major typhlosole descends into the food sorting caecum (FSC) to the left of the axial fold (AXF), bends back on itself and returns parallel to and above the descending limb. It thus forms a simple loop inside the food sorting caecum with its tip (TYT) facing the oesophageal opening (OES). In *M. striatulus* (Fig. 6), the food sorting caecum is not so well developed, and the major typhlosole is in the form of a drawn-out 'S' inside the food sorting caecum. In this species, the major typhlosole ends (TYT) a bit higher up on the left wall. The course of the major typhlosole in *M. modiolus* described by Nelson (1918) appears to be similar to that of *M. undulatus*, though Nelson has described the major typhlosole as ending within the 'caecum'. In *Arcuatula* sp. (Fig. 7) the form of the major typhlosole is broadly similar to that of *M. undulatus*. However, in *Arcuatula* sp., the 2 limbs of the typhlosole are disposed in the same plane (horizontal), and not vertically one above the other as in *M. undulatus*.

The ducts of the right side (DDR) in the modiolids are also associated with a lateral fold (LF), which originates, as in *Perna viridis*, in the food sorting caecum and follows the contours of the stomach parallel to the major typhlosole. The slight differences in the form of the lateral fold and the manner of opening of the ducts along the fold between *Modiolus* spp. and *Arcuatula* sp. are indicated in the respective figures. In both species of *Modiolus*, there is no posterior sorting area though there seems to be a small outpushing on the



7



8

FIG. 7 & 8. Stomach in the Mytilidae. Interior of the stomach of: 7. *Arcuatula* sp.; 8. *Lithophaga gracilis*. (For lettering, see p 229)

right wall. In *M. undulatus* it is in the form of a shallow depression; in *M. striatulus* it is more pronounced, and in *Arcuatula* there is a channel-like area (C) extending from the midgut into which the minor typhlosole (TYM) also extends.

In *Lithophaga gracilis* (Fig. 8) and *Botula cinnamomea*, the axial fold is well developed, particularly in the latter where the fold is more extensive and terminates near the oesophageal aperture. Purchon (1957) observes that in *Lithophaga nasuta* the major typhlosole "descends towards the apex of the food sorting caecum", and that the caecum itself projects downwards below the stomach. However, in both *L. gracilis* and *B. cinnamomea*, the arrangement of these structures is different. In *L. gracilis* the major typhlosole (TY) enters a depression of the wall to the left of the axial fold (AXF), ascends the anterior left wall where it ends (TYT). In *B. cinnamomea*, the major typhlosole accompanies the axial fold almost up to its termination on the right side. In both these species therefore, the food sorting caecum is not in the form of a downward outpushing of the stomach wall ventrally, nor is it a pronounced depression to the left of the axial fold as in other mytilids described earlier.

Unlike *L. gracilis* the major typhlosole of *B. cinnamomea* sends a small U-shaped limb into the region of the food sorting caecum.

About 8 or 9 ducts are distributed on the right side in both these species along the lateral fold. In *Botula cinnamomea*, the ducts are arranged at regular intervals along the lateral fold, but in *Lithophaga gracilis* the ducts are arranged in 3 groups (DDD, DDR). The lateral fold in *B. cinnamomea* tapers posteriorly, while in *L. gracilis* the fold embraces a cup-shaped depression posteriorly, where 6 ducts open. Between the terminal part of the lateral fold and the major typhlosole there is another flat ridge bearing minute grooves, which in *L. nasuta* Purchon has referred to as a sorting area (Purchon,

1957: Fig. 7, SA³). Purchon has compared this area to the posterior sorting area of Graham (1949).

Ciliary currents are indicated in Figs. 5, 6, 7 and 8, and it may be observed that the general pattern is very similar to that of *Perna viridis*.

Isognomonidae (= Vulsellidae)

Isognomon nucleus (Lamarck)

Fig. 9

The stomach, fashioned on the mytilid pattern, resembles that of *Malleus albus* described by Purchon (1957). The axial fold (AXF) is conspicuous, though the food sorting caecum does not seem to be well developed. The major typhlosole (TY) turns sharply into a pocket of the stomach wall, to the left of the common openings of the midgut and the style sac. It skirts the dorsal wall of this pocket and then extends forwards in the form of a thin pleat around the base of the axial fold. Anteriorly it curves towards the left and enters a shallow depression, which may be considered as the region of the food sorting caecum (FSC). However, unlike the food sorting caecum of the Mytilidae, some ducts of the digestive diverticula open (DDR) very close to it. The major typhlosole (TYT) ends on the anterior left wall in a groove along the axial fold. Purchon (1957) after describing a more or less similar course for the major typhlosole in *M. albus*, observes that a break occurs in the typhlosole inside a pocket ('E²') on the left wall. Such a break was not observed in the present species in the pocket on the left wall. Into this pocket-like area open 5 ducts of the digestive diverticula, and 6 more ducts (DDR) open along the ventral wall of the stomach. The ducts are placed along a fold which runs into this area from the region of the food sorting caecum, and which therefore corresponds to the lateral fold (LF). There is a groove external to the lateral fold which resembles a similar groove described in *M. albus*.

Ciliary currents are similar to those

of the Mytilidae and are indicated in Fig. 9. In the pocket-like region on the left wall into which the major typhlosole turns, ciliary currents are directed outwards, though along the posterior wall behind the pocket, cilia direct particles into the pocket.

Pteriidae

Pinctada anomoides (Reeve)

A detailed description of the stomach of *Pinctada vulgaris* is given by Purchon (1957). The axial fold is prominent and rises typically from the middle of the floor of the stomach, arching over the roof to end slightly on the right side. The major typhlosole ends on the left wall in an area, which may be described as an extension of the food sorting caecum.

In *Pinctada anomoides*, the dorsal hood is more extensive than in *P. vulgaris*, and the ridge on its posterior wall is well developed. The left pouch is a shallow area on the left posterior wall. The ducts of the right side are distributed at 4 centres, which roughly correspond to the regions marked 'E²', 'DDD²' and 'DDD¹' by Purchon (1957: fig. 6) in *P. vulgaris*. As in the Mytilidae, there is a lateral fold arising in the left wall and running parallel to the major typhlosole. The lateral fold ends posteriorly close to a group of ducts which open near the midgut.

Pinnidae

Pinna bicolor Gmelin

Fig. 10

The stomachs of *Pinna atropurpurea* and *Atrina vexillum* have been described and figured by Purchon (1957). In general, the stomach of *P. bicolor* is similar to that of *P. atropurpurea*. The axial fold (AXF) is well developed in both species, though in *P. bicolor* it pushes deeper into the right wall. As a result of this, the sorting area in *P. bicolor* extends in the form of a deep pocket (FSC') in front of the axial fold

on the right wall. The right wall bears a series of ridges which converge dorsally to a point towards the right of the dorsal hood (DH). The major typhlosole (TY) runs into an embayment of the stomach wall on the left immediately after it emerges from the midgut (MG). Behind the axial fold, there is a small ridge (R) which also runs into the dorsal hood. In both the species, 2 large embayments are found in the region corresponding to the left pouch (LP) of other species. Three or 4 large ducts open into each of these embayments; and into each large duct open as many small ducts from the digestive diverticula of the left side. Similarly, into a depression on the right wall, 3 large ducts (DDR) open, into each of which open 3 or 4 smaller ducts that penetrate the mass of the digestive diverticula of the right side.

Ostreidae

Ostrea (Crassostrea) madrasensis

Preston

Ostrea forskalii Gmelin, Fig. 11

The stomach of *Ostrea edulis* was first described by Yonge (1926) and later by Graham (1949), while Purchon (1957) has described the stomach of *O. parasitica*. The stomach of *O. madrasensis* is broadly similar to that of both these species, while that of *O. forskalii* resembles more that of *O. parasitica*. In the description given below, the course and arrangement of the major typhlosole and its relation to the axial fold are emphasized.

In *Ostrea madrasensis*, the axial fold arises centrally from the floor of the stomach and extends beyond the tip of the major typhlosole on to the right wall. The base of the axial fold shows faint ridges, which in *O. parasitica*, Purchon (1957) has referred to as a sorting area ('SA²'). The major typhlosole, after emerging from the midgut, skirts the base of the axial fold and, along its course dips into 2 depressions and enters a pocket in the wall anterior to the axial fold, where it forms a U-shaped loop.

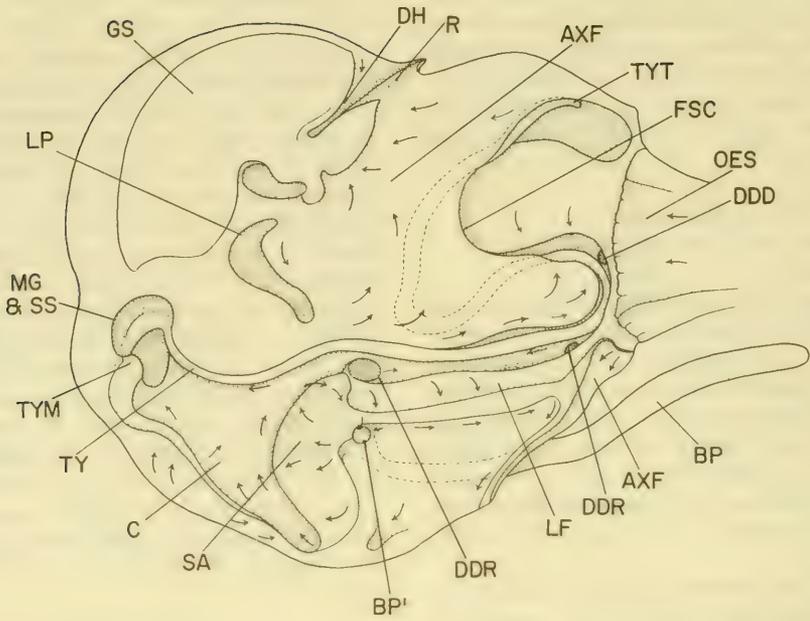


FIG. 11. Interior of the stomach of *Ostrea forskalii*. (For lettering, see p 229)

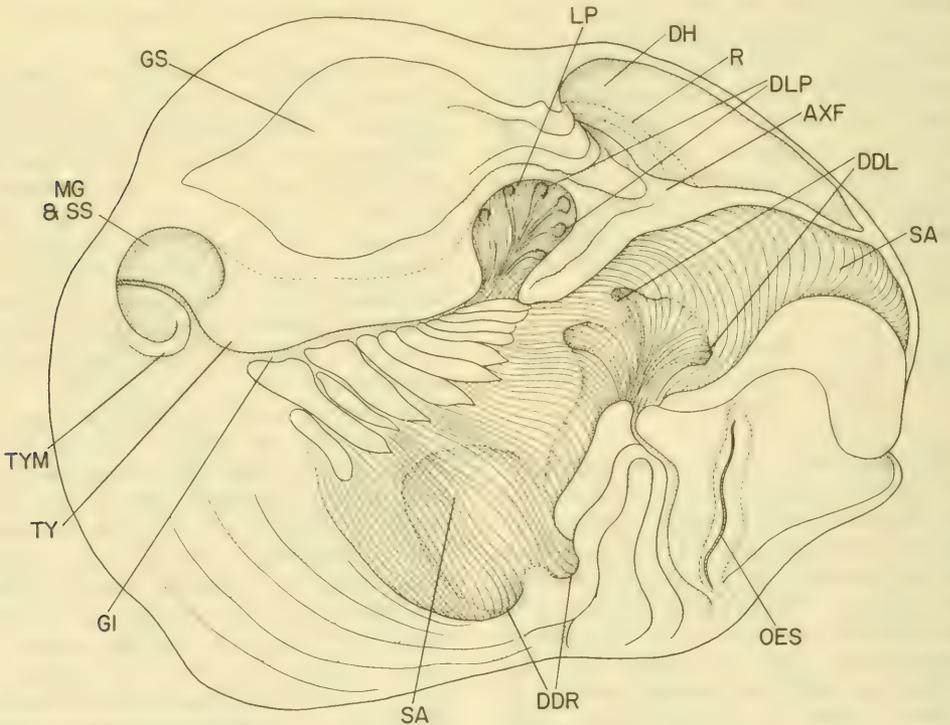


FIG. 12. Interior of the stomach of *Pecten crassicostatus*. (For lettering, see p 229)

The distal end of the major typhlosole then runs across the roof alongside the axial fold and ends on the right wall close to the oesophageal opening. In *O. forskalii* (Fig. 11), the major typhlosole (TY) does not accompany the axial fold (AXF) to the opening of the oesophagus but ends (TYT) dorso-laterally.

In *Ostrea madrasensis*, the ducts of the digestive diverticula are arranged in 3 or 4 groups along the course of the major typhlosole and to its right. Altogether about 15 ducts open in this manner. The wall to the right of the opening of the midgut forms a channel which runs laterally and ends blindly near the dorsal hood. This channel seems to correspond to the 'hollow' and rejection tract in *O. parasitica* (Purchon, 1957). In *O. forskalii*, the openings of the ducts of the right side are arranged in 3 groups on the ventral wall close to a fold, which corresponds to the lateral fold (LF) of the Mytilidae. Of these 3 groups of openings, the 2 lateral groups (DDR) correspond to the embayments of the stomach wall described in *O. parasitica* as 'E³' and 'E²'. The third group (DDD) is situated more towards the left, where the typhlosole dips into the food sorting caecum (FSC). Of the lateral groups of openings, the posterior one contains 3 large ducts, one of which leads into 8 or 9 ducts. In *O. forskalii*, there is a channel (C) on the posterior right wall which is more extensive than in *O. madrasensis*. The minor typhlosole (TYM) runs into this channel along its posterior boundary, as in *Arcuatula* sp., while the wall anterior to the channel rises prominently and bears numerous parallel grooves. Considering the nature of the ciliary action on it, this grooved area corresponds to an incipient sorting area (SA).

In *Ostrea madrasensis*, the left pouch lies beneath its gastric shield. It is a shallow incurving area in which are 4 main openings of the ducts of the left side. The posterior wall of the pouch is in the form of a high ledge which reaches up to the opening of the style

sac. In *O. forskalii*, there are 2 recesses in the stomach wall, of which only the basin-like lower recess (LP) contains the openings of 4 large ducts. In *O. parasitica*, where the configuration of the left wall is similar, Purchon (1957) considers both the concavities as parts of the left pouch.

In *Ostrea forskalii*, the dorsal hood (DH) bears a thin ridge (R) anteriorly, and the wall of the hood anterior to the ridge shows faint grooves and folds. This area seems to be similar to the sorting area, which in many Eulamellibranchia, extends into the dorsal hood. On the right wall in *O. forskalii*, near the posterior-most opening of the digestive diverticula, and approximately in the same position in which an isolated duct opening ('DDD¹') is shown in *O. parasitica* by Purchon (1957), there is a funnel-shaped aperture (BP'). This aperture leads into a narrow elongated pouch² (BP) which is directed anteriorly. In a specimen in which the stomach is 6 mm long, the pouch measures about 4 mm and has a diameter of 0.75 mm. The pouch has thin walls and its lining is thrown into longitudinal ridges. These are continuations of the ridges around the funnel-like openings, and appear to carry stiff cilia. In freshly collected animals, superfine particles are found inside the pouch.

Ciliary currents:

In both the species, particles entering the stomach move to the vicinity of the dorsal hood across the axial fold. From the base of the axial fold movement of particles is upwards, and they seem to be borne dorsally on a mucous sheet; mucus is secreted profusely in the basal region of the axial fold. On the floor of the stomach, and around the openings of the ducts on the right side, particles are wafted away laterally, particularly

²In a recent thesis, Reid (1964) reports a comparable pouch in *Lima hians*, *Ostrea edulis* and *Mytilus edulis*. ED.

over the surface of the lateral fold. In *Ostrea forskalii* many particles find their way from the right wall into the blind pouch (BP): thus, during the study of ciliary currents in this species, specimens of which were sexually mature, gonadial products from around the dissected stomach (and let into the stomach in small quantities for current studies), were later found in the blind pouch! In the grooved area (SA) on the right wall in *O. forskalii*, particles are directed backwards into the channel (C) leading into the midgut, though some particles move upwards and anteriorly towards the opening of the blind pouch (BP'). Particles from the posterior wall and those rejected from the hood also move into the channel (C), which thus serves as a wide rejection tract.

ANISOMYARIA - II

The stomach of the Pectinidae, Anomidae and Limidae differs from other Anisomyaria in 2 chief features: a) the position of the left pouch and b) the relation of the major typhlosole to it. The left pouch in the stomach of these forms is found anterior to the major typhlosole, and the typhlosole itself is in the form of a faint ridge extending between the opening of the midgut and the posterior margin of the pouch. In addition, the axial fold is not conspicuous in all these stomachs and the ducts of the digestive diverticula tend to be distributed in groups on the floor of the stomach.

Pectinidae

Pecten crassicosatus Sowerby

Fig. 12

The stomach of *Pecten maximus* has been described and figured by Graham (1949), while Purchon (1957) has described the stomach of *Spondylus hystrix*. The stomach of the present species is similar to that of *P. maximus*, and also shows resemblance to that of *Lima fragilis* (Purchon, 1957).

The oesophagus (OES) opens into the stomach antero-dorsally on a raised ridge. The midgut (MG) and the style sac (SS) open together, and the minor typhlosole (TYM) turns to the right in the form of a rim over the common opening. The major typhlosole (TY) emerges in the form of a wide ribbon that is more or less flush with the stomach floor and turns left into the left pouch (LP), where it terminates at the mouth of the nearest duct. The left pouch is an oval pocket in the middle of the left wall where 5 or 6 ducts of the digestive diverticula (DLP) open. Numerous small grooves and ridges line the floor of the pouch and converge towards its opening into the stomach. The shallow intestinal groove (GI) runs from the left pouch to the midgut on the anterior and right side of the major typhlosole.

The anterior wall of the dorsal hood (DH) carries a broad ridge (R) which bears fine grooves, and beyond the ridge is a thin fold of the stomach wall (AXF), which separates the dorsal hood from a grooved sorting area (SA) anteriorly. Graham (1949) describes this fold as the 'left caecal wall' in *Pecten maximus* and Purchon (1957) refers to it, in part, as the 'process separating the (sorting) area from the intestinal groove' in *Spondylus hystrix*. The fold extends ventrally up to the mouth of the left pouch, and appears to be a counterpart of the axial fold described earlier. It is not so well developed, but it extends up the left anterior wall and across the roof to a point above the oesophageal aperture. There is of course no fold of the major typhlosole anterior to the axial fold here.

The sorting area (SA) extends over the greater part of the floor of the stomach anterior to the major typhlosole and the axial fold. It invades the anterior left wall, crossing over the roof to end above the oesophageal aperture. It is limited anteriorly by an area of smooth ridges and pleats around the oesophageal opening. This grooved area is referred to

as the 'food sorting caecum' and 'sorting area of the food sorting caecum' by Purchon (1957) in *Spondylus hystrix*. The typhlosole, however, does not extend into this area; the sorting area may be divided into an anterior and a posterior region, the line of demarcation following the same course as that between 'SAC' and 'SAP' in *Pecten maximus* (Graham, 1949). Extending from the mouth of the left pouch to the midgut, there is a series of broad ridges placed at right angles to the major typhlosole. Beyond these ridges, the floor of the stomach inclines slightly and is marked by finer grooves and ridges.

Anomiidae

Placenta placenta (Linnaeus)

The stomach of this species has been fully described and figured as *Placuna placenta* by Purchon (1957), and the following note is added only to fix the identity of the minor typhlosole: Purchon has shown the minor typhlosole to be within the midgut and to the right of the major typhlosole. However, the midgut and the style sac are separate; and a small ridge, defined by a groove external to it, extends the length of the style sac along its line of separation from the midgut. This may be regarded as the minor typhlosole, placed as it is along the line of fusion of the style sac with the midgut.

The extension of the major typhlosole into the stomach is in the form of a very thin ribbon which curves immediately to the left and ends at the aperture of the nearest duct of the left pouch. This portion of the major typhlosole is quite insignificant and cannot be well differentiated from the floor of the stomach below the gastric shield.

EULAMELLIBRANCHIA

Unionidae

Lamellidens corrianus Lea

Fig. 13

The stomach of this species resembles

that of *Anodonta cygnea* described first by Graham (1949) and later by Purchon (1958), who, having found a close similarity between the stomachs of *A. cygnea* and *Hyridella australis*, observed that "the internal structure of the stomach is of high stability in the Unionacea". This is supported by the present studies on *Lamellidens corrianus*.

The oesophagus (OES) is a short, wide and much compressed tube which opens obliquely into the stomach. Its opening into the stomach is wide, and opposite the oesophageal aperture is a flat lobe on the anterior part of the stomach floor. It is clearly defined in fresh specimens, and is an area of intense ciliary activity, being provided with fine ridges and grooves. Purchon (1958), in *Anodonta cygnea*, has shown a few ridges in this region and has termed it a sorting area ('SA⁷'). The flat lobe continues laterally as a fairly broad fold over the right wall and runs across the roof into the dorsal hood (DH). This fold is also provided with fine grooves and ridges and, following the terminology adopted in this study, it is called the anterior fold (AF) (Owen, 1953; Dinamani, 1957). A fine groove, corresponding to the dorsal groove (DG), runs along the anterior fold into the dorsal hood.

The major typhlosole (TY) emerges from the common opening of the midgut and the style sac, adjacent to the minor typhlosole (TYM), and turns to the left in a wide arc, terminating just within the aperture of a common duct of the digestive diverticula on that side. Because of the short distance traversed, and the sweep it makes to the left after emerging from the midgut, the major typhlosole forms a prominent fold on the floor of the stomach near the combined opening of midgut and style sac. This corresponds to the 'conical mound' described by Purchon in *Anodonta cygnea*. Near the tip of the major typhlosole is a group of ducts opening close together through 2 large openings (LDC). The 2 openings lead off as duct-like extensions from the stomach into the substance of the digestive diver-

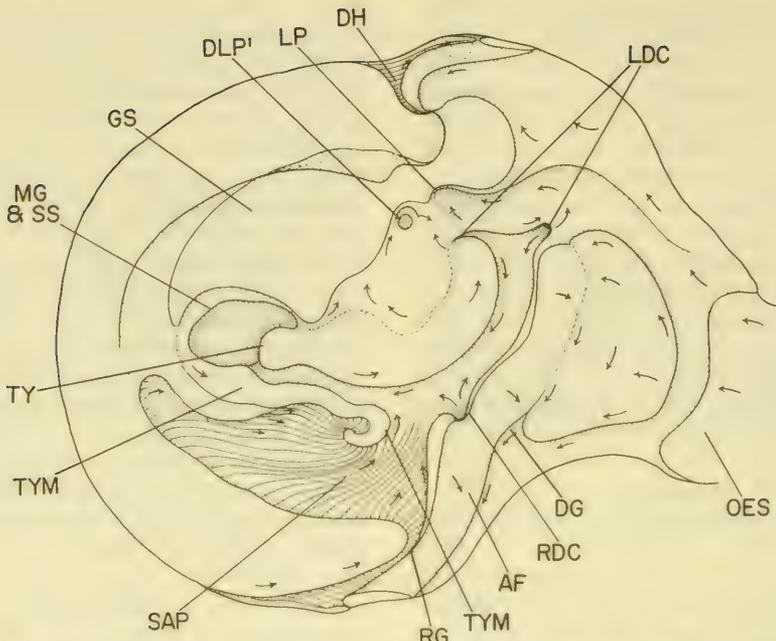


FIG. 13. Interior of the stomach of *Lamellidens corrianus*. (For lettering, see p 229)

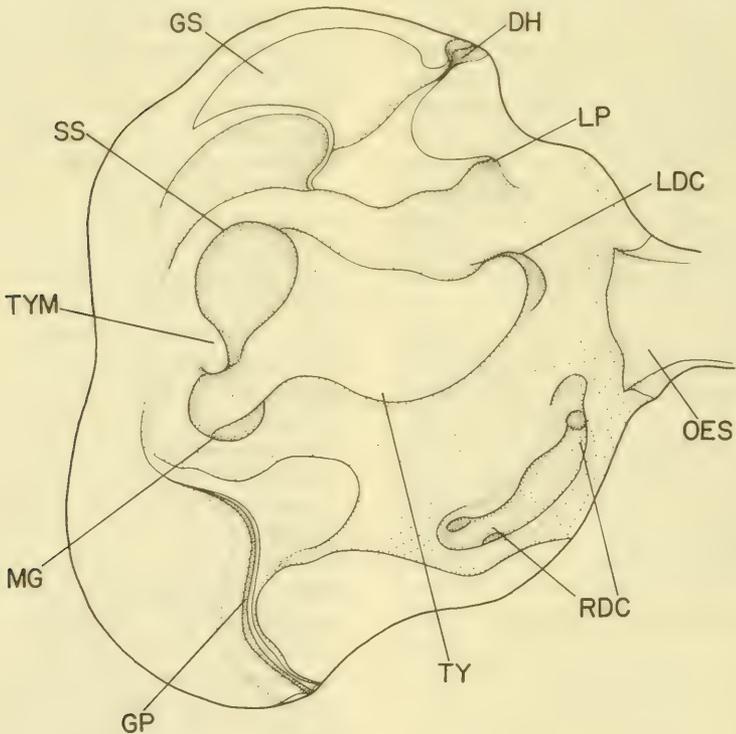


FIG. 14. Interior of the stomach of *Gastrochaena impressa*. (For lettering, see p 229)

ticula, and along their course numerous main ducts of the diverticula open.

Just above the region of the 2 large openings on the left side (LDC) there is a shallow depression in the left wall, which is skirted by the thickened border of the gastric shield (GS). Though a similar area is described as 'a broad shallow hollow' by Purchon (1958) in *Anodonta cygnea*, he does not consider it to be the left pouch; he describes another isolated opening ('ld') of the digestive diverticula, posterior to the above 'hollow', as the left pouch. It may be pointed out, however, that in other species such as *Ostrea parasitica*, *Neotrigonia margaritacea* and *Malleus albus*, in which there is a blind out-pushing below the gastric shield as well as a duct opening separately near it, Purchon (1957) has considered both as constituting the left pouch. The single isolated opening (DLP') in this area may or may not be regarded as part of the left pouch, though the region of the left wall in the form of a depression may be identified as the left pouch (LP).

The posterior sorting area (SAP) is well developed in this species, being folded into 2 sections, one above the other, as a result of the inpushing of the posterior wall. The sorting area is carried into the dorsal hood, where it occupies a small area of the wall, limited anteriorly by the anterior fold. A thin groove corresponding to the rejection groove (RG) appears to be present along the posterior margin of the fold.

Ciliary currents:

Particles entering the stomach through the wide oesophagus are at once subjected to the action of the cilia of the broad fleshy lobe which is placed across the entrance to the stomach. Cilia on this fold convey most of the particles across into the dorsal groove, which in turn conveys them to the tip of the dorsal hood. Particles which are thus brought into the dorsal hood are subjected to a process of sorting on the posterior sorting area within the hood.

Along the grooves of the posterior sorting area, most of the particles move towards the knob-like tip of the minor typhlosole and towards the opening of the midgut. Over the major typhlosole currents move forwards, and where the typhlosole forms a fleshy mound currents move upwards, conveying particles to the opening of the isolated duct (DLP') and to the posterior region of the left pouch. The ducts of the right side open into the stomach through a large common opening on the right side (RDC) and ridges lead into the opening. The ridges are richly ciliated and the action of these cilia deflects most of the particles out of the common opening of the ducts. Inbetween the ridges, some particles move into the opening. Similarly, on the left side (LDC) a system of currents exists near the common openings of the ducts, particles being mostly deflected either into the main intestinal groove or towards the left pouch. Cilia on the ventral wall of the left pouch lead particles inwards, while on its dorsal wall, they are passed upwards into the dorsal hood. Around the dorsal hood itself, cilia on the walls carry particles into the hood.

Gastrochaenidae (= Rocelliariidae)

Gastrochaena impressa Deshayes

Fig. 14

The stomach of *Rocellaria* (*Gastrochaena*) *cuneiformis* has been described by Purchon (1954), who restated the salient features of its structure in his later work (Purchon, 1958), questioning the validity of certain terms used by him in his earlier study. He observes that the sorting area on the anterior aspect of the stomach is more extensive than he had originally indicated. The figure given with his first description lacks in a few details, especially of the right wall, and some of the details observed in the present species are described and illustrated in Fig. 14.

The stomach is small and is situated superficially in the antero-dorsal region: in an animal of 30 mm shell length the

stomach measured only about 4 mm. The oesophagus (OES) opens anteriorly into the globular stomach. The midgut (MG) and the style sac (SS) are conjoined, though the openings are clearly separated from one another by the typhlosoles. The minor typhlosole (TYM) ends as a small projection at the entrance of the common opening. The major typhlosole (TY) is a wide ribbon-like structure which sweeps across the floor to the left wall and ends at the edge of a depression (LDC) into which groups of ducts of the digestive diverticula open. The typhlosole does not enter the duct, and this arrangement is similar to that of *Lamellidens corrianus*.

There are 3 main openings of the digestive diverticula placed around the terminal portion of the major typhlosole, and into each of these, 2 or 3 large ducts open together. Purchon (1954) recognized the areas where the ducts open in *Rocellaria cuneiformis* as corresponding to the left caecum. Above these openings is the small depression of the left pouch (LP), into which the gastric shield (GS) sends a small spur. There does not appear to be any duct opening into the left pouch. The gastric shield is small, and is attached to a pronounced thickening on the stomach wall by means of its 2 spurs. There is a set of ridges entering the dorsal hood from the left wall, but a sorting area corresponding to that of *R. cuneiformis* in the anterior wall of the hood is not very conspicuous.

There is a saucer-shaped depression in the anterior right wall (RDC) on one side of the major typhlosole. Ducts from the right side lead into this depression through 3 openings, 1 anterior and 2 posterior. The anterior opening is the largest, and leads into a duct-like passage along which 2 or 3 main ducts open; similarly, one of the posterior openings leads into a wide passage along which open as many main ducts from the posterior right side. The third opening is the smallest and represents a single large duct. To the right of the common opening of the midgut and the

style sac, the stomach wall shows a large depression with ridges rising behind and in front of it. A groove (GP), accompanied by 2 thin folds on either side, winds out of the depression and extends up to the dorsal hood. A pad-like thickening is present on the right wall anterior to this groove, occupying the same position as the structure described by Purchon (1958) in *Hiatella arctica*. The walls of the stomach around the common openings of the ducts on the right side are lined by fine ridges and grooves, which follow the contour of the floor of the stomach.

Carditidae

Beguina variegata (Bruguière)

Fig. 15

The stomach resembles that of *Beguina semiorbiculata* (Purchon, 1958) and shows only few differences from the latter species. An important difference is the presence, in *B. variegata*, of the minor typhlosole, which Purchon states to be absent in the species described by him. The chief feature of the stomach of the present species lies in the patchy distribution of the openings of the ducts of the digestive diverticula, numerous ducts being clustered together in particular areas of the stomach wall. On the left wall, numerous ducts open into two embayments of the wall placed one above the other, of which the dorsal embayment represents the region of the left pouch (LP) and the ventral the region (LDC) where the openings of many left side ducts are concentrated. On the right side there is a shallow basin (RDC), with 2 recesses, into which open the ducts of the right side. To the right of this region, there is a wide ledge on the stomach wall, with a groove running posterior to it. This ledge corresponds to the sorting area 'SA⁵' of Purchon (1958). The major typhlosole (TY) runs forward from the common opening of the midgut and the style sac and ends as a flat spiral coil (TYT) on the floor near the openings of the ducts

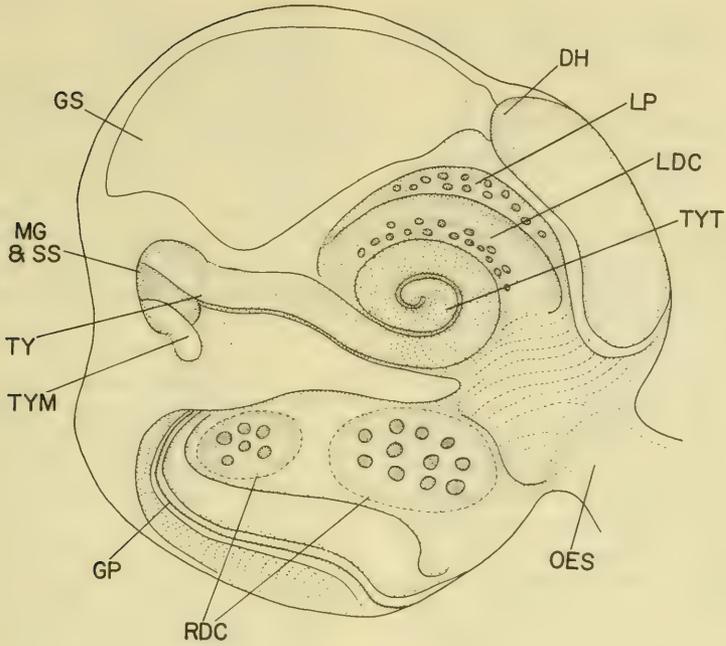


FIG. 15. Interior of the stomach of *Beguina variegata*. (For lettering, see p 229)

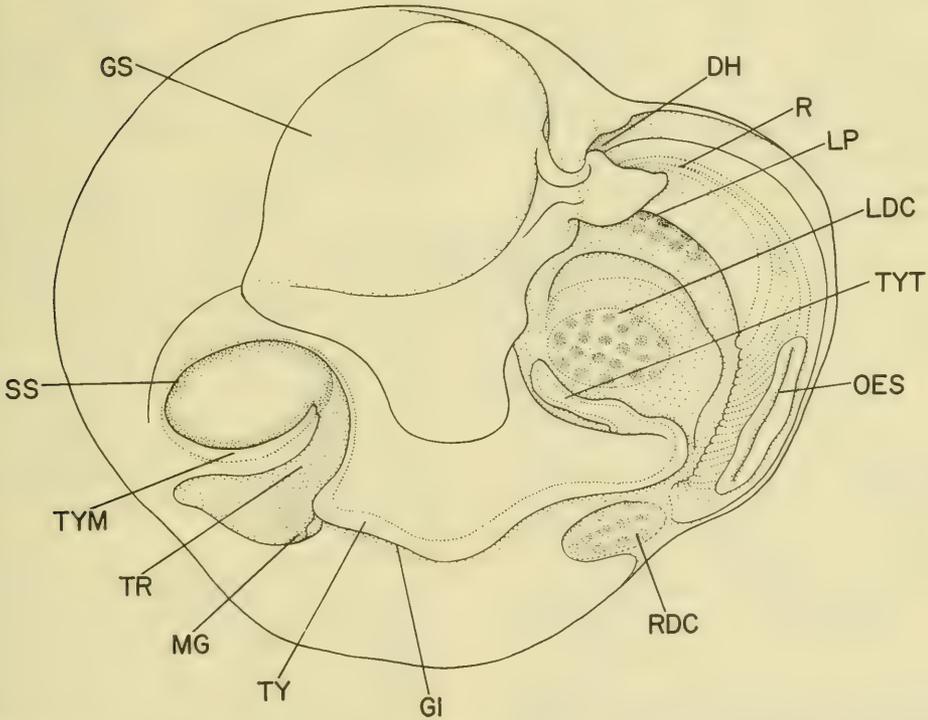


FIG. 16. Interior of the stomach of *Galeomma paucistriata*. (For lettering, see p 229)

on the left side. The typhlosole bears fine grooves and has a striated appearance.

Galeommidae (=Erycinidae)

Galeomma paucistriata Deshayes

Fig. 16

The stomach can be compared to that of *Scintilla hanleyi* described by Purchon (1958). It is fairly large when compared to the size of the animal. The oesophagus (OES) opens on a ledge which bears faint grooves and ridges ('SA'⁷ of Purchon). The openings of the midgut and the style sac are separate, and are placed to the right and left of a pronounced concavity (TR) on the postero-ventral wall. The opening of the midgut (MG) is hardly discernible, and the major typhlosole (TY) appears to arise from the region of the style sac (SS), and sweep over the minute opening of the midgut. However, as pointed out by Purchon, the major typhlosole is a thick fold inside the midgut, but is greatly attenuated as it nears the opening of the midgut into the stomach, and regains its original thickness only after entering the stomach. It extends across the floor of the stomach, and then turns to the left wall and ends in a depression there. In *S. hanleyi* the tip of the major typhlosole forms a slightly incurving lobe, but in *Galeomma paucistriata* the tip is bent on itself. Around the tip of the typhlosole (TYT) there are 2 recesses of the stomach wall into which many ducts open: that on the left is recognized as the left pouch (LP) and the recess nearer to the typhlosole as the area (LDC) of the openings of ducts of the left side. The gastric shield (GS) skirts the area of the left pouch posteriorly and a cusp of the shield fits into the recess. This vicinity of the gastric shield and the fact that a few ducts of the left side open here are reasons for recognizing the area as the left pouch. The dorsal hood (DH) lies above the gastric shield in the form of a deeply incurving pouch and 2 ridges (R) run out of the hood

anteriorly and end to the left of the oesophageal opening.

On the right wall, close to the point where the major typhlosole curves to the left, there is another embayment (RDC) into which open 10 or 12 ducts of the right side. The minor typhlosole (TYM) is a small lobe visible on the right side of the opening of the style sac. Purchon reports the minor typhlosole as absent in *Scintilla hanleyi*.

Veneridae

Meretrix casta (Chemnitz)

Cataleysia opima (Gmelin)

Chione tiara (Dillwyn)

Sunetta effosa Hanley

Venerupis macrophylla Deshayes

A large number of venerids were examined because of their easy availability in the backwaters and the littoral area. The structure of the stomach in all the species listed above is more or less uniform, and is very similar to that of *Gafrarium minimum* (Purchon, 1960), and of the corbiculid *Villorita cyprinoides* (Dinamani, 1957). The chief differences relate to the finer details of structure of the different regions of the stomach.

In all the species, the major typhlosole after emerging from the midgut crosses over to the right to invade a pocket of the stomach wall on that side, and then again crosses over to the left into another pocket of the stomach wall. These pockets are the right and left caeca respectively, into which open most of the ducts of the digestive diverticula. Attention is drawn to this configuration, which differs from that described above for the Unionidae, Carditidae and Erycinidae.

In *Cataleysia opima* the posterior globular part of the stomach is larger and the dorsal hood more spacious; the posterior sorting area also has wider ledges between the grooves. In *Chione tiara* the dorsal hood is in the form of a curved horn in the left wall. In *Sunetta effosa* the posterior sorting area

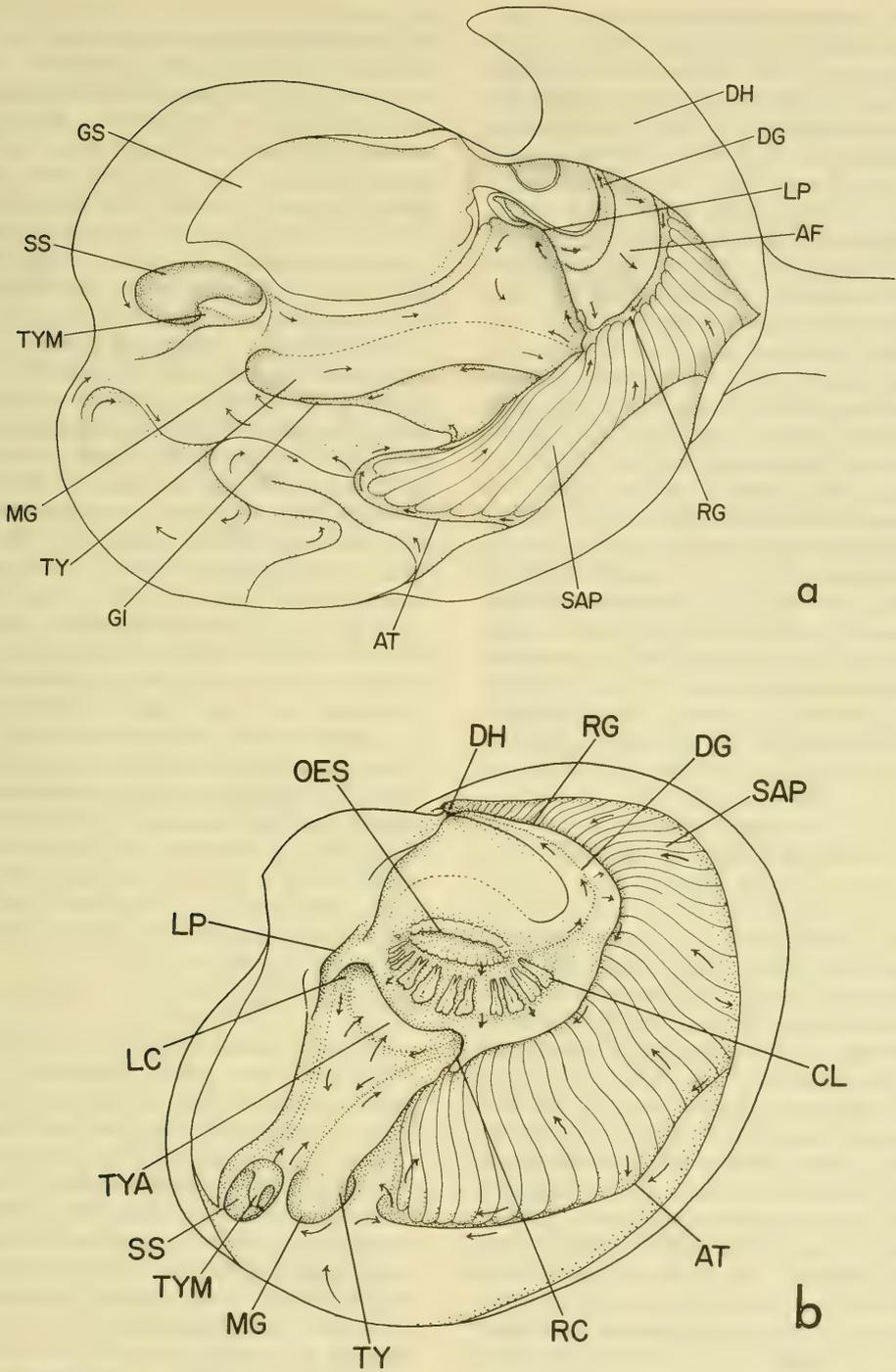


FIG. 17. Stomach of *Standella pellucida*. a, interior view; b, stomach opened behind the region of the dorsal hood and viewed posteriorly to show opening of oesophagus. (For lettering, see p 229)

is narrow but extensive and bears prominent ridges. In *Villorita cyprinoides* (Dinamani, 1957) it was not possible to detect the presence of ducts opening into the left pouch, but in all the species listed above 2 or 3 ducts of the digestive diverticula open into the left pouch. A lateral ridge, similar to that of *V. cyprinoides*, was found only in *Meretrix casta*, though a similar structure has been described by Purchon (1960: Fig. 8, BS) in *Gafrarium minimum*.

In the posterior region of the stomach the openings of the style sac and the midgut are conjoined in all the species examined, except in *Chione tiara* in which the openings, though placed together, lead into separate tubes. In *Venerupis macrophylla*, the minor typhlosole runs forwards for some distance parallel to the extension of the major typhlosole, and the tip of the latter ends as a small lobe ventral to the left pouch. The region of the caeca in all species is very similar to that of *Villorita cyprinoides*, except for small differences in the form of the major typhlosole within the caecal pockets.

Mactridae

Standella pellucida (Gmelin)

Fig. 17a, b

The structure of the stomach resembles closely that of *Mactra mera* (Purchon, 1960). The most noteworthy features of the stomach are the complete separation of the openings of the midgut and the style sac and their location in the postero-ventral region of the stomach, and the enlarged posterior sorting area. The openings of the midgut (MG) and the style sac (SS) are placed apart to the left and right of the median line. The minor typhlosole (TYM) arises from the style sac and ends in the form of a small knob projecting from the tube. Purchon (1960) regards the minor typhlosole as absent in *M. mera*, but has indicated a knob-like structure similar to that of the present species above the opening of the style sac.

The posterior sorting area (SAP) occu-

pies almost the whole of the right wall and arches over the anterior part of the stomach into the dorsal hood (DH). The sorting area is limited anteriorly by the rejection groove (RG), which joins the intestinal groove (GI) near the entrance of the right caecum (Fig. 17b, RC). The dorsal groove (DG), arising from near the oesophageal opening, and the rejection groove (RG) almost meet at the apex of the hood but are separated at the entrance to the hood by a broad fleshy fold (AF). Along the border of the posterior sorting area is a groove (AT) which ends posteriorly at the side of a ridge projecting from the posterior wall. The openings of the right and left caeca (RC, LC) are placed almost directly beneath the oesophageal opening (OES), and are separated from it by a sloping area of the anterior wall. This area bears a row of lobes (Fig. 17b, CL); each lobe is a key-like prominence, longer than broad and having a beaded appearance. These are similar to the papillae described by Purchon as 'ornamental' in *Mactra mera*. The left pouch (LP) is a small depression in the stomach wall immediately behind and slightly above the left caecum. It is very much reduced and appears to serve as an anchorage for the shield which sends a small spur into it. The shield is large and occupies most of the left wall behind the dorsal hood.

Ciliary currents:

Food particles coming in through the oesophagus are usually deflected to the right and pass into the dorsal groove, which takes them into the dorsal hood. Particles coming into the interior of the stomach, however, appear to come first into contact with a row of crenate lobes (CL); over the surface of the lobes cilia beat towards the grooves running between them, from whence the currents carry the particles into the main intestinal groove below, or to the rejection groove in the vicinity. If the lobes lie pressed together, either as a result of their turgescence or by movements of the stomach wall, they could form a con-

tinuous surface on which particles could move laterally, without entering the grooves between them; the lobes could alternatively move apart by relaxation of the stomach wall and allow particles to move into the grooves. Thus the crenate lobes appear to have a sorting function in the anterior region of the stomach.

Particles accumulating inside the dorsal hood pass out of it through 2 channels, the rejection groove and the acceptance tract (AT): the rejection groove drains into the main intestinal groove, but the acceptance tract takes particles to the ridge situated postero-ventrally, from where they are carried forwards over the major typhlosole towards the caeca. The major typhlosole runs through the 2 caeca and the portion (TYA) between the caeca runs immediately beneath the row of crenate lobes. This region of the typhlosole shows intense ciliary activity, with the majority of the currents deflected towards the crenate lobes and the main intestinal groove running anterior to the major typhlosole. The posterior wall of the stomach bears a series of ridges over which particles move mostly towards the openings of the midgut and the style sac.

Psammobiidae

Sanguinolaria (Soletellina) diphos (Gmelin)

Figs. 18a, b, c

The interior of the stomach of this species is fundamentally similar to that of *Gari togata* described by Purchon (1960). The oesophagus (Fig. 18a, OES) opens nearly in the middle of the anterior half of the stomach, which region carries a series of longitudinal ridges separated by wide grooves. The ridges do not appear to be ciliated and have only superficial resemblance to the crenate lobes described in *Standella pellucida*. The anterior fold (AF) runs in the form of a sinuous ribbon immediately behind

the row of ridges, and is unique in that it is flap-like: in some stomachs, the free edge of the flap is turned towards the ridges, while in others the edge is towards the interior of the stomach. The anterior fold reaches up to the opening of the dorsal hood (DH) (Fig. 18b).

The combined opening of the midgut (MG) and the style sac (SS) is to the left of the median line; the opening is situated on a slight elevation of the floor of the stomach. The greater part of the common opening is that of the style sac, the opening of the midgut being obscured by the fold of the major typhlosole (TY). The major typhlosole crosses the floor and invades the 2 caeca (RC, LC), and the free length of the typhlosole (TYA) between the caeca lies close to the anterior fold in the live condition. The typhlosole ends within the left caecum, with its tip (TYT) turned into the caecum in the form of a coil as in *Asaphis deflorata* (Purchon, 1960). The left pouch (LP) is placed immediately above the left caecum and is unique in that it is actually formed of 2 pouches, one above the other; this peculiar modification is reflected in the portion of the gastric shield entering the left pouch (Fig. 18c). Generally in bivalves a single spur of the shield enters the left pouch, but in the present species the gastric shield has 2 hook-like spurs penetrating the left pouch (Fig. 18c, GSL). The wall of the left pouch over which the spurs of the shield lie is smooth and thick, while the rest of the wall is thin and finely grooved.

Above the left pouch is a thick ridge on the wall (LP') which evidently provides mechanical support and anchorage for the strongly recurved spurs of the gastric shield. Above this ridge is the dorsal hood (DH) which is in the form of a flat recurved horn on the roof of the stomach (Fig. 18b). It is nearly 8 mm long in a stomach measuring 12 mm antero-posteriorly. The greater part of the wall of the hood is occupied by a spine-like spur of the gastric shield (Fig. 18c, GSD). Close to the opening

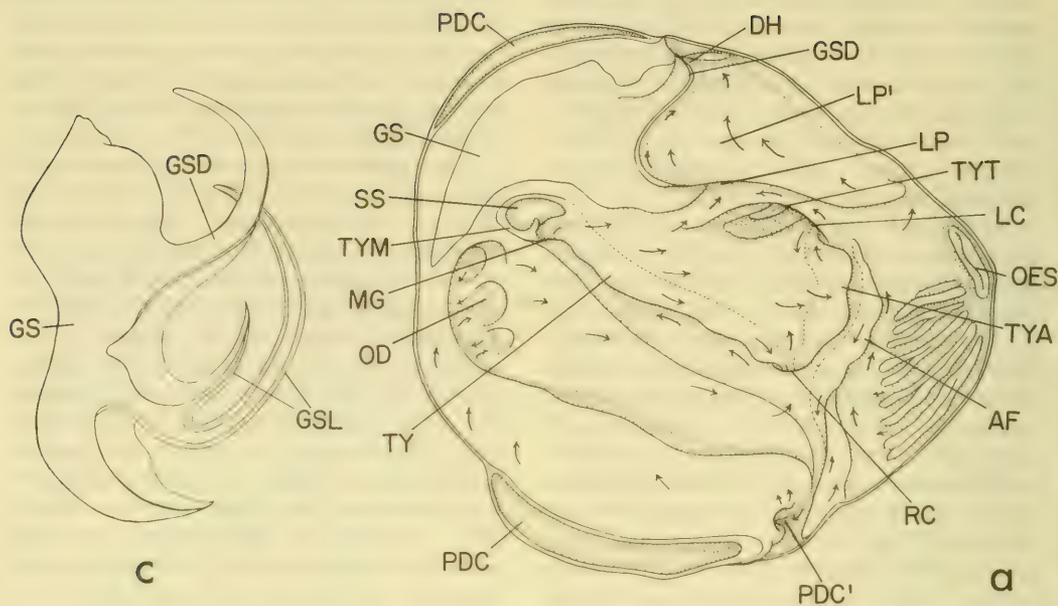
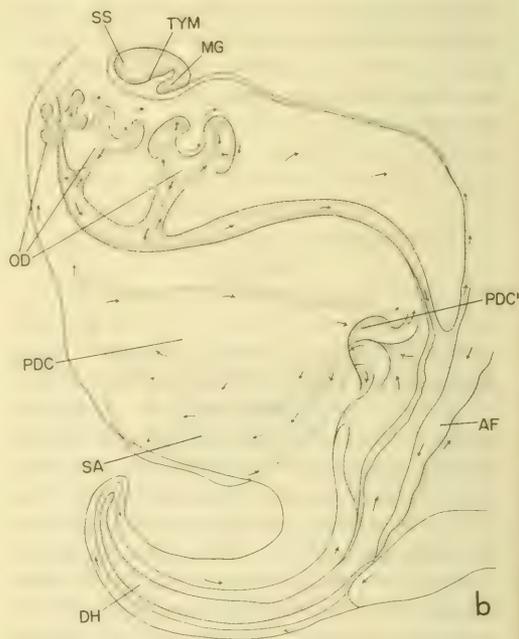


FIG. 18. Stomach of *Sanguinolaria (Soletellina) diphos*. a, interior view; b, dorsal region of the stomach to show the posterior dorsal caecum (caecum shown as a transparency); and c, gastric shield. (For lettering, see p 229)

of the dorsal hood into the stomach, is the opening of another pouch (Figs. 18a, b, PDC'), which extends over the entire width of the stomach in this region. The pouch (PDC) is thin-walled and is actually a double chamber, of which the right one bears folds and grooves (SA). The pouch is often full of sand grains and clearly is the postero-dorsal caecum described by Yonge (1949) in the Tellinacea. It also corresponds to the structure termed as 'appendix' in *Gari togata* and *Asaphis deflorata* by Purchon (1960).

Another peculiar region of the stomach is a series of oval depressions (Fig. 18a, b, OD) along the postero-ventral wall. Purchon (1960) has described similar structures in *Gari togata* as a series of



'blind pockets', and (1958), in the stomach of a member of the Pandoracea, *Laternula rostrata*, as a sorting area. No ducts of the digestive diverticula open into these depressions, but at each oval patch, there is a 'Y'-shaped channel in the middle, with the 2 arms leading upwards while the stem of the 'Y' leads ventrally into a channel. This channel runs forwards along the floor of the stomach and reaches a groove leading from the dorsal hood near the opening (PDC') of the postero-dorsal caecum. A posterior sorting area as such is absent, though the grooved area (SA) inside the postero-dorsal caecum may represent this structure. The gastric shield (GS) extends over the left posterior wall and occupies the cavernous portion of the stomach above the opening of the style sac. It is strongly recurved in the area between the dorsal hood and the left pouch and its 3 spurs (Fig. 18c) bend back sharply to support the shield on the wall.

Ciliary currents:

Most of the food material entering the stomach comes to the area of ridges in the anterior region. The ridges are not ciliated but the shallow grooves in between are ciliated and convey particles towards the anterior fold. The ridges may, by coming close to each other, occlude the grooves in between them, and particles entering the stomach may be directed over the dorsal wall; this current probably accounts for the large sand grains usually found within the postero-dorsal caecum. Particles moving in through the grooves collect in front of the anterior fold, where strong ciliary currents exist sweeping from right to left. This strong current conveys most of the accumulated particles to the region of the left pouch. From the anterior wall of the left pouch, cilia drive particles into the interior of the pouch, while along its posterior wall cilia convey particles towards the dorsal hood. The raised ridge on the left wall (LP') below the dorsal hood is an area

of strong ciliary activity; this ridge is faintly striated due to rows of cilia arranged regularly over its surface. The ridge has been found to secrete a large quantity of mucus, and particles are swept across the ridge into the dorsal hood in a film of mucus. Some of the particles which accumulate inside the hood are drawn to the tip of the revolving crystalline style, as shown in freshly opened stomachs.

As already stated, the anterior fold may be directed forwards towards the oesophagus or backwards to overlap a portion (TYA) of the major typhlosole. This capacity to change direction is evidently part of the natural functions of the fold, as is indicated by the difference in the direction of beat of the cilia on its two 'faces': over its posterior surface cilia beat to the right and eventually carry particles towards the postero-dorsal caecum and the dorsal hood; on the reverse side, which is uppermost when the fold is flapped towards the interior of the stomach, cilia beat in the opposite direction and carry particles to the left towards the left pouch and the tip of the crystalline style. In the series of oval depressions (OD) ciliary currents are diverse: in the Y-shaped channel particles move ventrally, while along the walls around the channel particles travel upwards towards the folds of the stomach wall. Ciliary action on particles does not, however, seem to yield any clue regarding the functions of these oval patches on the posterior right wall.

Tellinidae *Tellina ala* Hanley Fig. 19

Graham (1949) has described the stomach of *Tellina crassa*, and Yonge (1949) that of *T. tenuis*, and these both show broad similarities with the stomach of *T. ala* (Fig. 19). The stomach wall is thin and tough. Dorsally and posteriorly it is reinforced by a large and massive gastric shield (GS). Anteriorly,

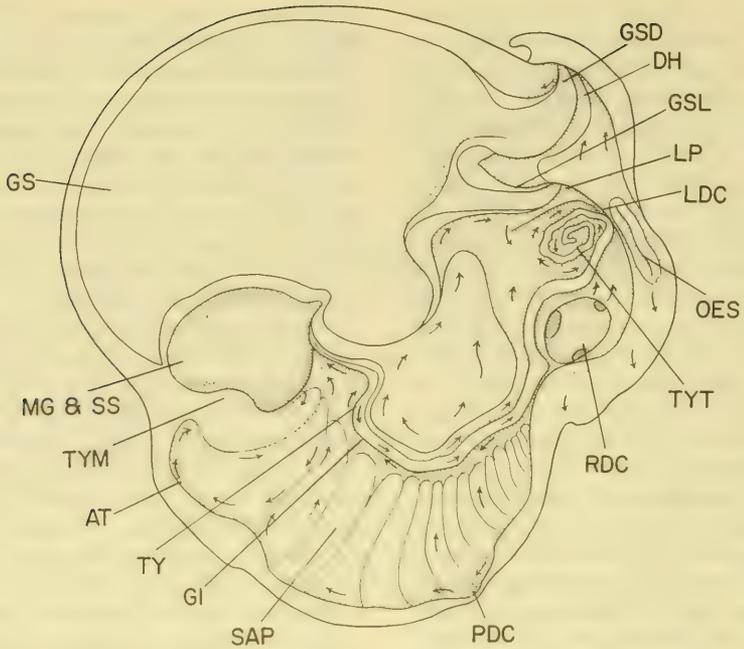


FIG. 19. Interior of the stomach of *Tellina ala*. (For lettering, see p 229)

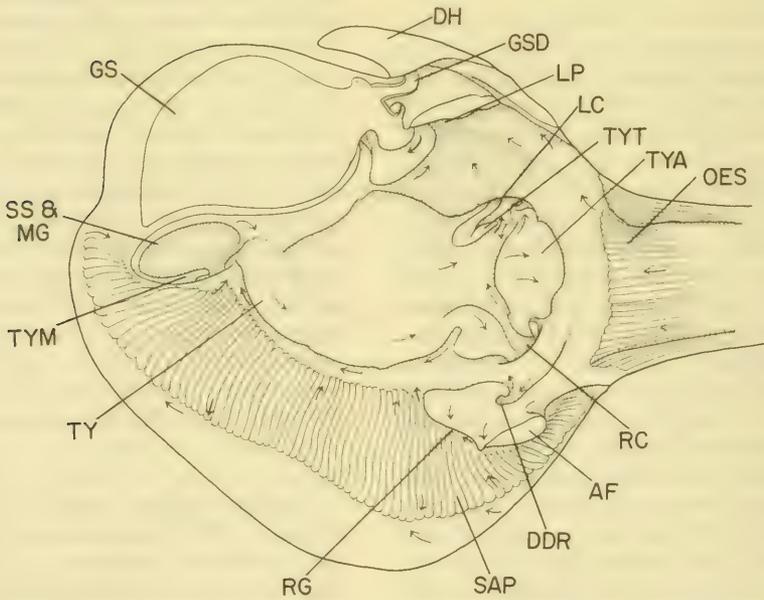


FIG. 20. Interior of the stomach of *Solen amandalei*. (For lettering, see p 229)

on the left side, is a well developed dorsal hood (DH) which curves deeply to the left, and a pointed spur of the gastric shield (GSD) extends into it. A similar spur of the shield (GSL) is thrust into the left pouch (LP), which is situated ventrally to the hood. Two wide ducts seem to open into the left pouch, with each duct giving off 3 or 4 subsidiary ducts.

The minor typhlosole (TYM) ends as a small prominence on the right wall immediately above the common opening of the midgut (MG) and the style sac (SS). The major typhlosole (TY) emerges from the midgut as a thin fold and curves to the right across the floor of the stomach. It turns anteriorly and dips into a shallow depression, and then turns left and forms a loop (TYT) in a recess on the left wall below the left pouch. In *Tellina crassa*, Graham (1949) shows a similar course for the major typhlosole, though here the major typhlosole also conspicuously loops in a depression on the right side, which Graham describes as the 'right half of the caecum'. In *T. ala* the major typhlosole does not form a conspicuous loop in the corresponding region, which is, however, indicated by a small depression which receives the openings of 3 large ducts and which region (RDC) may be considered as similar to those of other Eulamellibranchia described earlier in this study where ducts of the right side open together. In the recessed area of the left wall where the major typhlosole ends (TYT) 2 wide ducts open, each of which receives many subsidiary ducts along its course. This area (LDC) is similar to the 'left half of the caecum' of Graham (1949).

The epithelial lining of all wide ducts that open into the stomach is dark brown or black in colour, and the walls of these ducts seem to be suffused with a dark brown pigment; the stomach epithelium underlying the gastric shield is similarly pigmented. Pigmented epithelia have been reported by Owen (1956) in the ducts of the digestive diverticula in *Nucula*, and by Purchon (1958) in the stomach

of *Lucinoma (Phacoides) borealis*. The walls of the embayments and of the left pouch are finely grooved. The floor of the stomach below the gastric shield and in front of the style sac is in the form of an elevated pad which is bordered on its right and anterior sides by the winding major typhlosole. To the right of the major typhlosole and occupying the region of the right wall is a belt of broad ridges interspaced with grooves. This corresponds to a similar area described in *T. tenuis* by Yonge (1949), and obviously should be referred to as the posterior sorting area (SAP). Purchon (1960) regarded the posterior sorting area as present in all Type V stomachs, except that of the Tellinidae. However, he predicted that it would probably be demonstrated in the stomach of some member of the Tellinidae more clearly than was done for *T. tenuis*. His view is borne out in the species described here, where the posterior sorting area is distinct. There is also a depression (PDC?) in the middle of a ridge running along the dorsal boundary of the posterior sorting area; this depression occurs at a site corresponding in position to the opening of the postero-dorsal caecum described by Yonge (1949) in *Tellina tenuis*.

Ciliary currents:

Currents in the areas around the dorsal hood and the oesophageal opening are directed towards the hood. Within the stomach, maximum ciliary activity is observed on the elevation on the floor adjacent to the major typhlosole. In this region there is a circular motion of particles, which are eventually swept up towards the left pouch; in the walls of the left pouch and of the embayment below the pouch ciliary beat is also towards the dorsal hood. Behind the opening of the style sac, ciliary currents converge on a ridge near the minor typhlosole and there is a circular motion of particles in this region. In the posterior sorting area pronounced ciliary activity is towards the intestinal groove

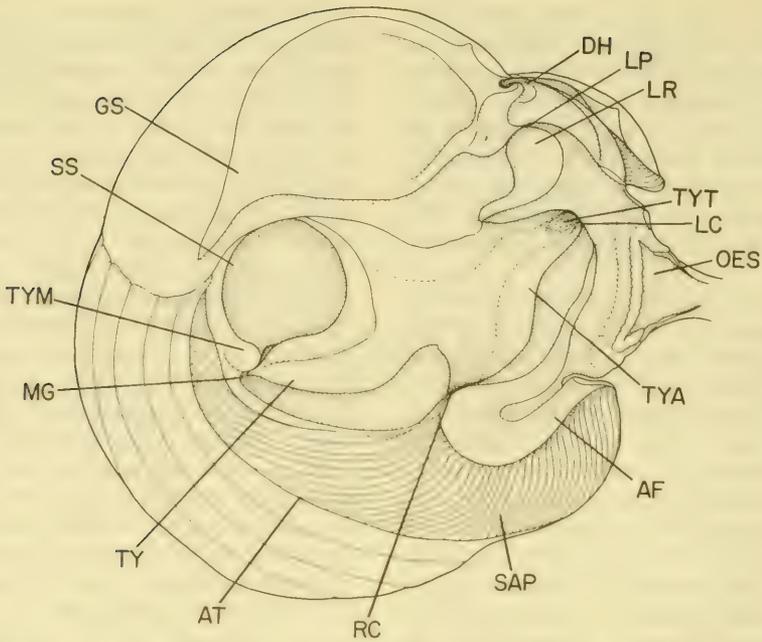


FIG. 21. Interior of the stomach of *Jouannetia cumingii*. (For lettering, see p 229)

(GI), though dorsally particles move towards the style sac along a groove (AT) extending along the boundary of the sorting area.

Solenidae

Solen annandalei Preston

Fig. 20

The stomach is simple and has the typical features of the family as described by Owen (1959a); it also resembles the stomach of *Pharella acutidens* described and figured by Purchon (1960). There is an anterior fold (AF) rising immediately above the right caecum, where an isolated opening (DDR) is present. This opening leads into a ciliated sinuous passage through the digestive diverticula along the course of which 8 ducts of the digestive diverticula of the right side open. Purchon (1960) has indicated an identical opening on the right wall of the stomach of *P. acutidens*. In *Solen annandalei*, the walls of the sinuous passage are unlike those of the ducts and resemble more those of the

stomach.

On the postero-ventral side of the stomach is the combined opening of the style sac (SS) and the midgut (MG); though this opening is single, it is really formed by 2 independent tubes. The major typhlosole (TY) bends sharply from the midgut, extends over the floor and invades the right and left caeca, and the tip of the typhlosole (TYT) is seen as a small lobe at the entrance of the left caecum (LC). The left pouch (LP) is a wide opening on the left wall which narrows distally and leads into 6 or 7 ducts of the digestive diverticula on the left side. The floor of the left pouch carries a wide striated ridge (SA⁶ of Purchon). The posterior sorting area (SAP) is extensive and is bounded anteriorly by a ridge, the anterior fold (AF), and the rejection groove (RG).

Ciliary currents:

It was very difficult to keep the animal alive outside its normal habitat, and in a dissected stomach ciliary currents are very feeble. However, a

number of dissections were made; and the ciliary currents were found to be essentially similar to those of other Eulamellibranchia. Most of the particles entering the stomach are deflected to the left, towards the region of the left pouch. On the walls of the pouch, cilia beat generally towards the dorsal hood. At the mouth of the isolated opening (DDR) on the right side, particles move into the passage, though posteriorly a current exists carrying particles out of the opening; a similar system of currents is indicated at the openings of the ducts into the passage. Currents in the posterior sorting area are more powerful and drive particles into the main intestinal groove or to the right dorsal walls.

Pholadidae

Jouannetia cumingii (Sowbery), Fig. 21
Martesia striata (Linnaeus)

The stomach of the pholads has been described by Graham (1949), and in more detail by Purchon (1955, 1956b). The 2 species examined during the course of the present study show great similarity of structure with those already described. However, in *Jouannetia cumingii* (Fig. 21), unlike those of other pholads, the style sac (SS) and the midgut (MG) open together into the stomach, and are separated only after leaving the stomach. The anterior fold (AF) is finely grooved throughout its length; no duct of the digestive diverticula opens into the left pouch; there is also a lateral ridge (LR) on the floor of the left pouch, very near the terminal portion of the typhlosole (TYT). In *Martesia striata*, 3-4 ducts of the digestive diverticula open into the left pouch, and the lateral ridge is in the form of 3 small lobes at the entrance of the pouch; within the left caecum the major typhlosole is arranged in 3 sections. A similar modification of the major typhlosole within the left caecum has been described in *Chama lazarus* by Purchon (1960).

DISCUSSION

Graham (1949) has summarized the main features of the bivalve stomach and has indicated that there is fundamental similarity of structure throughout the class. On the basis of stomach structure he has arranged the Bivalvia into 4 groups, and suggested that this grouping largely follows the classification of the class into Protobranchia, Filibranchia, Pseudolamellibranchia and Eulamellibranchia; his studies did not include the Septibranchia. The main purpose of Purchon's survey of stomach structure throughout the class (Purchon, 1956a *et seq.*) was "to discover reliable evidence for the recognition of phylogenies within the class", and his search revealed 'certain structures and relationships' which he regarded as 'trustworthy indicators' of phylogenetic relationship. The 2 typhlosoles and the main intestinal groove were considered as 2 such indicators, the disposition and relationship of which to other regions of the stomach were believed to be of sufficient significance as to yield on analysis 5 types of stomachs. Using these 5 types as basis for taxonomic division, Purchon (1960, 1963) proposed a new classification of the Bivalvia, splitting the class into 5 orders: I. Gastroprotea (= Protobranchia), II. Gastrodeutia (= Septibranchia), III. Gastrotriteia (= most of Filibranchia), IV. Gastrotetartika (= some Pseudolamellibranchia + many Eulamellibranchia) and V. Gastropempta (= remainder of Eulamellibranchia). He grouped these orders into 2 subclasses on the basis of the number of ducts of the digestive diverticula opening into the stomach, placing the first 2 orders under the Oligosyringia (with 2 or 3 simple ducts) and the latter 3 orders under the Polysyringia (with numerous ducts).

Regarding his proposal to unite the Protobranchia and the Septibranchia under a major taxon, based on the number of openings of the ducts of the digestive

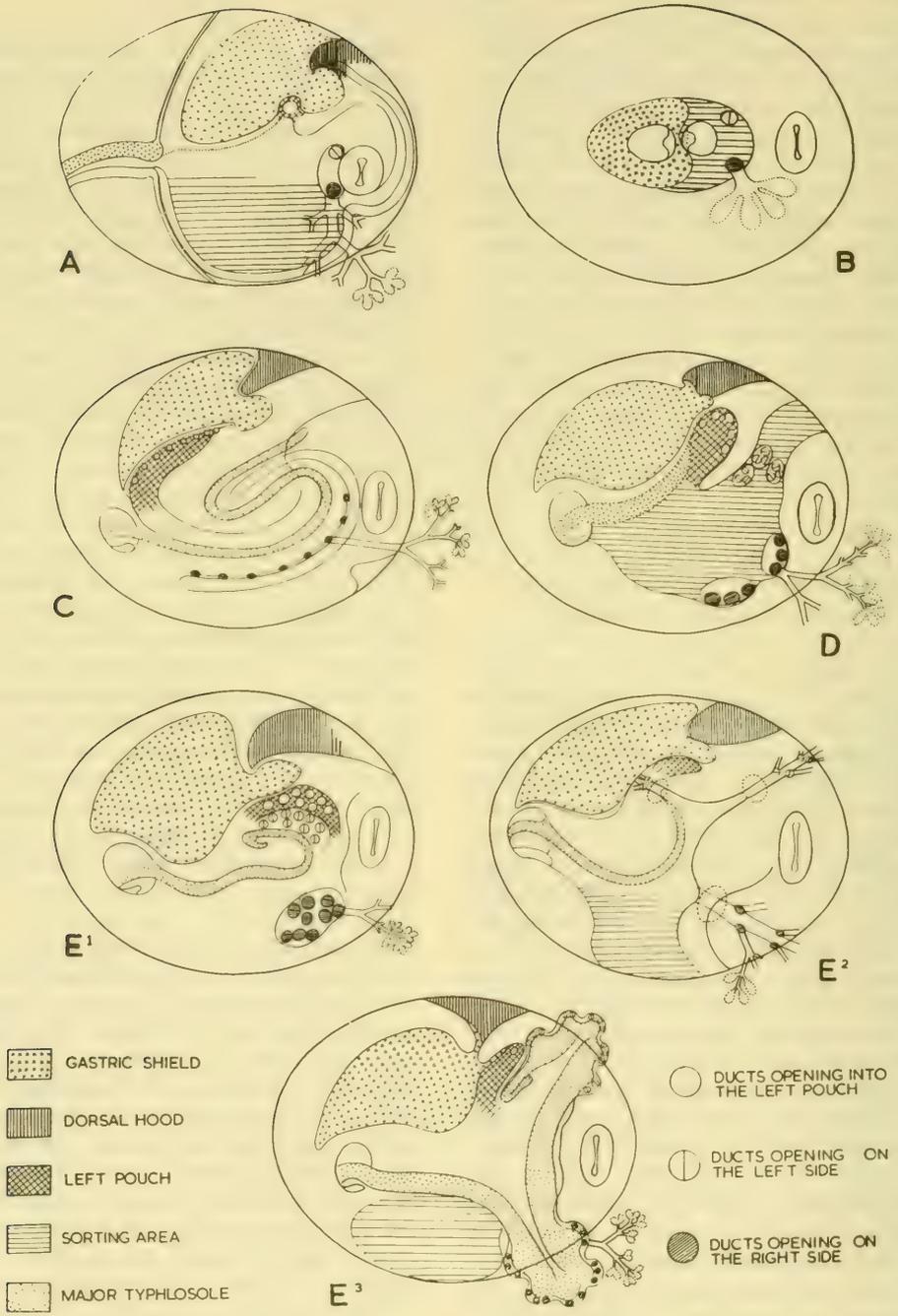


FIG. 22. Schematic representation of the variation in stomach structure and its association with the digestive diverticula, in A. Protobranchia; B. Septibranchia; C. Anisomyaria-I; D. Anisomyaria-II, E¹ to E³, three lines of Eulamellibranchia.

diverticula into the stomach, it may be mentioned that there are basic differences in the structure of the diverticula between the 2 groups. Those of the Nuculidae and the Nuculanidae possess a highly branching duct system (Owen, 1956), the main duct itself branching repeatedly within the mass of the digestive diverticula. In the Septibranchia, on the contrary, there is practically no duct system (Yonge, 1928), the 2 orifices in the stomach leading immediately into digestive tubules. Purchon's studies (1960) have also revealed that stomach types IV (Gastrotetartika) and V (Gastropempta), as he has defined them, occur not only with the same group of families (Sphaeriacea, Lucinacea, Tellinacea), but may even occur within the same genus (*Chama*). He therefore regards Type IV as ancestral to Type V, and accounts for the occurrence of the 'ancestral type' in the above examples as a case of 'reversion' or 'recapitulation' and 'juvenilisation'. At the same time he regards the differences between stomach Types IV and V as 'sharp and distinct'.

Therefore it is appropriate to give further consideration to the structural variation in the stomachs of bivalves, in order to throw more light on the occurrence of distinct types of stomach within the class. It may also be proper to examine whether these stomach types reflect a natural scheme of variation and modification.

Among the many features of the stomach that show basic variations in the bivalve stomach, the most significant appear to be those regions of the stomach wall which receive the ducts of the digestive diverticula. Related to this are changes in the manner of opening of the ducts into the stomach, as well as changes in the duct system itself, the last of which forms the subject matter of a forthcoming paper. As an index of changes within the stomach, particularly of its walls, 2 features are considered to be of importance. These are, (i) the site of the left pouch (indicative of changes

in the left wall of the stomach), and (ii) the modifications of the major typhlosole (corresponding changes in the right and ventral walls). This has been diagrammatically represented in Fig. 22, where the pattern of the duct system is also broadly indicated for each type.

The term 'left pouch' refers to an area of the left wall into which open some of the ducts of the digestive diverticula of the left side. Though there may be morphological differences in the form of the pouch, which in some cases may not even be in the form of a pouch, its identity in the stomach of the members of the different families of bivalves has been more or less uniformly established. The left pouch remains an important feature whose relationship to 2 other structures of the stomach, namely, the gastric shield and the dorsal hood, remains fairly constant (Graham, 1949). Therefore the degree of change occurring in the left wall of the stomach is reflected in these structures, and is indicated by the changes in the position and form of the left pouch. The number of ducts opening into the left pouch may also be correlated to these changes. The modifications of the major typhlosole are reflected in its relations to the group of ducts opening on the right wall of the stomach. The relationship of the major typhlosole to the left pouch also shows basic differences.

The left pouch has been identified in all the Bivalvia, except the Protobranchia and the Septibranchia. Therefore, the analysis, to begin with, is confined to those forms in which the left pouch is present. These can now be arranged under the following major sections:

Section I

The left pouch is an area on the left wall situated *posteriorly*, with many ducts opening into it; the major typhlosole runs into the stomach *anterior* to the left pouch; the ducts of the right side open singly along the floor of the stomach (Fig. 22C).

Section II

The left pouch is *anterior* to the major typhlosole, with many ducts opening to it; ducts of the digestive diverticula open individually on the right and left sides of the stomach floor, but are often distributed in definite groups (Fig. 22D).

Section III

The left pouch is *variable*, but developed on the left wall in an area *adjacent* to the tip of the major typhlosole, towards its left; openings of the ducts of the digestive diverticula tend to become aggregated anteriorly on right and left sides in definite embayments, recesses or pockets of the wall of the stomach (Fig. 22 E¹ - E³).

The above 3 sections represent the basic types of stomach in 3 main groups of bivalves: under Section I can be included the super-families Mytilacea, Ostracea, Pteriacea, Pinnacea and Arcacea; under Section II, the families Anomiidae, Pectinidae and Limidae; Section III comprises the Eulamellibranchia. Since that group contains a large assemblage of forms, further groupings become desirable. An attempt is made at grouping them according to a broad pattern of structural variation, which is indicated in outline below:

Group A

Forms in which the ducts of the digestive diverticula open close together in areas adjacent to the tip of the major typhlosole, where the stomach wall forms embayments; the left pouch forms one such embayment and receives numerous ducts (Fig. 22 E¹): e.g., Carditidae, Cyamidae, Erycinidae, Chamidae, Saxicavidae.

Group B

A further development to the above group: those forms in which the left pouch is definitely pocket-like, but has few ducts opening into it; definite caeca are not formed by the stomach wall; e.g.,

Clavagellidae, Pandoridae, Laternulidae, Chamostreidae, Myochamidae.

Group C

Forms in which the main ducts of the digestive diverticula open into 2-3 duct-like passages, each of which in turn enters the stomach adjacent to the major typhlosole; the left pouch proper is a small recess below the gastric shield with no duct entering it, but there may be one duct opening near to it (Fig. 22 E²): e.g., Unionidae, Trigonidae, Gastrochaenidae.

Group D

Forms in which the ducts are received in the majority of cases in the right and left caeca; left pouch is well formed, with only few or no ducts opening into it (Fig. 22 E³): e.g., Corbiculidae, Chamidae, Dreissenidae, Isocardiidae, Cyprinidae, Libitiniidae, Cardiidae, Tridacnidae, Veneridae, Mactridae, Tellinidae, Psammobiidae, Semelidae, Solenidae, Myidae, Pholadidae, Teredinidae.

It is to be understood that the above groupings are only indicative of certain trends, and no opinion is expressed here about primitive or basic types, or how the 'types' are phylogenetically related to each other. However, the suggested arrangement may indicate a line of structural modification that has as its basis the interaction between the stomach wall and the duct system of the digestive diverticula. Thus the greatest modification, and the most significant correlation between these 2 parts of the digestive system is seen in the development of the caecal pockets of some of the Eulamellibranchia, where the expanded major typhlosole invades the pockets of the stomach wall into which the ducts also open together. It is possible to regard the other lines of modification (Fig. 22 E¹ and E²) as different trends towards aggregation of ducts of the digestive diverticula in definite regions of the stomach wall.

The Protobranchia and the Septi-

branchia remain to be considered: the digestive diverticula in these groups open into the stomach through 2 or 3 apertures. However, the Protobranchia at least show features of a large sorting area and folds comparable to other Bivalvia. In the Nuculidae there is a single opening on the left wall close to the gastric shield and, in addition, there are 2 ducts opening on the ventral wall. The duct system is also well developed. The left side opening may be regarded as possibly representing the region of the left pouch, and if we are to accept the view that the fold extending to the opening of the left side represents an extension from the major typhlosole, especially in view of the presence of an intestinal groove in some species of *Nucula*, the relationship of the 'left pouch' to the 'typhlosole' would be very similar to that in the stomach of the Pectinidae and Anomiidae. It has already been indicated that traces of a fold resembling the axial fold of the Anisomyaria may be identified in the protobranch stomach also. However, in the Protobranchia the concentration of ducts of the digestive diverticula has been brought about by modifications of the duct system (Fig. 22 A) and the stomach wall has not 'responded' by forming embayments to receive the ducts. Thus the protobranch stomach shows affinities to those of other Bivalvia, and the left pouch may be regarded as represented in the group.

In the Septibranchia the 2 openings of the digestive diverticula on the floor of the stomach directly lead into many tubules and present an extreme simplification of both the stomach wall and the duct system (Fig. 22 B). It is at the same time possible to point to some Eulamellibranchia where the digestive diverticula have become similarly modified. The septibranch stomach appears to have, however, no relationship with any of the stomach types described above.

The foregoing analysis based on details of structure thus distinguishes 5 main types of stomach in 5 major groups of

bivalves. It may seem convenient to regroup the Eulamellibranchia under 2 major heads, based on the presence or absence of caeca, but it is suggested here that in those groups without caeca the embayments and recesses of the stomach wall, where numerous ducts open together, exhibit a trend similar to that in forms in which the stomach develops true caeca to accommodate the ducts. The grooved area described in some of the pseudolamellibranch families where ducts open in groups may also be considered as a development in the same direction, though no definite embayments are developed in the stomach wall. All these areas may, however, be said to be homologous in the stomach of the Bivalvia, and the degree of their development or modification may reflect differences in the mode of handling digestion by the stomach epithelia.

Further, if we assume these regions of the stomach where ducts become concentrated to be homologous, it would no longer become necessary to assume that a single caecum has originated in the Bivalvia, which later became halved, as suggested by Graham (1949) and Morton (1960). The double caeca arise by grouping of ducts independently on the right and left sides of the stomach. Evidence for this view is furnished by a recent study by Creek (1960) on the development of the gut in the young of *Cardium edule*. She has demonstrated that in the veliger the anterior part of the stomach increases in size in relation to the posterior part and that the digestive gland enlarges on each side of the anterior region. However, the left lobe separates into 2 portions, one dorsal and the other ventral, while the right lobe remains single. It is evident therefore that the right and left lobes of the digestive diverticula are separated early in development; the left lobe separates further into 2 lobes, which might represent those communicating with the left pouch and the left caecum, while the right lobe communicates with the right

caecum or its homologue.

CONCLUDING REMARKS

The association between the stomach and the digestive diverticula can not be estimated merely by counting the number of openings of the ducts into the stomach. Purchon (1963) regards the number of openings into the stomach as of immediate relevance to the initial division of the Bivalvia into 2 sub-classes. Thus Purchon unites the Septibranchia with the Protobranchia in the Oligosyringia. But the number of openings of the digestive diverticula is of minor significance, compared to basic differences in the structure of the digestive diverticula themselves between the Protobranchia and the Septibranchia. In some eulamellibranch families such as the Unionidae, Trigonidae and in genera such as *Tellina*, 3 large ducts of the digestive diverticula open into the stomach by as many openings. However, these openings connect with the digestive tubules through a highly branching system of main and secondary ducts. These instances, it is suggested here, may represent modifications of the duct system of the diverticula without the involvement of the stomach, whose walls usually develop pockets or embayments where duct openings are accommodated.

The functions of the digestive diverticula also can not be assessed, on present evidence, as presenting 'clear cut alternatives of intra-cellular and extra-cellular digestion' in the 2 sub-classes into which Purchon (1963) has divided the bivalves. In the first place, the functioning of the digestive system has not been clearly understood in the majority of the Bivalvia; the role ascribed to the digestive diverticula as organs of intra-cellular digestion in the majority of the bivalves is primarily based on observation of phagocytosis of small particles by tubule cells. Their role remains to be experimentally verified, particularly with reference to the behaviour of the tubule cells immediately

after phagocytosis (see, for example, behaviour of tubule cells in *Lasaea rubra*, Morton, 1956), apart from the basic question of how and what type of particles are conveyed through the duct system of the digestive diverticula (Owen, 1955; Morton, 1956). Correlated to this question are the problems of the occurrence, source, extent and nature of extra-cellular enzymes in the stomach. There is reasonable evidence for the presence of free enzymes in the stomach (Mansour, 1946; Mansour-Bek, 1946; Morton, 1956), and, in consequence, for extra-cellular digestion in the lumen of the stomach (Morton, 1956) in some bivalves, attributable to 'secretion' from the digestive diverticula. When it is remembered that Mansour (1946), Mansour-Bek (1945), Mansour & Zaki (1946) and Zaki (1951) worked on species of *Ostrea*, *Mytilus*, *Pinctada* and *Unio*, where the 'secretion' from the digestive diverticula may enter the lumen of the stomach (unlike those stomachs provided with caeca, where the major typhlosole may prevent such entry (see Owen, 1953; 1956)), the presence of free enzymes in these forms may be indicative of differences in the mode of digestion by the stomach. The role of the structures associated with the stomach, such as the ducts and tubules of the digestive diverticula, and the crystalline style, as well as those of the stomach itself such as the epithelia of the folds and ridges, particularly of the major typhlosole, has to be investigated in greater detail before the nature of the digestive activity can be properly assessed.

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RESUMEN

VARIACION EN LA ESTRUCTURA ESTOMACAL DE LOS BIVALVOS

P. Dinamani

Se describe la estructura del estómago en 36 especies de bivalvos, usando una terminología uniforme. Detalles de esa estructura en varios grupos se compara con anteriores estudios por Graham (1949) y Purchon (1956a, 1957, 1958, 1960).

Los aspectos más significativos de la variación estructural parecen ser aquellos que resultan de la interacción entre las paredes del estómago y los ductos de los divertículos digestivos. Estos se abren ya individualmente, o en grupos en las paredes del estómago, o se juntan para abrirse en grupos comunes grandes. En los Eulamellibranchia principalmente, la pared del estómago se ajusta para acomodar el ducto en área definidas, sacos o lagunas de la pared. Estas modificaciones de la pared en los sitios donde los ductos se abren, pueden también estar seguidas por otros cambios, de los cuales el más notable es el que se muestra en el ciego mayor del estómago, que invade las regiones donde los ductos de los divertículos digestivos se han juntado. Así, en la línea extrema de modificación, como se revela en algunos Eulamellibranchia, el ciego se expande ampliamente y llega a estar íntimamente asociado con las aberturas de los ductos en sacos especiales (caeca) de la pared estomacal.

El grado de relación entre el estómago y los ductos se cree que tiene significación para interpretar la evolución de la estructura estomacal. Esta relación es estimada en un diagrama (Fig. 22) el cual representa los más importantes aspectos de variación en la estructura del estómago. Se reconocen inicialmente, tres aspectos principales que representan otros tantos grupos mayores de bivalvos, como sigue: 1) Arcacea + parte de Anisomyaria (Fig. 22C), 2) el resto de Anisomyaria (Fig. 22D), y 3) Eulamellibranchia (Figs. 22E¹ a E³), los que presentan una secuencia o relación de fase entre el estómago y los divertículos digestivos. El estómago protobranquio revela un patrón estructural que incorpora los aspectos básicos de los grupos mayores señalados, pero en tal grupo el sistema de ductos de los divertículos digestivos ha sido modificado, sin cambios básicos en la pared del estómago (Fig. 22A) y una línea similar de modi-

ficación en una rama de los Eulamellibranchia (Figs. 22 E²). Los Septibranchia presentan un plan de extrema simplificación, en el estómago y los divertículos digestivos (Fig. 22B).

Para basar análisis estructurales en otras direcciones funcionales, es necesario un mayor conocimiento de las funciones de los túbulos digestivos y ductos, el estilete cristalino y epitelio del estómago.

АБСТРАКТ

ИЗМЕНЧИВОСТЬ СТРОЕНИЯ ЖЕЛУДКА У ДВУСТВОРЧАТЫХ МОЛЛЮСКОВ

П. ДИНАМАНИ

В работе описано строение желудка у 36 видов двустворчатых моллюсков; при этом все морфологические термины были унифицированы. Были проведены сравнения деталей строения желудка у различных групп двустворчатых с более ранними исследованиями по этому вопросу Греема (1949) и Пёрчон (1956a, 1957, 1958, 1960).

Наиболее значительными структурными изменениями, повидимому являются те, которые появляются в результате взаимодействия между стенками желудка и протоками пищеварительных дивертикул. Последние могут открываться в стенках желудка или в виде отдельных отверстий, или целыми группами, или могут сливаться, открываясь одним общим крупным протоком. У Eulamellibranchia стенки желудка большей частью приспособлены для вывода протоков в определенных местах, образуя углубления или карманы. Такие модификации стенок желудка в тех местах, где открываются протоки могут также сопровождаться и другими изменениями; из них наибольшего внимания заслуживают те, которые производит в желудке крупный тифлозоль, который обычно вдается в те места, где наблюдаются скопления протоков пищеварительных дивертикул. Крайнюю линию модификации (как это наблюдается у некоторых Eulamellibranchia), представляет случай, когда тифлозоль выдается наиболее далеко и приходит в тесное соприкосновение с отверстиями протоков в специальных карманах (саеса), образованных в стенках желудка.

Соотношение между желудком и протоками пищеварительных дивертикул имеет значение с точки зрения эволюции структуры самого желудка. Оценка этих соотношений представлена графически на рис. 22, на котором представлены основные направления изменений в структуре желудка. Можно различить три главных типа изменений строения желудка у трех основных групп двустворчатых: 1) Arcacea + часть Anisomyaria (рис. 22C), 2) остальные Anisomyaria (рис. 22D) и 3) Eulamellibranchia (рис. 22E). Внутри последних отмечены еще три дополнительных линии модификации (рис. 22E¹-E³), которые представляют собою последовательные или фазовые соотношения между желудком и

пищеварительными дивертикулами. Желудок у *Protobranchia* обнаруживает структурные особенности, включающие основные черты строения крупных групп, указанных выше, но у этой группы система протоков пищеварительных дивертикул, претерпела модификацию, но без основных изменений в стенке желудка. (рис. 22A); сходная линия модификации обнаружена у одной линии *Eulamellibranchia* (рис. 22E²). *Septibranchia* представляют собою исключительно упрощенный план строения, как желудка, так и пищеварительных дивертикул (рис. 22B).

Чтобы обосновать структурный анализ на функциональнй основе, необходимо больше знать о функциях пищеварительных трубочек и протоков, кристаллического стебелька и эпителия желудка.

PARTICULARITES HISTOCHIMIQUES DE LA GLANDE
ET DE LA SOLE PÉDIEUSES D'*ARION RUFUS*
(STYLOMMATOPHORA: ARIONIDAE)

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RESUME

L'étude histochimique de la glande et de la sole pédieuses d'*Arion rufus* (Linnaeus) aboutit aux conclusions suivantes:

La glande pédieuse, très développée, est constituée de 2 régions au niveau desquelles l'histochimie met en évidence 2 types de mucocytes, a et b, dont la localisation est en relation avec l'existence de ces régions. Les mucocytes de type a, situés antérieurement, sécrètent des mucopolysaccharides acides complexes associés à des lipides complexes. Les mucocytes de type b de la région postérieure émettent des mucopolysaccharides simples peu acides.

La sole pédieuse est très riche en mucocytes qui correspondent à 4 types cellulaires histochimiquement distincts. Les mucocytes de type I contiennent des mucopolysaccharides simples peu acides, des mucopolysaccharides acides complexes et des lipides complexes. Les mucocytes de type II produisent des mucopolysaccharides acides simples et complexes. Les mucocytes de type III élaborent des polysaccharides acides et des lipoprotéines. Enfin les mucocytes de type IV synthétisent des mucoprotides dont la partie muqueuse est peu acide.

Les résultats obtenus chez *Arion rufus* sont discutés et comparés avec ceux effectués par: Campion (1961) sur *Helix aspersa*, Arcady (1963) sur *Lehmannia poirieri*, et Binot (1965) sur *Onchidella celtica*. *O. celtica* et *L. poirieri* ne possèdent que 2 types de mucocytes pédieux, *Arion* présente donc une plus grande diversité dans la sécrétion de sa sole. Quant aux mucocytes pédieux d'*Helix*, bien que produisant 4 sécrétions distinctes ils ne sont pas réellement assimilables à ceux d'*Arion* tant par leur nature histochimique que par leur répartition.

INTRODUCTION

Les recherches récentes de Thompson (1960) sur les sécrétions tégumentaires de divers Prosobranches et Opisthobranches marins et celles de Campion (1961) sur les glandes cutanées d'*Helix aspersa* ont attiré l'attention sur les glandes tégumentaires des Mollusques en général. D'autre part, l'une de nous, dans un travail antérieur (Binot, 1965) a étudié la glande et la sole pédieuses d'un Pulmoné marin *Onchidella celtica* (Cuv.). Il nous a paru intéressant d'étudier histochimiquement ces sécrétions pédieuses chez un Pulmoné terrestre,

Arion rufus (Linnaeus) afin d'en déceler la nature et d'établir une comparaison avec les résultats précédemment obtenus chez *O. celtica*.

MATERIEL ET TECHNIQUES

De jeunes *Arion rufus* fixés par les liquides de Bouin, Gendre O⁰, Carnoy, Ciaccio, formol 10% et formol sublimé ont été débités en coupes sérieées transversales et longitudinales de 5 à 7 μ . D'autre part des coupes de tissus frais ont été pratiquées au kryostat.

Anatomie microscopique: Coloration à l'azan et à l'hémalun picro-indigo-

carmin.

Acides nucléiques: Réaction de Feulgen et technique de Unna-Brachet au vert de méthyle-pyronine suivie du test chlorydrique selon Vendrely.

Glucides: Réaction de Hotchkiss-Mac Manus (A. P. S.: Acide périodique-Schiff) avec coupes témoins soumises à la digestion salivaire. Mucopolysaccharides: Coloration au bleu alcyan, réaction de Kramer à l'azur avec et sans sulfatation, réaction de Lison au bleu de toluidine avec gamme de pH allant de 2,99 à 5 et contrôles appropriés.

Mucus: Colorations à la fuchsine paraldehyde avec et sans oxydation et au mucicarmin de Mayer. La métachromasie conférée par l'hémalun, le bleu de toluidine et la pyronine à certains glucides et notamment aux mucopolysaccharides acides a donné des indications utiles.

Protéines: Technique de Salazar (protéines tannophiles), réaction de Millon et azoréaction (radicaux, phénols), réaction de Glenner (radicaux indol et pyrrol), tétrazonium de Danielli (radicaux indol, pyrrol et phénol) et enfin, réactions de Chévremont et Frédéric et au dihydroxy-dinaphtyl-disulphide (D.D.D.) (groupements SH et SS).

Lipides: Réaction au noir Soudan B: sur tissus frais pour les lipides monophasiques et sur coupes à la paraffine pour les lipides hétérophasiques (Ciaccio).

GLANDE PÉDIEUSE

A. Anatomie microscopique: La glande pédieuse d'*Arion rufus* logée dans l'épaisseur de la sole, à la jonction des tissus du manteau et du pied, est formée par un massif glandulaire s'étendant sur les 2/3 de la longueur de l'animal. Cette glande affecte la forme d'un cul de sac dont le "plancher", seul glandulaire, est bordé par un épithélium cilié de type pédieux tandis que le "plafond" constitué par du tissu conjonctif est limité par un épithélium simple de type épidermique banal. Ces 2 épithéliums délimitent

ainsi une cavité dans laquelle est émise la sécrétion des mucocytes de la glande pédieuse, cette cavité se rétrécit de moitié de l'avant (140 μ environ) vers l'arrière (70 μ seulement).

Anatomiquement cette glande, constituée par des cellules glandulaires de grande taille groupées en flots par un emballage conjonctivo-musculaire, comporte deux régions distinctes, particulièrement nettes sur des coupes transversales:

la région antérieure, la plus longue, se subdivise elle-même en 2 zones comme le confirmeront ultérieurement les techniques histochimiques: une zone *médiane* qui se flanque rapidement de zones *latérales*, épaissies et symétriques, faisant totalement défaut à l'entrée de la glande (Fig. 1 et 2)

la région postérieure, démunie de bourrelets latéraux, se prolonge en s'effilant jusqu'au tiers postérieur de l'animal où elle se termine (Fig. 3)

B. Histochimie. Les techniques utilisées permettent de distinguer 3 types cellulaires respectivement nommés a₁, a₂ et b. La répartition de ces 3 types de mucocytes dans la glande pédieuse correspond très exactement aux régions définies anatomiquement: antérieures latérales, antérieure médiane et postérieure.

a. *Les régions antérieures latérales* sont constituées uniquement par des cellules a₁ de forme ovoïde, mesurant 20 à 30 μ de diamètre et dont le noyau arrondi possède un nucléole apparent, une chromatine granuleuse et mesure 8 μ environ. Le contenu de ces cellules, métachromatique à l'hémalun, présente les caractères histochimiques suivants: coloré fortement après la réaction à l'A.P.S., il prend aussi le bleu alcyan et est légèrement métachromatique au bleu de toluidine à pH 2,99 indiquant la présence d'une faible quantité de *mucopolysaccharides acides complexes* (Pl. I, Fig. 1). De plus ces cellules contiennent aussi un *mucopolysaccharide neutre* puisqu'elles deviennent fortement métachromatiques après sulfatation selon

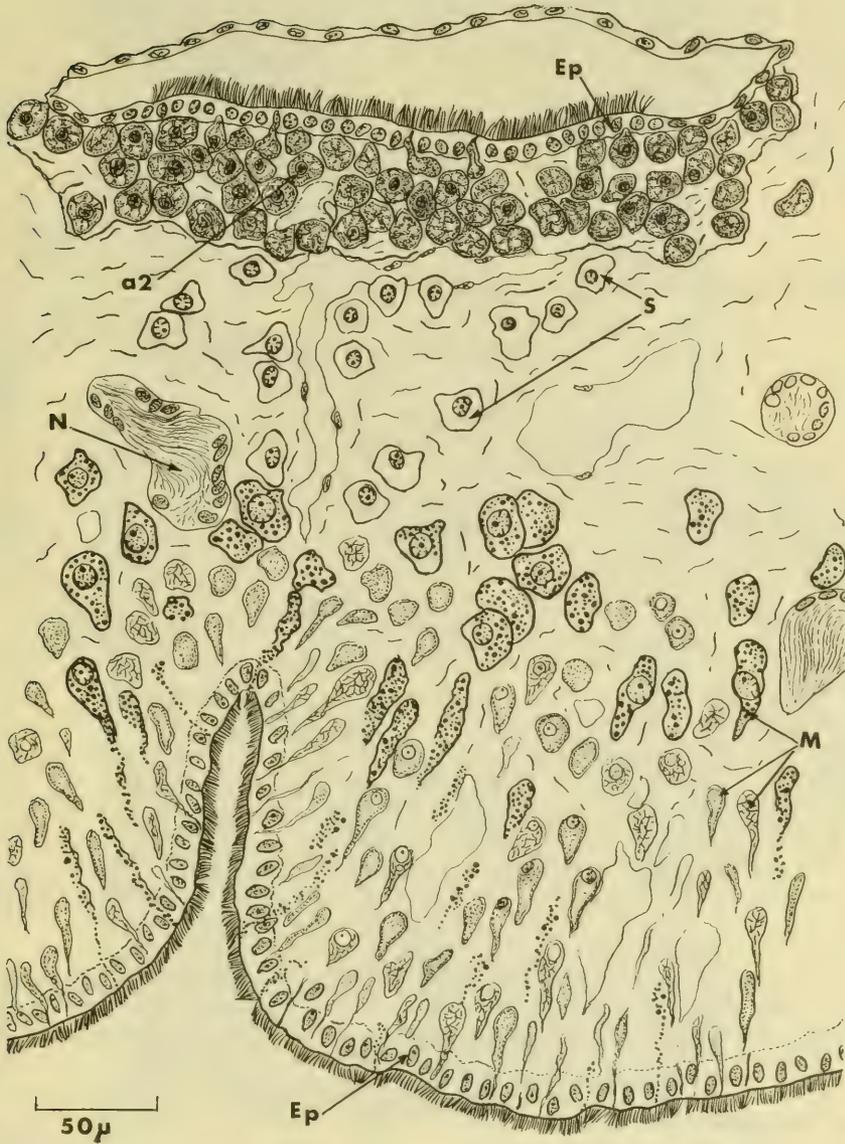


FIG. 1. Coupe transversale intéressant la glande pédieuse (entrée) et la sole pédieuse; a_2 : cellules de type a_2 ; Ep: épithélium cilié du canal de la glande pédieuse et de la sole; M: mucocytes de la sole pédieuse; N: nerf; S: stock de jeunes mucocytes.

Kramer (Pl. I, Fig. 2).

La réaction de Ciaccio y décèle la présence de quelques grains de lipides

hétérophasiques, mais seulement dans certaines cellules.

Enfin, la coloration au vert de méthyle-

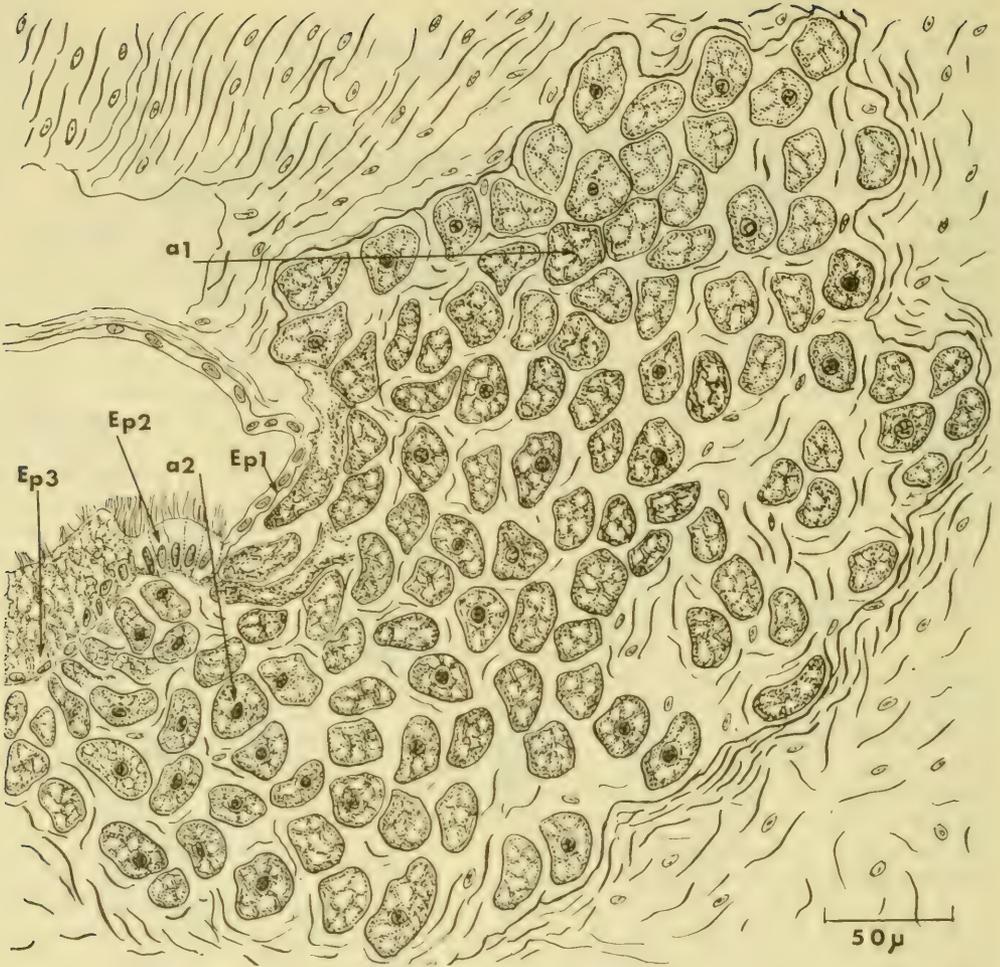


FIG. 2. Demi-coupe transversale de la région antérieure de la glande pédieuse (zone latérale et 1/2 zone centrale): a₁: jeunes mucocytes; a₂: mucocytes adultes; Canal de la glande pédieuse: Ep₁: épithélium des bords; Ep₂: épithélium des lèvres; Ep₃: épithélium de la région excrétrice centrale.

pyronine, complétée par le test chlorhydrique, montre que ces cellules contiennent des ribonucléines en quantité notable. En outre, nous n'avons jamais vu les cellules a₁ émettre leur produit de sécrétion à travers l'épithélium pédieux. Ce fait, joint à la présence d'un ergastoplasme abondant permet de penser que ces éléments ne représentent en fait que la forme jeune des cellules a₂, dont le produit de sécrétion possède,

mais en plus accentué, les mêmes caractères que celui des cellules a₁.

b) La région antérieure médiane est constituée uniquement par des cellules a₂ ayant le même aspect arrondi et la même taille que les cellules a₁, à l'exception des cellules émettrices qui prennent une forme allongée. Leur produit de sécrétion est un *mucopolysaccharide acide complexe* ainsi qu'en témoignent les caractères histochimiques: méta-

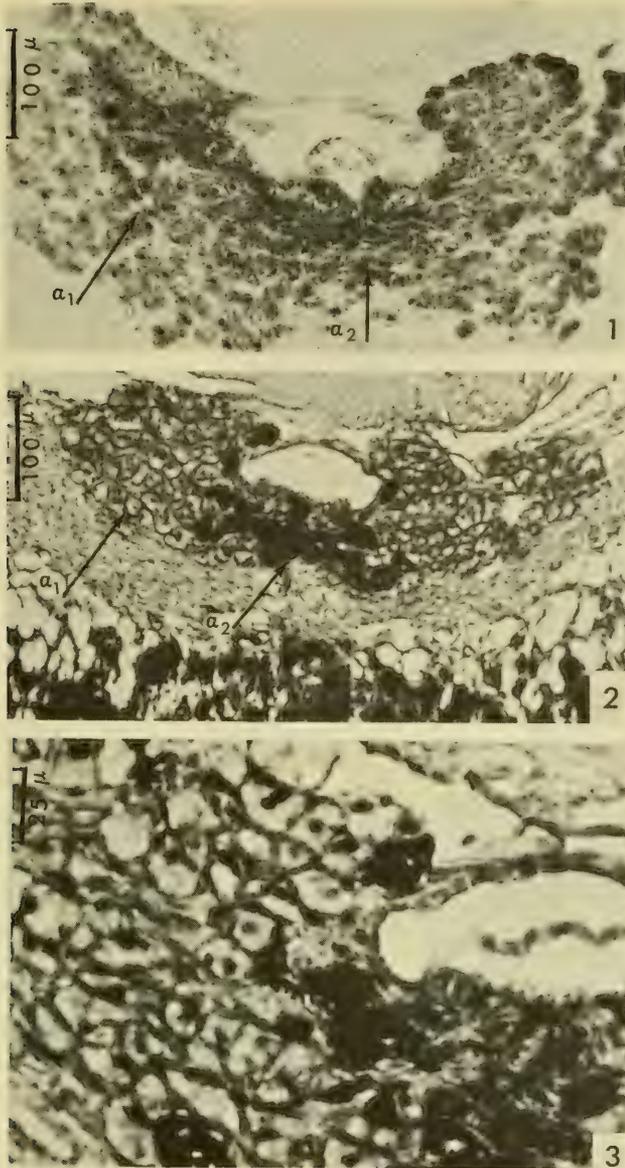


PLANCHE I. Glande pédieuse

1. Coupe transversale au niveau de la région antérieure; Bouin; hémalum picro indigo carmin; les cellules a_1 situées sur les côtés et les cellules a_2 au centre présentent le même aspect.
2. Coupe transversale au niveau de la région antérieure; Gendre O^0 ; réaction de Kramer après sulfatation; remarquez la métachromasie intense pour les cellules a_2 et plus légère pour les cellules a_1 .
3. Demi-coupe transversale au niveau de la région antérieure; Formol sublimé; Lison pH 2,99; métachromasie très intense des cellules a_2 et faible des cellules a_1 .

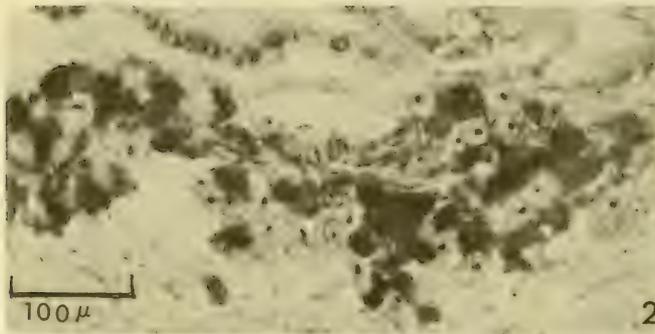
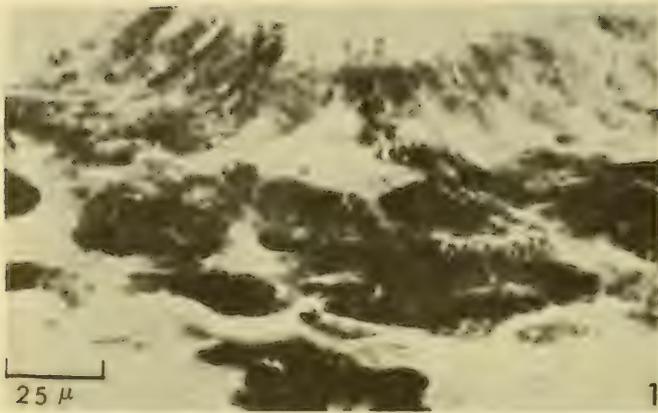


PLANCHE II. Glande pédieuse

1. Coupe transversale au niveau de la région antérieure médiane; Ciaccio; noir soudan B; les cellules a_2 seules visibles ici sont colorées en noir intense.
2. Coupe transversale au niveau de la région postérieure; Bouin; mucicarmin de Mayer; les cellules b qui ont pris le mucicarmin apparaissent en noir.
3. Coupe transversale au niveau de la région postérieure; Gendre; Salazar; certaines cellules b contiennent des grains colorés en noir par la technique de Salazar.

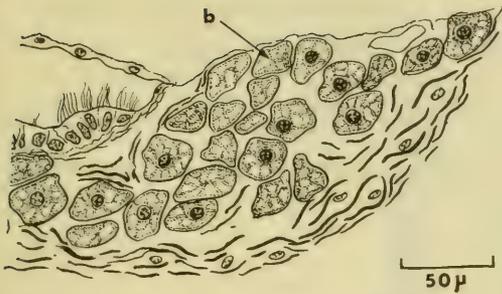


FIG. 3. Demi-coupe transversale de la région postérieure de la glande pédieuse: b: cellules de type b.

chromatique à l'hémalum et colorée en rose par l'A.P.S., cette sécrétion est aussi très métachromatique au bleu de toluidine à partir de pH 2,99 (Pl. I, Fig. 3), et prend fortement le bleu alcyan. La teinte éclatante conférée par la pyronine à ces cellules a_2 persiste après test chlorydrique, se superpose à la métachromasie au bleu de toluidine et, comme cette dernière, n'atteint que le produit de sécrétion lui même; il ne s'agit donc pas d'ergastoplasme.

La réaction de Ciaccio, très positive, prouve que ces cellules sont bourrées de lipides complexes (Pl. II, Fig. 1).

c) *La région postérieure* ne comporte que des cellules b, ayant le même aspect que les mucocytes a_1 et a_2 de la région antérieure, mais leur sécrétion est histochimiquement différente: non coloré par l'hémalum, le contenu de ces cellules b n'est métachromatique au bleu de toluidine qu'à partir de pH 4,53; il est très coloré par la fuchsine-paraldéhyde avec oxydation préalable et très faiblement sans oxydation. Cette sécrétion paraît donc composée de mucopolysaccharides simples peu acides auxquelles s'ajoutent en faible proportion des mucopolysaccharides neutres ainsi que l'indique la métachromasie discrète à l'azur, après sulfatation selon Kramer.

Quand aux réactions des protides, seules celles très peu spécifiques au mucicarmin pour les mucoprotides (Pl. II, Fig. 2) et de Salazar pour les protides tannophiles (Pl. II, Fig. 3) donnent des

résultats positifs, d'ailleurs absolument superposables. Etant donnés les résultats obtenus après ces 2 colorations, on était en droit d'espérer des réactions positives avec les diverses techniques spécifiques des protides mises en oeuvre ici, qui malheureusement sont toutes restées sans résultats, non seulement pour les cellules b mais aussi pour les cellules a_1 et a_2 . C'est pour quoi, dans le tableau I qui rappelle les observations histochimiques effectuées au niveau de la glande pédieuse d'*Arion rufus*, les réactions des protides ne sont pas mentionnées bien qu'elles aient été pratiquées.

d) *L'épithélium de la glande pédieuse* varie selon les divers niveaux de cette formation:

A l'entrée de la glande, région très courte où il n'y a que des cellules a_2 , cet épithélium est cubique avec noyaux arrondis et centraux; il possède une ciliature excepté sur les bords (Fig. 1).

Au delà de l'entrée, dans les régions antérieure et moyenne de la glande pédieuse où l'on trouve à la fois des cellules a_1 latérales et a_2 centrales, cet épithélium pédieux présente, des côtés vers le milieu, 3 aspects différents (Fig. 2): sur les bords il est cubique et non cilié (Ep 1); puis viennent 2 lèvres fortement marquées dont l'épithélium prismatique cilié possède des noyaux dressés selon le grand axe de la cellule (Ep 2); entre ces lèvres, une zone médiane où les noyaux sont refoulés vers la base des cellules épithéliales ciliées (Ep 3). Cette zone est privilégiée pour l'excrétion; en effet, c'est à cet endroit que sont émis les grains de sécrétion des cellules les plus évoluées, au travers des cellules épithéliales.

Dans la région postérieure, où il n'existe que des cellules b, l'épithélium pédieux présente les 3 aspects décrits dans les régions antérieure et moyenne, mais de façon beaucoup plus atténuée (Fig. 3).

SOLE PÉDIEUSE

A. Histochimie: L'analyse histo-

TABLEAU 1. Caractères histochimiques des cellules de la glande pédieuse d'*Arion rufus*

Techniques	cellules a ₁ -régions latérales	Cellules a ₂ région médiane	cellules b région postérieure
Hémalun-picro-indigo carmin	métachromatique	métachromatique	non métachromatique
Unna Brachet	+	++	+
Unna Brachet + test de Vendrely	-	++	-
A. P. S.	++	+	++
A. P. S. + digestion salivaire	++	+	++
Lison pH 2,99	+ métachromatique mauve	++ métachromatique violet foncé	non métachromatique
Lison pH 4,53	+	++	+
Bleu alcyan	+	++	E
Kramer (Azur)	bleu	métachromatique	bleu
Sulfatation + Kramer	+ métachromatique	++ métachromatique	E métachromatique
Fuchsine paraldéhyde sans oxydation	E	E	E
Fuchsine paraldéhyde avec oxydation	±	±	+
Mucicarmin	-	-	+
Salazar	-	-	+
Lipides monophasiques	-	-	-
Lipides hétérophasiques	+	++	-
Résultats:	M. P. S. acides complexes + M. P. S. neutres + un peu de lipides complexes	M. P. S. acides complexes + lipides complexes	M. P. S. simples peu acides et meme peut-etre neutres

A. P. S. = Acide périodique-Schiff

M. P. S. = mucopolysaccharides

E = en faible quantité

chimique prouve l'existence de 4 types de mucocytes numérotés de I à IV.

a. *Les mucocytes de type I*: sécrètent une substance hétérogène composée d'un mélange de *mucopolysaccharides acides complexes* et de *mucopolysaccharides neutres* auxquels s'associent des *lipides complexes* comme le montrent les réactions auxquelles ces cellules répondent

positivement, entre autres: la forte métachromasie après réaction de Lison au pH le plus bas, la métachromasie à l'azur accrue après sulfatation et une affinité intense pour le noir Soudan B après postchromisation selon Ciaccio (Fig. 4, I; Pl. III, Fig. 1, 2 et 3).

b. *Les mucocytes de type II*: présentent les mêmes caractères que les précé-

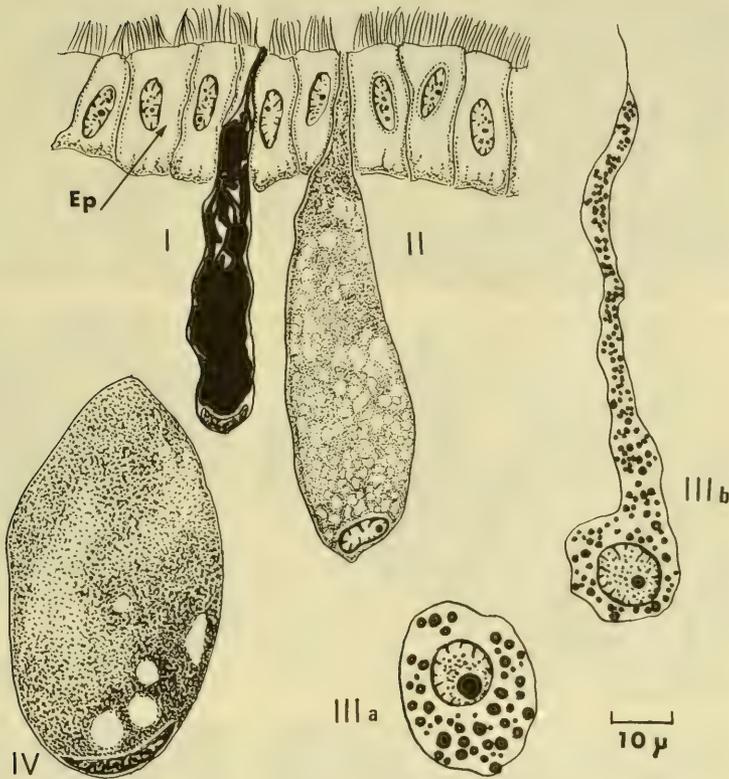


FIG. 4. Mucocytes de la sole pédieuse. Ep: épithélium cilié de la sole; I: cellule de type I; II: cellule de type II; IIIa: jeune cellule de type III; IIIb: cellule évoluée de type III; IV: cellules de type IV.

dents en ce qui concerne les mucopolysaccharides mais avec une intensité moindre. Cependant, ils s'en distinguent par l'absence de lipides hétérophasiques (Fig. 4, II; Pl. III, Fig. 2, 3).

c. *Les mucocytes de type III:* leur contenu toujours granuleux présente les caractères histochimiques suivants: rose après A.P.S., il prend la fuchsine paraldéhyde avec et sans oxydation. Quant aux protides, seules les réactions de Chévrement et Frédéric et au tétrazonium de Danielli sont positives, indiquant la présence de groupements réducteurs dont la nature n'a pu être définie malgré la mise en oeuvre des réactions spécifiques des radicaux.

D'autre part, la technique de Ciaccio permet d'y déceler un peu de lipides complexes. En résumé, ces cellules émettent une sécrétion hétérogène formée d'une substance mucolide associée à des lipides hétérophasiques (Fig. 4, III; Pl. III, Fig. 6).

d. *Les mucocytes de type IV:* ces derniers, de plus grande taille que les précédents élaborent un produit faiblement métachromatique au bleu de toluidine dès le pH 2,99 ainsi qu'à l'azur après sulfatation et qui, en outre, prend la fuchsine paraldéhyde après oxydation. Les réactions des protides concernant les groupements SH et les groupements indol, pyrrol et phénol sont positives;



PLANCHE III. Divers types de mucocytes de la sole pédieuse

1. Mucocytes de type I; Ciaccio; noir soudan B.
2. Mucocytes de type I (en noir) et II (en gris); formol-sublimé; Lison à pH 2,99.
3. Mucocytes de types I et II; formol-sublimé; bleu aleyan.
4. Deux mucocytes de type IV; Bouin; fuchsine paraldéhyde avec oxydation.
5. Détail d'un mucocyte de type IV; même technique.
6. Mucocytes de type III; Bouin; fuchsine paraldéhyde sans oxydation; noter l'aspect granuleux de la sécrétion.

TABLEAU 2. Caractères histochimiques des mucocytes de la sole pédieuse d'*Arion rufus*

Techniques	Mucocyte I	Mucocyte II	Mucocyte III	Mucocyte IV
Hemalun picro-indigo carmin	métachromatique	non métachromatique	non métachromatique	non métachromatique
Unna-Brachet	+	+	++	-
Unna-Brachet + test de Vendrely	+	+	-	-
A. P. S.	++	+	+ (rose)	rose
A. P. S. + digestion salivaire	++	+	+ (rose)	rose
Lison pH 2, 99	++	+	non métachromatique	E
Lison pH 4, 53	++	+	non métachromatique	+
Bleu Alcyan	+	+	-	E
Kramer (azur)	(+)	(+)	bleu	-
Sulfation + Kramer	++	++	bleu	légèrement métachromatique
Fuchsine paraldéhyde sans oxydation	(+)	(+)	++	-
Fuchsine paraldéhyde avec oxydation	+	+	++	++
Mucicarmin	+	+	-	+
Salazar	E	E	-	+
D. D. D.	-	-	-	+
Thioglycolate + D. D. D.	-	-	-	+
Iodacétate + D. D. D.	-	-	-	-
Chèvremont et Frédéric	-	-	+	+
Tetrazonium de Danielli	-	-	+	+
Glenner	-	-	-	+
Xanthidrol	-	-	-	+
Millon	-	-	-	E
Diazotation	-	-	-	-
Lipides monophasiques	-	-	-	-

Tableau 2 (continued)

Techniques	Mucocyte I	Mucocyte II	Mucocyte III	Mucocyte IV
Lipides hétéro-phasiques	++	-	+	-
Résultats:	M. P. S. simples peu acides + M. P. S. acides complexes + M. P. S. neutres + lipides complexes	M. P. S. acides simples + M. P. S. acides complexes	Polysaccharides acides + lipo- protéines	Mucoprotides dont la partie muqueuse est peu acide

A. P. S. = Acide périodique-Schiff
D. D. D. = dihydroxy-dinaphtyl-disulphide

M. P. S. = mucopolysaccharides
E = en faible quantité

par contre, la technique de Ciaccio demeure sans résultat. Ces cellules produisent donc un *mucoprotide* dont la partie muqueuse est *peu acide* (Fig. 4, IV; Pl. III, Fig. 4, 5).

Le tableau II résume ces résultats.

B. Répartition

Ces 4 types de mucocytes différents histochimiquement présentent en outre quelques variations quant à leur répartition tout au long de la sole pédieuse:

Les mucocytes de type I sont particulièrement nombreux sur les côtés et au milieu de la sole mais deviennent plus rares dans la région postérieure.

Les mucocytes de type II, les plus nombreux de tous, présentent une répartition homogène.

Les mucocytes de type III, beaucoup moins nombreux que ceux des 2 premiers types se localisent surtout dans la région la plus antérieure de la sole; en effet dans la région postérieure ils sont très clairsemés.

Quant aux mucocytes de type IV, les moins fréquents de tous, ils sont pratiquement absents des régions antérieure et postérieure, leur siège d'élection étant la région moyenne de la sole.

L'évolution de ces mucocytes, quelque soit leur type, se fait toujours de la profondeur du tégument vers sa périphérie; des cellules jeunes plus profondes, de forme arrondie ont un

noyau pourvu d'un nucléole bien visible et possèdent un ergastoplasme net; puis ces cellules se rapprochent progressivement de la surface en se chargeant de la sécrétion qui les caractérise; enfin en vieillissant les mucocytes, dont les noyaux se pycnosent, s'effilent et viennent déboucher entre les cellules épithéliales de la sole. Remarquons que pour les mucocytes de la glande pédieuse l'évolution est exactement la même, une différence existe cependant quant au mode d'excrétion. En effet, il semble que les cellules de la glande pédieuse n'émettent pas leur produit entre deux éléments épithéliaux comme ceux de la sole mais au travers de l'épithélium lui-même, bien qu'il soit difficile de l'affirmer. Une étude en microscopie électronique permettrait sans doute de répondre à cette question.

DISCUSSION

Certains des résultats obtenus appellent une discussion; c'est le cas notamment pour les régions latérales et centrales de la glande pédieuse antérieure où 2 types de cellules, a_1 et a_2 , ont été mis en évidence, types que nous n'avons pas cru devoir considérer comme 2 catégories distinctes mais plutôt comme 2 états évolutifs d'un même type cellulaire. En effet, une

filiation plus que probable entre les cellules a_1 et a_2 peut se déduire des faits suivants: les cellules a_1 que l'on ne voit jamais excréter contiennent comme tous les jeunes mucocytes beaucoup de ribonucléines pyroninophiles et sécrètent en faible quantité les produits que l'on trouve en abondance dans les cellules a_2 qui occupent la zone centrale de la glande pédieuse, seul endroit où la sécrétion est émise.

En comparant les résultats obtenus par Binot (1965) sur la glande pédieuse d'*Onchidella celtica* et nos propres observations sur cette même glande chez *Arion rufus*, on remarque que chez ces 2 espèces, la glande pédieuse renferme 2 catégories cellulaires qui élaborent en fin de compte des produits assez comparables histochimiquement. La seule différence notable concerne la taille de cette glande pédieuse qui est beaucoup plus développée chez *A. rufus* que chez *O. celtica*, ceci étant sans doute en relation avec le mode de vie terrestre de la première espèce.

Si l'on confronte les faits mis en évidence au niveau de la sole pédieuse d'*Arion rufus* et ceux décrits par Arcady (1963) sur *Lehmannia poirieri* et par Binot (1965) sur *Onchidella celtica* on constate que la sole pédieuse de *L. poirieri* ne comporte que 2 catégories de mucocytes granuleux se distinguant seulement par leur taille et non par la nature de leur sécrétion puisque dans les 2 cas le produit final est un mucopolysaccharide acide complexe. Chez *O. celtica*, les mucocytes de la sole, assez peu nombreux et localisés dans les sillons pédieux rappellent les 2 catégories cellulaires de la glande pédieuse de cette même espèce. Chez *A. rufus* la sole pédieuse est extrêmement riche en mucocytes, sans localisation privilégiée, qui appartiennent à 4 catégories distinctes ainsi que le prouvent leurs caractères histochimiques nettement tranchés. Il est intéressant de remarquer que chez *A. rufus* comme chez *O. celtica* les 2 types les mieux représentés numériquement dans la sole (I et II) présentent les mêmes caractères

histochimiques que les 2 types cellulaires (a et b) existant dans la glande pédieuse. Un point cependant différencie franchement *A. rufus* des 2 autres espèces envisagées, c'est la plus grande diversité des produits émis par les mucocytes de la sole.

En rapprochant nos résultats de ceux récemment obtenus par Campion (1961) on peut dire ce qui suit: il existe chez *Helix aspersa* 4 types de mucocytes (A, B, C, D) dont certains seulement peuvent être rapprochés avec prudence de ceux que nous avons décrit chez *Arion*; c'est le cas des cellules A et B d'*Helix* qui par leur contenu histochimique pourraient à la rigueur être comparés respectivement aux types II et I de la sole d'*Arion*. En ce qui concerne les mucocytes de type D on peut peut-être faire un rapprochement avec les cellules III. Quant aux mucocytes de type C, il semble impossible de les assimiler à notre catégorie IV. En résumé les points communs entre *Helix* et *Arion* portent surtout sur la présence de 4 catégories de mucocytes; mais la nature histochimique et la répartition de ces cellules, différentes pour ces 2 espèces rendent impossible toute assimilation véritable.

Nous inspirant des travaux de Thompson (1960) basés sur l'utilisation du papier pH pour mesurer le degré d'acidité des sécrétions tégumentaires de divers Prosobranches et Opisthobranches marins nous avons repris ces expériences chez *Arion* afin de connaître le pH des sécrétions tégumentaires de ce Pulmoné.

La réaction ionique effectuée au niveau du manteau et du pied se montre très proche de la neutralité tant pour les limaces au repos que pour celles soumises à une excitation mécanique. Rappelons que chez les nombreuses espèces testées par Thompson ces sécrétions présentent un pH très faible généralement compris entre 1 et 2 et que l'auteur considère l'acidité de ces sécrétions tégumentaires comme un moyen de dissuasion vis à vis des prédateurs. Il semble donc que Le

tegument d'*Arion* n'émet pas une sécrétion acide défensive comme c'est le cas pour de nombreux Prosobranches et Opisthobranches marins.

Enfin, une dernière remarque nous paraît importante avant de clore cette discussion: ayant noté la présence dans l'épaisseur du pied d'*Arion*, entre les tissus de la sole et de la glande pédieuse (Fig. 1), d'une zone occupée par des cellules claires rappelant de jeunes mucocytes, il n'est pas interdit de considérer cette zone comme un stock commun de cellules jeunes pouvant par différenciation engendrer les divers types de mucocytes décrits tant au niveau de la glande pédieuse que de la sole. Peut être une étude histochimique du développement embryonnaire de ces régions permettrait-elle de résoudre ce problème.

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ABSTRACT

THE HISTOCHEMICAL NATURE OF THE PEDAL GLAND AND SOLE OF THE FOOT IN *ARION RUFUS* (STYLOMMATOPHORA: ARIONIDAE)

M. Chétail and D. Binot

In the well developed pedal gland, 2 types of mucocytes, "a" and "b", can be demonstrated histochemically, whose location corresponds with 2 well defined parts of the gland. Type "a" mucocytes, located anteriorly, secrete acid complex mucopolysaccharides and complex lipids. Type "b", in the posterior part of the gland, discharge slightly acid simple mucopolysaccharides.

The pedal sole is crowded with mucocytes of 4 histochemically distinct cellular types: those of "type I" contain simple feebly acid mucopolysaccharides; those of "type II" both simple and complex mucopolysaccharides; those of "type III" acid polysaccharides as well as lipoprotids and, finally, those of "type IV" acid polysaccharides, the mucous part of which is slightly acid.

Comparing these results with those obtained by Campion (1961) in *Helix aspersa*, by Arcady (1963) in *Lehmannia poirieri* and by Binot (1965) in *Oncidiella celtica*, we find that the sole of *Arion rufus* show greater variety in its secretion than the 2 last-named species, which contain only 2 types of mucocytes. As for *Helix*, although the mucocytes of the pedal sole also produce 4 distinct secretions, these are not truly comparable either histochemically or in location, with those of *Arion*.

RESUMEN

NATURALEZA HISTOQUIMICA DE LA GLANDULA Y SUELA PEDAL
EN *ARION RUFUS* (STYLOMMATOPHORA: ARIONIDAE)

M. Chétail y D. Binot

En la glándula pedal bien desarrollada, dos tipos de mucocitos, "a" y "b" pueden demostrarse histoquímicamente, y cuya ubicación corresponde a otras dos bien definidas partes de la glándula. Tipo "a" de posición anterior es secretor de un complejo ácido de mucopolisacaridos y complejo lípido. Tipo "b" en la parte posterior, descarga ligeramente ácido mucopolisacarido simple.

La suela pedal esta coronada con mucocitos de cuatro histoquímicamente distintos tipos celulares: los del tipo "I" contienen ácido mucopolisacarido simple; tipo "II" contienen mucopolisacaridos simple y complejos; aquellos del tipo "III" ácido polisacarido asi como también lipotrópidos; y finalmente los del tipo "IV" ácido polisacarido, la parte mucosa del cual es ligeramente ácida.

Comparando estos resultados con aquellos obtenidos por Campion (1961) in *Helix aspersa*, por Arcady (1963) en *Lehmannia poirieri*, y por Binot (1965) en *Onchidella celtica*, encontramos que la suela de *Arion rufus* muestra mayor variedad en su secreción que las dos últimas especies nombradas las cuales contienen solamente dos tipos de mucocitos. En cuanto a *Helix*, aunque los mucocitos de la suela pedal también producen cuatro secreciones distintas, estas no son verdaderamente comparables, ya sea histoquímicamente o en ubicación, con aquellas de *Arion*.

ZUSAMMENFASSUNG

HISTOCHEMISCHE UNTERSUCHUNG DER FUSSDRÜSE UND DER FUSSOHLE
VON *ARION RUFUS* (STYLOMMATOPHORA: ARIONIDAE)

M. Chétail und D. Binot

Die stark entwickelte Fussdrüse von *Arion rufus* (L.) lässt 2 Teile erkennen, deren jeder auch histochemisch deutlich unterscheidbare Schleimzellen enthält: der vordere Teil solche vom Typ "a", der hintere vom Typ "b". Schleimzellen "a" scheiden komplexe, saure, mit komplexen Lipiden verbundene Mucopolysaccharide aus, diejenigen vom Typ "b" hingegen sondern einfache, nur schwach saure Mucopolysaccharide ab.

Die Fussohle ist reich an Schleimzellen, die 4 histochemisch verschiedenen Zellentypen entsprechen: Zellen vom Typ I enthalten einfache, schwach saure, sowie komplexe saure Mucopolysaccharide, wie auch komplexe Lipide; die vom Typ II saure Mucopolysaccharide einfacher wie auch komplexer Natur; die vom Typ III saure Polysaccharide und Lipoproteine; die vom Typ IV schliesslich Mucoproteide deren schleimige Komponente nur schwach sauer ist.

Ein Vergleich dieser Befunde mit denen von Campion (1961) für *Helix aspersa*, von Arcady (1963) für *Lehmannia poirieri* und von Binot (1965) für *Onchidella celtica* zeigt, dass die Ausscheidungen der Fussohle in *Arion* vielfältiger sind, als diejenigen der beiden letztgenannten Arten, die nur 2 Typen von Schleimzellen aufweisen. *Helix* erzeugt zwar auch 4 verschiedene Sekretionen, diese sind jedoch mit denen von *Arion* weder ihrer Verteilung nach, noch histochemisch, wirklich vergleichbar.

АБСТРАКТ

ГИСТОХИМИЧЕСКАЯ ПРИРОДА ПЕДАЛЬНОЙ ЖЕЛЕЗЫ И ПОДОШВЫ
НОГИ У *ARION RUFUS* (STYLOMMATORPHORA: ARIONIDAE)

Даниель Бино и Моника Четейл

В хорошо развитой ножной железе слизняка *Arion rufus* гистохимически хорошо различаются мукоциты типа "А" и типа "Б"; их местоположение связано с двумя хорошо развитыми частями железы. Мукоциты типа А, расположенные впереди, выделяют кислый комплекс мукополисахаридов и комплекс липидов. Мукоциты типа Б, находящиеся в задней части железы, выделяют слабо кислые простые мукополисахариды.

Подошва ноги наполнена мукоцитами четырех гистохимически-различных типов клеток: I тип, где клетки содержат простые слабо-кислые мукополисахариды; II тип, содержащие как простые, так и сложные мукополисахариды; III тип - с кислыми полисахаридами и с липопротеидами и, наконец, IV тип - с кислыми полисахаридами, слизистая часть которых имеет слабо-кислую реакцию.

Сравнивая приведенные выше данные с результатами работ Кемпшон (1961) по *Helix aspersa*, Эркеди (1963) по *Lehmannia poirieri* и вино (1965) по *Onchidella celtica*, авторы приходят к выводу, что подошва ноги у *Arion rufus* имеет большее разнообразие видов секреции, чем два последних вида, которые обладают лишь двумя типами мукоцитов. Что же касается *Helix aspersa* то хотя мукоциты подошвы ее ноги также выделяют 4 различных секрета, но они несравнимы с *Arion* ни гистохимически, ни по местоположению секреторирующих клеток.

CORRELATION BETWEEN NEUROSECRETORY CHANGES AND MATURATION
OF THE REPRODUCTIVE TRACT OF *ARION ATER*
(STYLOMMATOPHORA: ARIONIDAE)

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ABSTRACT

The nervous systems of *Arion ater* (Linnaeus) from animals at various known stages in the reproductive maturation cycle were examined for neurosecretory material by staining serial sections with Paraldehyde-fuchsin. Patterns of neurosecretory material in nerve cells and tracts of such material in the axons were mapped for the 3 main ganglion masses of the central nervous system (the supra-oesophageal, pleuro-parieto-visceral and pedal ganglion masses) and were obtained from the buccal ganglia and the optic tentacles. The main correlation shown between the maturation of the reproductive tract and neurosecretion occurred at a "critical point", the onset of maturation of the female reproductive glands. Certain small cells in the pleuro-parieto-visceral ganglion mass showed neurosecretory activity at this stage only. This stage is termed "critical", because experimental adverse conditions grossly retarded maturation if applied before this stage, but did not affect it much if applied after that stage had been reached. A possible control system for the maturation of the reproductive cycle is postulated from these results.

INTRODUCTION

Several workers have recently described various aspects of maturation of the pulmonate reproductive tract and suggested possible controlling factors. Laviolette (1954) described the role of the gonad in the maturation of the reproductive tract of the Arionidae, suggesting a hormonal system. Herlant-Meewis & van Mol (1959) suggested a relationship between neurosecretory activity and reproduction in 2 species of *Arion*, and Pelluet & Lane (1961) described the control of gamete maturation by brain and eyestalk substances in *Arion* species. Recently the present author (Smith, 1966a) described the

various maturation stages of the reproductive tract of *Arion ater* (Linnaeus) and found a close correlation between maturation and the season. The present study, based on the same specimens, was undertaken in order to ascertain the correlation, if any, between neurosecretory activity and maturation of the reproductive cycles. It should be noted that the work was carried out in England, and that months mentioned in connection with changes in the reproductive tract should be correlated with northern hemisphere seasons. As a foundation for the investigation of the patterns of neurosecretion, the anatomy of the central nervous system of *Arion ater* was described in detail (Smith,

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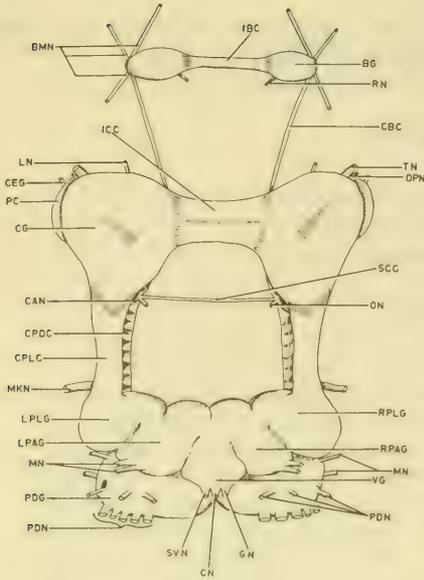


FIG. 1. A diagrammatic posterior view of the central nervous system showing the general anatomy and the origins of the nerves.

KEY TO ABBREVIATIONS

- | | |
|-------|---------------------------------------|
| ANT | anterior |
| AX | axon |
| BG | buccal ganglion |
| BMN | buccal mass nerve |
| CAN | cerebral anastomosis |
| CBC | cerebro-buccal connective |
| CEG | cephalic gland |
| CG | cerebral ganglion |
| CN | cardiac nerve |
| CPDC | cerebro-pedal connective |
| CPLC | cerebro-pleural connective |
| DCG | dorsal cell group |
| GN | genital nerve |
| IBC | inter-buccal commissure |
| ICC | inter-cerebral commissure |
| LN | lip nerve |
| LPAG | left parietal ganglion |
| LPLG | left pleural ganglion |
| MKN | mantle & kidney nerve |
| MN | mantle nerve |
| MSC | mesocerebrum |
| MTC | metacerebrum |
| NS | neurosecretory drops |
| OC | otocyst |
| OCN | otocyst nerve |
| ON | oesophageal nerve |
| OPN | optic nerve |
| PC | procerebrum |
| PCG | posterior cell group |
| PDG | pedal ganglion |
| PDN | pedal nerve |
| PLPDC | pleuro-pedal-connection |
| PPVG | pleuro-parieto-visceral ganglion mass |
| RN | radular nerve |
| RPAG | right parietal ganglion |
| RPLG | right pleural ganglion |
| SCC | subcerebral commissure |
| SVN | stomach and visceral nerve |
| T | tract of secretion |
| TN | tentacular nerve |
| VCG | ventral cell group |
| VG | visceral ganglion |
| VN | visceral nerve |
| VLCG | ventro-lateral cell group |
| VMCG | ventro-mesial cell group |

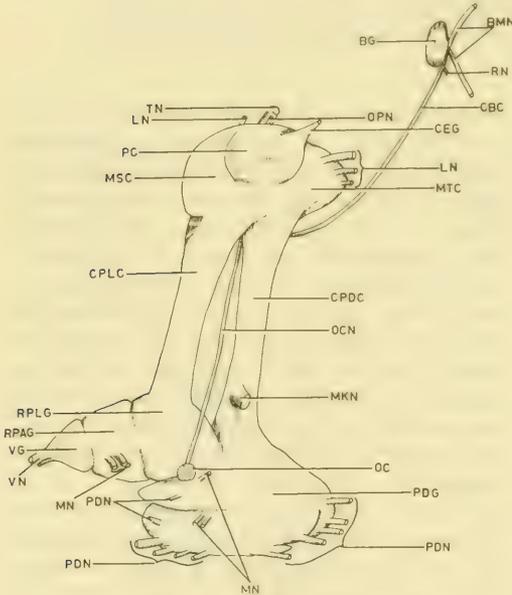


FIG. 2. A diagrammatic lateral view of the central nervous system from the right side showing the general anatomy and the origins of nerves.

1966b). For the purpose of this investigation, neurosecretory material is defined as paraldehyde-fuchsin positive material. The validity of this definition has been discussed elsewhere (Smith, 1966b).

MATERIALS AND METHODS

A total of 65 central nervous systems and optic tentacles from animals used in the determination of the maturation stages of the reproductive tract of *Arion ater* (Smith, 1966a) have been examined: the maturation stage of each individual is thus precisely known. These slugs comprized a natural population as well as individuals from controlled environment experiments. Since the techniques used were time consuming, the investigation was limited to a few specimens (2-4) representative of each maturation stage of the hermaphrodite gland, from each of the various groups.

The nervous systems were fixed in Susa for 6 hours or in Elftman-Dichromate-Sublimate for 3 days (Elftman, 1957). They were then transferred to Cellosolve, embedded in Ester Wax and serial sections were cut at 10μ . These were stained in Paraldehyde-fuchsin using a slight modification of the technique of Gabe (1953) (Smith, 1966b). The distribution of positive staining material in the nerve cell bodies and nerve fibres was then mapped at each hermaphrodite gland stage (see below) and the maps compared. Cell sizes were measured by means of a micrometer eye piece.

GENERAL ANATOMY OF THE CENTRAL NERVOUS SYSTEM

The detailed anatomy of the central nervous system of *Arion ater* has been described elsewhere (Smith, 1966b). It is sufficient here to give a brief outline. The pulmonate central nervous system consists of a highly complex circum-oesophageal ring formed by the amalgamation of the 9 primitive pulmonate ganglia (Bargmann, 1930). For con-

venience of description it is easier to divide the nerve ring in *Arion* into 3 large ganglion masses (Figs. 1 and 2):

1. The supra-oesophageal ganglion mass,
2. the pleuro-parieto-visceral ganglion mass,
3. the pedal ganglion mass.

Although the grooves on the surface of the ganglion masses do not necessarily indicate the positions of fusion of the original constituent ganglia, it is convenient to name the parts suggested by these grooves as if they were in fact the constituent ganglia.

PATTERNS OF NEUROSECRETION AT DIFFERENT STAGES OF MATURATION OF THE HERMAPHRODITE GLAND

Wherever possible animals were chosen from collections covering as wide a range of dates as possible in order to attempt to differentiate seasonal from maturation changes. Each part of the nervous system is considered separately, descriptions of neurosecretion being given with special reference to the states of activity of the secretory cells and to the presence and routes of any tracts of neurosecretion. Diagrams of each of the ganglion masses are given showing the distribution of the neurosecretory tracts and their relationships to the cell groups. The tracts are referred to in the text by numbers which correspond to those given in the diagrams.

The reproductive stages of the hermaphrodite gland, described in detail in an earlier paper (Smith, 1966a) are briefly summarized as follows:

STAGE

- | | |
|----|--------------------------------|
| A: | Spermatogonia and spermatocyte |
| B: | Early Spermatid |
| C: | Late Spermatid |
| D: | Early Spermatozoa |
| E: | Mid Spermatozoa |
| F: | Late Spermatozoa |
| G: | Early Oocyte |

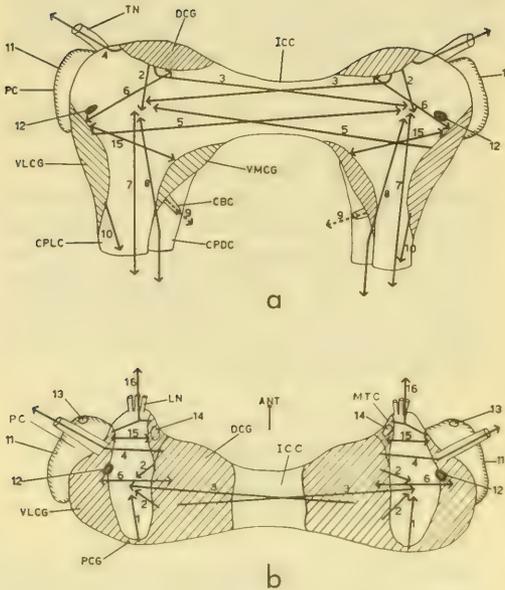


FIG. 3. Diagrams of the posterior (a) and dorsal (b) views of the supra-oesophageal ganglion mass showing the distribution of neurosecretory cells and tracts. For an explanation of the numbers see text.

H: Late Oocyte
I: Atrophy

The titles of these stages denote the predominant component in sections of the hermaphrodite gland. Where 2 or more stages go under the same gametogenesis designation but can be distinguished histologically, they are distinguished temporally. The main significant period in the maturation of the reproductive tract occurs during Stage E of the hermaphrodite gland, when mature sperm commence to leave the gland and copulation occurs. At that time all the female reproductive glands also differentiate and start to mature. The significance of this stage will be discussed in more detail later.

Supra-oesophageal Ganglion Mass

The pattern of neurosecretion in this ganglion mass is highly complex, but seems to be largely independent of the

state of the maturation of the reproductive system. The distribution of the neurosecretory cells and tracts, or paths, of secretion that are present for all or part of the maturation cycle is summarised in Fig. 3.

The posterior cell group (PCG, Fig. 3b) produces secretion which forms well defined tracts of neuro-secretory material (1) leading into the central fibrous ganglionic mass, where their course can no longer be followed. The dorsal group of cells (DCG, Fig. 3a, b; Fig. 4) produces a large amount of secretion which is passed out in tracts (2, Figs. 3a, b; T, Fig. 4) which also enter the central fibrous mass. Most of these tracts are short and connect with the mass of the same ganglion; some, however, pass through the inter-cerebral commissure (ICC) to the mass of the other cerebral ganglion (3). The cells in the dorsal group are usually full of secretion. A small tract of secretion arises from the small cells in the anterior-lateral part of the dorsal cell group and passes to the tentacular and optic nerves (4, Fig. 3a, b). A group of small cells (10-20 μ long) in the middle of the ventro-lateral cell group (VLCG) also produce secretion which passes along a tract to the fibrous mass of the opposite cerebral ganglion through the inter-cerebral commissure (5, Fig. 3a). Also associated with this group of cells is a well defined tract connecting it to the dorsal cell group (6). Whether the secretion in this tract originates in only one or both of these centres is not certain.

Tracts are seen in the cerebro-pleural (CPLC; 7, Fig. 3b) cerebro-pedal (CPDC; 8) and possibly in the cerebro-buccal connectives (BC; 9). These tracts connect the fibrous masses of the ganglia, but it is not certain in which direction the secretion is being transmitted. As much more secretion is present in the cerebral ganglia than in the others, it is considered possible that the secretion is passing out from the cerebral ganglia. There is also a small tract (10, Fig. 3a)

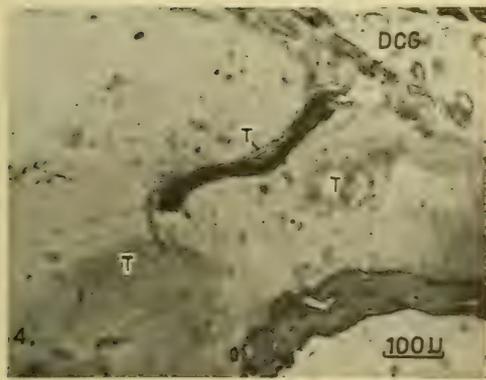


FIG. 4. Tracts of secretion (2, of Fig. 3) leading from the dorsal cell group of the cerebral ganglion. Susa, Paraldehyde-fuchsin.

from some small and medium sized cells ($15-35\mu$) in the ventro-lateral cell group (VLCG) down the cerebro-pleural connective (CPLC).

The procerebrum (PC) is largely empty of secretion. There is however a single layer of positive cells ($15-20\mu$ long) in some areas around the periphery of the lobe (11) towards its anterior end. At the ventral side of the procerebrum is a single large cell (12), $30-60\mu$ long, which is always full of large droplets of secretion, some $1-1.5\mu$ in diameter. The cephalic gland (CEG, Figs. 1,2) and vesicle at the anterior end of the procerebrum also usually have a few drops of secretory material associated with them (13).

The giant cell (14) in the mesial cell group of the metacerebrum (MTC) usually contains secretory droplets and the small cells ($10-20\mu$) surrounding it also have positive material. There is a tract (15) connecting these cells (VMCG, Fig. 3a) and cells of a similar size in the lateral cell group (VLCG). Finally, from the cells at the anterior end of the mesial and lateral groups originate tracts (16) which pass down the lip (LN) and inferior tentacular nerves.

The pattern of neurosecretory activity in these cells and tracts is largely similar throughout the year. There are, however, some differences in the distribution of neurosecretion related either directly or indirectly to the reproductive cycle or the season of the year or both.

In the spermatocyte stage (A) very early in the season there is little secretion present. Most of the tracts and positive cell groups, however, show some activity except for 3 areas; positive material is absent from around the vesicle (13) associated with the cephalic gland, from the lateral cells (VLCG) near the cerebro-pleural connective and from the tract (10) from these down the connective. One animal in this stage was from the end of the season (November 3rd) and was probably only 6-8 weeks old. This animal showed more secretion than animals at the same stage at the beginning of the year and the only deficiency it showed was the lack of secretion in the lateral cells and of the tract (10) originating from them.

More secretion is present at the subsequent early spermatid stage (B) but there is still no secretion around the vesicle (13) or in the lateral cells (VLCG).

In the late spermatid stage (C) still more secretion is present. The vesicle is much bigger and there are small drops of positive material surrounding it, as well as in some of the cells in the cephalic gland. In this stage the lateral cells also begin to secrete and tract (10) arising from them is seen.

In the early spermatozoa stage (D) there seemed to be less secretion than in the previous stage, but as only 2 animals were available, this result is uncertain. The vesicle is very large and seems to be filled with some refractile, paraldehyde-fuchsin negative material.

The mid-spermatozoa stage (E) has approximately the same amount of secretion as the late spermatid stage (C) but the distribution seems to be slightly different. There is slightly more secretion at the posterior end of

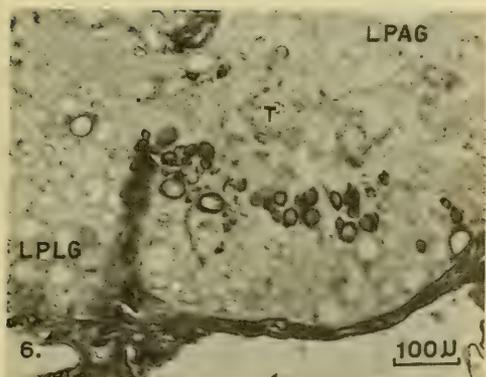


FIG. 6. A group of small cells in the ventral part of the parietal ganglion and around the parieto-pleural connection, full of secretion. Susa, Paraldehyde-fuchsin.



FIG. 7. The 2 mantle nerves of the parietal ganglion with small cells containing neurosecretory material and secretory tracts. Susa, Paraldehyde-fuchsin.

parts of this ganglion mass. The cells in the parietal ganglia are very active, neurosecretory material of varying quantity being present at all times of the year. Giant cells can be observed in all stages of secretion and storage, especially in the posterior and ventral parts of the ganglia. In certain periods the small cells ($15-35\mu$) surrounding the fibrous mass are very active, especially in the ventral parts of the ganglia and around the pleuro-parietal connection (Fig. 6). These cells give rise to tracts (24, Fig. 5a, b) which run into the fibrous mass. Large tracts (25, Fig. 5b) from the parietal ganglia run down the large mantle nerves (MN). At the points of origin of these nerves there occur a number of small cells which also become very active at certain times of the year and give rise to tracts apparently running into the tracts to the mantle nerves (26). Small positive cells (20μ) are also seen along the length of the mantle nerves near their origins from the ganglion (Fig. 7) in a few animals at certain times of the year.

The cells in the visceral ganglion (VG) usually show some secretory activity throughout the year. It is however very slight. The secretions of the giant cells

run in tracts to join the parietal-visceral tract in the fibrous mass (27). From this fibrous mass, tracts (28) run down all 3 of the visceral nerves.

As has been previously stated the amounts and distribution of neurosecretory material vary for different stages of reproductive maturation, and there seems to be some sort of relationship between the 2 as will be discussed later (p 295).

The spermatocyte stage (A) has very little secretion, but all the main tracts between the ganglia and the ganglion mass can be found. Most giant cells contain a few secretory droplets and 1-2 large cells ($50-80\mu$) in the posterior part of the parietal ganglia contain very many large drops of secretion. There are however, no small, positive cells surrounding the fibrous mass in the parietal ganglia, and no secretion at all in any of the cells of the pleural ganglia. This is also true of the animal collected in the very young stage, on November 3rd, although in the "frothy" cells, in the anterior lobe of the left parietal ganglion, there were many droplets. The droplets, however, occur in the cytoplasm of the cell and not in, or associated with, the large vacuoles.

The same distribution of neurosecretory material is seen in the early and late spermatid stages (B,C) though an increase in the amount of secretion in both the cells and tracts can be observed. In addition, in the late spermatid (C) and early spermatozoa (D) stages, a few cells surrounding the fibrous mass in the pleural ganglion are seen to contain a small amount of secretory material.

A major change is noticed in the distribution and amount of secretory material in this ganglion mass in the mid-spermatozoa stage (E), the stage when the first signs of maturation of the female part of the reproductive tract is observed. Much more secretion is found in all the tracts. Large amounts of neurosecretory material appear within the very small cells that occur in groups around the fibrous mass of the parietal ganglia, mainly in their ventral part, around the pleuro-parietal connection (Fig. 6) and around the origin of the mantle nerves. The tracts leading from these cells into the fibrous mass are filled with large amounts of secretion. In addition many small secretory cells occur in the lateral cell group of the pleural ganglia and around the pleuro-parietal and pleuro-pedal connections. These cells give rise to tracts which run into the pleural fibrous mass. The amount of secretion in the tracts running from the pleural into the pedal ganglia is also greatly increased.

The late spermatozoa stage (F) shows a further increase in secretory material, again with a slight increase in the number of small cells secreting. It seems to be the stage of maximum secretion, since the early and late oocyte stages (G, H) show a decrease in the amount of secretion and also a sharp decrease in the numbers of these small secreting cells. The greatest decrease is evident in the pleural ganglia, where, by the end of the late oocyte stage, only 1-2 small cells with secretory droplets are present. The larger secretory cells show little change in neurosecretory activity

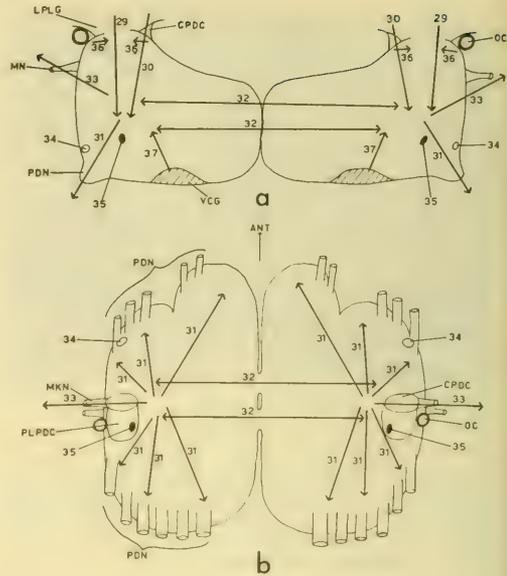


FIG. 8. Diagrams of the posterior (a) and dorsal (b) views of the pedal ganglion mass showing the distribution of neurosecretory cells and tracts. For an explanation of the numbers see text.

over this period. Both the animals in the atrophy stage (I) had a few of these small cells containing secretory material. In both cases the amount of secretion was greater than that in the young stages. In the animal collected in May, which had reproduced and over-wintered, there was still a large amount of secretion; one peculiar feature was the presence of many droplets of secretion between the very large vacuoles in the "frothy" cells of the left parietal ganglion.

Pedal Ganglion Mass

This ganglion mass has very little neurosecretory activity and there does not seem to be any correlation between the activity it does show and the reproductive maturation cycle. The distribution of neurosecretory cells and tracts are summarized in Fig. 8.

Large tracts of secretion enter the fibrous mass from the pleural ganglia

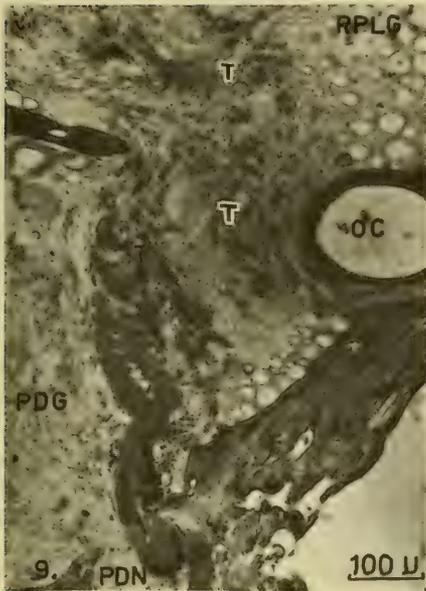


FIG. 9. Tracts of secretion (31) from the pleural ganglion through the pedal ganglion and into the pedal nerve. Susa, Paraldehydrofuchsin.

(29) and cerebro-pedal connectives (30). From this fibrous mass tracts (31) run down the pedal nerves (PDN, Fig. 8a, b; Fig. 9). Besides these, large tracts (32) run between the 2 pedal ganglia through the 2 inter-pedal commissures. Tracts (33) also lead from the fibrous mass down the lateral mantle nerves (MN, Fig. 8a; MKN, Fig. 8b). Very few cells in the pedal ganglia produce much secretion although a few secretory droplets can be found in practically all the cells. This is particularly true of the few giant cells in the anterior half of the ganglia where some positive droplets are nearly always found. One giant cell in particular (34), 80-120 μ in length, is always full of large droplets and is found in all the nerve rings examined. It is situated in the anterior third of the ganglion in the group of cells lateral to the central fibrous mass and usually just above the origin of one of the pedal nerves. Another cell (35) that is always full of large drops of secretion is a

medium sized cell (30-60 μ long) situated approximately half way along the length of the ganglion directly beneath the pleuro-pedal connection (PLPDC) and embedded in the fibrous mass immediately dorsal to the origin of one of the pedal nerves. There are a few secretory cells which only occur at certain times of the year. A few small cells (15-35 μ) occur around the pleuro-pedal connection (36) and similar cells (19 μ -20 μ) in the ventral cell group (VCG) give rise to tracts (37) which run into the fibrous mass.

The distribution of neurosecretion in the pedal ganglia is largely the same throughout the year. The amount of the secretion in the tracts changes according to the amount passed in from the pleural and cerebral ganglia, being a maximum in the late spermatozoa stage (F). The only exceptions to this are the 2 groups of small secretory cells which are empty of secretion until the late spermatozoa stage, when they commence secreting and continue for the rest of the reproductive cycle.

Buccal Ganglia

Contrary to the results reported by Hekstra & Lever (1960) for *Lymnaea stagnalis*, the cells of the buccal ganglia of *Arion ater* show a great deal of neurosecretory activity. Nearly all the cells contain some neurosecretory material and most cells usually contain considerable numbers of large positive droplets (Fig. 10). The lateral giant cells in particular show intense secretory activity. Another feature found in all the buccal ganglia examined is the presence of a few small cells (10-25 μ long) full of large secretory droplets around the origin of the radular nerve. Large amounts of secretion are found in tracts within the fibrous mass of the ganglion, in all nerves and connectives arising from the ganglia, and in the inter-buccal commissure.

This distribution of neurosecretory material is seen throughout the year with very little change in the amount of

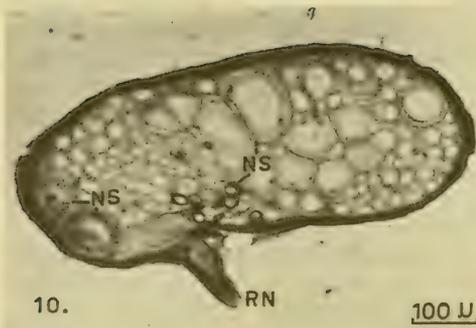


FIG. 10. Buccal ganglion showing neurosecretory material present in most of the cells. Susa, Paraldehyde-fuchsin.

secretion present. There seems, therefore, to be no correlation between the secretion produced in the buccal ganglia and reproductive maturation.

Optic Tentacles

Lane (1962) described the anatomy of the optic tentacles of a number of stylomatophorans and reported neurosecretion (paraldehyde-fuchsin positive material) in 'collar and lateral cells' in the optic tentacle. In the present investigation the optic tentacles of *Arion ater* were examined, collar and lateral cells as described by Lane were recognised and paraldehyde-fuchsin positive material was found in both types. Tracts of secretion were also found in the digitated ganglion and the tentacular nerve associated with it in the tip of the optic tentacle. These tracts, however, have not been connected with the collar and lateral cells. Paraldehyde-fuchsin positive material also occurs in considerable quantities at the ends of the finger-like processes of the digitated ganglion in or around the sensory endings in the tentacular epidermis. It also occurs in considerable quantities in the retinal cells of the eye. Pelluet & Lane (1961) postulated that neurosecretion is only produced in the optic tentacle

at certain periods during the reproductive cycle. This is at variance with the present investigation as tracts containing approximately the same amounts of secretion are observed in all stages of the maturation cycle. There also seems to be a large variation in the positive material in the collar and lateral cells within a single maturation stage.

EFFECT OF ENVIRONMENTAL FACTORS ON NEUROSECRETION AND REPRODUCTIVE MATURATION

In a previous paper it was shown (Smith, 1966a) that, although it was possible to advance or retard, with respect to the natural population, the maturation of the reproductive tract of animals by controlling one or more factors of their environment, it was not possible to alter the relationship between the hermaphrodite gland stage and the stage of maturation of the remainder of the tract. This means that a particular hermaphrodite gland maturation stage always corresponds to the same particular maturation stage of the remainder of the reproductive tract. As part of the present investigation, the pattern of neurosecretion was also mapped for the animals subjected to these environmental factors. In all cases exactly the same relationship between the neurosecretory activity and the hermaphrodite gland stage was observed as that already described above from animals of the natural population.

DISCUSSION

In the interpretation of the patterns of neurosecretion it is necessary to consider exactly what a large concentration of stainable material implies. One explanation might be that secretion is only seen in large quantities when it is being stored in the cell producing it, or in the axon, rather than being transported to the blood or target organ. If this were the case, a cell that was actively

secreting and constantly passing its secretion out down the axon would show little or no stainable material, as the material would be passed out as fast as it was being formed. The tracts from an actively secreting group of cells would then show little or no reaction, while the large prominent tracts would indicate material that was being stored and not sent out into the body. Because the greatest quantity of secretion is found at what can be assumed to be the animals' most active season physiologically (at the height of reproductive activity), and assuming that the positive staining material is in fact a neurohumour or a hormone, it follows that the above explanation would imply that the majority of substances produced by these cells are inhibitory in function; an unlikely explanation, that is probably incorrect.

An alternative explanation, probably nearer the truth, is that the amounts of secretion seen at any one time give an accurate guide as to the production and secretion rates of the cells and of the quantity of secretion actually passing through the axon. By this explanation the presence of secretion in a tract or cell implies active secretion of a substance into the blood or its passage down the axon to the target organ. It is also implied that the secretion present must be active at that time.

Although this second explanation seems to account for most of the secretions seen, it is possible that some of the cells may be storing the secretion. Krause (1960) has described storage cells, 'Sackzellen', in various parts of the central nervous system of *Helix pomatia*; while the author has been unable to demonstrate their presence in *Arion ater*, it is possible that the cells containing the large drops of secretion, that were found in the visceral and parietal ganglia, could be storage cells.

As described in a previous paper (Smith, 1966a), there appears to be one major period of change in the maturation of the reproductive system of *Arion ater* to which much of the activity of

the reproductive tract can be related. This critical period occurs during the mid- and late spermatozoa stage (E,F) of the hermaphrodite gland and marks the onset of maturation of all female accessory glands and also the beginning of copulation. In view of the difficulties of interpreting the results obtained by staining for neurosecretion with paraldehyde-fuchsin, it is of great interest to find that the only major change in the pattern of neurosecretion, that can be associated with reproduction, also occurs in the mid- and late spermatozoa stages. At that time large amounts of secretory material suddenly appear in various groups of small cells in the pleuro-parieto-visceral ganglion mass. This secretion is only found in animals in which the female glands have started to mature. It rises to a maximum by the late spermatozoa stage (F), when copulation occurs, and then decreases rapidly in quantity until very little is present by the late oocyte stage (H). The sudden appearance of large amounts of secretory material at this stage loosely agrees with Pelluet & Lane's (1961) theory that there is a brain hormone controlling egg production or the female stage. The relationship between the presence of secretion in these small cells and the maturation of the female glands is always found, even when the onset of the female stage has been artificially advanced.

As the secretion from the small cells in the pleuro-parieto-visceral ganglion mass only occurs for a fairly short period, at the time of the major change in the reproductive tract it would seem reasonable to consider it as a trigger substance which initiates the change rather than maintains it. The actual mechanism of action is not known and could only be elucidated by an experimental approach. It could have a direct action on the reproductive tract as a whole, or an indirect action on the secretory centres of the hermaphrodite gland, which then might secrete an active substance causing the enlargement

of the female glands, as suggested by Laviolette (1954).

There are many more groups of neurosecretory cells and tracts in the nervous system besides those mentioned above. Their secretion seems to vary with the season, in quantity, though not in distribution, and is probably concerned mainly with general metabolic processes. Thus when the pattern of neurosecretion is examined for each hermaphrodite gland stage it is seen that the general level of secretion is also at a maximum at mid- and late spermatozoa stage. Such a situation would, however, be compatible with the intense reproductive activity and possibly with the intensification of other metabolic processes occurring at that time. It is interesting to note here that van Mol (1961) describes the secretion of the cephalic gland in *Arion rufus* as attaining maximum secretion at the corresponding season. This fact was also noted for *Arion ater* in the present investigation; but, since the collapsing and emptying of the cephalic gland appears to be fairly closely correlated with the late spermatozoa stage, the function of the secretion of the gland may be more closely connected to reproductive activity than is suggested by van Mol. However, as the colloidal secretion of the gland is not stained by paraldehyde-fuchsin it is not known whether it is discharged before, or at the time of gland collapse. For this reason no firm conclusion can be drawn as to the role, if any, played by the cephalic gland in reproductive maturation.

No specific relationships could be established between neurosecretion and the onset of differentiation of the male glands and it is possible that this onset is directly connected with general growth and metabolism. Though tracts of positive material were found in the tentacular nerve and in the collar and lateral cells of the optic tentacle, a connection between these cells and the tracts, as suggested by Lane (1962) could not be found; nor was there a relationship established between this positive

material and the reproductive maturation as suggested by Pelluet & Lane (1961) for *Arion subfuscus* and *Arion ater*. The results of this investigation do not entirely agree with Pelluet & Lane's hypothesis of a dual neurosecretory control of reproduction. They postulated the production of a tentacular "hormone" which suppresses egg production while stimulating sperm production and a brain "hormone" which stimulates egg production. The theory that there are 2 substances involved is not incompatible with the present work. The brain "hormone" postulated by them could be equivalent to the secretion of the small cells described above. It is with the mechanism of action of these "hormones" suggested by these authors that this investigation is in disagreement. Pelluet & Lane (1961) suggest that both these "hormones" are produced continually, but that in the young animals the tentacular substance dominates over the brain substance, while after maturity the relationship is reversed. This part of their hypothesis does not agree with the present findings: if it is assumed that their 'brain hormone' is equivalent to the secretion from the small cells of the pleuro-parieto-visceral ganglion mass (see p 295) described in this investigation, then that hormone is not secreted continually but only over a short critical period.

As already mentioned, the stage of maturation in which the female glands start to mature coincides with a "critical point" in the maturation cycle (Smith, 1966a). When the animals were subjected to grossly adverse conditions, such as extremely low temperature, before this critical point was reached, then the maturation cycle was grossly affected; whereas the maturation cycle was affected very little if the critical point had been reached before the adverse conditions were applied.

From all these results it is possible to postulate a control mechanism for reproductive maturation in *Arion ater*. At the beginning of the year, in favour-

able environmental conditions, there is a growth of the animal and of the male part of the reproductive system. It is possible that the maturation of the male glands is influenced by secretion from the hermaphrodite gland. At the mid-spermatozoa stage of the hermaphrodite gland a secretion is possibly produced by the gland or tract, which stimulates the production of a "trigger" substance by the small cells in the pleuro-parieto-visceral ganglion mass. This substance triggers off the changes occurring subsequent to the "critical point". It is possible that this "trigger" substance acts on the hermaphrodite gland which then controls the maturation of the remainder of the tract (Lavolette, 1954).

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RESUMEN

CORRELACION ENTRE CAMBIOS NEUROSECRETORES Y MADUREZ DEL TRACTO REPRODUCTOR DE *ARION ATER*

B. J. Smith

Los sistemas nerviosos en varios individuos de *Arion ater* (L.) de diferentes estados de maduración conocidos en el ciclo reproductivo, se examinaron para localizar

muestras neurosecretoras, tífindo series seccionadas con paradelhido-fucsina. Muestras neurosecretoras en células nerviosas y ductos de tales materiales en los axones, fueron cartografiadas de las 3 masas principales de ganglios del sistema nervioso central (supraesofágico, pleuro-parieto-visceral y pedales), y de los ganglios bucales y tentáculos ópticos. La correlación principal entre maduración del ducto reproductor y neurosecreción, ocurrió en el "punto crítico", el comienzo de madurez de las glándulas reproductoras femeninas. Ciertas pequeñas células en la masa ganglionar pleuro-parieto-visceral, mostraron solamente actividad neurosecretora. Este estado es denominado "crítico" porque condiciones experimentales adversas retardaron groseramente la maduración cuando se aplicaron previamente a tal estado, pero sin afectar mucho cuando se aplicaron despues. Un posible sistema de control para la maduración del ciclo reproductivo se deduce de estos resultados.

АБСТРАКТ

КОРРЕЛЯЦИЯ МЕЖДУ ИЗМЕНЕНИЯМИ НЕЙРОСЕКРЕТОРНОЙ ДЕЯТЕЛЬНОСТИ И СОЗРЕВАНИЕМ ПОЛОВОЙ СИСТЕМЫ У *ARION ATER* (STYLOMMATORPHORA, ARIONIDAE)

Б. Д. Смит

Изучалась нейросекреторная деятельность у слизняка *Arion ater* (L.) на различных стадиях цикла созревания половой системы этих моллюсков, для чего использовались серии срезов, окрашенных паральдегидфуксином.

Нейросекреция была обнаружена как в нервных клетках, так и в аксонах трех главных ганглиозных масс центральной нервной системы моллюсков (надглоточной, плевро-париетально-висцеральной и ножной), а также в буккальном ганглии в оптических тентакулах.

В начале созревания женских половых гонад наблюдается наибольшая корреляция между стадиями созревания половой системы и нейросекрецией. Только в это время наблюдается нейросекреторная активность некоторых мелких клеток париетально-висцерального ганглия. Эту стадию автор назвал "критической", поскольку искусственно созданные неблагоприятные условия, действующие до начала этой стадии сильно замедляют половое созревание, но почти не влияют на него после наступления этой стадии. Отсюда постулируется возможность контролирования стадий полового созревания у указанных.

EMERGENCE OF VELIGER LARVAE FROM EGGS
IN GELATINOUS MASSES LAID BY SOME JAMAICAN
MARINE GASTROPODS^{1,2}

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ABSTRACT

The gelatinous egg masses, and the hatching mechanisms of the veligers, are described for 5 species of tropical marine gastropods, 1 prosobranch and 4 opisthobranchs, from Jamaica. In all of the species, hatching involved a preliminary softening or liquefying of the egg membrane, presumably through the action of enzymes secreted by the enclosed veligers, but final emergence in all instances was by physical struggle. Remnants of the egg membrane persisted after eclosion in the nudibranch *Phidiana lyncaeus*, the bivalved gastropod *Berthelinia caribbea*, and the sea hare *Bursatella pleii*, but all vestiges of the membranes disappeared in the Antillean paper bubble *Haminea antillarum* and the prosobranch *Cerithium algicola*. The membrane was torn in *Haminea* by a vigorous back-and-forth movement, whereas in all the other species the rupture occurred by action of the strong velar cilia. After emergence, the veligers swam away by use of their vela in *Haminea*, *Cerithium* and *Bursatella*; in *Berthelinia* the velum was used to rupture the egg membrane, but all larvae crawled away by use of the foot; in *Phidiana* about half of them swam away and half crawled away.

INTRODUCTION

During a continuing study of the hatching mechanisms of aquatic invertebrates (previous publications listed in Davis, 1966a, 1966b), the opportunity arose to study eclosion in several marine gastropods living in Jamaican waters. Emergence of the veligers was essentially similar in several unrelated species of gastropods that laid gelatinous egg masses, differing only in details. The egg masses and the hatching are described for 1 prosobranch: *Cerithium algicola* Adams, and for 4 opisthobranchs: the nudibranch *Phidiana lyncaeus* Bergh, the Antillean paper bubble *Haminea antillarum* d'Orbigny, the bi-

valved gastropod *Berthelinia caribbea* Edmunds and the sea hare *Bursatella pleii* Rang. For none of the species has the hatching been described before, in fact their egg masses, except for those of *Berthelinia caribbea*, were not known. Because most of these species are poorly known, adult specimens of all but the very common *Bursatella pleii* have been deposited with the U. S. National Museum, for future reference, under the following catalogue numbers: *Phidiana lyncaea*, 672548; *Haminea antillarum*, 672549; *Cerithium algicola*, 672547; *Berthelinia caribbea*, 672550.

The present investigation was undertaken at the Marine Laboratory of the University of the West Indies, Port Royal, Jamaica (W. I.) from February to May,

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²24th paper in a series on hatching mechanisms in aquatic invertebrates.

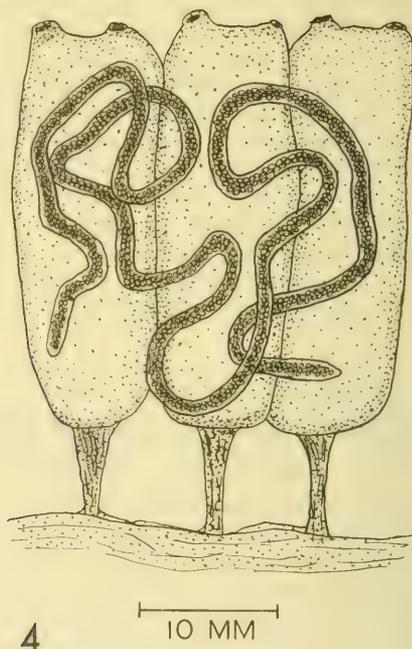
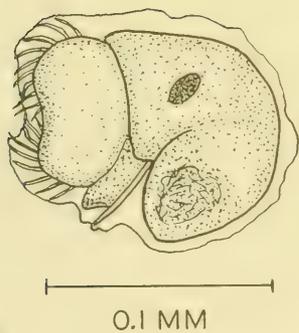
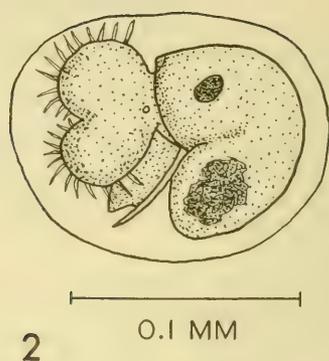
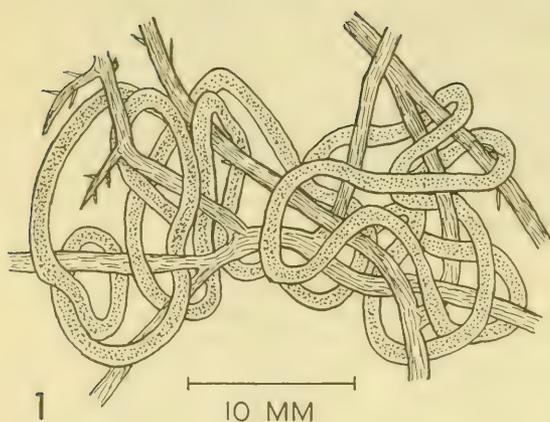


FIG. 1. Portion of an egg string of *Cerithium algicola*, collected in the field, twisted around the brown alga, *Acanthophora spicifera*.

FIG. 2. Veliger of *Cerithium algicola* ready to hatch. Note the large eye and the relatively small space between the veliger and the egg membrane.

FIG. 3. Hatching veliger of *Cerithium algicola*. The egg membrane has softened and collapsed and the velar cilia are penetrating through it.

FIG. 4. Egg string of *Phidiana lyncaeus*, as encountered in the field attached to specimens of ascidian, *Ecteinascidia turbinata*.

1965. Grateful acknowledgement is given for the unstinting cooperation of Dr. Ivan Goodbody, Director of the laboratory, who provided valuable advice in the project, and who provided space and facilities.

MATERIALS AND METHODS

All of the species under consideration were collected in mangrove areas near the laboratory at Port Royal, Jamaica; all of them were common. Eggs were collected in the field and observed until completion of hatching, or else observations were made on eggs laid by adults brought to the laboratory and maintained isolated from other gastropods until they oviposited. If eggs were brought in from the field, identity was ascertained later when adults laid eggs in the laboratory. Water temperatures in the air-conditioned laboratory remained mostly around 26.5° C.

Observations were made by stereoscopic and compound microscopes magnifying 27X and 100X, using both eggs isolated from the egg masses and also intact egg masses when the latter were not crowded and sufficiently transparent to allow adequate visibility.

OBSERVATIONS

1. *Cerithium algicola*. Throughout the period of study, egg strings of this prosobranch were common in a channel between 2 mangrove lagoons. After many failures, specimens of this snail, which was very common on the gravelly bottom of the channel, were induced to lay eggs in the laboratory, and hence there is no question regarding identity.

The egg strings were somewhat similar to those of *Phidiana*, described below, but they were firmer in consistency, much longer, and tangled together in a complex mass (Fig. 1). The width of the string approximated 1 mm. It contained a large number of eggs, tightly packed together in the center, but leaving approximately the

outer 80 μ free of eggs. These egg strings were very similar to those described by Lebour (1945) for *Cerithium ferrugineum* from Bermuda, except that the Bermuda strings had a diameter of only 0.32 mm. Individual egg membranes averaged 140 x 125 μ , and contained very small zygotes. The fully formed veligers, however, much more nearly filled the egg membranes (Fig. 2) than was the case for *Phidiana*. The veliger eye was very large, and the operculum extended some distance beyond the end of the foot. Veligers approaching the hatching stage were very active, swimming incessantly around within the membrane.

Hatching occurred 4 days after oviposition, and was similar both in separated eggs and in eggs within intact egg strings. First the egg membrane became thinner, usually beginning only on one side. Later it collapsed around the veliger completely, and the animal then beat its velar cilia vigorously against it, at the same time rolling over and over and shaking the body back and forth. Soon the membrane was broken by the velar cilia, which could be seen clearly extending individually through the now very flexible structure (Fig. 3), and the animal escaped. The time involved in hatching varied from a few minutes to as much as an hour. After emergence the remaining membrane became more and more faint, and finally (usually 10-15 minutes after eclosion) disappeared entirely.

2. *Phidiana lyncaeus*. An adult specimen of this aeolidiid species was collected on 19 February 1965 among semi-colonial ascidians (*Ecteinascidia turbinata*), that were growing abundantly on submerged hanging mangrove roots. Attached to a group of the ascidians was a long, contorted string of eggs. Both the eggs and the adult were taken to the laboratory, where the latter laid a second string of eggs, identical to that found previously on the ascidians, on some algae. *P. lyncaeus* adults were collected several other times in various habitats in the mangrove areas - usually

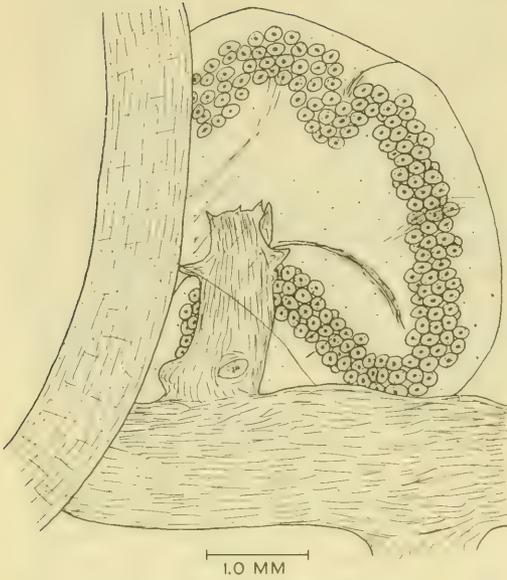


FIG. 5. Portion of a highly magnified egg string of *Phidiana lyncaeus*, laid in the laboratory on pieces of brown and green algae (*Acanthophora* and *Caulerpa*), showing details of the arrangement of the eggs and of the attachment of the string.

among bottom-living algae, but no additional specimens could be induced to lay eggs.

The egg strings (Fig. 4) differed greatly from the usual regularly arranged spiral ribbon of nudibranch spawn (Costello, 1938). They were approximately 1 mm wide in the portion containing the eggs, but there was in addition (Fig. 5) a thin very transparent gelatinous sheet of variable width, evident only under high magnification, by means of which the string was attached to the substratum. The eggs themselves were whitish and embedded in transparent gelatinous material, so that they were readily visible without dissection. The individual eggs were nearly spherical, and occurred singly within spheroid membranes whose dimensions averaged $298 \times 270\mu$. Even after full development the young nudibranchs within had a diameter of only somewhat over half the diameter of the egg membrane, so that

there was much fluid-filled space between the veliger and the membrane (Fig. 6).

Hatching took place 6 days after laying of the eggs. In individual eggs that had been dissected out of the egg mass the egg membrane gradually became softened and thinner, and subsequently it collapsed around the veliger inside (Fig. 7). At this stage 2 tiny red eyes were clearly visible at the head end of the veliger, and the foot also was well developed, with a distinct, horny operculum attached to it. The velum bore a group of very strong cilia around the edge. The transparent embryonic shell of the veliger exhibited 8 rows of very minute surface nodules, which were slightly darker than the surrounding shell.

After collapse of the membrane the veliger "played" with it by use of the velar cilia. These produced suction action on the membrane as the animal rotated around and around inside. Ultimately the membrane would break, always by action of the velar cilia, and the animal would escape. When the young nudibranchs were irritated, as by dissecting the eggs out of the gelatinous mass at this late stage, or by other rough treatment, the process of emergence was greatly hastened, and the rupture of the membrane occurred by a tearing action against the edge of the shell, caused by vigorous velar action, rather than by the velar cilia alone. In the intact egg string it could be seen that the egg membrane gradually became thinner and thinner, and that it did not collapse as in the separated eggs. In these intact eggs eventually (after from 35-45 minutes) the animal would push its way through the now very tenuous membrane with the foot. The emerging veliger was rather far along in its development, as suggested by the large size of the foot. In emerging from separated eggs, about half of the specimens swam away by velar action, and about half crept away on the foot.

3. *Haminea antillarum*. Numerous characteristic egg masses occurred

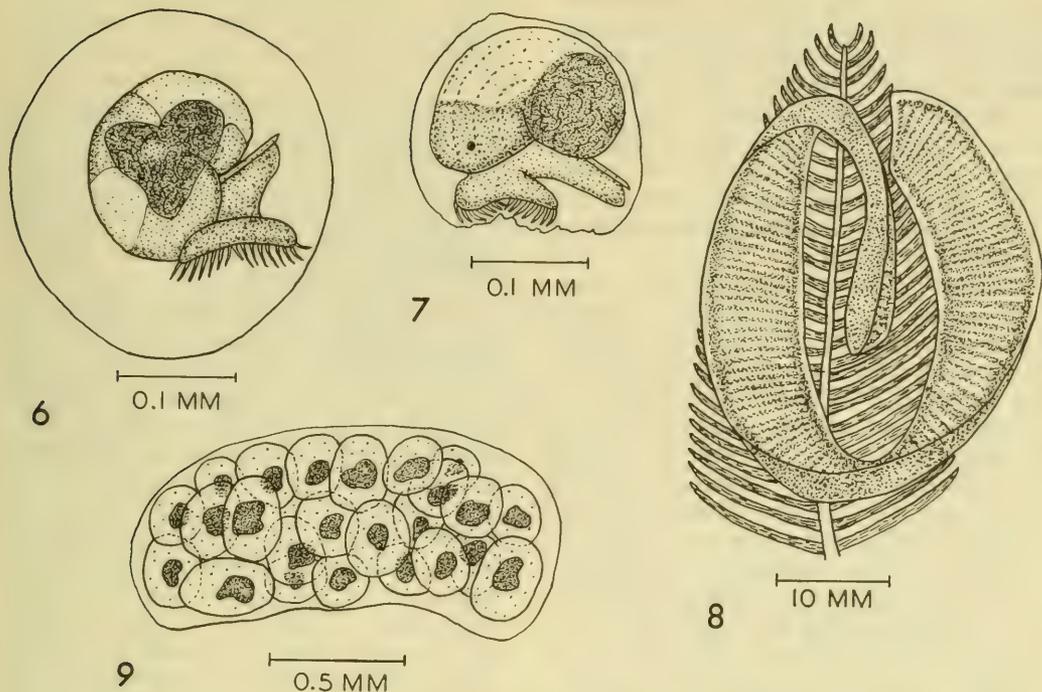


FIG. 6. An isolated egg of *Phidiana lyncaeus* to show the relative sizes of the veliger and the egg membrane. Development was not complete, as shown by the lack of a conspicuous eye.

FIG. 7. Eclosion in an isolated egg of *Phidiana lyncaeus*. The membrane has been softened and has collapsed around the veliger. The eye is present.

FIG. 8. Egg mass of *Haminea antillarum*, laid in the field on the green alga, *Caulerpa sertularioides*.

FIG. 9. Egg mass of *Berthelinia caribbea* laid in the laboratory. This egg mass contained 24 eggs.

throughout February to May, 1965, upon algae, twigs, etc. in the same location described above for egg masses of *Cerithium*. During the daytime collections, no gastropods were ever encountered closely associated with them, but living specimens of *H. antillarum* were collected from the accumulated debris at the base of algal growths, and during the night some of these laid egg masses in the laboratory that were identical to the field specimens. The egg masses of this tectibranch were large, forming a coiled ribbon of gelatinous material that occupied an

area usually around 20 x 25 mm. The ribbon itself was from 8-10 mm wide and about 2 mm thick (Fig. 8). It was attached to the substratum by an eggless gelatinous portion, while the outer edge of the ribbon also was without eggs, forming a thin flap of material. Within the ribbon the individual eggs were arranged in continuous spiral rows. These rows passed first across beneath the upper surface of the ribbon, then twisted back near the lower surface. Each row in most egg masses appeared to be 2 eggs wide, but in one egg mass, evidently laid by an animal near ex-

haustion of its egg supply, the eggs were more sparse than usual, and it was clear that each row consisted of only a single line of eggs. The row was, however, contorted both horizontally and vertically within the ribbon in such a manner that when more crowded the row would seem doubled. The dimensions of the membranes surrounding individual eggs averaged $141 \times 124\mu$.

Hatching occurred after 5 days. The mechanism was the same both in separated eggs and within intact egg masses, but the thickness of the egg mass, and the crowded condition of the eggs precluded a clear view of events except in single eggs removed from the mass. The formerly rather rigid egg membrane suddenly became flexible, as indicated by the distortions produced in it when the active veliger struck against it from within. Rather than manipulating the membrane with the velar cilia, the hatching animal performed a series of violent back-and-forth movements within it. Emergence always was velum first. The veligers emerged from the egg membrane 1 - 2 1/2 minutes after softening of the membrane. As the veliger hatched, it could be seen that the formerly rigid egg membrane had now become viscous and almost semi-liquid, for it was drawn out by the animal into long strings. After the animal had escaped, very little remained of the membrane, and 10 minutes later nearly every trace of it had disappeared. The gelatinous material of the egg mass outside of the individual eggs also liquified markedly so that the veligers could easily swim around in it.

4. *Berthelinia caribbea*. Specimens of this saccoglossan were collected from the type locality between February and May, 1965, on the alga *Caulerpa verticillata* in a channel between mangrove lagoons near Port Royal. In the laboratory numerous specimens laid eggs on algae, on small pebbles, or on the sides of the glass containers. A large number of additional egg masses were collected on the algae in the field. The eggs,

which were bright yellow, were grouped in small masses of very clear colorless gelatinous material. Egg masses laid in the laboratory contained from 3-32 eggs (average of 10 was 14.2 eggs) and those from the field contained from 7-21 eggs (average of 12 was 12.6 eggs). These findings contrast with those of Edmunds (1963), who described "yellowish egg masses ... usually with 40 to 80 eggs" for this species.

Egg masses varied in size depending upon the number of eggs. That illustrated in Fig. 9 contained 24 eggs and measured $1605 \times 640\mu$. Individual egg membranes also varied considerably in size. Most, however, were oval and approximately $240 \times 200\mu$; some were as small as $170 \times 156\mu$.

The mean time between oviposition and hatching was 18 days, which was long compared to the other species under consideration here. Development from the one-cell stage to a well developed veliger stage, however, was rather short (3 days). At the trochophore and early veliger stages the young snails were quite active, rolling constantly over and over, but during later life within the egg membrane they moved about only sporadically, and spent days at a time practically inactive. During hatching the egg membrane became less apparent and more flexible, disappearing completely some time after emergence. The enclosed veliger did not rotate incessantly as did the other species. The animals did not work very actively on the egg membrane and it took hours before they would finally wear through to the outside. As in other species the velar cilia sucked the membrane up into the cup formed by the velar surface, and hence the effectiveness of the ciliary action was enhanced. The young emerging from isolated eggs crept away by pedal action, rather than swimming away by use of the velum. Undoubtedly this trait helps to maintain the hatchlings in position on their specialized algal food supply, as described by Edmunds (1963), for the species has been encountered

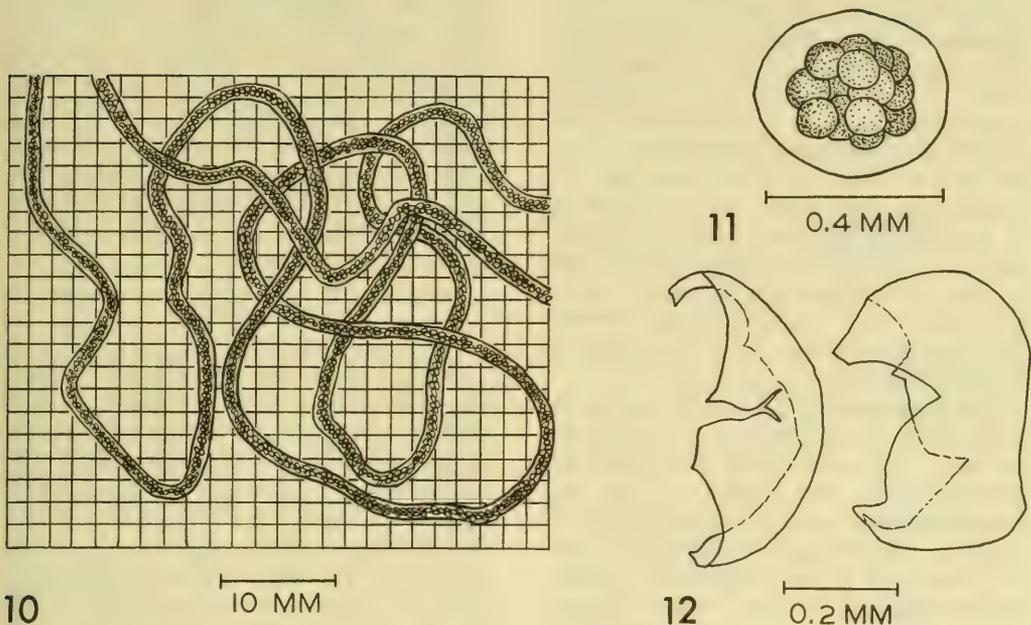


FIG. 10. Portion of an egg string of *Bursatella pleii*, laid in the laboratory on a piece of plastic screening.

FIG. 11. A single egg capsule of *Bursatella pleii* removed from the egg string, showing the several individual ova at the one-cell stage, clustered together in the center.

FIG. 12. Portions of 2 capsular membranes of *Bursatella pleii*, ruptured artificially prior to hatching, and showing characteristic jagged broken edges.

on one species of alga only, as is also true for certain other bivalved gastropods. Kawaguti & Yamasu (1960) have described a similar habit for the bivalved gastropod *Tamanovalva* (= *Berthelinia*) *limax*, which lives on *Caulerpa okamurai*. The egg masses of this species also are very similar to those of *B. caribbea*, although larger. On the other hand, the observations of Gonor (1961) on the eggs of another sacoglossan *Lobiger serradifalci*, emphasize that the egg masses of gastropod species that are closely related taxonomically, often differ greatly. In *Lobiger* the egg mass consists of a coiled gelatinous ribbon fastened to the *Caulerpa* frond flat side down.

Within an egg mass all of the individual eggs hatched before any of the young snails made their exit. Usually the young

soon emerged from the outer membrane enveloping the egg mass, but in one instance, they did so only after a lapse of over 30 hours. In the meantime the young were vigorously milling around inside the flexible membrane pushing against it from the inside. Eventually a hole was formed and the young escaped one by one.

5. *Bursatella pleii*. Specimens of this tectibranch were collected on 26 March 1965, creeping over the muddy bottom of a mangrove lagoon in about 1 m of water. In the laboratory they were placed in large finger bowls covered with plastic screening and maintained in running water. Egg strings were deposited upon the screening. The strings (Fig. 10) were for the most part about 1.2 mm wide, but in places up to 1.5 mm, and they tapered to very thin ends. Numerous

slightly angular, sub-spherical capsules measuring around 425-365 μ were crowded into the string. Most capsules (Fig. 11) contained 12-14 yellow ova, which were so crowded that exact measurements of their dimensions were difficult (but they approximated 85 - 70 μ). Except for their smaller diameter, the egg strings were very similar to those of another tectibranch, *Aplysia protea*,³ collected by Dr. I. Cornman at Cow Bay on the south coast of Jamaica. Also they were very similar to egg masses of *Aplysia californica*, as reported by MacGinitie (1934).

Development of the embryos was rapid. In 4 days the veligers were well developed, and swimming very actively around within the capsules. At this time some of the capsules were removed from the egg string for better visibility, and a number were accidentally broken. It was observed that they broke with very jagged, splintered edges (Fig. 12). Some of the capsules were tested under a magnification of 27X by pressure from insect pins. They resisted rupture, bending inwards under moderate pressure, but suddenly broke with jagged edges with somewhat greater exertion. The enclosed veligers soon found the holes thus artificially produced in the capsule and actively swam away.

When hatching normally, the membrane of the capsule softened greatly, as was indicated by the fact that it was bent each time one of the enclosed veligers touched it with the velar cilia. Whenever this happened, after the membrane had sufficiently softened, the veliger would briefly pause in its rapid gyrations to manipulate the membrane with the cilia. About 15 minutes after the beginning of the softening process, one of the veligers would break a hole through to the outside and escape. Then one by one the remaining young snails

would escape through the same opening. Capsules emptied within 5-12 minutes after the first rupture. At that time manipulation of the capsular membrane by insect pins showed it to be fully flexible. The membranes could be torn readily, but they did not rupture with jagged edges. Two days after emergence of the young the capsular membranes still were present, and in the same condition as during emergence.

DISCUSSION

In all 5 of the species considered here the hatching process included both a radical change of consistency of the egg membrane, and an active bursting of the membrane by action of the enclosed veliger. Except for *Haminea* the rupture was solely or mainly through the action of the strong velar cilia. Although its detailed nature was not demonstrated in the present study, it is clear that the softening of the membrane was caused by action of an enzyme or enzymes secreted by the veligers at the appropriate time in their development. In *Haminea antillarum* and *Cerithium algicola* the enzyme completely dissolved the membrane shortly after hatching was completed, but in the other species parts of it remained behind indefinitely.

Hatching of veligers from gelatinous egg masses has seldom been described, the only publications apparently being those of McGowan & Pratt (1954), Gohar & Abul-Ela (1957a, 1957b) and D'Asaro (1965). Gohar & Abul-Ela, without giving any details, suggested that hatching in the tectibranch *Berthellina citrina* and the nudibranch *Chromodoris pulchella* occurs by the action of a hatching enzyme secreted by the veligers. McGowan & Pratt (1954) were cautious. They described a disintegration of the gelatinous egg mass "related in some way to the state of development of the larvae" D'Asaro (1965) stated that the veliger of *Strombus gigas* escaped by "forcibly swimming" through the softened plug in

³In a recent publication by Eales (1960) it is stated that *A. protea* is synonymous with *A. dactylomela*.

an aperture of the egg capsule, and that the softening "may be related to the production of enzymes...." Many gastropods hatch from the egg in stages later than the veliger. Among these, the hatching process in the fresh-water tropical prosobranch *Amnicola hydrobioides* is strikingly similar to that described above for eclosion from the egg membranes (Davis, 1964), except that the tearing of the membrane is accomplished by action of the foot and proboscis. In that species the eggs are laid individually, without a gelatinous mass. On the other hand, in another annicolid, *A. limosa* (Davis, 1961) emergence is strictly mechanical by action of the radula. Bondesen (1950) has shown that eclosion of fresh-water pulmonates of the families Physidae, Lymnaeidae and Planorbidae is mainly physical, but that the lymnaeid *Myxas glutinosa* emerges from its egg mass after dissolving the egg membrane by action of enzymes. Other descriptions and reports of emergence from the tough egg capsules characteristic of many marine snails is surveyed by Davis (1961, 1964).

Aside from many descriptions dealing with the spawn of one, or of a few, gastropod species, there are some more general accounts, particularly by LoBianco (1888, 1889) on the spawn of animals (including gastropods) of the Gulf of Naples, Lamy (1928) on museum specimens of dried egg cases of prosobranchs, Lebour (1937, 1945) on eggs of British and Bermudan prosobranchs, Ostergaard (1950) on spawn of Hawaiian marine gastropods and Bondesen (1950) on egg masses of fresh-water pulmonates. As far as could be ascertained, the egg masses and eggs of none of the species considered herein except *Berthelinia caribbea* have been described previously. Some of the earlier scattered literature has been briefly summarized by Simroth (1896-1907). LoBianco (*op. cit.*) has described briefly the spawn of *Cerithium vulgatum* as white, delicate, ribbon-shaped, and irregularly folded on itself, which is in contrast to the almost cylindrical contorted egg strings of *C.*

algicola. On the other hand, judging by LeBour's (1945) description of the egg string of *C. ferrugineum*, it is very similar to that of *C. algicola* though more delicate.

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RESUMEN

EMERGENCIA DE LARVAS VELIGERAS DE MASAS OVIGERAS
GELATINOSAS PUESTOS POR ALGUNOS CARACOLES DE
JAMAICA

C. C. Davis

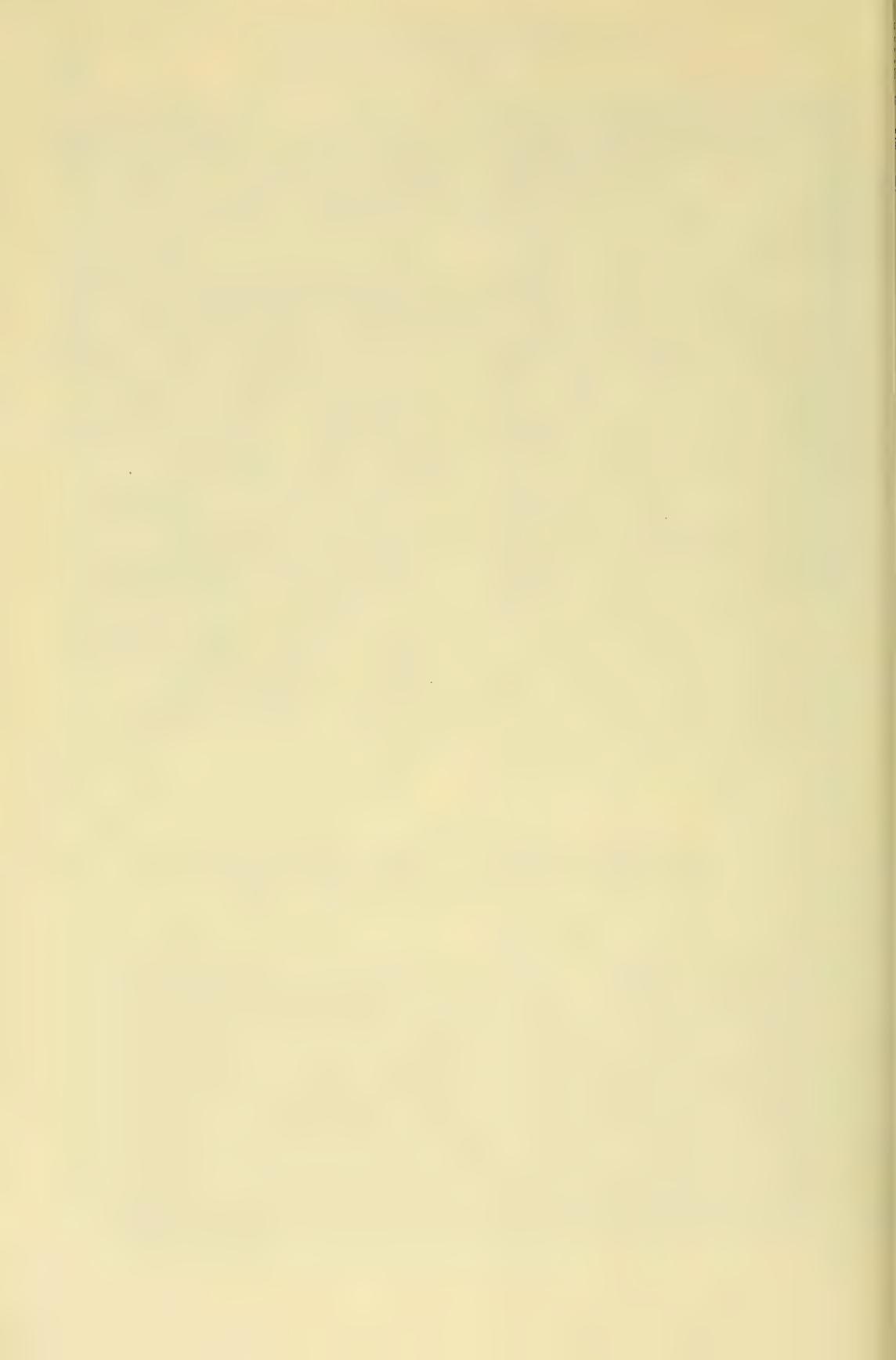
Las masas ovígeras gelatinosas y el mecanismo de eclosión de las veligeras, se describen para 5 especies de gastrópodos marinos, 1 prosobranquio y 4 opistobranquios de Jamaica. En todas las especies la eclosión fue precedida por un ablandamiento o licuación de la membrana ovígera, presumiblemente por acción de enzimas secretadas por las veligeras contenidas, pero emergencia final fue por esfuerzo físico. Restos de la membrana del huevo fueron retenidos después de la eclosión en el nudibranchio *Phidiana lyncaeus*, el gastrópodo bivalvo *Berthelinia caribbea*, y la "liebre marina" *Bursatella pleii*, pero desaparecieron en *Haminea antillarum* y el prosobranquio *Cerithium algicola* de Antillas. La membrana de *Haminea* fue rota por el vigoroso movimiento de vaivén, mientras que en las otras especies la ruptura ocurrió por la fuerte acción de cilias velares. Después de la emergencia, las veligeras nadaron usando los velos en *Haminea*, *Cerithium* y *Bursatella*; en *Berthelinia* el velum fue usado para romper la membrana del huevo, pero todas las larvas se dispersaron reptando por uso del pié; En *Phidiana* la mitad de ellas se dispersaron nadando y la otra mitad reptando.

АБСТРАКТ

ВЫХОД ЛИЧИНОК-ВЕЛИГЕР ИЗ ЯИЦ, ЗАКЛЮЧЕННЫХ В СЛИЗИСТЫХ
КЛАДКАХ НЕКОТОРЫХ МОРСКИХ ГАСТРОПОД
ОБИТАЮЩИХ В РАЙОНЕ ЯМАЙКИ

Ч. К. Дэвис

Описывается механизм выхода (вылупливания) личинок в стадии велигер из слизистых кладок пяти видов тропических морских гастропод (1 *Prosobranchia* и 4 *Opisthobranchia*), обитающих в районе Ямайки. У всех этих видов процесс вылупливания личинок начинается с размягчения или разжижения яйцевой оболочки, благодаря действию энзимов, выделяемыми заключенными внутри яиц велигерами; однако, окончательный выход личинок происходит далее благодаря их чисто-физическим усилиям. После выхода личинок у некоторых форм остатки яйцевой оболочки сохраняются, как например, у голожаберного моллюска *Phidiana lyncaeus*, у двустворчатых гастропод *Berthelinia caribbea*, и у морского зайца *Bursatella pleii*, в то же время у других - у антильской улитки *Haminoea antillarum* и у переднежаберного *Cerithium algicola* она полностью исчезает. У *Haminoea* яйцевая оболочка разрывается, благодаря энергичным движениям самой личинки, в то время как у остальных видов разрыв оболочки образуется благодаря действию сильных ресничек паруса личинки. После выклева и выхода личинки-велигер родов *Haminoea*, *Cerithium* и *Bursatella* уплывают при помощи работы своего паруса (*velum*); у *Berthelinia* парус служит лишь для разрыва яйцевой оболочкм, а личинки уползают прочь при помощи ноги. У *Phidiana* примерно половина личинок уплывает, а другая половина - уползает.



DISC ELECTROPHORETIC ANALYSIS OF MOLLUSCAN INDIVIDUALS AND POPULATIONS¹

George M. Davis² and Gene K. Lindsay³

ABSTRACT

The value of disc electrophoresis in providing insight into problems of molluscan systematics was investigated. Were electrophoretic patterns yielded by proteins from haemolymph or foot muscle extract stable? Did they differ in individuals of a single population or in different populations of the same species?

Proteins from haemolymph of individual *Helix pomatia* yielded 12, from foot muscle extract 20, components, which showed no qualitative changes with snails of different size (age). When size of the snail was correlated with protein density there was a significant inverse quantitative change with haemolymph, but not with foot muscle extract. Since the latter yielded a stable pattern over a variety of physiological conditions (induced by age, forced hibernation in a refrigerator and laboratory maintenance) and also provided more fractions, it was chosen for use in further experiments.

Comparing electrophoretic patterns of foot muscle extract from single snails of the same population of *Helix*, no significant differences were found.

Four different populations of the amphibious snail *Pomatiopsis lapidaria* were studied, also using foot muscle extract. Altogether 26 distinct fractions were found for the species, of which from 16-19 were present in any one population. But despite significant variation between populations, the species was characterized by a densitometric pattern clearly recognizable in each of them.

Several facts became evident: a) Although foot muscle extract provided a source of protein yielding stable electrophoretic patterns, not all fractions characterizing a population were routinely separated in 1 test; at least 10 tests per population were needed to encompass all variation and establish with certainty a stable, reliable pattern. b) The 2 species studied had very distinct electrophoretic patterns. Certain regions of the gel column provided densitometric patterns of greater value for comparison. In general, the region from about mid-gel to front can best be used to characterize a taxon. c) Disc electrophoresis has distinct advantages over techniques such as starch gel and paper electrophoresis: (1) the standard 7.5% polyacrylamide gel allows greater fractionation and resolution of components; (2) the pore size of the gel can be changed to permit selection for certain molecules; (3) a gel can be charged with a sample as small as 300-800 micrograms; (4) the actual "run" time is only about 30 minutes; (5) stained gels are easily stored for future reference. While the technique is very sensitive and small experimental errors in gel preparation have a profound negative effect, routine checks with human blood serum, whose pattern is well known, can indicate whether gel conditions are optimal.

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It should be noted in general that although electrophoretic profiles can reveal specific and subspecific characteristics, and are important in demonstrating genetic differences not evident anatomically, they do not suffice by themselves to define taxonomic categories, because the identity of the protein components observed remains unknown. Immunological tests with selected antigen-antibody systems can help in revealing homology of components from different taxa, located in analogous positions. However, biophysical data have their useful place only within an integrated framework of precise anatomy and cytology.

INTRODUCTION

The use of electrophoresis in systematic studies on many groups of organisms has been reviewed by Sibley (1960). Cheng (1964) reviews previous work pertaining to mollusks. Little has been done with this group of animals.

Deutch & McShan (1949) used paper electrophoresis to study electrophoretic patterns of blood plasma from 5 species of snakes, 2 turtles, a bullfrog, fish, land snail and the horseshoe crab. No attempt was made to relate their data to systematics.

Wood *et al.* (1958) utilized starch gel to study the electrophoretic properties of blood proteins of a number of crustaceans and 2 mollusks: a species of squid and an oyster. They found only 2 protein components for each of the mollusks. They stated that "the results of these studies indicate that the electrophoretic patterns of serum proteins of closely related invertebrates have a remarkable degree of specificity. These findings suggest that starch gel electrophoresis of serum proteins may be useful in certain racial studies, taxonomic problems..."

Wright & Ross utilized cellulose acetate. Their studies concerned electrophoretic separation of blood proteins (1959, 1963) and egg proteins (1963, 1965) to obtain patterns providing characters of use in the taxonomy of planorbid snails. They state (1963) that "the results of this work confirm earlier doubts concerning the taxonomic value of molluscan blood proteins and indicate that comparison of egg-protein patterns may be a more rewarding study." In 1965 they presented egg-protein patterns

for different populations of species of the planorbid genera *Bulinus* and *Biomphalaria*. They concluded from their results that "egg proteins of planorbid snails are extremely sensitive indicators of variation between populations."

Davis & Lindsay (1964) initiated the use of disc-gel electrophoresis in molluscan systematics. Using *Helix pomatia*, a large land pulmonate of the family Helicidae, they found that "growth and accompanying physiological changes only affected the blood protein density and that linear sequence of proteins of both foot muscle and blood were reliable characteristics for a population of a species." In studying populations of the amphibious prosobranch *Oncomelania hupensis formosana* (Hydrobiidae; Pomatiopsinae), they found that results with disc electrophoresis were sensitive enough to demonstrate variation between populations of a species.

Davis (1965a) showed that species of distinct genera have distinct electrophoretic patterns. In that study, *Oncomelania hupensis formosana* was compared with *Pomatiopsis lapidaria*, a snail of the same subfamily.

The purpose of this paper is to present more detailed data on *Helix pomatia*, previously discussed in an abstract only (Davis & Lindsay, 1964) and to add new information concerning the use of disc electrophoresis in the study of populations of another species, *Pomatiopsis lapidaria*.

The main questions with which we were concerned were: 1) Is foot muscle or circulating haemolymph more reliable for use in routine systematic comparison? 2) Does age (size) affect the

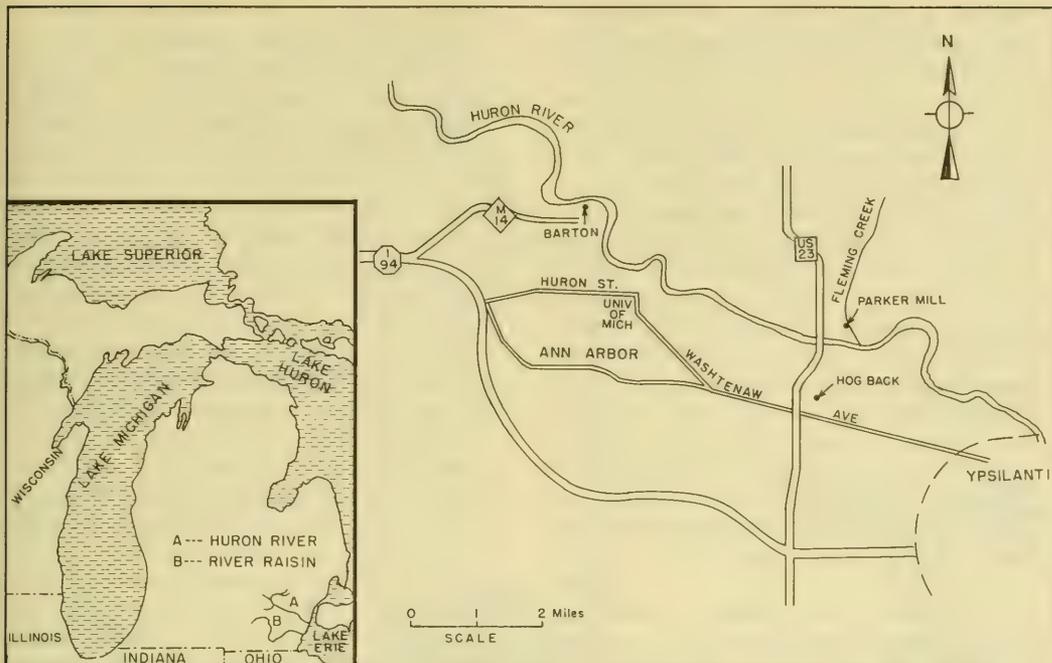


FIG. 1. Maps showing collection sites of the populations of *Pomatiopsis lapidaria* from Michigan.

electrophoretic patterns? 3) Do individuals from the same population differ significantly in electrophoretic patterns? 4) Do populations of the same species vary significantly in electrophoretic patterns?

MATERIALS AND METHODS

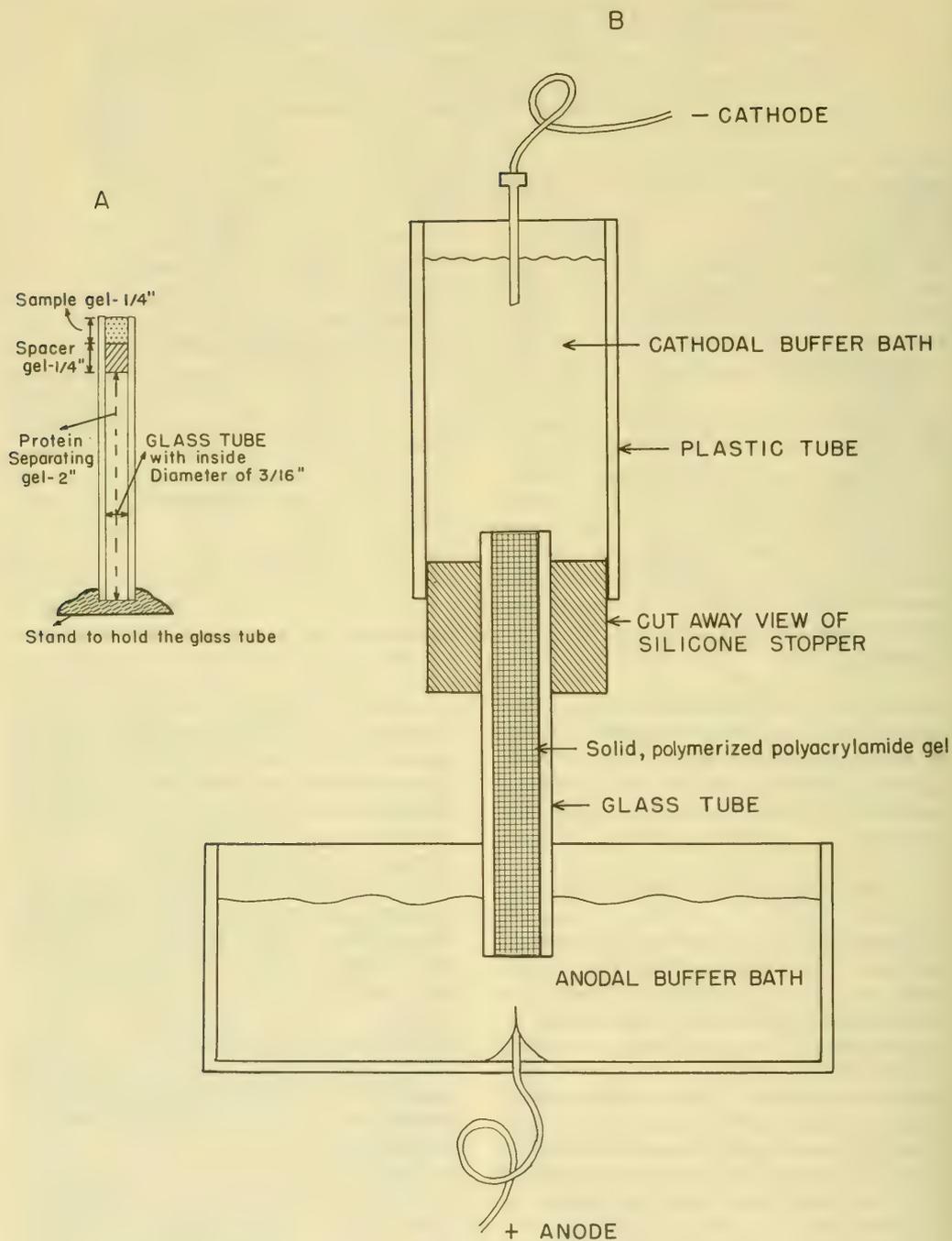
Large numbers of *Helix pomatia* were collected from Jackson, Michigan. Tests were made using snails fresh from the field, snails forced into hibernation in a refrigerator for a month, and with snails maintained in the laboratory several months. The animals were graded into 3 size categories: large, medium, small, which were arbitrarily defined in terms of a size index (Table 2). The size index was the product of shell length in mm by width in mm.

Four populations of *Pomatiopsis lapidaria* were chosen for study as shown in Fig. 1. The Clinton population was from the River Raisin drainage system. Hog Back, Parker Mill and Barton popu-

lations were from the Huron River drainage. The latter populations were separated by gaps of 1 or more miles where the species was absent. The snails utilized were collected from June to September. Only fully adult snails were used in the experiments.

Experiments with haemolymph were carried out with *Helix pomatia* only. Haemolymph was obtained by cutting (with scissors) into the pedal haemocoel and draining the haemolymph into a beaker. Care was taken to do the operation quickly and cleanly so as to avoid mucoid secretions from the foot mixing with the haemolymph. Almost immediately upon wounding the animal, mucus was secreted in response to the irritation.

Foot muscle was prepared from *Helix pomatia* and *Pomatiopsis lapidaria*. In large snails such as helicids, one snail provides enough foot tissue for 1 test or run (5-10 electrophoretic tubes). A run is defined as the duration of a test from the time of starting current



to cutting off current. In such a case, 0.3 gm of tissue (blotted with filter-paper to remove excess fluids) were homogenized in 2.0 ml of Carriker's (1946) physiological saline, using a Servall microhomogenizer (50,000 rpm). The tissue was homogenized for 30 seconds and then checked to see whether all the shreds of tissue were "taken." If some pieces remained the muscle was homogenized for another 30 seconds.

With small gastropods such as hydrobiid snails, 2 different procedures were employed depending upon the amount of material available. When 20-50 snails of *Pomatiopsis lapidaria* were available, 0.15 gm of foot muscle were homogenized in 1.0 ml of saline using the microhomogenizer. Often only a few snails were available, in which case 0.03 gm were homogenized by grinding the tissue in a small glass well with a small glass pestle, adding a "pinch" of fine silicon grit and 150 lambda of saline. All operations were carried out at temperatures of 2^o-3^o C attained by means of ice baths.

The homogenate was centrifuged in a refrigerated centrifuge at 1,200 rpm (250 x g) for 5 minutes (2^oC).

Disc electrophoresis was used. The name, according to Ornstein (1962) "was derived from the dependence of the new technique on discontinuities in the electrophoretic matrix, and coincidentally, from the discoidal shape of the separated zones of ions in the standard form of our technique" (The reader is also referred to Ornstein (1964) and Davis (1964) as well as bibli-

ographies on work pertaining to disc electrophoresis available through the Canalco Corporation, Rockville, Maryland, U. S. A.).

The technique has advantages over other electrophoretic procedures in that: 1) The standard 7.5% acrylamide gel makes possible greater fractionation and resolution of components than starch gel or paper electrophoresis. 2) The pore size of the gels can be adjusted to select certain molecular sizes. 3) The possibility of operating with a small amount of sample is a distinct advantage. Thus, a gel can be charged with a sample no larger than about 300-800 micrograms of protein. 4) The "run" time is only about 30 minutes. 5) Stained gels are easily stored for long periods of time in 7% acetic acid in small test tubes.

In this technique, the protein components to be separated migrate within a cylindrical column of gel; after separation the various fractions are stacked in the tube like coins and are visible as bands, in side view. The gel medium was a synthetic polymer made from stock solutions that had been prepared from basic chemicals (not from pre-mixed commercial solutions) according to formulae provided by the Canalco Corporation.

The standard technique utilizes 3 gel layers (Fig. 2A). Each is polymerized within a glass tube held vertically by a base stand. Starting at the bottom, 7.5% acrylamide solution is poured to a height of 2 inches; it polymerizes into a solid matrix in about 35 minutes. This

FIG. 2. Diagrammatic set-up for disc electrophoresis. A. A glass tube is set upright in a supporting base stand. Three gel solutions are poured into the tube, one at a time. Each solution is allowed to polymerize before the next is added. When the uppermost solution is polymerized the glass tube with internal gel column is removed from the base stand. B. The glass tube containing the gel column is held in place in the upper cylindrical cathodal buffer bath by means of a perforated silicone stopper, while the lower end hangs freely in the anodal buffer bath. In the "homemade" apparatus used, as many as 12 cathodal units, such as shown here, were used in a common anodal bath.

standard gel has an average pore size of 50 angstroms; the size of pore is dependent on the amount of acrylamide⁴ (Ornstein, 1962). Variance in pore size determines (in part) travelling speed, selection and distribution of different sized molecules.

After the lower or "separating" gel has polymerized, 1/4 inch of a 3% acrylamide solution is added, thus forming the "spacer" gel (with larger pores) which serves to sort, stack and concentrate the protein components in decreasing order of mobility prior to separation in the "separating" gel. When this gel has polymerized (about 15 minutes), the uppermost 1/4 inch or "sample" gel, containing the protein sample is added. This last gel has the same chemical composition as the "spacer" gel.

The glass tube with its enclosed solid cylinder of gel is then so placed as to bridge the gap between 2 buffer solutions (Fig. 2B). The sample end of each tube is inserted into its own cathodal buffer bath consisting of a plastic tube, while all gel tubes of one test (8-10 tubes) are immersed in a common anodal buffer bath (plexiglass container). The apparatus was easily assembled at a cost of less than 30 dollars.

The buffer was a solution of tris⁵ and glycine with a pH of 8.2-8.4. Prior to starting current through the gel, brom phenol blue dye was added to the cathodal buffer bath. A constant current of 5 milliamperes was passed through each tube simultaneously and was maintained by hand regulation of a Heathkit power supply. With the initiation of current through the gels, a visible blue phenol front formed at the tip of the sample gel. Progression of this front down the gel indicated the position of the leading band of protein. Current was cut off when the front had moved 1 and 1/4 inches

into the separating gel. The gel columns were removed from the glass tubes by "rimming" between the glass and the gel, under water, with a thin blunt-ended, polished steel rod. They were then immediately placed in Amido-Schwartz stain which fixed and stained the proteins. After 2 hours of staining the gels were destained in acetic acid.

In the experiments with haemolymph, the sample gels were prepared so that they contained 80 lambda of haemolymph per ml. Each tube was charged with a total of 12 lambda of haemolymph. When experimenting with foot muscle extracts, supernatant was mixed with sample gel in a ratio of 1:2.

Gel columns with stained protein bands (occupying a length of 33-35 mm) were analyzed in 2 ways. Densitometric tracings were made with the Canalco Model E microdensitometer, and Rf values for the various protein fractions were determined from the gels by direct measurement of the position of each band, using a ruler accurately calibrated in 0.5 mm units. An Rf value is the ratio of the distance from the origin to each band and to the front of the separation. This ratio tended to offset the slight shifts in band position due to small differences in length of the separation.

Rf values presented for the components are the averages for each component of all tubes of one test. It was found that aliquots of proteins from a single extract separated in several tubes yielded stable patterns and that the standard deviation for an average Rf value rarely exceeded the error observed when 2 different persons measured the same faint component, i.e., 3% or an Rf value of 0.014 at the most. When a single protein band was measured from a number of gels of different yet homologous experiments, the total variation rarely exceeded an Rf of 0.045 and was more commonly 0.020 or less.

Error in Rf determination often occurs for the following reasons. Although it was intended to terminate runs at 33 mm, they at times reached a length of

⁴CH₂: CHCONH₂

⁵tris = 2-amino-2-hydroxymethyl-1, 3-propanediol

TABLE 1. Three examples of statistical determinations serving to show whether the Rf values of 2 protein fractions of apparently homologous position in different gels are significantly different or not

Example	Average Rf value	Difference in Rf value	Total variation range for each mean value	Significant difference (t test)	Level of significance (P)
1	a*	0.546	0.045	No	0.10
	b	0.557	0.019		
2	a	0.230	0.015	Yes	0.01
	b	0.213	0.030		
3	a	0.923	0.038	No	0.10
	b	0.940	0.014		

*a and b represent 2 different gel series.

up to 35 mm. In the slightly longer runs, fractions did not necessarily migrate in proportion to the increased overall length, and some did not migrate beyond their position at 33 mm. Thus, the resulting Rf values for a band found to be stationary at 10 mm in 10 tubes of varying run length will vary. However, on the whole, Rf values tend to minimize differences in homologous bands due to different run lengths.

It was found that, on the whole, when average Rf values differed by 0.018 they indicated a significant statistical difference (using standard t test). Where Rf values were only 0.017 mm apart, it was necessary to determine whether or not there was a significant difference. Examples of such instances are given in Table 1.

The densitometric tracings were 4 times the actual run length. The position of a fraction in a tracing was readily evident when the fraction was dense. The position of faint fractions was not always clearly indicated on a tracing. In such a case, the position was determined by multiplying the Rf value by the length of chart (measured from origin to mid-point of last curve).

An integrating recorder attached to the densitometer marked off density units beneath the tracing. Each unit (a single line marked by pen) was equal to 9 mm² area under the curve. The area under each curve is a function of the amount (density) of dye which stains each protein component. Accuracy of quantitative densitometry relies on a linear relationship between protein concentration and stain uptake. This linear relationship is obtained when Amido-Schwartz dye is used (Hansl, 1964).

Estimates of relative protein concentration in each electrophoretic tube were made by totalling the density units for a predetermined length of gel. Total density units were compared for runs involving different size classes of snails to determine if increased size and/or age were correlated with varying density of blood or foot muscle extract proteins.

The densitometer and integrating recorder were checked for reproducibility of readings. A single gel of electrophoretically separated foot muscle protein was analyzed 30 times. The average value of 101.7 units showed a standard deviation of 4.3. A single

TABLE 2. Density of electrophoretically separated protein fractions from the haemolymph of *Helix pomatia*, for bands 3-6, determined by visual inspection of gels, as correlated with shell size. The results are from 35 snails (i. e., 35 tests)

Size index (Length in mm x width in mm)	Relative size	Visually observed density	No. of snails
40 - 110	Small	Distinct and dense Visible but faint Faint and fuzzy	17 2 2
200 - 800	Medium	Distinct but faint, especially band 4	3
960 - 1640	Large	Visible but faint Very faint, fuzzy, band 4 barely visible	3 8

electrophoretic separation of human blood serum was also analyzed 30 times; the mean number of density units for a predetermined length of gel was 46.3 and the standard deviation was 3.4. When 30 different gels of blood serum were likewise studied, the mean was 62.2 and the standard deviation was 3.7. It appears that a standard deviation of 3-4 units can be attributed to mechanical error.

EXPERIMENTS

1. Thirty five *Helix pomatia*, representative of 3 size classes (Table 2) were used: single specimens provided haemolymph for each of 35 tests, while simultaneous foot muscle tests were conducted with only 20 of these snails. Each haemolymph test was made with 2-3 gels and each test with foot muscle extract with 3-5 gels. In a later series of tests, foot muscle extract of an additional 10 snails of the large size class was studied, after the snails had been forced into hibernation in the refrigerator.

These tests were made to determine: (1) whether age and/or size caused variation in density of protein or shift in position of protein fractions; (2)

whether fractions were present at one stage of growth and absent in another; (3) whether haemolymph or foot muscle was more stable for routine electrophoretic analysis of species; and (4) whether the extent individuals of the same population varied in their electrophoretic patterns.

2. Ten tests were conducted with large sized *Helix pomatia* which had spent 1 month in hibernation in the refrigerator. In these experiments, foot muscle extract only was used.

3. Ten to 20 tests were conducted with each of the 4 populations of *Pomatiopsis lapidaria*. The data served to investigate the degree of variation between different populations of that species.

RESULTS

1. *Helix pomatia*

Haemolymph

In the 35 tests run (35 individual snails) a total of 12 components were found in the haemolymph (Table 3). Four of the fractions were always very faint. Densitometric tracings of the electrophoretically separated fractions are shown in Fig. 3 for small snails (I),

TABLE 3. Rf values for the 12 protein fractions separated electrophoretically from the haemolymph of *Helix pomatia* (mean for gels of 35 tests)

Band No. *	Corresponding Rf value
1	0.043
2	0.101
3	0.188
4	0.240
5	0.304
6	0.350
7	0.650
8	1.000

*The Rf values for another 4, very faint components are: 0.144, 0.550, 0.725, 0.942.

medium size snails (II) and large snails (III). The position of each of the 8 clear-cut fractions are marked below each tracing. It is graphically evident that blood density decreased with increased size (i.e., age) (Fig. 3, Table 2). This is particularly noticeable in the decreasing magnitude of band 7 with larger size (Fig. 3, I-III).

When haemolymph from small snails was analyzed, peaks A and D were very pronounced. With large snails and associated decrease in protein density per volume of haemolymph, the "A" fraction is visible in the gel but does not form a peak discernable in the densitometric tracings (Fig. 3, II, III). Likewise, individual peaks C, D become low and appear to lose their identity.

There was no qualitative change associated with increase in body size. There was, however, a significant decrease in protein density per volume of haemolymph with increase in body size. When the coefficient of correlation (r) was determined for variables of protein density (determined by total density units recorded by the integrating recorder for the entire length of protein separation in each gel) and body size, r was -0.88 and significant at $P < 0.01$. However, as

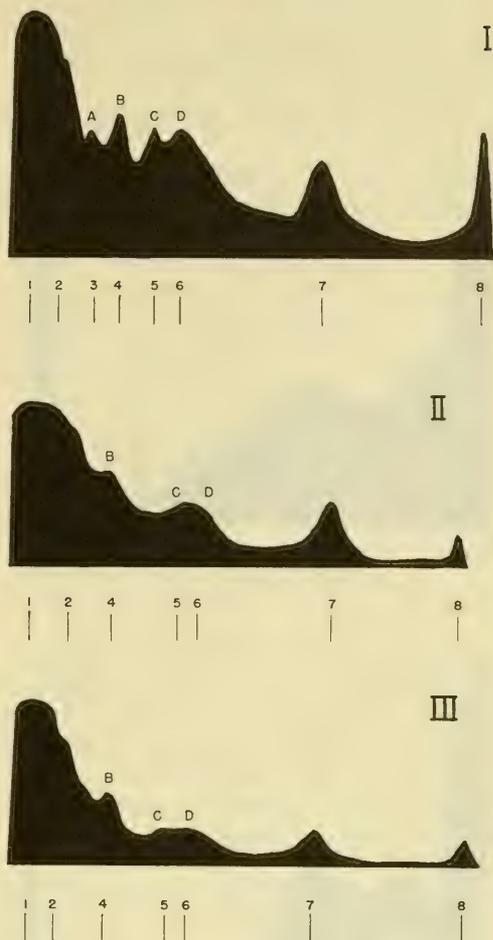


FIG. 3. Densitometric tracings showing electrophoretic patterns for *Helix pomatia* haemolymph from I) small snails, II) medium snails, III) large snails.

shown in Table 2, 4 of 21 small snails had faint components (bands 3-6) of similarly low density as those in large snails. This is, perhaps, due to experimental error.

Foot Muscle

A total of 20 components were resolved in the electrophoretic separation of foot muscle extract (Table 4). The 4 tracings in Fig. 4, and the 4 columns in Table 4 pertaining to the tracings, show the total range of variation en-

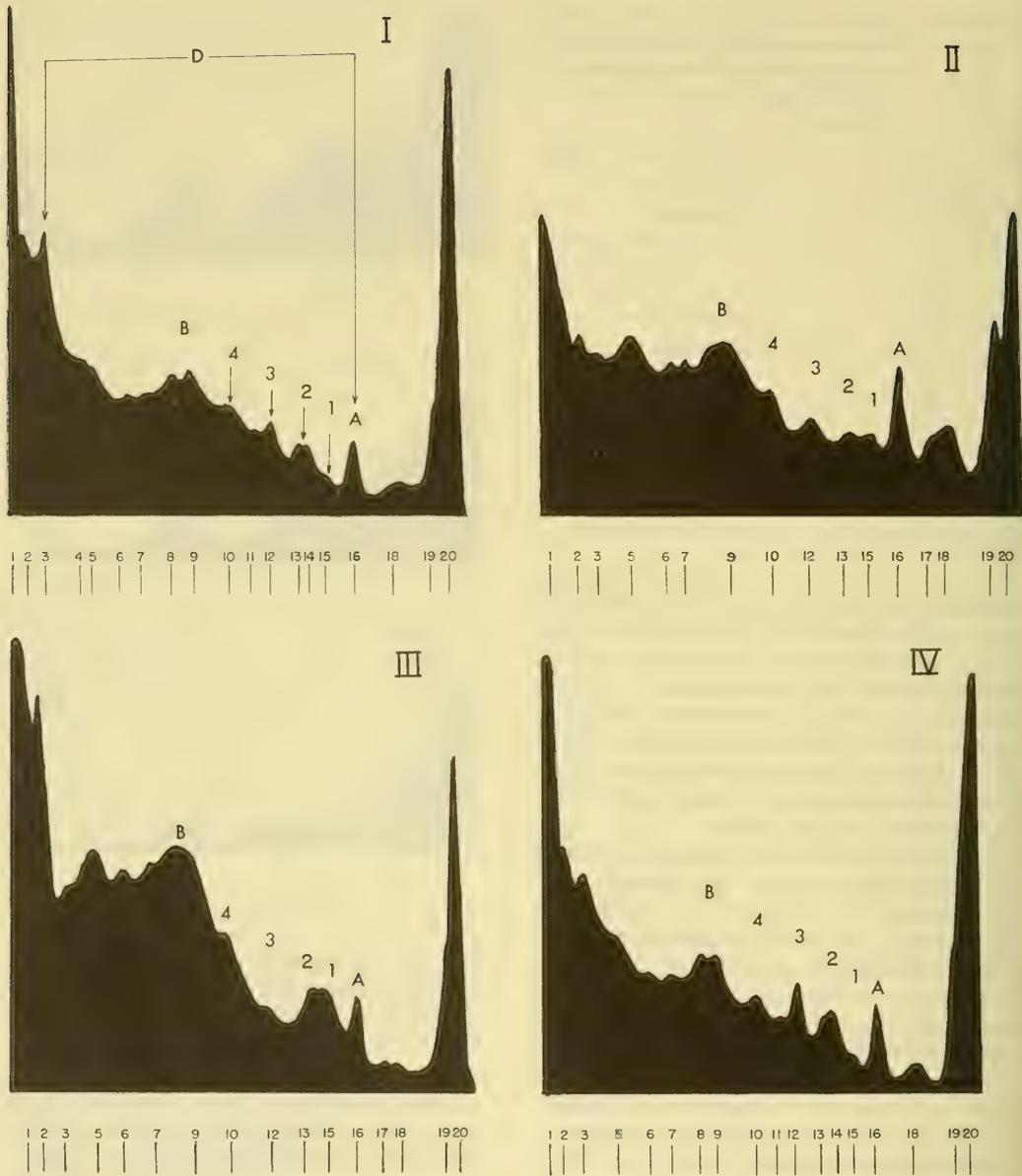


FIG. 4. Electrophoretic patterns showing the extent of variation in 4 individuals of *Helix pomatia*, I, II, III, IV, using foot muscle extract. The protein fractions discerned (20) are numbered below the densitometric tracings. Above each pattern the most characteristic components 1-4 are located between gel regions A, B. D represents the stretch of gel where density units were counted.

countered in 20 tests: from 1-3 components were not resolved in 4 snails. The 4 individuals revealing this range

were found within 10 tests and no further variation was found in the next 10 tests. The species is characterized by the

TABLE 4. Range of variation in the occurrence of a total of 20 distinct fractions* of protein extracted from the foot muscle of *Helix pomatia*

Band No.	Corresponding Rf value**	Individuals***			
		I	II	III	IV
1	0.022	+	+	+	+
2	0.075	+	+	+	+
3	0.091	+	+	+	+
4	0.167	+	-	-	-
5	0.182	+	+	+	+
6	0.255	+	+	+	+
7	0.304	+	+	+	+
8	0.376	+	+	+	+
9	0.415	+	+	+	+
10	0.496	+	+	+	+
11	0.548	+	-	-	+
12	0.582	+	+	+	+
13	0.661	+	+	+	+
14	0.684	+	-	-	+
15	0.714	+	+	+	+
16	0.763	+	+	+	+
17	0.823	-	+	+	-
18	0.867	+	+	+	+
19	0.965	+	+	+	+
20	1.000	+	+	+	+

*Not all fractions are necessarily found in every test, involving 1 snail each. It took 4 out of 10 snails to obtain total variation; examination of an additional 10 snails did not reveal any further variation. The 4 fractions involved are faint.

**Average from 20 runs with 3-5 tubes each.

***Compare with Fig. 4.

region of gel between and including peaks at gel region A and B (Fig. 4). Particularly characteristic are peaks 1-4. Frequently, differences in length of run of 1 mm affected sharpness of a component in the gel and the resulting tracing showed this in terms of lower and broader peaks or less distinct peaks. This situation is exemplified by the prominent peak B, appearing in tracings II and III, which actually represents 2 wide protein bands that are close together and slightly diffuse due to in-

creased length of runs. The diffuseness is such that 2 individual distinct peaks are not recorded as they are in tracings I and IV of Fig. 4, where the runs were slightly shorter and the fractions clear-cut.

As shown in Table 4, in some experiments a given fraction was not resolved, i.e., fractions 4, 11, 14, 17. These were low density components. This lack of resolution is attributed to slight variations in preparing gels for polymerization, slight error in protein sample concentration and changes in length of run. As mentioned above, such changes in length of run can cause adjacent components to appear as 1 wide band. From 15-20% of the components may vary in this manner. These are most often faint fractions very closely associated with other fractions.

The "fingerprint" (the linear pattern of protein bands in a gel characterizing a population or species) of one individual did not vary significantly qualitatively or quantitatively from that of another in the same population. When the size index ranged from 40-1056, the density units in a predetermined length of gel (Fig. 4, I, area bounded by D) were determined. The coefficient of correlation (r) for size against density units was -0.47 and was not significant ($P > 0.10$).

Ten adult snails with a size index of 1240-1260 were chosen to determine variability in density units between different preparations of foot muscle extract. These adults had spent 1 month in hibernation in the refrigerator. Ten tests were made with 3-4 tubes per test. The average number of density units within area D for all tubes was 73.8, with a standard deviation of 5.49. For any one experiment, the greatest total variation for tubes in that test was 14 units. As stated earlier, mechanical error accounted for a standard deviation of 3-4 units. Therefore, differences in total density units per homologous preparations and runs are very slight.

TABLE 5. Congruence (or lack of it) in the occurrence and location of a total of 26 distinct fractions of protein extracted from foot muscle from 4 populations of *Pomatiopsis lapidaria*

Band number	Corresponding Rf values* in Populations			
	Parker Mill	Clinton	Hog Back	Barton
1	0.014	0.014	0.014	0.014
2	0.056	0.056	0.056	0.056
3	0.104	0.104	0.104	0.104
4	0.148**	0.148	0.148	0.148
5	0.194	0.194	0.194	0.186
6	0.245	0.245	0.245	0.231
7	0.295**	0.305	0.309**	0.294**
8	++A 0.338	-	A 0.347**	A 0.355
9	A 0.368	A 0.385	-	A 0.383
10	0.424	0.425**	-	0.437
11	0.456**	-	B 0.472	-
12	B 0.494	B 0.505	C 0.507	B 0.493
13	-	-	-	C 0.520
14	C 0.554	-	D 0.565	-
15	-	-	E 0.585	D 0.586
16	D 0.600	D 0.616	-	-
17	E 0.634	-	-	E 0.631
18	-	E 0.656	0.661	-
19	-	-	-	0.695
20	0.725	0.716	-	0.714**
21	-	-	0.746	-
22	0.814	0.826	-	0.828
23	-	-	0.891	-
24	0.955	-	0.933	0.944
25	-	0.970	-	-
26	1.000	1.000	1.000	1.000

* Rf values averaged from 10-20 runs for the different populations.

** Indicates bands often difficult to resolve.

+ Fractions 8 and 9 often form a single wide band.

++ Letters indicate characteristic fractions as labelled in Figs. 5, 6.

The "fingerprint" from these snails was the same as that found for snails fresh from the field or snails maintained in the laboratory for several weeks.

2. *Pomatiopsis lapidaria*

Foot Muscle

A Study of Populations

From 10-20 tests were made for each of 4 populations. Rf values for the

components separated from snails of each population are listed in Table 5. As can be seen, there is considerable variation between populations when Rf values are considered by themselves. The different populations had from 16-19 fractions. These did not entirely correspond: any one population had 7-10 fractions that were missing in 1 or more of the other populations; a total of 26 fractions was found to characterize the

species as a whole.

Despite these variations in the number of distinct components, the species is especially and fully characterized by: (1) prominent components A-E (Figs. 5, 6); and (2) by the fact that the area between E and the front band is devoid of high density components. Considering components A-E, the twin dense bands D, E are prominent and characteristic for the species. Fraction C is a slight, faint component preceded by 2 dense, wide fractions, A and B (Fig. 5).

The densitometric patterns, in the characteristic region, for all populations studied, were similar and served to characterize the species. However, the most significant differences between populations involved the migrational characteristics of fractions A-E, i.e., their Rf values. As shown in Table 5, Rf values are listed and the characteristic fractions are designated by letters A-E, referring to the lettered fractions in Figs. 5, 6.

Parker Mill population is used as a reference population. One notes in Fig. 5, I & I', that fraction C is present as a strong, well defined component and that D, E are distinctly separated as to form 2 distinct peaks.

Clinton population (Fig. 6, IV) is characterized in having widely separated D-E fractions. As seen in Table 5, the Rf value for E is significantly greater than that for the same component in Parker Mill. The C fraction is absent. This population is further characterized by the lack of fractions between Rf 0.826 (band 22) and Rf 0.970 (band 25) while in all other populations 1 or 2 faint bands can be found in this region. Component 0.970 is hardly separated from the dense front band while in all other populations the fraction next to the front is widely separated from the front.

Barton population (Fig. 6, III, III') is characterized by lower Rf values for fractions C, D as compared to Parker Mill. C is present but very faint. A pronounced component at 0.695 (band

19) is typical for this population.

Hog Back snails (Fig. 5, II, II') differ the most in that components B-E have the lowest Rf values. D, E fractions are so close together that, with their diameters considered, they form a single dense wide peak in the densitometric tracing (Fig. 5, II). C is faint and difficult to resolve. There is a distinctive fraction at 0.746 (band 21).

Minor Variations. As already noted for *Helix pomatia*, experimental error does affect gel conditions in such a manner that variation in resolution may occur. Likewise, shifts in 1 or 2 mm in length of run affect resolution of components.

In the Parker Mill population, bands 7 and 11 were very faint and rarely encountered (Table 5). Band 4, i.e., component with an Rf of 0.148, is faint or absent.

In the Hog Back population, fraction 7 is sometimes resolved. Peak A is sometimes a result of fractions 8 and 9, not just 8 as shown in Fig. 5. Peak B is often represented by a wide, frequently diffuse band.

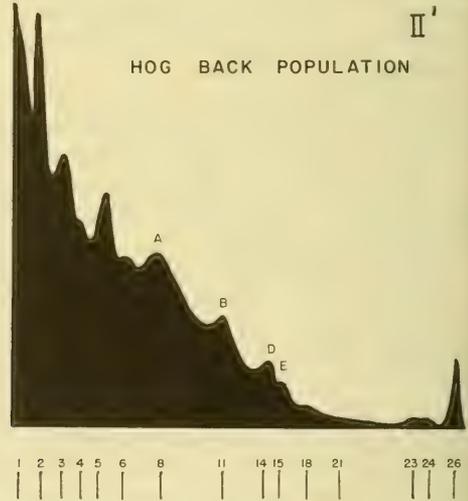
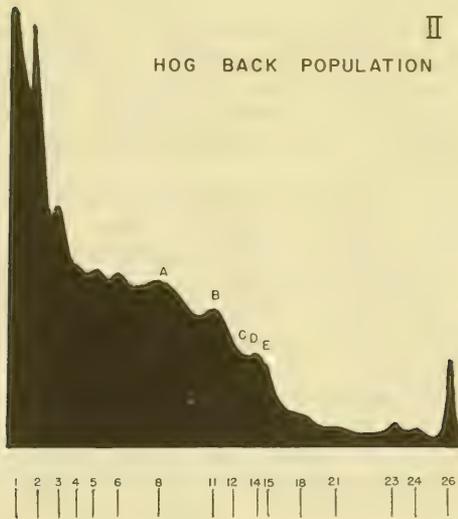
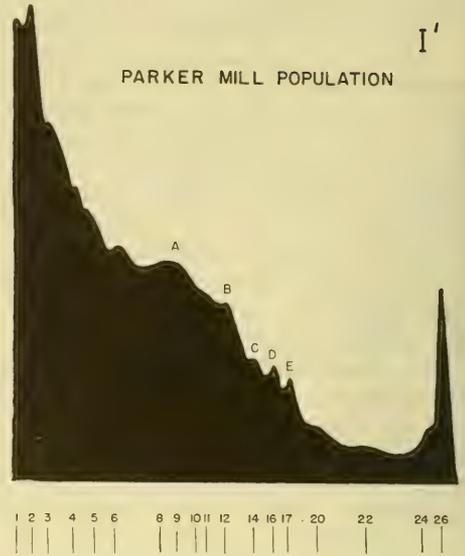
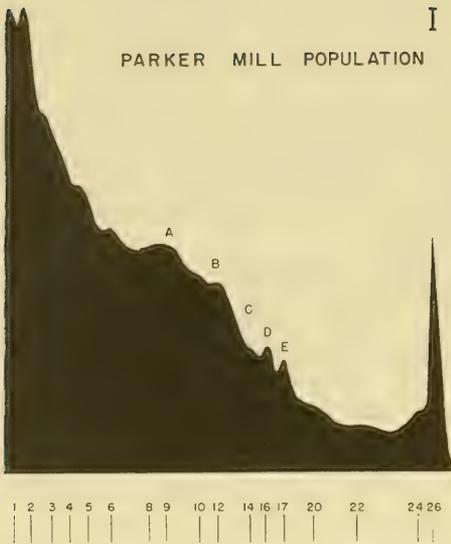
In the Clinton population, fraction 4 was always pronounced while band 9 was most often wide and blurred so that 2 distinct fractions (i.e., 0.338, 0.385) could not be clearly resolved. Band 10 (Rf 0.425) was not resolved in 50% of the tests.

As shown in Fig. 6, several fractions in the Barton population varied from absent to weak and poorly resolved. These included bands 7, 9, 20. Again, band 9 was often considered fused with band 8, in a wide, diffuse, dense band. Fig. 6, III, shows a tracing of a gel where these 3 fractions are not resolved.

DISCUSSION

The results from experiments with *Helix pomatia* haemolymph and foot muscle extracts showed that size (age), maintenance in the laboratory (change of diet, climate) and hibernation had no

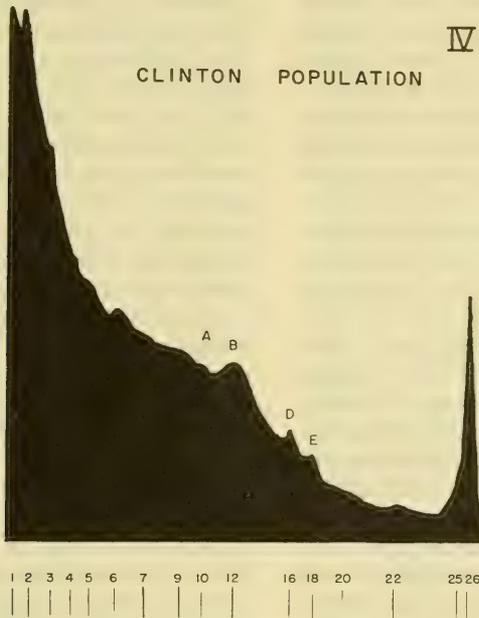
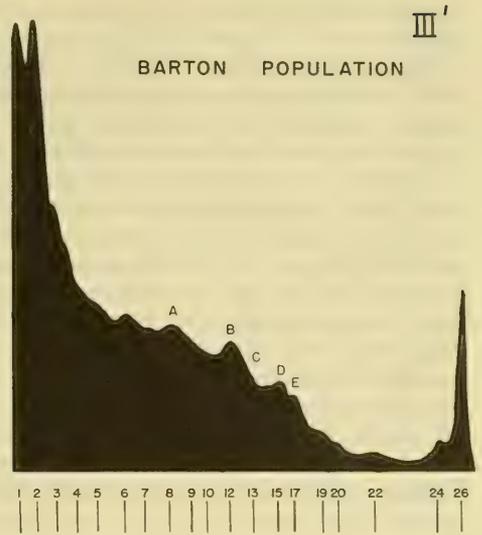
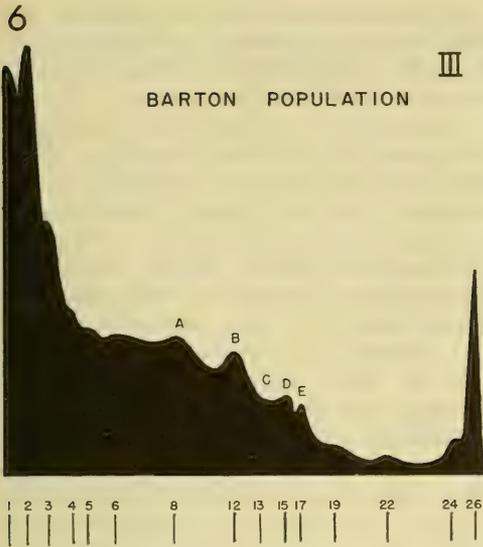
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FIGS. 5 & 6. Variation in the electrophoretic patterns of 4 populations of *Pomatiopsis lapidaria*. The fractions are numbered below the densitometric tracings, and the most characteristic components for the species are lettered A-E.

qualitative or quantitative effect on the electrophoretic "fingerprint" when foot muscle extract was used. Our results with *Helix* haemolymph differed somewhat from those of Wright & Ross (1963), who found that blood proteins varied considerably, both qualitatively and

quantitatively, in *Biomphalaria glabrata*, with progressive growth and development. *Helix pomatia* showed only a quantitative change with increment in size. It is noted that the blood (haemolymph) of the latter does not possess haemoglobin.



FIGS. 5 & 6. Variation in the electrophoretic patterns of 4 populations of *Pomatiopsis lapidaria*. The fractions are numbered below the densitometric tracings, and the most characteristic components for the species are lettered A-E.

Because of the variation occurring with haemolymph and because foot muscle tissue yielded reliable and reproducible qualitative and quantitative results, foot muscle extract was chosen

to routinely establish "fingerprint" patterns for populations and species. Comparisons between individuals showed no significant differences; therefore, the foot muscles of several individuals of

Pomatiopsis lapidaria were often combined.

Advantages of Foot Muscle. There are other advantages in using foot muscle tissue besides its apparent stability in electrophoretic characteristics. 1) The tissue is much more readily obtained from small mollusks than haemolymph; 2) foot muscle tissue is dense and yields relatively great weight of proteins per volume; 3) one can separate more distinct protein components from the tissue than from haemolymph; 4) the foot is comparatively homogeneous, i.e., it is predominantly muscle with mucus glands, a few nerve elements, and some circulating haemolymph; 5) the foot is rarely parasitized and is readily observed for parasites; 6) the foot is characteristic for the phylum mollusca.

In relation to the last point, it is noted that Wright & Ross (1963, 1965) used egg proteins, which yielded satisfactory results. While egg production is abundant enough for many pulmonate snails in culture, to assure numerous and sufficient runs, it is not as satisfactory for many other mollusks where eggs are laid singly and few in number, where eggs are especially minute, where adults resist reproduction in the laboratory or produce eggs seasonally.

Taxon Specific Fingerprints. As pointed out by Davis (1965a), certain gastropods which are known to be distinct in terms of correlated cytological, anatomical and ecological information, also have distinctive electrophoretic "fingerprints." Such differences were found for *Oncomelania hupensis formosana* and *Pomatiopsis lapidaria*, 2 species of closely related genera (Davis, 1965a) and for *P. cincinnatiensis* and *P. lapidaria*, 2 species of the same genus (Davis, unpublished).

Some groups of fractions or gel regions do distinctly characterize these taxa. Despite the differences found in different populations of *Pomatiopsis lapidaria*, this species was nevertheless definitely characterized by components A-E, the twin band D, E and the fast moving

section near the front, devoid of dense fractions. As expected, characteristic features were also associated with *Helix pomatia* (this paper).

It has been apparent that the more characteristic differences are usually found in the region of mid-gel to the front. The region near the origin is most commonly packed with a series of large molecules of slow migration. Sodeman & Meuwissen (1966) also reported that the area near the origin was "technically difficult to interpret".

It is suspected that a number of species that have been distinguished on insufficient data may not show distinct patterns, i.e., may not be true species. For instance, in a preliminary study (Davis, unpublished) the pulmonate lymnaeid snails *Stagnicola emarginata servata* and *Stagnicola catascopium* from Michigan, did not appear to have distinct differences. On the other hand, the degree of specificity of patterns may be less pronounced in other major molluscan groups than it is in the prosobranchs, and the lack of differentiation may be related to conditions obtained in another subclass. Possibly both factors are operative (see discussion below).

Population Differences. Our results with 4 populations of *Pomatiopsis lapidaria* show that the electrophoretic technique used is sensitive enough to demonstrate population variability in terms of migrational differences in identifiable components and of new or different fractions.

One of us (Davis, 1965a) studied in detail the internal anatomy of *Pomatiopsis lapidaria* from each of these populations and found no significant differences in structure, either qualitative or quantitative, with one exception. The central tooth from the radulae of snails from Parker Mill had a formula of 1-1-1/2-2 for 61% of the population while in Hog Back and Barton populations the formula was 1-1-1/3-3 in 80%.

While electrophoretic data show slight

but consistent differences in migrational characteristics, these differences were so slight that the densitometric patterns for the 4 populations were very similar. In another paper, Davis (1967) discusses populations of *Oncomelania hupensis formosana* in which differences revealed by disc electrophoresis are great enough to change a portion of the densitometric pattern. In this case, electrophoretic differences were associated with forms which differed in susceptibility to infection with different strains of *Schistosoma japonicum*, the oriental human blood fluke. No significant difference in anatomy has been demonstrated, that could be coupled with these electrophoretic or parasitological differences.

The ability to resolve population differences indicates the potential for providing information on subspecific genetic differences. As mentioned above, such differences can be used to characterize subspecific taxa with unique characteristics such as a distinctive relationship to parasitic infection.

Wright & Ross (1965) demonstrated in an electrophoretic study on African Planorbidae (using cellulose acetate matrix and egg proteins) that populations of species of *Bulinus* and *Biomphalaria* had characteristic electrophoretic patterns. They reported that patterns for different populations varied where no morphological characters had previously been proved capable of separating the populations. Their findings agree with ours (Davis & Lindsay, 1964; Davis, 1965a) in that electrophoretic patterns provided objective criteria for characterizing different taxon levels.

In their initial study with 7.5% polyacrylamide electrophoresis, Pace & Lindsay (1965) studied 15 populations of African bulinid snails as well as *Biomphalaria glabrata* from Puerto Rico and *Helisoma trivolvis* from Michigan. They state that "strikingly little variation was found" when the taxa were compared and that "the variation observed between populations of one species of *Bulinus* were often as great

as that between the three subfamilies represented in the study." They further stated that the "applicability of disc electrophoresis techniques to the solving of taxonomic and phylogenetic problems in the Planorbidae, much less the Bulinidae, appears unlikely."

The last statement was a little premature for several reasons. Although they give no details, it appears that they did find variation in electrophoretic patterns between different populations of 1 species of *Bulinus*. The source material should be re-evaluated to determine the significance of the variation found. Certainly, however, the results they obtained with 1 standard technique do not justify their statement: "Thus, genetic divergence as displayed by the divergence of protein composition is essentially absent in the Planorbidae relative to the Lymnaeidae and the Hydrobiidae."

It is well understood that electrophoretic patterns are subject to major changes with slight changes of experimental procedure. The use of 7.5% polyacrylamide provides a rather tight sieving action on proteins, as the average pore size in the gel is about 50 angstroms. Patterns obtained by 7.5% gels may be a series of consecutive bands with few distinguishing features. One reason for this picture is that the small pore size renders possible separation of rather closely related groups or populations of molecules into a series of bands. If the pore sizes were increased (e.g., by using 4.5% acrylamide) and the pH changed, a different pattern would result because of the decreased friction on large molecules and shift in the migrational potential of the molecule relative to change in charge. These changes might result in more characterizing "fingerprints." It might be that, with the current technique, the planorbid proteins extracted from the foot appear homogeneous, while, with larger pore gels in conjunction with different pH levels in the buffer solutions, undetected heterogeneity might

be revealed. It would, indeed, be strange if, within such a large, diverse and ancient group as gastropod mollusks, a single stereotyped experimental procedure would serve to adequately characterize species and population differences.

Disadvantages of Disc Electrophoresis. The method presents several problems which accompany the advantages of multicomponent separation. These are: 1) minute experimental error has a pronounced effect on gel resolution and protein fractionation; 2) species analysis is complicated by significant population differences; 3) routine separation of 17-25 components in a stretch as small as 35 mm maximally, complicates analysis and comparisons between species.

1) Concerning experimental error, a quote from Hansl (1964) should suffice. He stated, concerning the reproducibility of the sample material itself, "...disc electrophoresis, as designed by Ornstein and Davis, has been designed to avoid some of the reproducibility problems often presented by the single-layer starch and cyanogum procedures. Reproducibility is, nevertheless, not yet perfect. Matters of glassware cleanliness, care in gel preparation, storage, handling and shelf life of chemical solutions can cause differences between laboratories and even between work done at different times in the same laboratory. Although, in the hands of experienced disc electrophoresis users, these differences are today no worse than those encountered in paper electrophoresis..."

As a control for gel conditions, we have routinely used human blood serum. Since the pattern for human serum is well known, deviation from the pattern indicates that a group of gels is suspect and possibly producing results not correct in terms of patterns established when gel conditions are optimal.

2) While in our work we welcome methods of resolving population differences for a species, when such differ-

TABLE 6. A listing of Rf values for foot muscle protein fractions of 3 gastropod species, to reveal the number of corresponding components which are not significantly different.* (The value connected by a line is also not significantly different.)

<i>Oncomelania hupensis chiui</i>	<i>Pomatiopsis lapidaria</i> (All populations)	<i>Helix pomatia</i>
0.014	0.014	0.022
0.064	0.056	0.075**
-	-	0.091
0.117	0.104	-
-	0.148	-
0.174	-	0.167
-	0.194	0.182
0.247	0.245	0.255
0.276	-	-
0.303	0.295	0.304
-	0.338	-
0.373	0.368	0.376
0.427	0.424	0.415
-	0.456	-
0.477	-	-
-	0.494	0.496
-	0.520	-
0.544	0.554	0.548
-	0.585	0.582
0.601	0.600	-
-	0.634	-
0.652	0.656	0.661
-	-	-
-	0.695	0.684
0.721	0.725	0.714
-	0.746	-
-	-	0.763
0.780	-	-
0.831	0.826	0.823
0.896	0.891	0.965
-	0.933	1.000
-	0.970	0.965
1.00	1.000	1.000

*When the difference between 2 Rf values is 0.017 or more, the standard t test should be made to determine whether or not there is a significant difference (see p 317).

**The difference between 0.075 and 0.056 was not significant.

ences occur, such differences do entail a greater amount of work to fully characterize a species for adequate comparison with other species. More complex analysis also involves the necessity for an adequate supply of snails which are not always available. As mentioned for *Pomatiopsis lapidaria*, however, such differences between populations do not necessarily interrupt a species' specific pattern.

3) Numerous fractions packed in a short distance complicate comparisons between gels. Most often the region near the origin is packed with 6-7 components no matter what the tissue analyzed. In Table 6, Rf values of *Helix pomatia*, *Pomatiopsis lapidaria* and *Oncomelania hupensis chiuvi* are compared. The last species was formerly known as *Tricola chiuvi* and the justification for change in nomenclatural status is discussed by Davis (1965b, 1967). One finds that 63% of the fractions of *Helix pomatia* and 54% of the fractions of *Pomatiopsis lapidaria* are identical to those of *O. h. chiuvi*. *Pomatiopsis* is in the same hydrobiid subfamily as *Oncomelania* while the stylommatophoran *Helix* is in a different subclass.

It has become apparent that only specific regions of the gel characterized by fraction position and density are diagnostic for a species and can serve for interspecific comparisons.

Diagnostic Limits of "Fingerprints"
Pointing out population differences is an important first step in demonstrating genetic differences, when these exist, between populations of the same species. However, in evaluating relationships between snails, whether populations or species, snails and parasites, or when trying to understand the evolution of races, etc., the "fingerprint" alone is not sufficient.

The reasons for this are quite evident. The same Rf values for fractions, in different species, do not necessarily indicate homology. As shown for *Pomatiopsis lapidaria*, the same characteristic fractions may have different Rf values

in different populations and comparisons are best made in terms of the characteristic densitometric patterns. To quote Sibley (1960), "...because protein structure is genetically determined it seems reasonable to assume that two proteins are extremely similar if they are derived from closely related species and have identical electrophoretic characteristics. For example, it seems reasonable to assume electrophoretically identical peaks in different species of the same genus result from proteins nearly identical in basic structure."

Still, such identity must be proven. When a large number of genera and species (each species with a different "fingerprint," and populations of species with still further shifts in patterns) are investigated and compared, a large array of "fingerprints" may become confusing and the homology of components at the same Rf value less probable.

A natural next step, after the electrophoretic analysis of proteins, is the use of immunology to test identity, partial identity or nonidentity for selected antigen-antibody systems. To this end the preparation, on the one hand, of fraction specific antisera and, on the other hand, taxon specific antisera is to be encouraged. In the latter category, an antiserum discerning taxa at a level below that defined by morphology is desired.

Using such techniques, Davis (1967) was able to discuss the phylogeny and relationships involving a newcomer to the subspecies group of *Oncomelania hupensis*, i.e., *O. h. chiuvi*. The electrophoretic "fingerprints" were most useful in indicating which population of *O. h. formosana* on Taiwan was most closely related to *O. h. chiuvi*, while immunological results verified that the 2 above taxa were closely allied.

Integrated Approach. To define discrete genetic units or taxa, all knowledge available pertaining to those units must be utilized and various levels of knowledge integrated. An objective

systematic evaluation of molluscan species and races, especially those involved in the transmission of human disease, can be derived from anatomical, cytological and biophysical data. By themselves electrophoretic profiles do not serve to define categories (e.g., genera, species, subspecies) nor do the number of antigen-antibody systems define taxa.

At present, electrophoretic data are no more objective than precise anatomical data, when it comes to comparing taxa in terms of genetic relationships or phylogeny, because of the aforementioned lack of knowledge as regards the homology of protein fractions from different taxa. As already indicated, immunology can help bridge that gap at both levels, i.e., anatomical and electrophoretic.

There are those who feel that anatomical data are unsatisfactory in describing certain categories (genera, species) objectively. Two primary reasons are most commonly put forward: 1) the great variability in organs; 2) the lack of discrete characters useful for defining taxa at the specific and sub-specific levels, when physiological, ecological or parasitological differences have been observed.

In reviewing papers of molluscan anatomy, one finds adequate reasons why such opinions are voiced.

1) Too much anatomy is presented as precise, although what is actually involved, is organology indicative of family, subfamily or generic levels when the specific level was intended. With units of measurement omitted and figures roughly drawn, data are of comparatively little value.

2) Lack of uniformity in orienting organs in drawings makes comparisons from different papers difficult or impossible.

3) Stylized drawings have their value in pointing out general features of comparative interest but should not be used in place of precise detail of a structure's shape, substructure and relationship to

other structures.

4) Important systematic decisions are frequently made on partial anatomy. For example, the verge may provide data which are not balanced by data on the remainder of the reproductive tract or of the female system. Organs which are easy to study such as verge and radula should not be used without regard to structures more difficult to study and, perhaps, with greater fundamental information content.

5) Rarely do extrapolations from serial sections provide accurate anatomical information as to organ shape, relationships of coiled tubes, etc. While sectioning is most useful in demonstrating vague connections of tubes or delineating the origin of fine nerves closely associated with other nerves, etc., refined gross dissection is adequate for all but the smallest of mollusks.

Relative to these points the reader is referred to Walter (1963), who uses anatomical data to discuss a most crucial systematic issue. We feel that adequate objective definitions of genera and species (in the vast majority of cases) can be made with data derived from careful, precise anatomic study involving whole organ systems.

Cytological data have also proved to be important. In the *Bulininae* of the *Planorbidae*, Burch (1964) demonstrated the occurrence of a polyploid series, which not only serves to demarcate discrete genetic (and taxonomic) units in terms of chromosome number, but also to correlate susceptibility of infection with *Schistosoma haematobium* with the higher polyploid numbers.

Within a framework of precise anatomy and cytology, biophysical data have their useful place. Without this framework, however, one is caught in a morass of disjunct molecular populations.

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RESUMEN

ANALISIS DISCO-ELECTROFORETICO DE INDIVIDUOS
Y POBLACIONES DE MOLUSCOS

G. M. Davis and G. K. Lindsay

El valor de disco-electroforesis es investigado penetrando en problemas de la sistemática de moluscos. ¿Serian estables los patrones electroforéticos dados por proteínas de la hemolinfa o extracto del músculo pedal? ¿Difieren estos en individuos de la misma especie?

Proteínas de la hemolinfa de individuos de *Helix pomatia* dieron 12, y el músculo del pie 20, componentes, los cuales no mostraron cambios cualitativos en caracoles de tamaño (edad) diferente. Cuando el tamaño del caracol fue correlacionado con densidad proteínica, hubo un cambio cuantitativo inverso significativo, pero no con extractos del músculo pedal. Desde que el último ofreció un modelo estable sobre diversas condiciones fisiológicas (inducidas por edad, invernación forzada en refrigerador y mantenimiento en laboratorio), y también proveyó más fracciones, este fue elegido para otros experimentos.

Comparando los ferogramas del músculo pedal, en individuos de una misma población de *Helix*, no se encontraron diferencias significativas.

Cuatro poblaciones diferentes del caracol anfibio *Pomatiopsis lapidaria* fueron estudiadas también usando extractos del pie. En conjunto se encontraron 26 fracciones distintas para las especies, de las cuales 16-19 estaban presentes en cualquiera de esas poblaciones. Pero a pesar de significativa variación entre ellas, la especie se caracterizó por un modelo densitométrico claramente reconocible en cada una.

Varios aspectos se pusieron en evidencia: a) Aunque extracto muscular del pie fué la fuente protéica que dio ferogramas estables, no todas las fracciones caracterizando una población fueron rutinariamente separadas en una prueba; se necesitaron por lo menos 10 pruebas por población para abarcar todas las variaciones con certeza en patrones estables. b) Las dos especies estudiadas tenían patrones electroforéticos muy distintos. Ciertas regiones de la columna del gel dieron modelos densitométricos de mayor valor comparativo. En general la región mediana al frente puede usarse mejor para caracterizar un taxón.

c) Disco-electroforesis tiene distintas ventajas sobre otras técnicas como el gel almidón y electroforesis en papel: (1) el gel poliacrilamido standard de 7.5% permite mayor fraccionamiento y resolución de componentes; (2) el tamaño de poro del gel puede ser cambiado para permitir selección de ciertas moléculas; (3) el gel puede cargarse con una muestra tan pequeña como de 300-800 microgramos; (4) el tiempo necesario es solo de 30 minutos aproximadamente; (5) gels teñidos son fácilmente conservados para referencia futura.

Mientras la técnica es muy sensitiva y pequeños errores experimentales en la preparación del gel tienen un profundo efecto negativo, control rutinario con suero sanguíneo humano, cuyo patrón es bien conocido, puede indicar si las condiciones del gel son óptimas.

Debe notarse, en general, que aunque perfiles electroforéticos pueden revelar características específicas y subespecíficas, y son importantes para demostrar diferencias genéticas no anatómicamente evidentes, no son suficientes en sí mismas para definir categorías taxonómicas, porque la identidad de los componentes protéicos observados permanece desconocida. Pruebas inmunológicas con sistemas de antígenos-anticuerpos seleccionadas pueden ayudar a revelar homología de componentes de taxa diferentes, localizados en posiciones análogas.

Sin embargo, datos biofísicos tienen su lugar útil solo dentro de un marco integrado de anatomía y citología precisas.

АБСТРАКТ

АНАЛИЗ ОТДЕЛЬНЫХ ОСОБЕЙ МОЛЛЮСКОВ И ИХ ПОПУЛЯЦИЙ
С ПОМОЩЬЮ ДИСКОВОГО ЭЛЕКТРОФОРЕЗА

Г. М. ДЕВИС И Г. К. ЛИНДСЕЙ

В работе изучалась пригодность метода дискового электрофореза для целей систематики моллюсков. Являются ли электрофоретические картины, получаемые из экстрактов белков гемолимфы или из мускулов ноги моллюсков постоянными достаточно, чтобы их можно было бы использовать для целей систематики? Различаются ли эти картины у отдельных особей из одной и той же популяции или имеются различия у разных популяций одного и того же вида?

Вне зависимости от размеров (возраста) белки гемолимфы отдельных особей *Helix pomatia* имеют 12 компонентов, а белки из экстракта мускулов ноги - 20, не давая качественных различий. Но когда размер моллюсков коррелирует с количеством белка, то наблюдается значительное обратное количественное соотношение для гемолимфы, но не для мускула ноги. Поскольку последний дает наиболее постоянную картину при самых различных физиологических условиях (вызванных возрастом или усиленным содержанием в холодильнике и в лабораторных условиях) и дает наибольшее количество фракций, то экстракт из мускулов ноги и был выбран для дальнейших экспериментов.

При сравнении электрофоретических картин на экстрактах мускулов ноги, полученных от отдельных особей одной и той же популяции *Helix*, не было отмечено сколько нибудь существенных различий.

Изучались также экстракты из мускулов ноги, взятых у четырех различных популяций амфибионтного моллюска *Pomatiopsis lapidaria*. У этого вида было найдено 26 определенных фракций, из которых 16 - 19 имелись постоянно в каждой популяции. Однако, несмотря на значительные различия между популяциями, этот вид характеризовался денситометрической картиной, хорошо различимой в каждой популяции.

Авторы считают, что из проделанной работы становится очевидным следующее: а) хотя экстракт из мускулов ноги дает постоянную электрофоретическую картину, но не все фракции, характеризующие популяцию, могут быть выделены и разделены в одном опыте; необходим анализ не менее 10 проб на каждую популяцию, чтобы охватить все вариации и уверенно установить стабильные и постоянные компоненты; б) оба изученные вида дали очень различную электрофоретическую картину. Некоторые области колонки геля имели более ценные для сравнения денситометрические компоненты. В общем, для таксономической характеристики лучше всего использовать среднюю область колонки до ее фронтальной части. в) дисковый электрофорез имеет определенные преимущества перед такими методами, как электрофорез на крахмальном геле и бумаге: 1) стандартный 7,5% полиакриламидный гель обеспечивает лучшее фракционирование

компонентов; 2) для отбора некоторых молекул размер пор геля может быть изменен; 3) на гель можно нанести пробу, порядка 300 - 800 микрограмм; 4) время, необходимое для электрофореза составляет всего 30 минут; 5) окрашенные гели хорошо сохраняются для дальнейшего использования.

Поскольку этот метод очень чувствителен и малейшие ошибки при приготовлении геля влекут за собой глубокий отрицательный эффект, необходим обычный контроль на хорошо изученной сыворотке крови человека, электрофоретическая картина которой хорошо известна; такой контроль может указать на оптимальные условия приготовления геля.

Следует отметить, что хотя кривые, полученные при электрофорезе могут выявить видовые и подвидовые характеристики и очень важны для демонстрации генетических различий, незаметных анатомически, эти характеристики сами по себе недостаточны для установления таксономических категорий, т.к. идентичность компонентов белков пока остается неизвестной. Иммунологические пробы с избранными системами антигенов-антител могут помочь при установлении гомологии компонентов у различных таксонов, расположенных в аналогичных местах. Однако, использование биофизических данных может быть полезным лишь в рамках точных анатомических и цитологических характеристик.

GENETIC STUDIES ON *BIOMPHALARIA GLABRATA*
(BASOMMATOPHORA: PLANORBIDAE),
A THIRD PIGMENTATION ALLELE

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ABSTRACT

A mutant pigmentation variation in an albino colony of *Biomphalaria glabrata*¹ is reported; the mutant has black eyes and limited mantle pigment but is deficient in some of the pigment characteristic of the wild type. Experimental matings demonstrated that multiple alleles at the same locus determine the 3 pigmentation variations, with "black-eyed" dominant over albino, and wild type dominant over both "black-eyed" and albino. In view of the snail's ability for either self- or cross-fertilization, the presence of 3 pigmentation alleles particularly emphasizes the value of *B. glabrata* in genetic studies.

INTRODUCTION

Albino *Biomphalaria glabrata* have been found naturally occurring in Brazil and Venezuela. It was demonstrated by Newton (1954) that albinism in this species is determined by a single gene and is recessive to wild type pigmentation. This character has been used in mating experiments for systematic studies, and to demonstrate relations between reproduction and geographic separation of strains (Barbosa et al., 1956; Paraense, 1955, 1956, 1959; and Richards, 1962).

The appearance of black-eyed snails in aquarium colonies of albino *B. glabrata* was recently noted. These snails also had pale pigment spots in the mantle, but could be distinguished from the wild type pigmented snails. Studies were therefore carried out to determine the genetic relation of the 3 pigmentation variations.

MATERIALS AND METHODS

The albino colony of *B. glabrata* combining albinism and high susceptibility to infection with *Schistosoma mansoni*, was established by Newton (1955). The black-eyed mutant appeared in this colony. The wild type pigmented colony used in the present matings was from Bahia, Brazil.

Snails were reared in tap water dechlorinated by aeration, in 400 ml beakers with Petri dish covers, and fed romaine lettuce. They were reared in isolation and allowed to produce offspring by self-fertilization before mating. When 2 *B. glabrata* of common geographic origin were mated, cross-fertilization typically takes over almost completely (Paraense, 1959), this "dominance" over self-fertilization lasting about a month after re-isolation. In this study snails were mated for one week. The duration of egg laying in

¹In Opinion 735 of the International Commission on Zoological Nomenclature it has been ruled that the name *Biomphalaria* is to be given precedence over *Planorbina* (i. e. *Australorbis* of authors), etc., by any zoologist who considers these names to apply to the same taxonomic genus. ED.

B. glabrata is such that it is possible to induce self-fertilization in a snail and then cross it several times, in series.

"Black-eyed" mutant *B. glabrata* were isolated and allowed to reproduce by self-fertilization. True breeding individuals were then mated with albinos or with snails homozygous for wild type pigmentation, as demonstrated by prior self-fertilization. Offspring of the various pigment types after mating were isolated and allowed to reproduce by self-fertilization, then used in backcross matings. In the following results A indicates albino, B "Black-eyed", and C wild type pigmentation.

RESULTS

Examples of the various crosses and results are shown in Figs. 1 and 2. All the types of crosses were repeated several times with different snails with comparable results.

True-breeding albino and "black-eyed" snails (Fig. 1) were mated and then re-isolated. Seventy-five post-cross offspring from the albino parent and 35 from the "black-eyed" parent were examined, and all were "black-eyed". When these F_1 hybrids were reared in isolation, self-fertilization resulted in B and A offspring in a 3:1 phenotypic ratio. When they were backcrossed with albinos, both parents produced B and A offspring in 1:1 ratio. The same homozygous B parent was later mated with a homozygous C snail. The B snail subsequently produced 3 B F_1 offspring (presumably resulting from sperms remaining from the previous cross). Otherwise 50 F_1 offspring examined from each parent were all C's. Isolated F_1 hybrids produced by self-fertilization C and B F_2 snails in a 3:1 ratio. When hybrid F_1 individuals were backcrossed to homozygous "black-eyed" snails, both parents produced C and B offspring in 1:1 ratio.

A cross between CA and BA heterozygotes is shown in Fig. 2. Pre-cross

self-fertilization had produced F_1 generation snails in a 3:1 ratio of both C:A and B:A. After mating the CA snail produced C, B and A offspring in 2:1:1 ratio. When the post-cross F_1 generation snails from the CA parent were isolated, the A's bred true, the B's produced B and A F_2 offspring in 3:1 ratio, and the C's produced 3:1 ratios of either C and B or C and A.

The BA parent snail in Fig. 2 continued to produce B and A offspring in a 3:1 ratio after mating, indicating it was still self-fertilizing. Subsequent matings of each heterozygous parent snail with other snails demonstrated that the CA snail did not function as a male in cross-fertilization when mated, a condition not uncommon. The BA snail was successfully cross-fertilized in other matings.

DISCUSSION

From the results of matings performed it is concluded that multiple alleles at the same locus determine the 3 pigmentation variations with "black-eyed" dominant over albino, and a wild type pigmentation dominant over both "black-eyed" and albino. This third pigmentation allele in *B. glabrata* is being used in current studies. Thus, it is possible to mate an albino with both a "black-eyed" and a wild type snail, either concurrently or in consecutive series, and to distinguish in the albino's offspring which snail served as the male parent.

Both wild type and "black-eyed" snails show variations in mantle pigment: spots, diffuse background pigment, both or neither. Pigment variations were noted by Newton (1954). The characteristic difference between wild type and "black-eyed" snails is the occurrence of black pigment in the mantle collar and foot in the wild type, which is lacking in the "black-eyed" type. This pigment deficiency, most marked in young snails, facilitates observation of developing parasites and internal organs in "black-eyed" as well as albino

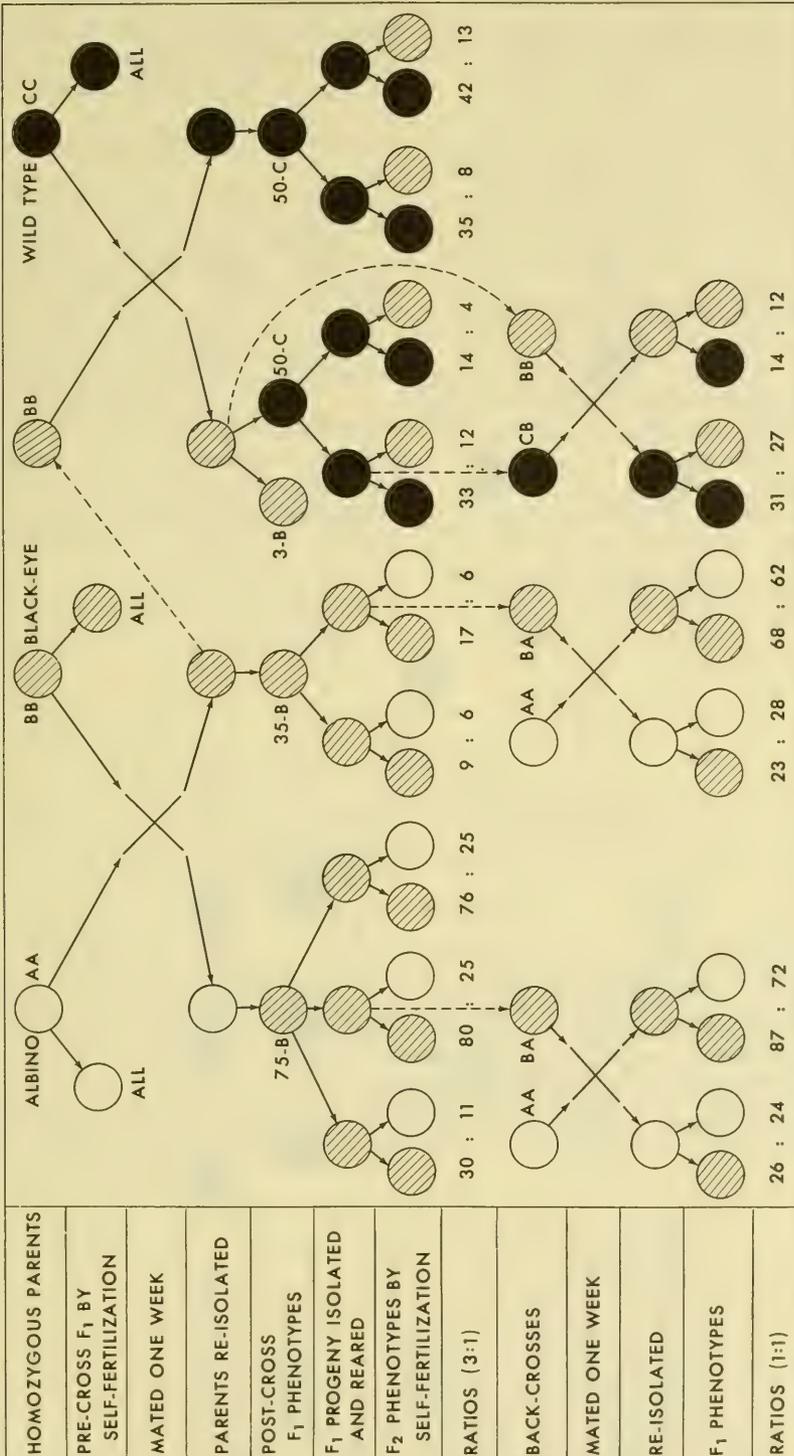


FIG. 1. Diagram of *Biomphalaria glabrata* matings employing 3 pigmentation alleles: "black-eyed" X albino, "black-eyed" X wild type, and backcrosses.

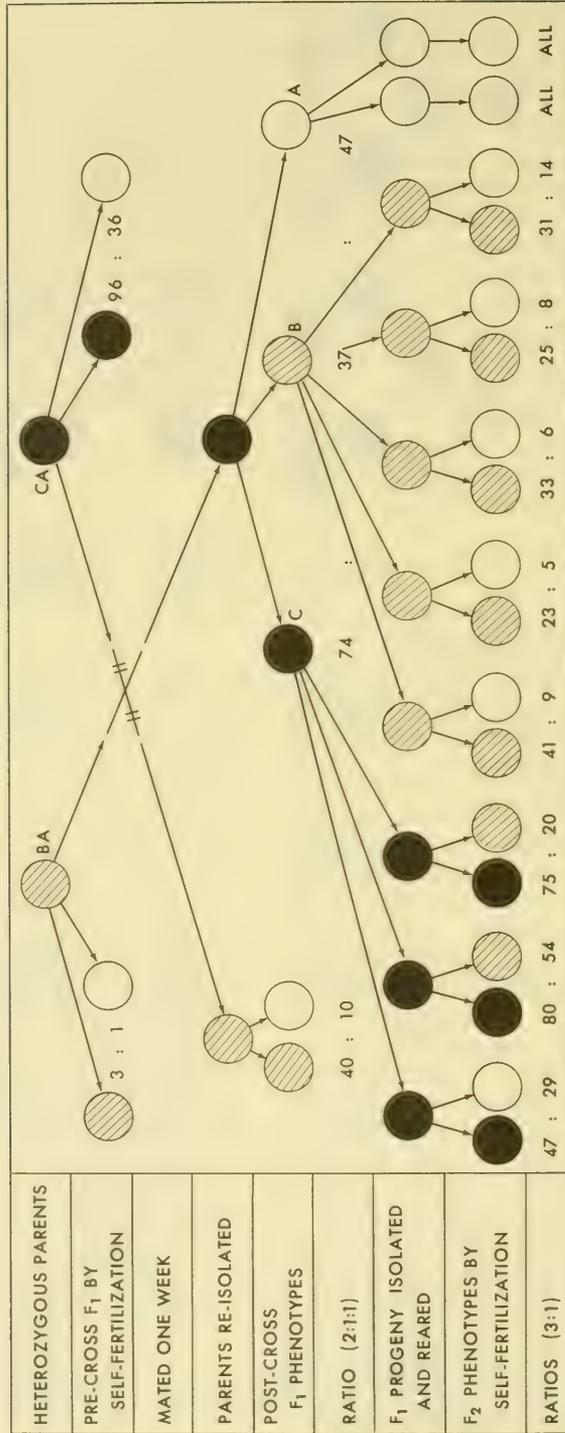


FIG. 2. Diagram of a cross between "black-eyed"/albino and wild type/albino heterozygotes of *Biomphalaria glabrata*.

snails. The "black-eyed" snails are as susceptible to infection with *Schistosoma mansoni* as the albino colonies in which they were discovered.

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RESUMEN

ESTUDIOS GENETICOS EN *BIOMPHALARIA GLABRATA*
(BASOMMATOPHORA: PLANORBIDAE)
UNA TERCERA PIGMENTACION EN ALELO

C. S. Richards

Una mutación variante en pigmentación de una colonia albina de *Biomphalaria glabrata* se registra. La mutación tiene ojos negros y pigmento del manto limitado, pero es deficiente en algunos de los pigmentos característicos del tipo silvestre. Acoplamientos experimentales comprobaron que los alelos múltiples del mismo locus, determinan las tres variaciones pigmentarias, con "ojos negros" dominantes sobre el albino, y tipo silvestre dominante sobre ambos, los de ojos negros y albinos. En vista de la capacidad del caracol para fertilización cruzada o autofertilización, la presencia de 3 alelos pigmentarios aumenta la importancia de *Biomphalaria glabrata* en estudios genéticos.

АБСТРАКТ

ГЕНЕТИЧЕСКИЕ ИССЛЕДОВАНИЯ ПИГМЕНТАЦИИ У
BIOMPHALARIA GLABRATA (*BASOMMATOPHORA: PLANORBIDAE*)

К. С. РИЧАРДС

Описывается изменчивость пигментации мутантов в популяции альбиносов *Biomphalaria glabrata*; мутанты имеют чёрные глаза и ограниченную пигментацию мантии, но это не проявляется у диких форм. Экспериментальное спаривание показало, что множественные аллеломорфы из одного и того же локуса хромосомы определяют три пигментных вариации: доминирование "черноглазых" над альбиносами, доминирование дикого типа и над "черноглазыми" и над альбиносами. С точки зрения способности улиток к само-и перекрёстному оплодотворению, наличие трёх аллелей пигментации особенно подчёркивает ценность *B. glabrata* для генетических исследований.

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CHROMOSOMENUNTERSUCHUNGEN AN GASTROPODEN
(STYLOMMATOPHORA)

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Schweiz

ZUSAMMENFASSUNG

Bei 33 chromosomal unbekanntem Stylommatophoren und 2 Prosobranchiern wurde die Chromosomenzahl ermittelt, bei 8 Heliciden, 4 Clausiliiden und einem Prosobranchier wurde sie bestätigt.

Die neu ermittelten Chromosomenzahlen (n) sind: *Pomatias elegans*, *P. costulatus*, 13; *Cochlicopa lubrica*, 26; *Abida secale*, *Chondrina avenacea*, *C. similis*, 30; *Ena montana*, *E. obscura*, *Zebrina dendrita*, 24; *Succinella oblonga*, 11 und 12; *Discus rotundatus*, 29; *Vitrea diaphana*, 20; *Laciniaria plicata*, 24; *Delima itala*, *Papillifera papillaris*, 30; *Graciliaria strobili*, 24; *Candidula unifasciata*, 27; *Helicella itala*, *H. cretica*, 26; *Monacha cantiana*, 23; *Trichia unidentata*, *T. sericea*, *T. villosa*, *Euomphalia arpatschaina*, 23; *Helicodonta obvoluta*, 27; *Helicigona achates*, 30; *H. pouzolzi*, *H. setosa*, 29; *Isognomostoma isognomostoma*, *I. holosericum*, 30; *Euparypha pisana*, 28; *Helix dormitoris*, *H. lucorum*, 27; *Eobania vermiculata*, 27; *Caucasotachea lenkoranea*, 30.

Arten aus Jugoslawien, Persien und der Türkei zeigten im Vergleich mit verwandten europäischen Arten keine chromosomalen Besonderheiten.

Die Zusammenhänge der Chromosomenzahlen mit der Systematik wurden geprüft: In einigen Familien (Chondrinidae, Enidae, Bradybaenidae) sind die Chromosomenzahlen einheitlich, in andern, vor allem bei den Helicidae variieren sie stark. Innerhalb der Unterfamilien der Helicidae können die Chromosomenzahlen entweder einheitlich sein, nur um 1-3 Chromosomen variieren oder wie bei den Helicinae und Helicellinae grössere Unterschiede zeigen. Gattungen weisen meist nur eine einzige Chromosomenzahl auf, wo nicht, ist die Gattung gewöhnlich in Untergattungen aufgeteilt.

Die Helicidae wurden genauer untersucht, wobei neben der Chromosomenzahl auf besondere Merkmale wie Chromosomengrösse und Verteilung der Chiasmata geachtet wurde. Auf Grund der Karyotypen ist die Unterteilung der Unterfamilie Helicellinae in die Tribus Helicelleae und Monacheae gerechtfertigt, wobei aber *Cochlicella acuta* dem Tribus Monacheae zuzuteilen wäre. Chromosomensätze von Arten verschiedener Untergattungen oder Gattungen mit gleicher Chromosomenzahl lassen sich durch die Grössenverhältnisse der Chromosomen und anhand von Chiasmata meistens unterscheiden (Helicigoninae). Bei den Helicinae bildet *Cepaea* einen Sonderfall. Innerhalb derselben Gattung kommen 2 verschiedene Chromosomenzahlen vor: 22 und 25. BALTZER (1913) vermutete einen Zerfall des auffallend grossen Chromosoms, das bei *C. nemoralis* und *C. hortensis* (beide n=22) vorkommt, in mehrere kleine Chromosomen bei *C. vindobonensis* (n=25). Bei *C. silvatica* (n=25) fand ich aber ebenfalls ein grosses Chromosom; nur bei *C. vindobonensis* könnte BALTZERs Theorie zutreffen. Auf Grund der verschiedenen Karyotypen scheint eine Unterteilung der Gattung *Cepaea* in 2 Untergattungen angebracht. Es wurde auch eine Lokalform von *C. hortensis* mit 30 statt 22 Chromosomen gefunden.

In einer Population von *Succinella oblonga* wurden 2 verschiedene Chromosomenzahlen gefunden (n=11 und 12).

Im allgemeinen stimmen die zytologischen Befunde mit der modernen Klassifizierung der Stylommatophoren überein.

EINLEITUNG

Mit der Zytotaxonomie der Gastropoden beschäftigten sich bisher nur wenige Autoren. M. PERROT (1938) leistete auf diesem Gebiet Pionierarbeit. Er ermittelte die Chromosomenzahlen von 20 Pulmonatenarten, von denen die meisten der Familie Helicidae angehörten. Die Ergebnisse prüfte PERROT auf ihre Eignung als Bestimmungsmerkmal. Ausserdem schloss er von den Chromosomenverhältnissen auf Verwandtschaft zwischen gewissen taxonomischen Einheiten. Frühere Autoren, z. B. J. L. PERROT (1930, 1934) oder KLEINERT (1909), beschrieben den Ablauf der Reifeteilungen und stellten die Chromosomenzahlen fest. Erst in den letzten Jahren wurden die zytologischen Befunde an Gastropoden auch taxonomisch ausgewertet. LE CALVEZ & CERTAIN (1950), INABA (1950, 1953, 1959b, c), STAIGER (1950a, b; 1954), BURCH (1959, 1960a, b, c; 1961) und NISHIKAWA (1962) behandelten zytotaxonomische Fragen an Prosobranchia, Nudibranchia und vor allem an Basommatophora.

BURCH diskutiert in verschiedenen Arbeiten (1960b, 1961) die Frage nach dem Wert der Chromosomenzahlen für die Schneckensystematik. Die Chromosomenzahl allein ist nach BURCH nur zur Unterscheidung höherer taxonomischer Einheiten von Bedeutung, während für niedrigere Einheiten weitere Merkmale zur Charakterisierung eines Chromosomensatzes beigezogen werden müssen.

Mit Stylommatophora beschäftigten sich ausser M. PERROT (1938), HUSTED & BURCH (1946), welche die Familie Polygyridae untersuchten und INABA (1959a), der 29 Spezies aus 6 Familien prüfte.

In der vorliegenden Arbeit wurden die Chromosomenzahlen von 19 Heliciden, 14 andern Stylommatophoren und 2 Prosobranchiern bestimmt. Ausserdem konnten die Chromosomenzahlen von 8 weiteren Heliciden, 4 Clausiliiden und einem Prosobranchier bestätigt werden. Bei *Cepaea hortensis* fand ich eine neue Variante.

Meine Arbeit bestätigt und ergänzt die Befunde PERROT's; die taxonomischen Zusammenhänge lassen sich auf Grund des grösseren Materials besser beurteilen. Dank neuer Präparations-technik schien es mir lohnend, auch einige von PERROT (1938) bereits untersuchte Arten nochmals vorzunehmen.

Von den 2 Möglichkeiten, anhand vieler Individuen und Populationen die Chromosomenverhältnisse weniger Arten zu untersuchen, oder an wenigen Tieren möglichst viele Arten zytologisch zu bestimmen, wählte ich die zweite. Deshalb habe ich gelegentlich ein einziges Tier als repräsentativ für die Art betrachtet. Ich bin mir bewusst, dass darin die Gefahr einer Fehlbestimmung liegt. Die Übereinstimmung der Chromosomenverhältnisse vieler Individuen vom selben Fundort oder von Individuen verschiedener Fundplätze, die ich gelegentlich feststellen konnte, sowie die Übereinstimmung meiner Resultate mit denen PERROT's sprechen gegen einen prinzipiellen Fehler dieses Vorgehens.

An dieser Stelle danke ich in erster Linie Herrn Prof. Dr. H. BURLA, der die Anregung zu dieser Arbeit gab und ihre Durchführung ermöglichte, für seine verständnisvolle Hilfe und für die Korrektur der Arbeit. Fr. Dr. E. HAUSCHTECK und besonders Frau A. GISMANN danke ich für wertvolle Ratschläge und für die Durchsicht des Manuskripts. Zu grossem Dank verbunden bin ich Herrn Dr. L. FORCART, Basel, für seine freundliche Unterstützung bei der Bestimmung etlicher Arten. Schliesslich danke ich für die Beschaffung von Material aus West-, Mittel- und Südeuropa Herrn Prof. H. BURLA, Herrn Dr. L. FORCART, Herrn H. JUNGEN und Herrn W. GÖTZ, Zürich; letzterem auch für die Funde aus Jugoslawien, Persien und der Türkei, die während zweier Expeditionen des Zoologischen Museums Zürich gesammelt wurden¹.

¹Unterstützt vom Schweizerischen Nationalfonds

MATERIAL UND METHODE

Zu Beginn meiner Arbeit untersuchte ich ausschliesslich einheimische Arten, später auch solche aus dem Ausland, die mir als Vergleich mit einheimischen Arten derselben Gattung oder Unterfamilie wertvoll schienen.

Fundorte und ihre Biotope, Fund- und Fixierungsdaten erwähne ich bei der Beschreibung der betreffenden Arten. Häufig konnte ich die Schnecken nicht sofort nach dem Einbringen verarbeiten. In solchen Fällen bewahrte ich sie auf feuchtem Papier in Bechern auf und fütterte sie mit Salat oder Karotten, die von den meisten Arten in grossen Mengen gefressen wurden. Unter diesen Bedingungen blieben die Tiere lange Zeit am Leben. Zur Untersuchung der Chromosomen verwendete ich hauptsächlich verschiedene Reifeteilungsstadien der Spermatogenese. Das hat die Vorteile, dass die Chromosomen grösser sind als in Mitosen und dass sich bei hohen Chromosomenzahlen die Tetraden sicherer zählen lassen als doppelt so viele Mitosechromosomen. Ausserdem findet man meistens zur Untersuchung geeignete Teilungsstadien in der Zwitterdrüse, wenn man die Tiere im Laufe des Sommers fixiert. Ich fand Reifeteilungen von April bis Oktober. Es kam vor, dass von 2 Individuen einer Art das eine Tier viele, das andere keine brauchbaren Reifeteilungsstadien aufwies, obwohl sie vom selben Ort stammten und gleichzeitig fixiert wurden.

Die folgende Methode eignete sich zur Herstellung der Präparate am besten: Die herauspräparierten Zwitterdrüsen wurden mit Essigsäure (45%) 1-3 Stunden lang fixiert. Wenn ich das Material nicht sofort weiterverarbeiten konnte, fixierte ich mit Essigsäure von ca. -8°C , um Mazerieren zu vermeiden. Bei dieser Temperatur lässt sich das Gewebe im Fixierungsmittel monatelang aufbewahren, ohne Schaden zu nehmen. Die fixierten Zwitterdrüsen wurden stückchenweise unter silikonisierten Deck-

gläsern im Fixierungsmittel gequetscht, anschliessend auf Trockeneis gefroren, das Deckglas abgelöst und das Präparat in Alkohol (96%) gestellt. Darauf hydrolysierte ich die Präparate 7-10 Minuten in 1n-HCl bei 60°C und färbte sie bei gleicher Temperatur 1/2 Stunde, bei Zimmertemperatur mindestens 12 Stunden, mit Hämatoxylin nach GOMORI (MELANDER & WINGSTRAND, 1953). Anschliessend differenzierte ich 1/2 Stunde in Essigsäure (50%). Dann wässerte ich die Präparate mindestens 2 Stunden mit fliessendem Wasser, führte sie nachher durch die aufsteigende Alkoholreihe in Xylol und deckte sie mit Caedax ein.

Zur Feststellung der Chromosomenzahl verwendete ich alle zur Zählung geeigneten Reifeteilungs- und Mitosestadien. Bevor ich die Chromosomen zählte, prüfte ich jeweils genau, ob sie sich nirgends überdeckten und ob keine Chromosomen durch das Quetschen abgesprengt worden waren. Lagen mehrere Platten nebeneinander, so achtete ich darauf, ob die Abgrenzung zwischen den Platten deutlich in Erscheinung trat. Zur Auszählung der Mitoseplatten mit hohen Chromosomenzahlen zeichnete ich die Platten mit dem Abbé'schen Zeichenapparat und zählte die Chromosomen auf der Zeichnung.

Die am häufigsten festgestellte Chromosomenzahl (Modalzahl) betrachtete ich als repräsentativ für die betreffende Art. Nach unten streuende Abweichungen von der Modalzahl ergeben sich, wenn Chromosomen beim Herstellen der Präparate verlorengehen oder so nahe nebeneinander liegen, dass sie als ein einziges Chromosom gezählt werden. Solche Stellen werden bei der Prüfung der Platte vor dem Zählen leicht übersehen. Seltener treten höhere Zahlen auf. Wahrscheinlich zerbrachen in diesen Fällen Chromosomen beim Quetschen. Die abweichenden Zahlen sind jeweils erwähnt, wenn sie von Bedeutung sind.

In einigen Fällen fand ich nur wenig brauchbare, d.h. den erwähnten

Bedingungen entsprechende Teilungsstadien. Erfahrungsgemäss ändert sich die durch wenige Zählungen ermittelte Modalzahl nicht, wenn mehr Kerne ausgezählt werden. Im günstigsten Fall lässt sich die Chromosomenzahl schon mit einer einzigen einwandfreien Platte bestimmen, doch stützen sich meine Angaben auf Zählungen von mindestens 3 Platten pro Tier. Nur bei 4 Arten beruht die gefundene Chromosomenzahl auf weniger als 5 übereinstimmenden Platten.

Zur Bestimmung der Chromosomenzahl sind Metaphasen der ersten Reifeteilung am zuverlässigsten, weil die Chromosomen dann am stärksten kontrahiert und am schärfsten gegeneinander abgegrenzt sind. Diakinesestadien eignen sich je nach Alter ebenfalls, während sich frühe Stadien nur dann auszählen lassen, wenn alle Chromosomen sichtbar sind. Das kommt bei den relativ hohen Chromosomenzahlen der *Stylommatophora* höchst selten vor.

In neueren zytologischen Arbeiten werden meistens neben der Anzahl die Gesamtlänge der Chromosomen und deren Armverhältnis zur Charakterisierung eines Karyotyps benützt. BURCH (1960b) konnte bei Spermatozoonteilungen der von ihm untersuchten *Basommatophora*-Arten mit wenigen Ausnahmen die Spindelfaseransatzstelle (SFA) beobachten. Da ich in meinem Material nur vereinzelte Stadien fand, bei denen die SFA sichtbar war, musste ich mich nach anderen Merkmalen umsehen. Ich wählte die Grössenunterschiede der Tetraden oder Bivalente innerhalb eines Satzes, benützte aber nur auffallende, abschätzbare Unterschiede. Messungen führte ich nicht durch, da sich Reifeteilungsstadien schlecht dafür eignen. Ein zweites Merkmal fand ich in der Verteilung der Chiasmata, die ich in den Diakinesestadien häufig beobachten konnte. Die Chiasmahäufigkeit lässt sich aber an Diakinesestadien nicht feststellen, weil

die Chiasmata dann grösstenteils terminalisiert sind und ihre Zahl nicht mehr feststellbar ist. In der Regel traten 3 Typen von Diakinesefiguren auf:

1. Kreuzförmige Bivalente, die ein nicht terminalisiertes Chiasma enthalten.

2. Ringbivalente, bei Terminalisation von mindestens 2 Chiasmata, wenn diese auf beide Armpaare verteilt sind. Die Zahl der Chiasmata eines Bivalents lässt sich nur im Diplotän bestimmen, solange kein Chiasma terminalisiert ist.

3. Bivalente, bei denen kein Chiasma erkennbar ist. Ob es sich dabei um Bivalente mit einem terminalisierten Chiasma handelt, oder um Bivalente mit mehreren terminalisierten Chiasmata im selben Armpaar, lässt sich nicht beurteilen.

Die Angaben über Zahl und Verbreitung der Arten stellte ich aus JAECKEL & ZILCH (1962), EHRMANN (1933), GERMAIN (1930) und WENZ & ZILCH (1959/60) zusammen.

Die Photographien stellte ich mit dem ZEISS-Ultraphot-Mikroskop her. Für die Abbildungen 15 und 21a verwendete ich die Hellfeldoptik mit dem ZEISS-Planobjektiv 100 (n.A. 1,25), für Abb. 6 und 12a die Phasenkontrastoptik mit Objektiv 40 (n.A. 0,65) und für alle übrigen Aufnahmen Phasenkontrastoptik mit ZEISS-Neofluar-Objektiv 100 (n.A. 1,30). Die Photos wurden mit der eingebauten Kleinbildkamera mit automatischer Belichtung aufgenommen.

DIE CHROMOSOMENVERHÄLTNISSE DER HELICIDAE

Die Familie ist mit 80 rezenten Gattungen und 104 Untergattungen die grösste der Ordnung *Stylommatophora*. Die morphologische Vielfalt innerhalb der Familie hat zur Unterteilung in 8 Unterfamilien geführt, von denen 5 mit 22 Gattungen und 54 Arten in Mitteleuropa vertreten sind.

Subfamilia Helicellinae

Im Mittelmeergebiet und im ozeanischen Westeuropa sind die Helicellinae mit grossem Formenreichtum verbreitet. Die wenigen Arten Mitteleuropas bevorzugen Kalkboden und kommen hauptsächlich an warmen Rasenhängen, Feldrainen und Hecken vor. Noch vor einigen Jahren (EHRMANN, 1933) wurden alle mitteleuropäischen Arten der Helicellinen in der Gattung *Helicella* zusammengefasst, die aber in Untergattungen unterteilt wurde. Heute umfasst die Unterfamilie die beiden Tribus Helicelleae und Monacheae, die zusammen 7 mitteleuropäische Gattungen (11 Arten) enthalten.

Tribus Helicelleae

Candidula unifasciata (POIRET, 1801)
[=*Helix candidula* STUDER, 1820
=*Helicella unifasciata* GERMAIN, 1929]

Die Art ist in ganz Frankreich und Deutschland bis nach Polen und der VSSR verbreitet. In der Schweiz wurde sie im Rhonetal, Jura, Engadin und im Kanton Appenzell nachgewiesen.

Zur Untersuchung kamen 6 Tiere dieser Art, die südlich von Blansingen (Schwarzwald) an den Rändern eines in Löss eingeschnittenen Hohlweges gesammelt wurden. Ich fixierte die Tiere am 19. September 1960.

Die Hälfte der Tiere enthielt viele analysierbare Teilungen in der Keimdrüse, ein Tier sehr wenige, und bei zweien fand ich keine brauchbaren Teilungen. Anhand von 35 untersuchten Diakinese- bzw. Metaphasekernen stellte ich die Chromosomenzahl $n=27$ fest. Diese Zahl trat bei 28 Kernen in Diakinesestadien und bei 6 Metaphaseplatten auf, wovon sich 2 in der ersten und 4 in der zweiten Reifeteilung befanden.

Bei den meisten der beobachteten Diakinesestadien konnte ich Chiasmata feststellen. In 20 von 28 Kernen fiel mir ein besonders grosses Bivalent mit

mindestens 2 Chiasmata auf, wobei wenigstens ein Chiasma terminalisiert war, ein anderes nicht (Nr. 1 in Abb. 1). Auch in anderen Teilungsphasen, in denen die Chiasmata nicht zu erkennen waren, fiel dieses Chromosomenpaar durch seine Grösse auf. Das zweitgrösste Bivalent (Nr. 2) zeigte bei 8 der untersuchten Kerne ein nicht terminalisiertes Chiasma. Ungefähr gleich häufig liessen sich die 3 nächstkleineren Bivalente identifizieren. Zwei davon enthielten ebenfalls ein nicht terminalisiertes Chiasma (Nr. 3 und 4), das dritte war durch mindestens 2 terminalisierte Chiasmata gekennzeichnet (Ringbivalent, Nr. 5). Diese Chiasmata waren bei einem

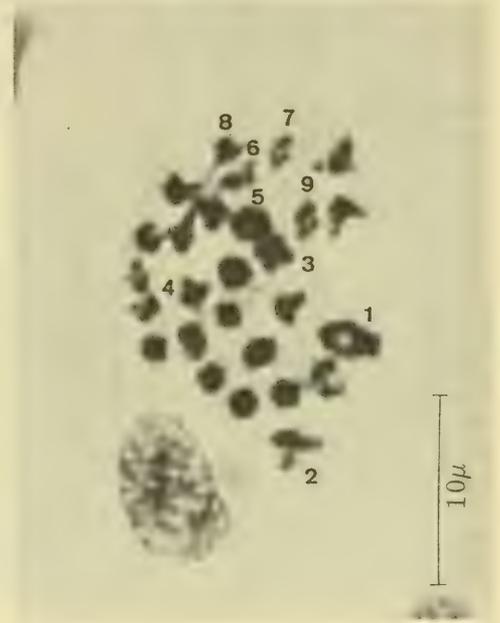


Abb. 1. *Candidula unifasciata*, $n=27$, spätes Diakinesestadium; Erklärungen zur Grösse und zur Zahl der Chiasmata im text.

Drittel der untersuchten Kerne erkennbar. Es folgte eine ganze Reihe nicht unterscheidbarer, aber mehr oder weniger deutlich kleinerer Bivalente, von denen einige ein Chiasma enthielten; bei anderen war keines zu erkennen. Die 4 kleinsten Elemente des Satzes

(Nr. 6-9) waren unterscheidbar kleiner als die Mehrzahl der Tetraden.

Helicella obvia (HARTMANN, 1840)
[=*H. candicans* PFEIFFER, 1842
=*Helix obvia* KOBELT, 1877]

Die Verbreitung dieser Art erstreckt sich über ganz Zentral- und Osteuropa. Der Mensch fördert die Ausbreitung, indem er viele Tiere mit Saatgut verschleppt. *Helicella obvia* ist an Trockenheit angepasst und nicht auf Kalkboden angewiesen.

Die 2 Tiere, die ich untersuchte, stammen von Colmar (Elsass), nur eines enthielt reichlich brauchbare Teilungsstadien. Die Art wurde bereits von M. PERROT (1938) zytologisch untersucht. In Kernen verschiedener Stadien der ersten Reifeteilung zählte er 26 Elemente, wobei er auf Schwierigkeiten bei der Untersuchung der Prophase dieser Art hinwies, weil die Kerne verhältnismässig klein und die Chromosomen zahlreich seien. Meine Zählungen an 31 Kernen in deutlich analysierbaren Diakinesestadien oder Metaphase bestätigten die Ermittlungen PERROT's.

PERROT beschrieb die Tetraden der Metaphase sowie die der etwas früheren Stadien als unter sich gleich gross (1938: Abb. 11 A). Tatsächlich liessen sich hier die Bivalente schwerer identifizieren als bei *Candidula unifasciata*. In Abb. 2 erkennt man zwar mehrere Chiasmata und kann einige Ringbivalente und kreuzförmige Bivalente unterscheiden. Bei anderen Kernen sind diese Merkmale aber weniger deutlich zu sehen. Hingegen stechen fast immer 2-3 Bivalente durch ihre Grösse von den übrigen, unter sich fast gleich grossen Bivalenten ab.

Helicella itala (LINNAEUS, 1758)
[=*H. ericetorum* O. F. MUELLER,
1774]

Die Art kommt in West- bis Mitteleuropa an trockenen, bewachsenen Hängen vor, fehlt jedoch südlich der Alpen. In der Schweiz lebt sie in den Kalkalpen, im Rhonetal und im Jura.

Das einzige von mir untersuchte Ex-

emplar dieser Art wurde am Fuss eines Kalkfelsens von Istein im Schwarzwald gefunden. Zur Untersuchung verwendete ich 33 Kerne in Diakinese oder in Metaphase. Davon enthielten 30 Kerne eindeutig 26 Bivalente. Bei fast allen Kernen fielen 2-3 grosse Elemente auf (Nr. 1-3 in Abb. 3). Bei 7 Platten hatte das grösste Bivalent 2 Chiasmata, wovon das eine terminalisiert war, so wie beim grössten Bivalent von *Candidula unifasciata*. Bei 16 Kernen war eines der grossen Elemente ein Ringbivalent, in 9 Fällen kam ein weiteres dazu. Das 4. auffallende, etwas kleinere Bivalent hatte ein Chiasma (Nr. 4). Ich beobachtete es bei 9 Platten.

Trochoidea (Xerocrassa) cretica
(PFEIFFER, 1841)

Die Verbreitung dieser Art erstreckt sich über die östlichen Mittelmeerländer.

Das untersuchte Tier wurde am 4. Mai 1962 30 km östlich von Silifke (Türkei), unter einem Stein in einem Buschbestand gefunden. Ich fixierte es am 12. Juni 1962. Das Tier war noch nicht voll ausgewachsen. Die kleine Zwitterdrüse enthielt ausschliesslich Mitosen.

Ich zählte bei 5 Platten 52 Chromosomen verschiedener Länge (Abb. 4).

Tribus Monacheae

Monacha cartusiana
(O. F. MUELLER, 1774)

[=*Theba cartusiana* GERMAIN, 1929]

Diese mediterrane Art ist vom Mittelmeerraum im Westen bis Belgien und Holland, im Osten durch den Balkan bis Mähren vorgestossen. In die Schweiz kam die Art von Frankreich her zuerst in die Westschweiz, dann dem Jura entlang bis Basel und in die übrige Nordschweiz.

Die beiden von mir untersuchten Tiere stammen vom grasbewachsenen Hang einer alten Kiesgrube bei Wangen (Zürich). Sie wurden am 26. Juli 1960 fixiert. Beide Tiere enthielten viele

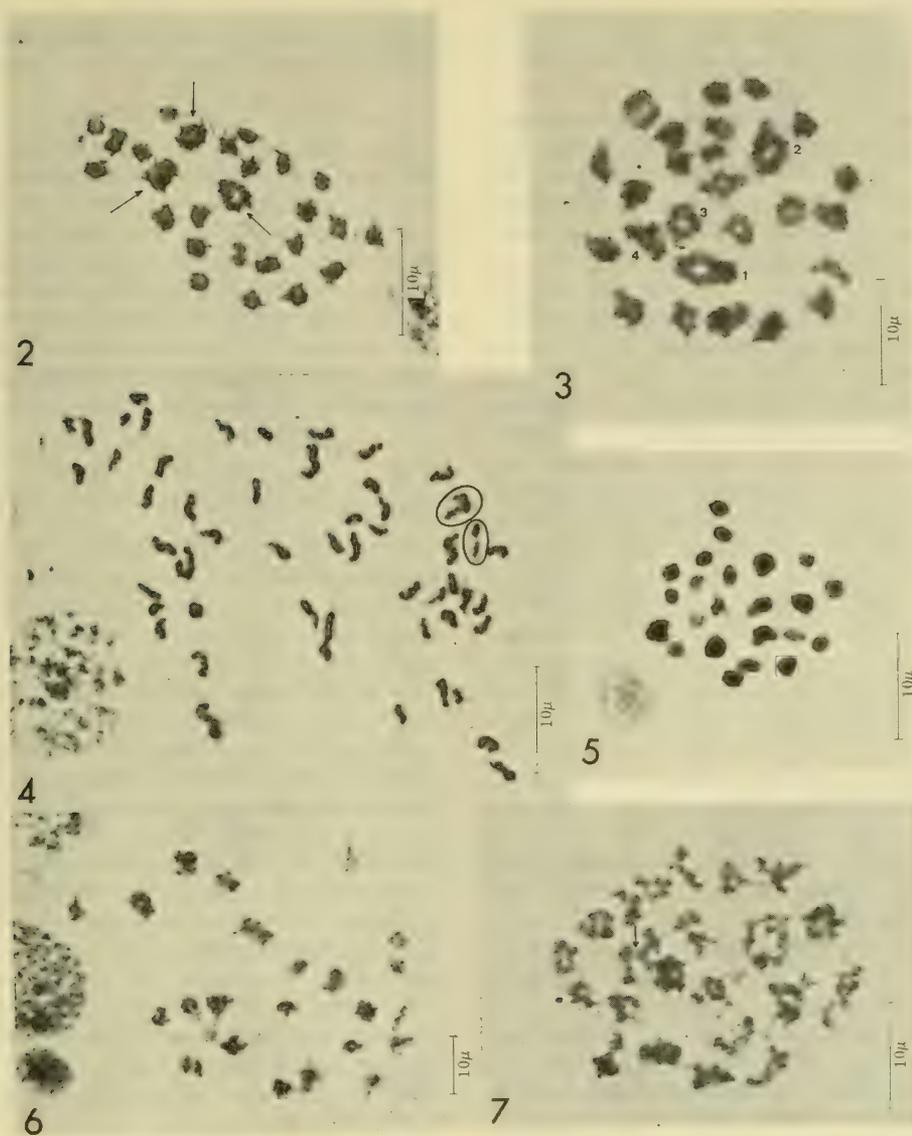


Abb. 2-7. Abb. 2. *Helicella obvia*, $n=26$, spätes Diakinesestadium. Die 3 grossen Bivalente sind mit Pfeilen bezeichnet. Abb. 3. *Helicella itala*, $n=26$, Diakinesestadium. 1-4: besonders grosse Bivalente. Abb. 4. *Trochoidea (Xerocrassa) cretica*, $2n=52$, Metaphase einer Spermatogonienteilung. Eingezeichnete Chromosomen sind als eines zu zählen. Abb. 5. *Monacha cartusiana*, $n=23$, Metaphase der ersten Reifeteilung. Abb. 6. *Monacha cantiana*, $n=23$, Diakinesestadium. Bei einigen Bivalenten sind Chiasmata zu erkennen. Die dunkle Stelle zwischen den 5 Bivalenten links unten ist kein Chromosom. Ihre Verbindung zu den 2 darüberliegenden Bivalenten vermag ich nicht zu erklären. Abb. 7. *Trichia unidentata*, $n=23$, frühes Diakinesestadium. Die Chromosomen sind wenig kontrahiert. Ganz links erkennt man ein Bivalent mit wahrscheinlich 3, ganz rechts eines mit vielleicht 4 Chiasmata. Der Pfeil bezeichnet eine Stelle, an der 2 Bivalente nahe aneinanderstossen.

Teilungen.

PERROT (1938) fand bei dieser Art 23 Tetraden, welche er als etwa gleich gross beschrieb.

Diese Zahl konnte ich an meinem Material bestätigen. In 38 von 40 untersuchten Kernen, wovon sich die meisten in Diakinesestadien befanden, stellte ich 23 Bivalente fest. Ein Kern schien 20, ein anderer 24 Elemente zu enthalten.

Bei allen Kernen zeigten die Tetraden unterschiedliche Grössen. Da aber zwischen der kleinsten und grössten Tetrade alle Zwischenstufen vorkamen, war eine Klassierung schwer zu treffen. In Abb. 5 fallen wie in den meisten Sätzen 3-4 Elemente als besonders gross und 4-5 als besonders klein auf. Die Zahl der Chiasmata liess sich nicht in allen Kernen ermitteln. Die erwähnten grossen Elemente erschienen kreuzförmig oder als Ringbivalente. Manchmal waren aber ein Kreuz und 2-3 Ringe vorhanden; andere Platten hatten 2 oder sogar 3 Kreuze und entsprechend weniger Ringe.

Monacha cantiana (MONTAGU, 1803)
[=*Theba cantiana* GERMAIN, 1929]

Von der Atlantikküste aus drang diese Art in Frankreich, Belgien und den Niederlanden ins Binnenland ein. Ausserdem ist sie in England allgemein verbreitet. In der Schweiz wurde sie bisher noch nicht gefunden.

Die beiden untersuchten Exemplare wurden am 28. Mai 1962 in Roda de Bara (Spanien) unter Holzstücken auf trockenem Weideland gefunden. Bisherige Angaben über Vorkommen der Art im Mittelmeergebiet sollen sich laut JAECKEL & ZILCH (1962) auf eng verwandte Arten beziehen. Da der Fundplatz in der Nähe eines Bahndammes lag, könnte es sich um verschleppte Tiere handeln. Beide Exemplare hatten relativ kleine Zwitterdrüsen. Diese enthielten wenig Reifeteilungen.

Die Grössenverhältnisse der Bivalente stimmten mit denen von *Monacha cartusiana* ungefähr überein. Bei den 5 analysierbaren Platten zählte ich 23

Chromosomenpaare (Abb. 6). Ausserdem konnte ich noch 2 Mitoseplatten mit 46 Chromosomen zählen.

Subfamilia Hygromiinae

Die früher unter dem Namen *Fruticicolinae* bekannte Unterfamilie umfasst 20 rezente Gattungen und ist über ganz Europa, Nordafrika und Südwest- bis Nordasien verbreitet. In Mitteleuropa ist sie durch 6 Gattungen vertreten. Mit Ausnahme einiger südlicher Formen, die trockene Plätze besiedeln, bevorzugen die mitteleuropäischen Arten feuchte, pflanzenreiche Biotope.

Trichia (Petasina) unidentata
(DRAPARNAUD, 1805)

[=*Fruticicola unidentata* HELD, 1837]

Das Verbreitungsgebiet dieser Art erstreckt sich über die Ostalpen und die Karpaten zum sächsisch-böhmischen Erzgebirge, über die Nord- und Zentralalpen westwärts bis zur Ostschweiz und vom Bodenseegebiet wieder nordöstlich gegen Böhmen.

Das untersuchte Tier, welches ich am 4. April 1960 fixierte, fand ich 14 Tage vorher am südlichen Stadtrand von Winterthur in unmittelbarer Nähe eines Baches unter Laub am Nordrand eines Buchenwaldes. Bei 20 untersuchten Kernen, von denen fast alle in frühen Diakinesestadien waren, zählte ich in 18 Fällen 23 Bivalente. Die nur schwach kontrahierten Bivalente waren verschiedenen gross und mehr oder weniger gleichmässig abgestuft (Abb. 7). In einigen Kernen fielen 3-4 Elemente durch besondere Grösse auf.

Trichia villosa (STUDER, 1789)
[=*Trochulus villosus* CHEMNITZ, 1786
=*Fruticicola villosa* HELD, 1837]

Diese Art ist nordwestalpin. Ihr Verbreitungsgebiet erstreckt sich über die ganze Schweiz mit Ausnahme des Wallis und Tessins und reicht vom Jura weiter in die Kalkgebiete Südwestdeutschlands. Die Linie Mainz-Augsburg bildet ungefähr die nördliche Begrenzung.

Das eine der untersuchten Tiere fand ich an einer feuchten Wegböschung im Zürichbergwald, einem lichten Buchenwald mit wenig Unterholz. Es wurde am 20. Mai 1960 fixiert. Drei weitere Exemplare stammen vom Klausenpass. Der Fundort liegt westlich der mit "Waldhüttli" bezeichneten Häusergruppe in einem Mischwald auf 1400 m.ü.M. und wurde Ende August 1961 besucht.

Die Zwitterdrüse des Zürichberg-tieres war voller feifeteilungen: 62 von 82 untersuchten Kernen enthielten eindeutig 23 Tetraden. Bei 10 Kernen zählte ich 22 Elemente; je 5 Kerne enthielten 21 bzw. 24 Bivalente. Fast alle diese Kerne befanden sich in der Metaphase der ersten Reifeteilung. Es fiel mir auf, dass häufig die kleinste Tetrade in der Teilung am weitesten fortgeschritten war, sodass die beiden Hälften schon auseinandergewichen waren, wenn die übrigen Tetraden noch Einheiten bildeten. Die grösste Tetrade erschien ungefähr 4-5 mal so gross wie die kleinste. Dazwischen waren alle Übergänge vorhanden. Bei den grössten Tetraden waren die Unterschiede klein, sodass etwa 3-4 besonders grosse Elemente in den meisten Platten auffielen. In den meisten der untersuchten Stadien liess sich nur in diesen grossen Bivalenten ein Chiasma erkennen. In frühen Diakinesestadien aber wurde es bei fast allen Bivalenten sichtbar (Abb. 8).

In einem der Tiere vom Klausenpass fand ich 12 analysierbare Kerne. Zehn davon enthielten 23, einer 22 und einer 24 Tetraden. Auch hier fielen die grossen kreuzförmigen Bivalente auf.

Trichia (Trichia) sericea
(DRAPARNAUD, 1801)

[=*Fruticicola sericea* HELD, 1837]

Trichia sericea kommt fast im ganzen Alpengebiet bis in die Südostalpen vor. Ferner findet man sie in Ostfrankreich, Belgien und England. Von den Ostalpen her breitet sich die Art nach Norden bis Böhmen aus. Sie besiedelt auch den Schweizer Jura und das Mittelland,

während sie in Süd- und Mitteldeutschland unregelmässig verteilt ist und in Nord- und Ostdeutschland fehlt.

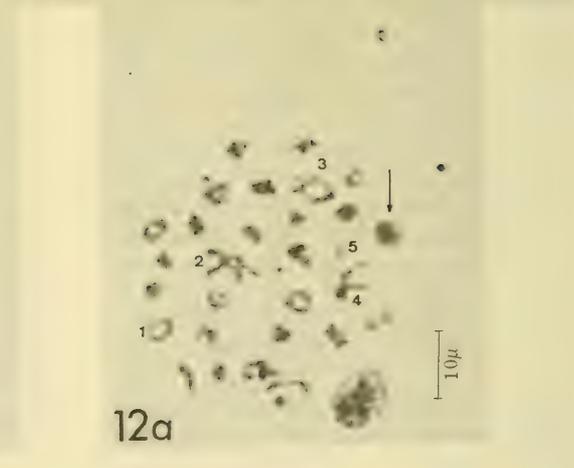
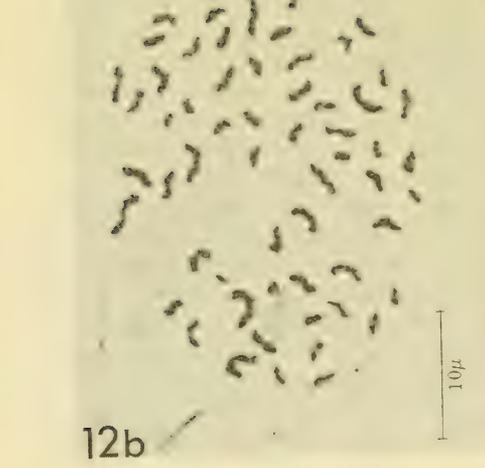
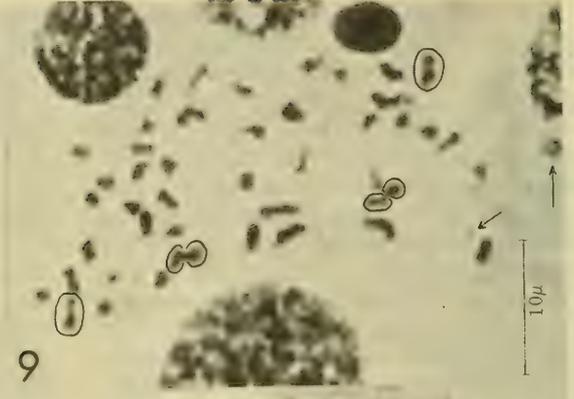
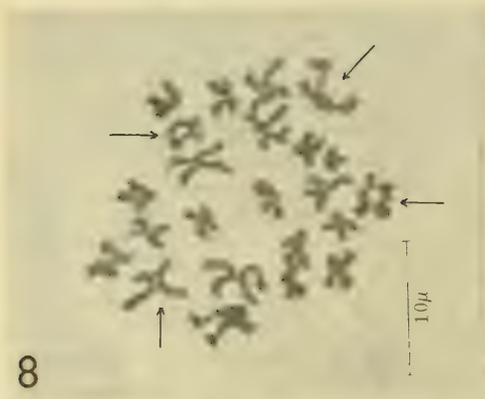
Zur Untersuchung gelangte ein Tier, welches in einem lichten Laubwald in der Nähe des Tössufers südlich von Winterthur gefunden wurde. Die Fixierung erfolgte am 4. April 1960. Die Keimdrüse des Tieres enthielt wenige analysierbare Teilungen. Bei 3 geprüften Metaphasestadien der ersten Reifeteilung zählte ich 23 Tetraden. Von den 9 Mitoseplatten zählten 2 Metaphasen 36, je eine 38, 40 und 44 Chromosomen, während 4 Platten 46 Chromosomen enthielten (Abb. 9). Daraus ergibt sich mit einiger Wahrscheinlichkeit die Chromosomenzahl $n=23$, die aber noch durch weitere Zählungen erhärtet werden muss.

Euomphalia (Harmozica) arpatchaina
sewanica (MARTENS, 1881)

Die beiden Exemplare dieser Unterart wurden unter Buxusgebüsch auf einer Weide ca. 20 km südlich von Shahi (Persien) am 31. Mai 1962 gefunden. Beide Zwitterdrüsen enthielten brauchbare Kerne, von denen sich die meisten in der Metaphase der ersten Reifeteilung befanden. Bei 31 Platten zählte ich 23 Elemente. Davon fielen 2 durch besondere Grösse auf, die übrigen waren gleichmässig abgestuft. Die bei diesen Präparaten immer stark kontrahierten Tetraden sahen alle mehr oder weniger rund aus. Neben diesen Reifeteilungsplatten fand ich noch 3 Mitosekerne, deren Chromosomenzahl übereinstimmend 46 betrug. Die Chromosomen zeigten grosse Längenunterschiede. Vier der Chromosomen waren deutlich länger als die übrigen, sie entsprachen den beiden grossen Tetraden der ersten Reifeteilung. Bei einigen Chromosomen lässt sich die SFA erkennen, die median oder submedian liegt (Abb. 10).

Subfamilia Helicodontinae

Von insgesamt 10 Gattungen sind 7 über die Mittelmeerländer verbreitet.



In Mitteleuropa ist die Unterfamilie nur durch eine Gattung mit einer Art vertreten.

Helicodonta obvoluta
(O. F. MUELLER, 1774)

Die Art ist über ganz Süd- und Mitteleuropa verbreitet. Sie lebt vorwiegend in Wäldern unter Laub, Holz und Steinen. Die untersuchten Tiere stammen von 2 verschiedenen Fundplätzen. Der erste liegt in einem Laubwald nordwestlich der Stadtgrenze von Winterthur; die Fixierung der Tiere erfolgte am 2. August 1960. Der 2. Fundort befindet sich am Kerenzberg etwa 1 km südwestlich der Passhöhe, oberhalb der Strasse in einem steil abfallenden Laubwald. Diese Tiere präparierte ich am 7. September 1961. Von jedem Fundplatz untersuchte ich 2 Tiere, von denen aber nur je eines ein gebrauchbares Teilchen aufwies.

Ich fand insgesamt 19 Zellen in Diakinesestadien. Vierzehn der Kerne enthielten 27 Bivalente, bei den übrigen 5 zählte ich 24-26 Elemente. Die Tetraden eines Kerns zeigten verschiedene Grössen. Die kleinste ist ungefähr 3-4 mal kürzer als die grösste Tetrade. Bei der abgebildeten Metaphaseplatte der ersten Reifeteilung (Abb. 11) erkennt man 10 deutlich kreuzförmige Bivalente, bei den übrigen lassen sich keine Chiasmata feststellen.

Subfamilia Helicogoninae

Die früher als Campylaeinae oder Ariantinae bezeichnete Unterfamilie umfasst heute 3 europäische Gattungen mit 14 Arten und ist vom Süden Europas bis nach Schweden verbreitet, haupt-

sächlich jedoch in Zentraleuropa und in den Alpen.

Helicigona (Helicigona) lapicida
(LINNAEUS, 1758)
[=*Chilotrema lapicida* FITZINGER,
1833]

Die Schnecken dieser Art ("Steinpicker") sind meist an nicht zutrockenen Mauern und Felsen zu finden, kommen aber auch in Laubwäldern, vor allem an Buchenstämmen vor. Das Verbreitungsgebiet der Art erstreckt sich von Skandinavien über ganz Europa bis zur Hauptkammlinie der Alpen.

PERROT (1938) hat auch diese Art untersucht und 29 Tetraden in der ersten Reifeteilung festgestellt. Er beschreibt sie als "tétrades de taille assez semblable".

Die beiden von mir untersuchten Vertreter von *Helicigona lapicida* stammen aus einem Buchenwald (leicht geneigter Nordhang) südlich von Winterthur. Sie wurden am 8. Juni 1960 fixiert. In den Keimdrüsen beider Tiere fand ich viele analysierbare Reifeteilungen, von denen ich 40 Kerne in Diakinese und in Metaphase prüfte. Sie enthielten ausnahmslos 29 Tetraden in mehr oder weniger gleichmässiger Grössenabstufung, wobei der Unterschied zwischen dem grössten und kleinsten Bivalent auffallend gross war. In fast allen frühen Diakinesestadien fielen die grössten Elemente durch ihre Form auf (Abb. 12a): 1-2 Ringbivalente (1), ein Bivalent mit 2 Chiasmata, die nicht terminalisiert sind (2), eines mit mindestens 2 Chiasmata, wovon eines nicht terminalisiert ist (3) und 1 oder 2 kreuzförmige Bivalente (4). Das kleinste Bivalent (5) ist bereits geteilt. Die 58 Chromosomen der

Abb. 8-12. Abb. 8. *Trichia villosa*, $n=23$, Diakinesestadium. Die Pfeile weisen auf Bivalente mit wahrscheinlich mehr als einem Chiasma hin. Abb. 9. *Trichia sericea*, $2n=46$, Metaphase der Spermatogonienteilung. Die eingekreisten Stellen sind als je 1 Chromosom zu zählen. → kein Chromosom. Abb. 10. *Euomphalia arpatschaina sewanica*, $2n=46$, mitotische Metaphase. Nahe beisammenliegende Chromosomen sind durch Kreise markiert. Abb. 11. *Helicodonta obvoluta*, $n=27$, frühe Metaphase. Daneben ein Ruhekern. Abb. 12. *Helicigona lapicida*. a) $n=29$, Diakinesestadium. 1 = Ringbivalente; 2, 3 = 2 Chiasmata; 4 = 1 Chiasma, 5 = kleinstes Bivalent bereits geteilt. → kein Chromosom. b) $2n=58$, mitotische Metaphase.

mitotischen Teilung sind in der Metaphase länglich. Der grosse Unterschied zwischen langen und kurzen Chromosomen ist auch hier auffallend. Die abgebildete Mitose (Abb. 12b) war die einzige, die ich auswerten konnte.

Helicigona (Chilostoma) achates rhaetica (STROBEL, 1859)

[=H. (*Chilostoma*) *cisalpina rhaetica* (MOUSSON, 1850) KOBELT, 1875]

Diese Subspecies lebt im Veltlin, Puschlav, Engadin, im Oberinntal bis Landeck, im Vintschgau und im Nauders-tal. Sie ist die westliche Rasse der ostalpinen *Helicigona (Chilostoma) achates achates* (ROSSMAESSLER, 1835), die bis nach Vorarlberg und über den Brenner ins Südtirol bis zur Etschlinie vorgedrungen ist.

Helicigona (Chilostoma) achates rhaetica kommt an Felsen, Mauern und in Geröllhalden vor und bevorzugt Kalkstein. Das untersuchte Tier wurde am 20. April 1963 an Steinen in einem Mischwald südlich der Station Fetan (Engadin) auf ca. 1250 m.ü.M. gefunden. Untersucht wurden 50 Kerne in Diakinese- oder Metaphasestadien: 42 davon enthielten 30 Bivalente; in einem Fall zählte ich 32, in 2 Fällen 31 und in 5 weiteren 24 bis 29 Tetraden. Die Tetraden eines Kerns waren verschieden gross, das Verhältnis des grössten zum kleinsten Bivalent betrug schätzungsweise 4:1. Auch bei dieser Art konnte ich in den Diakinesestadien bei den grössten Tetraden Chiasmata beobachten. In allen analysierbaren Kernen stellte ich 4-6 Ringbivalente und 3-5 kreuzförmige Bivalente fest. Bei der Metaphaseplatte der ersten Reifeteilung in Abb. 13 sind die Chiasmata nur bei wenigen Tetraden sichtbar.

Helicigona (Arianta) arbustorum (LINNAEUS, 1758)

Diese Art lebt in feuchten Laubwäldern, in Gebüsch, auf feuchten Wiesen, an Bachufern und in Rieden. In grossen Höhen kommt sie auf Matten und Geröllhalden, ja sogar an extrem

trockenen Südhängen vor. H. (*Arianta*) *arbustorum* ist hochgradig anpassungsfähig, variabel und bildet eine Menge ökologischer Rassen.

Ihr Verbreitungsgebiet erstreckt sich vom Südfuss der Alpen bis nach Skandinavien über den 70. Grad hinaus, von Island und den Britischen Inseln nach Ungarn, den Karpaten, der Ukraine, Polen und Lettland. H. (*Arianta*) *arbustorum* wurde bereits von PERROT (1938) untersucht. Er beschrieb Schwierigkeiten, die dadurch entstanden waren, dass einige kleine Tetraden die Tendenz hatten, sich zu verklumpen. Bei gut fixierten Metaphaseplatten stellte er 30 Elemente verschiedener Grösse fest.

Das von mir untersuchte Exemplar wurde im Juli 1960 am Nordufer des Mettmenhaslisees (bei Dielsdorf, Zürich) in einem lockeren Erlen- und Faulbaumbestand gefunden.

Meine Resultate stimmen mit den Ergebnissen PERROTs überein. In 21 Diakinese- und Metaphasestadien der ersten Reifeteilung zählte ich 30 Tetraden, in 2 Fällen nur 29, doch zeigten diese beiden Platten Überlagerungen. Der Unterschied zwischen dem kleinsten und grössten Element einer Platte war beträchtlich. Oft stach ein Bivalent durch besondere Grösse hervor, die übrigen zeigten regelmässige Abstufungen. In den Diakinesestadien konnte ich bei grösseren Bivalenten Chiasmata feststellen (Abb. 14). Es hatten 11-13 Bivalente pro Kern mindestens 2 Chiasmata, während ich 4-6 Bivalente pro Kern mit einem Chiasma feststellen konnte. Die von PERROT beschriebenen Schwierigkeiten waren bei meinen Präparaten nicht bemerkbar. Ich nehme an, dass sie auf die von PERROT verwendete Paraffintechnik zurückzuführen sind.

Helicigona (Dinarica) pouzolzi (DESHAYES, 1830)

Die Heimat dieser Art ist Dalmatien und Südserbien. Im April 1961 wurden 2 Exemplare dieser Art in der Nähe von Dubrovnik in einem trockenen felsigen

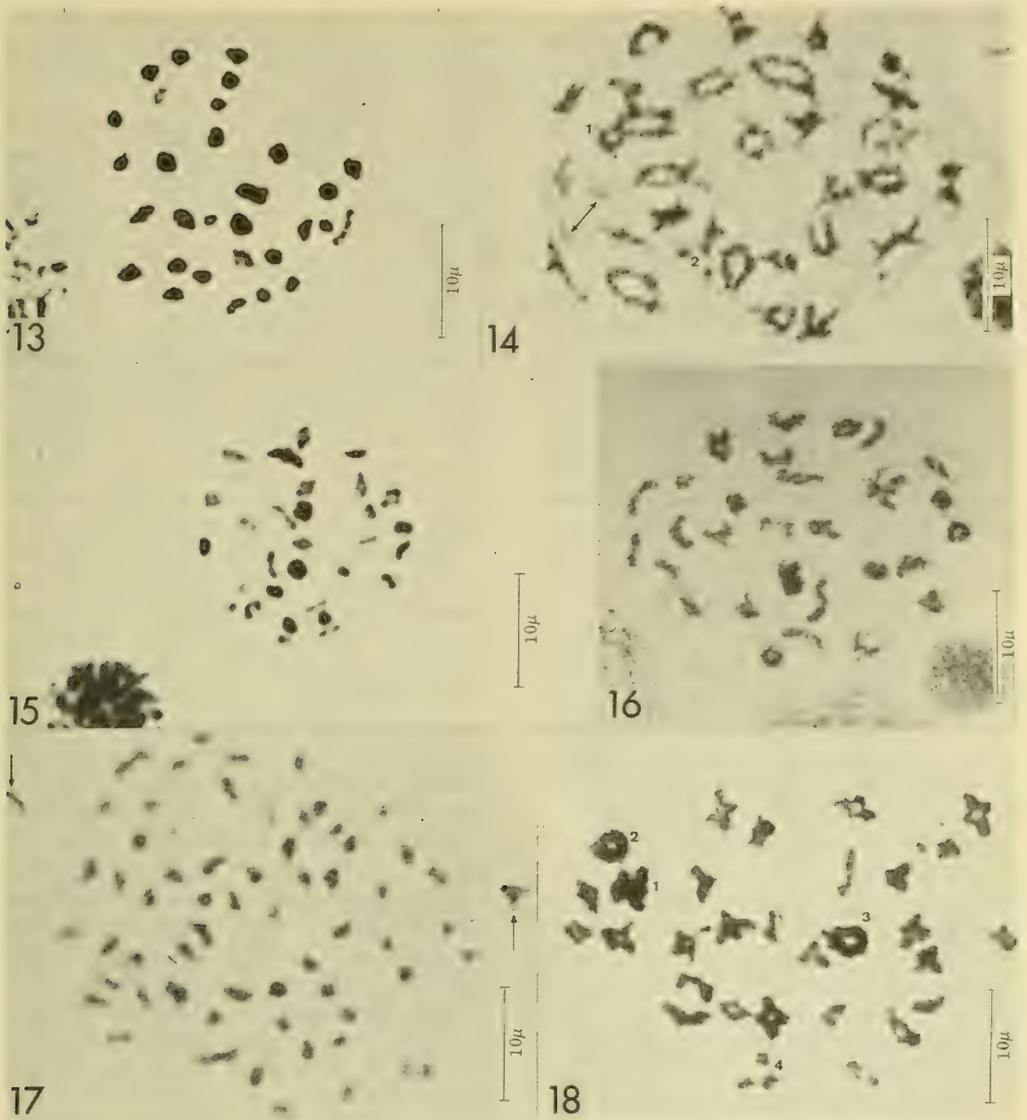


Abb. 13-18. Abb. 13. *Helicigona achates rhaetica*, $n=30$, Metaphase der ersten Reifeteilung. Daneben ein Kern in früher Prophase. Abb. 14. *Helicigona arbustorum*, $n=30$, frühes Diakinesisstadium. Eines der grossen Bivalente (1) hat mindestens 3 Chiasmata. Ferner fällt das kreuzförmige Bivalente (2) auf, bei dem in den längeren Armen ein Unterbruch zu erkennen ist. \longleftrightarrow keine Chromosomen. Abb. 15. *Helicigona pouzolzi*, $n=29$, frühe Metaphase. Eine kleine Tetrade links unten ist bereits geteilt. Abb. 16. *Helicigona setosa*, $n=29$, Diakinesisstadium. Erklärung im Text. Abb. 17. *Isognomostoma isognomostoma*, $2n=60$, mitotische Metaphase. \rightarrow keine Chromosomen. Abb. 18. *Isognomostoma holosericum*, $n=30$, Diakinesisstadium. Das grösste Bivalent (1) liegt in der Gruppe links im Bild. Darüber ein Ringbivalent (2), ein zweites (3) ist etwas rechts der Mitte zu erkennen. Am unteren Rand ein dreiteilig scheinendes Bivalent (4). Ich halte es für ein Bivalent mit einem fast terminalisierten Chiasma. Auffallend ist der Unterbruch im Chromosom.

Biotop gefunden.

Die Gonaden beider Tiere enthielten fast ausschliesslich schwer zählbare mitotische Metaphasen. Die Chromosomen waren zum grössten Teil punktförmig, einige länglich. Unter vielen Platten fand ich nur 11, bei denen ich die Chromosomen zählen konnte. Von ihnen hatten 9 die Chromosomenzahl 58, eine schien 56, eine andere 59 Chromosomen zu enthalten. Ausserdem fand ich 4 frühe Metaphaseplatten der ersten Reifeteilung, deren Tetraden klar gegeneinander abgegrenzt waren. Bei allen konnte ich eindeutig 29 Elemente zählen. Etwa 4 davon waren Ringbivalente, 5-6 erschienen kreuzförmig (Abb. 15). Die Tetraden eines Satzes zeigten starke Grössenunterschiede.

Helicigona (Liburnica) setosa
(ROSSMAESSLER, 1836)

Das Verbreitungsareal dieser Art umfasst die östlichen Küstenländer der Adria von Istrien bis Montenegro.

Zur Untersuchung gelangten 2 Tiere, die in der Nähe von Titograd, nördlich des Skutarisees in typisch mediterranem Biotop (Macchia) gefunden wurden.

Nur eine Keimdrüse wies brauchbare Teilungsstadien auf: 14 Kerne in Metaphase- oder verschiedenen Diakinesestadien enthielten eindeutig 29 Tetraden. Bei 8 Diakinesestadien konnte ich Chiasmata feststellen: 6-7 Bivalente pro Kern hatten mindestens 2, meist terminalisierte Chiasmata. Ein Bivalent, meistens aber 2 pro Kern, waren kreuzförmig (Abb. 16). Häufig fiel ein Bivalent durch besondere Grösse auf; in Abb. 16 ist dieser Grössenunterschied aber nicht deutlich.

Isognomostoma isognomostoma
(GMELIN, 1780) SCHROETER, 1784
[=*I. personatum* LAMARCK, 1792]

Diese Art ist alpin-karpatisch mit weiter mitteleuropäischer Ausbreitung. Im Westen reicht sie bis in die katalanischen Pyrenäen. Gegen Osten ist sie im ganzen Alpengebiet verbreitet, mit südlicher Grenze im Tessin. Gegen

Norden kommt die Art in ganz Mitteldeutschland bis nach Böhmen-Mähren und im polnischen Jura vor. Sie fehlt in der norddeutschen Ebene.

Man findet die Tiere hauptsächlich unter Steinen, altem Holz oder Laub, an schattigen, feuchten Stellen am Boden von Wäldern, oder an bewachsenen Felsen.

Die beiden untersuchten Tiere wurden an verschiedenen Orten gefunden. Das erste stammt vom Tössufer südlich von Winterthur. Es wurde anfangs April 1960 eingebracht. Das zweite Exemplar wurde im August 1961 am Nordhang eines Buchenwaldes, 2 km westlich von Baden (Aargau) gefunden. Obwohl die beiden Tiere zu ganz verschiedenen Zeiten fixiert wurden, enthielten beide Zwitterdrüsen fast keine brauchbaren Reifeteilungsstadien, sondern nur schwer analysierbare Spermatogonienteilungen. Unter den 100 geprüften Metaphaseplatten fand ich in jedem Tier nur wenige, bei denen die Chromosomen klar genug abgegrenzt waren. Beim Winterthurer Tier zählte ich bei 14 Platten 60 Chromosomen (Abb. 17), 6 Platten ergaben Zahlen zwischen 53 und 59, und eine Platte schien 62 Chromosomen zu enthalten. Ausserdem fand ich zufällig 2 Pachytänstadien, bei denen alle Chromosomenenden sichtbar waren, so dass ich sie zählen konnte. Beide hatten 30 Chromosomen.

Beim Badener Exemplar stellte ich bei 8 Platten 60 Chromosomen fest, während ich bei 7 Platten 55-59 Chromosomen zählte.

Isognomostoma holosericum
(STUDER, 1820)

Das Verbreitungsareal dieser Art erstreckt sich über den Jura und das ganze Alpengebiet mit Ausnahme der südlichen Westalpen. Im Südosten reicht es bis zu den Karstländern, im Osten bis in die Westkarpaten und von dort nordwestwärts über Böhmen-Mähren zum Erz- und Riesengebirge; von dort wird die Verbindung zu den Alpen durch den Böhmerwald hergestellt.

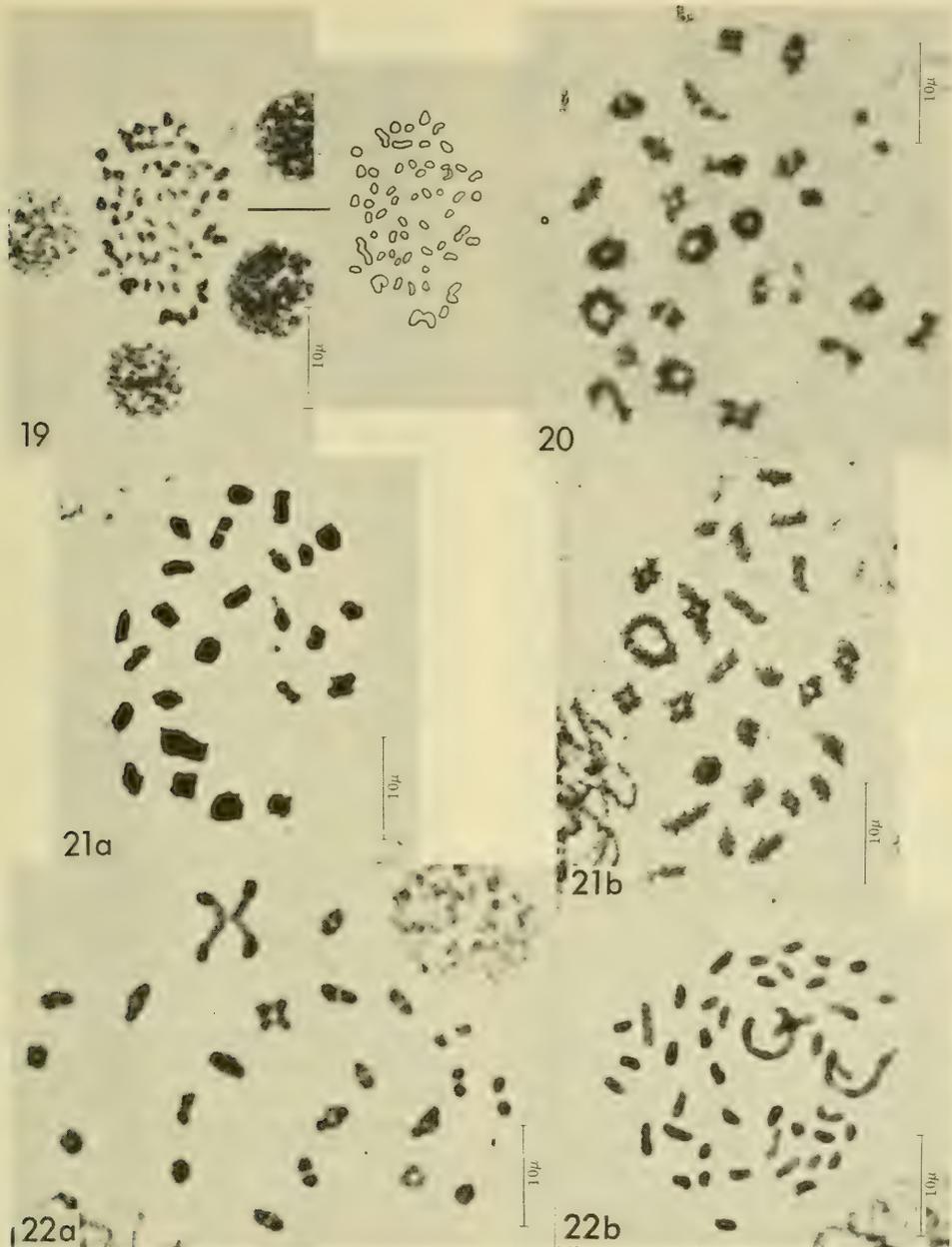


Abb. 19-22. Abb. 19. *Euparypha pisana*, $2n=56$, mitotische Metaphase. Da die Chromosomen nicht alle in einer Ebene liegen und deshalb einige Stellen auf dem Foto undeutlich sind, diene die danebenstehende Zeichnung dem besseren Verständnis. Abb. 20. *Cepaea vindobonensis*, $n=25$, Diakinesestadium. Zwei kleine Bivalente rechts im Bild sind bereits geteilt. Abb. 21. *Cepaea silvatica*, $n=25$, a) Metaphase der ersten Reifeteilung. Die grösste Tetrade ist unten links erkennbar. b) Diakinesestadium. Das grösste Bivalent bildet einen Ring. Das rechts darüberliegende kreuzförmige Bivalent ist hier kaum kürzer. Abb. 22. *Cepaea nemoralis*, a) Diakinesestadium, $n=22$. b) mitotische Metaphase, $2n=44$.

Die Art ist gesteinsindifferent und lebt vor allem unter morschem Holz, totem Laub und unter Steinen in Gebirgswäldern. Sie kommt aber auch oberhalb der Baumgrenze unter Geröll und auf Grasheiderasen vor.

Das untersuchte Exemplar stammt aus einem Mischwald eines Nordhanges oberhalb Promotogno im Bergell (Graubünden). Es wurde im August 1961 auf ca. 950 m.ü.M. gefunden. Von 25 Kernen in verschiedenen Diakinesestadien enthielten 2 Kerne 29, die übrigen 30 Bivalente. Eines der Bivalente war oft merklich grösser und hatte meistens ein Chiasma. Unter den übrigen, der Grösse nach regelmässig abgestuften Elementen fand ich bis zu 15 kreuzförmige Bivalents (Abb. 18). Hingegen waren Bivalente mit deutlich mehreren Chiasmata seltener als bei anderen Arten. Meistens stellte ich 1-2 Ringbivalente fest, nur in einem Kern waren es 3. Nebst den 25 Diakinesestadien fand ich noch 7 Metaphaseplatten der Spermatogonienteilungen mit klar gegeneinander abgegrenzten Chromosomen. Bei 6 Platten konnte ich 60 Chromosomen feststellen, bei einer Platte zählte ich 58.

Subfamilia Helicinae

Die Helicinae sind die grössten und höchstorganisierten Heliciden. Mit 21 Gattungen und 37 Untergattungen (ca. 300 Arten) ist diese Unterfamilie die umfangreichste der Helicidae. Ihr Hauptentwicklungsgebiet liegt rings um das Mittelmeer. Im Westen kommen Helicinae bis zu den Kanarischen Inseln, im Osten bis Persien vor. In Mitteleuropa sind aber nur 4 Gattungen mit zusammen 7 Arten vertreten. Alle gehören dem Tribus Heliceae an. Der Tribus Murelleae ist nicht vertreten.

Tribus Heliceae

Euparypha pisana

(O. F. MUELLER, 1774)

[=*Theba pisana* RISSO, 1826]

Die Art ist xerophil, sie lebt an

trockenen, heissen Stellen von Gärten, Feldern und Böschungen, oft an der prallen Sonne. Häufig findet man sie in grosser Zahl an dünnen Stengeln krautiger Pflanzen.

Die Art ist in allen Mittelmeerländern heimisch. Sie breitet sich von der iberischen Halbinsel der atlantischen Küste entlang nach Norden bis Belgien und England aus. Von der Küste her ist sie ins Innere Frankreichs eingedrungen.

Die beiden untersuchten Exemplare stammen von Roda de Bara (Spanien), vom selben Fundplatz wie *Monacha cantiana*, und wurden im Mai 1962 gesammelt und fixiert. Beide Tiere waren nicht ganz ausgewachsen. Ich fand in den Gonaden keine Reifeteilungen, das Material enthielt aber ziemlich viele Metaphaseplatten von Spermatogonienteilungen. Von 40 geprüften Platten erwiesen sich nur 9 als zählbar. Dabei stellte ich 5 Platten mit 56 Chromosomen fest (Abb. 19), bei den restlichen 4 Platten zählte ich 50, 54, 57 und 58 Chromosomen. Die Modalzahl 56 müsste durch weitere Zählungen bestätigt werden.

Cepaea vindobonensis

(FERUSSAC, 1821)

[=*Tachea vindobonensis* LEACH, 1818

=*Helix (Cepaea) austriaca*

ROSSMAESSLER, 1835]

Die Art ist südosteuropäisch und ihr Verbreitungsgebiet erstreckt sich vom Kaspischen Meer westwärts durch Südrussland, die Ukraine, über die Karpaten und die nördliche Balkanhalbinsel bis zu den Steirischen und Kärntner Alpen. Gegen Norden reicht sie von den Karpaten bis nach Mähren und zum Böhmerwald sowie nach Südpolen.

BALTZER (1913) beschrieb bereits die Spermatogenese von *Cepaea vindobonensis*. Er stellte die Chromosomenzahl $n=25$ fest. Die Tetraden der ersten Reifeteilung wurden als verschieden gross geschildert, keine der Tetraden fiel durch besondere Merkmale

auf. PERROT (1938) bestätigte BALTZERS Ergebnisse. Auch meine eigenen Resultate stimmten mit denen von BALTZER überein. Der Fundplatz der beiden von mir untersuchten Individuen liegt in Jugoslawien, 30 km südlich von Mostar, bei Hutova-Blato. Es handelt sich um ein weitgehend trockengelegtes Sumpfgelände.

Ich kontrollierte 30 Kerne in Diakinese- und Metaphasestadien und konnte bei 27 von ihnen 25 Tetraden feststellen. Zwei Kerne schienen 26 und einer 24 Bivalente zu enthalten. Innerhalb eines Kerns war die Grösse der Tetraden regelmässig abgestuft. In den Stadien, in denen ich Chiasmata feststellen konnte, fiel mir auf, dass viele Bivalente mindestens 2 Chiasmata hatten. In den meisten Kernen konnte ich 3-6 Ringbivalente und nur 1-3 kreuzförmige Bivalente erkennen (Abb. 20). Ausser diesen Stadien der Reifeteilung konnte ich noch 3 Metaphaseplatten der Spermatogonien auszählen, welche 50 Chromosomen enthielten.

Ein weiteres Tier stammt von Neusiedel (Österreich), wo es Ende Mai 1960 im Gebüsch am Rand eines Hohlweges in der Nähe des Dorfes gefunden wurde. Ich fand verhältnismässig wenig Teilungen. Zehn von 12 untersuchten Kernen in Diakinese- und Metaphasestadien enthielten 25 Tetraden, die sich auch in Bezug auf Grösse und Verteilung der Chiasmata nicht von denen der jugoslawischen Tiere zu unterscheiden schienen.

Cepaea silvatica (DRAPARNAUD, 1801)

Die Art ist mit verschiedenen Subspecies über den Französischen und Schweizer Jura sowie in den Westalpen verbreitet und dringt durch die Westschweizer Kalkalpen in die Zentralschweiz und bis zum Walensee vor. *Cepaea silvatica* kommt auf kalkhaltigem Grund in Wäldern und auf Alpweiden, an Felsen und Mauern bis zu Höhen von 2600 m vor.

PERROT (1938) fand auch bei dieser Art 25 Tetraden, wovon eine oft viel

grösser war als die übrigen. Er beschrieb auch die Spermatogonienteilung, bei der er zahlreiche V-förmige Chromosomen erkannte, von denen ein Paar deutlich länger war.

Ich untersuchte 2 Tiere vom Südhang des Augstmatthorns, nördlich des Brienersees (Berner Oberland), welche am 17. August 1961 auf 1400 m.ü.M. in einem Buchenwald nahe der Waldgrenze gefunden wurden.

Das Material enthielt reichlich Reifeteilungsstadien. Ich untersuchte 40 Kerne in Diakinese- bis Metaphasestadien und stellte ausnahmslos 25 Tetraden pro Kern fest. In fast allen Fällen war ein Bivalent wesentlich grösser als das nächstkleinere, die übrigen waren regelmässig abgestuft (Abb. 21a). In 19 Kernen hatte dieses grosse Bivalent mindestens 2 Chiasmata, in 6 andern nur eines. Unter den kleineren Elementen waren meistens 3-6 kreuzförmige und 2-4 Ringbivalente zu erkennen (Abb. 21b).

Cepaea nemoralis (LINNAEUS, 1758)

Die Art ist über ganz Mittel- und Westeuropa verbreitet und sehr häufig, vor allem in Kulturland wie Gärten und Parks, in Hecken und an Mauern. Man trifft sie aber auch in lichten Wäldern an. Die Spermatogenese von *C. nemoralis* wurde erstmals von KLEINERT (1909) untersucht. In den Spermatogonienteilungen fand er 48 Chromosomen, wovon 2 besonders gross waren. Dementsprechend stellte er in den Reifeteilungen 24 Bivalente fest, von denen eines durch seine Grösse auffiel. Auf den Abbildungen zeigt dieses meistens 2 Chiasmata. Im Widerspruch zu KLEINERT stehen die Ergebnisse PERROTs (1938), der in der ersten Reifeteilung 21 gleich grosse Elemente und ein deutlich grösseres fand. Die Zahl der Mitosechromosomen ermittelte PERROT nicht, hingegen beobachtete er ein Paar grosser V-förmiger Chromosomen, dem grossen Bivalent der Reifeteilung entsprechend. Meine eigenen Resultate bestätigen die Ergebnisse

PERROTs.

Ich untersuchte ein Exemplar der Art aus einem lichten Erlenwald vom Nordufer des Mettmenhaslisees (bei Dielsdorf, Zürich). Die Zwitterdrüse des Tieres enthielt viele analysierbare Reifeteilungen und einige mitotische Metaphasen mit klar abgegrenzten Chromosomen. Ich prüfte 60 Kerne der ersten Reifeteilung, die fast ausnahmslos 22 Tetraden enthielten; nur 2 Kerne mit 20 Tetraden wichen davon ab. In allen Kernen fiel eine besonders grosse Tetrade auf. Sie war immer kreuzförmig und behielt diese Form bis in die Metaphase bei. Auch bei den kleinen Bivalenten liessen sich kreuzförmige erkennen, und zwar in jedem Kern etwa 4-7. Bivalente mit mehreren Chiasmata beobachtete ich seltener und nur bei kleinen Elementen.

Bei dem abgebildeten Diakinesestadium (Abb. 22a) erkennt man im Chiasma des grössten Bivalenten die Kreuzung zweier Chromatiden. Bei den andern kreuzförmigen Bivalenten ist sie nicht vorhanden. Diese haben die Rotation (WHITE, 1954: 90) bereits hinter sich. Das grosse Bivalent braucht also etwas länger für die Umbildung vom Diplotän-Chromosomenpaar zur fertigen Tetrade. Das konnte ich bei dieser Art in vielen Fällen feststellen. Diese Beobachtung passt zur Aussage WHITEs (1954: 93), dass sich kurze Chromosomen in der Anaphase zuerst teilen, was man bei den 2 kleinsten Bivalenten rechts erkennen kann. Auch bei anderen Arten liess sich diese Erscheinung beobachten.

In der Metaphase der Spermatogonienteilung (Abb. 22b) fand ich 44 verschiedene lange Chromosomen, wovon ein Paar, dem grossen Bivalent der Reifeteilung entsprechend, 2-3 mal so lang war wie das zweitlängste.

Cepaea hortensis
(O. F. Mueller, 1774)

Das Verbreitungsgebiet dieser Species deckt sich weitgehend mit dem von *C. nemoralis*, ist aber im Ganzen etwas nach Norden verschoben. So fehlt die

Art in den Südalpen und Italien, reicht aber andererseits bis weit nach Skandinavien. Man findet *C. hortensis* im selben Biotop wie *C. nemoralis*, sie ist aber weniger Kulturfolger. Auch *C. hortensis* wurde schon mehrmals untersucht. Sowohl KLEINERT (1909) als auch PERROT (1938) stellen völlige Übereinstimmung mit den Verhältnissen bei *C. nemoralis* fest. Das ist an sich auch zu erwarten, da die beiden Arten wenig verschieden sind und sich kreuzen lassen (LANG, 1908). Angeblich bastardieren die Arten auch in der Natur (BOETTGER, 1921). BALTZER (1913), der *C. hortensis* untersuchte, fand, wie später PERROT, die Chromosomenzahl $n=22$, während KLEINERT 24 Chromosomen zählte.

Ich untersuchte 4 Tiere vom Klausenpass. Der Fundort liegt südlich von Schwanden, am Südufer des Vordereschächen, auf 1080 m.ü.M., in einem ziemlich dichten Mischwald mit feuchtem Boden, der viel Moos, Farne und kleinere Sträucher aufwies. Die Tiere wurden Ende August 1961 gesammelt und fixiert.

Zur Untersuchung kamen 50 Kerne in Diakinese- bis Metaphasestadien der ersten Reifeteilung; die Metaphasen der Spermatogonienteilungen waren wegen vieler Chromosomenüberlagerungen nicht analysierbar. 32 Kerne enthielten übereinstimmend und eindeutig 30 Tetraden. Bei einem Kern zählte ich 32 Elemente und bei 11 andern Kernen stellte ich verschiedene Zahlen von 20-29 fest. Die restlichen 7 Kerne erwiesen sich als nicht analysierbar. In keiner der 43 ausgezählten Platten zeichnete sich eine der Tetraden durch Grösse oder charakteristische Form aus. Die Grösse der Tetraden war regelmässig vom grössten bis zum kleinsten Element abgestuft, der Unterschied beträchtlich (Abb. 23). Bei Stadien, in denen ich Chiasmata erkennen konnte, beobachtete ich 4-7 Bivalente mit deutlich 2 Chiasmata und nur etwa 3-4 kreuzförmige pro Kern. Bei allen 4 Tieren dieses Fundortes stimmten die Chromosomenverhältnisse überein. Meine Er-



Abb. 23. *Cepaea hortensis* vom Klausenpass, $n=30$, Metaphase der ersten Reifeteilung.

gebnisse diskutiere ich auf S. 368.

Helix (Cryptomphalus) aspersa
(O. F. MUELLER, 1774)

Die Art ist mediterran westeuropäisch. Sie kommt in allen Küstenländern des Mittelmeeres vor, hat sich aber über ganz Westeuropa bis nach

Südholland verbreitet. Von Frankreich aus drang sie in den letzten Jahren in die Schweiz und ins Rheingebiet ein. Durch Obst- und Gemüsehandel werden immer wieder Exemplare verschleppt.

PERROT (1938) fand in den Spermatozyten erster Ordnung 27 Tetraden, die er als einander sehr ähnlich beschreibt. Das untersuchte Exemplar dieser Art stammt von Tour de Vallas (Camargue, Frankreich); ich fixierte es am 24. August 1960. Bei 32 Kernen der ersten Reifeteilung konnte ich deutlich 27 Tetraden feststellen, bei 2 Kernen zählte ich 26 und bei einem nur 25 Bivalente. In vielen Fällen schien ein Bivalent besonders gross zu sein, doch war der Unterschied gegenüber dem nächstkleineren nicht so ausgeprägt, dass ich ihn immer mit Sicherheit feststellen konnte. Im übrigen waren die Elemente wieder regelmässig abgestuft, bis zu etwa einem Viertel des grössten Bivalents. Bei 20 Kernen in Diakinesestadien liessen sich Chiasmata beobachten, wobei ungefähr



Abb. 24. *Helix aspersa*, $n=27$, Diakinesestadium. 1 = Bivalent mit mindestens 2 Chiasmata; 3-6 = Ringbivalente; 2, 7-12, ev. 13 = je 1 Chiasma.

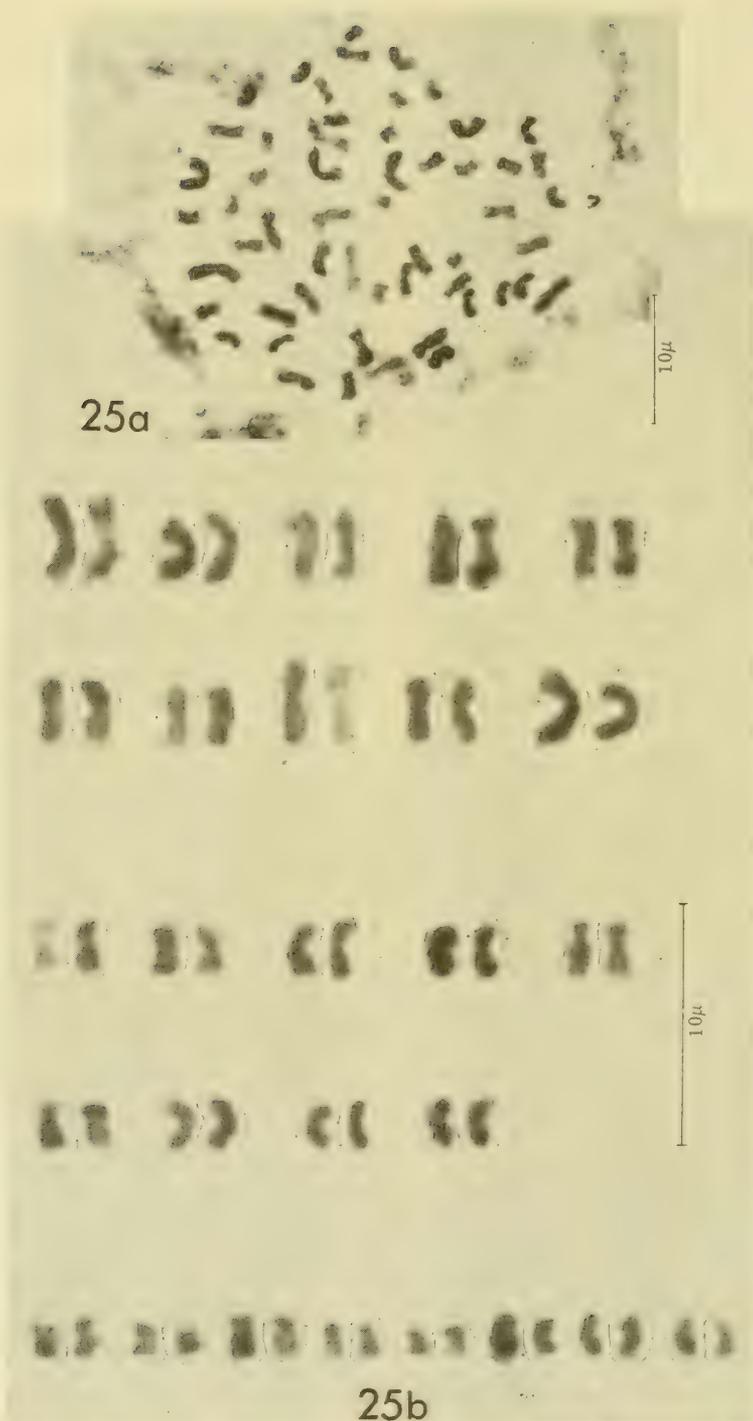


Abb. 25. a) *Helix aspersa*, $2n=54$, mitotische Metaphase. b) Chromosomensatz von *Helix aspersa*. Erklärung im text.

gleich viele kreuzförmige Bivalente wie Ringbivalente zu erkennen waren. Je eines gehörte zu den grössten, die übrigen zu den mittelgrossen Elementen.

In Abbildung 24 erkennt man beim grössten Bivalent (No. 1) mindestens 2 Chiasmata, wovon eines nicht terminalisiert ist. Das zweitgrösste Bivalent ist kreuzförmig (No. 2). Weiter lassen sich 2 deutliche Ringbivalente (No. 3 und 4) identifizieren; die beiden runden Bivalente (No. 5 und 6) sind stärker kontrahierte Ringbivalente. Bei den Bivalenten 7-12, wahrscheinlich auch bei 13, erkennt man je ein Chiasma. Die Bivalente 14-17 haben vermutlich 2 Chiasmata, wovon das eine terminalisiert ist.

Zwei Metaphaseplatten der Spermatozyonteilung hatten eindeutig 54 Chromosomen. Die eine der beiden Platten war die einzige im gesamten von mir untersuchten Material, bei der man bei vielen Chromosomen die SFA erkennen konnte (Abb. 25a). Die Chromosomen lassen sich der Grösse nach in etwa 3 Gruppen ordnen. Zu den grössten Chromosomen gehören 20, zu den mittleren 18 und zu den kleinsten 16 Chromosomen (Abb. 25b). Die Lage der SFA ist bei 14 grossen Chromosomen median oder submedian, bei 2 Paaren subterminal und bei einem Paar nicht zu erkennen. In der mittleren Gruppe sind 4 Chromosomenpaare metazentrisch; bei 2 Paaren liegt die SFA submedian bis subterminal und bei 3 Paaren ist sie nicht sichtbar. Bei 5 Paaren der kleinsten Chromosomen liegt die SFA ebenfalls submedian bis subterminal; bei den restlichen lässt sie sich nicht erkennen. Akrozentrische Chromosomen beobachtete ich keine.

Helix (Helix) dormitoris bosnica
(KOBELT, 1906)

Die Vertreter dieser Unterart, die in Bosnien und der Herzegowina beheimatet ist, wurden während der bereits erwähnten Expedition im Sommer 1961 ca. 10 km SSE von Mostar (Jugoslawien)



Abb. 26. *Helix dormitoris bosnica*, $n=27$, frühes Diakinesestadium.

nahe der Strasse nach Blagaj gefunden.

Die Gonaden der beiden untersuchten Tiere enthielten viele Reifeteilungen. Bei 48 von 60 untersuchten Kernen konnte ich 27 Tetraden feststellen, bei den andern 12 Kernen zählte ich viermal 28, viermal 26, dreimal 25 und einmal 24. Die Tetraden unterschieden sich in der relativen Grösse nicht sichtlich von denen von *Helix aspersa*. Was die Zahl der Chiasmata betraf, so hatte *Helix dormitoris* durchschnittlich mehr Bivalente mit mehreren Chiasmata. Das grösste Element erschien häufig als Ringbivalent, doch war ein kreuzförmiges Bivalent oft fast gleich gross, ähnlich wie bei *Helix aspersa*, aber nicht immer so deutlich (Abb. 26).

Helix (Helix) lucorum trapezuntis
(FORCART, 1963)

[=*Helix (Helicogena) moussoni*
boettgeri KOBELT, 1905]

Diese Unterart ist bisher nur von Trabzon (Trapezunt, Türkei) bekannt. Die Nominatform *Helix lucorum* soll in Südrussland, der Türkei und Zentralitalien vorkommen (GERMAIN, 1930).

Der Fundort liegt in einem kleinen

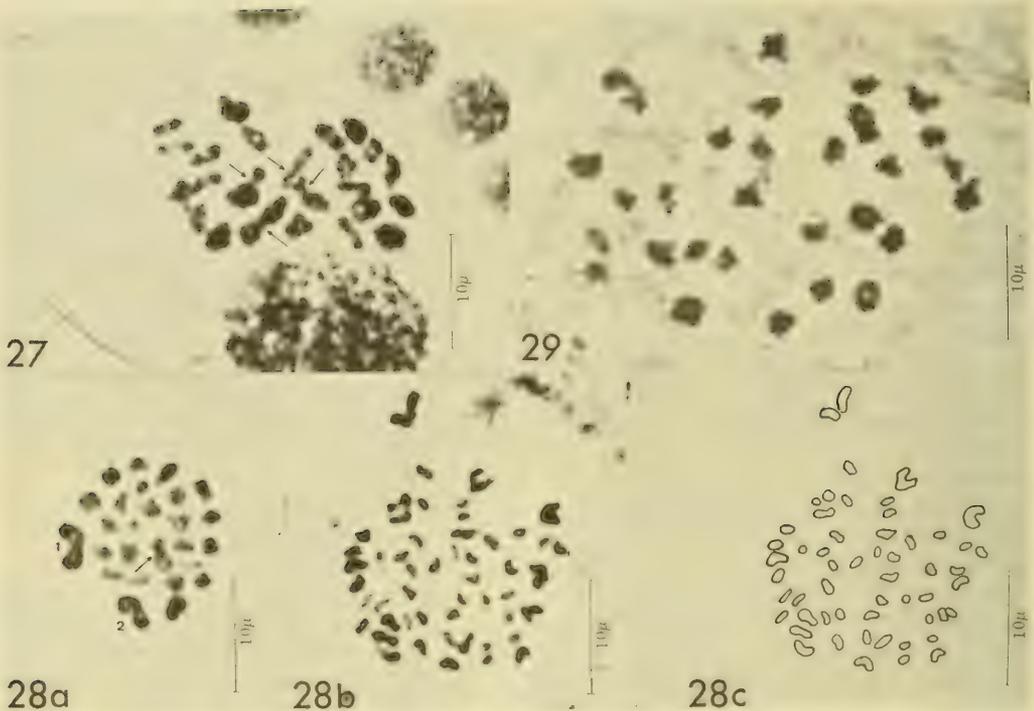


Abb. 27-29. Abb. 27. *Helix lucorum trapezuntis*, $n=27$, späte Diakinese bis Metaphase. Die Pfeile deuten auf Stellen, an denen Tetraden nahe zusammenstossen. Abb. 28. *Eobania vermiculata*, a) $n=27$, frühe Metaphase. Der Pfeil weist auf eine Überlagerung zweier Tetraden hin. b) $2n=54$, mitotsche Metaphase. c) Zeichnung zum besseren Verständnis von b. Abb. 29. *Caucasotachea lenkoranea*, $n=30$, spätes Diakinesestadium.

Wald ca. 10 km östlich von Trabzon, 500 m von der Küste des Schwarzen Meeres entfernt. Das Tier wurde am 19. Juli 1962 fixiert.

Die 41 untersuchten Kerne dieses Individuums befanden sich hauptsächlich in der Metaphase der ersten Reifeteilung, nur 5 davon in Diakinesestadien. Bei den Metaphaseplatten, vor allem bei späten Stadien, kamen gelegentlich Chromosomenüberlagerungen vor. Deshalb zeigte die Chromosomenzahl bei relativ vielen Kernen eine leichte Abweichung (24-26 Elemente); 29 Platten enthielten jedoch eindeutig 27 Tetraden. Die Tetraden waren wie üblich der Grösse nach abgestuft (Abb. 27). Bei den wenigen Stadien, bei denen Chiasmata zu beobachten waren, stellte ich 4-6 Bivalente mit 2 Chiasmata und 3-4 kreuzförmige Bivalente pro Kern fest.

Eobania vermiculata
(O. F. MUELLER, 1774)

Diese Art ist in allen Mittelmeerlandern verbreitet, vor allem in den östlichen. Sie lebt vorwiegend auf Feldern, in Gärten und Weinbergen.

Im Juli 1961 erhielt ich 6 Exemplare, welche in Jugoslawien zwischen Golubovci und Plavnica, nördlich des Skutarisees gesammelt wurden. Alle Tiere enthielten wenig brauchbare Teilungsstadien. Im Ganzen konnte ich 34 Kerne untersuchen, hauptsächlich Metaphasestadien. Bei 23 Kernen zählte ich 27 Tetraden, die übrigen schienen bis 5 Elemente weniger zu haben. In der frühen Metaphase fielen häufig 1-2 besonders grosse, oft schleifenförmige Bivalente auf (Abb. 28a, 1, 2). Es könnte sich um Bivalente mit einem Chiasma in der Mitte und

TABELLE 1. Chromosomenzahlen verschiedener terrestrischer Gastropoden (ausser Helicidae)

Taxonomische Einheit	Fundort	Z	n	Anzahl d. Platten in RT. I.	2n	Anzahl d. Mitoseplatten
Stylommatophora						
Cochlicopidae						
<i>Cochlicopa lubrica</i>	Linthal Regensburg	2 5	26	19(24)	52	3(4)
Chondrinidae						
<i>Abida secale</i>	Grindelwald	3	30	4(4)	-	-
<i>Chondrina avenacea</i>	Klausenpass	6	30	11(13)	60	1(1)
<i>Chondrina similis</i>	Albenga (It.)	1	30	7(11)	60	1(3)
Enidae						
<i>Ena montana</i>	Linthal Ütliberg Klausenpass	1 1 1	24	14(15)	48	16(17)
<i>Ena obscura</i>	Baden	1	24	1(1)	48	27(39)
<i>Zebrina detrita</i>	Rüdlingen	2	24	28(28)	48	11(12)
Succineidae						
<i>Succinella oblonga</i>	Baden Baden	1 1	11 12	62(67) 21(28)	22 -	1(1) -
Endodontidae						
<i>Discus rotundatus</i>	Winterthur	1	29	30(31)	-	-
Zonitidae						
<i>Vitrea diaphana</i>	Baden	5	20	20(22)	-	-
Clausiliidae						
Cochlodininae						
<i>Cochlodina laminata</i>	Ütliberg Istein (D.)	1 1	24	6(7)	48	5(7)
Clausiliinae						
<i>Clausilia parvula</i>	Huttingen (D.)	6	24	13(17)	48	17(23)
<i>Iphigena ventricosa</i>	Baden Kerenzerberg	1 1	24	3(5)	48	33(51)
<i>Iphigena plicatula</i>	Zürich Klausenpass Baden	1 2 5	-	-	48	27(37)
<i>Laciniaria plicata</i>	Winterthur	3	24	4(4)	48	10(12)
Alopiinae						
<i>Delima itala</i>	Gandria	1	ca 30	12	ca 60	40
<i>Papillifera papillaris</i>	Roda de Bara (Sp.)	4	-	-	ca 60	6
Fusulinae						
<i>Graciliaria strobili</i>	Gandria	1	-	-	48	3(3)
Prosobranchia						
Pomatiasidae						
<i>Pomatias elegans</i>	Rabac-Labin (Ju.)	5	13	44(44)	-	-
<i>P. costulatus hyrcanus</i>	Shahi (Persien)	2	13	68(84)	26	5(5)
Cyclophoridae						
<i>Cochlostoma septemspirale</i>	Baden	1	13	21(25)	-	-

Z = Anzahl der untersuchten Tiere. RT. I. = Erste Reifeteilung. Die Zahlen der Kolonnen 5 und 7 geben an, wie viele Platten die Modalzahl zeigten; in Klammern ist das Total der geprüften Platten angegeben. It. = Italien; D. = Deutschland; Sp. = Spanien; Ju = Jugoslawien

Chiasmata an beiden Enden handeln. Ausserdem fand ich in 3 Tieren je eine zählbare Metaphaseplatte der Spermatogonienteilungen. Eine davon hatte ziemlich viele Chromosomenüberlagerungen, in ihr zählte ich 51 Chromosomen. Die beiden andern Platten enthielten je 54 Elemente (Abb. 28b, c).

Caucasotachea lenkoranea
(MOUSSON, 1863)

Zur Untersuchung kamen 3 Tiere aus Persien, welche etwa 20 km südlich von Shahi (nordöstlich von Teheran, in der Nähe der Küste des Kaspischen Meeres) gefunden wurden. Bei 2 Tieren fand ich keine analysierbaren Teilungsphasen. Das 3. enthielt wenigstens so viele Metaphaseplatten, dass ich die Chromosomenzahl $n=30$ mit einiger Sicherheit feststellen konnte. Ich untersuchte 34 Kerne, 20 davon enthielten 30 Bivalente. Die übrigen Zählungen ergaben häufig 28 oder 29, selten 24, 25 oder 31 Elemente. Keine der Tetraden war besonders gross. Chiasmata konnte ich zwar in einzelnen Kernen feststellen (Abb. 29), doch war ihre Verteilung häufig undeutlich.

CHROMOSOMENZAHLEN BEI
ANDEREN FAMILIEN

Ausser bei den untersuchten Vertretern der Helicidae stellte ich bei 18 Stylommatophoren anderer Familien und bei 3 Prosobranchiern die Chromosomenzahlen fest. Ich fasse die Ergebnisse in Tabelle 1. zusammen.

Bei den 4 Clausiliiden *Cochlodina laminata*, *Clausilia parvula*, *Iphigena ventricosa* und *I. plicatula* bestätige ich damit die Befunde THALERS (1963). Die Angabe ANKELs (1925) $n=12-13$ für *Cochlostoma septemspirale* (Cyclophoridae) präzisiere ich auf $n=13$.

Besonders erwähnenswert ist das Ergebnis der Zählungen an *Succinella oblonga*. Ich untersuchte 2 Tiere aus einem Buchenwald westlich von Baden (Aargau). Eines der Tiere enthielt 62 Kerne in Diakinese oder Metaphase-

stadien, alle hatten 11 Bivalente. Bei 5 weiteren Kernen schienen es 10-12 Elemente zu sein. Die einzige mitotische Metaphase hatte 22 Chromosomen. Der Befund lässt auf die haploide Zahl 11 schliessen. Im andern Tier fand ich 21 Kerne mit 12 Bivalenten, 7 andere Kerne schienen 10 und 11 Bivalente zu enthalten oder waren nicht eindeutig. Hier ist die Modalzahl 12. Dieses Resultat ist doppelt überraschend: Erstens kommt es in meinem Material sonst nirgends vor, dass Tiere derselben Population verschiedene Chromosomenzahlen aufweisen. Zweitens weichen die Chromosomenzahlen von denen der andern Succineiden stark ab (Tab. 3). Sollte sich durch spätere Untersuchungen die Zahl $n=11$ als die normale von *Succinella oblonga* erweisen, so wäre eine Verbindung zu *Succinea putris* mit $n=22$ durch Polyploidie denkbar. Parallelfälle sind bei Basommatophoren bekannt (BURCH, 1960a; BURCH, BASCH & BUSH, 1960).²

DIE BEDEUTUNG DER
CHROMOSOMENVERHÄLTNISSE
FÜR DIE TAXONOMIE DER
GASTROPODEN

HUSTED & BURCH (1946) wiesen als erste auf die deutliche Trennung von Basommatophoren mit niederer und Stylommatophoren mit höherer Chromosomenzahl hin und schlossen daraus auf eine Zunahme der Chromosomen im Lauf der progressiven Evolution. INABA (1959a) erklärte diese Zunahme durch Fragmentation. Auf Grund seiner Untersuchungen an Bradybaeniden schlug INABA eine Revision der Systematik überall dort vor, wo innerhalb einer Gattung verschiedene Chromosomenzahlen auftreten.

²Nach Abschluss dieser Arbeit stellten BUTOT & KIAUTA (1964) an holländischen Tieren von *Succinella oblonga* $n=12$ und $2n=24$ fest. Ausserdem sind von BURCH (1964) an anderen Succineiden sehr niedrige Chromosomenzahlen ($n=5$ und 6) nachgewiesen worden.

goninae; Tab. 4) oder es kommen größere Abweichungen vor wie bei den Helicellinae und Helicinae (Tab. 4). Verschiedene Gattungen der selben Unterfamilie lassen sich trotz gleicher Chromosomenzahl zytologisch unterscheiden, wenn andere Merkmale des Chromosomensatzes charakteristisch sind (z.B. *Eumophalia* und *Trichia*, s.S. 348-349). Das trifft aber nicht immer zu (*Monacha* und *Cochlicella*, s.S.367, Helicellinae).

TABELLE 4. Chromosomenzahlen der bisher untersuchten Helicidae

Taxonomische Einheit	n
Helicellinae	
Tribus Helicelleae	
<i>Candidula unifasciata</i>	27*
<i>Helicella obvia</i>	26
<i>H. itala</i>	26*
<i>Trochoidea cretica</i>	26*
<i>Cochlicella acuta</i>	23
Tribus Monacheae	
<i>Monacha cantiana</i>	23*
<i>M. cartusiana</i>	23
Hygromiinae	
<i>Perforatella incarnata</i>	24
<i>Trichia unidentata</i>	23*
<i>T. villosa</i>	23*
<i>T. sericea</i>	23*
<i>Eumophalia arpatschaina</i>	23*
<i>Hygromia cinctella</i>	21
Helicodontinae	
<i>Helicodonta obvoluta</i>	27*
Helicigoninae	
<i>Helicigona (Arianta) arbustorum</i>	30
<i>H. (Chilostoma) achates rhaetica</i>	30*
<i>H. (Helicigona) lapicida</i>	29
<i>H. (Liburnica) setosa</i>	29*
<i>H. (Dinarica) pouzolzi</i>	29*
<i>Isognomostoma isognomostoma</i>	30*
<i>I. holosericum</i>	30*
Helicinae	
<i>Eobania vermiculata</i>	27*
<i>Helix (Helix) pomatia</i>	27
<i>H. (H.) dormitoris</i>	27*

Tabelle 4 (Forts.)

Taxonomische Einheit	n
<i>H. (H.) lucorum trapezuntis</i>	27*
<i>H. (Cryptomphalus) aspersa</i>	27
<i>H. aperta</i>	27
<i>H. cincta</i>	27
<i>H. melanostoma</i>	27
<i>Pseudotachea litturata</i>	22
<i>Cepaea nemoralis</i>	22
<i>C. hortensis</i>	22, 30*
<i>C. silvatica</i>	25
<i>C. vindobonensis</i>	25
<i>Archelix dupotetiana</i>	26
<i>A. hieroglyphicula</i>	26
<i>A. punctata</i>	26
<i>Euparypha pisana</i>	28*
<i>Caucasotachea lenkoranea</i>	30*

Die mit* versehenen Zahlen sind neu ermittelt worden, die übrigen übernahm ich von PERROT (1938) und MAKINO (1951).

Gattungen weisen in den meisten Fällen homogene Chromosomenzahlen auf: *Helix*, *Trichia*, *Helicella* (Tab. 4), *Ena* (Tab. 1), *Agriolimax* (4 Species mit 30 Chromosomen; BEESON, 1960). Wo verschiedene Zahlen innerhalb einer Gattung auftreten, ist diese meistens in Untergattungen unterteilt: *Helicigona* (Tab. 4), *Arion* (5 Untergattungen mit 4 verschiedenen Chromosomenzahlen; BEESON, 1960).

Arten der selben Gattung (oder Untergattung) haben bei Stylommatophoren gleiche Chromosomenzahlen. Ausnahmen sind die 3 von BEESON (1960) untersuchten *Milax*-Arten (33, 33 oder 34, 34 Chromosomen) und die Arten der Gattung *Cepaea*, von denen noch die Rede sein wird.

Die einzige Familie, bei der sich mehrere Arten mit Hilfe der Chromosomenzahl eindeutig unterscheiden lassen, ist die Familie Viviparidae (Prosobranchia). Ihre 3 europäischen Vertreter weisen die haploiden Chromosomenzahlen 7, 9 und 10 auf (RAINER, 1963). Interessant sind solche Unterscheidungsmöglichkeiten vor allem dann,

wenn morphologische Merkmale nicht oder schwer verwendbar sind. Das trifft aber hier nicht zu.

Zusammenfassend lässt sich sagen, dass die Befunde über Chromosomenzahlen die heutige Klassifizierung der Stylommatophora unterstützen.

BESPRECHUNG DER HELICIDAE

Betrachten wir nun die Helicidae etwas genauer, und vergleichen wir deren Chromosomenverhältnisse unter Beachtung der Systematik.

Helicellinae

Candidula unifasciata (n=27) unterscheidet sich von den übrigen Helicellen (n=26) in Bezug auf Chromosomenzahl und besondere Merkmale des Chromosomensatzes. *Candidula* hat 1 grosses und 4 besonders kleine Bivalente, während die *Helicella*-Arten 3 grosse, aber keine kleinen Elemente enthalten. Die heutige Einteilung in verschiedene Gattungen ist also auch vom zytologischen Standpunkt aus gerechtfertigt. Die *Helicella*-Arten lassen sich auf Grund des Chromosomenbildes nicht unterscheiden, ebensowenig wie die beiden Arten der Gattung *Monacha* (n=23).

Die von PERROT (1938: Fig. 11b) untersuchte *Cochlicella acuta* gleicht in Zahl (n=23) und Aussehen der Chromosomen den beiden *Monacha*-Arten: Alle Platten enthalten 3-4 besonders grosse und 4-5 sehr kleine Elemente. Die Unterteilung der Unterfamilie in die Tribus Helicelleae und Monacheae ist durch die verschiedenen Chromosomenzahlen gerechtfertigt. Nach dem Karyotyp beurteilt müsste *Cochlicella acuta* aber dem Tribus Monacheae zugeteilt werden.

Hygromiinae

Bei den 3 von mir untersuchten *Trichia*-Arten (n=23) konnte ich keine Unterschiede im Chromosomenbestand finden. Der Vertreter der Gattung *Euomphalia* (n=23) hingegen unter-

scheidet sich trotz gleicher Chromosomenzahl von *Trichia* dadurch, dass nur 2 statt 3-4 Elemente des Satzes grösser sind als die übrigen. *Hygromia cinc-tella* (PERROT, 1938) weicht mit n=21 von den übrigen Hygromiinae (n=23 und 24) auffallend ab. Nach PERROT sind 2 der 21 Chromosomen grösser als die übrigen. Es wäre denkbar, dass diese grossen Elemente aus mehreren kleinen Chromosomen zusammengesetzt wären, wie das BALTZER (1913) für *Cepaea hortensis* annahm (s.S.368), sodass der Chromosomensatz den übrigen der Familie entspräche.

Helicodontinae

Da die Unterfamilie bei uns nur eine Art enthält, lässt sich nichts über die Helicodontinae als Ganzes sagen. Ich vergleiche *Helicodonta* (n=27) mit den anderen Heliciden gleicher Chromosomenzahl. *Helicodonta* hat mehr Bivalente mit einem Chiasma und die Grössenunterschiede innerhalb eines Chromosomensatzes sind kleiner als bei *Helix* und *Eobania*. Ausserdem fällt bei diesen beiden Gattungen meistens ein Chromosom durch besondere Grösse auf, bei *Helicodonta* nicht.

Helicigoninae

Von dieser Unterfamilie untersuchte ich 2 Gattungen, wobei von der Gattung *Helicigona* 5 Untergattungen geprüft wurden. Diese Unterteilung spiegelt sich in den Chromosomenverhältnissen. Die Vertreter der Gattung *Helicigona* zeigen 2 verschiedene Chromosomenzahlen. Auch die Chromosomensätze gleicher Zahl lassen sich unterscheiden:

H. (Arianta) arbustorum (n=30) enthält ein grosses Element und viele Bivalente mit 2 Chiasmata.

H. (Chilostoma) achates (n=30) hat kein merklich grösseres Chromosom und nur etwa halb so viele Bivalente mit 2 Chiasmata.

H. (Helicigona) lapicida (n=29) weist wenig Bivalente mit 2 Chiasmata auf; der Grössenunterschied zwischen dem grössten und kleinsten Chromosom ist

beträchtlich.

H. (Dinarica) pouzolzi (n=29) enthält zwar ungefähr gleich viele Ringbivalente wie *H. lapicida*, aber etwa doppelt so viele kreuzförmige Bivalente. Der Grössenunterschied ist geringer.

H. (Liburnica) setosa (n=29) hat doppelt so viele Ringbivalente wie die beiden vorigen Arten, ausserdem ist eines davon besonders gross.

Die Spermatocyten von *Isognomostoma holosericum* (n=30) enthalten ein grosses kreuzförmiges Bivalent und nur 1-2 kleine Ringbivalente und unterscheiden sich dadurch deutlich von den *Helicigona*-Kernen mit 30 Bivalenten.

Helicinae

Die zytologisch untersuchten Arten dieser Unterfamilie gehören alle demselben Tribus, Heliceae, an. Trotzdem sind sie in Bezug auf die Chromosomenzahl weniger einheitlich als die Vertreter der anderen Unterfamilien.

Wir treffen 6 verschiedene Chromosomenzahlen zwischen 22 und 30 an. Homogenität zeigt die Gruppe *Helix-Eobania* mit 27 Chromosomen. Soweit ich die Chromosomen selbst untersuchte, konnte ich zwischen den einzelnen *Helix*-Arten immerhin geringe Unterschiede finden: *H. aspersa* und *H. dormitoris* unterscheiden sich durch ein grosses kreuzförmiges Bivalent von *H. lucorum*. *H. dormitoris* ihrerseits ist an der grossen Zahl von Ringbivalenten zu erkennen. Ich teile deshalb PERROT's (1938: 556) Meinung nicht, der die Unterteilung in Untergattungen als "reichlich künstlich" betrachtete. Die 2. einheitliche Gruppe umfasst 3 *Archelix*-Arten mit 26 Chromosomen (PERROT, 1938). Die 3. Gruppe wird vom heterogenen *Pseudotachea-Cepaea*-Komplex gebildet.

Eine bereits erwähnte Besonderheit zeigt die Gattung *Cepaea*, von der 2 Species 22 Bivalente und 2 andere 25 Bivalente enthalten. Bei *C. nemoralis* und *C. hortensis* ist eines der 22 Bivalente viel grösser als die übrigen (vergl. PERROT, 1938; BALTZER, 1913; KLEINERT, 1909). BALTZER

nahm an, dass das grosse Chromosom einigen kleinen entspräche. Diese These stützte er durch 2 Beobachtungen:

1. Ein durch Selbstbefruchtung entstandenes Exemplar von *C. hortensis* enthielt neben typischen Kernen auch solche mit 24 bis 26 Elementen, aber ohne grosse Tetrade.

2. Ein Exemplar von *C. nemoralis* aus Bern enthielt nur Spermatocyten mit 28 bis 29 Bivalenten, wobei alle etwa die gleiche Grösse zeigten.

Zu diesen Beobachtungen kann ich eine weitere hinzufügen:

Bei den von mir untersuchten *Cepaea hortensis*-Tieren vom Klausenpass betrug die Chromosomenzahl ausnahmslos 30. Kein Chromosom war besonders gross. Es handelt sich offenbar um eine Lokalform und es wäre interessant zu untersuchen, obneben den von mir gefundenen Tieren auch solche mit 22 Chromosomen vorkommen, ferner wie weit die Lokalform verbreitet ist und ob sich die beiden Formen von *C. hortensis* kreuzen lassen. Die gleichen Fragen gelten für den Berner Fundplatz von *C. nemoralis* (n=28-29). Der von BALTZER postulierte Zerfall des grossen Chromosoms verleitet zur Annahme, dass die beiden *Cepaea*-Arten mit 25 Chromosomen auf diese Weise entstanden wären. Dagegen spricht der Befund an *C. sylvatica*, in deren Karyotyp eines von 25 Chromosomen wesentlich grösser ist als die übrigen. Bei *C. vindobonensis* hingegen sind alle 25 Chromosomen regelmässig abgestuft. Da verschiedene Chromosomenzahlen innerhalb derselben Gattung bei *Stylommatophoren* von grösster Seltenheit sind, schlage ich vor, die Gattung *Cepaea* in 2 Untergattungen aufzuteilen. Zur einen gehören *C. nemoralis* und *C. hortensis* mit 22, zur anderen *C. vindobonensis* und *C. sylvatica* mit 25 Chromosomen.

Von den beiden letzten Gattungen, *Euparypha* (n=28) und *Caucasotachea* (n=30), standen mir nur je eine Art zur Verfügung, sodass eine Aussage über Zusammenhänge zytologischer Art unmöglich ist.

Die Tiere aus Jugoslawien, Persien

und der Türkei zeigten im Vergleich mit verwandten europäischen Arten keine chromosomalen Besonderheiten.

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ABSTRACT

CYTOLOGICAL STUDIES OF GASTROPODS (STYLOMMATOPHORA)

M. Rainer

The chromosome numbers of 33 stylommatophoran snails and 2 prosobranch snails have been determined and those of 8 helicid snails, 4 clausiliids and 1 prosobranch snail were confirmed.

The newly determined numbers (n) are: *Pomatias elegans*, *P. costulatus*, 13; *Cochlicopa lubrica*, 26; *Abida secale*, *Chondrina avenacea*, *C. similis*, 30; *Ena montana*, *E. obscura*, *Zebrina dendrita*, 24; *Succinella oblonga*, 11 and 12; *Discus rotundatus*, 29; *Vitrea diaphana*, 20; *Laciniaria plicata*, 24; *Delima itala*, *Papillifera papillaris*, 30; *Graciliaria strobili*, 24; *Candidula unifasciata*, 27; *Helicella itala*, *H. cretica*, 26; *Monacha cantiana*, 23; *Trichia unidentata*, *T. sericea*, *T. villosa*, *Euomphalia arpatschaina*, 23; *Helicodonta obvoluta*, 27; *Helicigona achates*, 30; *H. pouzolzi*, *H. setosa*, 29; *Isognomostoma isognomostoma*, *I. holosericum*, 30; *Euparypha pisana*, 28; *Helix dormitoris*, *H. lucorum*, 27; *Eobania vermiculata*, 27; *Caucasotachea lenkoranea*, 30.

Species from Yugoslavia, Persia and Turkey did not show any chromosomal differences from related European species.

The relation of chromosome numbers to systematics was examined: within some families (Chondrinidae, Enidae, Bradybaenidae) chromosome numbers were uniform while in others, especially in the Helicidae they varied considerably. Within the helioid subfamilies they may either be equal, or differ by only 1-3, or show larger differences, as in the Helicinae and Helicellinae. Within a single genus the chromosome number is mostly constant; when there is divergence, the genus usually is also divided into subgenera.

The Helicidae were examined with special attention to size of chromosomes and distribution of chiasmata. The division of the subfamily Helicellinae into the tribes Helicelleae and Monacheae is supported by karyotype differences, but, by these criteria, *Cochlicella acuta* should be assigned to the Monacheae. In species of genera or subgenera that have equal chromosome numbers, the chromosome sets can usually be distinguished (Helicigoninae) by the above cytological features. In the Helicinae, *Cepaea* forms an exception in that 2 chromosome numbers, 22 and 25, occur within the same genus. BALTZER (1913) suggested that the conspicuously large chromosome observed in the species with $n=22$ (*C. nemoralis* and *C. hortensis*) might have fragmented into several smaller chromosomes in *C. vindobonensis* ($n=25$). His theory might apply to that species, but not to *C. sylvatica* ($n=25$) where I also found a very large chromosome. On a cytological basis, a division of the genus *Cepaea* into 2 subgenera would seem appropriate. In addition a local form of *C. hortensis*, with 30 instead of 22 chromosomes, was also discovered.

In a population of *Succinella oblonga*, 2 different chromosome numbers were found ($n=11$ and 12).

In general, the cytological findings correlate well with the modern classification of the Stylommatophora.

RESUMEN

ESTUDIOS CITOLÓGICOS DE GASTROPODOS (STYLOMMATOPHORA)

M. Rainer

Se ha determinado el número cromosómico de 33 gastrópodos y 2 prosobranquios, y confirmado el de otros 8 helicidos, 4 clausilidos y un prosobranquio.

Los números determinados ahora son: *Pomatias elegans*, *P. costulatus* 13; *Cochlicopa lubrica* 26; *Abida secale*, *Chondrina avenacea*, *C. similis* 30; *Ena montana*, *E. obscura*, *Zebrina dendrita* 24; *Succinella oblonga* 11 y 12; *Discus rotundatus* 29; *Vitrea diaphana* 20; *Laciniaria plicata* 24; *Delima itala*, *Papillifera papillaris* 30; *Graciliara strobili* 24; *Candidula unifasciata* 27; *Helicella itala*, *H. cretica* 26; *Monacha cantiana* 23; *Trichia unidentata*, *T. sericea*, *T. villosa*, *Euomphalia arpatschiana* 23; *Helicodonta obvolvata* 27; *Helicigona achates* 30; *H. pouzolzi*, *H. setosa* 29; *Isognomostoma isognomostoma*, *I. holocericum* 30; *Euparypha pisana* 28; *Helix dormitoris*, *H. lucorum* 27; *Eubania vermiculata* 27; *Caucasothacha lenkoranea* 30.

Especies de Yugoslavia, Turquía y Persia, no mostraron diferencia cromosómica con otras europeas relacionadas.

La relación entre el número cromosómico y la sistemática fué examinada dentro de algunas familias (Chondrinidae, Enidae, Bradybaenidae) en las que los números eran uniformes, mientras que en otros, especialmente los Helicidae, variaron considerablemente. Entre las subfamilias de Helicidae el número puede ser igual o diferir sólo por 1-3, y también mostrar grandes diferencias como en los Helicinae y Helicellinae.

En un mismo género el número tiene mayor constancia, y cuando existe divergencia, esta generalmente se muestra en la división subgenérica.

Se ha prestado especial atención al tamaño de los cromosomas y distribución de los quiasmas (torsión espiral) en los Helicidae. La división de la subfamilia Helicellinae en las tribus Helicellae y Monachae se mantiene por diferencias del krotipo, pero bajo este criterio *Cochlicella acuta* debe ser asignada a los Monachae. En especies de géneros o subgéneros que tienen igual número de cromosomas, el juego puede distinguirse generalmente (Helicigoninae) por los aspectos citológicos recién mencionados. En los Helicinae, *Cepaea* es una excepción, porque los números 22 y 25 ocurren dentro de un mismo género. Baltzer (1913) sugirió que los grandes cromosomas observados en las especies con $n=22$ (*C. nemoralis* y *C. hortensis*), pueden tener varios cromosomas fragmentados en *C. vindobonensis* ($n=25$); su teoría puede aplicarse a esas especies, pero no a *C. sylvatica* ($n=25$) en la cual he encontrado también un cromosoma muy largo. Una división del género *Cepaea* en dos subgéneros, basados en la citología, parece ser apropiada. Adicionalmente, una forma local de *C. hortensis*, con 30 en lugar de 25 cromosomas, fue descubierta. En una población de *Succinella oblonga*, se observaron números diferentes ($n=11$ y 12).

En general, los resultados citológicos correlacionan bien con la clasificación moderna de los Stylommatophora.

АБСТРАКТ

ЦИТОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ GASTROPODA (STYLOMMATOPHORA)

М. Рейнер

Определялось число хромосом у 33 видов Stylommatophora и двух видов Prosobranchia; у 8 видов Хелицид, у 4 видов Клаузилиид и у 1 вида Prosobranchia. число хромосом было подтверждено. Вновь определенное количество хромосом (n) у различных видов оказалось следующим: *Pomatias elegans*, *P. costulatus* 13; *Cochlicopa lubrica* 26; *Abida secale*, *Chondrina avenacea*, *C. similis* 30; *Ena montana*, *E. obscura*, *Zebrina dendrita* 24; *Succinella oblonga* 11 и 12; *Discus rotundatus* 29; *Vitrea diaphana* 20; *Lacinaria plicata* 24; *Delima itala*, *Papillifera papillaris* 30; *Graciliaria strobili* 24; *Candidula unifasciata* 27; *Helicella itala*, *H. cretica* 26; *Monaca cantiana* 23; *Trichia unidentata*, *T. sericea*, *T. villosa*, *Euomphalis arpatschaina* 23; *Helicodonta obvolvata* 27; *Helicigona achates* 30; *H. pouzolzi*, *H. setosa* 29; *Isognomostoma isognomostoma*, *I. holoserivicum* 30; *Euparypha pisana* 28; *Helix dormitoris*, *H. lucorum* 27; *Eobania vermiculata* 27; *Caucasotachea lenkoranea* 30.

Виды из Югославии, Персии и Турции не имели отличий в количестве хромосом от родственных им видов.

Исследовалось соотношение количества хромосом с систематическим положением моллюсков. В некоторых семействах (Chondrinidae, Enidae, Bradybaenidae) число хромосом было одинаково, в то время, как у других, особенно у Helicidae, оно значительно изменялось. В подсемействах семейства Helicidae оно или одинаково или различается на 1-3, или же - больше (как у Helicinae и Helicellinae). Внутри одного рода число хромосом большей частью постоянно; если имеются различия, то обычно род распадается на подрода. Сем. Helicinidae было исследовано,

уделяя особое внимание размерам хромосом и распределению хиазма. Деление подсемейства *Helicellinae* на трибы *Helicellae* и *Monacheae* подтверждается различиями кариотипов; в соответствии с этими критериями *Cochlicella acuta* следует отнести к *Monachene*. У видов родов или подродов, имеющих одинаковое число хромосом, наборы их обычно могут различаться (*Helicigoninae*) по указанным выше цитологическим признакам. У *Helicinae Cepaea* является исключением, поскольку у одного и того же рода встречаются 2 набора хромосом ($n=22$ и 25).

Бальтцер (1913) предполагал, что более крупные хромосомы, которые наблюдаются у видов с 22 хромосомами (*C. nemoralis* и *C. hortensis*) могут разделиться на несколько более мелких (как у *C. vindobonensis*, где $n=25$). Эта теория может быть применена к указанным видам, но не к *C. sylvatica* ($n=25$), у которой хромосомы также очень крупные. Судя по цитологическим данным, род *Cepaea* можно разделить на 2 подрода. Кроме того, была обнаружена локальная форма *C. hortensis* с 30 хромосомами, вместо 22.

В популяции *Succinella oblonga* было обнаружено 2 набора хромосом ($n=11$ и 12).

В общем, найденные цитологические данные хорошо коррелируются с современной классификацией *Stylommatophora*.

THE HISTOLOGY OF THE ALIMENTARY SYSTEM OF
MARISA CORNUARIETIS
(MESOGASTROPODA: AMPULLARIIDAE)¹

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ABSTRACT

Little information exists on the histology of the Ampullariidae and the Proso-branchia in general. This study deals with a detailed histological investigation of the alimentary system of the ampullariid snail *Marisa cornuarietis* (L.). It was made in order to provide a sound basis for subsequent histochemical investigation, indispensable for understanding the mode of functioning of that system.

Various cuticularized, ciliated and richly glandular regions are differentiated in the lining epithelium of the buccal cavity. The peristome is rich in unicellular subepithelial glands. The buccal vestibule has a cuticularized lining, and is followed by the mandibular region of the buccal cavity, of which the lining secretes the 2 jaws. The jaw material is differentiated into 2 superimposed distinct layers, an upper lamellated cuticular layer and a lower one of upright columns of a different chemical nature. The lining of the odontophoral part of the buccal cavity presents certain specialized regions with distinct characteristics such as the subradular organ, the 2 dorsal buccal ridges which enclose the dorsal food channel, and the postradular ledge, besides the sub- and supra-radular epithelia. A special type of mucus-secreting cell predominates in the epithelia of the subradular organ, the outer regions of the dorsal buccal ridges and the postradular ledge. The same cell type also abounds in certain regions of the lining epithelia of the oesophageal pouches.

The oesophagus is histologically differentiated into a pro-, mid- and post-oesophagus. The lining epithelia of the 3 regions are largely composed of ciliated columnar cells. Those of the mid-oesophagus, in particular, show a high secretory activity.

The stomach comprises a proximal U-shaped gizzard and a distal tubular style sac. The gizzard is largely lined with a columnar non-glandular cuticularized mucosa. Its most distal part has a ciliated lining and acts as a sorting region. Two blind gastric pouches open in this region, and a prominent directing lappet projects from its floor and serves to direct the excretory material issuing from the digestive gland. The gastric pouches as well as the style sac are lined with columnar cells of various length, which are ciliated and at the same time show a high secretory activity. Their secretion enwraps the faecal rod, which is moved spirally distalwards along the style sac and intestine. The lining mucosa of the caecum is histologically closely similar to that of the pro-intestine.

A pro-, mid- and post-intestine can be distinguished, each with its charac-

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teristic inner folds, and its characteristic types of secretory as well as ciliated and/or absorptive cells. Of these cell types, 4 are described in the mid-intestine and 5 in the post-intestine. Absorptive cells with distinct brush borders exist in different parts of the intestine and rectum, which fact indicates that absorption does take place in these organs.

The salivary glands are of the branched tubular type. Their tubules possibly have 2 types of secretory cells showing different phases of secretory activity. The salivary gland ducts are lined with both secretory and ciliated cells.

The 2 lobes of the digestive gland consist of a complicated system of branching tubules with 2 main cell types, the digestive cells, which contain secretory and absorbed bodies of various nature, and the excretory cells, which accumulate in their bodies certain excretory substances in the form of brown concretions.

INTRODUCTION

Since the discovery of its potentialities as a biological control agent against some snail transmitters of schistosomiasis, *Marisa cornuarietis* (L.) has attracted a great deal of attention. For the last few years, the present writers have been carrying out a series of investigations on this ampullariid snail with the aim of accumulating fundamental basic information on its morphology, histology and mode of functioning of its different organ systems. The authors also demonstrated, in a series of laboratory experiments, that this snail deliberately attacks and preys upon some snail intermediate hosts of the trematodes *Fasciola* and *Schistosoma*, such as *Lymnaea caillaudi* Bourguignat, *Bulinus truncatus* (Audouin) and *Biomphalaria alexandrina* (Ehrenberg) (Demian & Lutfy, 1964, 1965 a, b).

The fact that *Marsia cornuarietis*, unlike some other ampullariids, is capable of consuming animal food besides its vegetable diet, made a thorough investigation of the mode of functioning of its alimentary system all the more interesting. However, it was soon realized that a knowledge of the detailed histology of this system is indispensable for working out its histochemistry, which would, in turn, help in understanding the mode of its functioning.

Most of the previous studies on the functioning of the digestive system in the Gastropoda were, at best, incomplete,

even when based on some histochemical information, evidently because their histological bases were not detailed enough. For this reason, the present work is intended to deal with the histological structure of the alimentary system of *Marsia cornuarietis* in as much detail as possible. The writers are confining themselves to a descriptive account of the different histological elements which go into the formation of this system and have, in the most part, refrained from giving interpretations of the functioning. The work is meant to furnish a basis for detailed histochemical studies now in progress. The histology of the radula and the radular sac, as well as that of the odontophoral cartilages, have been given in earlier publications (Lutfy & Demian, 1964a, b). The work of Demian (1964) furnishes the gross anatomical basis for this series of histological investigations.

The present work was carried out in the Department of Zoology, Faculty of Science, Ain Shams University, Cairo.

HISTORICAL

Comparatively little work has been done on the histology of the Gastropoda in general. Most of the previous histological studies made on this group of molluscs were concerned with pulmonate snails, particularly those which act as intermediate hosts for some trematode parasites of man and domestic animals,

and some widely-distributed familiar forms such as *Helix*. An almost complete review of the studies made on the histology of the alimentary system of such pulmonates was included in the work of Carriker & Bilstad (1946) on *Lymnaea stagnalis appressa* Say. Of the most important investigations which followed the latter work are those of Marcuzzi (1950) and of Pan (1958) on *Australorbis* (= *Biomphalaria*) *glabratus* (Say), and that of Rigby (1963) on *Oxychilus cellarius* (Müller).

The histology of the Prosobranchia received much less attention. In their excellent book, Fretter & Graham (1962) summarized most of the histological information available for this group in an illuminating comparative way.

As far as the Ampullariidae are concerned, the paucity of the literature in this field is quite evident. Some brief accounts of the histology of the alimentary system of various ampullariids were included in the works of Prashad (1925) on *Pila globosa* (Swainson), of Meenakshi (1954) on *P. virens* (Lamarck) and of Michelson (1955) on *Ceratodes* (*Marisa*) *cornuarietis*. Perhaps the most detailed study so far made on the functioning of the digestive system in the Ampullariidae is that of Andrews (1965) which was mainly concerned with *Pomacea canaliculata* (d'Orbigny). Her histological description, however, did not cover some important organs and tissues of the system and was rather brief for some others.

Thus, the general lack of detailed histological accounts in the previous literature precludes detailed comparisons between the structures which are encountered in *Marisa* and corresponding ones in other prosobranchs.

MATERIAL AND TECHNIQUES

Specimens used in the present study were reared in the laboratory from a stock of Puerto Rican origin. Live snails were fixed directly without previous anaesthetisation as described in

a previous publication (Lutfy & Demain, 1964a). Their alimentary systems were then rapidly dissected out under the fixative and transferred to a fresh amount of the fixing fluid. Zenker's sublimate-bichromate mixture with formalin gave the best results with cytological details, and chrome-osmium mixtures such as Flemming's strong fluid and Champey's fluid were most helpful in demonstrating nuclear patterns. Bouin's picro-formol fluid and Duboscq-Brazil's fluid were used as general fixatives, and alcohol formol and Carnoy's fluid were employed for special purposes such as subsequent PAS staining.

The material was dehydrated and cleared in dioxan and embedded in paraffin wax. Serial sections were cut to the thickness of 4-7 μ .

The stains used were Delafield's haematoxylin counterstained with eosin or phloxine, Heidenhain's iron haematoxylin counterstained with orange G or Van Gieson's mixture, and Mallory's Triple stain. Among the various other stains used for special purposes are Mayer's mucicarmine, toluidine blue and periodic acid Schiff (PAS).

Drawings were made with the aid of a camera lucida. No absolute measurements are given in the text as reference can be made to the scales given with the drawings and photomicrographs. It should be noted, however, that all figured sections were taken from snails of 2-2.2 cm shell diameter which provided better material for sectioning and study than average sized adults of about 3.2 cm shell diameter.

OBSERVATIONS

The histology of the different organs in the alimentary system are dealt with here in the following order. Only in the buccal mass, which has a very complicated structure, are the different component tissues and regions described under separate headings:

Buccal mass

A. Lining epithelium

KEY TO LETTERING ON FIGURES

(all illustrations are of *Marisa cornuarietis*)

a. dgl.	anterior lobe of digestive gland	fr.	furrow on dorsal buccal ridge
a. dgl. d.	anterior digestive gland duct	gb. c.	globulocyte
ac. bd.	acidophilic bodies in digestive cells	gl. c.	gland cell (of suspected nature)
ac. gl.	special acidophilic gland cells on post-intestinal raphe	gn. bd.	"green bodies" in digestive cells
ac. gr. c.	various acidophilic granular cells	gr. c.	granulocyte
ac. nl.	acidophilic nucleolus	gr. gl.	special gland cells with refractile granules in post-intestinal mucosa
an. gl.	anal gland	gs. p.	gastric pouches
bc. l.	lateral recess of buccal cavity	gz.	gizzard
bc. m.	various buccal muscles	h. vs.	hepatic vestibule
bc. md.	lumen of mandibular region of buccal mass	h. vs. o.	opening of hepatic vestibule into gizzard
bc. od.	lumen of odontophoral region of buccal mass	hy. c.	hyalocyte
bc. vs.	lumen of buccal vestibule	in. col.	intercolumnar binding material
br. b.	brush border	int. gr.	intestinal groove
bs. c.	basophil	itg.	covering integument of visceral mass
bs. nl.	basophilic nucleolus	len.	blood lacuna
cae.	caecum	l. m. f.	longitudinal muscle fibres
chb. bd.	chromophobic bodies in digestive cells	m.	mouth opening
chb. s.	chromophobic spherules	mc. c.	mucus cells of goblet type
cil.	cilia	mc. gl.	special type of unicellular mucus glands in the epithelia of subradular organ, outer region of buccal ridge and outer wall of oesophageal pouch
cil. fr.	ciliated furrows	mc. glc.	special mucus cells characteristic of mid-intestinal mucosa
cil. gr.	ciliated groove	mc. sep.	subepithelial mucus glands
cl. c.	columnar epithelial cells	mc. stg.	mucus string on major typhlosole of style sac
c. m. f.	circular muscle fibres	md. gt.	mid-intestinal gutter
cn. t. d.	dense connective tissue	md. fl.	mid-intestinal folds
cn. t. l.	loose or vascular connective tissue	md. int.	mid-intestine
col.	columns of inner layer of jaw	m. f.	various muscle fibres
col. j.	columnar layer of jaw	mfl.	myofibrils
con.	crystalline concretions in connective tissue	mj. ty.	major typhlosole
ct. f.	connective tissue fibres	m. n.	mitotic nucleus
cut.	cuticle	mn. ty.	minor typhlosole
cut. j.	cuticular layer of jaw	mt. ep.	covering epithelium of mantle
cut. lm.	newly secreted cuticular lamella	mvi.	microvilli
d. cae. fl.	dorsal caecal folds	n.	nuclei
dg. c.	digestive cells	n. fil.	nuclear filaments
dgl. tb.	digestive gland tubules	nk. sep.	neck of subepithelial mucus gland
d. lp.	directing lappet	nr.	nerve
drp.	secretory droplets	oes. o.	opening of post-oesophagus into gizzard
ep. j.	jaw-secreting epithelium	p. dgl.	posterior lobe of digestive gland
ex. bd.	excretory bodies in excretory cells of digestive gland	p. dgl. d.	posterior digestive gland ducts
ex. c.	excretory cells	pg.	pigment granules
ex. s.	excretory spherules	pl. gnd.	pallial gonoduct
fb. c.	fibrocyte	pr. gr.	pro-intestinal groove
fd. ch.	dorsal food channel	pr. int.	pro-intestine
fl. k.	floor of posterior renal chamber	pr. oes.	pro-oesophagus

pr. ty.	pro-intestinal typhlosole	sl. sec.	secretory spherules in salivary gland cells
pt. int.	post-intestine	sn. cn.	supranuclear cone
pt. oes.	post-oesophagus	sph. 1	gizzard sphincter
pt. rph.	post-intestinal raphe	sph. 2	proximal sphincter of style sac
rect.	rectum	sph. 3	distal sphincter of style sac
rect. fl.	rectal folds	sr. rd. ep.	supraradular epithelium
rd. al.	alary process of radula	srd. o.	subradular organ
rd. al. ^{1,2}	inner and outer layers of alary process of radula	stl. s.	style sac
rd. cl.	radular collostyle	stl. sec.	acidophilic secretion in lumen of style sac
rdg. i.	inner region of dorsal buccal ridge	str. l.	layer of vertical striations underlying cuticle
rdg. o.	outer region of dorsal buccal ridge	trn. j.	transitional zone of jaw
rd. mb.	radular membrane	vc.	vacuoles
rd. th.	radular teeth	v. cae. fl.	ventral caecal folds
sb. rd. ep.	subradular epithelium	vn. gs. r.	ventral gastric ridge
sep.	granular sarcoplasm	v. spt.	vertical septum
scm.	sarcolemma		
sl. gl.	salivary gland		
sl. gl. d.	salivary gland duct		

B. Subepithelial glands

C. Connective tissue

D. Muscles

Oesophagus and oesophageal pouches

Stomach

Intestine

Rectum and anal gland

Salivary gland

Digestive gland

BUCCAL MASS

A. Lining Epithelium

The mouth opening is surrounded by a continuous circular lip or peristome. It leads into the buccal cavity which is differentiated into 3 more or less distinct regions: the buccal vestibule or oral tube, a mandibular region and an odontophoral region (Demian, 1964). In all previous studies made on the histology of ampullariid snails, the lining epithelium of the corresponding regions of the buccal cavity was either neglected altogether or very inadequately described.

1. Peristome

The peristome (Fig. 28) is covered by a simple epithelium which is thrown into a variable number of folds that point mainly towards the centre of the

oral opening (m.). It is composed of tall narrow columnar cells (Fig. 1, cl. c.) and secretes a very thin cuticular covering (cut.) which is perforated at the points where epithelial and subepithelial mucus glands open on to the surface. The cells rest on a distinct basement membrane and are markedly taller at the tips of the folds than they are at their bases. They have elongated oval nuclei (n.) which are poor in chromatin; each has 1 or 2 distinct nucleoli. The nuclei are located in the basal halves of the cells and are topped each with a differentiated portion of cytoplasm assuming the form of an elongated cone (sn. cn.). This structure stains darker than the rest of the cytoplasm which is generally acidophilic in reaction and contains, in the distal part of each cell, numerous highly acidophilic granules and rodlets. Below the nucleus, the cytoplasm shows numerous fine fibrillae.

Mucus-secreting cells (mc. c.) of various forms abound in this epithelium. The majority are goblet-like, with swollen middle portions and attenuated basal regions which harbour the nuclei. Some are nearly as narrow as the normal epithelial cells, while others show a constriction half way their length. All these cells react positively towards

specific mucin stains. Their contents show in the form of the characteristic network that acquires a dark blue colour in haematoxylin-eosin (H - E) preparations, a bright red colour after Mayer's mucicarmine stain and an intense violet colour after toluidine blue. Their nuclei may be oval or elliptical, and are rich in coarse chromatin granules.

2. Buccal vestibule

The folds marked on the peristome are continued backwards along the buccal vestibule (Fig. 30, bc.vs.) referred to as oral tube by Andrews (1965) in *Pomacea canaliculata*. That author does not consider it part of the buccal cavity, nor do Fretter & Graham (1962) in various other prosobranchs. The greater part of the vestibule is lined by a chitinogenic epithelium which is devoid of mucus-secreting elements. It is made up of narrow columnar cells (Fig. 2, cl.c.) of much the same structure as those covering the peristome. However, they have more pronouncedly acidophilic apices and secrete a thicker layer of cuticle (cut.) which gains gradually in thickness posteriorly. This layer assumes a red colour in H - E preparations and appears formed of closely packed, thin, stratified lamellae. It is especially more developed over the summits of the epithelial folds which therefore appear sharply pointed.

As one moves backwards along the buccal vestibule, the lumen gradually assumes a triangular cross sectional outline. The apex of the triangle is directed upwards, and its sides and base are interrupted by the epithelial foldings. Towards the posterior end of the vestibule, however, the lumen takes the form of an inverted T. The epithelium on either side of the vertical limb of the T flattens out gradually, while its cuticular covering thickens and becomes continuous with that of the 2 jaws. The epithelial lining of the transverse limb of the T, on the other hand, keeps its folded nature, although the folds become gradually simpler and less numerous

posteriorly. The largest and most conspicuous of these folds are 2, lying one on either side of the median line.

Non-epithelial cellular elements of different forms are always seen wedged among the bases of the epithelial cells in both the peristomal and vestibular regions. The majority possess a hyaline cytoplasm and a large oval eccentric nucleus which is poor in chromatin. Other cells present a smaller ovoid eccentric nucleus, with coarser chromatin granules, and more abundant cytoplasm that contains distinctly acidophilic granules and rodlets. Both types have their parallels among the connective tissue cells (p 389), and are therefore assumed to be wandering elements from the subepithelial connective tissue.

3. Mandibular region of buccal cavity and jaws

In the mandibular region of the buccal cavity, the lumen still appears in the form of an inverted T in cross section. However, the vertical limb of the T is relatively wider, biconvex and bounded on both sides by the 2 jaws. The transverse limb of the T extends laterally below the lower edges of the jaws which project considerably into the lumen (Figs. 33, 34). The epithelial lining of the latter part of the cavity is composed of normal chitinogenic cells of the same nature as those lining the buccal vestibule, and is similarly thrown into a number of sharply pointed cuticularized folds. A similar epithelium also lines the narrow mid-dorsal strip of the lumen which is embraced between the upper edges of the 2 jaws. This epithelium always forms 3 downwardly projecting folds which are covered by very thick cuticle.

The rest of the epithelial lining of this mandibular region is responsible for the secretion of the jaws (Figs. 31, 33, 34, ep.j.). Its cells are tall columnar (Fig. 3A, cl.c.) and have oval or elliptical nuclei, which are generally rich in chromatin; each contains 1 or 2 prominent spherical nucleoli. The cytoplasm

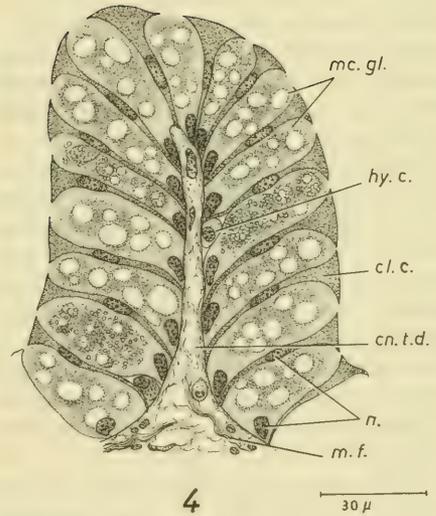
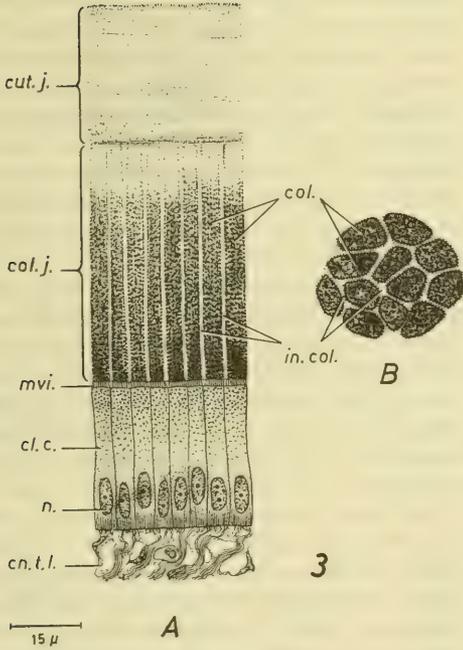
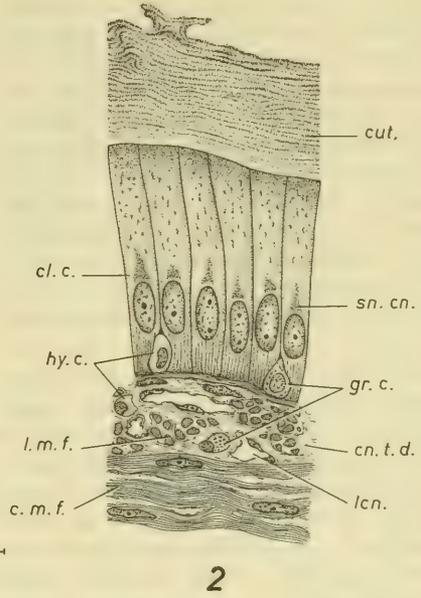
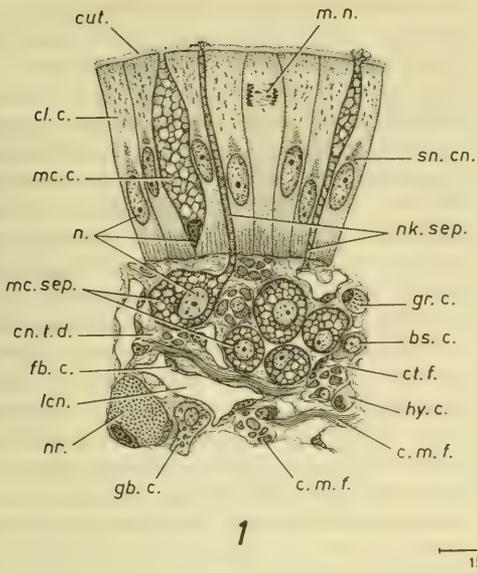


FIG. 1. Part of a cross section through the peristome. FIG. 2. Part of a cross section through the buccal vestibule. FIG. 3. (A) Part of a cross section through the jaw and its epithelium. (B) Part of a cross section passing transversely through the columnar layer of the jaw. FIG. 4. Epithelial fold of the subradular organ.

in the larger part of the cell is distinctly acidophilic and holds tiny spherical granules of higher acidophilia, especially in its apical region. The very bases of the cells exhibit numerous short and dense fibrillae and are moreover extended into fine root-like processes by which the cells are firmly affixed to the underlying basement membrane. A narrow zone of delicate microvilli (mvi.) intervenes between the apical cell membranes and the overlying jaw material.

Two distinct layers can be histologically differentiated in the substance of each jaw. The one which is found directly adjacent to the epithelium (col.j.) is apparently denser than the other layer and is composed of narrow columns which are regularly disposed in a direction parallel to that of the long axes of the underlying epithelial cells. Each column evidently corresponds to and is secreted by the individual cell lying below it. Horizontal sections of the jaw show the individual columns (Fig. 3B, col.) as quadrangular or polygonal structures, separated from each other by a thin chromophobe material (in.col.). The columns are most intensely coloured and well-defined proximally, and become gradually chromophobic towards their distal ends, which remain almost colourless with all stains used.

The second or surface layer of the jaw (cut. j.) is of a widely different nature and is apparently much more flexible than the inner layer of columns. It is mainly constituted of thin compact stratified lamellae which extend in a direction parallel to the surface of the jaw. It is continuous with, and shows the same staining reactions as, the normal cuticular layer that is secreted by the epithelium around the jaws.

The 2 layers differ widely also in their staining reactions. Their staining properties are given here in detail so as to render comparisons between the histology of the jaws and that of the radula easier, and to aid in understanding the modes of secretion of both structures.

Thus, in Mallory's triple stained preparations, the inner layer acquires an orange-red colour that turns gradually into faint yellow towards the apices of the columns. The outer cuticular layer, on the other hand, assumes a homogeneous light blue color. In ordinary H - E preparations, the apices of the columns assume a faint yellowish colour, or are almost colourless, while the major proximal parts of the columns are pink. The substance found in between the columns takes an intense red colour which similarly fades away distally. The cuticular layer acquires a bright red colour in the same preparations. After staining with iron haematoxylin - Van Gieson's, the columns appear black, while the outer layer assumes a yellowish tint. In PAS stained preparations, the columns take an orange colour, while the cuticular layer gives a PAS-positive reaction (purplish-red) as does the cuticular covering of other parts of the buccal cavity epithelium.

The 2 layers also vary in their relative thickness throughout the length of the jaw with the result that each jaw becomes roughly divisible, both morphologically and histologically, into 3 regions. The changes in the relative thickness of the 2 layers can best be studied in a series of transverse sections of the buccal mass.

The thickest most anterior 1/3 of each jaw, which appears macroscopically dark brown in colour, is predominantly made up of the layer of columns (Fig. 33, col.j.), the cuticular layer (cut.j.) being extremely thin and even disappearing completely at the free anterior edge of the jaw.

As one moves backwards along the middle 1/3 of the jaw, the cuticular layer gains more and more in thickness, while the layer of columns thins out gradually until it fades away completely (Fig. 34). Thus, the posterior 1/3 of the jaw is solely made up of one layer, viz. the cuticular layer, which again thins out gradually backwards as it approaches and joins the general cover-

ing of the buccal cavity epithelium behind the jaws.

The arrangement described above prevails along the major central region of the jaw. Along the 2 lateral edges of each jaw, however, the 2 component layers are not sharply demarkated from one another (Fig. 32, trn.j.) and instead of a lower layer of upright columns and an upper layer of transversely disposed lamellae, there appears one continuous lamellated layer of the same staining reaction as the normal cuticular layer. But the proximal zone of this layer shows numerous rows of fine infiltrating granules corresponding to the underlying epithelial cells and exhibiting staining reactions characteristic of the material of the columns in other parts of the jaw.

The above histological observations may suggest that each jaw first appears as an exaggeration of the cuticular covering of the buccal cavity epithelium. Subsequently, the epithelium underlying the anterior 2/3 of the jaw contributes a secretion of a different nature. This secretion is most probably elaborated in the form of fine threads and minute irregular spherules which are arranged in vertical rows and forced below or into the cuticular layer. The threads and spherules elaborated by each cell then coalesce into a rod or column. The individual columns become bound together by a less chromophilic material and thus the dense inner layer of the jaw is differentiated, and the original cuticular layer persists above it. This process, however, does not take place in the posterior 1/3 of the jaw which, throughout the snail's life, remains solely composed of a single layer, viz. the cuticular layer. But there are strong indications that the cuticle secreted in this region shifts slowly forwards over the columnar layer of the anterior 2/3 of the jaw, thus replacing what may be worn off the surface cuticle there.

Along the 2 lateral edges of the jaw, are found what may be considered as transitional zones, where the material

of the columns is not as actively elaborated as elsewhere. Thus, the columns are not well-defined and, instead, there appear only rows of irregular spherules penetrating through the thick cuticular layer.

Distinctive staining reactions, similar to those of the 2 layers of the jaw material, are observed in the substance of the radula as well as in the 2 layers constituting its alary processes (Lutfy & Demian, 1964a). The inner layer in each alary process (Fig. 5, rd.al.¹) appears homologous with the outer cuticular layer of the jaw, while the outer layer of the alary process (rd.al.²), although not distinctly formed of columns, corresponds to the inner layer of the jaw. This reversed disposition may be explained by assuming that 2 epithelial layers contribute to the formation of the alary processes of the radula as is the case with the radula itself. The subradular epithelium secretes the lower cuticular layer of each process, while the supraradular epithelium forms its upper layer.

4. Odontophoral region of buccal cavity

The floor of the buccal cavity in this region is raised into a large spheroidal prominence, the odontophore, which is free dorsally, laterally and ventrally near its anterior tip. Dorsally, it carries the radular ribbon as well as the 2 alary processes of the radula; a mulberry-shaped mass, the subradular organ, projects from its anterior surface.

The epithelial covering of the *subradular organ* (Fig. 35, srd.o.) is thrown into a large number of folds which are secondarily folded, and fine protrusions of the underlying connective tissue (Fig. 36, cn.t.d.) pass into them. The dominant elements in this epithelium are elongated club-shaped or sacciform unicellular glands (Fig. 4, mc.gl.) which have attenuated bases, by which they rest on the basement membrane, and which open on to the surface through fine pores. Mayer's mucicarmine and toluidine blue

tests leave no doubt that these gland cells are engaged in the secretion of mucus. This finding confirms that the organ is principally concerned with the production of a lubricating mucus secretion to facilitate the movements of the odontophore within the buccal cavity (Demian, 1964).

The nucleus (n.) in these cells is located at their very bases and thus often acquires an elongated conical shape, with the base of the cone directed distally. Its chromatin material is very dense and intensely chromophilic. The cytoplasmic contents are largely basophilic in nature and vary considerably in shape from one gland cell to the other. In some, they appear in the form of minute spherules among which lie few small colourless vacuoles. In others, the spherules are bigger and the vacuoles are larger and more numerous. Still in some other cells the contents assume a completely foamy appearance. Various intermediate conditions are also met with, which fact may indicate that the differences noticed represent various stages in the elaboration of the secretion produced by these cells.

The presence of these mucus cells in such great abundance in the epithelial covering of the subradular organ, their relatively large size and saccular form have a marked effect on the frequency and shape of the normal columnar epithelial cells (cl.c.) found between them. The latter are quite few and highly squeezed, with a narrow elongated spindle shape, but often retaining broad apices. Seldom does one come across a group of more than 2 or 3 of these epithelial cells, and the majority lie singly. Non-epithelial wandering cells from the underlying connective tissue are also encountered among the bases of the cells in this epithelium.

The space found below the subradular organ and enclosed between the ventral surface of the odontophore and the floor of the buccal cavity is the *sublingual cavity*. This space is lined by an epithelium which resembles to some extent

that of the subradular organ. It is similarly rich in mucus-secreting elements and poor in normal columnar cells.

The epithelium covering the anterior surface of the odontophoral mass above the level of the subradular organ is made of tall columnar chitinogenic cells, similar to those lining the buccal vestibule and the mandibular region of the buccal cavity. It is devoid of gland cells and forms a number of irregular folds of various sizes.

The dorsal surface of the odontophore is largely covered by the *subradular epithelium* (Fig. 5, sb.rd.ep.) which contributes to the secretion of the radular ribbon and the 2 alary processes of the radula. Towards the posterior end, a median evagination passes almost vertically downwards from this epithelium into the odontophoral mass. This evagination forms the radular sac. The part of the epithelium that overlies the radula in the latter sac constitutes the so-called *supraradular epithelium* (sr.rd.ep.). Detailed histological descriptions have been given in a previous publication for the radular sac and the radula (Lutfy & Demian, 1964a), and also for the *odontophoral cartilages* (Lutfy & Demian, 1964b).

Two long prominent dorsal buccal ridges hang down from the roof of the buccal cavity on the odontophoral region, one on either side of the median line. A long *dorsal food channel* (Figs. 5, 37, fd.ch.) is enclosed between the 2 ridges; starting anteriorly between the 2 jaws and reaching posteriorly to the oesophagus. The epithelium lining this channel is mainly composed of ciliated columnar cells (Fig. 6, cl.c.), the cilia of which beat strongly backwards. It is not of uniform thickness but presents some depressions and a few longitudinal folds. The most conspicuous of these is a median fold which increases gradually in height towards the rear and merges with the major dorsal fold of the pro-oesophagus. The columnar cells have oval or elliptical nuclei, each with

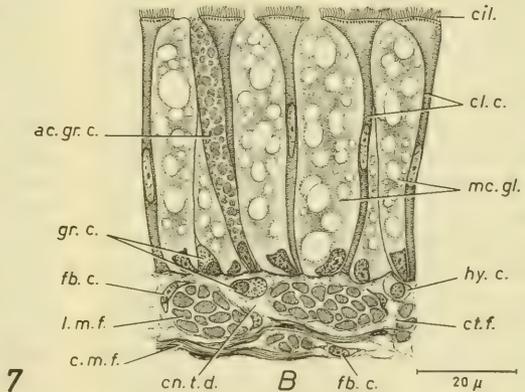
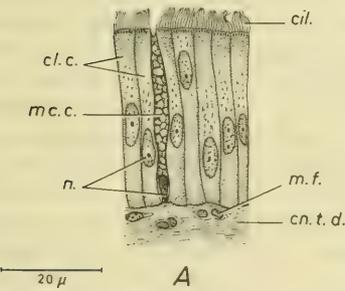
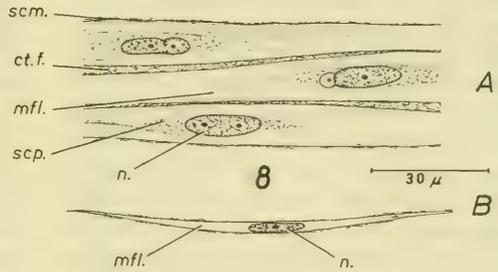
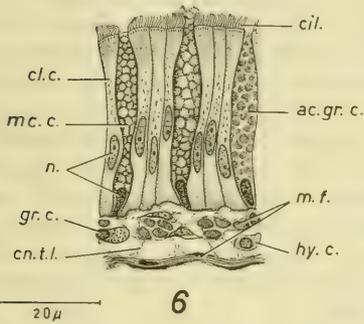
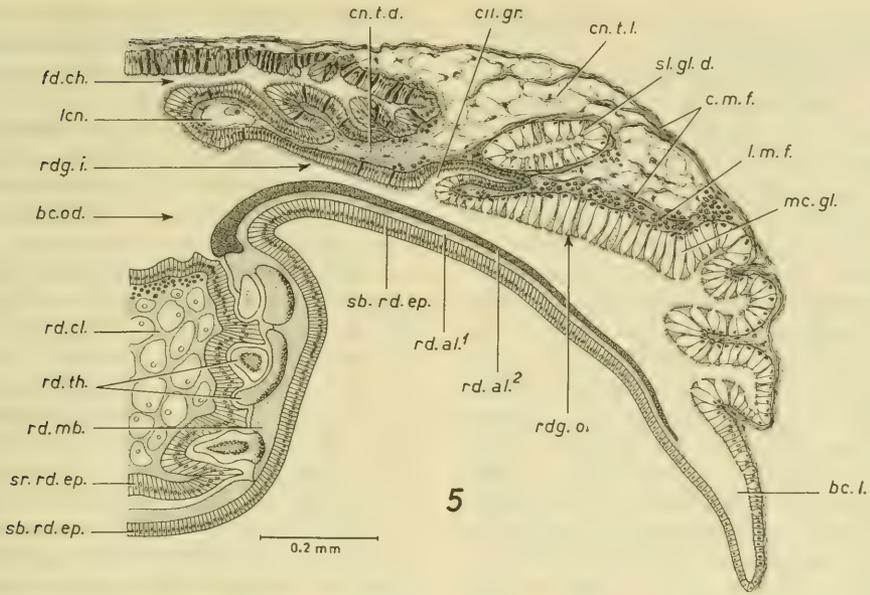


FIG. 5. Part of a cross section through the odontophoral region of the buccal mass. FIG. 6. Lining epithelium of the dorsal food channel (enlarged from Fig. 5). FIG. 7. (A) Epithelium of the inner region of the dorsal buccal ridge (enlarged from Fig. 5). (B) Epithelium of the outer region of the dorsal buccal ridge (enlarged from Fig. 5). FIG. 8. (A) Longitudinal section of some intermediate granular muscle fibres. (B) A smooth muscle fibre.

1 or 2 nucleoli and numerous irregular chromatin granules.

Among these cells there lie numerous mucus-secreting cells (mc.c.) which present a great diversity in form but all react positively towards Mayer's mucicarmine and toluidine blue stains. Some are saccular, while others are regularly cylindrical. Still in others the apical or basal portions are considerably broader than the cell body. In the large majority of these mucus cells the nucleus is elongated conical in form.

Another type of unicellular glands (ac.gr.c.) is also encountered in this epithelium, but in much smaller numbers than the preceding mucus cells. These are invariably fusiform, with broad middle portions, narrow distal necks by which they open on to the surface, and attenuated bases which harbour the nuclei. The reaction of these cells towards the stains used contrasts with that exhibited by the mucus cells. Their cytoplasm contains large spherical granules of uniform size which take a bright blue colour after toluidine blue and a red colour in H-E preparations, in contrast to the purple and dark blue colour assumed by the contents of the mucus cells after these 2 techniques respectively. The distinction between the 2 kinds of gland cells is also clear in Mallory's triple stained preparations where the mucus-secreting elements take a light blue shade and show largely vacuolated contents, while the other gland cells assume a dark blue tint and present closely packed spherical granules.

Each of the *dorsal buccal ridges* is morphologically and histologically differentiated into 2 distinct regions, separated by an oblique groove (Figs. 5, 37, cil.gr.). The inner or medial region (rdg.i.), which abuts on the dorsal food channel, is covered by a ciliated epithelium that contains only few mucus-secreting elements and is thrown into conspicuous oblique folds which project to greater or lesser distances towards the median line. The cilia beat strongly in a diagonal direction (backwards and

inwards) towards the food channel. The epithelial covering of the outer or lateral region of the ridge (rdg.o.) is, on the other hand, highly glandular and far less folded. At the extreme outer edge of the ridge, the epithelium merges with that lining the corresponding lateral recess of the buccal cavity (bc.l.) of the same side.

In the epithelium of the inner medial region, the columnar cells (Fig. 7A, cl.c.) are very tall and narrow on the summits of the folds and much shorter at their bases. Except for carrying longer cilia, these cells do not represent any marked differences from those lining the food channel. Their nuclei lie mostly in the basal halves of the cells, but sometimes occupy distal positions. Therefore the epithelium appears pseudostratified in several places. Not infrequently one comes across some of the apically situated nuclei undergoing mitotic division. The cytoplasm is more acidophilic towards the apices of the cells and a number of basophilic granules and rodlets are often seen in it just above the nucleus. Mucus cells (mc.c.) do exist in this epithelium but in much smaller numbers than in the epithelial lining of the food channel, and also present a lesser variety of form.

The epithelial covering of the outer lateral region of each dorsal buccal ridge is markedly different from that of the medial region. Large unicellular glands (Fig. 7B, mc.gl.) dominate this epithelium, squeezing between them a few inconspicuous normal epithelial cells (cl.c.) which have extremely slender bodies, but broad apices carrying short cilia (cil.). They have spindle-shaped nuclei which lie half way along their lengths. The majority of the glandular cells are, on the other hand, club-shaped or saccular and resemble closely those which dominate the covering epithelium of the subradular organ (p 383). Their nuclei are always basal, dense in chromatin and of elongated conical or elliptical form. The cyto-

plasm presents a faint network surrounding numerous vesicles of various sizes. In Mallory's triple stained preparations, the network assumes a faint blue tint. However, numerous fine granules of a bright red colour are often seen in these preparations clinging to the network. These gland cells react positively towards Mayer's mucicarmine and toluidine blue, but less strongly than do the mucus cells found in the epithelia of both the food channel and the medial region of the dorsal buccal ridge.

In toluidine blue preparations, a few other gland cells (ac.gr.c.) are also distinguished in this epithelium and contain distinct spherical granules of a bright blue colour. The same cells are also distinguishable in H-E preparations where the granules appear red. Consequently these cells are assumed to be engaged in the elaboration of some different substance and are comparable to the above described granular gland cells found in the epithelium of the dorsal food channel (Fig. 6, ac.gr.c.).

Prashad (1925), Michelson (1955) and Andrews (1965) referred to the dorsal buccal ridges in various ampullariids as the buccal glands, apparently because of the highly glandular nature of their covering epithelia. However, it is clearly evident from the above description that the different types of gland cells encountered in the covering epithelia of the dorsal buccal ridges of *Marisa cornuarietis* can be matched with similar elements in other regions of the buccal cavity epithelium, such as those covering the subradular organ and the postradular ledge. For this reason, and because the terms "buccal glands" and "buccal gland cells" have been used for some kind of non-localized subepithelial mucus glands in the buccal masses of some other gastropods (Carriker & Bilstad, 1946; Pan, 1958), which have no counterparts in the dorsal buccal ridges of *Marisa*, the term "dorsal buccal ridges" seems more appropriate for these structures. In all

probability these ridges are mainly engaged in the production of a lubricant which facilitates the feeding movements and acts as an adhesive for ingested food particles. They simultaneously serve in directing the food stream and keeping it within the limits of the dorsal food channel.

Two compressed *lateral recesses of the buccal cavity* (Figs. 5, 37, bc.l.) extend on either side of the odontophore. Corresponding spaces in other gastropods are sometimes referred to as the buccal pouches (Fretter & Graham, 1962). Two sheets can be distinguished in the epithelium lining each recess, an outer sheet, which is continuous with the epithelium covering the outer region of the dorsal buccal ridge of the same side (rdg.o.), and an inner sheet which is continuous with the subradular epithelium underlying the neighbouring alary process of the radula (sb.rd.ep.). The epithelium constituting each of the 2 sheets generally resembles that with which it is continuous. Thus, the outer sheet carries short cilia and is rich in mucus-secreting and granular acidophilic gland cells. The inner sheet, on the other hand, constitutes a subcolumnar to cuboidal epithelium which is almost completely devoid of gland cells and is not covered with cuticle. Both sheets thin out gradually towards the lower corner of the recess.

A wide semilunar fold of the floor of the buccal cavity, the *postradular ledge*, projects horizontally forward above the posterior region of the odontophore. It corresponds to the oesophageal valve described by Fretter & Graham (1962) in some other prosobranchs. Its free anterior edge is concave and its lateral portions are fused on either side with the walls of the buccal cavity, reaching points anterior to the median portion of the ledge. As a result, successive transverse sections of the buccal mass first show 2 lateral horizontal folds which approach the median line gradually until they form a continuous horizontal shelf.

The epithelial covering of this ledge is highly glandular and structurally similar to those of the outer portions of the dorsal buccal ridges; being predominantly formed of large saccular mucus cells and a few acidophilic granular gland cells. The normal columnar epithelial cells are present in small numbers and are highly squeezed between the glandular elements. However, on the mid-dorsal part of the ledge and on the whole of its free anterior margin, the epithelium is exclusively formed of non-glandular columnar cells.

Below the postradular ledge, there projects from the posterior wall of the buccal cavity a compressed finger-like process which extends medially forwards above the collostylar hood. This process has a central connective tissue core and is covered with an epithelium of the same nature as that covering the major part of the postradular ledge. The same histological structure is also found in the collostylar hood, or the epithelial sheet that rests on the upper end of the radular sac.

B. Subepithelial Glands

Numerous large flask-shaped mucus-secreting glands, which give a strong positive reaction with Mayer's mucicarmine and toluidine blue stains, are encountered in the subepithelial connective tissue around the peristome (Fig. 28, mc.sep.). They give a distinct yellowish colour to this region in fresh specimens.

Each gland is a single cell (Figs. 1, 29, mc.sep.) that has an ovoid or spheroidal body and opens to the surface through a long narrow neck (nk.sep.). The nucleus is large, oval and with one spherical nucleolus and a number of irregular chromatin granules. The necks of these glands pass between the covering epithelial cells of the peristome and can be easily differentiated from the mucus cells of this epithelium

(mc.c.), whether of the goblet or the narrow cylindrical type, as they are much more slender, lack a nucleus and do not usually acquire as intense a colour as that assumed by the latter cells. The cytoplasm in these glands, however, reacts to H-E and Mallory's triple stain in much the same way as the goblet cells of the epithelium.

These subepithelial gland cells are either found singly or, more frequently, form groups or nests of 3-6 cells each. The individual cells of each group may or may not be separated from each other by some connective tissue and thin muscle bundles.

Probably these subepithelial mucus cells correspond to the buccal gland cells described by Carriker & Bilstad (1946) in *Lymnaea stagnalis appressa* and to the mucus-secreting elements reported by Pan (1958) as constituting ductless buccal glands in *Biomphalaria glabrata*. In both these snails, these mucus-secreting elements were described as being scattered over the entire buccal mass and not localized around the oral aperture as in *Marisa cornuarietis*.

C. Connective Tissue

The main components of the connective tissue, namely the structureless ground substance, the fibres and the cellular elements, are all basically similar in constitution not only within the different regions of the buccal mass but also within all parts of the alimentary system examined. The connective tissue may however vary in appearance and abundance of one or the other of its elements in the different organs. Thus, while it is dense and rich in its fibrous elements in some places, it appears wide-meshed and presents fewer fibres and cells in others. A full description of the connective tissue found in the respiratory organs of *Marisa cornuarietis* has already been given by the writers (Lutfy & Demian, 1965). This description applies equally well here.

The same kind of collagen-like fibres and the same 5 types of connective tissue cells are also encountered in the alimentary system and in other organ systems of the snail as well. Thus the writers can safely assume that these fibres and cell types (shown for instance in Figs. 1, 9, 13) are basic and common to the connective tissue of *Marisa* in general, and can now suggest a name for each cell type derived from one or the other of its characters.

1. The *fibrocytes* (or fibroblasts, fb. c.) are fusiform or spindle-shaped cells with elongate oval or elliptical nuclei and scanty homogeneous cytoplasm that is drawn out into several fine branching processes. Corresponding elements in *Helix pomatia* and *Biomphalaria glabrata* were referred to by Baecker (1932) and Pan (1958) respectively as the fibroblasts and were assumed to have the ability to transform into a variety of other cell types.

2. The *hyalocytes* (hy.c.) are spheroidal, saccular or tear-drop-shaped cells, with kidney-like or partially divided nuclei and slightly acidophilic hyaline cytoplasm.

3. The *granulocytes* (gr.c.) are spheroidal, ovoid or irregular cells, with extremely eccentric small nuclei and highly acidophilic cytoplasm full of distinct granules and rodlets.

4. The *basophils* (bs.c.) are large irregular cells, with characteristically large vesicular nuclei and basophilic cytoplasm.

5. The *globulocytes* (gb.c.), which comprise the largest cell type, are somewhat irregular, with small spheroidal or oval nuclei and scanty basophilic basic cytoplasm that holds numerous spherical refractive globules of various sizes.

A full description of all these cell types and their staining reactions has been given in connection with the respiratory organs (Lutfy & Demian, 1965).

In addition, a 6th type of cell, the

pigment cell, which is known to be abundant in several pulmonates, was also encountered in the present study. These cells (not figured) are scattered singly in the connective tissue of the buccal mass, have no fixed form and possess irregular, inconspicuous nuclei; their cytoplasm is crammed with spherical granules of a dark brown or black colour.

The connective tissue fibres (Figs. 1, 9, 13, ct.f.) are collagen-like; they assume a red colour in iron haematoxylin - Van Gieson's preparations, and a blue colour after Mallory's triple stain. Crystalline concretions (con.) are encountered in various numbers in the meshworks of the connective tissue.

Within the buccal mass, in particular, the connective tissue appears quite dense. It is relatively rich in its cellular and fibrous elements in the region of the peristome (Figs. 1, 28, cn.t.d.) and holds a large number of thin diffuse muscle fibres which constitute the labial retractor muscles and labial sphincter (Demian, 1964). Small scattered blood sinuses (lcn.) of irregular shape but definite outlines are enclosed in this tissue. They are usually lined with fibrocytes and increase in size in the antero-posterior direction, giving the connective tissue the general appearance of erectile tissues. In the region of the buccal vestibule (Figs. 2, 30), the subepithelial connective tissue is also dense, but the haemocoelic sinuses in it are more numerous and lie closer to the basement membrane of the epithelium.

A pad of loose or vascular connective tissue (cn.t.l.) with an open network of slender fibres lies below each jaw (Figs. 31, 33, 34). This tissue holds numerous muscle bundles which mainly belong to the buccal sphincter.

The subradular organ has a core of dense connective tissue (Fig. 36, cn.t.d.) which penetrates into its epithelial foldings along with some muscle fibres (m.f.).

A thin layer of subepithelial connective

tissue extends the whole length of the dorsal food channel in the dorsal wall of the buccal mass. This layer is followed by a distinct layer of thin bundles of longitudinal and circular muscle fibres.

On both sides of the food channel, there are much thicker masses of loose vascular connective tissue (Fig. 5, cn. t.l.). These are continuous with the connective tissue cores of the dorsal buccal ridges which, however, are denser and hold larger amounts of muscle fibres running in various directions. Haemocoelic spaces (lcn.) abound within the folds of the dorsal buccal ridges. The largest of these sinuses appear filled with a homogeneous acidophilic substance, apparently the haemolymph, and a few cells. Some of the latter have spheroidal eccentric nuclei and their cytoplasm is crowded with coarse acidophilic granules. Others have bean-shaped nuclei and hyaline cytoplasm, and still others appear as elongate ovals with elliptical nuclei. The first 2 cell types have exact parallels in the connective tissue.

D. Muscles

Two types of muscle fibres are recognized in the alimentary system. The first is mainly found within the walls of the buccal mass, constituting an elaborate system of extrinsic and intrinsic buccal muscles (see Demian, 1964). The rest of the musculature of the alimentary system is chiefly made up of the 2nd type. Both types lack any periodic striations and approach, in their general morphology, the smooth muscle fibres of vertebrates. They correspond to the intermediate granular fibres and the smooth fibres respectively described by Pan (1958) in *Biomphalaria glabrata*.

1. Intermediate granular muscle fibers (Fig. 8A)

These comprise long spindle-shaped cells with strongly refractive delicate outer membranes or sarcolemmae

(scm.). In longitudinal section, 2 distinct regions can be identified in the cytoplasm of each fibre: a thicker and denser outer acidophilic zone, and an inner one of much less acidophilic cytoplasm (scp.). Extremely thin myofibrils (mfl.) of an intense acidophilic reaction are packed lengthwise in the outer zone. The granules of the inner zone are basophilic in nature and vary considerably in number, size and form, being rod-shaped, spheroidal or irregular. Sometimes they appear connected by fine threads forming a sort of reticulum without special pattern. In other cases, they appear arranged in longitudinal rows. The nucleus (n.), which generally lies in the thickest middle portion of the fibre, is either oval or elliptical and has a distinct nuclear membrane, 1 or 2 conspicuous nucleoli and a few chromatin granules which are often linked together by thin chromatin threads. In the majority of cases, a small spheroidal lump is seen attached to the outer surface of the nuclear membrane at one pole of the nucleus. This lump often contains a small spherical granule which is nearly as basophilic as the chromatin bodies of the nucleus. Apart from this structure, the nucleus itself occasionally presents some peculiar phenomena such as having constrictions in the nuclear membrane which seem to grow inwards into the nucleoplasm separating off a part of the nucleus, either at its middle or nearer to one of its poles. In this case, numerous chromatin granules are seen attached to the ingrowing membrane, and occasionally also one nucleolus is found on either side of the partitioning membrane, or at least in close proximity of it. The whole picture gives the impression that the nucleus is about to split into 2 daughter nuclei, but there is no definite proof for this peculiar nuclear division. Moreover, some nuclei present a strange outgrowth which may take a different direction from that of the long axis of the nucleus.

In cross sections, these muscle fibres

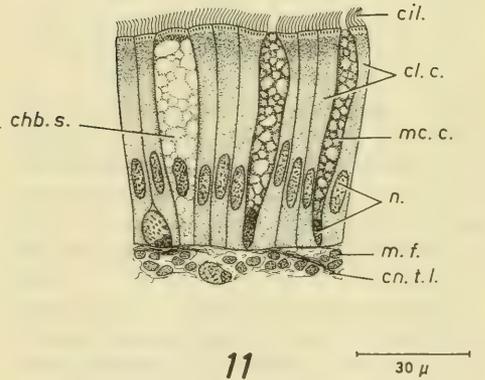
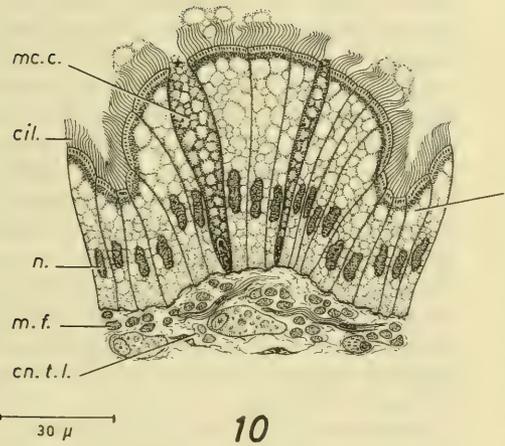
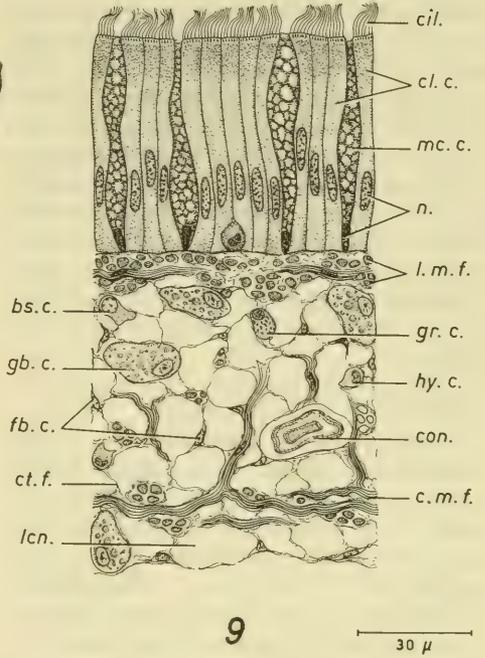
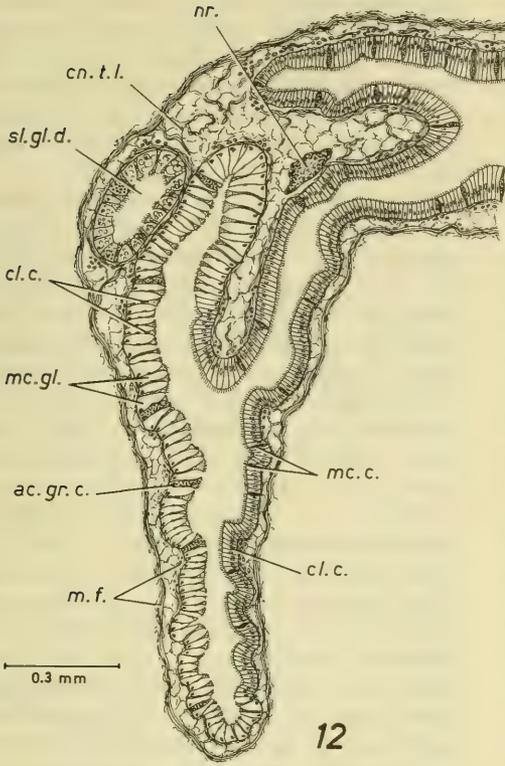


FIG. 9. Part of a cross section through the pro-oesophagus. FIG. 10. Lining epithelium of the crop. FIG. 11. Lining epithelium of the post-oesophagus. FIG. 12. Longitudinal section of an oesophageal pouch.

appear circular, oval polygonal or even irregular in outline. The 2 characteristic zones of the cytoplasm in each are well-defined in the sections which pass near the middle of the fibre. The outer zone presents closely packed tiny dots, which correspond to the myofibrils cut transversely, while the inner lighter zone shows some basophilic granules.

Each of the buccal muscles is formed of a larger or smaller number of muscle fibres of this type, bound together in bundles by scanty collagen-like fibres constituting an endomysium. The neighbouring bundles are held together by a perimysium of similar connective tissue.

2. Smooth muscle fibres (Fig. 8B)

This 2nd type of muscle fibres includes much smaller and thinner fusiform cells with a homogeneous acidophilic cytoplasm and extremely delicate sarcolemmae. Fine myofibrils (mfl.) run lengthwise in each fibre. The nucleus is elongatedly oval and usually located at about the middle of the fibre; it is poor in chromatin material, but often presents a conspicuous nucleolus. These smooth fibres may be scattered singly in the connective tissue or may form groups of larger or smaller size. They prevail in the walls of the alimentary tract from oesophagus to rectum.

OE SOPHAGUS AND OE SOPHAGEAL POUCHES

The oesophagus of *Marisa* is differentiated into 3 distinct regions: a short and relatively narrow pro-oesophagus, a much longer and markedly dilated mid-oesophagus or crop, and a long post-oesophagus which leads into the stomach.

At the beginning of the pro-oesophagus (Fig. 38, pr.oes.), the lumen appears more or less X-shaped in cross section. The organ presents a large dorsal internal fold, a ventral fold, and 2 main lateral folds. The dorsal and ventral folds gradually gain in height towards

the rear, and 1 or 2 smaller foldings appear on either side of each. At the same time the 2 lateral folds each divide into 2-3 smaller ones. The whole of the epithelial lining of the pro-oesophagus carries long cilia which beat backwards.

The crop (Fig. 39) presents a large and considerably wide dorsal fold, 3 smaller ventral ones and 2 minor folds on either side. Although this is a constant morphological feature of the crop, it is not always demonstrable in cross sections of the organ, because numerous transverse and oblique impressions appear readily on all these major folds upon the slightest contraction of the organ during fixation, with the result that several secondary folds show in cross sections.

The largest dorsal fold of the crop has a thick connective tissue core and a broad bifid summit formed by a conspicuous depression or shallow tract extending lengthwise along its middle. The long and powerful cilia on the 2 subdivisions beat obliquely backwards, in 2 opposite directions, thereby creating a continuous flow down the crop, along the mid-line of this dorsal fold. This ciliary beat provides a continuation along the crop of the flow that starts in the buccal mass by the ciliary beat of the dorsal food channel and dorsal buccal ridges. Thus the dorsal fold of the crop in *Marisa* and the median tract on it correspond to the 2 dorsal folds and the food channel found between them in the mid-oesophagus of other ampullariids and various other prosobranchs (Fretter & Graham, 1962; Andrews, 1965). The remainder of the lining of the crop carries weaker cilia which beat in a more or less transverse direction driving secretions produced by the epithelium towards the dorsal fold to join the food stream.

The dorsal fold of the crop continues down the post-oesophagus until it fades away on the left surface of the vertical septum that stands between the 2 limbs of the gizzard. Other longitudinal folds

which run along the post-oesophagus carry shorter cilia.

The walls of all 3 regions of the oesophagus are formed of a lining mucosa of simple epithelium, followed by a thick coat of vascular connective tissue, but they have no outer serous epithelial coverings. The mucosa contains mucus cells which vary in frequency from one region to the other. A thin layer made up largely of longitudinal muscle fibres runs close below the mucosa. Another thicker outer layer, of mostly circular fibres, exists near the periphery of the connective tissue coat. Some fibres from the latter layer cross the connective tissue and reach the inner muscular layer.

The mucosal lining of the *pro-oesophagus* consists largely of simple columnar cells (Fig. 9, cl.c.) which vary considerably in height, attaining their maximal length at the tips of the folds. They are uniformly provided with remarkably long cilia (cil.). Their nuclei are elliptical, basal, highly basophilic and rich in chromatin granules but without discernable nucleoli. The nuclear membrane is distinct and presents a smooth surface. The cytoplasm is more chromophilic towards the apices of the cells and presents no secretory granules or vacuoles.

The mucosa of the *crop* (Figs. 10, 39) is evidently a seat of extensive secretory activity. The ciliated columnar cells which dominate in this mucosa differ markedly from those of the pro- and post-oesophageal mucosae in being engaged in active secretion. They present various pictures which apparently correspond to different phases of the secretory activity in them. An apical zone of dense acidophilic cytoplasm is always seen underlying their distal membranes. In preparations taken from normally fed snails, some of these cells appear narrow and their cytoplasm hardly shows any secretory products. Others are broader and full of moderately chromophobic small spherules (chb.s.). However, the

majority are much distended and crowded with colourless secretory spherules which gain gradually in size and seem to coalesce into larger globular bodies towards the apices of the cells. These bodies are occasionally seen pouring out of the cells into the lumen of the organ. They are colourless, refractile and clear in both H-E and Mallory's triple stained preparations (after fixation in Zenker's fluid), but assume a homogeneous grey colour in preparations stained with iron haematoxylin - orange G (after fixation in Flemming's strong fluid). The nucleus appears roughly ovoid, with no discernable nucleolus, and its chromatin material is not easily identifiable as distinct granules. It presents a corrugated nuclear membrane, a feature often encountered in secretory cells. In sections taken from starved snails, the cells do not show such variety of secretory phases and therefore their cilia show up more clearly.

In the *post-oesophagus* (Fig. 40; Fig. 45, pt.oes.), the mucosa is predominantly formed of normal ciliated columnar cells (Fig. 11, cl.c.). Interspersed among these are a few secretory cells like those found in the mucosa of the crop. The epithelium covering the dorsal fold in both crop and post-oesophagus is characterized by carrying longer cilia and holding a larger number of mucus cells.

Mucus-secreting cells of the goblet type (Figs. 9-11, mc.c.) are encountered in the mucosa of all 3 regions of the oesophagus but are relatively more numerous in the pro- than in the mid- and post-oesophagus. They have narrow apical regions, thick middle portions and attenuated bases which harbour the nuclei. These are either elliptical or in the form of elongated cones. The cytoplasm presents the characteristic meshwork that stains dark blue in H-E preparations, the spaces within the meshes assuming a faint bluish colour. In preparations fixed in Flemming's strong fluid and stained with iron

haematoxylin - Van Gieson's stain, the cytoplasmic contents appear as yellowish spherules.

The subepithelial connective tissue coat is relatively thicker in the crop than in the pro- and post-oesophagus (see Figs. 38, 39, 40). This tissue is of the wide-meshed vascular type and is especially rich in some cellular elements such as fibrocytes and globulocytes. The latter are remarkably numerous in the walls of the crop where they lie in between and directly below the inner layer of longitudinal muscle fibres.

Two *oesophageal pouches* (Fig. 12) arise as elongated lateral evaginations of the alimentary tract at the anterior limit of the pro-oesophagus. Two distinct regions can be differentiated in the epithelial lining of each pouch. The greater part of the outer wall of the pouch is lined with a thick glandular epithelium of the same nature as that covering the outer regions of the dorsal buccal ridges (p 383). The dominant elements in this epithelium are saccular or club-shaped cells (mc.gl.) with basal triangular nuclei. They contain a slightly basophilic vacuolated secretion which gives a mildly positive reaction to Mayer's mucicarmine and toluidine blue stains. This reaction is weaker than that usually given by ordinary mucus cells to the same stains. Squeezed in between these glandular elements are some acidophilic granular cells (ac. gr.c.) and a few normal slender columnar cells (cl.c.) with more or less broad apices.

The rest of the lining epithelium of the oesophageal pouch is thrown up into a number of longitudinal folds and is mainly formed of narrow ciliated columnar cells (cl.c.) with some goblet cells (mc.c.) in between.

Below the epithelium, there is a relatively thin layer of loose vascular connective tissue (cn.t.l.) which is continuous with the connective tissue coats of the pro-oesophagus and salivary glands. It holds numerous muscle fibres,

most of which run lengthwise close below the epithelium.

STOMACH

The stomach (Fig. 17) is divisible into 2 main regions (see Demian, 1964), a proximal U-shaped cardiac portion (gz.) and a distal tubular pyloric one (stl.s.). Corresponding subdivisions of the stomach in some other ampullariids have been recently termed gizzard and style sac respectively (Andrews, 1965) on the basis of their homologies with parallel regions in the stomach of some closely related prosobranchs (Fretter & Graham, 1962). This reasonable terminology is also adopted in the present work.

The stomach is covered on its dorsal side by the thin general covering integument of the visceral mass (Figs. 13, 42, 43, itg.), while its ventral side abuts mostly on the digestive gland. This integument, as mentioned below in connection with the digestive gland (p 407), is composed of a single-layered surface epithelium, followed by a thin layer of circularly disposed muscle fibres (c.m.f.). Below these layers there is connective tissue which forms a continuous coat all round the stomach and binds it to the digestive gland tissue. The connective tissue (cn.t.l.) is of the loose wide-meshed type and holds the musculature proper of the stomach that lies below the lining mucosa of the organ.

The epithelial lining of the greater portion of the 2 limbs of the U-shaped cardiac portion or *gizzard* is of the simple columnar cuticularized type, and forms a large number of prominent folds of more or less regular form and height. This epithelium (Fig. 13) is devoid of mucus-secreting elements and its cells (cl.c.) are generally shorter and broader than those of the oesophagus. Their nuclei are oval or spheroidal and short of basal (subbasal) in position. Some of these are occasionally seen undergoing mitotic division, during which process

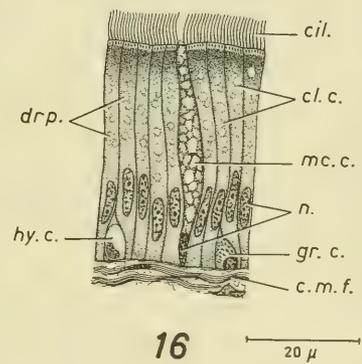
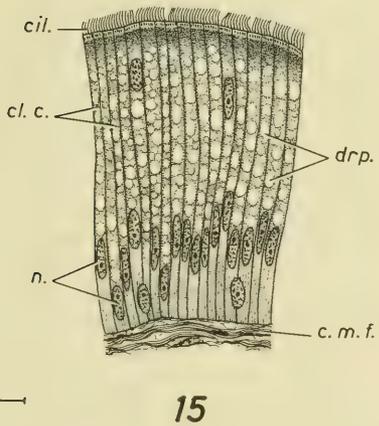
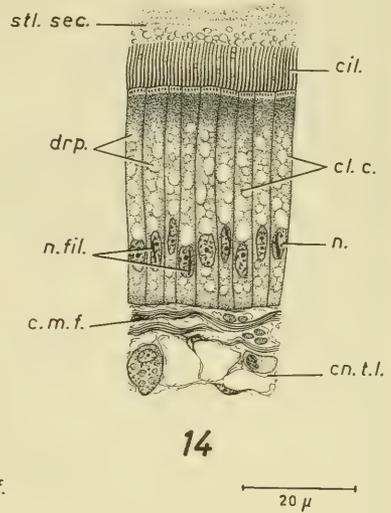
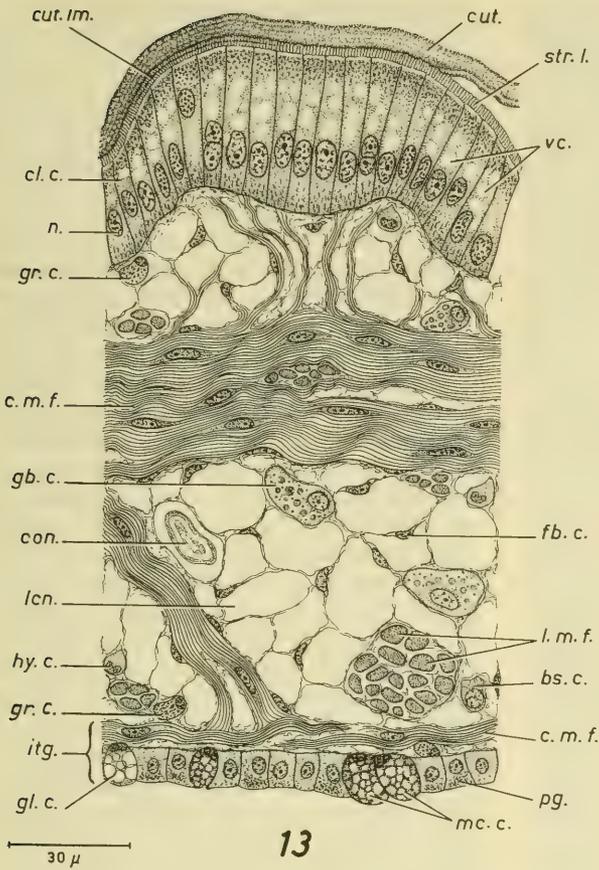


FIG. 13. Part of a cross section through the gizzard. FIG. 14. Lining epithelium of the style sac. FIG. 15. Lining epithelium of a gastric pouch. FIG. 16. Lining epithelium of the caecum.

they come to lie near the distal margins of the cells. Each nucleus has 1 or 2 conspicuous nucleoli and several irregular chromatin granules.

Just below the apical cell membrane, there appears in these cells a very narrow zone of clear and slightly acidophilic cytoplasm. This zone is followed inwards by a wider zone of more acidophilic cytoplasm loaded with coarse granules. Similar granules and some distinct threads are encountered at the very basal portions of the cells and all assume a deep red colour in both H - E and Mallory's triple stained preparations. The rest of the cytoplasm is richly vacuolated; the vacuoles (vc.) containing a colourless and seemingly liquid substance.

This epithelium secretes a thick layer of cuticle (cut.) which is distinctly composed of closely packed lamellae extending in a direction parallel to the surface of the epithelium. A narrow conspicuous zone (str.1.), which stains a light purplish-blue in Mallory's preparations and a faint red after H - E, intervenes between the cuticle and the free surface of the epithelium. This layer presents fine vertical striations. According to Fretter & Graham (1962), a corresponding layer interposed between the distal surface of the gizzard epithelium and the overlying cuticle seems to be of common occurrence in several prosobranchs. The presence of such a layer has led to the suggestion that the cuticular secretion escapes from tiny pores in the cell surface in the form of fine threads which later coalesce to form a continuous sheet over the epithelium. However, another suggestion entailing a different method of secretion of this cuticle, could be advanced from the present investigation.

After careful examination of the layer in question in *Marisa cornuarietis* the writers are more inclined to believe that it is constituted of fine microvilli which project from the free borders of the epithelial cells, rather than of fine threads of cuticular secretion issuing from pores

located on these free borders. In this case, the cuticular material would be secreted in the form of thin sheets among the bases of the microvilli, each sheet being gradually pushed upwards and added to the overlying cuticular lamellae. The following facts could be advanced in support of this hypothesis: (i) A thin horizontal dark bluish sheet (cut.lm.), which presumably represents the most recently secreted cuticular lamella, is occasionally encountered at some level or other within the layer of microvilli. (ii) The sections which pass obliquely through the cuticular layer reveal the presence, in the lamellae constituting it, of regular rows of tiny lighter-coloured circles which were possibly occupied by the microvilli of the epithelial cells. (iii) The cuticular layer is easily detached from the underlying epithelium under the effect of mechanical factors involved in sectioning. In such cases, the layer of supposed microvilli retains its identity and connection with the distal borders of the cells, a phenomenon which would not be expected if the layer in question was formed of thin fragile cuticular threads. The same explanation may hold true also for a corresponding layer of microvilli described in the epithelium responsible for the secretion of the jaws (p 380).

The epithelium on both surfaces of the *vertical septum* (Figs. 17, 43, v.spt.), which stands between the 2 limbs of the gizzard, is of the same cuticularized type common to the gizzard mucosa. It is only along a narrow longitudinal strip on the left face of the septum that that epithelium differs in being formed of ciliated columnar cells with intervening goblet cells. This strip represents a posterior continuation of the major dorsal fold of the post-oesophagus. Between the 2 surface epithelia of the septum, there is vascular connective tissue which is continuous with the general connective tissue coat of the stomach.

A prominent *ventral gastric ridge* (Figs. 17, 42, vn.gs.r.), of a more or

less triangular outline, projects from the floor of the gizzard between its 2 limbs. The hepatic vestibule opens into the gizzard through a wide semilunar opening (h.v.s.o.) situated just in front of this ridge. The ridge has a thick connective tissue core into which penetrate a few muscle fibres. Its covering epithelium has an even surface. It does not present any conspicuous structural differences from the epithelium constituting the general lining of the gizzard and is covered by a cuticular layer of the same thickness.

Close below the lining epithelium of the gizzard, there is a thick muscle layer (Figs. 13, 42, c.m.f.) whose fibres run mostly in a circular direction. Thin bundles from this layer penetrate into the foldings of the mucosa, while others run in various directions towards the outer muscle layer of the covering integument of the visceral mass. The mixture of food and digestive secretion in the gizzard is acted upon by this elaborate musculature. Anteriorly, the circular muscle layer of the gizzard penetrates into the vertical septum so as to form 2 independent muscular coats around the 2 limbs of the organ. The one which encircles the right limb is somewhat more developed and constitutes a more or less distinct sphincter (sph.l.). This muscle does not only serve in squeezing fluids of partially digested material out of the food pulp found in the lumen of the gizzard, but also controls the passage of the secretion of the digestive gland from the hepatic vestibule into the gizzard. Its contraction causes the vertical septum to press against the ventral gastric ridge and at the same time it apposes the 2 edges of the hepatic vestibule opening one against the other, thus effecting closure of that opening.

The most distal part of the right limb of the gizzard (Figs. 17, 43, 44, gz.), in front of the gizzard sphincter, has relatively thin and not very muscular walls. Its epithelial lining is non-cuticularized and ciliated. As suggested

by Andrews (1965), this part most probably represents a vestige of an originally large sorting area of the stomach. The lining of the right wall of this region has its cilia beating obliquely backwards towards the hepatic vestibule opening. The partly digested soluble material, which is pressed out of the food mass under the influence of the gizzard sphincter, is directed by these cilia towards the hepatic vestibule to reach the digestive gland where it is taken up by the digestive cells. The residue of indigestible material is then gradually moved from the gizzard into the style sac. This residue forms what is often referred to as the stomach string after Carriker (1946).

Two adjacent gastric pouches (Figs. 17, 44, gs.p.) arise as blind evaginations on the left dorsolateral wall of the same region of the gizzard and a thick and highly prominent fold, or directing lappet (d.lp.), projects from the ventral wall. The gastric pouches correspond to the so-called glandular pouch of the stomach of *Pomacea canaliculata* (Andrews, 1965). The lappet coalesces at one end with the ventral wall of the hepatic vestibule, between the openings of the 2 posterior digestive gland ducts, and merges at the other end with the major typhlosole of the style sac (mj.ty.). It carries powerful cilia and overhangs, on its left side, a few ciliated furrows which radiate out fan-wise from the hepatic vestibule towards the gastric pouches and the intestinal groove (int. gr.) running along the style sac. The epithelial coverings of the directing lappet and these ciliated grooves approach very much that of the major typhlosole of the style sac (p 398) in their histological structure. Minute dark brown spherules of excretory matter from the digestive gland are carried by ciliary currents along the digestive gland ducts and the hepatic vestibule towards the opening into the gizzard. There they come under the effect of the powerful cilia of the directing lappet and the ciliated furrows, and become

intermingled with mucus in a continuous string, the so-called liver string. The string is driven along the furrows towards the intestinal groove of the style sac.

The lining mucosa of the *style sac* (Fig. 14; Figs. 17, 45, 46, *stl.s.*) forms a regular series of closely arranged low transverse foldings. This lining is formed of slender ciliated columnar cells and generally lacks mucus-secreting elements except in certain specialized regions, namely the major and minor typhlosoles of the organ. The ciliated columnar cells (*cl.c.*) have basal elliptical nuclei, each with a large number of extremely fine chromatin granules and a definite eccentric spherical nucleolus. Distally the cytoplasm is denser and shows higher acidophilia. Between the dense apical zone and the nucleus, the cytoplasm is richly vacuolated. The vacuoles are filled with nearly colourless secretion droplets (*drp.*) and the ground cytoplasm occupies the interstices between them. In Mallory's triple stained preparations, the cytoplasm stains blue distally and violet to reddish in the larger proximal part of the cell. In material fixed in Flemming's strong fluid and stained with iron haematoxylin-orange G, the basic cytoplasm acquires a dark blue colour while the secretory material in the vacuoles ranges from colourless to light grey or blue. The cilia are long and seem to beat in unison as they all take one and the same direction in prepared sections.

Following the basement membrane on which this epithelium rests, there is a thin circular muscle layer (*c.m.f.*) which is far less developed than that of the gizzard. The connective tissue coat following this layer is relatively thick and binds the organ to the post-oesophagus and digestive gland.

The mucosa of the style sac, although completely devoid of special gland cells, is apparently engaged in active secretion of some slightly acidophilic substance which is liberated from the free tips of

the cells, among the bases of the cilia. Acidophilic secretion droplets (*stl.sec.*) of a reddish colour and tiny vacuoles of a lighter hue are often encountered among and above the cilia in H-E preparations. These droplets appear blue after staining with Mallory's triple stain. The combination of ciliation and secretion, although unusual, has been recorded for the lining epithelial cells of the gut of other gastropods (Graham, 1952) and is here also met with in different parts of the crop and intestine.

The epithelial linings of the 2 blind *gastric pouches* (Fig. 15) do not seem to present marked differences from the above described general mucosa of the style sac except in having taller and more slender columnar cells carrying shorter and comparatively weaker cilia (*cil.*). Neither here nor there are any mucus-secreting glands found. The gastric pouches produce a copious viscous non-mucoid secretion (of a whitish colour in fresh specimens) which gives the same reaction to stains as that developed by the style sac mucosa. This secretion apparently contributes to the consolidation of the stomach string.

At the entrance to the style sac, there is a conspicuous sphincter (Fig. 17, *sph.2*) which is marked internally by a prominent circular ridge projecting at the very proximal end of the sac. Along the left wall of the style sac, there runs a conspicuous longitudinal intestinal groove (*int.gr.*), formerly named the pyloric groove (Demian, 1964), bordered by 2 longitudinal ridges, the *major typhlosole* (*mj.ty.*) to the right and the *minor typhlosole* (*mn.ty.*) to the left side (the major and minor pyloric folds of Demian, 1964).

The major typhlosole (Figs. 45, 47) has a thick connective tissue core, and below its base the thin muscle coat of the style sac is continuous. The epithelial covering of this typhlosole consists largely of elongated cylindrical or saccular mucus cells (*mc.c.*) with basal ovoid or cone-shaped nuclei. The normal columnar cells in this epithelium are

highly squeezed between the mucus-secreting elements and carry long and powerful cilia which beat forwards, in a direction different from those of the style sac mucosa. The epithelium of the minor typhlosole is, on the other hand, largely composed of normal ciliated columnar cells.

The lumen of the style sac is always filled with a viscous secretion (stl.sec.) of a milky white colour that is mixed with the food pulp coming from the gizzard, the stomach string. This secretion, to which the gastric pouches richly contribute, is added on the periphery of the stomach string so that in cross sections the string appears as a tightly coiled spiral. This configuration indicates that the stomach string is continuously rotated within the lumen of the style sac, evidently in an anti-clockwise direction (viewed from the distal end of the style sac). As it receives additive secretion issuing from the gastric pouches and the style sac mucosa, the stomach string becomes consolidated into a faecal rod, while it is being moved spirally forward into the intestine, bypassing the caecum. The style sac also receives another string, the liver string, which later becomes associated with the faecal rod.

Mucus issues profusely from the epithelial covering of the major typhlosole (Fig. 47). This mucus retains its identity and does not mix with the acidophilic secretion (stl.sec.) of the mucosa of both style sac and gastric pouches. Thus a thick continuous string of mucus (mc. stg.) is always seen over the major typhlosole, moving forwards under the effect of its cilia.

A small compressed sinuous *caecum* arises as an evagination at the distal end of the style sac. A series of dovetailing dorsal and ventral folds (Fig. 17, d.cae.fl., v.cae.fl.) project into its lumen which is therefore reduced to a narrow winding ciliated channel. The major typhlosole of the style sac becomes distally confluent with a transverse fold that projects circumferentially at the

distal end of the sac and marks the position of a sphincter (sph.3) which controls the opening between the style sac and pro-intestine. The minor typhlosole fades away at the mouth of the caecum, while the intestinal groove becomes continuous with the winding lumen of the caecum, which in turn is continuous distally with the pro-intestinal groove.

In cross sections of the caecum, the lumen is seen to be divided into several compressed chambers. These are separated by narrow partitions which correspond to the dovetailing internal folds of the organ. The thick wide-meshed connective tissue coat of the caecum penetrates into these folds and holds some diffuse muscle fibres running in different directions. The epithelial lining of the caecum (Fig. 16) consists mainly of narrow ciliated columnar cells (cl.c.) and holds numerous gland cells which bear great similarity to those found in the lining epithelium of the pro-intestine (Fig. 18). The ciliated columnar cells have basal elliptical nuclei. Secretory droplets (drp.) of the same nature and staining reactions as those found in the lining epithelial cells of the style sac are seen in the middle regions of these cells.

INTESTINE

Two morphologically distinct regions were previously identified in the intestine of the snail under investigation (Demian, 1964). The present histological study, however, reveals beyond doubt that this organ is divisible into 3 structurally different segments which are referred to here as the pro-intestine, mid-intestine and post-intestine. Andrews (1965) recognized 3 corresponding regions in the intestine of *Pomacea canaliculata*, but she did not describe their histology and her morphological account of these regions differs in some respects from that given here.

The first segment of the intestine, or the *pro-intestine* (Figs. 17, 48, 49), starts by a relatively wide opening at the distal

end of the style sac. It immediately turns backwards to run on the right side of this sac, along the perimeter of the posterior renal chamber. A short distance before the intestine completes its course along the periphery of the kidney, and before it disappears below it, the pro-intestine ends and the mid-intestine starts.

Of the 3 divisions of the intestine of *Marisa cornuarietis*, the pro-intestine is the shortest. Its most characteristic feature is a highly prominent and fairly broad internal fold, the pro-intestinal typhlosole (pr.ty.), which runs the whole length of the organ, diminishes gradually in height distally and ends abruptly. It projects from the dorsal wall of the pro-intestine and has its crest inclined to one side. Thus, it delimits a narrow channel or groove, the pro-intestinal groove (pr.gr.), from the major part of the lumen found on the other side of the typhlosole. A few smaller longitudinal and oblique folds project into the major lumen. The pro-intestinal groove continues the caecal channel, while the main cavity of the pro-intestine receives the faecal rod from the style sac.

The internal lining of the pro-intestine is ciliated all over, the cilia being pronouncedly longer on the typhlosole and on the lining of the pro-intestinal groove. In that groove, the cilia beat towards the distal end of the pro-intestine, while in the rest of the lumen, the cilia beat transversely and a little obliquely backwards, thereby causing the faecal rod to rotate in an anti-clockwise direction (viewed from the distal end) and to proceed into the mid-intestine.

The lining mucosa of the pro-intestine is generally composed of narrow columnar cells (Fig. 18, cl.c.) which carry long cilia (cil.) and at the same time present distinct brush borders (br.b.) which acquire a red colour in H - E preparations and a blue colour after Mallory's triple stain. Similar occurrences of columnar cells with both cilia and brush borders have been reported

by Carriker & Bilstad (1946) in the intestinal epithelium of *Lymnaea stagnalis appressa*. Colourless droplets of various sizes appear crowded in these cells above the nucleus, while the cytoplasm below it is nearly free of such droplets. The nuclei are elliptical and lie at various levels in the lower halves of the cells. Each has a distinct nuclear membrane, a tiny nucleolus, which is not always discernable, and abundant chromatin granules. The cytoplasm is largely acidophilic, especially in the apical region of the cell, where it also presents fine fibrillae running lengthwise.

The most common of the gland cells encountered in this mucosa are mucus cells of the goblet type (Figs. 18, 49, mc.c.). They are present in abundance everywhere except in that part which lines the pro-intestinal groove, where they are scanty or even missing.

The mucosa rests on a thick connective tissue basis of the loose vascular type (Fig. 48, cnt.l.) which holds 2 distinct muscle layers. The 1st is very thin, lies directly below the mucosa and consists of small bundles of inner longitudinal and outer circular muscle fibres (m.f.). The outer muscle layer (c.m.f.) is much more developed; it continuously surrounds the intestinal tube and lies within the connective tissue coat. This layer is largely formed of circular fibres, which indicates that the pro-intestine contributes to the compression of the faecal rod. Diffuse muscle bundles run between the outer and inner muscle coats.

The distal end of the pro-intestinal typhlosole marks the end of the pro-intestine and the start of the *mid-intestine* (Fig. 17). The latter proceeds for a short distance along the posterior edge of the posterior renal chamber then disappears below it, making a circular loop, and then continues to the post-intestine. The mid-intestine is morphologically distinct from the 2 other segments, as it has more translucent walls. Two distinct muscular coats, similar to those of the pro-intestine,

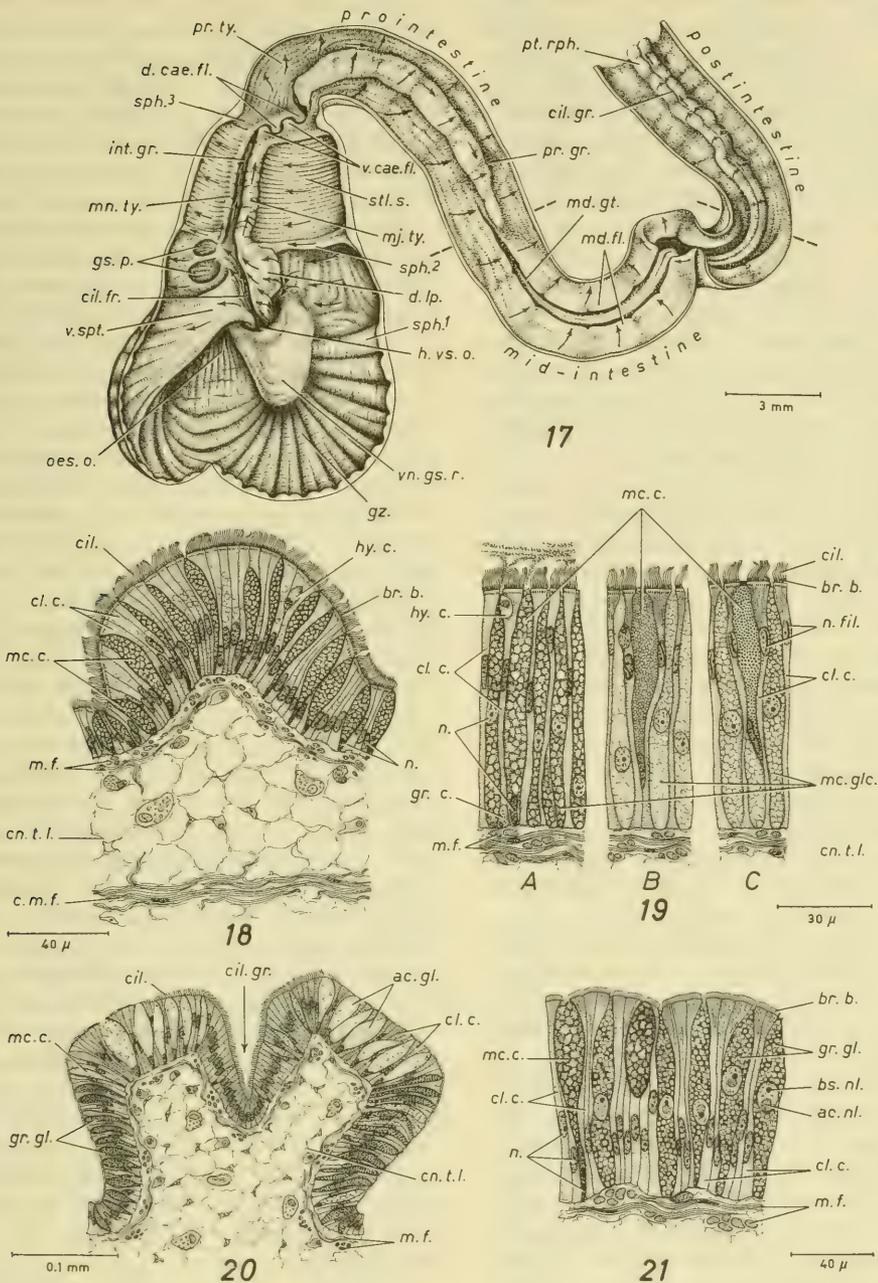


FIG. 17. The stomach and intestine opened by a longitudinal incision to show their inner structure. The arrows indicate the direction of ciliary currents. FIG. 18. Part of a cross section through the pro-intestine. FIG. 19. Lining epithelium of the mid-intestine. (A) stained with mucicarmine, (B) stained with H - E, and (C) stained with Mallory's. FIG. 20. Cross section through the post-intestinal raphe. FIG. 21. General lining epithelium of the post-intestine (H - E).

are found in the walls of the mid-intestine although the inner layer is relatively thicker. The greater part of its walls is lined with a remarkably high, slightly folded and richly glandular epithelium, which forms an incompletely closed cylinder, with a horse-shoe-shaped cross sectional area (Figs. 50, 51). In cross sections, the 2 ends of the horse-shoe are seen to overlies a padding of connective tissue, thus projecting considerably into the lumen. These projections represent 2 longitudinal folds (md.fl.) which run the whole length of the mid-intestine delimiting a narrow compressed channel or gutter from the lumen of the mid-intestine.

The gutter (md.gt.) is lined with a much lower and less glandular epithelium. It first makes its appearance as a shallow groove at the distal end of the pro-intestine. It is quite independent of the pro-intestinal groove and lies on the other side of the pro-intestinal typhlosole. As one moves distally, the gutter gains gradually in width at the expense of the major part of the lumen. Along the middle of the gutter, the epithelium shows 2 median small folds which also increase gradually in height towards the distal end of the mid-intestine and merge into the bifid post-intestinal raphe (pt.rph.).

The peculiar structure of the mucosa of the mid-intestine, when combined with certain macroscopic observations on freshly dissected snails, indicates that the organ, besides displaying active secretory and absorptive functions, is engaged in compressing the faecal rod and moulding it into successive pellets. As the faecal rod passes into the main lumen of the mid-intestine, it becomes subjected to considerable compression while being spirally rotated as it was in the pro-intestine. The muscular activity of the walls of the mid-intestine can bring about an approximation of the 2 mid-intestinal folds, whereby the diameter of the main lumen is greatly reduced and the faecal rod is much compressed. The compression of the rod

at the angles where the mid-intestine curves along its course apparently results in dividing the rod into successive pieces or pellets. The fluid material which is normally squeezed out of the faecal rod as a result of these muscular activities, collects in the gutter where it is probably partially absorbed.

Histologically, the thickened major part of the mid-intestinal mucosa (Figs. 19A-C, 52, 53) is made up of extremely tall and narrow ciliated columnar and glandular cells which may reach 100μ in length. The ciliated columnar cells (cl.c.) have broad apices which carry long cilia (cil.) as well as distinct brush borders (br.b.). Their cytoplasm is highly acidophilic, especially in the distal regions of the cells where it presents fine fibrillae, which are apparently connected with the basal granules of the cilia. The nuclei are elongatedly oval and lie mostly in the distal halves of the cells. They are rich in chromatin and usually present one spherical nucleolus each. Besides, they show a peculiar structure which is most clearly demonstrable in Mallory's triple stained and H - E preparations after fixation with Zenker's fluid. In these preparations there appears a conspicuous red refractive long filament (n.fil.) curved about the nuclear membrane parallel to the long axis of the nucleus. Similar nuclear filaments are also occasionally encountered in the columnar epithelial cells lining other parts of the alimentary tract. They are here described and photographed in the mid-intestinal mucosa only because they can best be demonstrated in its large elongated cells. To the authors' knowledge, no mention was made before of any comparable structures in the Gastropoda.

Enormous numbers of unicellular mucus glands of 2 main types are found among these ciliated columnar cells. The first comprises elongated mucus cells of the ordinary goblet type (mc.c.) common to other regions of the alimentary tract. These have swollen

apical regions and gradually attenuating basal halves which harbour elliptical or conical nuclei, with dense chromatin. These cells react positively towards both Mayer's mucicarmine and toluidine blue (Fig. 19A). In H - E preparations, their cytoplasmic contents form a fine meshwork of a dark blue colour (Fig. 19B), while in Mallory's triple stained preparations the cytoplasm assumes a light blue tint and contains extremely fine red granules, homogeneously distributed all over the cell (Fig. 19C). In the latter preparations, the cells also show a small apical bright red mass which looks like a plug situated at the cell opening. The 2nd type of gland cells (mc.glc.) comprises narrow elongated elements with attenuated necks by which they open on to the surface through minute pores. Copious secretion is poured out of these openings and accumulates around the faecal rod in the lumen. These cells have large oval or spheroidal nuclei which lie at different levels within the basal halves of the cells. Each has a very conspicuous spherical nucleolus and a moderate amount of chromatin material. The cytoplasmic contents react positively towards Mayer's mucicarmine, revealing a meshwork similar to that of the first type or goblet cells found in the same preparations (Fig. 19A). But when stained with toluidine blue, only the distal parts of the cells show positive metachromasia, which is, however, much weaker than that exhibited by the cell contents of the neighbouring goblet cells. They also react differently towards certain other stains. Thus, in H - E preparations they present a wide meshwork of a very faint bluish-red tint (Fig. 19B), and in Mallory's triple stained preparations their meshwork assumes a clear bright violet colour. Such wide differences between the staining properties of these 2 types of gland cells, when added to other morphological differences in their general form and nuclear characteristics, confirm that they represent 2 distinct categories of

mucus cells, not merely 2 different phases of the secretory cycle in one and the same type.

A further peculiarity of this epithelium is the presence among its cells of large numbers of hyalocytes (hy.c.) and granulocytes (gr.c.) which make their way from the underlying connective tissue and may reach levels close to the free surface of the epithelium.

The lining of the mid-intestinal gutter (Figs. 50, 51, md.gt.) consists of much shorter columnar cells which vary in height from 8-35 μ . They are non-ciliated and have distinct striated borders. They reach their maximal length in the 2 longitudinal folds which project into the middle of the gutter. The cells have basal elliptical nuclei and lightly acidophilic cytoplasm. Among these absorptive cells, there is a relatively small number of mucus cells of the goblet type.

The *post-intestine* (Figs. 17, 20, 21, 54, 55) is the longest division of the intestine. It makes 2-3 loops below the posterior renal chamber. A single muscle coat of circular and longitudinal fibres (m.f.) is present in its walls close below the mucosa. The latter is thrown into a large number of more or less transverse folds. Besides, a large prominent longitudinal fold with a bifid crest, the post-intestinal raphe (pt.rph.), extends along the whole length of the organ. It has a thick connective tissue core, and a deep groove (cil.gr.) runs medially on top of it. The epithelium lining the groove and covering a part of the summit of the raphe carries long cilia (cil.) which beat transversely inwards towards the groove. Elsewhere in the post-intestinal mucosa, the epithelium is non-ciliated and composed of absorptive as well as secretory cells.

The absorptive cells (Figs. 20, 21, 56, 57, cl.c.), which constitute the dominant element in the post-intestinal mucosa, are narrow, elongated and columnar, with distinct brush borders. They have sub-basal elongate elliptical nuclei, each with one small nucleolus and abundant

chromatin. The cytoplasm is highly acidophilic and finely granular, and presents fine fibrillae in its apical zone. In Mallory's triple stained preparations, the tiny granules of these cells take a faint red hue.

Three main types of secretory gland cells can be distinguished in this epithelium. The first type is the ordinary goblet cell (mc.c.). The 2nd and most abundant type of the gland cell consists of elongated sacciform or clavate cells (gr.gl.) with narrow necks. The nucleus is remarkably large and may be situated anywhere in the lower 2/3 of the cell. It is either ovoid or spheroidal, is rich in chromatin and harbours at least 2 conspicuous nucleoli. The largest of these (ac.nl.) is always spherical and distinctly acidophilic, acquiring a blue colour after Mallory's triple stain and a red one in H-E preparations. It occasionally appears surrounded by a circle of coarse chromatin granules. The other nucleolus (bs.nl.), or nucleoli, are either spheroidal, oval or elongated and distinctly basophilic and stain bright red with Mallory's triple stain and blue with H-E. A basic nucleolus may be partly embedded in, or sticking to, the surface of the larger acidophilic nucleolus. The cytoplasmic contents appear in the form of distinct, spherical, refractile granules of a bright red colour. The ground cytoplasm is distinctly basophilic and forms compact strands and masses between the granules. In Mallory's triple stained preparations, the secretory granules are extremely fuchsinophilic and the ground cytoplasm is colourless and not discernible. The 3rd type of gland cell (ac.gl.) is localized on the sides of the raphe. It comprises large saccular cells with spheroidal and very basal nuclei. The larger part of the cell body is occupied by a homogeneous slightly acidophilic secretion which stains a bright red in Mallory's triple stained preparations. The basic cytoplasm is scanty and is confined to a small area around the nucleus, as well as to a very thin peripheral strip sur-

rounding the secretory mass. In some of these cells, the secretion seems to undergo a process of dissolution and is evacuated into the lumen, leaving the cell almost empty.

RECTUM AND ANAL GLAND

The *rectum* (Fig. 58, rct.) differs from the post-intestine in having a relatively wider lumen and less pronounced internal foldings. The most conspicuous of the latter are 2 adjacent ciliated rectal folds (rct.fl.) which run longitudinally in continuation of the post-intestinal raphe. The connective tissue and muscular coats of the rectal walls are similar to those of the post-intestine.

The lining mucosa of the rectum is largely formed of non-ciliated columnar cells (Fig. 22, cl.c.) which have distinct brush borders and are closely similar to those which dominate in the mucosa of the post-intestine. Mucus-secreting cells of the goblet type (mc.c.) are also encountered in this epithelium.

Ciliated columnar cells are confined to the 2 major rectal folds on which the cilia beat in 2 opposite directions towards the groove enclosed between the 2 folds. The epithelial coverings of these folds also contain few acidophilic gland cells similar to those encountered on the sides of the post-intestinal raphe (see Fig. 20, ac.gl.).

The presence of various absorptive cells with distinct brush borders in the different regions of the mucosal lining of the intestine and rectum indicates that, regardless of what has been reported by Andrews (1965) for *Pomacea canaliculata*, absorption does take place in these organs in *Marisa cornuarietis*.

It is worth noting that the different types of connective tissue cells, and in particular the globulocytes (gb.c.), are more abundant in the walls of the intestine and alimentary tract in general than they are in many other organs.

A well-developed *anal gland* (Fig. 58, an.gl.), in the form of a long compressed diverticulum, opens in the rectum near

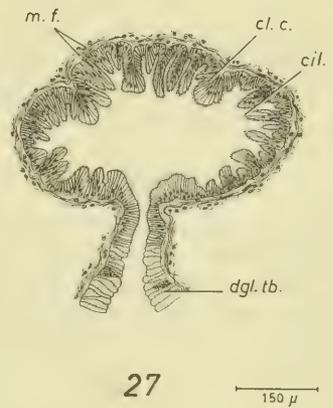
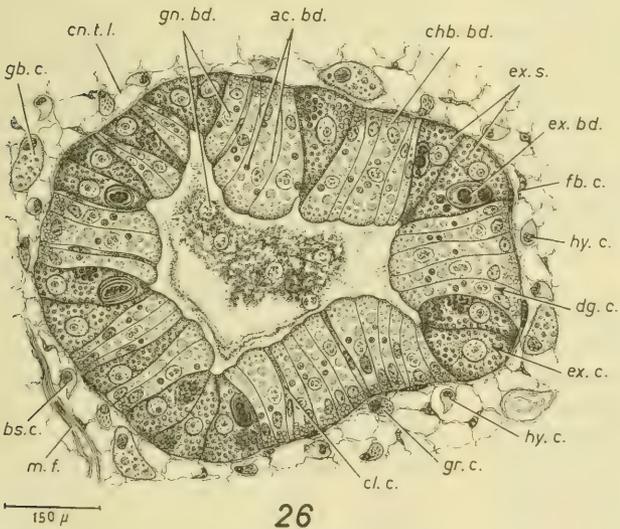
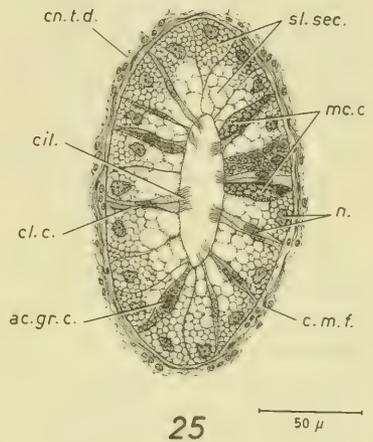
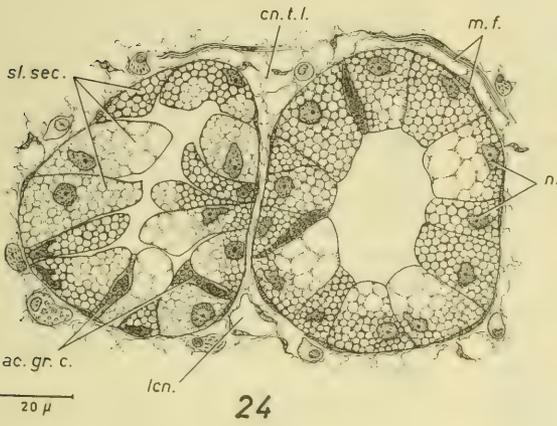
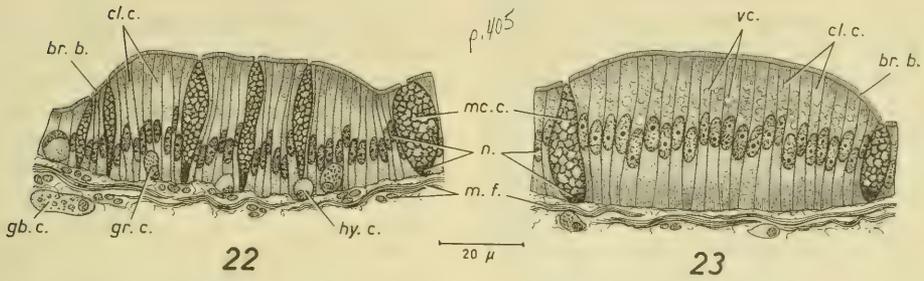


FIG. 22. General lining epithelium of the rectum. FIG. 23. Lining epithelium of the anal gland. FIG. 24. Part of a cross section through the salivary gland showing 2 of its tubules (H - E). FIG. 25. Cross section through a salivary gland duct. FIG. 26. Cross section through a salivary gland tubule (Mallory's). FIG. 27. Cross section through a digestive gland duct.

the base of the anal papilla. It lies within the mantle roof above the pallial gonoduct, and extends more or less longitudinally parallel to the rectum. It is highly lobulated and is often externally conspicuous by virtue of its reddish or brownish colour, which varies in intensity from one snail to the other.

The gland is embedded in the connective tissue of the mantle and surrounded by a layer of circular muscle fibres. It is lined with a simple epithelium of non-ciliated columnar cells (Fig. 23, cl.c.) which vary greatly in height; thus forming a number of longitudinal ridges. The cells have brush borders (br.b.) and oval or elliptical nuclei situated near the middle of the cells. Each contains 1 or 2 spherical nucleoli and abundant chromatin granules. The apical cytoplasm is dense and fibrillar. Below it, and above the nucleus, there is a region of coarsely granular and less acidophilic cytoplasm with small vacuoles (vc.). The basal cytoplasm is devoid of such vacuoles and presents a large number of fibrillae.

Among the columnar cells of this epithelium there are mucus-secreting cells of the goblet type (mc.c.), which are mostly saccular and have basal ovoid nuclei. They are relatively sparser than the corresponding mucus elements of the rectal mucosa. The lumen of the anal gland contains a vacuolated substance which acquires a blue colour in H - E preparations.

SALIVARY GLANDS

Two salivary glands (Fig. 38, sl.gl.) of a branched tubular nature lie on either side of the pro-oesophagus and lead into the buccal cavity by 2 long narrow ducts (sl.gl.d.). They are enveloped by dense connective tissue which is connected in several places with the connective tissue coating of the pro-oesophagus and oesophageal pouches. Within the glands themselves, there are thin strands of loose vascular connective tissue which extend between the glandular tubules.

In section, the salivary gland tubules (Figs. 24, 59, 60) show distinct lumina around which the secretory cells are arranged in one layer. These cells vary widely in form in the wall of any one tubule, apparently representing different phases in the process of elaboration of the salivary secretion. They may be tall columnar, short pyramidal, large globular or irregular. The accumulation of secretion in some of these elements causes the neighbouring cells to acquire the pyramidal or irregular forms.

In some cells the cytoplasm is crowded with distinct secretory spherules (sl. sec.) of moderate size. These spherules do not stain well with routine stains but take on a dark blue colour in toluidine blue preparations and give a positive PAS reaction. The scanty ground cytoplasm lying in between the spherules gives a basophilic reaction. The blue and violet colours it assumes after H - E and Mallory's triple stains respectively help to define the outlines of the secretory spherules.

As the amount of secretion increases in the cell, the ground cytoplasm gradually becomes scantier, with the result that the cell becomes less basophilic and its secretory spherules become ill-defined. This gradual change may take place synchronously all over the cytoplasm of the cell, or may proceed in one part of the cell at a faster pace than it does in another. In later stages, the secretory spherules coalesce into larger vacuolated masses. Eventually the cell appears distended with a large amount of vacuolated liquid secretion which is poured out into the lumen of the tubule.

The nucleus in these secretory cells is usually oval and basal, has a conspicuous nuclear membrane and contains a large spherical nucleolus. The nucleoplasm is outstandingly rich in coarse chromatin granules. Certain changes in the position and form of the nucleus take place during the above mentioned sequence of changes noticed in the cells. The pressure exerted by the accumulated secretory spherules cause the nucleus to

become pyramidal, polygonal or of irregular shape, and the nuclear membrane to show some inward indentations. At the same time the nucleus becomes less chromophilic and its chromatin granules become less well-defined. Sometimes the whole nucleoplasm stains homogeneously and does not show any structural detail.

Not all the glandular cells, however, can be fitted into the above mentioned sequence of changes. Some smaller elements (ac.gr.c.) of various forms appear crammed with small, spherical and distinctly acidophilic granules which acquire a bright red colour after H - E staining and a bright blue colour in Mallory's triple stained preparations. No basophilic ground cytoplasm is discernible in these cells and the nucleus does not present any marked difference from those of the preceding dominant elements except smaller size. Whether these cells constitute an independent type of secretory elements in the salivary glands, or only represent an early stage in the sequence of changes displayed by the 1st type, is not known. It is noteworthy, however, that most of the recent workers believe that there is only one type of gland cell in the gastropod salivary glands, whose aspect varies with the different functional phases of the cells (Carriker & Bilstad, 1946).

A *salivary gland duct* arises from several tributaries in the posterior 1/3 of each gland. It presents a remarkably wide regular lumen near its origin (Fig. 38, sl.gl.d.), but narrows gradually as it proceeds forwards through the anterior 2/3 of the gland, reaching its minimum diameter as it issues from the anterior edge of the gland (Figs. 5, 12, 37) and runs forwards to open into the buccal cavity. The duct is surrounded throughout its length by a thin layer of dense connective tissue (cn.t.d.), and as it courses along the dorsal wall of the buccal mass it acquires a thin coating of the circular muscle fibres.

The salivary gland ducts are not purely conducting but also secretory as is the case in most prosobranchs (Fretter & Graham, 1962). The same cellular elements that are encountered in the walls of the glandular tubules are found in the lining epithelium of each duct (Fig. 25), together with ciliated columnar cells and a few unicellular mucus glands. Although of widely different forms, all the cells lining the duct are of nearly the same height, with the result that the lumen retains a regular outline throughout the length of the duct. The dominant secretory type of cells shows a similar variety of form and presents the same secretory phases that have been described above for the secretory cells of the glandular tubules. The 2nd secretory type, i.e. the acidophilic granular cells (ac.gr.c.), are relatively more abundant in the walls of the tubules. The mucus-secreting elements (m.c.) are of the ordinary goblet type, while the columnar cells (cl.c.) are highly squeezed between the glandular elements, but have broad apices which carry long cilia (cil.). Cilia are found throughout the whole salivary gland duct as well as in its smaller tributaries.

DIGESTIVE GLAND

This large dark brownish or greenish-brown gland occupies a considerable part of the visceral hump and consists of 2 very unequal lobes, a small right anterior lobe (Figs. 42-45, a.dgl.), and a much larger left posterior lobe (p.dgl.), which extends from below the stomach backwards as far as the apex of the hump. Both lobes are externally enveloped by the general covering integument of the visceral mass (itg.). The latter is composed of an outer single-layered epithelium (Fig. 13), which rests on a distinct basement membrane, followed by a thin layer of circular muscle fibres. The epithelial cells range from subcolumnar to cuboidal or even subcuboidal and contain some

brown or black pigment (pg.). They have oval, spherical or discoidal nuclei, each with a distinct nucleolus and scanty chromatin.

Among the normal epithelial cells there are spherical or saccular mucus-secreting cells (mc.c.). These are occasionally far larger than the normal epithelial cells. Their nuclei may be located at the base or at one side of the cell, very close to the cell membrane. Another type of gland cell (gl.c.) is also encountered in this epithelium and comprises elements which have nearly the same shape as the mucus cells but are generally smaller in size. They contain distinct acidophilic spherules which sometimes coalesce to form larger acidophilic globules. Thus they contrast with the mucus cells in the form of their contents and in their reaction towards Mayer's mucicarmine and toluidine blue stains. Both types of unicellular glands have their exact parallels in other parts of the outer epithelial covering of the body, such as that of the pallial region (Lutfy & Demian, 1965).

Inside of the muscle layer there exists a layer of loose connective tissue (cn.t.l.) which varies widely in thickness around the circumference of the gland and which is continuous with the connective tissue intervening between the glandular tubules. This tissue is especially rich in blood spaces (lcn.) and concretions (con.). All 5 types of connective tissue cells are encountered in it in great abundance. They exhibit a high amoeboid activity, especially the hyalocytes (hy.c.), granulocytes (gr.c.) and globulocytes (gb.c.). The pseudopodia developed by these cells are exceptionally numerous and frequently gain direct contact with the digestive gland's tubules.

The digestive gland is of the compound branched tubular type. Each of its 2 lobes is formed of a highly complicated system of branching tubules (Fig. 61, dgl.tb.) which open into a relatively small number of fine ductules that collect into 3 main digestive gland ducts, a single

duct from the anterior lobe (Figs. 44, 45, a.dgl.d.) and 2 ducts from the posterior lobe (Fig. 42, p.dgl.d.).

A very thin layer of circular muscle fibres surrounds each hepatic tubule. The cells constituting the wall of the tubule are arranged around wide irregular lumina and are easily recognizable as 2 main types: acidophilic "digestive cells" and basophilic "excretory cells". A review of the literature shows that there frequently is some confusion regarding the identity of the corresponding digestive gland elements of different gastropods and the functions attributed to them.

i) Digestive cells

The digestive cells (Figs. 26, 62-64, dg.c.) correspond to the gland cells of Prasad (1925), the absorbing cells of Graham (1932), the hepatic cells of Michelson (1955), the digestive cells of Carriker & Bilstad (1946), Cleland (1954), Pan (1958) and Fretter & Graham (1962), and the secretory and digestive cells of Andrews (1965). These recently adopted terms are also synonymous with such older terms as liver cells, palisade cells, resorption cells, secretion cells and vacuolar cells (Carriker & Bilstad, 1946). They were claimed to be occasionally ciliated (Graham, 1932), to elaborate some digestive enzymes for extracellular digestion, and to be engaged either in the ingestion and intracellular digestion of particulate food, or in the absorption of digested food in solution, the secretory and digestive activities being synchronized throughout the gland (Carriker & Bilstad, 1946; Andrews, 1965). Moreover they are believed by some authors to store fat and glycogen (Hurst, 1927).

The digestive cells are by far the most numerous elements in the walls of the digestive gland tubules. They are long columnar or club-shaped, with domed distal apices and flat bases by which they rest on a very thin basement membrane. They vary greatly in length within one and the same tubule. The

nucleus is basal, usually oval, but may be elliptical, spheroidal or even irregular; it presents one conspicuous nucleolus. The nuclear chromatin is usually difficult to make out, but occasionally it appears in the form of tiny spherical granules. In many cases, especially when the cell is crowded with secretion, the nuclear membrane shows some depressions and indentations.

Inside the major part of the cell body, the cytoplasm shows various degrees of vacuolation and different kinds of contents. The vacuolation is usually so pronounced and the cell contents are so numerous that the ground cytoplasm appears confined to thin strips between these structures. However, a lump of dense ground cytoplasm is usually seen around the nucleus and/or in other parts of the cell.

In the distal part of each cell is a narrow marginal zone of more or less dense and strongly acidophilic cytoplasm that acquires a deep violet colour in Mallory's triple stained preparations. This zone is serrated on its inner surface because of a number of small or moderate-sized vacuoles below it. The free borders of the cells are frequently covered by one or more thin lamellae of an acidophilic material, which has the same staining reactions as the substance of the ground cytoplasm. The lamellae usually extend over several adjacent cells.

Three different types of cytoplasmic contents are encountered in the different digestive cells which may contain more than one of these components:

(a) The first component is represented by small spherical highly refractive bodies (chb.bd.) between which the cytoplasm forms a close-meshed network. These are more frequently encountered in the basal halves of the cells and do not take up any of the stains used but remain always colourless. Some of them coalesce, forming larger spheroidal colourless refractive droplets. These bodies seem to correspond to what Andrews (1965) recognized as lipid deposits in the digestive cells of *Pomacea*

canaliculata, and to the fatty and lipid droplets described by Graham (1932) in *Patella vulgata*.

(b) The 2nd component (gn.bd.) consists of a lightly chromophilic, finely granular and slightly refractive material occurring either in groups of spheroidal bodies or in larger lumps, which are faint blue when treated with Mallory's triple stain and red after H - E staining. These may occupy different sites in the cell and are occasionally also found in large numbers in the lumina of the hepatic tubules as well as in the cavity of the gizzard. Each group of such spheroidal bodies, or single large lump, shows a more or less definite acidophilic limiting rim. They probably correspond to the so-called "green bodies" of Andrews (1965).

(c) The 3rd component is formed of large homogeneously acidophilic bodies (ac.bd.) of spheroidal, oval or irregular form, which lie within colourless vacuoles in the digestive cells. These bodies assume a bright red colour in H - E preparations and a blue colour after Mallory's stain. Sometimes the lumina of the tubules are seen to contain large amounts of a liquid substance which shows the same staining reactions as these bodies. This is especially true in preparations taken from starved snails. These bodies most probably correspond to the small spherules of liquid deposits described by Andrews (1965) in the secretory phase of the digestive cells in *Pomacea canaliculata*. She suggests that these spherules represent some enzymatic secretion.

No fragments of the digestive cells were ever encountered in the lumina of the digestive gland tubules, which fact indicates that the type of secretion here is neither apocrine nor holocrine as described for other gastropods (Krijgsman, 1925; Carriker & Bilstad, 1946; Pan, 1958).

ii) Excretory cells

The excretory cells (Figs. 26, 62-64, ex.c.) correspond to the ferment cells

of Prashad (1925) and Michelson (1955), the secreting cells of Graham (1932), the lime cells of Carriker & Bilstad (1946) and Pan (1958), the calcium secreting cells of Cleland (1954), and the excretory cells of Fretter & Graham (1962) and Andrews (1965). They are also synonymous with such older terms as calcareous and chalk cells (Carriker & Bilstad, 1946).

The excretory cells are present in much smaller numbers than the digestive cells. They are quite distinct and easily distinguished from the latter by virtue of their different morphological characteristics and staining reaction. They are short pyramidal or cone-shaped, but may sometimes be columnar, and are either present singly or in groups of 2-4. They are also markedly shorter than the digestive cells and therefore appear wedged in between groups of the latter cells. The difference in length between the 2 types of cells is partly responsible for the irregularity of the lumina of the digestive gland tubules which, in cross section, send narrow branches towards the shorter excretory cells. At the same time the basal portions of these cells look as if they were pressing against the underlying basement membrane; they bulge out into the surrounding connective tissue (Figs. 63, 64) and give the tubule a more or less angular cross sectional outline.

The excretory cell has a characteristically large nucleus, with a distinct vesicular appearance, a very distinct nuclear membrane and a large conspicuous spherical nucleolus. The latter usually occupies a slightly eccentric position and has many chromatin granules attached to it. Up to 3 nucleoli may be encountered in the nucleus. The nucleoli invariably assume a homogeneous blue colour in H - E preparations and each shows a brightly coloured central sphere or nucleolenema.

The body of the excretory cell is usually crowded with a large number of spherules (ex.s.) of regular form but

different sizes. The general colour of the cell, which is largely blue with H-E and reddish-purple with Mallory's triple stains, is mainly due to these spherules. The smallest spherules are blue after H - E, very faint violet after Mallory's triple stain and meta-chromatically red after toluidine blue (Fig. 64). Larger spherules each presents a central core of the same staining properties, surrounded by a zone of lighter colour and an outer rim of the same nature as the central core. The lighter-coloured zone increases in thickness with the increase in size of the spherule and therefore the spherules grow gradually paler.

The ground cytoplasm forms some acidophilic masses around the nucleus and in the apical portions of the cells, as well as thin strands which show between the spherules and assume a bluish-red colour in H - E preparations. Thicker strands of this cytoplasm occasionally surround groups of spherules with the result that they appear in the cell in more or less distinct aggregates.

Each excretory cell contains also 1 - 2 large, dark brownish, oval or spherical excretory bodies (ex.bd.), each composed of 1-3 large brown spherules which are enveloped by a few lamellae of the same substance, but of a lighter colour. These excretory bodies are generally chromophobic, usually retain their natural colour and appear located inside colourless vacuoles. They are apparently released into the lumina of the hepatic tubules by the rupture of the distal membranes of the excretory cells. They are frequently encountered in the lumina in prepared sections, and can moreover be macroscopically seen in freshly dissected specimens, under a binocular microscope, flowing out of the opening of the hepatic vestibule into the gizzard, where they become entangled with mucus to form the liver string.

These bodies definitely represent the final stage in the elaboration of some excretory material within the excretory cells. All stages in their formation can

be followed in detail. The process starts in the excretory cells by the formation of the above described small metachromatic spherules, which gradually grow in size, as they receive additional material from the ground cytoplasm, and at the same time become aggregated in groups or nests. The spherules of each group then gradually lose their chromophilia and become yellowish, brownish, then dark brown in colour, the process starting at the circumference of each spherule and then proceeding inwards. Meanwhile some spherules fuse together forming larger spheres which are further surrounded by a common lamellar covering of the same substance. The excretory material which goes into the formation of these bodies is in all probability absorbed by the excretory cells through their basal portions which bulge into the surrounding connective tissue and therefore come very close to the blood sinuses in it. A similar hypothesis was advanced by Andrews (1965) in *Pomacea canaliculata*.

Besides the 2 main types of cells described above, which constitute the walls of the digestive gland tubules, very narrow elongated fusiform elements (Fig. 26, cl.c.) are occasionally encountered in these walls. These cells usually give a general reaction similar to that of the cells among which they lie, being acidophilic when present between the digestive cells and basophilic when encountered very close to the excretory cells. Thus it seems reasonable to assume that they are undifferentiated epithelial cells on their way to transform into either one or the other of the 2 major types.

The *digestive gland ducts* (Figs. 42-45, a.dgl.d. and p.dgl.d.) and their tributaries are easily distinguished from the tubules proper. Their lining epithelia are highly folded and made up of elongated columnar cells (Fig. 27, cl.c.). The oval or elliptical nuclei of these cells are either distinctly basal or lie in the lower half of the cell, depending

on the position of the cell on the fold. Below the basement membrane, there is a distinct muscle layer of alternating circular and longitudinal fibres (m.f.).

The hepatic tubules open directly into the hepatic ductules without the intervention of distinct non-secretory necks. A large number of tubules also open in the same manner into the 3 main ducts of the digestive gland.

The lining epithelium of the *hepatic vestibule* (Fig. 43, h.vs.) is thrown into a large number of oblique ridges and grooves which carry strong cilia. The majority of these folds converge towards a longitudinal ridge which runs along the middle of the floor of the vestibule. The cilia carried by the latter ridge help to direct the stream of excretory bodies along the hepatic vestibule into the gizzard.

The wall of the vestibule has the same histological structure as that of the digestive gland ducts. It is lined with tall ciliated columnar cells, followed by a thin muscular coat, both of which are of much the same nature as the corresponding structures in the walls of the digestive gland ducts. But, while the lining of these ducts is completely devoid of gland cells, a number of goblet-shaped cells are encountered among the columnar cells lining the vestibule. These cells are restricted to the ventral ridge and give a negative reaction after Mayer's mucicarmine and toluidine blue. They scarcely take any colour with any of the stains used, and their contents show in the form of colourless granules with a very faint meshwork in between the granules.

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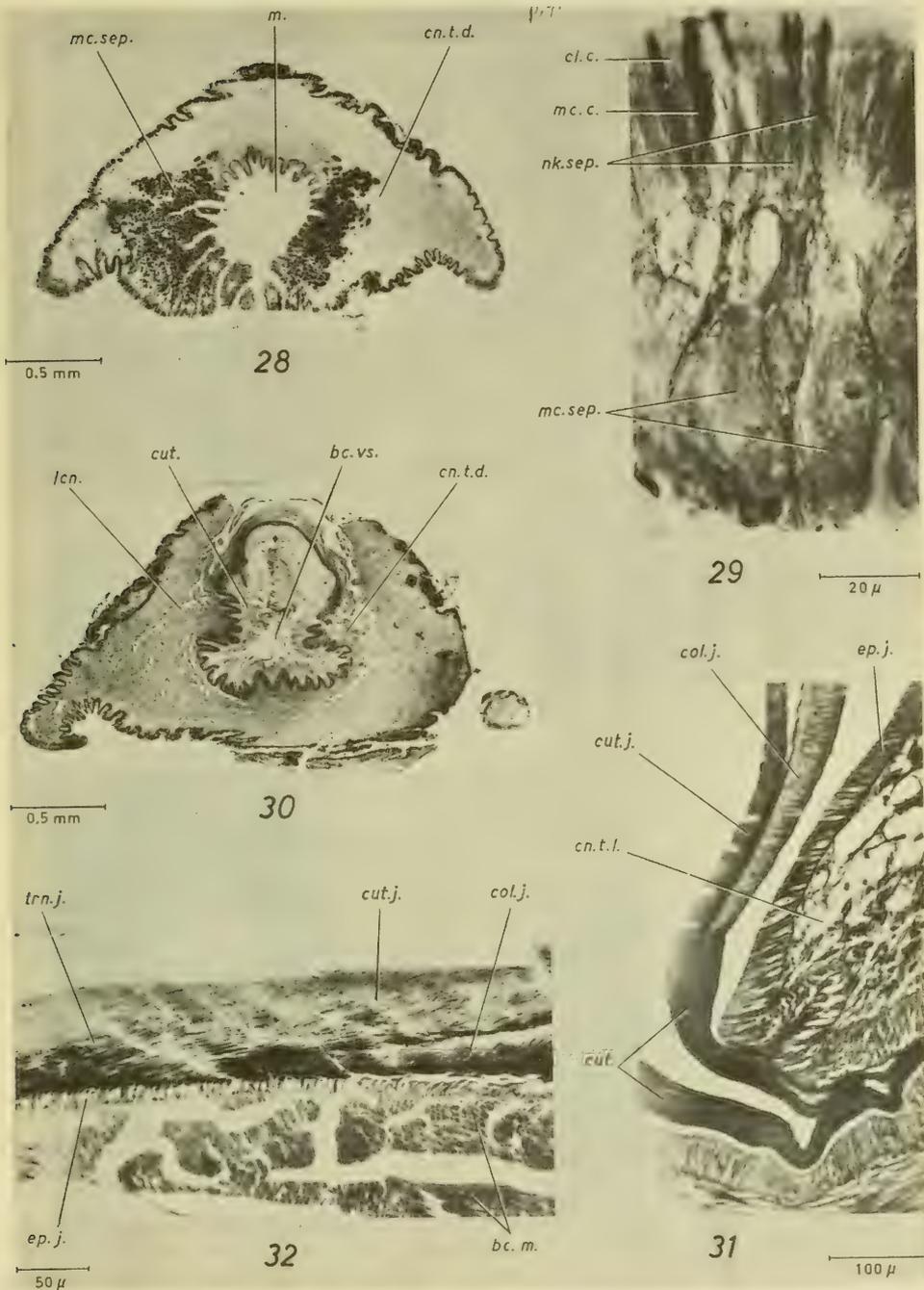


FIG. 28. Cross section through the periostomal region (Zenker, Mucicarmine). FIG. 29. Epithelium and subepithelial mucus glands of the peristome (Zenker, Toluidine blue). FIG. 30. Cross section through the region of the buccal vestibule (Zenker, Mucicarmine). FIG. 31. Part of a cross section through the mandibular region of the buccal mass (Zenker, Iron H - Van Gieson's). FIG. 32. Cross section through the transitional region of the jaw (Zenker, Mallory's).

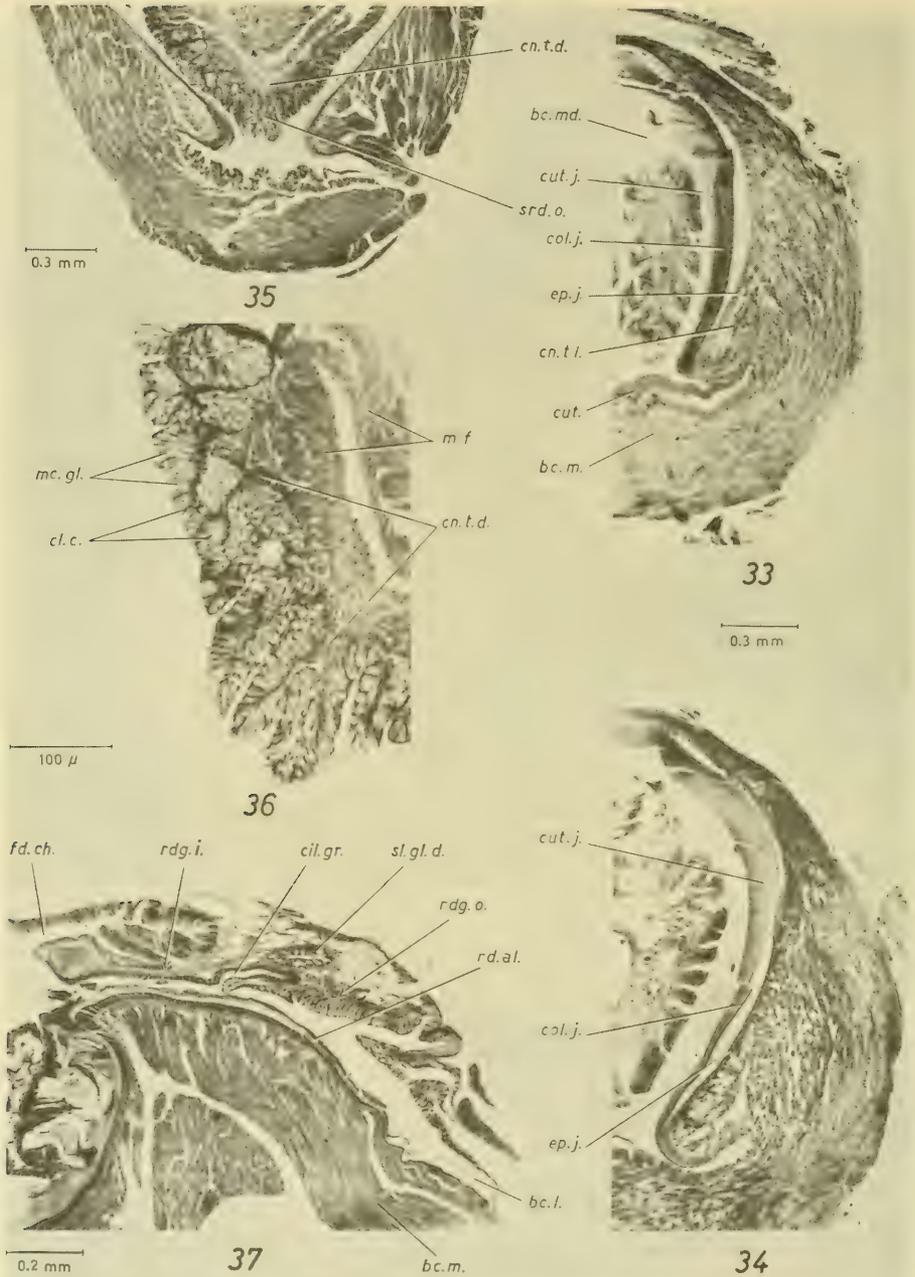


FIG. 33. Half of a cross section of the buccal mass passing through the anterior third of the jaw (Flemming's, Iron H - Van Gieson's). FIG. 34. Half of a cross section of the buccal mass passing through the posterior third of the jaw (Flemming's, Iron H - Van Gieson's). FIG. 35. Part of a cross section of the buccal mass showing the subradular organ (Zenker, H - E). FIG. 36. Enlarged portion of Fig. 35 showing some epithelial folds of the subradular organ. FIG. 37. Part of a cross section of the buccal mass through the odontophoral region (Zenker, H - E). (see Fig. 5).

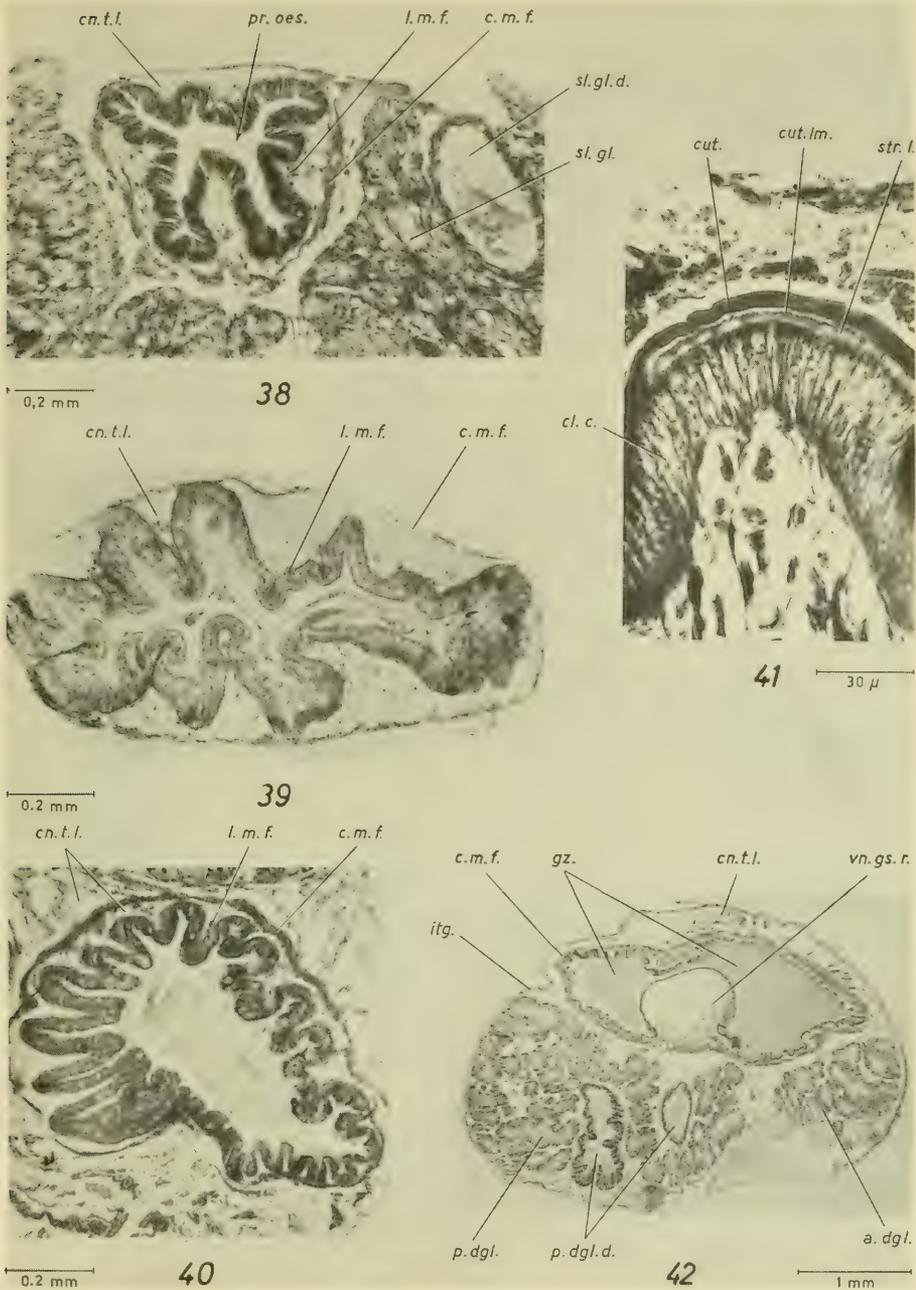


FIG. 38. Part of a cross section through the pro-oesophagus and salivary glands (Zenker, H - E). FIG. 39. Cross section through the crop (Flemming's, Iron H - Van Gieson's). FIG. 40. Cross section through the post-oesophagus (Zenker, Mallory's). FIG. 41. Lining epithelium of the gizzard (Zenker, Mallory's). FIG. 42. Cross section of the visceral mass passing through the posterior region of the gizzard (Zenker, H - E).

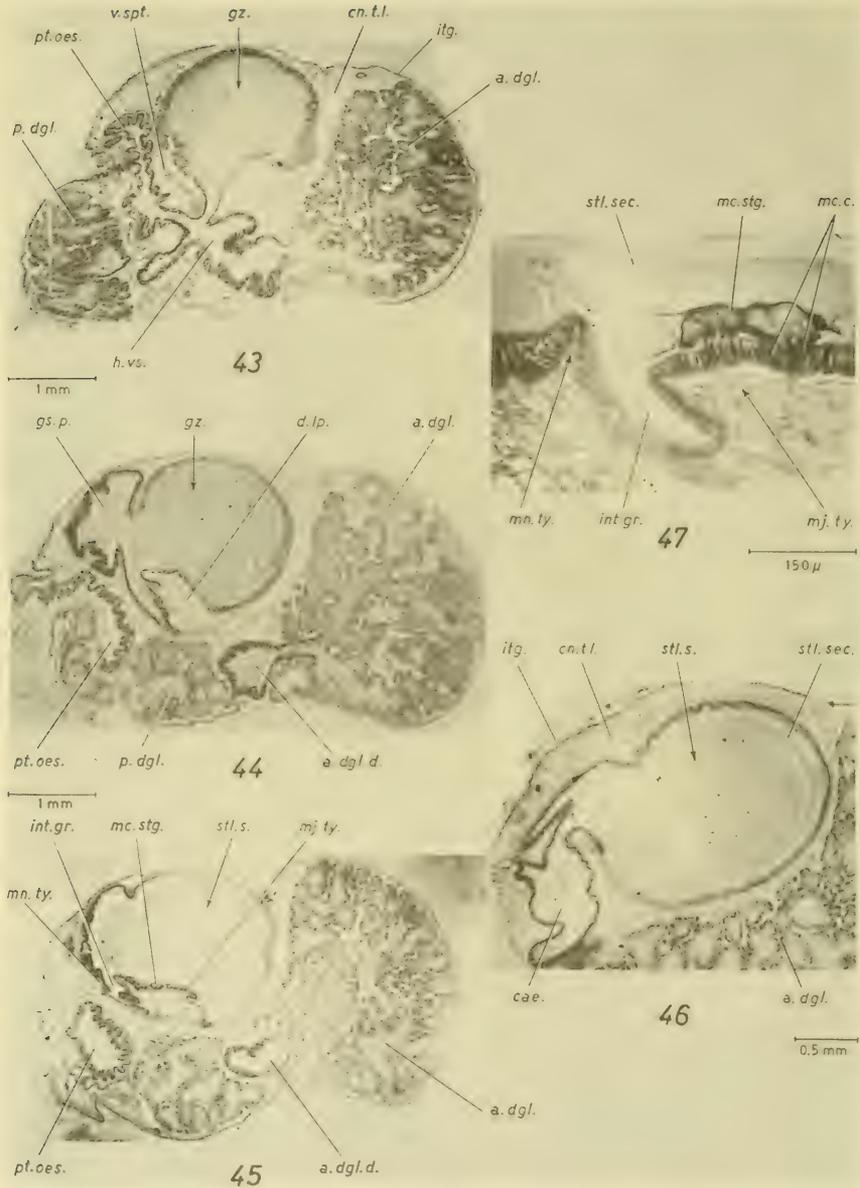


FIG. 43. Cross section of the visceral mass passing about the middle of the gizzard, where the hepatic vestibule opens (Zenker, H - E). FIG. 44. Cross section of the visceral mass passing through the distal region of the gizzard and showing the posterior gastric pouch and the directing lappet (Zenker, H - E). FIG. 45. Cross section of the visceral mass passing through the style sac (Zenker, Toluidine blue). FIG. 46. Part of a cross section of the visceral mass passing through the most distal part of the style sac and the caecum (Zenker, Mucicarmin - haemalum). FIG. 47. Part of a cross section of the style sac showing its major and minor typhlosoles (Zenker, Mucicarmin).

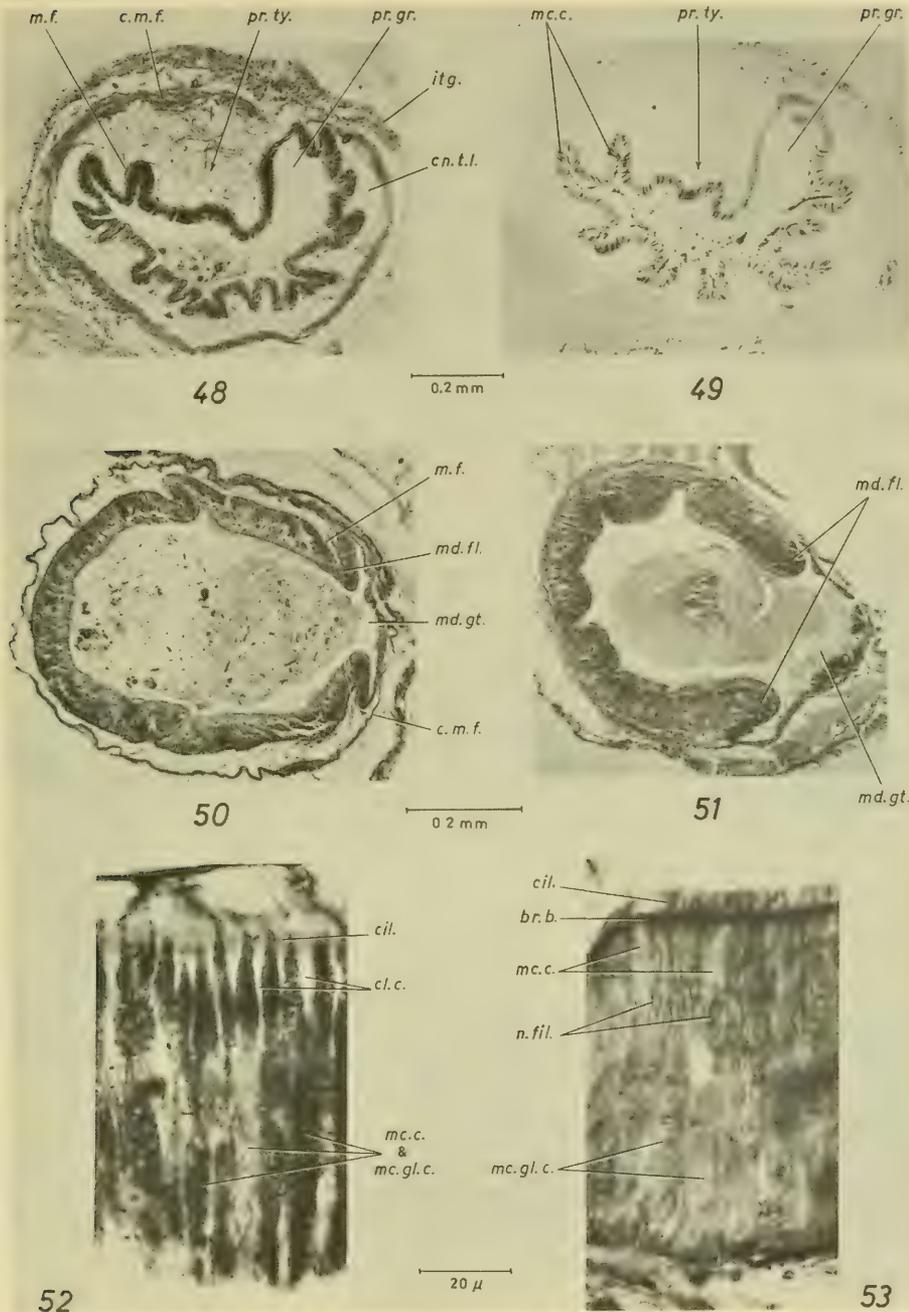


FIG. 48. Cross section through the pro-intestine (Zenker, H - E). FIG. 49. Same as in Fig. 48 but stained with toluidine blue to show the mucus cells. FIG. 50. Cross section through the proximal part of the mid-intestine (Zenker, Mallory's). FIG. 51. Cross section through the distal part of the mid-intestine (Duboscq-Brazil, H - E). FIG. 52. Lining epithelium of the mid-intestine (Zenker, Mucicarmine). FIG. 53. Lining epithelium of the mid-intestine (Duboscq-Brazil, Mallory's).

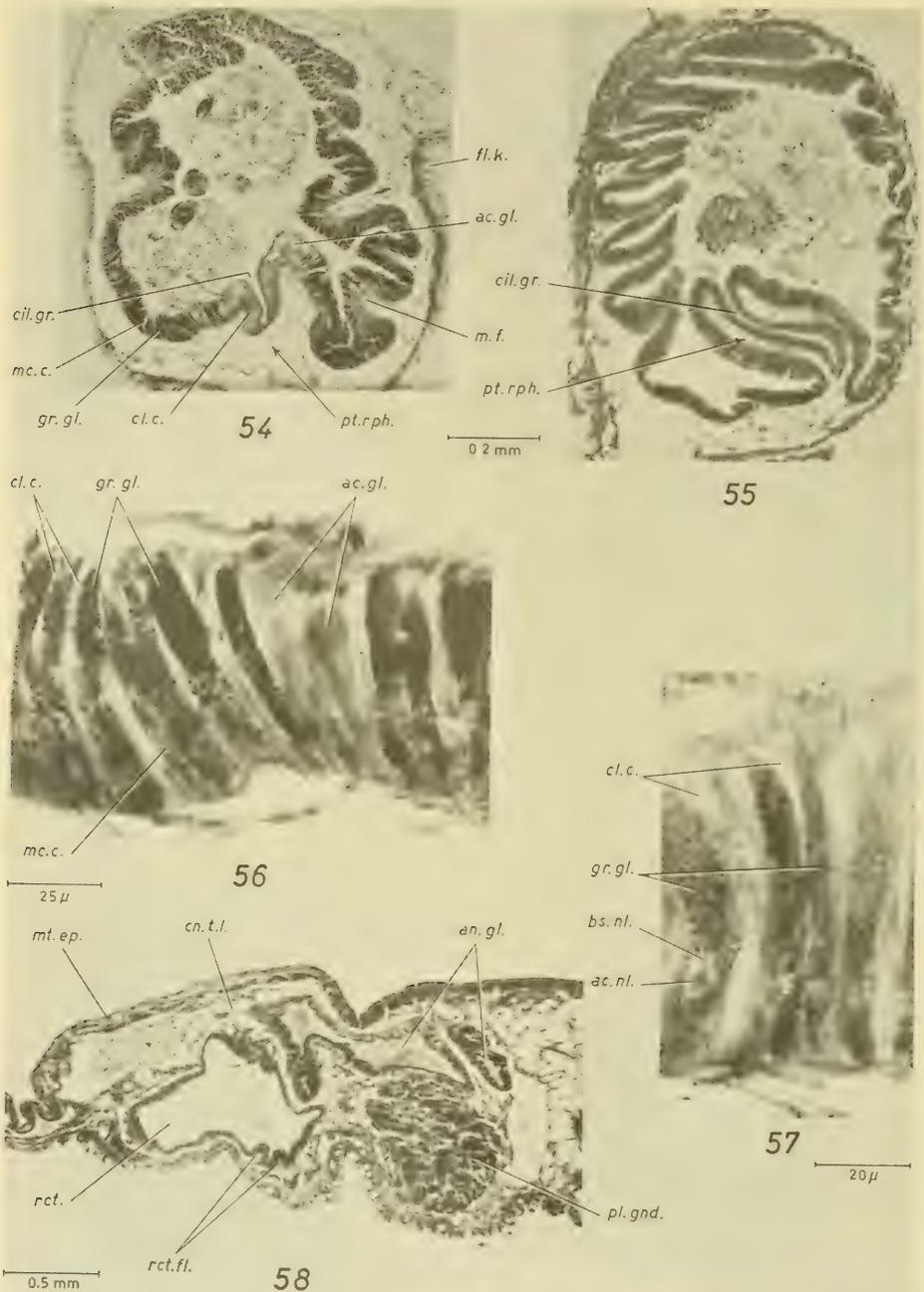


FIG. 54. Cross section through the proximal part of the post-intestine (Zenker, Mallory's). FIG. 55. Cross section through the distal part of the post-intestine (Duboscq-Brazil, H - E). FIG. 56. Enlarged portion of Fig. 54 showing the epithelium on one side of the post-intestinal raphe. FIG. 57. General lining epithelium of the post-intestine (Zenker, H - E). FIG. 58. Part of a cross section of the mantle roof passing through the rectum and the anal gland (Zenker, H - E).

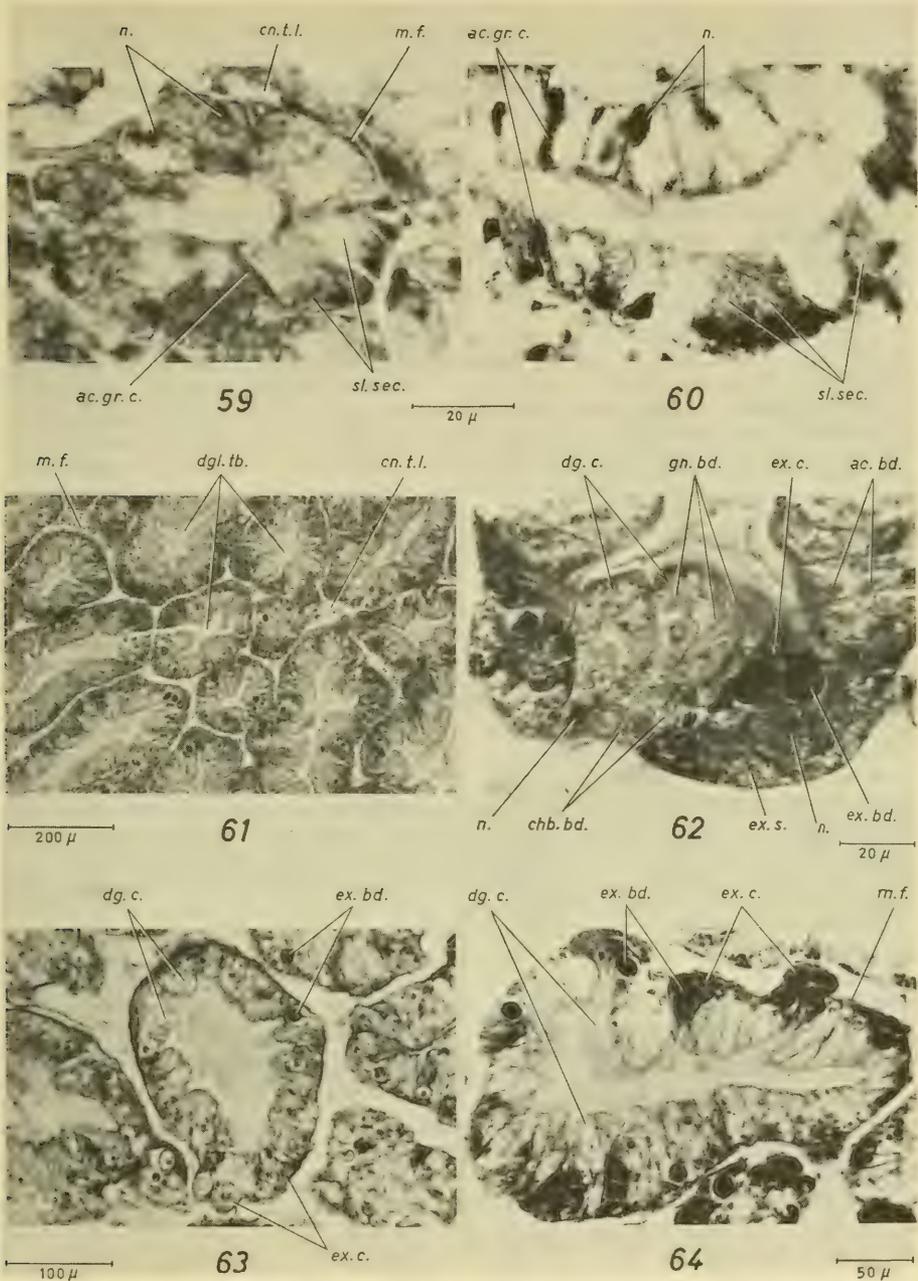


FIG. 59. Cross section through a salivary gland tubule (Zenker, H - E). FIG. 60. Cross section through a salivary gland tubule (Flemming's, Iron H - Van Gieson's). FIG. 61. Part of a cross section through the digestive gland (Zenker, H - E). FIG. 62. Epithelium of a digestive gland tubule (Zenker, H - E). FIG. 63. Cross section through a digestive gland tubule (Zenker, Mallory's). FIG. 64. Cross section through a digestive gland tubule (Zenker, Toluidine blue).

RESUMEN

HISTOLOGIA DEL SISTEMA DIGESTIVO DE *MARISA CORNUARIETIS*
(MESOGASTROPODA-AMPULLARIIDAE)

R. G. Lutfy and E. S. Demian

Existe muy escasa información sobre la histología de los Ampullariidae, y de los Prosobranchia en general. Este trabajo trata de una investigación histológica detallada del sistema digestivo del caracol *Marisa cornuarietis* (L.), que se hizo para encontrar una base mejor para otras investigaciones histoquímicas, indispensables para interpretar la función del sistema.

El epitelio de la cavidad bucal esta diferenciado en varias regiones cuticularizadas y ciliadas. El peristoma es rico en glandulas unicelulares subepiteliales. El vestibulo bucal tiene un forro epitelial y se continúa en la región maxilar de la cavidad, en la cual tal epitelio secreta las dos mandibulas. El material de estas maxilas está diferenciado en dos capas superimpuestas, la superior laminada-cuticular, y la inferior de naturaleza química distinta dispuesta en columnas verticales. El epitelio de la parte odontófora de la boca presenta regiones especializadas con caracteres distintos, como el órgano subradular, los dos lomos bucales que envuelven el canal alimenticio dorsal, la barra del borde post-radular, y los epitelios sub y supranodulares. El mismo tipo de célula también abunda en ciertas regiones del epitelio del bulbo esofágico.

El esófago se diferencia histologicamente en pro-medio y post-esófago. El forro epitelial de las tres regiones se compone en su mayoría de columnas celulares ciliadas. Los del esófago medio particularmente, muestran una actividad secretora muy elevada.

El estómago consiste en una molleja proximal en forma de U y un saco distal tubular del estilete. La molleja está forrada con una mucosa cuticularizada no glandular. Dos sacos, o bulbos gástricos ciegos, se abren aquí, y una extensión en forma de solapa prominente se proyecta desde su fondo, la cual sirve para dirigir la materia secretada usada por la glandula digestiva. Estos sacos gástricos, así como el del estilete, están forrados con células columnares de variada longitud y ciliadas, y al mismo tiempo muestra gran actividad secretora. La secreción envuelve los cuerpos cilíndricos fecales, como varitas, que se mueven distal y espiralmente a lo largo del saco del estilete e intestino. La capa mucosa del ciego es histologicamente muy similar a aquella del saco del estilete.

Un intestino anterior, medio y posterior puede distinguirse, cada uno con sus característicos pliegues internos y tipo de células excretoras, así como ciliadas y absorbentes. De tales tipos celulares se describen 4 del intestino medio y 5 del posterior. Células absorbentes, pinceladas y distintas, existen en diferentes partes del intestino y recto, un hecho que indica que la absorción tiene lugar en esos órganos.

Las glándulas salivares son del tipo ramoso tubular. Los tubos tienen, posiblemente, dos tipos de células secretoras mostrando diferentes fases de actividad. Los ductos están revestidos con células secretoras ciliadas.

Los 2 lóbulos de la glándula digestiva consisten de un complicado sistema de tubos ramificados con 2 tipos principales de células, las digestivas que contienen cuerpos secretados, y las células excretoras las cuales acumulan en sus cuerpos ciertas sustancias excretoras en forma de concreciones marrones.

АБСТРАКТ

ГИСТОЛОГИЯ ПИЩЕВАРИТЕЛЬНОЙ СИСТЕМЫ
MARSIA CORNUARIETIS (MESOGASTROPODA: AMPULLARIIDAE)

Р. Г. Латфи и Е. С. Демьян

О гистологии моллюсков *Ampullariidae* и *Prosobranchia* вообще очень мало известно. В настоящей работе рассматривается гистологическое строение пищеварительной системы брюхоногого моллюска *Marsia cornuarietis* (L.). Это исследование должно было послужить прочным основанием для последующих гистохимических исследований, необходимых для понимания способа функционирования пищеварительной системы.

В покровном эпителии, выстилающем буккальную (глоточную) полость, различаются кутикуляризованные, реснитчатые и богатые железами области. Перистом богат одноклеточными субэпителиальными железами.

Буккальное (глоточное) преддверие имеет кутикуляризованную выстилку, переходящую в мандибулярную область буккальной полости, покровы которой секретируют две челюсти. Вещество, из которого образованы челюсти, дифференцировано на два накладываются друг на друга слоя: верхний-пластинчатый кутикулярный слой и нижний, состоящий из вертикальных столбиков иной химической природы. Выстилка одонтофорной (языковой) части буккальной полости весьма специализирована, с хорошо различимыми участками, такими, как субрадулярный орган, два спинных буккальных гребня, окружающих спинную пищевую бороздку, пострадулярный край, а кроме того суб- и супра-радулярный эпителий. В эпителии субрадулярного органа, на наружных участках спинных буккальных гребней и на пострадулярном крае преобладают клетки особого типа, выделяющие слизь. Клетки такого же типа изобилуют и в некоторых участках покровного эпителия, выстилающего карманы пищевода.

Сам пищевод гистологически дифференцирован на передний, средний и задний отделы. Покровный эпителий этих трех областей в большей своей части состоит из реснитчатых столбчатых призматических клеток. Клетки средней части пищевода имеют особо высокую секреторную активность.

Желудок включает в себя проксимальную U-образно изогнутую часть и дистально расположенный трубчатый мешок для кристаллического стебелька (*style sac*). Проксимальная часть в основном выстлана столбчатым нежелезистым кутикулизованным слизистым эпителием. Ее самый дистальный участок имеет реснитчатую выстилку и действует уже как сортирующий орган. В этой области открываются два слепых желудочных кармана, со дна которых выдается направляющая складка, служащая для направления частиц, выделяемых пищеварительной железой. Желудочные карманы, как и трубчатый мешок для кристаллического стебелька (*style sac*) выстланы столбчатыми клетками различной высоты, которые несут реснички и одновременно показывают высокую

секреторную активность. Их секреция обволакивает фекальные палочки, которые спирально продвигаются в дистальном направлении вдоль мешочка кристаллического стебелька и кишечника. Слизистый эпителий, выстилающий слепой вырост прямой кишки гистологически очень близок к таковому же, выстилающему мешочек для стебелька (**style sac**).

Передняя, средняя и задняя кишка могут быть различимы, поскольку обладают характерными внутренними складками, характерными типами как секреторных, так и реснитчатых и всасывающих клеток. Из этих типов клеток четыре описаны для средней и пять для задней кишки. Всасывающие клетки, с хорошо различимой каемкой щетинок по краю, наблюдаются в различных частях кишечника и в прямой кишке (ректуме), что свидетельствует о том, что в этих органах действительно происходит абсорбция.

Слюнные железы разветвленно-трубчатого типа. Эти трубочки, возможно, имеют два типа секреторных клеток, показывающих разные фазы секреторной активности. Протоки слюнной железы выстланы как секреторными, так и реснитчатыми клетками.

Две лопасти пищеварительной железы (печени) состоят из сложной системы разветвленных трубочек с двумя главными типами клеток: пищеварительными клетками, которые содержат секреторные и абсорбированные вещества различной природы и выделительными клетками, накапливающими в своем теле некоторые экскреторные вещества в форме коричневых конкреций.

ADAPTIVE SIGNIFICANCE OF GASTROPOD TORSION

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ABSTRACT

An important aspect of Garstang's theory of the evolutionary origin of gastropod torsion has not been questioned. This part deals with the advantages supposed to accrue to the veliger larva immediately after the postulated caenogenetic origin and rapid incorporation of torsion. The theory rests here upon 2 basic assumptions. (1) During defensive retraction of a gastropod veliger larva the mantle cavity serves to accommodate and protect components of the cephalopedal mass. (2) Some parts of the cephalopedal mass are more fragile than others; the "head" is considered to be more vulnerable to attack than the foot.

New observations on gastropod larvae show these assumptions to be incorrect. Little remains in the embryology of modern gastropods which can be used in support of an adaptive theory of the caenogenetic origin of gastropod torsion.

Gastropod asymmetry results from 2 separate anatomical phenomena, helical coiling of the visceral mass, and torsion between the cephalopedal and visceral masses. Visceral coiling clearly results in a compact and relatively stable disposition of the dorsal visceral hump. The adaptive significance and evolutionary history of gastropod torsion are more controversial topics; it is with aspects of these that this communication deals.

Yonge (1947) and most other authorities (Eales, 1950; Knight, 1952; de Beer, 1958; Fretter & Graham, 1962) accept a theory advanced by Garstang (1928, 1929), according to which torsion may have arisen in the remote ancestors of modern Gastropoda through a mutation affecting the larval retractor muscle or muscles of the planktonic larva. Contraction of the then asymmetrical cephalopedal musculature necessarily brought about a shift in the position of the larval mantle-cavity, with the result that cephalopedal retraction was brought

about in a more advantageous sequence of priority. After such a rapid torsion, it was argued, the head might, during retraction, be brought first into the shelter of the mantle-cavity. Before torsion, the less fragile foot had been given this privilege. As Eales (1950) put it, the significance of gastropod torsion was "to enable the larva to withdraw head first instead of head last into its nautiloid shell".

Garstang's theory was an attractive one, for several reasons. First, it avoided the difficulty of visualizing the evolutionary acquisition of torsion in a phylogenetic sequence of adult gastropods. It postulated an immediate selective advantage consequent on the incorporation of torsion into the larval body. Second, it fitted into the prevailing climate of opinion (itself largely a product of Garstang's brilliant argument) which was eager to explore the theoretical possibilities of caenogenesis in metazoan phylogeny. Third, it explained the total lack of adult forms

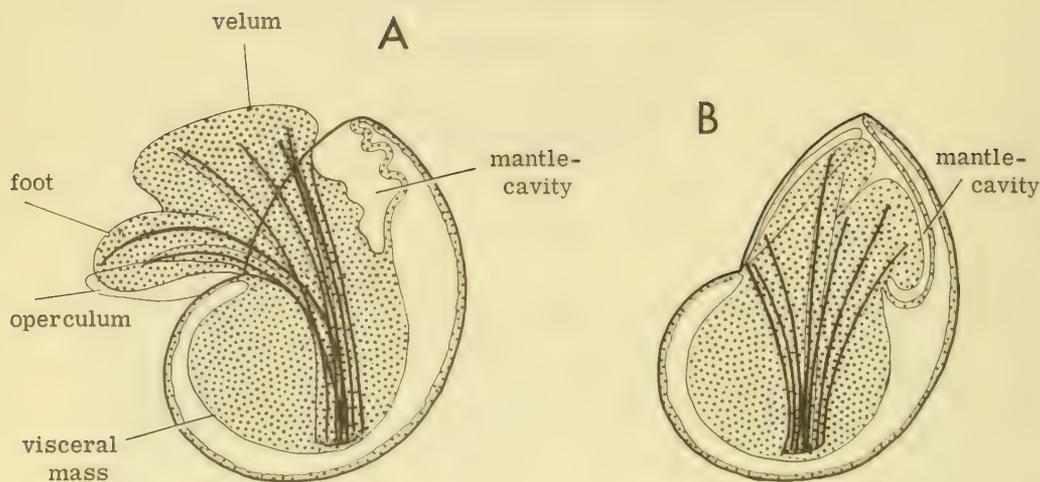


FIG. 1. Hypothetical veliger larva of a gastropod, from the left lateral aspect; A, extended, B, retracted. It can be seen that the mantle-cavity serves, in this model, to accommodate the head and foot during contraction of the cephalopedal retractor musculature.

showing intermediate stages of torsion. The theory was helped by Crofts' (1937) discovery that ontogenetic torsion in the prosobranch *Haliotis* is initiated by the contraction of an asymmetrical, precociously developed, larval cephalopedal retractor muscle. Many zoologists agreed with Knight (1952) that "undoubtedly when torsion first appeared in the remote ancestors of *Haliotis* the same mechanism was responsible". Yonge (1947) elaborated the obvious corollary of the theory, that the original selective advantage of torsion in the veliger larva was so great that its consequent acquisition by the adult gastropod might be considered to have been neutral or even disadvantageous. The subsequent evolution of the gastropods, he suggested, testified to the ways in which some unwelcome morphological components of torsion were nullified in the adult phase. To Yonge (Morton & Yonge, 1964) it still seems 'impossible to conceive of the initial selective value of torsion except in terms of immediate advantage to the

larva'. Other authors (Borradaile *et al.*, 1951) recalled Pelseneer's (1906) claim that opisthobranchiate gastropods, which have discarded most of the 'unwelcome' effects of torsion in the adult phase, nonetheless retain torsion in the larval phase; the torsion was said to be reversed during metamorphosis.

Recently this unanimity has been challenged. Morton (1958) has presented strong evidence against the view that gastropod torsion is or was wholly disadvantageous for the adult gastropod. He points out that an anterior post-torsional situation of the mantle-cavity in the adult is more efficient from the point of view of ctenidial ventilation. In addition, Pelseneer's claim that opisthobranch embryos and larvae exhibit a torsion like that of prosobranchs has been denied (Thompson, 1958, 1962).

One aspect of Garstang's theory that has not yet been criticized is that part which deals with the advantages supposed to accrue to the veliger larva after the postulated caenogenetic origin and rapid

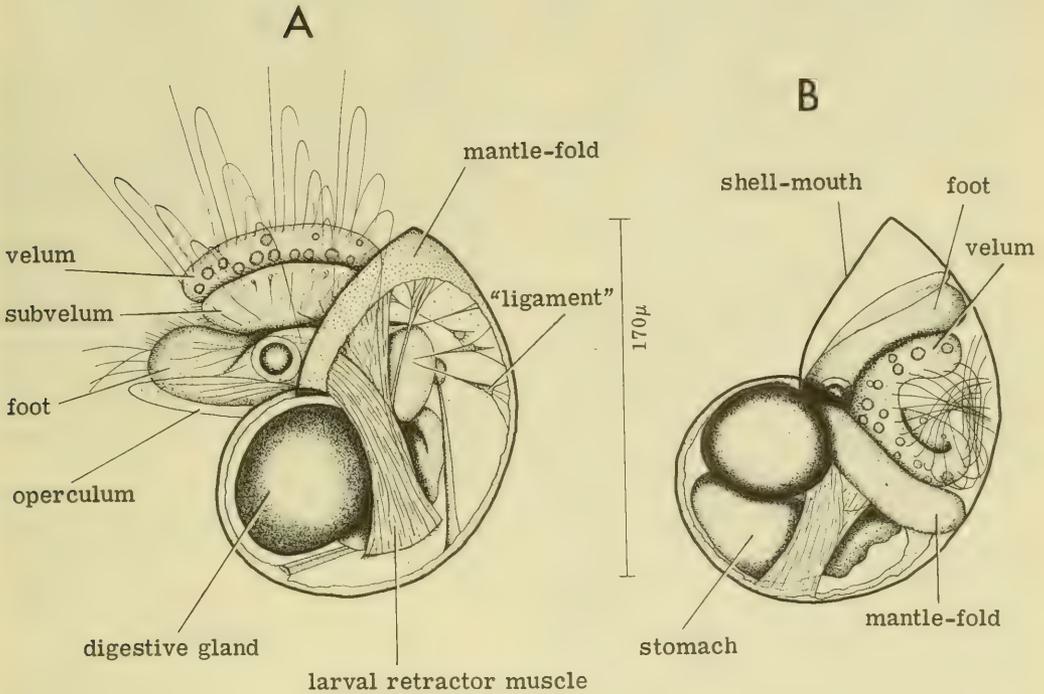


FIG. 2. Newly hatched larvae of *Aplysia punctata*; camera lucida drawings of live specimens, from the left lateral aspect. A, fully extended during swimming; B, retracted following an abrupt disturbance. Note that during cephalopodal retraction the mantle-fold is detached from the shell-mouth and the velum and foot are not accommodated by the mantle-cavity.

incorporation of torsion. The theory at this point rests on a number of assumptions. These may be summarized as follows.

1. During defensive retraction of a gastropod veliger larva the mantle cavity serves to accommodate and protect components of the cephalopodal mass.

2. Some parts of the cephalopodal mass are more fragile than others. Garstang and many other authors accept that the "head" of the veliger is more vulnerable to attack than the foot. For instance, Morton & Yonge (1964) speak of the head as "sensitive and vulnerable", while the larval foot is "tougher".

It is not difficult to prove these as-

sumptions false. To take them in sequence:

1. The mantle-cavity could function in the way Garstang and others imply only if the mantle-fold remained firmly attached to the shell-mouth. Figure 1A & B attempts to show such a situation; in this case the volume and position of the mantle-cavity clearly limit the amount of the cephalopodal mass which may be brought within the shelter of the shell during defensive retraction. But gastropod veliger larvae are not like the fiction shown in Fig. 1. Observations on the behaviour of larvae of *Littorina littorea* and of a number of other gastropods have convinced me that

in all cases the mantle-fold is, on abrupt disturbance, immediately withdrawn from the shell mouth, as the larval retractor musculature contracts to bring the head and foot into the shell. Fig. 2 shows the positions of various organs in the extended and retracted veligers of an opisthobranch gastropod, *Aplysia punctata*. Much the same sequence of events was seen in *Littorina* larvae, confirming an earlier statement (Thompson, 1958) that, during retraction in a gastropod veliger, the cephalopedal mass enters, not the mantle-cavity, but simply the shell-cavity. In this situation the position and volume of the mantle-cavity are irrelevant to the efficiency of retraction.

2. When the cephalopedal mass is extended for swimming and feeding, the horny operculum of the veliger is carried on the posterior face of the foot. It plays no part in defence except during retraction into the shell. The soft parts of the foot and the velum are vulnerable to attack by small carnivores (decapod larvae, ciliate Protozoa, chaetognaths, coelenterates, etc.) which have been seen in the laboratory to rapidly devour enfeebled veligers. Neither in such tests, nor in histological investigations, has any observation been made which supports the theory that the head, i.e., the velum and subvelum, is any more (or any less) vulnerable to attack than the soft parts of the foot. Damage to any part of the cephalopedal mass results rapidly in death. The electron microscope confirms that the foot and velum are equally fragile, and reveals an interesting and hitherto undescribed external strengthening 'basketwork', which may be seen in the photograph (Fig. 3) to invest much of the otherwise naked epithelium of the cephalopedal mass. This basketwork is somewhat similar to the relatively rigid brush-border of the gill epithelium of *Mytilus* (Afzelius, 1960).

These observations remove the ontogenetic basis upon which Garstang's theory rested. It is not my purpose here

to criticize Garstang for his attempt to base arguments about events in the Palaeozoic upon observations on present-day animals, but solely to aver that the observations themselves were mistaken. Little in the embryology of modern gastropods remains which can be used in support of an adaptive theory of the caenogenetic origin of gastropod torsion. Any new theory, to account for the evolutionary origin and incorporation of torsion in these molluscs, must, in my view, be based upon palaeontological and neontological studies of adult gastropods. It is certainly no longer true that, as Allen (1963) writes, "nobody doubts the basic idea of larval protection put forward by Garstang".

It is a pleasure to record the kindness of Professor Alastair Graham in reading a draft of this paper during its preparation.

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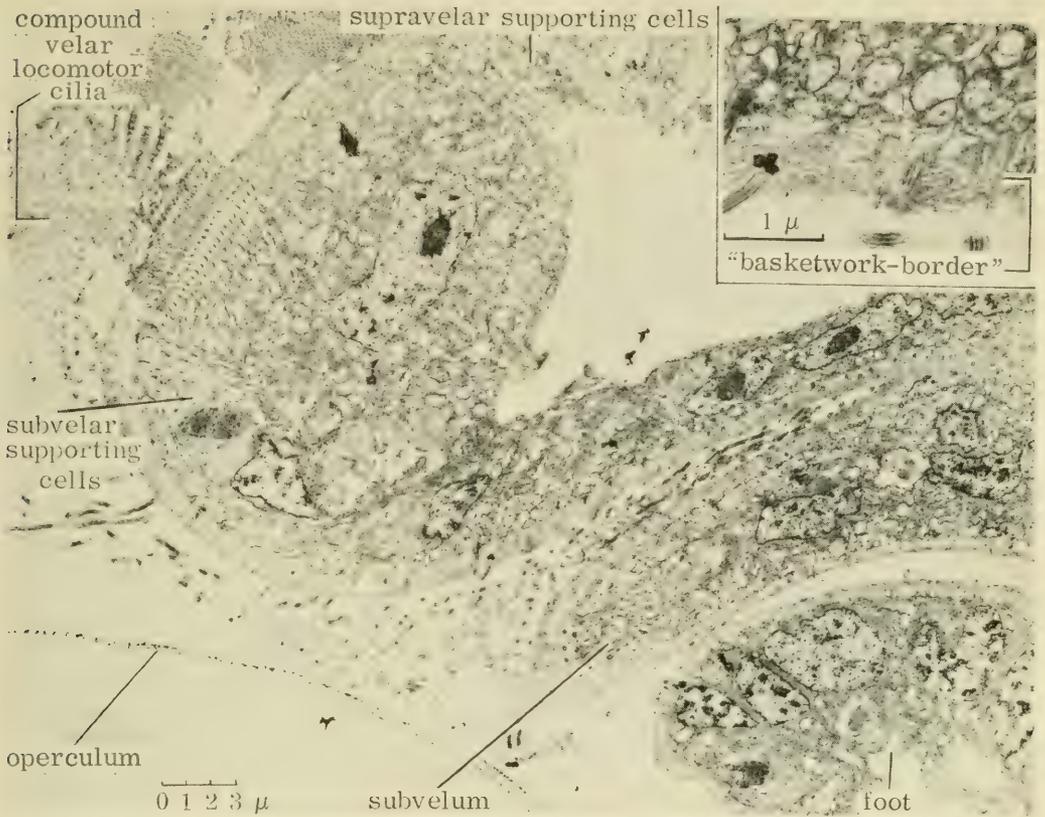


FIG. 3. Portions of Akashi electron micrographs of araldite sections through a newly hatched, veliger larva, fixed in osmic acid, of the opisthobranch gastropod *Archidoris pseudoargus*. The plane of the section is approximately sagittal; anterior is to the top of the page, ventral to the left. The larger micrograph shows the similarity of the epidermal armour of various parts of the cephalopodal mass. Inset is a small portion of another micrograph, showing the 'basketwork-border' at greater magnification.

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ADDENDUM

In a stimulating review of theories of gastropod torsion published recently by Ghiselin (1966), further serious criticisms of Garstang's theory are advanced. To Ghiselin, gradual change seems more reasonable than any theory which provides no adaptive adult intermediates and must rely upon a saltation.

GHISELIN, M. T., 1966, The adaptive significance of gastropod torsion. *Evolution*, 20: 337-348.

RESUMEN

EL SENTIDO ADAPTIVO DE LA TORSION EN GASTROPODOS

T. E. Thompson

Un aspecto importante de la teoría de Garstang, acerca del origen evolutivo de la torsión en los gastrópodos, nunca se había puesto en duda: la parte que trata del aumento favorable en la larva veliger inmediatamente después que el origen cenogénico se manifiesta, y la rápida incorporación de la torsión. Tal teoría descansa sobre dos suposiciones básicas. (1) Que durante la retracción defensiva de la larva, la cavidad del manto sirve para acomodar, y proteger, los componentes de la masa cefalopedal. (2) Que algunas partes de esta masa cefalopedal son más frágiles que otras: la "cabeza" se considera más vulnerable a un ataque, que el pié.

Nuevas observaciones en larvas de gastrópodos muestran ahora que tales suposiciones son incorrectas. Poco queda en el conocimiento embriológico de los gastrópodos modernos, que se puede utilizar para sostener una teoría adaptiva del origen cenogénico de la torsión en gastrópodos.

АБСТРАКТ

АДАПТИВНОЕ ЗНАЧЕНИЕ
ТОРСИОННОГО ПРОЦЕССА У БРЮХОНОГИХ МОЛЛЮСКОВ

Т. Е. Томпсон

Важное значение теории Гарстанга об эволюционном происхождении торсионного процесса у брюхоногих моллюсков не подлежит сомнению. Это связано с теми преимуществами, которые приобретаются личинкой велигер после постулируемого ценогенетического происхождения и быстрого возникновения торсии. Теория основывается на двух основных предположениях: (1) при защитном сокращении личинки брюхоногих моллюсков-велигер-ее мантийная полость способствует приспособлению и защите цефалопедальной области; (2) некоторые части цефалопедальной области являются более слабыми и переходящими по сравнению с другими; "голова" считается более уязвимой для воздействия, чем нога.

Новые наблюдения над личинками брюхоногих моллюсков показывают, что эти предположения неправильны. Мало что из эмбриологии современных брюхоногих моллюсков может быть использовано для поддержания адаптивной теории ценогенетического происхождения торсионного процесса у гастропод.

THE EFFECT OF AMPHENONE B
ON THE EGG PRODUCTION OF *LYMNAEA STAGNALIS*

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ABSTRACT

Amphenone B [3, 3'-bis (p - aminophenyl) - butanone - 2 - dihydrochloride] produces many physiological effects in vertebrates. In the present study it was used on adult *Lymnaea stagnalis* at a concentration of 0.001% (=10 ppm), higher concentrations having proved lethal. From the start the number of snails laying eggs, as well as the number of egg-cells per capsule, was considerably smaller than for the controls. The shape of the eggs was also abnormal. Frequently eggs had more than 1 egg-cell (maximum 23). Ultimately the egg-membranes were creased, incomplete or even absent.

Many apparently normal egg-cells proved to be sterile. When more than 3 embryos per egg were present, they died before hatching. Egg-cells with abnormal or lacking membranes showed no development. It is estimated that the fertility of treated snails on the 6th day of the experiment, was only 4-7% that of the controls.

Placing the snails into an amphenone B free medium after 1 week of treatment did not result in recovery. Although the number of egg-capsules per snail became normal again, the number of egg-cells per capsule and the shape of the eggs were still abnormal after several months.

Very likely amphenone B interferes with the processes in the ovotestis and in the oviductal tract as well. Its effect on *L. stagnalis* may be regarded as molluscicidal, since it causes a particular kind of sterility.

INTRODUCTION

Amphenone B was first synthesized by Allen & Corwin (1950). Its chemical structure is 3,3'-bis (p-aminophenyl)-butanone-2-dihydrochloride (Bencze & Allen, 1957; Korman & Olson, 1957). The compound affects several organs in vertebrates, e.g. the uterus and ovary of mammals, the thyroid gland in mammals, birds and fishes, and the adrenal cortex in mammals and teleosts (comp. Allen et al., 1950; Hertz et al., 1950, 1955; Hogness et al., 1952; Lever & Van Overbeeke, 1956; Vlijm, 1956, 1958; Van Overbeeke, 1960).

The variety of effects caused by amphenone B in vertebrates, together with our interest in the physiology and

endocrinology of molluscs, led us to study its effect on the basommatophoran *Lymnaea stagnalis* (Linnaeus).

It soon appeared that snails treated with amphenone B produced highly abnormal egg-capsules. Evidently, the female genital organs were severely affected by the chemical. Some of the results obtained are presented in this paper.

MATERIALS AND METHODS

Adult specimens of *L. stagnalis* (shell height 30-35 mm), 8 months old, all of the same generation and taken from stock reared in the laboratory for many generations, were kept in 400 ml glass jars in tap water. They were fed lettuce

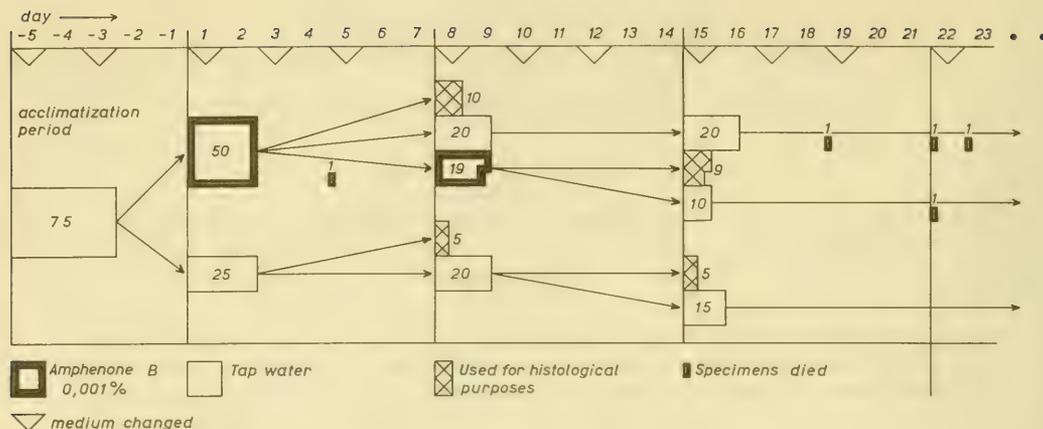


FIG. 1. Diagram of the experimental set up. Temperature 25°C; 12 hrs of light per day.

ad libitum. The temperature was constant at 25°C and the animals were illuminated 12 hours per day (from 9 a.m. until 9 p.m.). The egg-capsules¹ were collected daily at 9 a.m. and the water was changed 3 times a week (Monday, Wednesday and Friday) at 10 a.m. Amphenone B² was dissolved in tap water.

EXPERIMENTAL

Preliminary tests revealed that many of the snails died within a few days when treated with amphenone B solutions of 0.02, 0.01 or 0.005% (at 0.02%, over 60% of the animals died within 10 days). Viability was not notably affected, however, when a 0.001% (10 ppm) amphenone B concentration was used. Therefore, a more extensive experiment was carried out at this concentration. The experimental set up is presented in Fig. 1. The

¹The terminology of Bondesen (1950: 21) is used: the entire egg mass is called *egg-capsule*, the egg-cell with its white and the egg-membranes *egg*.

²The amphenone B used in the experiments was kindly supplied by Dr. M. J. Allen of Ciba Pharmaceutical Products Inc., Summit, N. J., U. S. A.

experiment was started with 75 animals. After an acclimatization period of 5 days - the snails were taken from the stock room where conditions such as temperature and illumination were not constant - amphenone B was administered to 50 specimens, while 25 were kept as controls. After one week, 10 of the snails exposed to amphenone B and 5 of the controls were killed and examined histologically. The 39 remaining experimental animals (1 had died) were divided into 2 groups. The first group (19) was treated with amphenone B for another week, the second (20) was transferred to tap water to study whether recovery of the egg production would occur. Two weeks after the beginning of the experiment, again some snails were killed for histological use. From now on all animals were kept in tap water (Fig. 1). Observations were continued for 8 1/2 weeks at 25°C and 12 hrs of light per day. The snails were then transferred back to the stock room. Collection of egg-capsules was continued for several months.

RESULTS

1. Total egg production

Because changing the water stimulated the laying activity of *L. stagnalis*, the

TABLE 1. Egg production of *Lymnaea stagnalis* treated with amphenone B and their controls

Day	No. of snails		No. of capsules		% of snails producing an egg capsule		Mean No. of egg-cells per capsule	
	C	E	C	E	C	E	C	E
2	25	50	14	17	56	34	103 ± 23.2	98 ± 33.9
4	25	50	20	27	80	54	129 ± 24.5	87 ± 34.5
6	25	49	18	27	72	55	138 ± 37.8	57 ± 29.0
9	20	19	15	6	75	32	125 ± 41.0	32 ± 16.5
11	20	19	15	6	75	32	118 ± 29.7	23 ± 11.2
13	20	19	13	7	65	37	125 ± 16.0	20 ± 11.6

C, controls; E, experimental snails

largest numbers of capsules were always found on days following a water change day. It therefore seemed most reliable to focus the attention on those days when comparing the egg production of experimental animals and controls. It then appeared that amphenone B depressed the laying activity considerably (Table 1). The percentage of the snails which produced an egg-capsule on any day was much lower for the snails treated with amphenone B than for the controls.

That the experimental animals laid fewer egg-capsules does not, in itself, prove the inhibition of the production in, or the release of egg-cells from, the ovaria, because the capsules of the treated snails might have contained more egg-cells than those of the controls. This, however, was not the case, as appears from Table 1 and Fig. 2. These show that during the course of the experiment the mean number of egg-cells³ per "amphenone B-capsule" gradually decreased, being only 16% of the control number at the end of the second week.

It can therefore be concluded that exposure to 0.001% amphenone B is highly effective in depressing egg production in *L. stagnalis*. Calculations

show that, for example on the 13th day of the experiment, it lowered the mean number of egg-cells per snail to less than 10% of the control value.

2. Morphology of the eggs and egg-capsules after exposure of parent to amphenone B

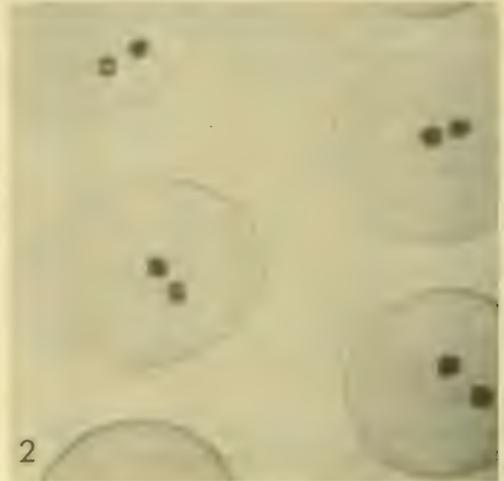
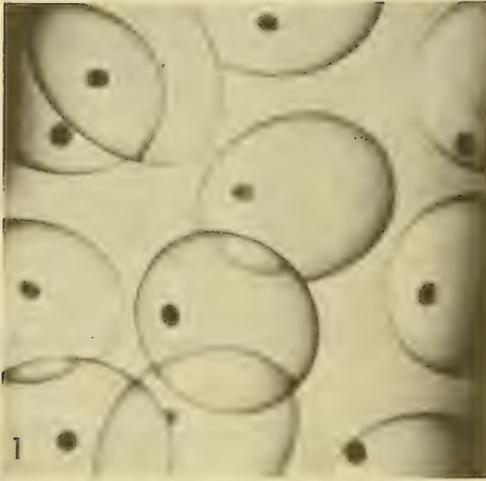
The decrease in the number of egg-capsules and of egg-cells per capsule is not the only recognizable effect caused by amphenone B. As already mentioned, the shape of the eggs and egg-capsules was also affected by the chemical.

As early as the 2nd and 3rd day of the experiment abnormal egg-capsules were already present. Many of these had eggs containing more than the single egg-cell normally found (Plate I, Fig. 1). Admittedly, in control capsules also, 1-2 eggs with more than one egg-cell may sometimes occur. However, these eggs are almost always the last eggs of the capsule and they usually do not contain more than 2 egg-cells. In some of the experimental capsules, on the other hand, the majority of the eggs contained 2 egg-cells (Plate I, Fig. 2). In yet other capsules the number of egg-cells per egg was even much higher (Plate I, Fig. 3), the maximum observed being 23.

Not all treated animals produced this type of abnormality during the 1st days of the experiment; quite a number of them began laying egg-capsules abnormal in a different way. The normally

³Because the egg-membranes were frequently damaged or even absent (see below), the number of egg-cells was determined instead of the number of eggs.

PLATE I



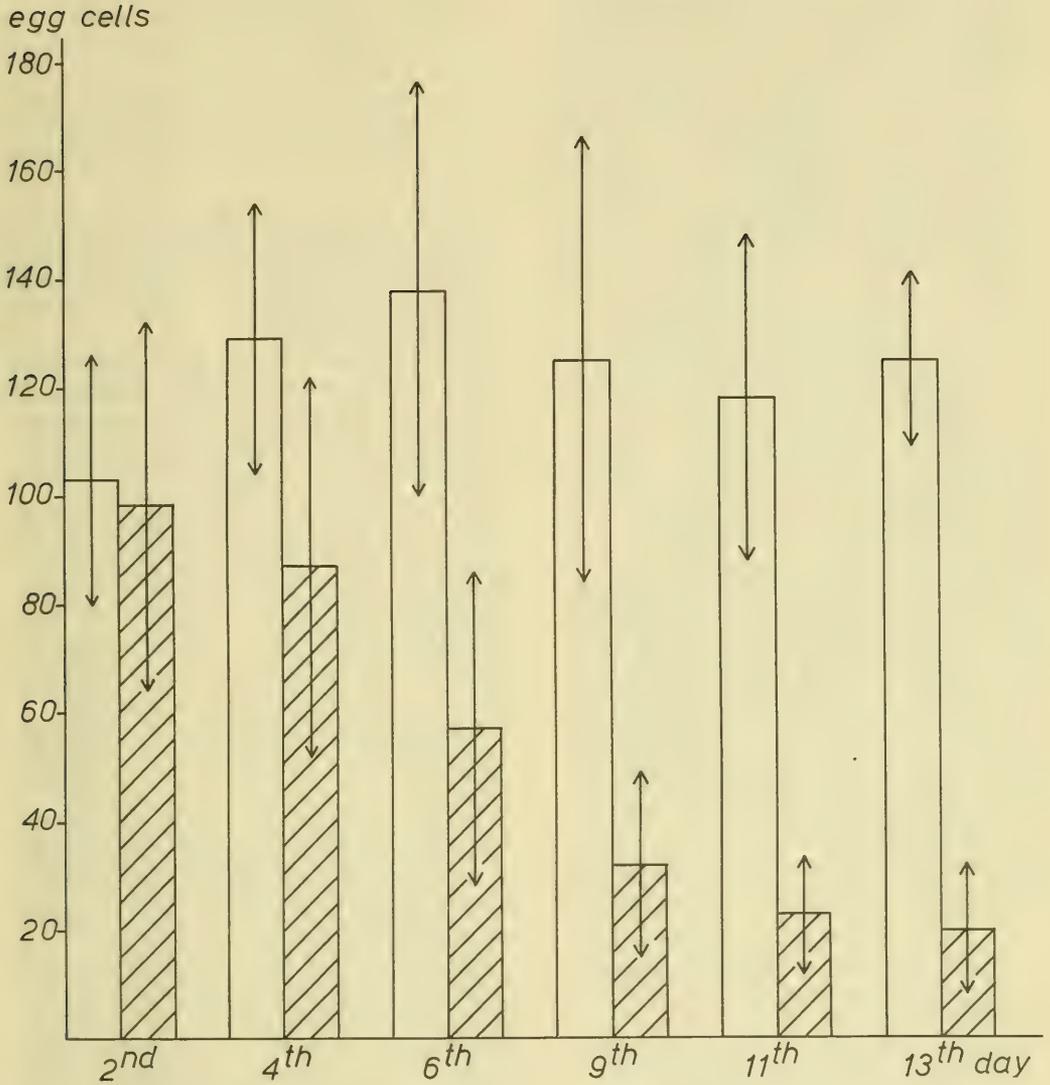


FIG. 2. Mean number of egg-cells per capsule. Blank columns: controls; hatched columns: amphenone B 0.001%; arrows: standard deviations.

PLATE I (facing page)

Photographs of parts of egg-capsules of *Lymnaea stagnalis* (x 20). Fig. 1, normal capsule. Figs. 2-6, damaged capsules, laid by snails exposed to amphenone B.

closely appressed internal and external egg-membranes surrounding each egg (cf. Bondesen, 1950) were severely deformed in many eggs. This type of malformation was regularly seen from the 4th day onwards. The membranes (and the egg-strings) could be seen separately, and the internal membrane showed creases (Plate I, Fig. 4). In other capsules only remnants of the egg-membranes were left (Plate I, Fig. 5). Frequently they were entirely absent (Plate I, Fig. 6). The egg-cells were then only surrounded by the jelly mass of the capsule and the capsular membrane. This membrane also seemed to be altered since dust particles and microorganisms were more easily attached to it than to control capsules (Plate I, Fig. 3). Eggs with incomplete egg-membranes also frequently contained more than one egg-cell. Even capsules without any egg-cells were occasionally observed. The incomplete egg-membranes are probably the most characteristic effect of amphenone B on the morphology of the egg-capsules. An animal, which had once started to produce this type of capsules, continued to do so, even after it was returned to an amphenone B free medium (see below).

It is possible to classify the egg-capsules on the basis of their degree of deformation, as follows (see Fig. 3).

0. Normal capsules. Eggs with intact egg-membranes (neither separated, nor perforated) and one egg-cell per egg (Fig. 3, 0);
1. Only 1-2 eggs contain 2 egg-cells. Egg-membranes intact (Fig. 3, 1);
2. The majority of the eggs (intact membranes) contain more than one egg-cell (Fig. 3, 2);
3. Eggs with intact, and eggs with creased membranes. More than one egg-cell per egg may occur (Fig. 3, 3);
4. Capsules containing eggs with intact and eggs with creased or incomplete membranes, but also having areas with egg-cells lacking egg-membranes. More than one egg-cell per egg may occur. (Fig. 3, 4);
5. Only deformed eggs. Again more than one egg-cell per egg may occur

(Fig. 3, 5);

6. Incomplete eggs (multiple egg-cells per egg may occur) and areas with egg-cells lacking egg-membranes (Fig. 3, 6);
7. Capsules containing egg-cells without egg-membranes only (Fig. 3, 7);
8. Capsules without eggs or egg-cells (Fig. 3, 8).

In the main, the succession of categories represents a continuously graded series of deformation. Clearly, only capsules of categories 0 and 1 can be qualified as normal.

As soon as 1 and 2 days after amphenone B treatment (capsules collected on the 2nd and 3rd day of the experiment) a number of egg-capsules were already abnormal. Fig. 4 shows that then over 20% of them had deformation degree 2 or 3. The 4th and 5th days show a continuation of the trend. Almost all of the capsules collected on the 6th day were seriously deformed: as many as 40% showed deformation of the 7th degree, i.e., the egg-membranes were entirely lacking. In the 2nd week of amphenone B treatment the percentage of highly deformed egg-capsules was still larger (Fig. 4).

These results show that treating *L. stagnalis* for about 1 week with 0.001% of amphenone B is effective in causing severe damage to the majority of the egg-capsules laid.

From Fig. 4 it can be seen that, even during the 2nd week of the experiment, there was a small number of capsules (2) having eggs with only 1 egg-cell and intact egg-membranes (category 0). However, these 2 capsules were not normal in regard to the number of egg-cells per capsule: they contained only 33 and 31 egg-cells respectively, while the control capsules contained over 100 each (see Fig. 2).

3. After-effects of amphenone B treatment on egg production and morphology

It seemed of interest to determine whether treated snails, after they had started to lay abnormal egg-capsules,

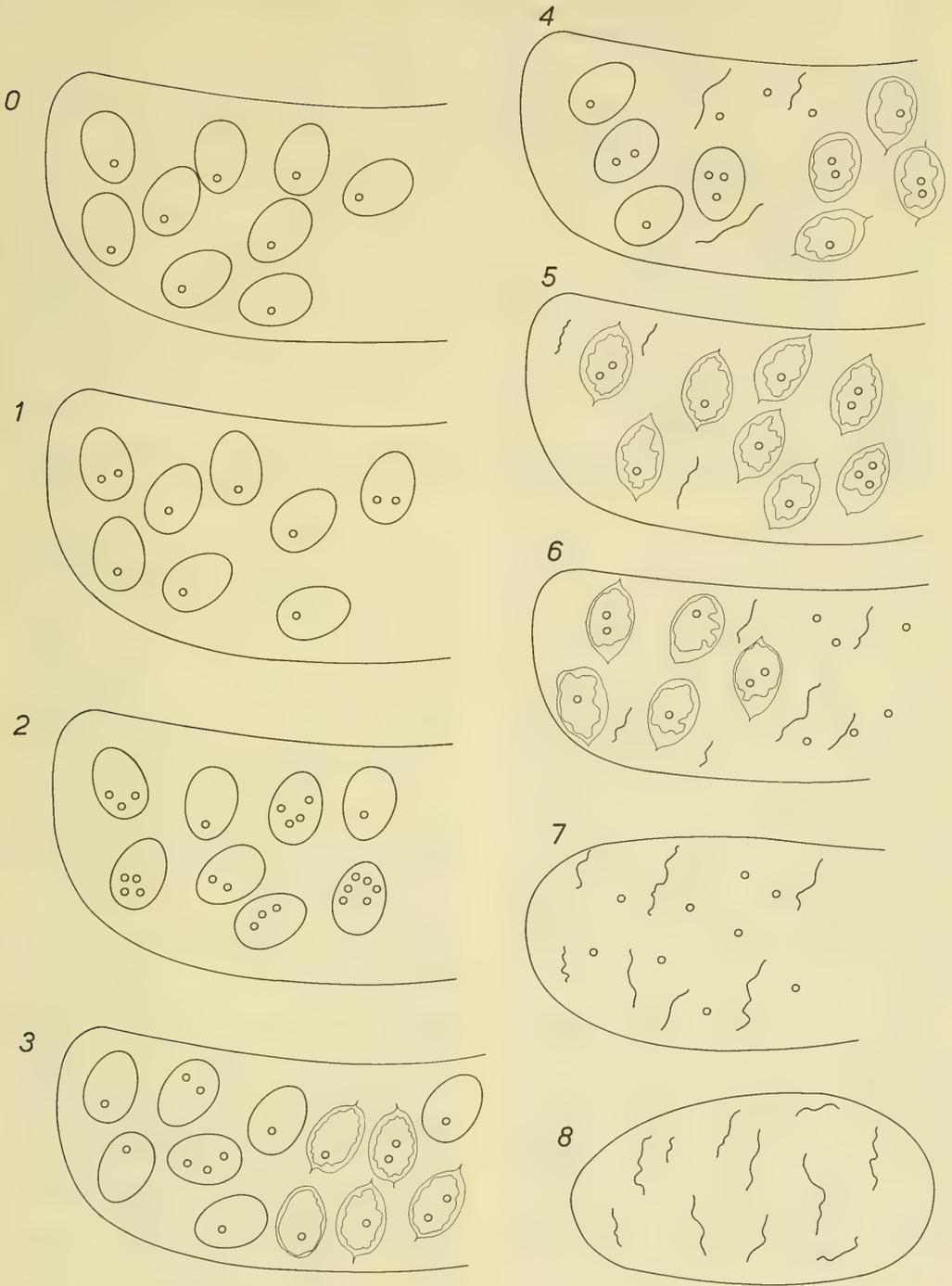


FIG. 3. Degrees of deformation in the eggs of *Lymnaea stagnalis*, caused by amphenone B. The wavy lines in categories 4-8 represent remnants of egg-membranes (see also text).

TABLE 2. Egg production of *Lymnaea stagnalis* kept in tap water for 6 weeks after 1 week of treatment with amphenone B at 0.001%

Week of experiment*	No. of snails		No. of capsules		Mean No. of capsules per snail		Mean No. of egg-cells per capsule	
	C	E	C	E	C	E	C	E
2	20	20	62	38	3.10	1.90	119 ± 34.2	48 ± 30.9
3	15	19	29	35	1.93	1.75	107 ± 34.6	46 ± 31.9
4	15	17	22	27	1.47	1.58	97 ± 28.3	39 ± 24.0
5	15	17	8	19	0.53	1.12	66 ± 31.9	23 ± 13.2
6	15	17	11	18	0.73	1.06	85 ± 20.7	20 ± 11.4
7	15	16	6	14	0.40	0.75	101 ± 17.0	24 ± 14.2

C, controls; E, experimental snails

*Note that the 2nd week of the experiment corresponds to the 1st week of observation after transfer of the snails to the normal medium (the 3rd to the 2nd, and so forth).

would revert to normal when returned to an amphenone B free medium. A group of 20 snails, transferred to tap water after 1 week of amphenone B treatment (Fig. 1) was used for studying the after-effects. This group was thought to be more suitable than that of 10 specimens which had been exposed to amphenone B for 2 weeks, because recovery of the capacity for egg production, if it were to occur, was expected to take place more readily after shorter exposure. Collection of the capsules was continued for several months, but detailed results (Table 2, Fig. 5) are given only for the first 6 weeks, because by then the egg-production, in the controls also, had almost stopped.

During the 6-week period 4 experimental animals died. Of the controls 5 specimens were killed for histological investigation at the end of the 1st week of post-exposure observations (i.e., the 2nd week of the experiment; compare Fig. 1). Table 2 shows that egg laying decreased during the course of the experiment for both controls and snails exposed to amphenone B. The number of capsules per snail in the experimental animals decreased less sharply than that of the controls and during the last 4 weeks even exceeded the control value.

Thus, as far as the number of capsules per snail is concerned, improvement of egg laying capacity was evident. The mean number of egg-cells per capsule, however, remained low for the experimental snails (Table 2; Fig. 5), being markedly lower in the last 3 weeks of observation than in the 1st 3 weeks, both absolutely and in relation to the controls. In other words, although the mean number of capsules per snail was relatively high in the amphenone B snails, the mean number of egg-cells fell short of the control value.

Fig. 6 presents the degree of deformation of the capsules laid during the 6-week "recovery period". It shows that the percentage of normal capsules (categories 0 and 1) did not increase during this period. At its end the majority of the eggs were still severely damaged.

It is therefore concluded that the deleterious effects of one week's exposure to amphenone B at 0.001% are not cancelled by returning the snails to the normal medium for 6 weeks.

Because egg production almost stopped in the 7th week of the experiment, the snails were again transferred to the stock room in the 9th week. There, though egg-capsules were laid only incidentally, they served to demonstrate

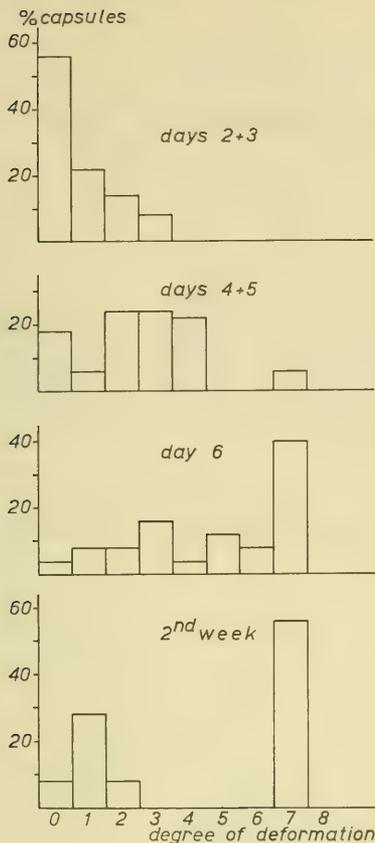


FIG. 4. The degree of deformation of the egg-capsules of *Lymnaea stagnalis* during 2 weeks of amphenone B treatment at 0.001%.

that the treated snails had not recovered. In the 17th week, for example, when 7 amphenone B snails and 8 controls were still alive, 2 egg-capsules were laid in each group. The control capsules contained 92 and 95 normal eggs (with 1 egg-cell per egg), whereas the amphenone B capsules contained only 54 and 60 egg-cells, with a deformation degree of 5 and 7 respectively. Neither was recovery noted in the group treated with amphenone B for 2 weeks. In this group the last capsule was laid 9 months after the start of the experiment. This capsule was still abnormal.

A small percentage of capsules observed during the "recovery period" was

morphologically normal, as was also the case during treatment (see previous section). However, the mean number of egg-cells in these capsules was again small, being 26 ± 16.8 for the capsules laid during the 4th, 5th and 6th week after transfer to tap water. The control value was 3-4 times higher (see Table 2).

In this connection it is worth noting that nearly all capsules laid by an individual snail during the entire period of observation had the same degree of deformation as the capsules it laid at the end of the first week, even when amphenone B had been administered for 2 weeks. For most of the snails this was degree 5, 6 or 7. Two individuals, however, never laid morphologically deformed capsules. They produced the normal capsules (degree 0) conspicuous in Fig. 6.

4. Fertility of snails exposed to amphenone B

To determine whether egg-cells in abnormal egg-capsules were able to develop, some capsules of each of the categories of deformation were incubated in tap water at 25°C and regularly examined under a dissection microscope.

The observations can be summarized as follows: (1) Frequently normal looking eggs, with intact membranes, from snails exposed to amphenone B, showed no development at all. Probably they were not fertilized. It also occurred that "normal eggs" showed only development for the first 1-2 days and then died. (2) In some cases eggs with 2 or even 3 egg-cells developed normally. However, when more than 3 egg-cells per egg were present, the embryos always died before hatching, probably due to lack of food. (3) Eggs without or with creased or incomplete egg-membranes showed viability only during the first day, or none at all.

Thus, egg-capsules with deformation of the 5th, 6th, 7th and 8th degree (see Fig. 3) yielded no young snails whatso-

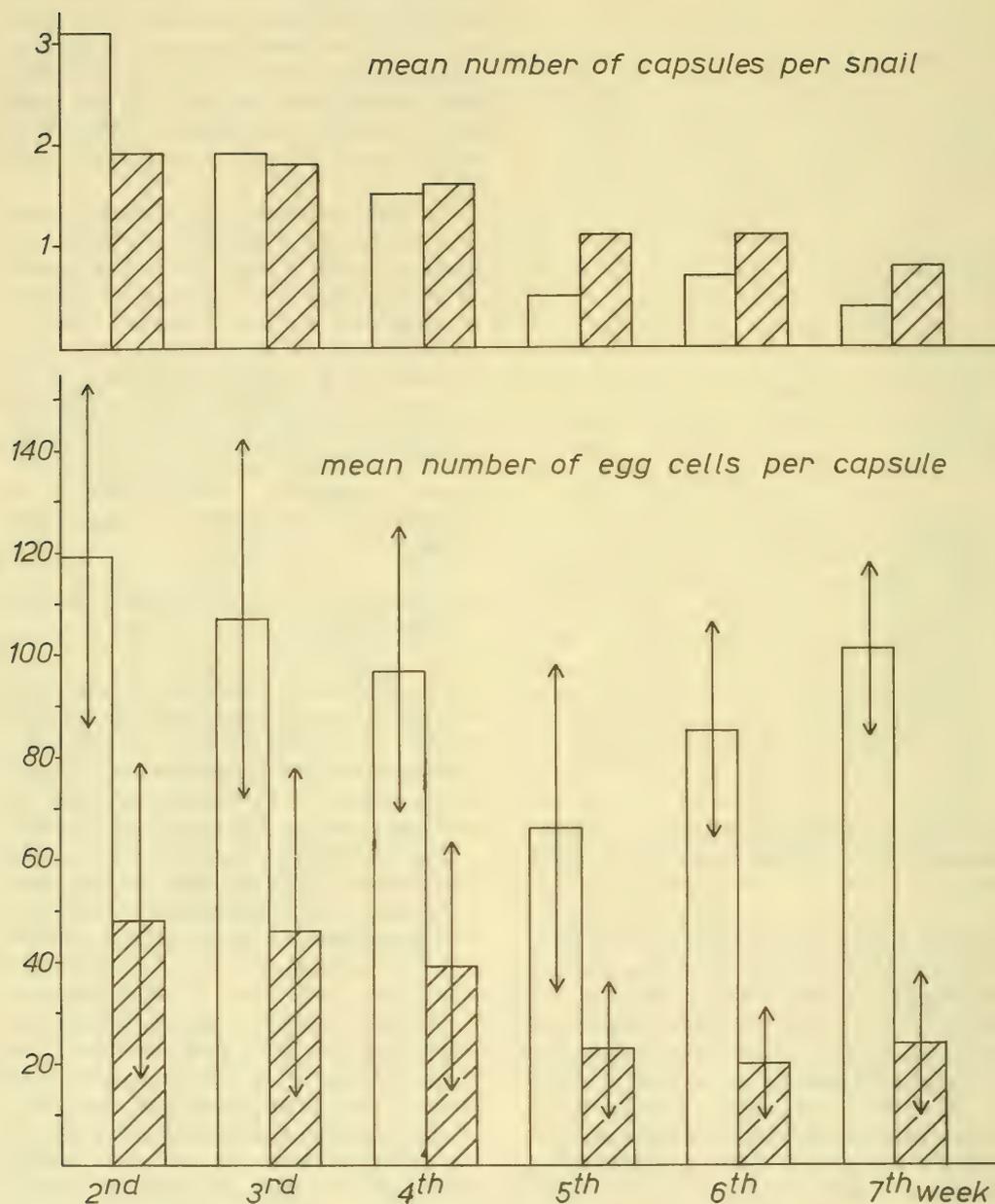


FIG. 5. Egg-production of *Lymnaea stagnalis* during 6 weeks in tap water, following 1 week of amphenone B treatment at 0.001%. Blank columns: controls; hatched columns: experimental snails; arrows: standard deviations.

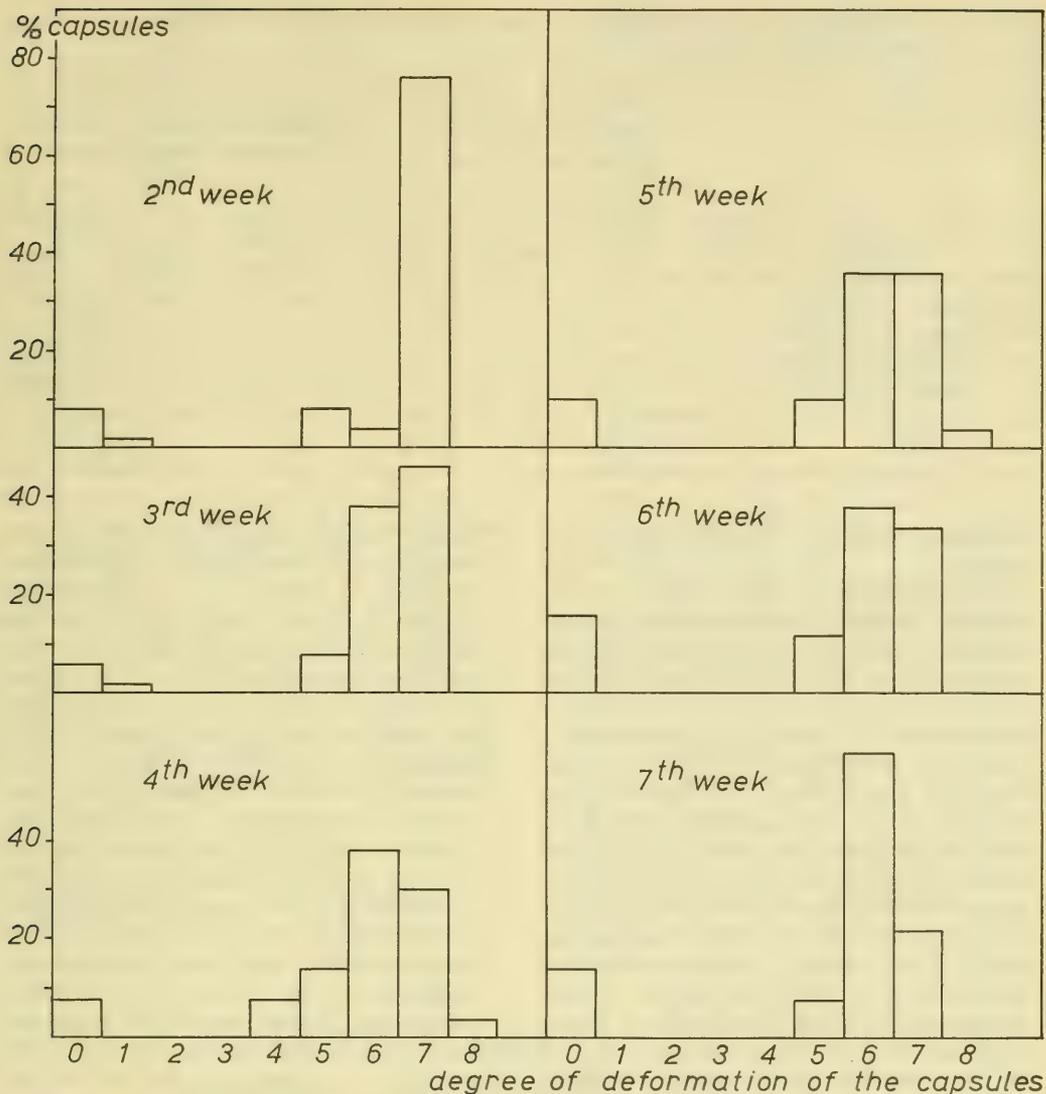


FIG. 6. Degree of deformation of the egg-capsules of *Lymnaea stagnalis* laid during 6 weeks' maintenance in tap water, following 1 week of amphenone B treatment at 0.001%.

ever, and those of the 2nd, 3rd and 4th degree only a limited number. Furthermore, not every egg from capsules of degrees 0 and 1 developed into a young *Lymnaea*.

To obtain an idea about the fertility of snails treated with amphenone B, the number of egg-cells (per animal) that might be expected to produce normal young snails was estimated as a per-

centage of that of the controls (Table 3). On the basis of the observations described above, it may be assumed that the number of developing egg-cells of treated snails will probably not be larger than the sum of the egg-cells of capsules belonging to categories 0, 1 and 2, and, more likely, not greater than the sum of categories 0 and 1 only. For the estimation of the fertility it was

TABLE 3. Fertility of *Lymnaea stagnalis* exposed to amphenone B at a concentration of 0.001% (For details see text)

	Mean No. of egg-cells per snail on day			Degree of fertility as a per- centage of control value on day		
	2	4	6	2	4	6
Controls	58	103	99	100	100	100
Amphenone B snails						
Totals	32	47	31	55.1	45.6	31.3
Degree of deformation						
0 + 1 + 2 + 3	32	37	12	55.1	36.4	12.2
0 + 1 + 2	32	27	7	55.1	26.2	7.1
0 + 1	31	14	4	53.4	13.6	4.0

assumed that all control eggs were viable.

As can be seen from Table 3, the number of eggs from the experimental animals that can be expected to be viable decreased rapidly, both because of a decrease of the total egg-cell production and because of an increase in the degree of deformation of the capsules. Thus, on the 6th day, the fertility of the treated snails was probably only 4-7% that of the controls. From Figs. 5 and 6 and Tables 2 and 3 it is easily understood that the degree of fertility did not increase very much after returning the snails to tap water. It can therefore be concluded that 0.001% amphenone B administered to *L. stagnalis* for 1 week, decreases the fertility to less than 10% of the control value for several months, and perhaps for even longer.

DISCUSSION

The results of this study indicate that amphenone B has diverse effects on the egg-production of *L. stagnalis*. The number of egg-cells released from the ovarium is decreased. Moreover, many egg-cells seemed to be unfertilized. This situation suggests an influence on oogenesis and spermatogenesis and/or perhaps on ovulation. However, the

former action seems the more likely. Preliminary histological observations revealed a decrease in the relative number of young oocytes (for the method used see Joosse, 1964). Very probably, therefore, oogenesis is retarded. Spermatogenesis also seemed to be abnormal: for example, spermatids appeared to be absorbed by the Sertoli cells. Other tissues, such as the digestive gland, also showed damage. That the egg-membranes were often creased, incomplete or absent, indicates that parts of the oviductal tract are affected, probably in particular the pars contorta (cf. Bretschneider, 1948) which forms these membranes. Since transfer of the snails to tap water caused no evident improvement, the injury seems to be rather serious. Its nature could not be established.

The resultant effect might be described as a particular type of sterility. For, although the egg-cells may in some cases be fertile, they fail to develop, due to secondary causes, such as, for example, malformation of the egg-membranes. Therefore amphenone B, used at a concentration of 0.001% (10 ppm) for 1 week, can be regarded as a molluscicide for *L. stagnalis*, even though the adult animals are not killed, since the viability of the ova is decreased considerably.

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RESUMEN

EL EFECTO DE AMFENONE B SOBRE LA PRODUCCION OVULAR
LYMNAEA STAGNALIS

H. H. Boer y J. Lever

Amfenone B [3, 3'-bis (p-aminofenil) -butanone-2-dihidroclorido] produce muchos efectos biológicos en vertebrados. Para el presente estudio fue usado sobre individuos adultos de *L. stagnalis* en una concentración de 0.001% (=10 ppm), y concentraciones más elevadas resultaron fatales. Desde el principio el número de individuos en la puesta, así como el número de células ováricas por cápsula, fue considerablemente menor que en los controles. La forma de los huevos era también anormal. Con frecuencia los huevos tenían más de una célula ovárica (máximo 23). Al final las membranas se agrietaron, quedando incompletas o ausentes.

Muchas células ováricas resultaron aparentemente estériles. Cuando había más de 3 embriones presentes por huevo, murieron antes de eclosionar. Células ovulares con membranas anormales o ausentes no desarrollaron. Se estima que la fertilidad de

los caracoles tratados en el sexto día de experimentación, fue sólo de 4-7% de los controles.

Colocando las caracoles en un medio libre de anfenone B, y despues de una semana de tratamiento, no se produjo restablecimiento. Aunque el número de capsulas ovígeras por individuo volvió a ser normal, el número de células ováricas por cápsula, y la forma de los huevos, permanecieron anormales por varios meses.

Muy probablemente amfenone B interfiere con el proceso en el ovotestis y también en el tracto oviductal. Su efecto sobre *L. stagnalis* puede considerarse como el de un "molluscida", desde que causa una condición particular de esterilidad.

АБСТРАКТ

ВЛИЯНИЕ АМФЕНОНА-В НА ПРОДУКЦИЮ ЯИЦ У *LYMNAEA STAGNALIS*

Х. Бер и Т. Левер

Амфенон-В [3,3'-бис (р-аминофенил)-бутанон-2-дигидрохлорид] производит различное физиологическое воздействие на позвоночных. В настоящем исследовании он применялся для воздействия на взрослых *L. stagnalis* в концентрации 0,001% (=10 ppm); более высокие концентрации являлись уже летальными. С самого начала опыта количество улиток, откладывающих яйца, как и количество яйцеклеток в одной капсуле были значительно меньше, чем в контроле. Форма яиц также отклонялась от нормы. Часто яйца имели более одной яйцеклетки (максимум до 23). Наконец, яйцевая оболочка становилась сморщенной, неразвитой или даже совсем отсутствовала. Многие с виду нормальные яйцеклетки оказывались стерильными. Если в яйце находилось более 3 эмбрионов, они отмирали еще до вылупления. Яйцеклетки с ненормальными оболочками или без них - не развивались. Было установлено, что количество оплодотворенных яиц у подопытных улиток на шестой день опыта составлял всего 4-7%, по сравнению с контролем. Перенос улиток после 1 недели опыта, в среду, не содержащую амфенона-В, уже не давал их восстановления. Хотя количество яйцевых капсул в каждом моллюске снова становилось обычным, но количество яйцеклеток в каждой из них, как и общая форма яиц, оставалась ненормальной еще в течение нескольких месяцев. Сходным образом амфенон-В действует на процессы образования гермафродитной железы и на процессы в яйцеводах. Действие амфенона-В на *L. stagnalis* может рассматриваться, как "моллюскоцидное", поскольку оно вызывает особый вид стерильности.

STIMULATION OF EGG PRODUCTION IN A PHYSID AND A LYMNAEID¹

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ABSTRACT

Physa pomilia Conrad, hatched in the laboratory and reared in total darkness, began egg production at the same time as those reared under normal daylight conditions, in September, at the approximate age of 40 days. All individuals came from the same egg mass. The numbers of eggs produced per individual were at least 3 X greater for the snails reared in the dark. The viability of eggs was of the same order of magnitude (83-85%) for both series.

In another simultaneous experiment, *Physa pomilia* and the lymnaeid *Pseudo-succinea columella* (Say), reared under normal daylight conditions, began producing eggs at the same age and time, i. e. at a season when daylength was decreasing. The suggestion that reproduction (in *Lymnaea*) might be induced by lengthening of the days is thus not supported.

It is concluded that in the species of snails investigated, initiation of egg production is a function of temperature rather than of light, provided the snails have reached sexual maturity. When the temperature is maintained above a certain minimum level, oviposition takes place regardless of length of day.

Maximum egg production for individuals of both species reared in daylight did not coincide with long daylight periods but tended to be inversely proportional to the length of day. This fact together with the much greater egg production of *Physa pomilia* reared in total darkness suggests that light may inhibit egg production.

INTRODUCTION

Light and temperature are the most obvious environmental variables possibly involved in the initiation of and subsequent control of egg production in freshwater snails. This paper presents evidence that the stimulus for initial egg production in *Physa pomilia* Conrad is not dependent upon the presence of light. It also presents data from the literature which indicate that temperature may be of primary importance in triggering the first oviposition in various freshwater snails.

Jenner (1951) has suggested that reproduction in *Lymnaea* [= *Stagnicola*] *palustris* (Müller) is induced in response to lengthening days; his snails required a daily minimum of 13 1/2 hours of light (fluorescent lamp) before they began to lay eggs at room temperature. He demonstrated that following the initiation of egg production, exposure of snails to an 11-hour-day was more effective in eventually stopping egg laying than a 6 3/4-hour-day or darkness. It was observed, however, that egg production once initiated, can be stopped completely by decreasing the temperature below a

¹This investigation was supported by a research grant E-1019 from the National Institutes of Health, U. S. Public Health Service.

certain critical level regardless of day length. He also found that oviproduction can be initiated by a rise in temperature even under short-day conditions.

Odum (1957) counted the eggs laid by *Pomacea paludosa* (Say) throughout the year at Silver Springs, Florida. He found positive correlation between numbers of eggs and vernal increases in length of day and suggested that photoperiodism was implied, although not proved, by these data. Although the water temperature is essentially constant at Silver Springs, eggs of *Pomacea* are deposited above the surface of the water; thus the possible control of oviposition by temperature, in particular that of air, cannot be ignored.

It is known that certain species occupying a wide north-south range will breed only during the warmer periods in the northern limits of their range but reproduce throughout the year in its southern portions (Hunter, 1964). A study group on the ecology of snails which are intermediate hosts of bilharziasis has made the following observation: "An important point is the effect of annual range of temperature upon the length of the breeding season. In certain areas the period of time during which the water is warm enough to permit oviposition, development and growth is only sufficient for the reproduction of one generation. In more southerly latitudes, where the warm season is longer, several generations may be produced, or breeding may even continue all the year round. Thus *Bulinus truncatus* in northern Iraq produces only a single generation annually, while in central and southern Iraq and in Egypt it produces two or more generations each year. *Australorbis glabratus* in north-east Brazil continues to breed all the year round. A similar situation exists with regard to those species of *Oncomelania* which produce only one generation annually in the northern part of their range, but breed all the year round in warm southern areas" (WHO Study Group, 1956).

Pulmonate snails collected from icy waters during winter months began ovipositing shortly after being brought into the laboratory (Cheatum, 1951). *Physa gyrina* Say living in warm waters discharged from a starch factory in Illinois reproduced throughout the year (Agersborg, 1932). *Physa gyrina* collected from beneath the ice of ponds, where the water temperature was 4-5°C, began to lay eggs within 24 hours after being brought into the laboratory (DeWitt, 1954a). Van Nieuwenhoven & Lever (1946) state that a gradual rise in temperature (18-28°C) serves as a strong stimulus to egg production in *Lymnaea stagnalis* (L.).

The reports quoted illustrate the well known importance of temperature in the reproduction of freshwater snails, but the possible effects of daylight, if any, are not clearly revealed in the literature.

In view of the evidence already available with regard to the effects of light and temperature on reproduction it was of interest to investigate whether or not *Physa pomilia*, an easily reared form, could live and reproduce in complete darkness, and, if so, to determine whether or not the absence of light would have an adverse effect upon the initiation of oviproduction and upon the total reproductive capacity of the snail.

It also seemed appropriate to compare the egg-laying responses, under controlled conditions, of this species with those of another pulmonate species. The common lymnaeid *Pseudosuccinea columella* (Say) was chosen for this purpose because previous studies (Dewitt & Sloan, 1958) had indicated that this snail resembled *Physa* in the time needed for attaining sexual maturation and in other aspects.

METHODS

A stock of *Physa pomilia* and *Pseudosuccinea columella* was collected from streams in the Devil's Millhopper in Alachua County, Florida, during the month of July 1962. Experiments were carried out with snails hatched from

eggs laid within a month from the time the parents had been collected in the field.

Two experiments, I and II, involving 12 snails each, were conducted simultaneously.

I. Two groups of 6 *Physa pomilia* each were reared in the laboratory, one under conditions of normal daylight, the other in total darkness. All snails came from the same egg mass. Each group comprized 2 single snails that had been isolated soon after hatching, and 2 pairs. Isolated and paired snails were used because a previous study (DeWitt & Sloan, 1959) indicated that under daylight conditions single *Physa pomilia* took a longer time to become sexually mature and laid fewer less viable eggs per individual than snails reared together in pairs. Conditions of daylight consisted in a northern exposure without direct sunlight. Total darkness was achieved by painting jars black on the outside and moreover placing them inside a wooden frame covered with black cloth. To make sure that light did not penetrate the painted jars, a 100 Watt bulb was placed inside each jar, with the cord running through a hole in the lid. The hole was carefully taped and the lid loosely screwed on. This assembly was then tested in a photographic darkroom: when the bulb was lighted, no light was detected coming from the jar. All jars contained 2 liters of spring water and were loosely capped;

none were aerated. The water was changed in all jars once every 2 months. The snails were fed a calcium alginate diet prepared according to the formula of Lee & Lewert (1956). Eggs were removed from all containers once a week and records kept of the numbers laid and hatched. The snails of the dark series were exposed to light only briefly, less than 1 minute, at the time when the eggs were removed. Both the light and dark series were maintained in the same air conditioned room and temperatures (Table 1) were the same in all containers. Temperature data represent the mean of the maximum and minimum daily (24 hours) air temperatures of the room for each month. The maximum occurred during the day, the minimum at night. There was little change from month to month and the difference between daytime and nighttime temperatures was of the same order of magnitude for each month. For this reason recording of temperatures was discontinued after a 9 month period. Water temperatures of the containers were not taken regularly but in each of the several instances when they were checked the temperature was near the average of the maximum and minimum for that particular month.

II. For purposes of comparison 3 pairs each of *Physa pomilia* and *Pseudosuccinea columella* were reared concurrently. These snails were maintained under normal daylight conditions, but were otherwise treated as described above. Individuals of *Physa pomilia* used in this series were obtained from a different egg mass than those used in experiment I.

Observations on the snails of experiments I and II were made from the time of hatching, August 1962, until the death of the last individual, July 1964.

RESULTS

Data from the paired snails of the light-dark experiment (I) are shown in Fig. 1, together with the daylight duration

TABLE 1. Mean monthly room temperature in °C

Month	Maximum	Minimum
July 1962	24	19
August	24	19
September	23	20
October	23	18
November	23	18
December	23	18
January	24	17
February	24	18
March 1963	24	17

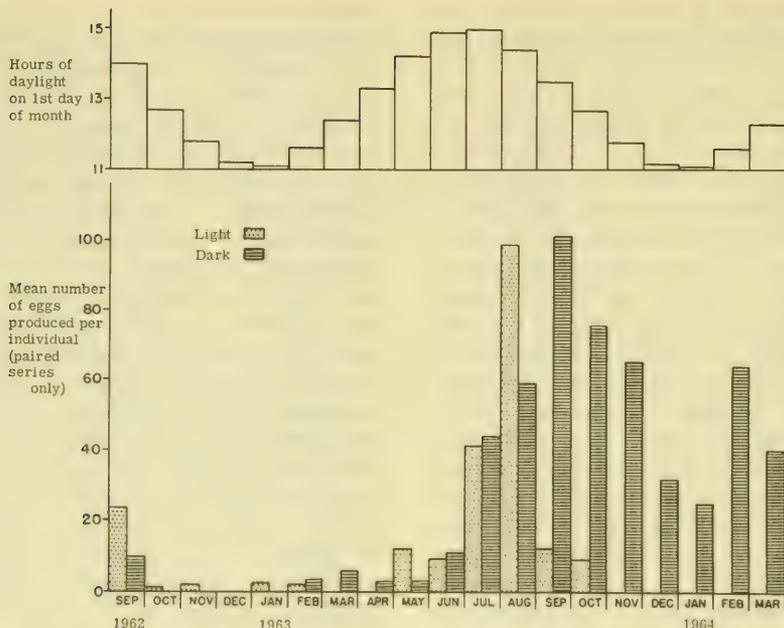


FIG. 1. Egg production of *Physa pomilia*, kept in daylight and in total darkness, from the beginning (September 1962) to the end (March 1964) of egg-laying (monthly means for 4 snails each).

curve. The isolated snails are not included since, under daylight conditions, 1 failed to reproduce and 1 produced only a single egg mass of 10 eggs, while the 2 kept in the dark produced but a total of 309 eggs and only over the 4 month period of June through September, 1963. Peak production was in August for one of them and in September for the other.

The pairs reared under normal daylight conditions and those reared in the dark began to lay eggs at approximately the same time in September 1962 when they were around 40 days old. Eggs were produced more or less continuously in both light and darkness; definite peaks occurred in each case in late summer of the following year. After the peak was achieved, oviproduction rapidly decreased and permanently stopped in the snails of the light experiment but declined rather gradually and was subject to pulses of productivity in those living in darkness. Total egg production per individual was considerably greater in

the dark, both in the case of pairs (3:1) and of isolated snails (30:1). The viability of eggs was similar in both groups, amounting to 83% for snails in the light series and to 85% for those in the dark series.

The data obtained from experiment II for *Physa pomilia* and *Pseudosuccinea columella* under normal daylight conditions are presented in Fig. 2. As in experiment I, the snails showed fluctuation in egg production. The viability of the eggs produced by *Physa pomilia* in this experiment was 94%, that of *Pseudosuccinea columella* was 93%.

The time span shown in Figs. 1 and 2 does not represent the total life span of the snails, but only that interval of their life during which eggs were produced. The snails used in all experiments were hatched on approximately the same date, varying by only days (around August 1, 1962). The length of time for maturation was about the same (approximately 40 days) for all groups. All paired individuals of both species began laying

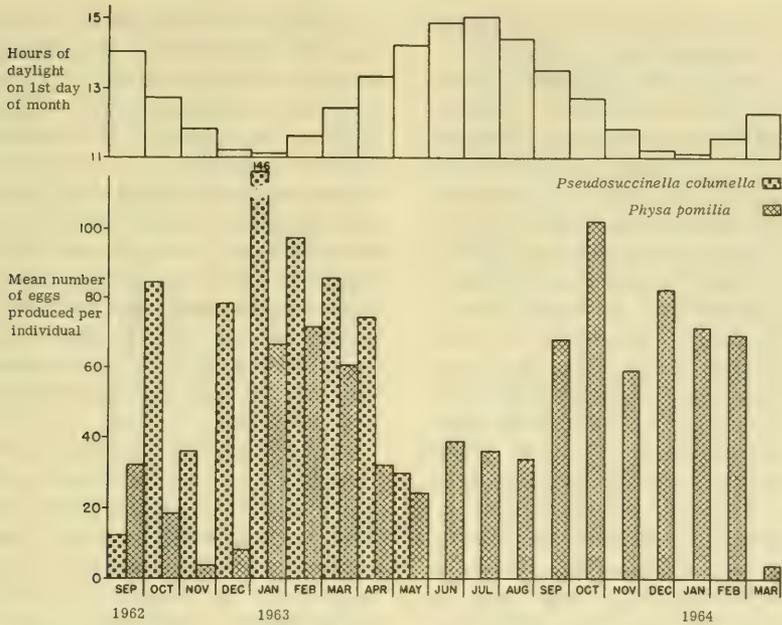


FIG. 2. Egg production of 3 pairs each of *Pseudosuccinea columella* and *Physa pomilia* from beginning to the end of egg-laying (September 1962–March 1964).

eggs within the month of September when the day-length declined. The different individuals lived for varying periods of time after the last egg masses had been produced. In all cases one of each pair of snails died while eggs were being laid. Thus the length of post-reproductive survival could be determined for only one of each pair.

The last eggs were laid by the 2 surviving *Physa pomilia*, reared in experiment I under normal daylight conditions, during the month of October 1963; following this event the snails lived for 62 and 86 days. Those reared in darkness laid the last eggs in September 1963 and March 1964 respectively and lived for 92 and 155 days following the cessation of oviposition.

For the 3 pairs of *Physa pomilia* reared in experiment II (under normal daylight conditions) egg laying ceased in April 1963, December 1963 and March 1964. The post-reproductive periods were 5, 82 and 100 days. The *Pseudosuccinea columella* lived for 1, 6 and 7 days after the last eggs had

been laid in January, March and May of 1963.

DISCUSSION

The results of a previous study on isolated and paired individuals of *Physa pomilia* (DeWitt & Sloan, 1959) were corroborated by those in this investigation in that the pairs produced more eggs per individual than did the isolated snails. In that earlier study, the same tendency was much more pronounced in 4 individuals of *Helisoma duryi* Weatherby, hatched from the same egg mass, and reared in isolation: one of these snails lived for 434 days without producing a single egg; another lived for 790 days and laid 4 eggs on the 647th day; when the remaining 2, isolated for 379 days without laying an egg, were paired on the 380th day, egg production began within a few hours. Together these 2 snails produced a total of 2,522 eggs before death. Thus, copulation may perhaps serve as a stimulus to greater egg production. However, there also

exists conflicting evidence as regards other pulmonate species: isolated individuals of *Physa gyrina* (DeWitt, 1954a) and *Pseudosuccinea columella* (DeWitt & Sloan, 1958) laid an appreciably greater number of eggs per individual than did paired individuals of these 2 species.

All paired *Physa pomilia* hatched from the same egg mass and used in experiment I (light-dark) began oviproduction in September within a few days of each other. This timing demonstrates that initiation of egg production was not triggered by increase in day-length. Indeed, light conditions appeared to play no role in the reproductive cycle of the snails reared in darkness. Further evidence supporting the contention that, in the species investigated, the initiation of oviproduction is not dependent upon long day-light conditions is found in experiment II, where both *Physa pomilia* and *Pseudosuccinea columella* kept under natural light also began egg production in September.

The difference in total egg production between the 2 groups of *Physa pomilia* reared from 2 different egg masses obtained from 2 different parents (see Figs. 1 and 2) was probably due to normal genetic variation. Similar variation between individuals and groups of individuals has been noted in *Physa gyrina* (DeWitt, 1954b) and *Pseudosuccinea columella* (DeWitt & Sloan, 1958).

Agersborg's (1932) report that *Physa gyrina* reproduced the year around, even under winter light conditions, indicates that in nature adequately high temperature alone is sufficient to maintain continuous reproduction. Jenner's (1951) results show that egg production (in *Lymnaea*) can be initiated by a rise in temperature and, once initiated, can be stopped completely by lowering the temperature. In both cases day-length was not critical. These observations indicate that oviproduction can be controlled by temperature alone.

That the various species have well defined temperature requirements and will not oviposit below their specific

temperature thresholds has been repeatedly observed. Thus Precht (1936) reported that *Planorbis* [= *Planorbarius*] *corneus* L. commenced oviposition at 12°C. Bondesen (1950) working with *Ancylus fluvialis* L. found that these snails would not begin to lay eggs at a temperature below 7°C. In a natural population of *Physa gyrina* (DeWitt, 1955) egg-laying did not occur until the water temperature had reached 10°C. W. F. DeWitt (1955) found that a minimum temperature of 7-8°C is required before egg production could begin in *Physa fontinalis* L.

It is generally agreed that, in a natural population of freshwater gastropods, the individuals are in phase as regards their reproductive cycle; that is, the rhythm of the reproductive cycle corresponds to the temperature cycle of the environment. In the north, where winter conditions are severe, reproduction ceases when the temperature falls below a certain level. In those areas where temperature remains more or less constant at a relatively high level, reproduction is carried on throughout the year; but, although the daylight period varies in length under various field conditions, it has not been shown to affect egg production.

Under laboratory conditions, when the temperature is maintained above a certain required level, reproduction is demonstratedly continuous in a number of freshwater snails. The present investigation has demonstrated that oviproduction in a physid and a lymnaeid was not stimulated in response to lengthening periods of daylight. After attainment of sexual maturity, the release of ripe gametes presumably depends upon favorable temperatures. When these temperature conditions are met experimentally, oviproduction begins and continues regardless of light conditions and length of day.

The greater productivity of *Physa pomilia* reared in the dark, in experiment I, may be due to a tendency of some freshwater gastropods to lay their

eggs during the night hours. This observation has been reported for *Physa gyrina* (DeWitt, 1954c) which, although it oviposited throughout a 24 hour period, showed a definite tendency to lay egg masses during the early morning hours. The fact that productivity of those snails reared in daylight, in the same experiment, tended to be inversely proportional to the length of day lends support to this argument. Further support is found in experiment II where maximum egg production by the 2 species did not coincide with maximum day-length. In both *Pseudosuccinea columella* and *Physa pomilia* the first peak production occurred during the winter months of January and February 1963. A second peak occurred in *Physa pomilia* in October 1964 when day-length was declining.

There is the possibility that egg production may be controlled by a light/dark ratio. Such control has been well authenticated in some birds. It is also conceivable that total darkness may mimic the effect of a light/dark ratio favorable to egg production. Future experiments involving larger numbers of snails and well controlled photoperiods are planned.

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RESUMEN

ESTIMULO DE PRODUCCION OVIGERA
EN UN PHYSIDO Y UN LYMNAEIDO

R. W. DeWitt

Individuos de *Physa pomilia* Conrad, nacidos en laboratorio y desarrollados en obscuridad total, comenzaron la puesta al mismo tiempo que otros criados en luz diurna normal, en septiembre, y aproximadamente a los 40 días después del nacimiento. Todos los individuos procedían de una misma masa ovígera. El número de huevos puestos por individuos criados a oscuras fué por lo menos 3 veces mayor. La capacidad de supervivencia de los huevos fué en el mismo orden de magnitud (83-85%) en ambos casos.

En otros experimentos simultáneos, *P. pomilia* y *Pseudosuccinea columella* (Say), criados bajo condiciones de luz normal, comenzaron la puesta a la misma edad y tiempo, en la estación del año cuando la luz diurna decrecía en duración. La sugerencia que la reproducción (en *Lymnaea*) puede estar inducida por la prolongación de los días, no se mantiene. Se concluye, en cambio, que en las especies investigadas, la iniciación de la puesta es una función de temperatura más bien que de luminosidad, siempre que los individuos hayan alcanzado madurez sexual. Cuando la temperatura se mantiene sobre el límite mínimo, la puesta tiene lugar sin estar afectada por la duración del día.

Producción máxima de huevos por individuo en ambas especies, criados en luz, no coincide con largos periodos diurnos, sino que hay una tendencia a una proporción inversa a la luz del día. Este hecho, junto con la mayor puesta de *P. pomilia* criados en total obscuridad, sugiere que la luz puede inhibir producción ovígera.

АБСТРАКТ

СТИМУЛИРОВАНИЕ ПРОДУКЦИИ ЯИЦ У ФИЗИД И ЛИМНЕИД

Р. М. Деви́тт

Physa pomilia Conrad, выведенные и содержавшиеся в лаборатории в полной темноте, начали продуцировать яйца в одно время с теми *Physa*, которые содержались при обычном дневном свете - в сентябре, в возрасте, примерно 40 дней. Все индивидуумы подопытных моллюсков вышли из одной и той же кладки. Количество яиц на 1 экземпляр у моллюсков, живших в темноте, было, по крайней мере в 3 раза больше, чем у моллюсков, живших на свету. Выживаемость яиц была одинакова в обоих сериях опытов (83-85%). В другом, одновременно поставленном опыте, *Physa pomilia* и *Pseudosuccinea columella* (Say) (Lymnaeidae), содержавшиеся при нормальном дневном освещении начали продуцировать яйца одновременно и в одинаковом возрасте - т.е. в тот сезон года, когда количество дневного света уменьшается. Таким образом, предположение, что начало размножения (у *Lymnaea*) может вызываться удлинением дня - данным опытом не подтверждается. У исследованных видов моллюсков начало продуцирования яиц зависит скорее от температуры, чем от света, принимая, что моллюски уже достигли половозрелости. Если температура поддерживается выше некоторого минимального уровня - начинается откладывание

яиц, вне зависимости от продолжительности дня.

Максимальная продукция яиц у индивидуумов обоих видов, содержащихся при дневном свете, не только не совпадает с периодом наиболее длинного дня, но имеет тенденцию быть обратно-пропорциональной к продолжительности дня. Этот факт, вместе с данными о большей продукции яиц у *Physa pomilia*, содержащихся в полной темноте, заставляет предположить, что свет может задерживать продукцию яиц.

ОГЛАВЛЕНИЕ*

Дж. Б. БЁРЧ	О хромосомах у промежуточных хозяев человеческого билхарциозиса	127
Х. БЕР И Т. ЛЕВЕР	Влияние амфенона-в на продукцию яиц у <i>Lymnaea stagnalis</i>	431
Д. БИНО И М. ЧЕТЕЙЛ	Гистохимическая природа печеночной железы и подшвы ноги у <i>Arion rufus</i> (Stylommatophora: Arionidae)	269
Ч. К. ДЭВИС	Выход личинок-велигер из яиц, заключенных в слизистых кладках некоторых морских гастропод обитающих в районе ямайки	299
Г. М. ДЕВИС И Г. К. ЛИНДСЕЙ	Анализ отдельных особей моллюсков и их популяций с помощью дискового электрофореза	311
Р. М. ДЕВИТТ	Стимулирование продукции яиц у физид и лимнеид	445
П. ДИНАМАНИ	Изменчивость строения желудка у двустворчатых моллюсков	225
Р. Г. ЛАТФИ И Е. С. ДЕМЬЯН	Гистология пищеварительной системы <i>Marisa cornuarietis</i> (Mesogastropoda: Ampullariidae)	375
С. М. ПАТТАРСОН	Число хромосом и систематика брюхоногих моллюсков из <i>Streptoneura</i>	111
М. РЕЙНЕР	Цитологическое исследование Gastropoda (Stylommatophora)	341
О. РАВЕРА	Влияние рентгеновских лучей на характеристику населения <i>Physa acuta</i> (Gastropoda: Basommatophora)	95

*Does not include abstracts in Vol. 5, No. 1.

К. С. РИЧАРДС

Генетические исследования пигментации у
Biomphalaria glabrata (Basommatophora: Planorbidae) 335

Г. П. СЕЛЛМЕР

Функциональная морфология и экологическая
характеристика двустворчатого моллюска
Gemma gemma (Eulamellibranchia: Veneridae) 137

Б. Д. СМИТ

Корреляция между изменениями нейросекреторной
деятельности и созреванием половой системы у
Arion ater (Stylommatophora: Arionidae) 285

Т. Е. ТОМПСОН

Адаптивное значение торсионного процесса у
брюхоногих моллюсков 423

INDEX TO SCIENTIFIC NAMES

- Abida*, 341, 363
secale, 341, 363
Abra, 36
abyssicola, *Lyonsiella*, 183
Acanthophora, 300, 302
spicifera, 300
achates, *Chilostoma*, 367
achates achates, *Chilostoma*, 352
achates, *Helicigona*, 341, 367
achates achates, *Helicigona*, 352
achates rhaetica, *Chilostoma*, 352, 366
achates rhaetica, *Helicigona*, 352, 353, 366

aciculata, *Ocinebrina*, 70
Acmaeidae, 37, 113, 114
Acoela, 10
Actiononaias, 78
ellipsiformis, 78
acuta, *Cochlicella*, 341, 366, 367
acuta, *Physa*, 51, 95-109
acutidens, *Pharella*, 258
Adacnarca, 179
nitens, 179
adranocerca, *Cercaria*, 138, 206, 208
Aenigma, 156
africanus ovoideus, *Bulinus*, 128
Agriolimax, 366
Agriolimax, 73
reticulatus, 73
ala, *Tellina*, 227, 255-257
albus, *Malleus*, 239, 247
alexandrina, *Biomphalaria*, 31, 376
alexandrina alexandrina, *Biomphalaria*, 26, 131

algicola, *Cerithium*, 299-307
Aligena, 182
elevata, 182
Alopiinae, 363, 365
alterniflora, *Spartina*, 143
Amalthea, 117, 119
conica, 117, 119
Amnicola, 307
hydrobioides, 307
limosa, 307
Amussium, 227
pleuronectes, 227
Ampullariidae, 375
Anachis, 118
miseria, 118
Anadara, 231
granosa, 231

Anatina, 183
elliptica, 183
Anatinacea, 160, 162
Anatinidae, 183
Ancistrum, 35
mytili, 35
Ancylus, 42, 450
fluviatilis, 42, 450
angasi, *Ostrea*, 180
angolensis, *Bulinus tropicus*, 25, 128
angulata, *Tulotoma*, 38, 115, 120
Anomomyaria, 225-227, 230, 244, 260, 263

annandalei, *Solen*, 227, 256, 258
Anodonta, 184, 245, 247
cygnea, 245, 247
imbecillus, 77
Anodontinae, 77
Anomalodesmata, 160
Anomiidae, 226, 227, 230, 244, 245, 262, 263

anomoides, *Pinctada*, 226, 241
Anomia, 156
antillarum, *Haminea*, 299-307
aperta, *Cantareus*, 65
aperta, *Helix*, 65
Aplysia, 425, 426
californica, 306
dactylomela, 306
protea, 306
punctata, 425, 426
appressa, *Lymnaea stagnalis*, 377, 388, 400

arbustorum, *Arianta*, 64, 352, 366, 367
arbustorum, *Helicigona*, 352, 353, 366, 367

arbustorum, *Helix*, 64
Arca, 226, 231, 232, 235
concamera, 226, 231, 232
frieli, 179
gracialis, 179
inaequivalvia, 226, 231
pectuncutoides grandis, 179
rhombea, 226, 231
vivipara, 179
Arcacea, 225, 226, 262
Archaeogastropoda, 37, 111-114, 121
Archelix, 368
Archidoris, 83, 427
pseudoargus, 83, 427

- Arcidae, 179, 226, 231
Arcuatula, 226, 237, 238, 243
arctica, *Hiatella*, 248
arenaria, *Mya*, 36, 141, 143, 150, 209
arenosa, *Lyonsia*, 183
Arianta, 64, 352, 366, 367
 arbutorum, 64, 352, 366, 367
 Ariantinae, 351
Arion, 269-284, 285-298, 366
 ater, 285-298
 rufus, 269-284, 296
 subfuscus, 296
 Arionidae, 269, 285, 265
 Ariophantacea, 63
arpatschaina, *Euomphalia*, 341, 366
arpatschaina sewanica, *Euomphalia*,
 349, 351
arpatschaina sewanica, *Harmozica*,
 349
artica, *Portlandia*, 179
Asaphis, 253, 254
 deflorata, 253, 254
asianus, *Chicoreus*, 118
Aspergillum, 183
 javanicum, 183
aspersa, *Cryptomphalus*, 64, 359
aspersa, *Helix*, 64, 73, 269, 359, 360
Assimineae, 116, 117, 123
 castanea, 116, 117
 grayana, 123
 japonica, 116, 117
 parasitologica, 116, 117
 yoshidayukioi, 116, 117
Assimineidae, 37, 116, 117, 123
Astarte, 180
 borealis, 180
 crenata, 180
 elliptica, 180
 montagu, 180
 sulcata, 180
 Astartidae, . 180
Astraliium, 114
 haematragum, 114
aureus, *Staphylococcus*, 45
Australorbis, 17, 29, 31, 33, 107, 131,
 335, 377, 446
 glabratus, 29, 31, 107, 377,
 446
australis, *Hydriddella*, 245
austriaca, *Cepaea*, 356, 357
austriaca, *Helix*, 356
ater, *Arion*, 285-298
ater, *Viviparus*, 115
Atrina, 241
 vevillum, 241
atropurpurea, *Pinna*, 227, 241
avenacea, *Chondrina*, 341, 363
aviculooides, *Philobrya*, 179
Axinopsis, 181
 orbiculata, 181
Babylonia, 118, 119
 japonica, 118, 119
Babinka, 9
balthica, *Macoma*, 36, 81, 143
barvicensis, *Trophonopsis*, 71
Basommatophora, 75, 95, 335, 342, 365
Batillaria, 116
 zonalis, 116
beccarii, *Bulinus*, 25, 128
Bedevina, 118
 birileffi, 118
Beguina, 227, 248, 249
 semiorbiculata, 248
 variegata, 227, 248, 249
belcheri, *Limopsis*, 226, 231, 232
Bellamyia, 113, 115
 bengalensis, 115
 dissimilis, 115
Bellamyinae, 113, 115, 117, 121
bengalensis, *Bellamyia*, 115
Berthelinia, 299-307
 caribbea, 299-307
 citrina, 306
 limax, 305
Bertiae, 63
bicineta, *Pyrene*, 118
bicolor, *Pinna*, 227, 240, 241
bidentata, *Mysella*, 182
Bilharzia, 21
Biomphalaria, 17, 21, 25, 26, 31-33,
 130, 131, 312, 324, 327, 335, 339,
 339, 376, 377, 388-390
 alexandrina, 31, 376
 alexandrina alexandrina, 26, 131
 choanomphala choanomphala, 131
 glabrata, 26, 131, 324, 327, 335, 339,
 377, 388-390
 pfeifferi gaudi, 26, 131
 pfeifferi pfeifferi, 26, 131
 pfeifferi madagascariensis, 26, 131
 sudanica tanganyicensis, 26, 131
birileffi, *Bedevina*, 118
Bithynia, 116, 117
 leachi, 123
 tentaculata, 123
 usseriensis, 116, 117

- Bithyniidae, 37, 116, 117, 123
 Bivalvia, 7, 67, 225-268
blainvillei, *Muricopsis*, 70
boettgeri, *Helix moussoni*, 361
Boetgerilla, 57
 vermiformis, 57
Bolinus, 70
 brandaris, 70
bolteni, *Lanistes*, 115
borealis, *Astarte*, 180
borealis, *Lucinoma*, 257
borealis, *Parvatrema*, 138, 206-208
borealis, *Phacoides*, 257
Boreotrophon, 71
 clathratus, 71
 truncatus, 71
borinquēnae, *Parvatrema*, 206
Bornia, 182
 corbuloides, 182
 longipes, 182
bosnica, *Helix dormitoris*, 361
Botula, 226, 237, 239
 cinnamomea, 226, 237, 239
 Bradybaenidae, 341, 365
brandaris, *Bolinus*, 70
brevicula, *Littorina*, 115
bronni, *Purpura*, 118
brunnea, *Psephidia*, 182
 Buccinidae, 38, 118, 119
Buccinum, 118
 undatum, 118
 Bucciniacea, 38, 112, 118, 119
 Buliniinae, 128
Bulinus, 15, 16, 21, 25, 31, 32, 127-130,
 312, 327, 376, 446
 africanus, 15
 africanus ovoideus, 128
 becarii, 25, 128
 coulboisi, 25, 42, 128, 129
 forskalii, 16, 25, 128, 129
 globosus, 23, 25, 128
 guernei, 25, 128
 jousseaumei, 25, 128
 livatus, 130
 nasutus nasutus, 23, 128
 nasutus productus, 128
 natalensis, 25, 128, 129
 reticulatus, 25, 128
 schackoi, 128, 129
 senegalensis, 25
 tropicus, 21, 129
 tropicus angolensis, 25, 128
 tropicus natalensis, 21
 tropicus tropicus, 25, 128
 tropicus zanzibaricus, 25, 128
 truncatus, 16, 31, 42, 376, 446
 truncatus rohlfsii, 25, 128, 129
 truncatus sericinus, 25, 127
 truncatus truncatus, 25, 128, 129
 ugandae, 128
Bursatella, 299-307
 pleii, 299-307
caillaudi, *Lymnaea*, 31, 376
californianus, *Mytilus*, 150
californica, *Aplysia*, 306
californica, *Pomatiopsis*, 116
Callinectes, 143
 sapidus, 143
Cambridium, 8, 9
Camenae, 63
Campeloma, 113, 115
 decisum, 115
 ponderosum, 115
 ponderosum coarctatum, 115
 subsolidum, 115
 Campylaeinae, 351
canaliculata, *Pomacea*, 377, 380, 397,
 399, 404, 409
candicans, *Helicella*, 346
candidula, *Helix*, 345
Candidula, 341, 345, 346, 366, 367
 unifasciata, 341, 345, 346, 366, 367
Cantareus, 65
 aperta, 65
Cantharidus, 114
 collichroa, 114
Cantharus, 118
 subrubiginosus, 118
cantiana, *Monacha*, 341, 347, 348, 356,
 366
cantiana, *Theba*, 348
cantoni, *Katayama*, 131
capgrandi, *Phyllonotus*, 70
carbonnieri, *Murex*, 70
Carcinides, 143
 maenas, 143
cardiformis, *Pseudokellyna*, 182
 Cardiidae, 182
Cardita, 181
 concamerata, 181
 Carditidae, 180, 181, 227, 248, 262
carditoides, *Petricola*, 151
Cardium, 36, 141, 182, 195, 263
 edule, 36, 195, 263

- elegantulum*, 182
exigium, 182
lamarcki, 36
caribbea, *Berthelinia*, 299-307
carpenteri, *Tegula pfeifferi*, 114
carthusiana, *Helix*, 65
cartusiana, *Monacha*, 65, 346, 347, 366
cartusiana, *Theba*, 346
Carunculina, 77, 78
parva, 77, 78
casina, *Venus*, 150
casta, *Meretrix*, 227, 250, 252
castanea, *Assiminea*, 116, 117
Cataleysia, 227, 250
opima, 227, 250
catascopium, *Stagnicola*, 326
Caucasotachea, 341, 364, 368
lenkoranea, 341, 364
Caulerpa, 302-305
okamurai, 305
sertularioides, 303
verticillata, 304
Ceratodes, 377
cornuarietis, 377
Cercaria, 138, 206, 208
adranocerca, 138, 206, 208
milfordensis, 208
Ceriantharia, 9
Cerithiacea, 37, 112, 113, 116, 117
Cerithidea, 116, 206
cingulata, 116
costata, 206
djadjariensis, 116
rhizophorarum, 116
Cerithiidae, 37, 117, 119
Cerithium, 299-307
algicola, 299-307
ferrugineum, 301, 307
vulgatum, 307
certica, *Xerocrassa*, 346, 347
Cetoconcha, 183
Cetoconchidae, 183
Cellana, 114
eucosmia, 114
nigrolineata, 114
toreuma, 114
cellarius, *Oxychilus*, 377
celtica, *Onchidella*, 269, 281
Cepaea, 64, 341, 355-359, 366, 368
austriaca, 356, 357
hortensis, 341, 342, 358, 359, 368
nemoralis, 341, 355-368
nemoralis-etrusca, 64
silvatica, 341, 355, 368
vindobonensis, 341, 355, 356, 368
Cephalopoda, 7, 10
Chama, 259, 261
lazarus, 259
Chamidae, 262
Chamostreidae, 262
Chicoreus, 70, 118
asianus, 118
dujardini, 70
Chilostoma, 352, 366, 367
achates, 367
achates achates, 352
achates rhaetica, 352, 366
cisalpina rhaetica, 352
Chilotrema, 351
lapicida, 351
Chione, 227, 250, 252
tiara, 227, 250, 252
chiui, *Oncomelania hupensis*, 328, 329
chiui, *Tricula*, 329
Chlamys, 67
Chlorella, 158
choanomphala choanomphala, *Biomphalaria*, 131
Chondrina, 341, 363
avenacea, 341, 363
similis, 341, 363
Chondrinidae, 341, 363, 365
Chromodoris, 306
pulchella, 306
cincinnatiensis, *Pomatiopsis*, 18, 19, 38, 111, 115, 120
cinctella, *Hygromia*, 366, 367
cingulata, *Cerithidea*, 116
cinnamomea, *Botula*, 226, 237, 239
Cipangopaludina, 113, 115
malleata, 115
cisalpina rhaetica, *Chilostoma*, 352, 366, 367
cisalpina rhaetica, *Helicigona*, 352
Cistenides, 143
gouldii, 143
citrina, *Berthelinia*, 306
clarkiae, *Montacuta*, 181
clathratus, *Boreotrophon*, 71
Clausilia, 363, 364
parvula, 363, 364
Clausiliidae, 363, 365
Clausiliinae, 363, 365
Clavagellidae, 183, 262

- clavigera*, *Purpura*, 118
Clinocardium, 67
Clithon, 114, 117
retropictus, 114, 117
Clypina, 113, 114
picta, 113, 114
coarctatum, *Campeloma ponderosum*,
115
Cochlicella, 341, 366, 367
acuta, 341, 366, 367
Cochlicopa, 341, 363
lubrica, 341, 363
Cochlicopidae, 363
Cochlodina, 363, 364
laminata, 363, 364
Cochlodininae, 363
Cochlostoma, 363, 364
septemspirale, 363, 364
Coelenterata, 10
collichroa, *Cantharidus*, 114
Columbella, 118, 119
rustica, 118
versicolor, 118, 119
columella, *Pseudosuccinea*, 445-451
communis, *Phragmites*, 143
complanatus, *Elliptio*, 161
compressa, *Enteromorpha*, 144
compressa, *Lasmigona*, 77
concamera, *Arca*, 226, 231, 232
concamerata, *Cardita*, 181
concinna, *Notacmea*, 114
Condylocardia, 181
Condylocardiidae, 181
conica, *Amalthea*, 117, 119
consona, *Helix*, 65
consona, *Theba*, 65
contectoides, *Viviparus*, 115
contectus, *Viviparus*, 115
Contumax, 117
kobelti, 117
Corbiculidae, 262
corbuloides, *Bornia*, 182
Corculum, 67
coreensis, *Lunella coronata*, 114
cornea, *Euthria*, 118
corneus, *Planorbarius*, 450
corneus, *Planorbis*, 450
corniculatus, *Muricopsis*, 70
cornuarietis, *Ceratodes*, 377
cornuarietis, *Marisa*, 31, 115, 375-411
cornutus, *Turbo*, 114
coronata coreensis, *Lunella*, 114
corrianus, *Lamellidens*, 227, 245, 246,
248
costata, *Cerithidia*, 206
costata, *Philobrya*, 179
costigera, *Mysorella*, 116, 117
costulata, *Oncomelania*, 131
costulatus hyrcanus, *Pomatias*, 363
costulatus, *Pomatias*, 341
coulboisi, *Bulinus*, 25, 128, 129
Crago, 143
septemspinus, 143
crassa, *Oncomelania*, 131
crassa, *Tellina*, 255, 257
crassicostatus, *Pecten*, 227, 242, 244
Crassostrea, 140, 143, 148, 178, 188,
241
madrasensis, 241
virginica, 140, 143, 148, 178, 188
crenata, *Astarte*, 180
Crenella, 180
decussata, 180
glandula, 180
crenulata, *Melania*, 38, 116, 118, 120
crenulata, *Radina*, 116, 118
cretica, *Helicella*, 341
cretica, *Trochoidea*, 346, 347, 366
Cryptomphalus, 64, 359
aspera, 64, 359
Cumberlandia, 77
monodonta, 77
Cumingia, 64
cumingii, *Jouannetia*, 227, 258, 259
cuneiformis, *Gastrochaena*, 247
cuneiformis, *Rocellaria*, 247, 248
Cuspidaria, 183
glacialis, 183
obesa, 183
subtorta, 183
Cuspidariidae, 183
Cyamiidae, 181, 262
Cyamium, 181, 182
minutum, 181, 182
Cyclas, 184
Cyclophoridae, 363
cygnea, *Anodonta*, 245, 247
Cyprinidae, 262
cyprinoides, *Villorita*, 250, 252
Cyrella, 179
minuta, 179
Dacrydium, 180
vitreum, 180
dactylomela, *Aplysia*, 306

- Daudebardia*, 57
 dobrogica, 57
decipiens, *Semisulcospira*, 116
decisum, *Campeloma*, 115
decussata, *Crenella*, 180
deflorata, *Asaphis*, 253, 254
Delima, 341, 363
 itala, 341, 363
delphinodonta, *Nucula*, 179
demissus, *Modiolus*, 143
dendrita, *Zebrina*, 341, 363
Deroceras, 57, 58
 forcarti, 57
 melanocephalus, 57
 schleschi, 57
 subagrestis, 57
 transcaucasicus, 57
devexa, *Thracia*, 183
dextrorsum, *Limnaea*, 63
diaphana, *Vitrea*, 341, 363
dibranchiata, *Glycera*, 143
didyma, *Glossoulax*, 119
didyma, *Neverita*, 117, 119
diegensis, *Teredo*, 182
Digenea, 15
dilatata, *Macroschisma*, 113, 114
Dinarica, 352, 366, 368
 pouzolzi, 352, 366, 368
Dinocardium, 67
diphos, *Sanguinolaria*, 227, 253, 254
diphos, *Soletellina*, 227, 254
Diplodontidae, 181
directus, *Ensis*, 143
discors, *Modiolaria*, 180
Discus, 341, 363
 rotundatus, 341, 363
dissimilis, *Bellamya*, 115
distorta, *Thracia*, 183
djadjariensis, *Cerithidea*, 116
dobrogica, *Daudebardia*, 57
dobrogicus, *Limax*, 57
donacis, *Postmonorchis*, 207
Donax, 85, 195, 197, 207
 gouldii, 195, 197, 207
 vittatus, 85
dormitoris bosnica, *Helix*, 361
dormitoris, *Helix*, 341, 361, 366, 368
Dostia, 114, 117
 violacea, 114, 117
dufresnoyi, *Ocenebra*, 70
dujardini, *Chicoreus*, 70
duplicatus, *Polinices*, 209
 duryi, *Helisoma*, 449
Dreissenidae, 262
Drupinae, 70
Ecteinascidia, 300, 301
 turbinata, 300, 301
Edotea, 143
edule, *Cardium*, 36, 195, 263
edulis, *Mytilus*, 35, 36, 140, 143, 150,
 163, 188, 208, 233, 235, 243
edulis, *Ostrea*, 157, 180, 184, 243
effosa, *Sunetta*, 227, 250
elatospira, *Poiriera*, 70
elegans, *Pomatias*, 61, 73, 341, 363
elegantulum, *Cardium*, 182
elevata, *Aligena*, 182
elliptica, *Anatina*, 183
elliptica, *Astarte*, 180
Elliptio, 161
 complanatus, 161
elongata, *Oncomelania*, 131
elongata, *Pandora*, 183
emarginata serrata, *Stagnicola*, 326
Ena, 341, 363, 366
 montana, 341, 363
 obscura, 341, 363
Endodontidae, 363, 365
Enidae, 341, 363, 365
Ensis, 143
 directus, 143
Enteromorpha, 144
 compressa, 144
Entovalva, 182
 mirabilis, 182
Eobania, 64, 341, 362, 366-368
 vermiculata, 64, 341, 362, 366
equalis, *Thysaria*, 181
equestris, *Ostrea*, 180
ericetorum, *Helicella*, 346
erinacea, *Ocenebra*, 70
erinaceus, *Tritonalia*, 118
Erycinidae, 250, 262
eucosmia, *Cellana*, 114
Eulamellibranchia, 137, 180, 225, 227,
 235, 243, 245, 257, 259, 260,
 262, 263
Euomphalia, 341, 349, 351, 366, 367
 arpatschaina, 341, 366
 arpatschaina sewanica, 349, 351
Euparypha, 341, 355, 356, 368
 pisana, 341, 355, 356
Euplica, 119
 versicolor, 119

- Euthria*, 118
cornea, 118
Euthyneura, 37, 111, 121
excoelata, *Ocinebrina*, 70
exigium, *Cardium*, 182
expansa, *Nucula tenuis*, 179
Fasciola, 31, 376
gigantica, 31
Fasciolaria, 118, 119
lignaria, 118, 119
Fasciolariidae, 38, 118, 119
fausti, *Katayama*, 131
ferrea, *Pisania*, 118
Ferrissia, 42
parallela, 42
tarda, 42
ferrugineum, *Cerithium*, 301, 307
ferruginosa, *Montacuta*, 181, 184
festiva, *Hiria*, 119
festiva, *Tritia*, 118, 119
Filibranchia, 179, 259
Fissurellacea, 37, 112-114 114
Fissurellidae, 37, 113, 114
flava, *Fusconaia*, 77
fluviatilis, *Ancylus*, 42, 450
fluctuosa, *Gomphina*, 182
Foraminifera, 55
forcarti, *Deroceras*, 57
formosana, *Katayama*, 131
formosana, *Oncomelania*, 19, 26, 116, 132
formosana, *Oncomelania hupensis*, 26, 132
fontinalis, *Physa*, 450
forskalii, *Bulinus*, 25, 128, 129
forskalii, *Ostrea*, 227, 241-244
fragilis, *Lima*, 244
Fragum, 67
fraterna, *Portlandia*, 179
fretensis, *Gemma gemma*, 139
frieli, *Arca*, 179
frons, *Ostrea*, 180
Fruticicola, 348, 349
sericea, 349
unidentata, 348
villosa, 348
Fruticicolinae, 348
Fusconaia, 77
flava, 77
fuscoviridis, *Notacmea*, 114
Fusulinae, 363
Gafrarium, 150, 250, 252
minimum, 150, 250, 252
Galeomma, 182, 227, 249, 250
paucistriata, 227, 249, 250
turtoni, 182
Galeommidae, 182, 250
gallina, *Tegula*, 45
Gari, 253, 254
togata, 253, 254
Gastrochaena, 227, 246, 247
cuneiformis, 247
impressa, 227, 246, 247
Gastrochaenidae, 226, 227, 247, 262
Gastrodeutia, 259
Gastropempta, 259, 261
Gastropoda, 7, 57, 95, 123, 376, 402, 423
Gastroproteia, 259
Gastrotetartika, 259, 261
Gastrotriteia, 259
gaudi, *Biomphalaria pfeifferi*, 26, 131
Gemma, 137-223
gemma, 137-223
gemma fretensis, 139
gemma manhattensis, 139
gemma purpurea, 139, 140
purpurea, 206
gemma fretensis, *Gemma*, 139
gemma, *Gemma*, 137-223
gemma manhattensis, *Gemma*, 139
gemma purpurea, *Gemma*, 139, 140
georgianus, *Viviparus*, 115
gigantea, *Saxidomus*, 150
gigantica, *Fasciola*, 31
gigas, *Strombus*, 306, 45
glabrata, *Biomphalaria*, 26, 131, 324, 327, 335-339, 377, 388, 389, 390
glacialis, *Cuspidaria*, 183
glacialis, *Pandora*, 183
glabratus, *Australorbis*, 29, 31, 107, 377, 446
glandula, *Crenella*, 180
globosa, *Pila*, 377
globosus, *Bulinus*, 25, 128
globosus, *Physopsis*, 23
Glossoulax, 119
didyma, 119
glutinosa, *Myxas*, 307
Glycera, 143
dibranchiata, 143

- Glycymeris*, 233
violascens, 233
Gomphina, 182
fluctuosa, 182
gouldii, *Cistenides*, 143
gouldii, *Donax*, 195, 197, 207
gouldi, *Thysaria*, 181
gracialis, *Arca*, 179
Graciliaria, 341, 363
strobili, 341, 363
gracilis, *Lithophaga*, 226, 237-239
grandis, *Arca pectuncutoides*, 179
granosa, *Anadara*, 231
granularis, *Nodilittorina*, 115, 117
granulata, *Poromya*, 183
grayana, *Assimineae*, 123
groenlandicum, *Propeamusium*, 180
guernei, *Bulinus*, 25, 128
Gyraulus, 42
parvus, 42
gyrina, *Physa*, 450, 451
habei, *Semisulcospira*, 116
haemastoma, *Stramonita*, 71
haemastoma, *Thais*, 71
haematobium, *Schistosoma*, 15, 23, 25,
127, 130, 330
haematragum, *Astraliium*, 114
Haliotidae, 37, 113, 114
Haliotis, 9, 45, 113, 114, 424
japonicus, 113, 114
rufescens, 45
Haminea, 299-307
antillarum, 299-307
hanleyi, *Nucula*, 229
hanleyi, *Scintilla*, 250
Harmozica, 349
arpatschaina sewanica, 349
haudmuticus, *Haustellum*, 70
haudmuticus, *Murex*, 70
Haustellum, 70
haudmuticus, 70
Helaplex, 118
trunculus, 118
Heliceae, 356, 368
Helicella, 341, 345-347, 366, 367
candicans, 346
cretica, 341
ericetorum, 346
itala, 341, 346, 347, 366
obvia, 346, 347, 366
unifasciata, 345
Helicelleae, 341
Helicellinae, 341, 345, 366, 367
Helicidae, 312, 341, 342, 363, 365, 367
Helicigona, 341, 351-353, 366-368
achates, 341, 367
achates achates, 352
achates rhaetica, 352, 353, 366
arbustorum, 352, 353, 366, 367
cisalpina rhaetica, 352
lapicida, 351, 366-368
pouzolzi, 341-353, 366, 368
setosa, 341, 353, 354, 366, 368
Helicigoninae, 351, 365, 367
Helicinae, 341, 356, 366, 368
Helicodonta, 341, 351, 366, 367
obvoluta, 341, 351, 366
Helicodontinae, 349, 366, 367
Helicogena, 361
moussoni, 361
Helisoma, 327, 449
duryi, 449
trivolvus, 327
Helix, 63-65, 73, 269, 281, 295, 311-333
341, 345, 346, 356, 359-368, 377, 389
aperta, 65
arbustorum, 64
aspersa, 64, 73, 269, 359, 360
austriaca, 356
candidula, 345
carthusiana, 65
consona, 65
dormitoris, 341, 361, 366, 368
dormitoris bosnica, 361
lucorum, 341, 361, 368
lucorum trapezuntis, 361, 362
moussoni boettgeri, 361
nemoralis-etrusca, 64
obvia, 346
peninsularis, 65
pomatia, 295, 311-333, 366, 389
profuga-etrusca, 65
surrentina, 65
vermiculata, 64
Heminerita, 117
japonica, 117
Hemipecten, 156
heptagonatus, *Homalocantha*, 70
herbstii, *Panopeus*, 143
herculana, *Lytopenete*, 57, 58
heros, *Polinices*, 143
hians, *Lima*, 243
Hiatella, 248
arctica, 248

- Hipponicacea, 37, 112, 117, 119
 Hipponicidae, 37, 117
Hira, 119
 festiva, 119
histrica, *Sinotaia*, 115
hizenensis, *Pusia*, 118, 119
Hochstettaria, 179
holosericum, *Isognomostoma*, 341, 353, 354, 366, 367
Homalocantha, 70
 heptagonatus, 70
 pauli, 70
hortensis, *Cepaea*, 341, 342, 358, 359, 368
hupensis chiui, *Oncomelania*, 328, 329
hupensis formosana, *Oncomelania*, 26, 132
hupensis hupensis, *Oncomelania*, 26
hupensis nosophora, *Oncomelania*, 26, 132
hupensis, *Oncomelania*, 26, 116, 131, 132
hupensis quadrasi, *Oncomelania*, 26, 132
Hydrobia, 123
 neglecta, 123
 stagnorum, 123
 ulvae, 123
 Hydrobiidae, 37, 111, 117, 123
hydrobioides, *Amnicola*, 307
Hygromia, 366, 367
 cinctella, 366, 367
 Hygromiinae, 348, 365, 366, 367
hyperborea, *Yoldia*, 179
hyrcanus, *Pomatias costulatus*, 363
Hyridella, 245
 australis, 245
hystrix, *Spondylus*, 244, 245
imbecillus, *Anodonta*, 77
imbriferum, *Propeamussium*, 180
impressa, *Gastrochaena*, 227, 246, 247
inaequivalvia, *Arca*, 226, 231
incarnata, *Perforatella*, 366
Indoplanorbis, 15
intermedius, *Typhis*, 70
intertextus, *Viviparus*, 115
Iphigena, 363, 364
 plicatula, 363, 364
 ventricosa, 363, 364
Isidorella, 15
 Isocardiidae, 262
Isoconcha, 181
 sibogai, 181
Isognomon, 67, 226, 227, 239, 240
 nucleus, 226, 239, 240
 Isognomonidae, 226, 239
isognomostoma, *Isognomostoma*, 341, 353, 366
Isognomostoma, 341, 353, 354, 366, 367
 holosericum, 341, 353, 354, 366, 367
 isognomostoma, 341, 353, 366
 personatum, 354
itala, *Delima*, 341, 363
itala, *Helicella*, 341, 346, 347, 366
japonica, *Assimineae*, 116, 117
japonica, *Babylonia*, 118, 119
japonica, *Heminerita*, 117
japonica, *Mysella*, 182
japonica, *Puperita*, 114, 117
japonica, *Schistosoma*, 130
japonica, *Thalotia*, 114
japonicum, *Schistosoma*, 17, 327
japonicus, *Haliotis*, 113, 114
javanicum, *Aspergillum*, 183
jenkinsi, *Potamopyrgus*, 42, 117
Jouannetia, 227, 258, 259
 cumingii, 227, 258, 259
jousseaumei, *Bulinus*, 25, 128
Jousseaumiella, 181
Katayama, 131
 cantoni, 131
 fausti, 131
 formosana, 131
 lii, 131
 nosophora, 131
 nosophora yoshidae, 131
 tangi, 131
Kellia, 181, 197
 suborbicularis, 181, 197
kelseyi, *Milneria*, 180
Kidderia, 181
kobelti, *Contumax*, 117
kochi, *Proclava*, 117
kurodai, *Semisulcospira*, 116
labio, *Monodonta*, 114
Laciniaria, 341, 363
 plicata, 341, 363
lactea, *Lucina*, 181
lamarcki, *Cardium*, 36
Lamellidens, 227, 245, 246, 248
 corrianus, 227, 245, 246, 248

- laminata*, *Cochlodina*, 363, 364
lampanicola, *Patelloida*, 114
 Lampsilinae, 77
Lanistes, 113, 115
 bolteni, 115
lanx, *Patelloida saccharina*, 114
lapicida, *Chilotrema*, 351
lapicida, *Helicigona*, 351, 366-368
lapidaria, *Pomatiopsis*, 18, 19, 38, 111,
 115, 116, 120, 311-333
lapillus, *Nucella*, 70
lapillus, *Purpura*, 38, 113, 119, 122
lapillus, *Thais*, 38, 119
Lasaea, 160, 182, 264
 rubra, 182, 264
Lasmigona, 77
 compressa, 77
 subviridis, 77
lateralis, *Modiolaria*, 180
Laternula, 255
 rostrata, 255
 Laternulidae, 262
Laviosa, 139
layardi, *Nucula*, 226, 228, 230
lazarus, *Chama*, 259
leachi, *Bithynia*, 123
Leanchoilea, 8
Leda, 179
 minuta, 179
 pernula, 179
Lehmannia, 269, 281
 poirieri, 269, 281
lenticula, *Portlandia*, 179
lenkoranea, *Caucasotachea*, 341, 364
Lepton, 181
 parasiticum, 181
 Leptonidae, 181, 182
leucophaeta, *Mytilopsis*, 183
libertina, *Semisulcospira*, 116, 118, 119
 Libitinidae, 262
Liburnica, 354, 366, 368
 setosa, 354, 366, 368
lignaria, *Fasciolaria*, 118, 119
lii, *Katayama*, 131
Lima, 243, 244
 fragilis, 244
 hians, 243
 Limacidae, 57, 365
limax, *Berthelinia*, 305
Limax, 57
 dobrogicus, 57
 zilchi, 57
limax, *Tamanovalva*, 305
limbata, *Nereis*, 143
 Limidae, 226, 230, 244, 262
Limnaea, 63
 dextrorsum, 63
 peregra, 63
 sinistrorsum, 63
 Limopsidae, 179, 226, 231
Limopsis, 179, 226, 231, 232, 233
 belcheri, 226, 231, 232
 vaginatus, 231, 233
limosa, *Amnicola*, 307
Limulus, 8, 143, 205
 polyphemus, 143
lineatus, *Melampus*, 143
lineatus, *Melanoides*, 42, 116, 118
lineatus, *Tarebia*, 116, 118
linguabovis, *Vitularia*, 70
Liolytopelte, 57
 Lioplacinae, 113, 115, 117, 121
Lioplax, 113, 115
 subcarinata, 115
livatus, *Bulinus*, 130
lischkei, *Tegula*, 114
Lithophaga, 226, 227, 235, 237-239
 gracilis, 226, 237-239
 nasuta, 235, 237, 239
Littoraria, 115
 strigata, 115
littorea, *Littorina*, 73, 209, 425
Littorina, 73, 115, 209, 425, 426
 brevicula, 115
 littorea, 209, 425
 Littorinacea, 37, 112, 117
 Littorinidae, 37, 116, 117
livescens, *Nassarius*, 118, 119
livescens, *Niotha*, 119
Lobiger, 305
 serradifalci, 305
Loligo, 45
 pealii, 45
longipes, *Bornia*, 182
longiscata, *Oncomelania*, 131
Lopadorhynchus, 10
lordi, *Psephidia*, 182
loscombiana, *Pholadidea*, 151
loveni, *Macoma*, 182
lubrica, *Cochlicopa*, 341, 363
Lucina, 160, 181
 lactea, 181
 Lucinacea, 261
 Lucinidae, 181

- Lucinoma*, 257
 borealis, 257
lucorum, *Helix*, 341, 361, 368
lucorum trapezuntis, *Helix*, 361, 362
Lunella, 114
 coronata coreensis, 114
lurida, *Ostrea*, 180, 184
luteostoma, *Purpura*, 118
Lutraria, 148
 lutraria, 148
Lymnaea, 21, 31-33, 47, 49, 73, 293,
 376, 377, 388, 400, 431-442,
 445, 446, 450
 caillaudi, 31, 376
 natalensis, 21
 palustris, 445
 stagnalis, 47, 49, 73, 293, 431-442, 446
 stagnalis appressa, 377, 388, 400
Lymnaeidae, 41
lyncæus, *Phidiana*, 299-307
Lyonsia, 183
 arenosa, 183
Lyonsiella, 183
 abyssicola, 183
Lyonsiidae, 183
Lytopenete, 57, 58
 herculana, 57, 58
 moldavica, 57, 58
 occidentalis, 57
 olteniana, 57
 suboccidentalis, 57, 58
Macoma, 36, 81, 143, 182, 208
 balthica, 36, 81, 143
 loveni, 182
 moësta, 182
 torelli, 182
macrophylla, *Venerupis*, 227, 250, 252
Macroschisma, 113, 114
 dilatata, 113, 114
 sinensis, 113, 114
Mactra, 252
 mera, 252
Mactridae, 227, 252, 262
maculosa, *Pisania*, 118
madagascariensis, *Biomphalaria pfeifferi*,
 26, 131
madrasensis, *Crassostrea*, 241
madrasensis, *Ostrea*, 227, 241, 243
maenas, *Carcinides*, 143
magellanica, *Modiolarca*, 181
magnifica, *Tulotoma*, 115
 malleata, *Cipangopaludina*, 115
Malleus, 239, 247
 albus, 239, 247
manhattensis, *Gemma gemma*, 139
mansonii, *Schistosoma*, 15, 17, 130, 335
margaritacea, *Neotrigonia*, 247
margaritifera, *Margaritana*, 77
Margaritana, 77
 margaritifera, 77
Marisa, 31, 32, 113, 115, 375-411
 cornuarietis, 31, 115, 375-411
Martesia, 227, 259
 striata, 227, 259
maximus, *Pecten*, 163, 244, 245
Melampus, 143,
 lineatus, 143
Melania, 38, 116, 118, 120
 crenulata, 38, 116, 118, 120
melanocephalus, *Deroceras*, 57
Melanooides, 42, 116-118, 118
 lineatus, 42, 116, 118
 scabra, 42
 tuberculatus, 42, 116, 118
mera, *Mactra*, 252
Mercenaria, 45, 139, 143, 150, 152, 164,
 188, 195, 208
 mercenaria, 45, 139, 143, 150, 152,
 164, 188, 195
mercenaria, *Mercenaria*, 139, 143, 150,
 152, 164, 188, 195
Meretrix, 227, 250, 252
 casta, 227, 250, 252
Mesogastropoda, 37, 111-113, 115-117,
 121, 375
Metazoa, 10
Micropogon, 175
 undulatus, 175
Milacidae, 365
Milax, 366
milfordensis, *Cercaria*, 208
Milneria, 180
 kelseyi, 180
minima, *Schistosomophora*, 131
minimum, *Gafrarium*, 150, 250, 252
minuta, *Cyrella*, 179
minuta, *Leda*, 179
minuta, *Turtonia*, 181
minutum, *Cyamium*, 181, 182
mirabilis, *Entovalva*, 182
misera, *Anachis*, 118
Mitracea, 38, 112, 118, 119

- Mitridae, 38, 118, 119
 Mobilina, 35
Modiolarca, 181
 magellanica, 181
 trapezina, 181
 Modiolarcidae, 181
Modiolaria, 180
 discors, 180
 lateralis, 180
 nigra, 180
modiolus, *Modiolus*, 233, 237
Modiolus, 143, 156, 226, 233, 236-239
 demissus, 143
 modiolus, 233, 237
 striatulus, 226, 236-239
 undulatus, 226, 236-239
moellendorffi, *Oncomelania*, 131
moldavica, *Lytopenete*, 57, 58
moësta, *Macoma*, 182
mollissima, *Somateria*, 207
 Mollusca, 7, 55, 67, 68, 111, 123
Monaca, 65
 carthusiana, 65
Monacha, 341, 346-348, 356, 366, 367
 cantiana, 341, 347, 348, 356, 366
 cartusiana, 346, 347, 366
Monacheae, 341, 346, 366
monodonta, *Cumberlandia*, 77
Monodonta, 114
 labio, 114
 neritoides, 114
 Monoplacophora, 10
Montacuta, 160, 181, 184
 clarkiae, 181
 ferruginosa, 181, 184
 phascalionis, 181
 Montacutidae, 181
montagui, *Astarte*, 180
montana, *Ena*, 341, 363
moussoni boettgeri, *Helix*, 361
moussoni, *Helicogena*, 361
multicosta, *Oncomelania*, 131
multigranosa, *Semisulcospira*, 116
Murex, 70
 carbonnieri, 70
 haudmuticus, 70
 spincosta, 70
 Muricacea, 38, 112, 118, 119
muricatus, *Trophonopsis*, 71
 Muricidae, 38, 69, 70, 118, 119
 Muricinae, 70
Muricopsis, 70
 blainvillei, 70
 corniculatus, 70
Mya, 36, 141, 143, 150, 208, 209
 arenaria, 36, 141, 143, 150, 209
Myalina, 67
 Myalinidae, 67
 Myidae, 262
 Myochamidae, 262
myopsis, *Thracia*, 183
Mysella, 182
 bidentata, 182
 japonica, 182
Mysorella, 116, 117
 costigera, 116, 117
 Mytilacea, 67, 262
mytili, *Ancistrum*, 35
mytili, *Peniculistoma*, 35
 Mytilidae, 180, 226, 231, 233, 236, 239, 241, 243
Mytilopsis, 183
 leucophaeta, 183
Mytilus, 35, 36, 140, 143, 150, 154, 163, 188, 208, 233, 235, 243, 264, 426
 californianus, 150
 edulis, 35, 36, 140, 143, 150, 163, 188, 208, 233, 235, 243
Myxas, 307
 glutinosa, 307
nakasekoeae, *Semisulcospira*, 116
 Nanine, 63
nasuta, *Lithophaga*, 235, 237, 239
nasutus nasutus, *Bulinus*, 128
nasutus, *Physopsis*, 23
nasutus productus, *Bulinus*, 128
 Nassariidae, 38, 118, 119
Nassarius, 118, 119, 143, 209
 livescens, 118, 119
 obsoletus, 143, 209
natalensis, *Bulinus*, 25, 128, 129
natalensis, *Lymnaea*, 21
 Naticacea, 37, 112, 117, 119
Natica, 85
 Naticidae, 38, 117, 119
navalis, *Teredo*, 182, 184
neglecta, *Hydrobia*, 123
nemoralis, *Cepaea*, 341, 355-368
nemoralis-etrusca, *Cepaea*, 64
nemoralis-etrusca, *Helix*, 64
 Neogastropoda, 38, 111-112, 118, 119, 121, 122

- Neopilina*, 7-9
Neotrigonia, 247
 margaritacea, 247
Nereis, 143
 limbata, 143
 virens, 143
Nerita, 79
Neritacea, 37, 79, 112, 114, 117
Neritidae, 37, 79, 114, 117
neritoides, *Monodonta*, 114
Neverita, 117, 119
 didyma, 117, 119
nigerrima, *Tegula*, 114
nigra, *Modiolaria*, 180
nigrolineata, *Cellana*, 114
Niotha, 119
 livescens, 119
niponica, *Semisulcospira*, 116
nitens, *Adacnarca*, 179
Nodilittorina, 115, 117
 granularis, 115, 117
 picta, 115, 117
nosophora, *Katayama*, 131
nosophora, *Oncomelania*, 19, 26, 116,
 131, 132
nosophora, *Oncomelania hupensis*, 26,
 132
nosophora slatteri, *Oncomelania*, 131
nosophora yoshidae, *Katayama*, 131
Notacmea, 114
 concinna, 114
 fuscoviridis, 114
 schrenckii, 114
notatum, *Sphaerium*, 156, 169
Nucula, 226, 228-230, 257, 263
 hanleyi, 229
 layardi, 226, 228, 230
 sulcata, 228, 230
Nucella, 70
 lapillus, 70
nucleus, *Isognomon*, 226, 239, 240
nucleus, *Nucula*, 179
Nucula, 9, 161, 179, 195
 delphinodonta, 179
 nucleus, 179
 proxima, 195
 tenuis, 179
 tenuis expansa, 179
Nuculanidae, 179
Nuculidae, 179, 226, 228, 230, 263
Nudibranchia, 342
nuttalli, *Schizotherus*, 150
obesa, *Cuspidaria*, 183
oblonga, *Succinella*, 341, 363, 364
obscura, *Ena*, 341, 363
obsoletus, *Nassarius*, 143, 209
obvia, *Helicella*, 346, 347, 366
obvia, *Helix*, 346
obvoluta, *Helicodonta*, 341, 351, 366
occidentalis, *Lytopelte*, 57
ocellatus, *Ovalipes*, 143, 175, 205
Ocenebra, 70, 71
 dufresnoyi, 70
 erinacea, 70
 torosa, 70
Ocinebrina, 70, 71
 aciculata, 70
 excoelata, 70
okamurai, *Caulerpa*, 305
Oligosyringia, 259, 264
olteniana, *Lytopelte*, 57
Oncomelania, 111, 116, 119, 127, 130-
 132, 312, 327-329, 446
 costulata, 131
 crassa, 131
 elongata, 131
 formosana, 19, 26, 132
 hupensis, 26, 116, 131, 132
 hupensis chiui, 328, 329
 hupensis formosana, 26, 116, 132, 312,
 327, 329
 hupensis hupensis, 26, 132
 hupensis nosophora, 26, 132
 hupensis quadrasi, 26, 132
 longiscata, 131
 moellendorffi, 131
 multicosta, 131
 nosophora, 19, 26, 116, 131, 132
 nosophora slatteri, 131
 quadrasi, 26, 116, 132
 yaoi, 131
Onchidella, 269, 281
 celtica, 269, 281
Opabinia, 8
opercularis, *Pecten*, 163
Opica, 65
 surrentina, 65
opima, *Cataleysia*, 227, 250
Opisthobranchia, 365
 orbiculata, *Axinopsis*, 181
 ornata, *Semisulcospira*, 116
Ostracea, 262
Ostrea, 45, 156, 157, 177, 180, 184, 227,
 241-244, 247, 264

- angasi*, 180
edulis, 157, 180, 184, 243
equestris, 180
frons, 180
forskalii, 227, 241-244
lurida, 180, 184
madrasensis, 227, 241, 243
parasitica, 241, 243, 247
permollis, 180
virginica, 45
Ostreidae, 178, 180, 227
Ovalipes, 143, 175, 205
ocellatus, 143, 175, 205
ovalis, *Psephidia*, 182
ovata, *Pila*, 115
ovata, *Venus*, 150
ovoideus, *Bulinus africanus*, 128
Oxychilus, 377
cellarius, 377
Palaemonetes, 143
vulgaris, 143
Pahudomus, 38, 116, 118, 120
tanschaurica, 38, 116, 118, 120
paludosa, *Pomacea*, 446
palustris, *Lymnaea*, 445
palustris, *Stagnicola*, 445
Pandora, 183
glacialis, 183
elongata, 183
rostrata, 183
Pandoracea, 255
Pandoridae, 183, 262
Panopeus, 143
herbstii, 143
papillaris, *Papillifera*, 341, 363
Papillifera, 341, 363
papillaris, 341, 363
Paractis, 143, 205
rapiformis, 143, 205
parallela, *Ferrissia*, 42
parasitica, *Ostrea*, 241, 243, 247
parasiticum, *Lepton*, 181
parasitologica, *Assimineae*, 116, 117
parksi, *Teredo*, 182
parva, *Carunculina*, 77, 78
Parvatrema, 138, 206-208
borealis, 138, 206-208
borinqueña, 206
parvula, *Clausilia*, 363, 364
parvus, *Gyraulus*, 42
Patella, 73, 409
vulgata, 73, 409
Patellacea, 37, 112-114, 121
Patellidae, 37, 113, 114
Patelloida, 114
lampanicola, 114
pygmaea, 114
saccharina lanx, 114
patula, *Siliqua*, 195, 197
paucistriata, *Galeomma*, 227, 249, 250
pauli, *Homalocantha*, 70
pealii, *Loligo*, 45
Pecten, 68, 163, 169, 227, 242, 244, 245
crassicostatus, 227, 242, 244
maximus, 163, 244, 245
opercularis, 163
tenuicostatus, 163, 169
Pectinacea, 68
Pectinidae, 226, 227, 230, 244, 262, 263
pectuncutoides grandis, *Arca*, 179
pellucida, *Standella*, 227, 251, 252
Penaeus, 175
setiferous, 175
Peniculistoma, 35
mytili, 35
peninsularis, *Helix*, 65
peninsularis, *Xerolauta*, 65
peregra, *Limnaea*, 63
Perforatella, 366
incarnata, 366
Peritrichida, 35
permollis, *Ostrea*, 180
Perna, 226, 233-235, 237
viridis, 226, 233-235, 237
pernula, *Leda*, 179
personatum, *Isognomostoma*, 354
Petasina, 348
unidentata, 348
Petricola, 151
carditoides, 151
pholadiformis, 151
pfeifferi carpenteri, *Tegula*, 114
pfeifferi gaudi, *Biomphalaria*, 26, 131
pfeifferi madagascariensis, *Biomphalaria*, 26, 131
pfeifferi pfeifferi, *Biomphalaria*, 26, 131
Phacoides, 257
borealis, 257
Pharella, 258
acutidens, 258
phascolionis, *Montacuta*, 181
Phaseolicama, 181

- Phidiana*, 299-307
lyncaeus, 299-307
Philobrya, 179
aviculoides, 179
costata, 179
setosa, 179
Philobryidae, 179
Pholadidae, 227, 259, 262
Pholadidea, 151
loscombiana, 151
pholadiformis, *Petricola*, 151
Phragmites, 143
communis, 143
Phyastra, 15
Phyllonotus, 70
capgrandi, 70
Physa, 51, 95-109, 445-451
acuta, 51, 95-109
fontinalis, 450
gyrina, 450, 451
pomilia, 445-451
Physopsis, 21, 23
globosus, 23
nasutus nasutus, 23
picta, *Clypina*, 113, 114
picta, *Nodilittorina*, 115, 117
Pila, 113, 115, 377
globosa, 377
ovata, 115
virens, 377
Pilidae, 37, 113, 115, 117, 121
Pinctada, 226, 227, 241, 264
anomoides, 226, 241
vulgaris, 227, 241
Pinna, 227, 240, 241
atropurpurea, 227, 241
bicolor, 227, 240, 241
Pinnacea, 262
Pinnidae, 227, 241
pisana, *Euparypha*, 341, 355, 356
pisana, *Theba*, 356
Pisania, 118
ferrea, 118
maculosa, 118
Placenta, 227, 245
placenta, 227, 245
placenta, *Placenta*, 227, 245
Planorbidae, 41, 128, 335
Planorbina, 131, 335
Planorbis, 15, 450
corneus, 450
pleii, *Bursatella*, 299-307
Pleuroceridae, 37, 113, 116, 118
pleuronectes, *Amussium*, 227
Pleurotomariacea, 37, 112-114
plicata, *Laciniaria*, 341, 363
plicatula, *Iphigena*, 363, 364
poirieri, *Lehmannia*, 269, 281
Poirieria, 70
elatospira, 70
Polinices, 143, 204, 205, 209
duplicatus, 209
heros, 143
Polygyridae, 342, 365
polynyma, *Spisula*, 150
polyphemus, *Limulus*, 143
Polyplacophora, 7, 10
Pomacea, 377, 380, 397, 399, 404, 409, 446
canaliculata, 377, 380, 397, 399, 404, 409
paludosa, 446
pomatia, *Helix*, 295, 311-333, 366, 389
Pomatias, 61, 73, 341, 363
elegans, 61, 73, 341, 363
costulatus, 341
costulatus hyrcanus, 363
Pomatiasidae, 363
Pomatiopsis, 17-19, 38, 111, 115, 116, 120, 131, 133, 311-333
californica, 116
cincinnatiensis, 17-19, 38, 111, 115, 120
lapidaria, 18, 19, 38, 111, 115, 116, 120, 311-333
Pomatopyrgus, 117
jenkinsi, 117
pomilia, *Physa*, 445-451
ponderosum, *Campeloma*, 115
ponderosum coarctatum, *Campeloma*, 115
Poromya, 183
granulata, 183
Poromyidae, 183
Portlandia, 179
arctica, 179
fraterna, 179
lenticula, 179
Postmonorchis, 207
donacis, 207
Potamididae, 37, 116, 119
Potamopyrgus, 42
jenkinsi, 42
pouzolzi, *Dinarica*, 352, 366, 368
pouzolzi, *Helicigona*, 341-353, 366, 368

- Proclava*, 117
kochi, 117
productus, *Bulinus nasutus*, 128
profuga-etrusca, *Helix*, 65
profuga-etrusca, *Xerocincta*, 65
 Propeamussidae, 180
Propeamussium, 180
groenlandicum, 180
imbriferum, 180
 Prosobranchia, 79, 342, 363, 365, 366, 375

Prosothenia, 131
quadrasi, 131
protea, *Aplysia*, 306
 Protobranchia, 178, 179, 226, 228, 230, 259, 260, 262-264
 Protozoa, 426
proxima, *Nucula*, 195
 Psammobiidae, 227, 253, 262
Psephidia, 182
brunnea, 182
lordi, 182
ovalis, 182
pseudoargus, *Archidoris*, 83, 427
Pseudokellyna, 182
cardiformis, 182
 Pseudolamellibranchia, 259
Pseudosuccinea, 445-451
columella, 445-451
Pseudotachea, 368
 Pteriacea, 67, 262
 Pteriidae, 226, 241
Pterynotus, 70
tricarinatus, 70
pugnax, *Uca*, 143
pulchella, *Chromodoris*, 306
pullastra, *Venerupis*, 152
 Pulmonata, 365
punctata, *Aplysia*, 425, 426
Puperita, 114, 117
japonica, 114, 117
Purpura, 38, 111, 113, 119, 121, 122, 132
bronni, 118
clavigera, 118
lapillus, 38, 111, 113, 119, 121, 122, 132
luteostoma, 118, 113, 119, 121, 122,
purpurata, *Venericardia*, 181
purpurea, *Gemma*, 206
purpurea, *Gemma gemma*, 139, 140
 Purpurinae, 70

Pusia, 118, 119
hizenensis, 118, 119
putris, *Succinea*, 75, 364
pygmaea, *Patelloida*, 114
pyogenes, *Streptococcus*, 45
Pyrene, 118, 119
bicineta, 118
testudinaria tylerae, 118, 119
 Pyrenidae, 38, 118, 119
quadrasi, *Oncomelania*, 26, 116, 132
quadrasi, *Oncomelania hupensis*, 26, 132
quadrasi, *Prosothenia*, 131
quadrasi, *Schistosomophora*, 131
Radina, 116, 118
crenulata, 116, 118
Rapana, 70
 Rapaninae, 70
rapiformis, *Paractis*, 143, 205
reticulata, *Semisulcospira*, 116
reticulatus, *Agriolimax*, 73
reticulatus, *Bulinus*, 25, 128
retropictus, *Clithon*, 114, 117
rhaetica, *Chilostoma achates*, 352, 366
rhaetica, *Chilostoma cisalpina*, 352, 366, 367
rhaetica, *Helicigona achates*, 352, 353, 366
rhaetica, *Helicigona cisalpina*, 352
rhizophorarum, *Cerithidea*, 116
rhombea, *Arca*, 226, 231
 Rissoacea, 37, 112, 115, 117, 123
robertsoni, *Schistosomophora*, 131
Rocellaria, 247, 248
cuneiformis, 247, 248
 Rocellariidae, 247
rohlfsi, *Bulinus truncatus*, 25, 128, 129
rostrata, *Laternula*, 255
rostrata, *Pandora*, 183
rotundatus, *Discus*, 341, 363
rubra, *Lasaea*, 182, 264
rufescens, *Haliotis*, 45
rufus, *Arion*, 269-284, 296
 Rugosa, 9
rustica, *Columbella*, 118, 119
rustica, *Tegula*, 114
saccharina lanx, *Patelloida*, 114
Salmonella, 45
typhi, 45
Sanguinolaria, 227, 253, 254
diphos, 227, 253, 254
sapidus, *Callinectes*, 143
 Saxicavidae, 262

- Saxidomus*, 150
 gigantea, 150
scabra, *Melanoides*, 42
scabra, *Thiara*, 116, 118
schackoi, *Bulinus*, 128, 129
Schistosoma, 15, 17, 23, 25, 127, 130,
 327, 330, 335, 376
 haematobium, 15, 23, 25, 127, 130,
 330
 japonicum, 130, 327
 mansoni, 15, 17, 130, 335
Schistosomophora, 131
 minima, 131
 quadrasii, 131
 robertsoni, 131
 slatteri, 131
Schizotherus, 150
 nutalli, 150
schleschi, *Deroceras*, 57
schrenckii, *Notacmea*, 114
Scintilla, 250
 hanleyi, 250
Scioberetia, 160, 182
secale, *Abida*, 341, 363
Semelidae, 160, 262
semidecussata, *Venerupis*, 188
semiorbiculata, *Beguina*, 248
Semisulcospira, 116, 118, 121
 decipiens, 116
 habei, 116
 kurodai, 116
 libertina, 116, 118, 119
 multigranosa, 116
 nakasekoeae, 116
 niponica, 116
 ornata, 116
 reticulata, 116
 trachea, 116
senegalensis, *Bulinus*, 25
septemspinus, *Crago*, 143
septemspirale, *Cochlostoma*, 363, 364
septentrionalis, *Thracia*, 183
Septibranchia, 183, 225, 259, 260, 262-
 264
sericea, *Fruticicola*, 349
sericea, *Trichia*, 341, 349, 351, 366
sericinus, *Bulinus truncatus*, 25, 127
serradifalci, *Lobiger*, 305
serrata, *Stagnicola emarginata*, 326
serrulata, *Serrulina*, 57
Serrulina, 57
 serrulata, 57
sertularioides, *Caulerpa*, 305
setiferous, *Penaeus*, 175
setosa, *Helicigona*, 341, 353, 354, 366,
 368
setosa, *Liburnica*, 354, 366, 368
setosa, *Philobrya*, 179
sewanica, *Euomphalia arpatschaina*, 349,
 351
sewanica, *Harmozica arpatschaina*, 349
sibogai, *Isoconcha*, 181
Siliqua, 195, 197
 patula, 195, 197
silvatica, *Cepaea*, 341, 355, 368
similis, *Chondrina*, 341, 363
sinensis, *Macroschisma*, 113, 114
sinistrorsum, *Limnaea*, 63
Sinotaia, 113, 115
 histrica, 115
slatteri, *Oncomelania nosophora*, 131
slatteri, *Schistosomophora*, 131
Solen, 67, 227, 256, 268
 annandalei, 227, 256, 258
Solenidae, 156, 227, 258, 262
Solenomya, 161
Soletellina, 227, 254
 diphos, 227, 254
solidissima, *Spisula*, 148, 150
Somateria, 207
 mollissima, 207
sowerbyi, *Typhis*, 70
Spartina, 143
 alterniflora, 143
Sphaeriacea, 261
Sphaeriidae, 42
Sphaerium, 156, 163, 169
 notatum, 156, 169
 sulcatum, 163
spicifera, *Acanthophora*, 300
spinicosta, *Murex*, 70
spinicosta, *Tubicauda*, 70
Spisula, 148, 150, 195
 polynyma, 150
 solidissima, 148, 150
 subtruncata, 148, 195
Spondylus, 244, 245
 hystrix, 244, 245
stagnalis, *Lymnaea*, 47, 49, 73, 293,
 431-442, 446
stagnalis appressa, *Lymnaea*, 377, 388,
 400
Stagnicola, 326, 445
 cataeoptium, 326

- emarginata serrata*, 326
palustris, 445
stagnorum, *Hydrobia*, 123
Standella, 227, 251, 252
pellucida, 227, 251, 252
Staphylococcus, 45
aureus, 45
Stramonita, 71
haemastoma, 71
Streptococcus, 45
pyogenes, 45
Streptoneura, 37, 111-125
striata, *Martesia*, 227, 259
striatulus, *Modiolus*, 226, 236-239
strigata, *Littoraria*, 115
strobili, *Graciliaria*, 341, 363
Strombus, 45, 306
gigas, 45, 306
stultorum, *Tivela*, 150, 197
Stylommastopora, 42, 75, 269, 285,
341-344, 363, 365
subagrestis, *Deroceras*, 57
subcarinata, *Lioplax*, 115
subfuscus, *Arion*, 296
suboccidentalis, *Lytopenete*, 57, 58
suborbicularis, *Kellia*, 181, 197
subrubiginosus, *Cantharus*, 118
subsolidum, *Campeloma*, 115
subtorta, *Cuspidaria*, 183
subtruncata, *Spisula*, 148, 195
subviridis, *Lasmigona*, 77
Succinea, 75, 364
putris, 75, 364
Succineidae, 42, 75, 363, 365
Succinella, 341, 363, 364
oblonga, 341, 363, 364
sudanica tanganyicensis, *Biomphalaria*,
26, 131
sulcata, *Astarte*, 180
sulcata, *Nucula*, 228, 230
sulcatum, *Sphaerium*, 163
Sunetta, 227, 250
effosa, 227, 250
surrentina, *Helix*, 65
surrentina, *Opica*, 65
Synapticola, 181
Tachea, 356
vindobonensis, 356
Tagelus, 156
Tamanovalva, 305
limax, 305
tanganyicensis, *Biomphalaria sudanica*,
26, 131
tangi, *Katayama*, 131
tanschaurica, *Paludomus*, 38, 116, 118,
120
tantilla, *Transennella*, 161, 182, 190
tarda, *Ferrissia*, 42
Tarebia, 116, 118
lineatus, 116, 118
Tegula, 45, 114
gallina, 45
lischkei, 114
nigerrima, 114
pfeifferi carpenteri, 114
rustica, 114
Tellina, 195, 197, 227, 255-257, 264
ala, 227, 255-257
crassa, 255, 257
tenuis, 195, 197, 255, 257
Tellinacea, 261
Tellinidae, 160, 182, 227, 255, 262
tentaculata, *Bithynia*, 123
tenuicostatus, *Pecten*, 163, 169
tenuis expansa, *Nucula*, 179
tenuis, *Nucula*, 179
tenuis, *Tellina*, 195, 197, 255, 257
Teredinidae, 178, 182, 262
Teredo, 160, 177, 182, 184
diegensis, 182
navalis, 182, 184
parksii, 182
testudinaria tylerae, *Pyrene*, 118, 119
Tetracorallia, 9
Thais, 38, 71, 119
haemastoma, 71
lapillus, 38, 119
Thaisinae, 70
Thalotia, 114
japonica, 114
Theba, 65, 346, 348, 356
cantiana, 348
cartusiana, 346
consona, 65
pisana, 356
Thecalia, 180
Thiara, 116, 118
scabra, 116, 118
Thiaridae, 37, 111, 113, 116, 117
Thigmotrichida, 35
Thracia, 162, 183
devexa, 183
distorta, 183
myopsis, 183
septentrionalis, 183

- thraciaeformis*, *Yoldia*, 179
 Thraciidae, 183
Thysaria, 181
 equalis, 181
 gouldi, 181
tiara, *Chione*, 227, 250, 252
Tivela, 150, 197
 stultorum, 150, 197
togata, *Gari*, 253, 254
torelli, *Macoma*, 182
toreuma, *Cellana*, 114
torosa, *Ocenebra*, 70
trachea, *Semisulcospira*, 116
transcaucasicus, *Deroceras*, 57
Transennella, 161, 182, 190
 tantilla, 161, 182, 190
trapezina, *Modiolarca*, 181
trapezuntis, *Helix lucorum*, 361, 362
tricarinata, *Valvata*, 115
tricarinatus, *Pterynotus*, 70
Trichia, 341, 347, 348, 349, 351, 366,
 367
 sericea, 341, 349, 351, 366
 unidentata, 341, 347, 348, 366
 villosa, 341, 348, 351, 366
Trichodina, 36
Tricula, 329
 chiui, 329
Tridacna, 67, 68
 Tridacnidae, 68, 262
 Trigonidae, 226, 262, 264
Tritia, 118, 119
 festiva, 118, 119
Triton, 63, 118
Tritonalia, 118
 erinaceus, 118
trivolvus, *Helisoma*, 327
 Trochacea, 37, 112-114, 121
 Trochidae, 37, 113, 114
Trochoidea, 346, 347, 366
 cretica, 346, 347, 366
Trochulus, 348
 villosus, 348
Trophonopsis, 71
 barvicensis, 71
 muricatus, 71
Tropicorbis, 33
tropicus angolensis, *Bulinus*, 25, 128
tropicus, *Bulinus*, 129
tropicus tropicus, *Bulinus*, 25, 128
tropicus zanzibaricus, *Bulinus*, 25,
 128
truncatus, *Boreotrophon*, 71
truncatus, *Bulinus*, 42, 376, 446
truncatus rohlfsi, *Bulinus*, 25, 128, 129
truncatus sericinus, *Bulinus*, 25, 127
truncatus truncatus, *Bulinus*, 25, 128,
 129
Trunculariopsis, 70
 trunculus, 70
trunculus, *Helaplex*, 118
trunculus, *Trunculariopsis*, 70
 Tryblidiacea, 7
tuberculatus, *Melanoides*, 42, 116, 118
Tubicauda, 70
 spinicosta, 70
Tulotoma, 38, 113, 120
 angulata, 38, 115, 120
 magnifica, 115
turbinata, *Ecteinascidia*, 300, 301
 Turbinidae, 37, 113, 114
Turbo, 114
 cornutus, 114
turtoni, *Galeomma*, 182
Turtonia, 181
 minuta, 181
tylerae, *Pyrene testudinaria*, 118, 119
typhi, *Salmonella*, 45
Typhis, 70
 intermedius, 70
 sowerbyi, 70
Uca, 143
 pugnax, 143
ugandae, *Bulinus*, 128
ulvae, *Hydrobia*, 123
undatum, *Buccinum*, 118
undulatus, *Micropogon*, 175
undulatus, *Modiolus*, 226, 236-239
unidentata, *Fruticicola*, 348
unidentata, *Petasina*, 348
unidentata, *Trichia*, 341, 347, 348, 366
unifasciata, *Candidula*, 341, 345, 346,
 366, 367
unifasciata, *Helicella*, 345
Unio, 169, 264
 Unionidae, 77, 226, 227, 245, 262, 264
 Unioninae, 77
usseriensis, *Bithynia*, 116, 117
uva, *Doto*, 351
vaginatus, *Limopsis*, 231, 233
Valvata, 115
 tricarinata, 115
 Valvatacea, 37, 112, 117
 Valvatidae, 37, 115, 117

- variegata*, *Begonia*, 227, 248, 249
Venericardia, 181
purpurata, 181
Veneridae, 137, 139, 182, 227, 250, 262
Venerupis, 152, 188, 227, 250, 252
macrophylla, 227, 250, 252
pullastra, 152
semidecussata, 188
ventricosa, *Iphigena*, 363, 364
Venus, 150
casina, 150
ovata, 150
vermiculata, *Eobania*, 64, 341, 362, 366
vermiculata, *Helix*, 64
vermiformis, *Boetgerilla*, 57
versicolor, *Columbella*, 118, 119
versicolor, *Euplica*, 119
verticillata, *Caulerpa*, 304
Verticordiidae, 183
vexillum, *Atrina*, 241
Villorita, 250, 252
cyprinoides, 250, 252
villosa, *Fruticicola*, 348
villosa, *Trichia*, 341, 348, 351, 366
villosus, *Trochulus*, 348
vindobonensis, *Cepaea*, 341, 355, 356, 368
vindobonensis, *Tachea*, 356
violacea, *Dostia*, 114, 117
violascens, *Glycymeris*, 233
virens, *Nereis*, 143
virens, *Pila*, 377
virginica, *Crassostrea*, 140, 143, 148, 178, 188
virginica, *Ostrea*, 45
viridis, *Perna*, 226, 233-237
Vitrea, 341, 363
diaphana, 341, 363
vitreum, *Dacrydium*, 180
vittatus, *Donax*, 85
Vitularia, 70
linguabovis, 70
vivipara, *Arca*, 179
Viviparacea, 37, 111-115, 117, 121
Viviparidae, 37, 113-115, 117, 366
Viviparinae, 113-115, 117, 121
Viviparus, 113, 115
ater, 115
contectoides, 115
contectus, 115
georgianus, 115
intertextus, 115
viviparus, 115
viviparus, *Viviparus*, 115
vulgaris, *Palaemonetes*, 143
vulgaris, *Pinctada*, 227, 241
vulgata, *Patella*, 73, 409
vulgatum, *Cerithium*, 307
Xerocincta, 65
profuga-etrusca, 65
Xerocrassa, 346, 347
certica, 346, 347
Xerolauta, 65
peninsularis, 65
yaoi, *Oncomelania*, 131
Yoldia, 179
hyperborea, 179
thraciaeformis, 179
yoshidae, *Katayama nosophora*, 131
yoshidayukioi, *Assimineia*, 116, 117
zanzibaricus, *Bulinus tropicus*, 25, 128
Zebrina, 341, 363
dendrita, 341, 363
zilchi, *Limax*, 57
zonalis, *Batillaria*, 116
Zonitidae, 363

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