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MALACOLOGIA

PROCEEDINGS of the SIXTH
EUROPEAN
MALACOLOGICAL
CONGRESS



Amsterdam 1977

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UNIVERSITY

PROCEEDINGS
of the
SIXTH EUROPEAN MALACOLOGICAL CONGRESS
(Amsterdam, 15-20 August 1977)

Edited by A. C. VAN BRUGGEN, Ph.D.

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PREFACE

The sixth international congress of *Unitas Malacologica Europaea* was held in Amsterdam, Holland, from 15-20 August 1977. It was attended by ca. 200 malacologists from all over the world representing ca. 30 nations; between them they read 11 major and ca. 135 contributed papers, and displayed ca. 15 posters.

Apart from the above, the programme featured an informal get-together in the bar of the Alpha Hotel on August 14, a reception organized by the Free University on August 15 (attended by the rector of the university, Prof. J. de Ruiter), a museum curators meeting under the chairmanship of Dr. Van Bruggen after this reception, a reception organized by the Zoological Museum of the University of Amsterdam on August 16 (attended by the director, Dr. C. A. W. Jeekel, and held in the exhibition halls of the museum and in the famous aquarium of the Zoo), a European Invertebrate Survey meeting under the chairmanship of Dr. Gittenberger on August 18, a congress dinner in the Alpha Hotel on August 19, and the general assembly of U.M.E. on August 20. The companion's programme offered an introduction to the city of Amsterdam. On Wednesday August 17 there was a choice of 3 excursions, viz. to the oceanographic research institute (N.I.O.Z.) on the island of Texel, to Zeeland with an opportunity to meet Mrs. Dr. Van Benthem Jutting, and to the surroundings of Amsterdam in order to collect and study freshwater molluscs. Much to our disappointment the weather was not what we expected it to be.

The Amsterdam congress has featured 2 innovations, viz. 11 invited lectures (which have been published separately under the title *Pathways in Malacology*, editor S. van der Spoel, Bohn, Scheltema & Holkema, Utrecht, 1979) and the introduction of posters. Both innovations have been well received by the congress participants.

The 1977 meeting was the last congress of *Unitas Malacologica Europaea*. On August 20 the general assembly decided to convert U.M.E. to a truly worldwide body: *Unitas Malacologica*.

Acknowledgments are due to the Free University, Amsterdam, for considerable financial assistance and free use of their magnificent congress facilities.

A. C. van Bruggen

INTRODUCTION

The Proceedings of the Sixth European Malacological Congress, Amsterdam, 15-20 August 1977, as here presented contain 87 papers of varying length, quality and subject matter. A total of ca. 135 papers was read and ca. 15 posters were exhibited. The 11 invited lectures have been published separately (see Preface) and a number of participants have declined to submit papers for the Proceedings. From the beginning it has been our policy that the Proceedings of the Amsterdam congress should not contain abstracts, but only papers; we have adhered to this rule, although there are a few exceptions. The abstracts have been published in a limited edition for congress participants (Abstracts, Sixth European Malacological Congress, Amsterdam, 152 p.). Congress participants exhibiting posters have also been asked to submit manuscripts if desired. Therefore this volume contains both contributed papers and posters. In addition and by way of exception 2 papers are published although their authors were unable to attend the congress. Editing the proceedings has been a task of some magnitude; on the whole editing has been mild and restricted. Some authors have (considerably) overstepped the mark as regards the length of their contributions; editing has resulted in some shortening and more modest authors have made up the difference in pages. The editor owes a debt of gratitude to many colleagues and others who have assisted in getting these Proceedings through the press. Without detracting from the valuable contributions by others, there would have been no Proceedings but for the help of Dr. George Davis and his editorial staff, Dr. E. Gittenberger, Dr. H. H. Boer, Mrs. Wendy van Bruggen, Mrs. J. B. Smit, H. Heijn and A. 't Hooft. The papers have been arranged according to subject; in the contents authors names have been enumerated alphabetically.

Once again we should like to stress our gratitude to the Free University, Amsterdam, for generous support in many respects.

A. C. van Bruggen

PRESIDENTIAL ADDRESS

A. C. van Bruggen

President of Unitas Malacologica Europaea

Ladies and Gentlemen,

I have the honour to welcome you to the Sixth International Malacological Congress organized on behalf of Unitas Malacologica Europaea. The preceding congresses of London (1962), Copenhagen (1965), Vienna (1968), Geneva (1971), and Milan (1974) have been a great success, each with its own atmosphere and achievements. I do hope the Amsterdam congress will be as memorable as the others have been.

Thanks to co-operation from many sides we have been able to look forward to today without undue trepidation. Our acknowledgements are due to the Comité d'Honneur consisting of Dr. Vera Fretter and Drs. Lever, Raven, Riedel and Wilbur. I owe a great and sincere debt of gratitude to the Organizing Committee consisting of Drs. Coomans, Gittenberger (Secretary-General), Joosse, Van der Spoel, Stoll (Treasurer), Verdonk and Dr. Lever as advisory member, and in addition Mrs. Van Bruggen. The congress bureau consisting of Mrs. Van Urk and Mr. Spittje have done their best to help us for which we are grateful. We have been liberally assisted financially by the Free University, indeed without their support there would have been no congress at all; further financial assistance has been graciously given by Her Majesty's Government (Minister of Education and Sciences) and by Shell Nederland. The Dutch malacological society has supported us from the beginning under the leadership of Drs. Joosse, past president, and Van der Spoel, the present president. Our thanks are due to all these people and institutions.

Ladies and Gentlemen, this is a long list of acknowledgments, but let me sound a warning here: a congress is not effected by organizing talent and money, a congress is made by the participants. The Concise Oxford Dictionary defines the word 'congress' as follows: "formal meeting of delegates for discussion, esp. of persons engaged in special studies." You will notice that in this wording the participants are mentioned twice, viz., as 'delegates' and as 'persons.' Let me emphasize that it is up to you all to transform this particular meeting into a real congress—we have done our best, now it is your turn.

As regards congresses the Netherlands are not altogether without experience in the field, particularly as regards biological congresses. Ornithologists and entomologists have repeatedly convened in our modest country and we have hosted a regular international zoological congress as far back as 1895 in Leiden, at a time when congresses were not as fashionable as they are today. Of course, there have been thematic meetings on various zoological subjects, but this is definitely the first time that a large international gathering of malacologists meets in this country. Allow me to recall that at a meeting on the occasion of the 25th anniversary of the Dutch malacological society in Amsterdam in 1959 in the presence of a limited number of foreign malacologists the idea of European congresses was born. The first congress in London in 1962 was conceived in this very city where we are convening today.

Malacology is the science of molluscs in as wide a context as possible and today you are honoured guests in a country where malacology has had very early beginnings and where a great deal of malacological research is conducted at present. According to Solem (1974) Swammerdam in the mid 1600's was the first person ever to meticulously dissect, describe and depict a snail, although his results were not published until almost a hundred years later (Swammerdam, 1737-1738). Nowadays the Free University is a well-known centre of experimental malacological research and it is therefore fitting that we convene here. Somebody once told me that here one finds the highest permanent concentration of malacologists in the world; today their numbers have temporarily multiplied. As regards malacology in the Netherlands I may refer to the booklet published by the Dutch malacological society on the occasion of our congress, which booklet graciously has been made available free to all full members of the congress (Van

Bruggen, 1977). This publication will give you a concise impression of malacological research being conducted in our country, at the same time making a lengthy discourse on the subject superfluous. Suffice it to say that malacology just now prospers in the Netherlands.

Every local organizing committee tries to bring something different; we have been guided by the principle that more attention should be paid to non-taxonomic malacology than has been done before. We have tried to set the themes for this congress by inviting 12 prominent malacologists from all over the world, who have been asked to give comprehensive lectures on their own particular subjects. Much to our regret Prof. Sakharov of the U.S.S.R. cannot be present at our conference. Apart from the main lectures, to be published separately in book form (Van der Spoel, 1979) next year, there are the usual contributed papers. These contributed papers, more than a hundred in number, reflect the wide diversity of molluscan studies on a world-wide basis. The papers have been organized in 3 simultaneous sessions in such a way that there are no clashes of interest, we hope. In addition we have introduced posters on a much larger scale than before. The response has been gratifying and we are able to announce about 15 posters of a very diverse nature. Production of the Proceedings of the preceding congress has been consistently dogged by ill-fortune, but these will now be available at this congress. I sincerely promise to see to quick publication of the Proceedings of the present congress, reason why we have asked you to bring the finalized manuscripts along with you to Amsterdam. Apart from invited and contributed papers and posters, the congress will feature the now customary meetings of the European Invertebrate Survey and of the museum curators.

It is somewhat risky to organize a congress in times of economic depression; our financial difficulties have been considerable and we regret the absence of potential participants because of financial stringency in their respective countries. Mrs. Dr. Van Benthem Jutting, formerly of the Amsterdam museum and our senior malacologist, will not attend the congress because it would be too much of a strain to her. Nevertheless she is looking forward to meeting the participants of the field trip to Zeeland. Also, some of our colleagues have passed away since we convened in Milan in 1974. I regret to have to inform you that Dr. Lemche of Copenhagen, president of the 1965 congress, has suddenly died very recently. Dr. Lemche will be remembered as a great scientist and a powerful force behind the European malacological congresses (see Knudsen, 1977). Two others have passed away, Dr. Van Regteren Altena of the Netherlands, a former council member of Unitas (Vice-President 1965-1968) and Prof. Caesar Boettger of Germany. All in all there are now in Amsterdam about 200 scientific participants of which ca. 30 from outside Europe, the total representing ca. 30 nations.

The time has come to review the position of Unitas. The main function of the U.M.E. has been to organize international malacological congresses in Europe. In my opinion this should be the paramount aim, next to furthering international projects such as the European Invertebrate Survey and world-wide molluscan conservation. Gradually a feeling has developed that working in a purely European context is perhaps somewhat restrictive and at the council meeting of Unitas in Frankfurt am Main in February 1977 we have decided to propose the conversion of U.M.E. into a world-wide international body, Unitas Malacologica, with the stipulation that every 3 years a congress will be held in Europe. Unitas has always had ties outside Europe; apart from lively participation by our corresponding members from outside Europe (particularly the Americans), the international journal *Malacologia*, based in the United States of America, has produced or assisted with the production of our Proceedings right from the beginning and we have their promise that they will continue to do so. We feel that the voice of a world-wide international body will be heard throughout the world where necessary, which particularly for co-operation in the field of conservation has its advantages. Moreover, international bodies may be able to obtain funds from United Nations agencies so that our financial position may improve, which in turn will place us on a par with other international biological congresses. After all, I do not have to remind you that the Mollusca are the second most diverse phylum in the animal kingdom. The tremendous economic, medical and veterinary importance of these fascinating animals warrants them being considered on an equal footing with groups like birds and insects, taxa that have had their own international congresses on a world-wide basis already over a considerable period. Our own congresses have always considered the economic importance of molluscs and indeed here in Amsterdam various aspects will be discussed in both invited and contributed papers. Our ambition is to further malacology, the study of molluscs, and we feel that a measure as discussed before would be beneficial to both malacology in

general and malacologists in particular. Herewith I declare the Sixth International Malacological Congress organized on behalf of *Unitas Malacologica Europaea* open.

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ALLOCUTION PRESIDENTIELLE

Résumé en Français

Mesdames et Messieurs,

Je vous souhaite la bienvenue à Amsterdam au sixième congrès international malacologique organisé sous les auspices de l'*Unitas Malacologica Europaea*. L'Université Libre à Amsterdam est un centre de la malacologie expérimentale et pour cela nous sommes enchantés de nous assembler ici. En outre, l'Université Libre nous a bien aidé en manière de finances; nous pouvons dire sans exagération qu'il n'y aurait nullement de congrès sans ce secours en argent.

Chaque comité d'organisation tend à faire quelque chose nouvelle ou différente. Nous nous sommes efforcés d'accentuer un peu plus les aspects non-taxonomiques de la malacologie. On retrouve les sujets du congrès dans les 11 conférences principales auxquelles nous avons invité des malacologistes du monde entier. A côté de cela il y aura environ 100 conférences normales et nous présentons aussi environ 15 'posters,' une innovation dont nous vous prions de bien prendre acte. En outre le congrès a organisé des séances de l'European Invertebrate Survey et des conservateurs malacologiques des musées.

Malheureusement les comptes rendus du congrès précédent (Milano, 1974) ont été publiés avec un tel retard que nous sommes à même de les distribuer au congrès actuel! Pour prévenir une répétition de cet événement nous vous avons prié de bien vouloir apporter à Amsterdam vos manuscrits complets; les conférences des malacologistes invités seront publiées comme un livre séparé.

Je vous prie aussi de bien vouloir penser avec nous sur l'avenir de l'*Unitas*. Le conseil de l'U.M.E. a conclu qu'une position internationale soit à préférer à une position européenne, d'une part afin que notre voix soit apprise dans le monde entier, ce qui est très important au point de vue de la protection des mollusques, etc., d'autre part afin que nous soyons à même d'obtenir de l'argent des organisations des Nations Unies. Après tout l'U.M.E. a toujours eu une interprétation plus ou moins mondiale, vu nos liens cordiaux avec les membres correspondants et la publication de nos comptes rendus. Pourtant nous nous faisons un devoir de continuer sans interruption la série des congrès en Europe.

Je vous souhaite un congrès productif.

ANREDE DES VORSITZENDEN

Deutsche Zusammenfassung

Sehr verehrte Damen und Herren,

Seien Sie herzlich willkommen beim Sechsten Internationalen Malakologenkongress, organisiert in Amsterdam im Namen der *Unitas Malacologica Europaea*. Die Freie Universität in Amsterdam ist bekannt als Mittelpunkt experimenteller Malakologie und deshalb freuen wir uns darüber heute hier zusammenkommen zu können. Ausserdem ist die Freie Universität uns auch

finanziell sehr zu Willen gewesen; man kann ohne Uebertreibung sagen, dass es ohne diese Unterstützung überhaupt keinen Kongress in den Niederlanden gegeben hätte.

Jedes Organisationskomitee bemüht sich etwas Anderes oder Neues zu bringen. Wir haben versucht die nicht-taxonomischen Aspekte der Malakologie etwas mehr zu betonen. Die 11 Hauptvorträge zeigen die Thema's des Kongresses an; zu diesen Hauptvorträgen haben wir Malakologen aus der ganzen Welt eingeladen. Daneben gibt es über 100 kleinere Beiträge und etwa 15 'Posters,' eine Neuigkeit welche hoffentlich starke Beachtung finden wird. Ausserdem gibt es wie immer eine Tagung der Museums-Kustoden und der 'European Invertebrate Survey.'

Leider sind die Abhandlungen des vorigen Kongresses (Mailand, 1974) so sehr verspätet, dass wir diese erst heute austeilen können. Damit sich so etwas nicht wiederholt, wurden die Redner gebeten ihre Manuskripte druckfertig nach Amsterdam mitzubringen. Die Hauptvorträge werden separat in Buchform veröffentlicht werden.

Auch möchte ich bitten dem Vorstand der U.M.E. Hilfe zu leisten bei der Politik der Zukunft. Der Vorstand hat beschlossen, dass eine internationale Organisation einem europäischen vorzuziehen ist, einerseits weil wir unsere Stimme weltweit hören lassen möchten (was immer wichtig ist, zum Beispiel beim internationalen Molluskenschutz), andererseits weil wir so auch finanziell von Organisationen der Vereinigten Nationen unterstützt werden könnten. Die Unitas hat immerhin schon seit Jahren mehr oder weniger weltweit gearbeitet, wie sich aus der Liste der korrespondierenden Mitglieder und aus unseren Verhandlungen klar ergibt. Man muss jedoch Sorge tragen für eine ununterbrochene Fortsetzung der Reihe europäischer Kongresse.

Ich wünsche Ihnen einen angenehmen und erfolgreichen Kongress.

UNITAS MALACOLOGICA EUROPAEA

Unfortunately the Proceedings of the Milan congress did not include reports on U.M.E.; these are included in this volume for the sake of continuity.

Preparations for the Milan congress were initially handicapped by a number of strikes, so that circulars and presidential messages had to be distributed mainly by the secretariat (Dr. O. Paget) in Vienna. The congress itself was well organized by the president Dr. Toffoletto and his committee and there were no difficulties. Ca. 125 participants took part in lectures and excursions.

During the General Assembly the president, Dr. F. Toffoletto, gave a short summary of the congress activities. The secretary, Dr. Paget, deputizing for the treasurer, Dr. Jung, gave a report on the financial situation of U.M.E. (summary see below). This report led to vivid discussion as the expenses of council meetings were criticized. The small number of members and the modest membership fees make it almost impossible to cover the expenses for the necessary meetings of the council. In this connection Dr. Paget stated that the expenses of the secretariat (secretary, paper, printing, stamps, etc.) and even part of the costs of the circulars were paid by the Austrian Ministry of Science and Research. In addition to this \$1000 had to be paid (as from the Milan congress) by Unitas as well as the congress as a contribution to printing the Proceedings. We are grateful for the contribution of *Malacologia*, but this is a heavy financial burden for the small budget of Unitas. A vote decided with a small majority that the membership fee should be increased to Sfr. 20 annually for ordinary members and Sfr. 10 for corresponding and collective members. This decision, incidentally, has very much improved the financial situation of Unitas.

Dr. Paget unfortunately had to cancel his offer of the Vienna congress (1968) to distribute annual lists of publications since almost no response and cooperation could be obtained from the European malacologists.

For the election of a new council and the place of the next congress there were no other candidates and suggestions than those proposed by the old council. Therefore the following council was elected with only 46 of the 132 members eligible to vote casting their vote:

President:	Dr. A. C. van Bruggen
Vice-president:	Dr. J. Gaillard
Secretary:	Dr. O. E. Paget
Treasurer:	Dr. P. Jung
Member of council:	Prof. Dr. A. Grossu

The new president declared in a first statement that he wanted to strengthen Unitas particularly by trying to attract more members.

At the meetings of the new council in 1974-1977 the basis for a complete reorganisation of Unitas was laid. The necessity of internationalisation on a worldwide basis was agreed on, particularly with respect to the possibility of raising funds from international organisations to improve the financial situation. As this could only be approved by voting at a congress the final decisions were postponed until the Amsterdam meeting.

The secretariat in Vienna contributed both financially and personally as regards the Amsterdam congress in order not to burden the budgets of both Unitas and the congress too heavily.

The Amsterdam congress (15-20 August 1977) took place at the Free University and was very well organized by the president, Dr. Van Bruggen, his Secretary-General Dr. E. Gittenberger, and the many other members of the committee (see list) and proved to be a great success.

During this congress the proceedings of the Milan congress were received and partially distributed.

A number of changes in the organisation of the congress were initiated in Amsterdam. One of these, the 'Poster Sessions,' was very successful indeed and certainly will play an important role in future congresses. Furthermore there was a number of invited lectures; these will be published in a separate volume available upon payment of an additional sum. The proceedings of the Amsterdam congress will be distributed free of charge *only* to members of Unitas and not (as formerly) to all congress participants. This decision caused a considerable number of congress participants to join U.M.E.

Another important decision was the above-mentioned intention to convert Unitas Malacologica Europaea to Unitas Malacologica on a worldwide rather than purely European basis. All corresponding members automatically become ordinary members. In the preliminary new rules it was proposed to hold congresses in Europe every 3 years, while in addition congresses outside Europe may be held on request in the periods in between. The retiring president should serve on the council for another 3 years. This will doubtlessly ensure continuity in the development and council of Unitas. The council will be enlarged so as to include a number of members from outside Europe. This decision was made to strengthen the international position and to enlarge the basis of our society. These important changes will not become effective before October 1, 1978. All above-mentioned decisions were taken at the final session, the General Assembly of Unitas; voting showed large majorities in favour of these changes.

Voting for the new council was done in the summer of 1977 and the proposals of the old council were almost unanimously accepted:

President:	Dr. J. Gaillard
Vice-President:	Prof. Dr. A. Grossu
Secretary:	Dr. O. E. Paget
Treasurer:	Dr. P. Jung
Member of council:	Prof. Dr. J. Joosse

Therefore Dr. Gaillard is president of Unitas for the period 1977-1980 and president of the next congress to be held in 1980 in France. This time 58 out of 125 members have used their right to vote.

Ca. 200 malacologists from ca. 30 countries attended the Amsterdam congress. A total of ca. 135 lectures was held and excursions gave the opportunity to see something of the Netherlands.

At the General Assembly the retiring president, Dr. Van Bruggen, reported on the state of Unitas and expressed the expectation that the society will now be able to work even more effectively and successfully. Then the treasurer, Dr. Jung, gave his report (see summary below). The secretary's report presented by Dr. Paget showed that in August 1977 Unitas had 170 members in 23 countries (134 ordinary, 26 corresponding, and 10 collective members). Unitas mourned the death of 3 foundation members (Prof. Dr. C. R. Boettger, Dr. C. O. van Regteren Altena, and Dr. H. Lemche, the latter president of the 1965 Copenhagen congress).

Besides supporting the European Invertebrate Survey, one of the main aims of Unitas is the worldwide conservation of molluscs.

Members of Unitas normally receive the proceedings of the European congresses; some other projects are in preparation in order to supply members with additional publications at a reduced price. Projects on the bibliography of European malacologists and on literature have been taken in hand and members will hear about this before the next congress.

Finally it may be stated that the development of Unitas Malacologica Europaea towards Unitas Malacologica on an international (worldwide) basis is without doubt a most important step on the way to a successful worldwide cooperation of all malacologists and we all hope that existing cooperation will be continued and improved.

We wish to thank all those who have supported Unitas before and we should like to invite all malacologists throughout the world to join us by applying for membership. Please contact Dr. O. E. Paget, Naturhistorisches Museum, Burggring 7, Postfach 417, A-1014 Wien, Austria.

O. E. Paget
Secretary

Nous regrettons que les Comptes rendus du Congrès de Milan ne renferment pas des rapports sur l'U.M.E.; ceux-ci ont été insérés dans ce tome pour assurer la continuité.

Au début des grèves ont rendu difficile le travail des personnes chargées de préparer le Congrès de Milan: les circulaires et les bulletins du Président ont dû être distribués principalement par le secrétariat (le dr. O. Paget) à Vienne. Le Congrès lui-même fut bien organisé par le Président le dr. Toffoletto et son comité. Il n'y a pas eu de difficultés. Environ 125 personnes ont participé aux conférences et excursions.

Pendant l'Assemblée Générale le Président le dr. Toffoletto a donné un résumé sommaire des activités du Congrès. Le secrétaire le dr. Paget remplaçant le trésorier le dr. Jung a rapporté sur la situation financière de l'U.M.E. (voir le résumé ci-après). Une discussion animée s'engagea au sujet des dépenses des réunions du Conseil. Le nombre restreint de membres et la modicité des cotisations rendent presque impossible de couvrir les frais des réunions du Conseil. En rapport avec ce qui précède le dr. Paget mentionna que les dépenses du secrétariat (secrétaire, papier, imprimés, timbres, etc.) et même une partie des frais des circulaires ont été payés par le Ministère de la Recherche Scientifique de l'Autriche. De plus (à partir du Congrès de Milan) l'Unitas aussi bien que le Congrès doivent payer \$1000 pour la publication des Comptes rendus. Nous acceptons avec reconnaissance la contribution de *Malacologia* mais le budget est trop restreint pour faire des frais si élevés. On a décidé à une petite majorité des voix que les cotisations s'augmentent à Sfr. 20 par an pour les membres ordinaires et à Sfr. 10 pour les membres correspondants et collectifs. Entretemps cette décision a beaucoup amélioré la situation financière de l'Unitas.

Malheureusement le dr. Paget dû révoquer son offre du Congrès de Vienne (1968) de distribuer des listes annuelles des publications parce qu'il n'a pas pu obtenir la coopération de la part des malacologistes européens.

En ce qui concerne l'élection du nouveau Conseil et le choix du lieu du congrès prochain on s'accorda avec l'ancien Conseil. Ainsi le Conseil suivant fut élu avec seulement 46 des 132 membres ayant droit de vote:

Président:	Dr. A. C. van Bruggen
Vice-Président:	Dr. J. Gaillard
Secrétaire:	Dr. O. E. Paget
Trésorier:	Dr. P. Jung
Membre du Conseil:	Prof. Dr. A. Grossu

En premier lieu le nouveau Président déclara qu'il souhaite consolider l'Unitas particulièrement en essayant d'attirer plus de membres.

Lors des réunions du nouveau Conseil dans la période 1974-1977 la base d'une réorganisation complète fut mise. On s'accorda à reconnaître la nécessité de l'internationalisation à base mondiale. Ceci en particulier en rapport avec la possibilité de pouvoir obtenir l'appui financier de la part des organisations internationales afin d'améliorer la situation financière. Pour pouvoir obtenir l'approbation de ces projets il a fallu les mettre aux voix au Congrès. Pour cela on a remis les décisions définitives à la réunion d'Amsterdam.

Au point de vue financier et personnel le secrétariat à Vienne a contribué au Congrès d'Amsterdam afin de ne pas trop charger le budget de l'Unitas aussi bien que celui du Congrès.

Le Congrès d'Amsterdam (du 15 au 20 août 1977) eut lieu à l'Université Libre et fut très bien organisé par le Président le dr. Van Bruggen, le Secrétaire Général le dr. E. Gittenberger et beaucoup d'autres membres du Comité (voir la liste) et se trouva avoir un grand succès.

Pendant le Congrès les Comptes rendus du Congrès de Milan ont été reçus et distribués en partie.

Un nombre de modifications dans l'organisation du Congrès a été initié à Amsterdam. En effet une d'entre elles, les "Poster Sessions," eut un grand succès et jouera sans doute un rôle important aux congrès futurs. De plus il y a eu des conférenciers invités; leurs communications seront publiés dans un tome à part, qu'on peut obtenir en payant un supplément. Les Comptes rendus du Congrès d'Amsterdam ne seront distribués gratuitement qu'aux adhérents de l'Unitas et non pas (comme autrefois) à tous ceux qui ont pris part au Congrès. Cette décision a persuadé un nombre considérable de congressistes à s'affilier à l'U.M.E.

Une autre résolution importante fut le projet déjà mentionné de convertir l'U.M.E. en Unitas *Malacologica* à base mondiale plutôt qu'à base purement européenne. Tous les membres

correspondants deviennent automatiquement membres ordinaires. Dans le nouveau règlement préliminaire on a proposé de tenir des congrès en Europe tous les 3 ans; en outre—sur demande—des congrès hors de l'Europe peuvent être tenus dans les intervalles. Le Président du Conseil ne démissionnera pas et restera encore 3 années. Cela assurera sans doute la continuité du développement et du Conseil de l'Unitas. Le Conseil sera agrandi de sorte qu'un nombre de membres hors de l'Europe peuvent accéder au Conseil. On a pris cette décision pour consolider la position internationale de notre société et pour en élargir la base. Les modifications importantes n'entreront pas en vigueur avant le premier octobre 1978. Toutes les décisions citées plus haut ont été prises à la séance finale, l'Assemblée Générale de l'Unitas. On a voté à la majorité des voix en faveur de ces modifications.

Dans l'été de 1977 on a voté pour l'élection du nouveau conseil; les propositions de l'ancien conseil ont été acceptées presque à l'unanimité des voix. Le conseil fut ainsi constitué:

Président:	Dr. J. Gaillard
Vice-Président:	Prof. Dr. A. Grossu
Secrétaire:	Dr. O. E. Paget
Trésorier:	Dr. P. Jung
Membre du Conseil:	Prof. Dr. J. Joosse

Le dr. Gaillard sera donc Président de l'Unitas pour la période 1977-1980 ainsi que président du congrès prochain qui doit avoir lieu en France en 1980. Cette fois 58 des 125 membres ont profité de leur voix délibérative.

A peu près 200 malacologistes d'environ 30 pays ont assisté au Congrès d'Amsterdam. Au total environ 135 conférences ont été présentées. Les congressistes ont eu l'occasion de faire des excursions en Hollande.

A l'Assemblée Générale le Président démissionnaire le dr. Van Bruggen rapporta sur la position de l'Unitas et exprima son espoir que dès maintenant la société peut fonctionner encore plus efficacement. Après le trésorier le dr. Jung donna un compte rendu (voir ci-après). Le rapport du secrétaire présenté par le dr. Paget montra qu'au mois d'août 1977 l'Unitas avait 170 membres en 23 pays (134 membres ordinaires, 26 membres correspondants et 10 membres collectifs). L'Unitas regretta la mort de 3 membres fondateurs (le prof. dr. C. R. Boettger, le dr. C. O. van Regteren Altena et le dr. H. Lemche; ce dernier a été président du Congrès à Copenhague en 1965).

Un des principaux buts de l'Unitas—en dehors de l'aide à la cartographie des invertébrés européens—est la conservation mondiale des mollusques.

Normalement les membres de l'Unitas reçoivent les Comptes rendus des congrès européens. On est en train de préparer d'autres projets afin de pouvoir procurer aux membres des publications complémentaires à prix réduit. Des projets pour composer des bibliographies des malacologistes européens et des listes de littérature ont été entrepris. On tiendra au courant les membres avant le congrès prochain.

Pour conclure on peut constater qu'avec le développement de l'U.M.E. pour devenir l'Unitas Malacologica à base internationale (mondiale) nous sommes sans doute sur la bonne voie pour ce qui est d'une coopération effective et efficace de tous les malacologistes. Nous espérons tous que la coopération existante sera continuée et améliorée.

Nous tenons à remercier tous ceux qui ont soutenu l'Unitas jusqu'à présent et nous aimons à inviter tous les malacologistes du monde entier à se faire inscrire comme membre de l'Unitas. Prière de s'adresser au dr. O. E. Paget, Naturhistorisches Museum, Burgring 7, Postfach 417, A-1014 Wien, Autriche.

O. E. Paget
Secrétaire

Nachdem durch eine Reihe von unglücklichen Zusammenhängen in den Proceedings des Mailänder Kongresses keine Berichte der U.M.E. aufgenommen wurden, sollen (um die Kontinuität zu wahren) diese Berichte in diesem Band nachgeholt werden.

Die Vorbereitung des Mailänder Kongresses war ursprünglich durch eine Reihe von Streiks sehr behindert, so dass vom Wiener Sekretariat (Dr. O. Paget) der Grossteil der Aussendungen

der Rundschreiben und alle Mitteilungen des Präsidenten durchgeführt werden mussten. Der Kongress selbst war vom Präsidenten Dr. Toffoletto und seinen Mitarbeitern sehr gut vorbereitet gewesen und in seiner Durchführung gab es keine Schwierigkeiten. Insgesamt ca. 125 Teilnehmer aus 25 Ländern nahmen an Vorträgen und einigen Exkursionen teil.

In der Generalversammlung gab Dr. Toffoletto ein kurzes Resümee des Kongresses und seiner Aktivitäten. Anschliessend gab der Sekretär Dr. Paget in Vertretung des Schatzmeisters Dr. Jung den Bericht über die finanzielle Situation der U.M.E. (Zusammenfassung siehe unten). Dieser Bericht gab Anlass zu Diskussionen, da von einem Teil der anwesenden Mitglieder die Ausgaben für die Treffen des Vorstandes kritisiert wurden, da sie (aufgrund der geringen Mitgliederzahl und der geringen Beiträge) einen beträchtlichen Teil der Einnahmen ausmachten. In diesem Zusammenhang stellte Dr. Paget fest, dass die gesamten Kosten des Sekretariats und selbst ein Teil der Aussendungen und Druckkosten ausschliesslich durch das österreichische Bundesministerium für Wissenschaft und Forschung getragen wurden. Darüber hinaus müssen ab dem Mailänder Kongress (diesen eingeschlossen) jeweils \$1.000.- sowohl von der Unitas als auch vom jeweiligen Kongressbudget zum Druck der Proceedings beigetragen werden. Wenngleich die grosse Hilfe von *Malacologia* anerkannt wird, bedeutet das doch eine schwere Belastung für das kleine Budget der Unitas.

In einer leider knappen Abstimmung wurde dann beschlossen, den jährlichen Mitgliedsbeitrag auf sfr. 20.- für Ordentliche auf jeweils sfr. 10.- für Korrespondierende und Kollektive Mitglieder zu erhöhen. Wie sich in der Zwischenzeit herausgestellt hat, war diese Massnahme geeignet, die finanzielle Situation der Unitas bedeutend zu verbessern.

Dr. Paget musste ferner bekanntgeben, dass es ihm nicht möglich war, die von ihm beim Kongress Wien 1968 freiwillig übernommenen Aufgaben der Herausgabe eines jährlichen Literaturverzeichnisses durchzuführen, da trotz mehrfacher Aufforderung keine ausreichende Beteiligung erreicht werden konnte.

Bei der Wahl des neuen Vorstandes und des Platzes des kommenden Kongresses konnte ausser dem Vorschlag des Vorstandes kein weiterer aus den Reihen der Mitglieder erhalten werden. Daraufhin wurde folgender Vorstand für die nächste Periode gewählt:

Präsident:	Dr. A. C. van Bruggen
Vizepräsident:	Dr. J. Gaillard
Sekretär:	Dr. O. E. Paget
Schatzmeister:	Dr. P. Jung
Vorstandsmitglied:	Prof. Dr. A. Grossu

Von 132 wahlberechtigten Mitgliedern haben nur 46 von ihrem Wahlrecht Gebrauch gemacht!

Der neue Präsident erklärte in seiner Antrittsrede, dass er versuchen werde, die Unitas durch verstärkte Mitarbeit zu stärken und auch insbesondere sich bemühen werde, neue Mitglieder zu werben.

Bei den zwischen 1974 und 1977 abgehaltenen Treffen des neuen Vorstandes wurden die Grundlagen für eine völlig neue Ordnung der Unitas gelegt. Allgemein wurde festgestellt, dass es notwendig wäre, die Unitas auf eine internationale Basis zu stellen, um für ihren Unterhalt auch Geldmittel internationaler Gesellschaften erhalten zu können. Da diese Frage jedoch nur durch eine Abstimmung der Mitglieder entschieden werden kann, wurden alle Vorbereitungen dafür getroffen, dieses Problem in Amsterdam zu lösen.

Auch bei der Vorbereitung des Amsterdamer Kongresses hat das Sekretariat in Wien sowohl in finanzieller als auch arbeitsmässiger Hinsicht beigetragen, das Unitas-Budget und jenes des Amsterdamer Kongresses nicht zu sehr zu belasten.

Der Amsterdamer Kongress (15-20 August 1977) fand in den Räumen der Freien Universität statt und war von seinem Präsidenten Dr. Van Bruggen, seinem General-Sekretär Dr. E. Gittenberger, sowie zahlreichen anderen Mitarbeitern (siehe Liste) bestens vorbereitet und ein voller Erfolg.

Während des Kongresses trafen die Proceedings des Mailänder Kongresses ein, die teilweise gleich verteilt werden konnten.

Eine Reihe von Änderungen wurde von den Organisatoren des Kongresses eingeführt. Vor allem hatten die zahlreichen "Poster-Sessions" grossen Erfolg und werden wohl auch bei künftigen Tagungen eine wesentliche Rolle spielen. Ferner gab es eine Reihe von eingeladenen Reden, die in einem eigenen Band erscheinen und gegen zusätzliche Bezahlung bezogen werden können. Die Proceedings des Amsterdamer Kongresses werden nur Mitglieder der Unitas gratis

erhalten, während alle übrigen Teilnehmer des Kongresses hfl. 30.- zu zahlen haben. Für die übrigen Bezieher ist der endgültige Preis noch nicht festgelegt. Diese Entscheidung hatte zur Folge, dass eine grosse Anzahl von Kongressmitgliedern der Unitas beiträt.

Ein weiterer wesentlicher Punkt war die schon angeführte Entscheidung, die "Unitas Malacologica Europaea" auf eine internationale Basis zu stellen, ihren Namen auf "Unitas Malacologica" zu ändern und automatisch alle bisherigen "Korrespondierenden Mitglieder" zu "Ordentlichen Mitgliedern" zu machen. In einem Entwurf der neuen Statuten wurde ferner festgestellt, dass zwar wie bisher alle 3 Jahre ein Kongress in Europa stattfinden wird, dass aber auf Antrag auch zwischen diesen Kongressen ausserhalb Europas weitere abgehalten werden können. Ferner wurde beschlossen, dass der scheidende Präsident für eine weitere Periode von 3 Jahren Mitglied des Vorstandes bleibt, wodurch Zweifellos eine grössere Kontinuität in der Entwicklung und Führung der Unitas gewährleistet ist. Darüber hinaus werden in den Vorstand der internationalen Organisation auch aussereuropäische Mitglieder aufgenommen werden. Diese Entscheidung soll dazu beitragen, die internationale Zusammenarbeit zu fördern und unsere Gesellschaft auf eine breitere Basis zu stellen. Um die Umstellung auf diese neue Situation zu erleichtern, wurde beschlossen, erst im Oktober 1978 die endgültige Umwandlung vorzunehmen. Dieser und alle übrigen Vorschläge wurden in der Abschlussitzung bzw. in der Generalversammlung mit grosser Mehrheit angenommen.

Die in Sommer 1977 durchgeführte Wahl für den neuen Vorstand (zu der neuerlich nur der Vorschlag des alten Vorstandes eingebracht wurde) ergab eine fast einstimmige Bestätigung des Vorschlages:

Präsident:	Dr. J. Gaillard
Vizepräsident:	Prof. Dr. A. Grossu
Sekretär:	Dr. O. E. Paget
Schatzmeister:	Dr. P. Jung
Vorstandsmitglied:	Prof. Dr. J. Joosse

Damit ist Dr. Gaillard Präsident der Unitas Malacologica für 1977-1980 und Präsident des Kongresses 1980 in Frankreich. Diesmal haben von 125 Mitgliedern 58 von ihrem Wahlrecht Gebrauch gemacht.

Am Kongress in Amsterdam nahmen insgesamt ca. 200 Malakozoologen aus etwa 30 Ländern teil. Es wurden ungefähr 135 Vorträge gehalten und einige Exkursionen durchgeführt.

Bei der abschliessenden Generalversammlung gab der scheidende Präsident Dr. A. C. van Bruggen eine Übersicht über die geänderten Verhältnisse der Unitas und gab seiner Hoffnung Ausdruck, dass diese Organisation noch wirkungsvoller und erfolgreicher wird arbeiten können.

Dann gab der Schatzmeister Dr. P. Jung seinen Bericht (Zusammenfassung siehe unten).

Der Bericht des Sekretärs Dr. O. Paget ergab dass die Unitas mit 1. August 1977 insgesamt 170 Mitglieder (134 Ordentliche, 26 Korrespondierende, 10 Kollektive Mitglieder) aus 23 Ländern hatte. Mit grossem Bedauern wurde der Tod von 3 Gründungsmitgliedern der Unitas erwähnt (Prof. Dr. C. R. Boettger, Dr. C. O. van Regteren Altena und Dr. H. Lemche, wobei letzterer Präsident des 2. Kongresses in Kopenhagen 1965 war).

Neben der Unterstützung des Projektes "European Invertebrate Survey" wird auch dem weltweiten Schutz der Mollusken grössere Bedeutung beigemessen werden müssen.

Um den Mitgliedern über den Bezug der Proceedings hinaus auch jenen weiterer Publikationen zu einem reduzierten Preis zu ermöglichen, sind einige Projekte in Vorbereitung. Vor allem eine Bibliographie europäischer Malakologen und über Literatur. Sie sollen vor dem nächsten Kongress näheres darüber erfahren.

Abschliessend kann festgestellt werden, dass die Entwicklung der "Unitas Malacologica Europaea" zur "Unitas Malacologica" auf weltweiter internationaler Basis zweifellos einen bedeutenden Schritt darstellt auf dem Weg zu einer wirkungsvollen weltweiten Zusammenarbeit aller Malakologen und wir alle hoffen, dass die bisherige allgemeine gute Zusammenarbeit auf diese Weise noch verstärkt werden wird.

Wir danken allen, die sich bisher so tatkräftig für die U.M.E. eingesetzt haben und laden alle Malakologen der Welt ein, durch ihren Beitritt diese Organisation zu stärken. Diesbezügliche Anfragen sind an den Sekretär, Dr. O. E. Paget, Naturhistorisches Museum, Burgring 7, Postfach 417, A-1014 Wien, Österreich, zu richten.

SUMMARY OF U.M.E. ACCOUNTS FOR 1971-1974

Income	
Subscriptions	Sfr. 3356.19
Interest	734.80
Income tax recovered	75.40
Reimbursements	<u>1050.00</u>
	Sfr. 5216.39
Expenditure	
Income tax	Sfr. 220.50
Council meetings	3569.30
Grants	1108.00
Sundries	<u>245.30</u>
	Sfr. 5143.10
Excess of income	<u>73.29</u>
	Sfr. 5216.39
Balance at 29.VIII.1974	
Assets Schweizerische Bankverein	Sfr. 7093.41
Balance 26.VIII.1971	7020.12
Excess	73.29

SUMMARY OF U.M.E. ACCOUNTS FOR 1974-1977

Income	
Subscriptions	Sfr. 5336.05
Interest	645.75
Income tax recovered	<u>348.50</u>
	Sfr. 6330.30
Expenditure	
Income tax	Sfr. 203.40
Council meeting	418.10
Contribution Proceedings Milan Congress	2525.00
Sundries	<u>328.50</u>
	Sfr. 3475.00
Excess of income	<u>2855.30</u>
	Sfr. 6330.30
Balance at 8.VIII.1977	
Assets Schweizerische Bankverein	Sfr. 9948.71
Balance 29.VIII.1974	7093.41
Excess	2855.30

P. Jung
Treasurer

FEINSTRUKTUR DES AUGES DER BERNSTEINSCHNECKE
SUCCINEA PUTRIS (L.) (GASTROPODA, STYLOMMATOPHORA)

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ABSTRACT

The structure and some aspects of the development of the eye of *Succinea putris* were studied with the aid of the electron microscope. The eye is of the closed vesicle type and is composed of retina, cornea, vitreous body, lens and optic nerve. Three different types of cell are to be found in the retina:

(1) the small elongated pigment cell with an avoid nucleus, many pigment granulae and short microvilli at the apical end of the cell;

(2) the sensory cell type I with a large irregular nucleus, long microvilli, which extend to under the surface of the lens, a large number of light-cored vesicles, 700 Å in diameter and the axon;

(3) the elongated slender sensory cell type II with many dense cored vesicles, several pigment granulae in the distal region of the cell and short irregular microvilli at the apical end of the cell. This type is few in number.

Two results of the study of the embryonic eye are described: the cornea cells differ from those in the adult eye in the nucleus-cytoplasm relation and the optic nerve is smaller than in the adult eye.

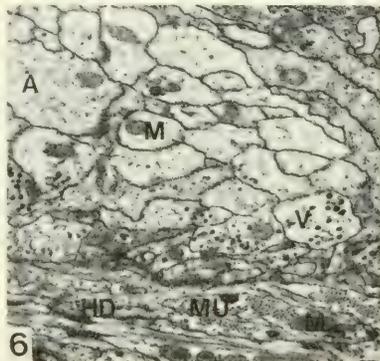
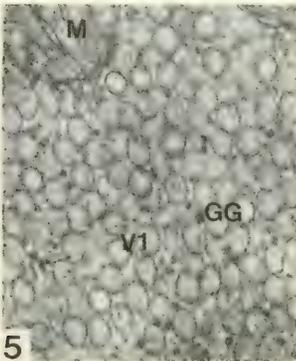
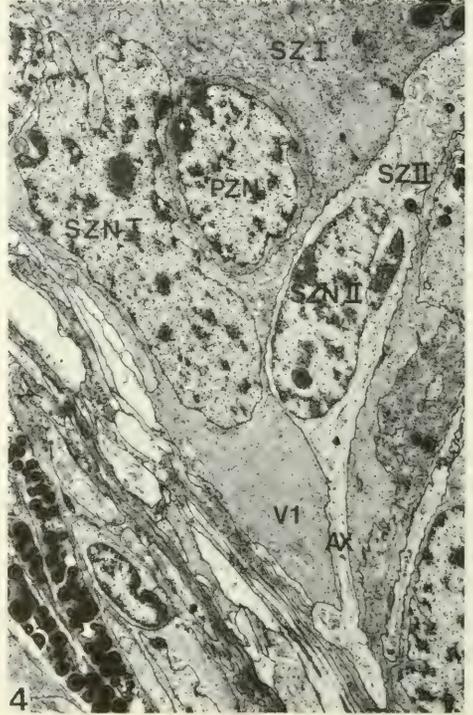
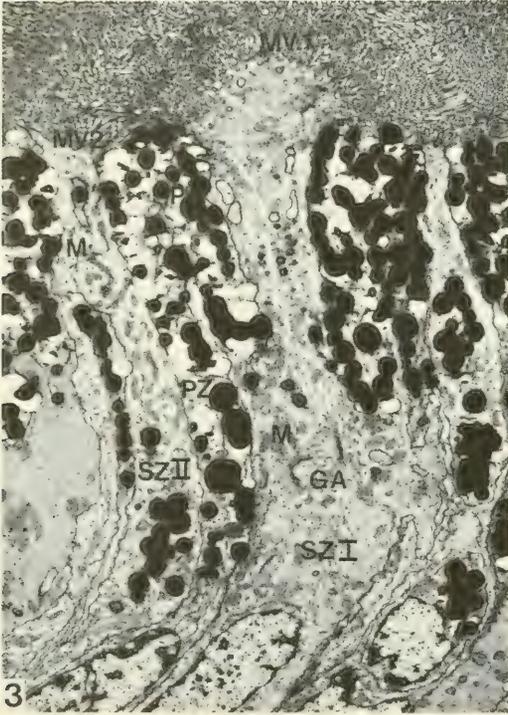
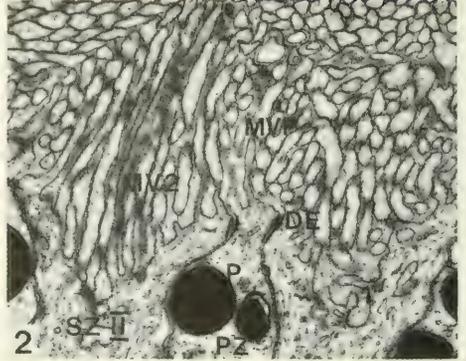
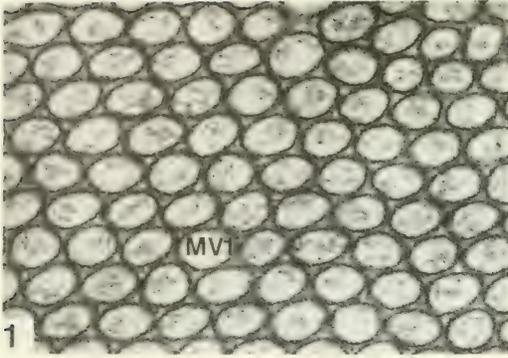
EINLEITUNG

Verschiedene Arbeiten befassten sich in der letzten Zeit mit der Feinstruktur der Augen von Gastropoden. Dabei wurden bei *Helix aspersa* (siehe Brandenburger, 1974) u.a. zwei Sehzelltypen beschrieben. Der Sehzelltyp II wurde bisher nur durch die Untersuchungen an den Augen von *Agriolimax californicus* (siehe Eakin & Brandenburger, 1975) und *Limax flavus* (siehe Kataoka, 1975) bei den Stylommatophora bestätigt. Es stellt sich die Frage, ob der Sehzelltyp II auch bei niederen Stylommatophora zu finden ist. Dieser Frage wird im Rahmen einer Beschreibung des Auges der Bernsteinschnecke *Succinea putris* (Sigmurethra) nachgegangen. Hier konnten mit Hilfe lichtmikroskopischer Methoden nur 2 Retinazelltypen differenziert werden (Zunke, 1978). Ausserdem werden an den Beispielen des Nervus opticus und der Corneazellen eines Embryoauges erstmalig Unterschiede zum adulten Auge belegt.

MATERIAL UND METHODEN

Als Material wurden Exemplare der Art *Succinea putris* (L.) aus dem Gebiet des Vogelsberges/Hessen verwendet. Die adulten Tiere wurden zur Eiablage in einem Terrarium gehalten. Die abgelegten Eier wurden datiert, um das Alter der Embryonen angeben zu können. Zwölf Tage alte Embryonen wurden wie die Augen der adulten Tiere in 2%-igem Glutaraldehyd 2 Stunden fixiert; als Puffer diente 0,05 M Kakodylatpuffer. Die Nachfixierung geschah nach gründlichem Auswaschen im Puffer in 1%-igem OsO₄. Eingebettet wurde in Vestopal W. Die am Reichert Mikrotom OmU2 gewonnenen Dünnschnitte wurden mit Uranylacetat und Bleicitrat kontrastiert. Zur Durchsicht und Fotografie der Dünnschnitte diente ein Zeiss EM 9A.

¹Mit dankenswerter Unterstützung der Deutschen Forschungsgemeinschaft.



ERGEBNISSE

Das Auge gehört zum Grundtyp des geschlossenen Blasenauges. Es differenziert sich in die Cornea, Retina, Linse und einen Glaskörper. Das Auge wird von einer Bindegewebskapsel umschlossen, die sich aus Muskulatur und Kollagenfasern zusammensetzt (Fig. 6).

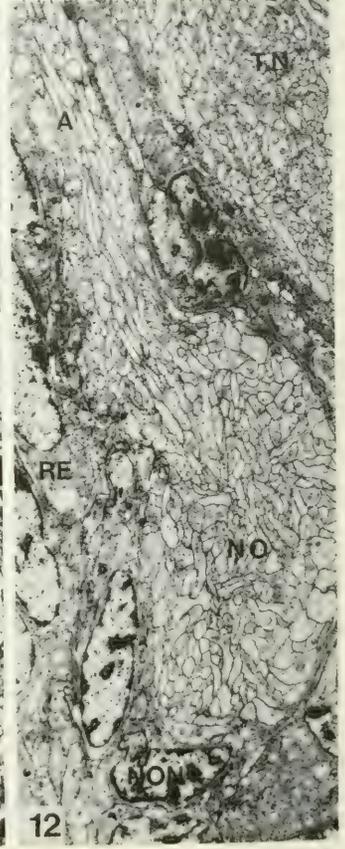
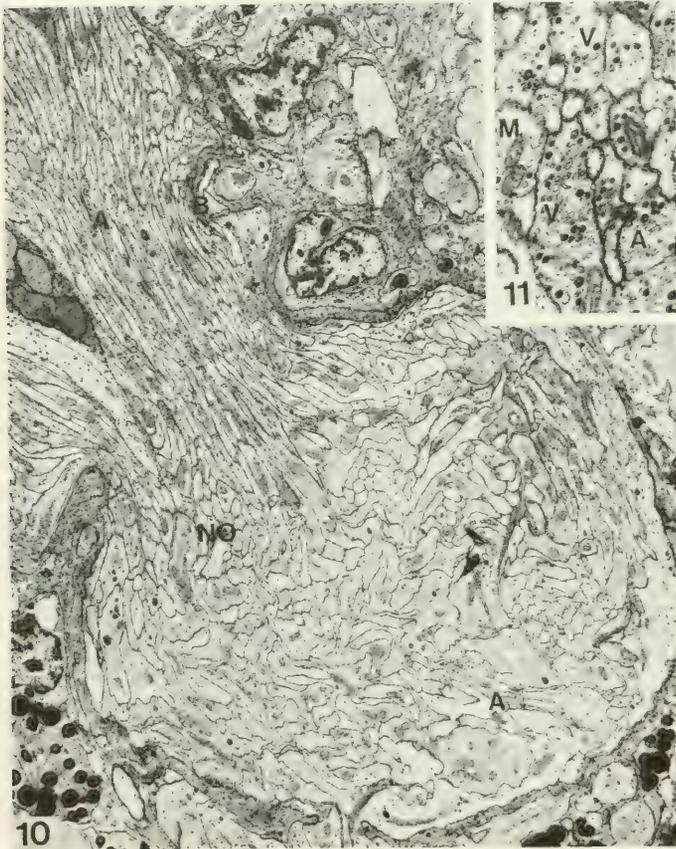
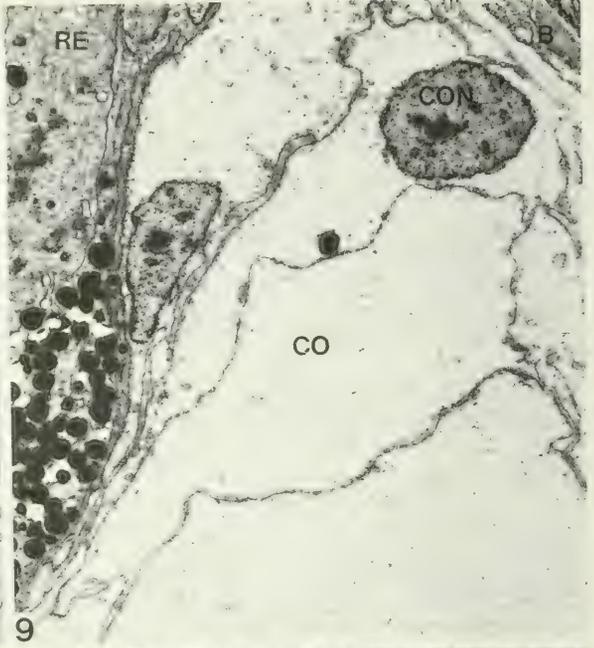
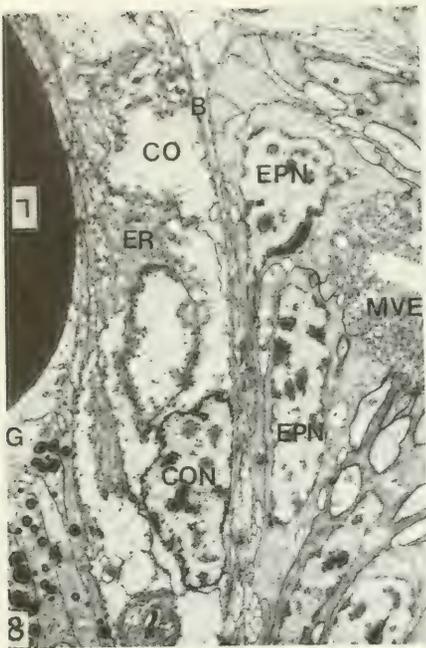
RETINA. Drei Zelltypen kann man bei einer Übersicht über die Retina erkennen: die Pigmentzellen und 2 unterschiedliche Sehzelltypen (Fig. 3, 4). Weiterhin fallen an der Retinabasis Bündel von quergeschnittenen Axonen der beiden Sehzelltypen auf (Fig. 6). Sehzelltyp I ist durch folgende charakteristische Merkmale gekennzeichnet: Von einem apikalen, bis in den Glaskörper hineinreichenden Zellfortsatz (Fig. 3) aus ragen lange, relativ gleichmässig geformte Mikrovilli bis an die Peripherie der Linse heran. Im Querschnitt (Fig. 1) zeigt sich ein homogenes Muster. Mitochondrien findet man im distalen Abschnitt der Zelle zwar auch, doch zahlreicher sind sie im mittleren Teil der Zelle (Fig. 3, 4). Auffallend sind die Golgi-Apparate (Fig. 3, 4), die in unterschiedlich hoher Anzahl vorhanden sind. Der Zellkern ist mindestens doppelt so gross wie die Zellkerne der anderen Zelltypen. Er weist starke Einbuchtungen auf und ist reich an Chromatin (Fig. 4). Umgeben wird der Kern von einer grossen Anzahl gleichgrosser, elektronenlichter Vesikeln, die einen durchschnittlichen Durchmesser von 700 Å haben (Fig. 4, 5, 7). Diese Vesikeln findet man in geringerer Anzahl in den übrigen Abschnitten der Zelle.

Diese für den Sehzelltyp I so charakteristischen Vesikeln sind im Sehzelltyp II nicht nachzuweisen. Hier fallen im Zytoplasma überwiegend elektronendichte Vesikeln (Fig. 7) neben vereinzelt elektronenlichter Vesikeln auf. Alle Vesikeln haben sehr unterschiedliche Durchmesser (700 Å-1200 Å). Der Zellkern liegt weiter von der Zellbasis entfernt als der Kern des Sehzelltyps I. Er ist von schollenförmiger Gestalt und ebenfalls mit Chromatin ausgefüllt. Starke Einbuchtungen sind selten (Fig. 4). Das Axon führt bis zur Basis der Retina (Fig. 4). Die Mikrovilli sind bei diesem Zelltyp sehr unregelmässig und kürzer als bei dem Sehzelltyp I (Fig. 2). Verbindungen zwischen einer Pigmentzelle und Sehzelltyp II sind Desmosomen (Fig. 2). Im Gegensatz zur Pigmentzelle hat der Sehzelltyp II in diesem apikalen Abschnitt kein Pigment. Es liegt im mittleren Bereich und hat den gleichen Durchmesser wie das Pigment der Pigmentzellen, ist aber nicht so zahlreich. Der Sehzelltyp II ist im Auge relativ selten vertreten. Die Pigmentzellen sind langgestreckt und schmal. Sie senden oft Fortsätze in die umliegenden Zellen. Der Kern ist von ovoider Gestalt (Fig. 4) und mit Chromatin angereichert. Starke Einbuchtungen fehlen ganz. Die Zelle ist fast vollständig mit Pigmentgranula ausgefüllt. Die Mikrovilli sind kurz (Fig. 2) und werden meistens von den Mikrovilli der anderen Retinazelltypen verdeckt.

Die CORNEA (Fig. 9) unterscheidet sich von den übrigen Zellen des Auges durch ein besonders elektronenlichtes Zytoplasma. Die birnenförmigen bis kreisrunden Kerne sind nur randständig zu finden. Organellen sind im Gegensatz zur embryonalen Corneazelle reduziert. Mitochondrien, Golgi-Apparate, Endoplasmatisches Retikulum findet man nur vereinzelt im basalen Bereich der Zelle. Zellgrösse und Kernform unterscheiden sich bei adulten Corneazellen sehr stark. Manche Corneazellen scheinen kernlos. Die Kern-Plasma Relation ist zugunsten des Zytoplasmas verschoben (vergleiche Fig. 8, 9). In Fig. 9 konnte nur der Randbereich der Cornea eines adulten Tieres abgebildet werden, Fig. 8 dagegen zeigt die Cornea des Auges eines 12 Tage alten Embryos an ihrer breitesten Stelle. Auffallend ist hier die Menge des rauhen und glatten Endoplasmatischen Retikulums. Mikrovilli sind nur gering ausgebildet.

Der everse Charakter dieses Gastropoden-Augentyps wird durch den Austritt der Axone der Sehzellen in Form eines Nervus opticus verdeutlicht (Fig. 10, 12). Zur Bildung des Nervus opticus muss die Bindegewebskapsel des Auges von den Axonen durchbrochen werden (Fig. 10, 12). Im Nervus opticus sind unterschiedliche Vesikeln (Fig. 11), die denen in den Axonbündel gleichen (Fig. 6) vorhanden. In den Axonen sind Mitochondrien nicht selten. Bemerkenswert sind die randständigen Kerne der den Nervus opticus umgebenden Bindegewebshülle (Fig. 12).

FIG. 1-7. Ausschnitte aus der Retina. 1. Mikrovilli des Sehzelltyps I (×53.600). 2. Mikrovilli des Sehzelltyps II und der Pigmentzelle (×15.600). 3. Apikaler Abschnitt der 3 Retinazelltypen (×3.700). 4. Distaler Abschnitt der 3 Retinazelltypen (×3.700). Man achte auf das Axon des Sehzelltyps II und die unterschiedliche Kerngrösse der verschiedenen Zelltypen. 5. Elektronenlichte Vesikeln des Sehzelltyps I (×53.600). 6. Axonbündel, an die Bindegewebskapsel grenzend (×9.400). 7. Ausschnitt aus Sehzelltyp II, Vesikeln und Golgi-Apparat (×15.600).



Dieses Merkmal findet man bei den embryonalen, wie auch bei den adulten Augen. Der Nervus opticus des embryonalen Auges ist schmaler als der des adulten Auges (Fig. 12), da die Anzahl der Axone geringer ist.

DISKUSSION

Die Untersuchungen am Auge von *Succinea putris* bestätigen das Vorhandensein eines zweiten Sehzelltyps. Er weist die gleichen Strukturen und ähnlich angeordnete Organellen auf, wie der Sehzelltyp II nach Brandenburger (1974). Nur konnten im Sehzelltyp II bei *Succinea putris* keine Cilien oder auch nur ein Basalkörper nachgewiesen werden. Weitere Bauelemente des Auges von *Succinea putris* decken sich mit den Untersuchungen von Brandenburger (1974) und Eakin & Brandenburger (1975) und Kataoka (1975). Es sind die für die Gastropodenaugen schon typischen elektronenlichtigen Vesikeln im Sehzelltyp I, die von Eakin als "photic vesicles" bezeichnet werden.

Eine geringere Anzahl der Axone im Nervus opticus des embryonalen Auges von *Succinea putris* lässt die Vermutung der Zellvermehrung im Verlaufe des Wachstums des Auges zu.

Desmosomen findet man bei allen bisher beschriebenen Augen im apikalen Bereich der Retina.

DANKSAGUNG

Ich danke Herrn Prof. Dr. K. J. Götting und Herrn Dr. C. J. Stoll für die freundliche Unterstützung zur Verwirklichung des Posters.

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ABKÜRZUNGEN ZU FIG. 1-12

A	Axone der beiden Sehzelltypen mit Vesikeln (V);	MVE	Epidermis, Mikrovilli;
AX	Sehzelltyp II, Axon;	MVP	Pigmentzelle, Mikrovilli;
B	Bindegewebkapsel;	NO	Nervus opticus;
CO	Corneazelle;	NON	Nucleus der Bindegewebshülle des Nervus opticus;
CON	Corneazelle, Nucleus;	P	Pigmentzelle, Pigmentgranula;
EPN	Epidermis, Nucleus;	PZ	Pigmentzelle;
ER	Endoplasmatisches Retikulum;	PZN	Pigmentzelle, Nucleus;
G	Glaskörper;	RE	Retina;
GA	Golgi-Apparat;	SZ I	Sehzelltyp I;
GG	Sehzelltyp I, Glykogengranula;	SZ II	Sehzelltyp II;
HD	Hemidesmosomen;	SZN I	Sehzelltyp I, Nucleus;
L	Linse;	SZN II	Sehzelltyp II, Nucleus;
M	Mitochondrium;	TN	Tentakelnerv;
MU	Bindegewebkapsel, Muskulatur;	V 1	Sehzelltyp I, elektronenlichtige Vesikeln;
MV 1	Sehzelltyp I, Mikrovilli;	V 2	Sehzelltyp II, Vesikeln verschiedener Dichte und Grösse.
MV 2	Sehzelltyp II, Mikrovilli;		

FIG. 8-12. Ausschnitte aus der Cornea und dem Nervus opticus. 8. Corneabereich des Auges eines 12 Tage alten Embryos (X3.700). 9. Corneabereich des Auges eines adulten Tieres (X3.700). 10. Nervus opticus eines adulten Auges, die Bindegewebkapsel durchbrechend (X3.700). 11. Unterschiedliche Vesikeln im Nervus opticus eines adulten Auges (X15.600). 12. Nervus opticus des Auges eines 12 Tage alten Embryos (X4.300).

THE SPECIALIZATION OF THE APLYSIID GUT

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University of Reading, England

ABSTRACT

Members of the subfamilies Aplysiinae and Dolabellinae contain the largest of living opisthobranchs. They are essentially herbivorous, ingesting relatively large fragments of a variety of algae. The food does not enter the stomach directly, but passes to a gizzard where it is triturated by teeth and digested by secretion from the digestive gland. Such small fragments as a filter chamber allows to pass and the products of digestion enter the stomach; the rest of the food is transferred directly to the intestine. In *Aplysia punctata* Cuvier and *Dolabella auricularia* (Lightfoot) a ciliary sorting mechanism within the stomach ensures that the largest particles found there do not enter the ducts of the digestive gland, but, together with waste from the gland, are compacted into pellets. The pellets, which incorporate the total waste from the stomach, are manufactured and routed to the intestine in areas isolated from the sorting areas and the ducts of the digestive gland; they retain their identity amongst the rest of the faecal matter, which has come from the foregut, and is relatively voluminous and loosely packed. Particles of food which will enter the digestive gland are maintained in the lumen of the stomach by opposing ciliary currents on folds of the wall, whilst the rejected ones pass along grooves between the folds and are directed posteriorly away from the openings to the gland. The successful functioning of the gut is achieved in similar ways in the two species. Posterior to the conducting region of the oesophagus are the crop, gizzard and filter chamber where there is considerable mechanical activity and which are confined to a ventral part of the anterior haemocoel by a septum. The stomach, which may be closed to oesophagus and intestine by sphincters and valvular folds, receives ducts of the digestive gland ventrally and laterally, whilst dorsally a channel, separated by typhlosoles, leads to the intestine. This channel comes from a caecum in the posterior wall where the faecal pellets are formed.

Members of the order Aplysiomorpha are the largest of living opisthobranchs. They are herbivorous, eating large fragments of algae, though if weed is not immediately available *Dolabella auricularia* (Lightfoot, 1786) (Bebbington, 1974) eats small invertebrates from sandy mud (Ko, 1976). The oesophagus and stomach (Eales, 1921, 1944, 1946) have certain characters that are found only in a few other gastropods, all opisthobranch. The initial part of the oesophagus is a tube leading from the buccal cavity to the vicinity of the visceral mass, where it opens to a crop followed by a gizzard (Fig. 1). Peristaltic movements of the crop force food into the gizzard for trituration by large teeth on the wall. Anterior to these are small sharp teeth, elongated and recurved, which are directed forward on dilatation of the gizzard, grip the weed passing from the crop and drag it into the gizzard as they are rotated backwards on its contraction (at). Crop and gizzard contain a brown digestive fluid from the digestive gland which is mixed with the food. The similarity in size of particles in these 2 chambers indicates that the food is passed to and fro between them. The backward passage of the food from the gizzard is regulated by a filter chamber (Howells, 1942; 'posterior crop' of Eales, 1921, 1944) which leads to stomach and intestine. Its epithelium forms setae which are directed forwards and extend across the lumen when the chamber is constricted and prevent the escape of large particles. Fluid, with the products of digestion and the finest particles, passes through the filter to the stomach. The bulk of the food intake, however, is directed from the gizzard to the intestine.

The stomach, a diverticulum arising between filter chamber and intestine, is relatively insignificant in size. It receives the broad ducts of the digestive gland (dd) which are long and much branched, and extends distally into a caecum (ce) which is comma-shaped and exposed on the surface of the gland. The walls of the stomach and the ducts are ciliated and have a

precise arrangement of folds. Along the dorsal gastric wall are two longitudinal typhlosoles (t_1 , t_2) which pass into the caecum (though at times they may block its entrance) and subdivide the lumen into dorsal (dc) and ventral (vc) channels: the ventral communicates with that part of the stomach into which the ducts of the digestive gland open, and the dorsal with the channel between the two gastric typhlosoles (t_1 , t_2) which leads to the intestine. The caecum is distinguished by its thick muscular coat of mainly circular fibres and by numerous epithelial glands in the dorsal channel. This channel is blocked at its inner end by a transverse bridge of tissue (tv).

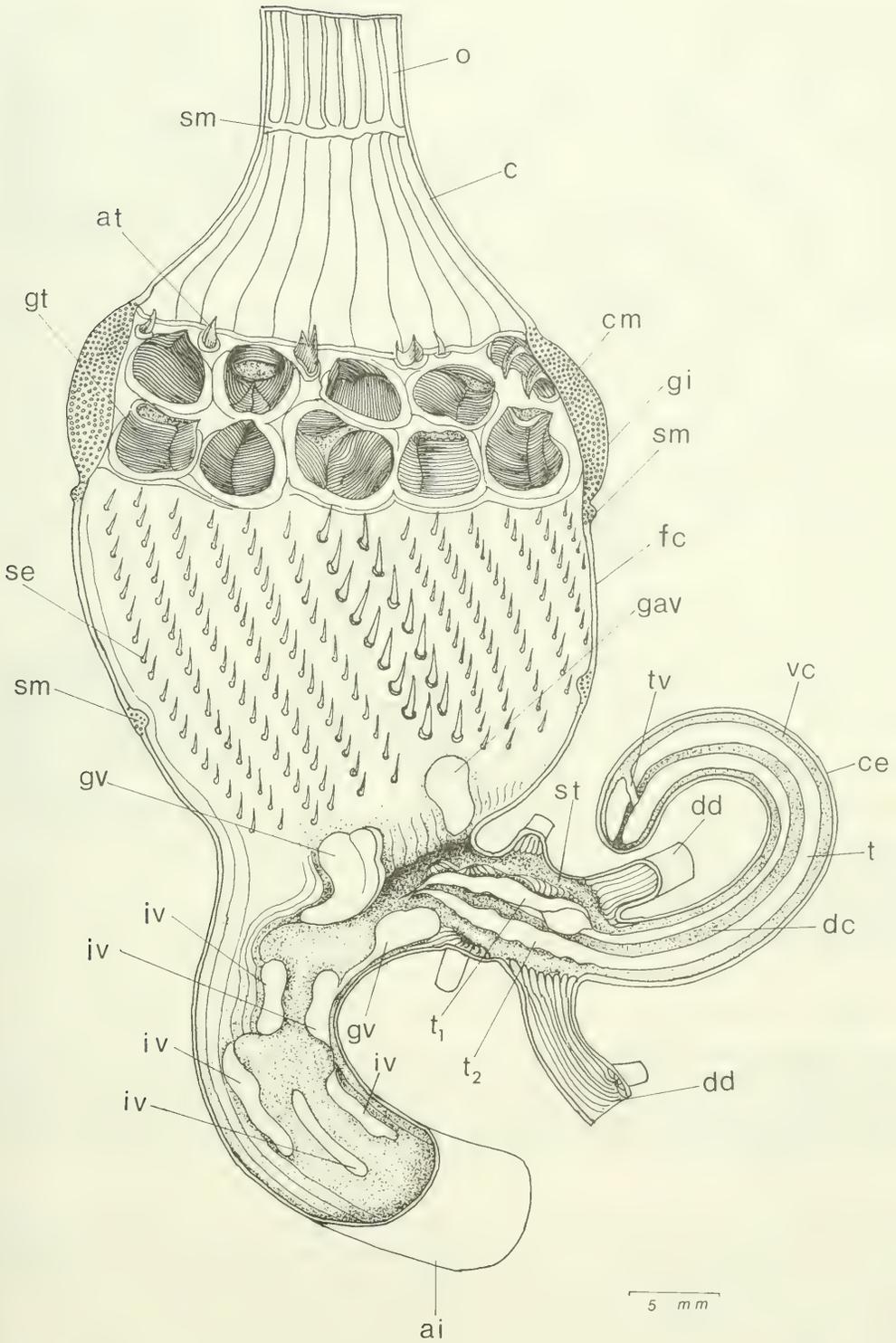
Details of gut structure are best known for *Aplysia punctata* (cf. Howells, 1942) and *Dolabella auricularia* (cf. Ko, 1976). Differences between them relate to the number of teeth in the gizzard, setae in the filter chamber and valves at the junction of filter chamber, stomach and intestine. In *Aplysia* Howells describes 4 or 5 rows of gizzard teeth (total number 50-60), small teeth comprising the 2 anterior rows. In *Dolabella* there are 6-8 small teeth in a single anterior row and an occasional one posterior to 2 rows of massive grinding teeth which total 9 or 10. The filter chamber of *Aplysia* has fewer setae (about 24) not arranged in regular rows as in *Dolabella* (Fig. 1, se) where also 2 longitudinal rows are enlarged and directed towards the gastric opening. Valves which isolate the stomach from the filter chamber and intestine are figured for *Dolabella*. The 2 gastro-intestinal valves (gv) are present in *Aplysia punctata* ('intestinal folds' of Howells), but no gastric valve (gav). In this species the gastric typhlosoles are longer, extending between the gastro-intestinal valves to the intestine (Fig. 2B, t_1 , t_2) and there are no intestinal valves; in *Dolabella* these may represent the remains of gastro-intestinal typhlosoles (Fig. 1, iv).

The muscular action of the walls of the gut maintains a constant exchange of material between crop and gizzard, a forward flow of digestive fluid which mixes with the food and the backward passage of this mixture. The filter chamber and valves at the entrance to the stomach ensure that only fluid and finely divided particles enter the stomach. Large pieces are prevented from escaping from the crop and gizzard by the filter and, if trapped there, are driven forward with digestive fluid giving the possibility of their digestion. Periodically the contents of the gizzard are sucked through the distended filter chamber to the anterior intestine.

It has been concluded (Howells, 1942) that ciliary currents on the gastric walls of *Aplysia* are concerned only with the transfer of waste from the digestive gland to the caecum. A re-investigation of these currents shows that they provide a sorting mechanism for particulate matter entering the stomach. This results in the larger particles, apparently too big to be ingested by cells of the digestive gland, being directed into the caecum. The epithelial folds of the lateral and ventral gastric walls (Fig. 2B) are separated by deep troughs which can be widely opened over a limited area or closed by subepithelial muscles. The largest and heaviest particles (tested by carborundum) coming into contact with the summits of the folds are initially carried posteriorly with the smaller ones, but come under the influence of currents directing them into the troughs which open and allow them to enter (Fig. 2E). They pass along the troughs which lead to the caecal entrance and are joined by waste from the digestive gland. The small particles are raised from the surface of the epithelium by opposing ciliary currents at the sides of the closed troughs and sucked into the ducts of the gland. In *Dolabella* diatom frustules and sponge spicules are found in the stomach and caecal contents, but there is no evidence that such particles are taken up by the cells of the digestive gland. The caecum also contains an abundance of brown spherules from the excretory cells of the gland.

The faecal matter in the stomach passes to the broad entrance to the ventral channel of the caecum where it accumulates in the lumen. It is carried posteriorly by ciliary currents and muscular activity and transferred to the dorsal channel (Fig. 2A, C, D). Here the contents are coated with secretion from epithelial glands and moulded into a faecal rod. Hashimoto *et al.* (1953) regarded this rod as a style with a weak amylase, but its ultimate fate refutes this idea. It is passed back into the stomach along the dorsal passage which leads to the intestine and is

FIG. 1. *Dolabella auricularia*, gut opened mid ventrally. Scale does not allow longitudinal, epithelial folds of stomach and ventral channel of caecum to be indicated. ai, anterior intestine; at, gripping tooth; c, crop; ce, caecum; cm, circular muscles of gizzard; dc, dorsal channel; dd, duct of digestive gland; fc, filter chamber; gav, gastric valve; gi, gizzard; gt, grinding tooth; gv, gastro-intestinal valve; iv, intestinal valve; o, oesophagus; se, seta; sm, sphincter; st, stomach; t, caecal typhlosole; t_1 , t_2 , gastric typhlosoles; tv, transverse valve; vc, ventral channel.



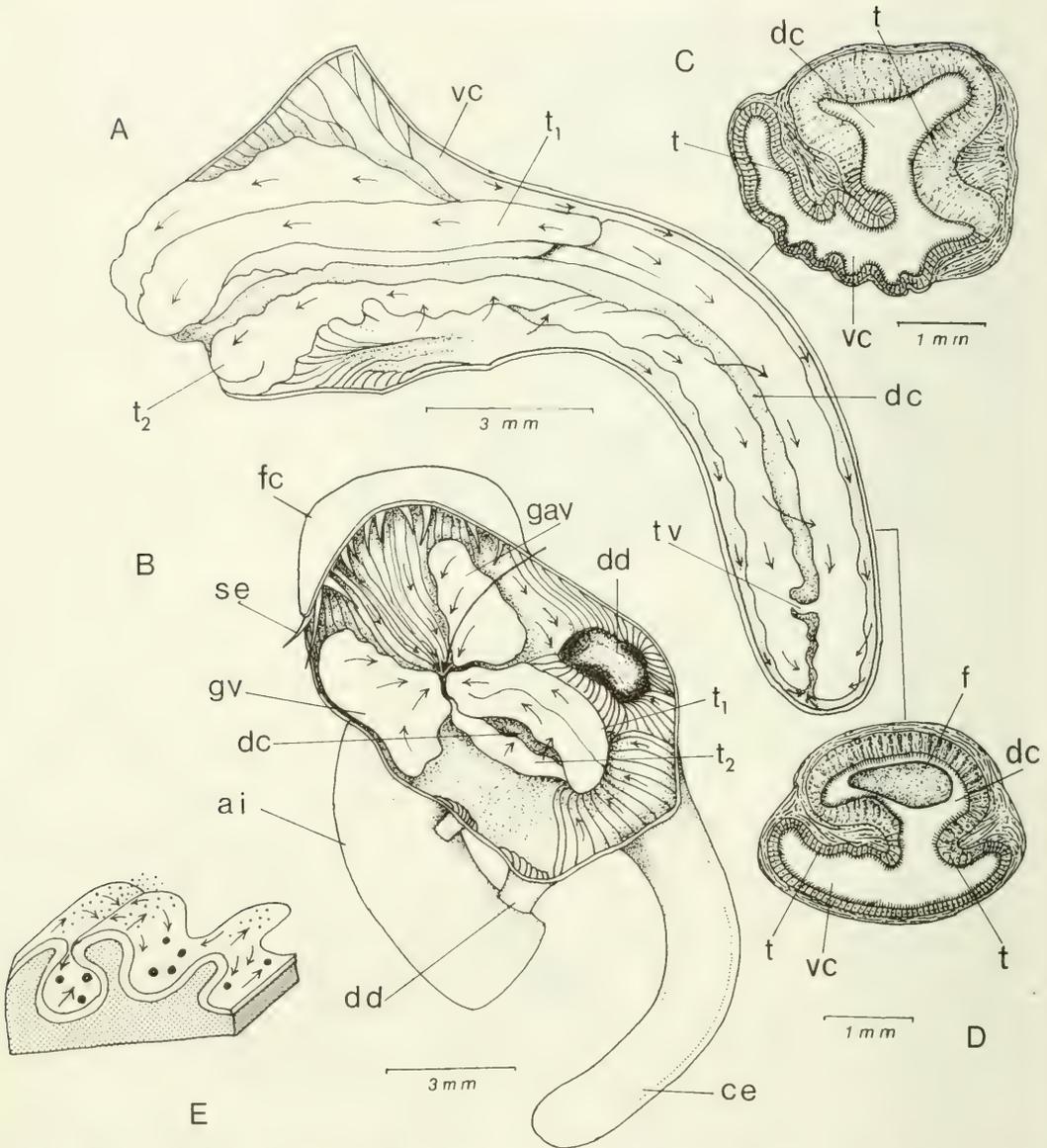


FIG. 2. *Aplysia punctata*. A, caecum and adjacent areas of stomach opened mid ventrally; arrows indicate ciliary currents on gastric typhlosoles and ventral channel of caecum. B, stomach opened mid ventrally; the gastric typhlosoles block the entrance to the caecum and intestine (large arrow). C, D, transverse sections of caecum at levels indicated by lines; note the thick muscular coat. E, diagram of piece of stomach wall to show folds separated by deep troughs, small particles (dots) driven into lumen by opposing ciliary currents (arrows), large particles carried into troughs and then towards caecum. f, faecal rod. Other letters as in Fig. 1.

isolated from the rest of the stomach by the gastric typhlosoles. In the intestine of *Dolabella* rods up to 10 mm long, slightly shorter than the caecum, are found alongside unutilized food passed into it by way of the filter chamber. The intestinal contents include strips of *Zostera* up to 12 mm long, retaining their colour since only cells with injured walls are emptied. Similar observations have been made for *Aplysia* feeding on *Ulva*.

The herbivore *Akera bullata* O. F. Müller regarded by Guiart (1901) and Morton & Holme

(1955) as an aplysiomorph, has similar specializations of the gut: a gizzard, filter chamber ('2nd gizzard' of Morton & Holme), reduced stomach with a caecum and broad intestine which receives the bulk of the food directly from the filter chamber. The presence of plant fragments in faecal rods moulded in the caecum, in addition to waste from the digestive gland, indicates that there is a ciliary sorting mechanism in the stomach. A remarkably similar gut is also present in the closely allied thecosomatous pteropods. They are ciliary feeders taking mainly vegetable food, but there is no evidence that cellulose walls are weakened except by the crushing action of gizzard plates. The cone-shaped chamber posterior to the gizzard, which opens to intestine and stomach, has no filter (Yonge, 1926; Howells, 1936)—an unlikely requirement for a ciliary feeder. The gastric caecum has been regarded as a style sac (Morton, 1954) and its contents as a style (Yonge, 1926; Howells, 1936). However, Howells (1942) re-investigated its structure in *Cymbulia peronii* (Blainville) and concluded that it was similar to that of *Aplysia* and concerned with consolidating faecal waste. Earlier (1936) he had suggested that there might be a sorting mechanism in the stomach with unwanted particles being directed to the intestine and not to the caecum as has now been shown for aplysiomorphs.

Herbivorous prosobranchs rasp the plant tissue and ingest small fragments, but aplysiomorphs crop larger pieces gripping the weed with the radula and cutting it with jaws. In the absence of a cellulase and with a gizzard action which, partly on account of the speedy passage of food through the gut, results in poor fragmentation, a very high percentage of food remains undigested. This food by-passes the stomach leaving it free to deal with the filtrate of semi-digested food from the filter chamber. Certain characteristic functions of the molluscan stomach are retained, a ciliary sorting mechanism related to particulate matter and the elaboration of waste. Although the gut is wasteful of herbage its success is reflected in the speedy growth and size which some animals attain.

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SEXUAL DIMORPHISM IN *BUCCINUM UNDATUM* L.

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ABSTRACT

The study of the variability in shape of fossil Gastropoda is limited to that of its only remnant, the shell. The aim of this study is to determine whether sexual dimorphism exists in the shell of *Buccinum undatum* L. Specimens of *Buccinum* have been taken from living populations. After experimenting on various ways of describing the shell shape quantitatively, it was found that discriminant analysis proved a rather successful method. This analysis revealed that, within a sample from one locality a high proportion (up to 90%) of shells of unknown sex can be classified correctly according to its sex. Differences in shape between the shells of different localities do occur and generally interfere with the sexual shape characteristics.

The problem how to interpret shape features in shells, the only fossil remnants of most gastropods, is often raised among palaeontologists (Morse, 1876; Klähn, 1920; Pelseneer, 1935; Makowski, 1962; Gould, 1966; Raup, 1966; Sohl, 1969). One of the topics in this field is sexual dimorphism. It is seldom possible to tell the sex of a mollusc specimen from its shell. Usually we need the body in order to determine the sex. It will be shown in this paper, however, that from a detailed study of the shape of certain shells using quantitative measurements it is possible to determine the sex with surprising reliability.

A suitable shell for investigations on this matter is *Buccinum undatum* L., the common whelk. *Buccinum* is large enough to provide measurements of good reliability. Samples of animals for this study were collected from the seabed, at various North European localities, mainly by scuba diving. The shells were marked according to sex, as determined by examining the soft body.

As there is little and contradictory information on the aspects of the shell which might show sexual differentiation, the shape of the shell has to be analysed in as many details as possible, and preferably in 3 dimensions. A geometric system naturally suited to describe the shell is a cylindrical coordinate system in which the columella is used as zero-axis (see Fig. 1). A mechanical measuring device with electronic output was constructed in order to measure the following coordinates: φ —the rotation angle in the shell from zero onwards; r —the horizontal distance from the zero-axis onwards; z —the vertical distance in the direction of the zero-axis. The shell can be turned around its axis to yield φ . Both r and z are measured by a system from outside the shell (see Fig. 2). The measuring was performed in points along the frame-pattern of the shell, the frame-pattern being a series of lines containing the most characteristic information about the shape of the shell (see Fig. 3). Among the shape elements the aperture is regarded as a most important feature of the whelk, this being the area where the animals might need differences in shape in order to perform their sexual acts.

The measurement data of the lines were processed by computer. Initially all the measured lines were plotted 2-dimensionally and visually inspected to determine whether differences could be detected between male and female shells. For this purpose the coordinate system was converted into a cartesian one. Differences seemed to exist in the aperture, the aperture of the male being slightly longer than that of the female. Also some differences in aperture form appeared, although there is a big overlap between the 2 sexes. The other measured tracts did not show differences between the sexes.

The 2-dimensional plots of the aperture in the horizontal and vertical planes suggested that differences might become more pronounced if the aperture would be projected onto an oblique plane. To investigate this possibility the coordinates were converted into coordinates arranged in

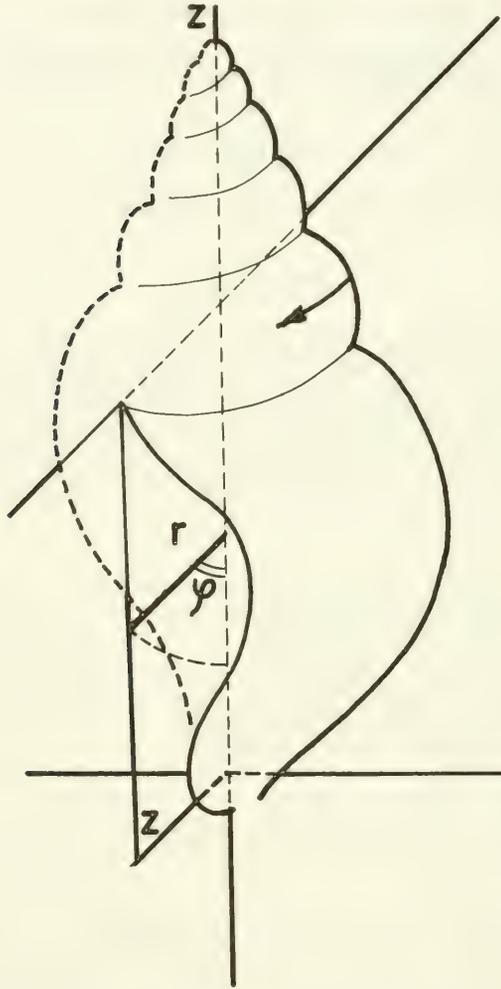


FIG. 1. Whelk with coordinate system.

slanting systems. Projections in 3 of the askew planes show a better pronounced difference between male and female shells, pointing towards the upper part of the aperture as the area of the shell showing most differences. The direction and change of direction in this part of the shell is not the same between males and females.

Now the shells themselves were examined again, in order to see how this shows in the actual shell. The direction of the upper part of the aperture and the way of curving of the concave part just below it seems to differ in males and females. The dominating aspect is that the aperture of the female shell has a rounder concave part, which is also located higher up (see Fig. 4). When guessing whether the shell belonged to a male or a female whelk, on the basis of this information, I guessed right 7 or 8 times out of 10. When this score is possible by eye there must be a way to teach the computer to discriminate between the sexes on the base of a method which can be generally applied.

Because of the shape and the great individual differences a mathematical description of the aperture in a way that makes sexing possible is very complicated, if not impossible. Therefore a statistical approach was adopted. The most promising method seemed to be discriminant analysis, a technique in multivariate statistics in which data of members of groups are analysed

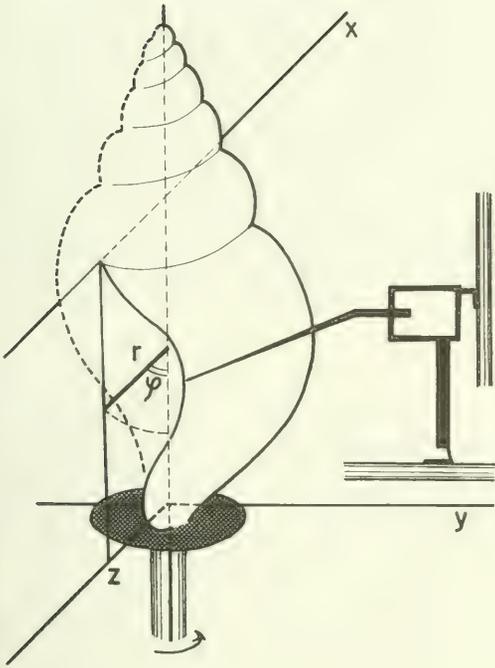
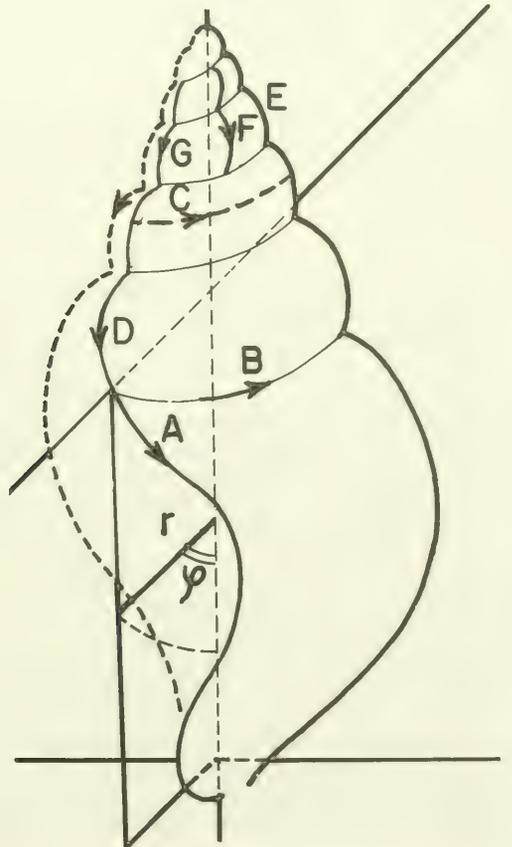


FIG. 2. Measuring equipment.

FIG. 3. The frame-pattern.



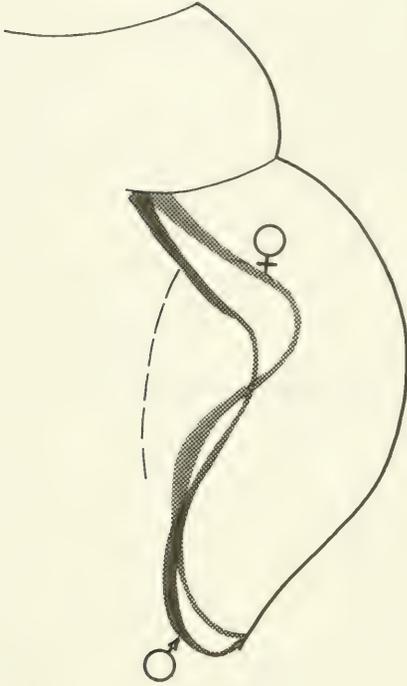


FIG. 4. The character of the differences between ♀ and ♂.

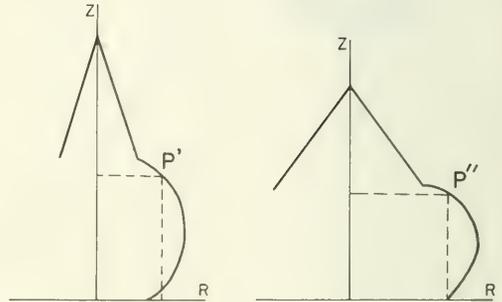


FIG. 5. Differences in shape of the shell of 2 populations.

in order to draw from them discriminatory coefficients. Individuals that belong to one of the groups, but of unknown affinity can then be classified using the discriminatory coefficients.

As direction and change of direction in the aperture seems characteristic of the differences between the sexes, the direction must be expressed in the data. In order to get comparable data on the aperture 16 points at equal distance were identified. Coordinates of these points were obtained by interpolating between the more than 16 measured points. Direction is expressed by vectors of unit length along the chords between adjoining points. The projections of these vectors in x-, y- and z-direction act as input parameters for the discriminant analysis program, together with the height of the aperture and a number indicating the sex of the whelk.

Two samples of 140 whelks each, from 2 different localities, containing equal numbers of male and female shells, were subjected to this analysis. One third of each sample, randomly chosen, was not numbered according to sex, these were of "unknown" membership. Two thirds were used for the analysing part. All shells were then classified afterwards, applying the thus found discriminant coefficient. Of the "unknown" individuals of the two samples 75% and 90% was classified correctly. Of the "known" ones classification was correct in 92% and 99% of the cases. The better performance in the latter classification is probably due to a sampling effect. It is seen that this way of measuring and processing is quite successful.

When discriminant analysis was applied to the combined samples of 280 whelks, the number of correctly classified cases decreased both for the "unknown" and for the "known" individuals to 70% and 90% respectively. As this lower score might be due to the different origin of the samples, the same method was used to determine whether differences between the 2 samples

existed. Indeed discrimination, based on factors independent of sexual dimorphism, between the 2 samples from different localities proved possible, indicating that some effect of microgeographical variation had entered the shape of the shell.

This effect of location on the shell shape, whether genetical or controlled by environmental factors, might interfere with the method applied (see Fig. 5). For instance, the slenderness of the shell, at least one of the factors in which populations of 2 localities differ, influences the dimensions of the aperture. P' and P'' (Fig. 5) are comparable points, but their coordinates do not show this. I am developing an adjusted computer program, accomodating the influence of slenderness on the aperture shape. To do this I concentrate on the upper part of the whelk, grown when the animal was sexually immature, and showing no visible differences between the sexes. I assume that from this part a measure of shell slenderness can be derived. Using this measure, the data of the aperture can be corrected for the effect of general shape of the shell.

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L'UTILISATION DU MICROSCOPE ELECTRONIQUE À BALAYAGE DANS L'EXAMEN DES TISSUS MINÉRALISÉS CHEZ LES LAMELLIBRANCHES

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ABSTRACT

Mineralized tissues of bivalves were studied with the aid of the Scanning Electron Microscope. Bivalve shells have a layered structure underneath the organic periostracum. Three structural groups of mineralized tissue may be distinguished: (1) nacro-prismatic structures with an external prismatic layer (Fig. 1) and an internal nacreous layer (Fig. 3), e.g. in Mytilidae and Unionidae; (2) foliated structures (Figs. 2 and 4), e.g. in Ostreidae, Pectinidae, Anomiidae, etc.; (3) crossed-lamellar structures (Figs. 5, 6 and 7), e.g. in Cardiidae, Glycymeridae, Veneridae, etc. Certain species also exhibit little canals or 'tubules,' see Fig. 8. Evolutionary trends in the various bivalve families, both Recent and fossil, still have to be elucidated.

Au cours du développement des organismes animaux et végétaux, les matériaux entrant dans la formation des parties dures (squelettes, carapaces, tests, etc.) sont toujours composés de 2 types de substances: une substance minérale et une substance organique. Ces substances existent en proportion variable. Leur conservation est possible sous certaines conditions chez les fossiles. Les phénomènes biologiques et cytologiques, qui font que la matière minérale se constitue à partir de l'activité organique au niveau des tissus épithéliaux, se traduisent dans la microstructure des organismes.

Tandis que chez les vertébrés, la phase organique est principalement constituée de collagène et la phase inorganique de CaCO_3 , les matrices organiques des invertébrés présentent une grande variation et les composants inorganiques sont nombreux.

Les Mollusques possèdent une coquille calcaire secrétée par le manteau. Le processus de formation de la coquille de Lamellibranche consiste essentiellement en un dépôt de cristaux de carbonate de calcium sur une matrice organique protéique ou conchyoline. Ces cristaux prennent naissance dans des sites de "nucléation" localisés à la surface de la matrice où existent des groupements chimiques adéquats aptes à permettre la constitution cristalline. Au cours de ce processus, 3 phénomènes dynamiques sont particulièrement remarquables:

—les réactions métaboliques associées à la formation du carbonate de calcium et la synthèse de la matrice organique à partir d'un germe.

—la sécrétion des constituants de la coquille par le manteau. La coquille est formée à partir d'une mince couche de liquide extra-palléal compris entre le manteau et sa surface interne. Le gaz carbonique nécessaire est puisé soit dans le milieu, soit lors de la décarboxylation du cycle de Krebs.

—la formation des couches cristallines.

La croissance de la coquille est liée à l'accroissement du manteau en surface, poids et épaisseur; cet accroissement est fonction de la quantité de carbonate de calcium et de matrice organique. Le carbonate de calcium se dépose sous 3 formes: calcite, aragonite et moins fréquemment vaterite. La forme cristalline adoptée par le calcaire, d'origine alimentaire, dépend du milieu dans lequel le carbonate se trouve dissous.

Les coquilles de Lamellibranches ont une disposition stratifiée en couches en dessous d'un revêtement superficiel ou periostracum. Le nombre et la nature de ces couches sont variables; on en distingue habituellement 2 ou 3 (externe, moyenne et interne). L'étude fine de ces tissus minéralisés (couches) réalisée au Microscope Electronique à Balayage a permis d'observer les principales structures suivantes: prismatique, nacré, foliée, lamellaire-croisée simple ou complexe.

L'association de ces types de structures détermine des groupes structuraux:

(1) Le groupe nacro-prismatique a fondamentalement une couche externe prismatique (Fig. 1), c'est à dire formée de lames de calcaire noyées dans de la conchyoline, et une couche interne nacrée (Fig. 3) comme chez les *Mytilidés* et *Unionidés*.

(2) Le groupe folié (Fig. 2 et 4) où l'on observe une distribution tout à fait variable des lamelles croisées et des prismes (*Ostréidés*, *Pectinidés*, *Anomiidés*, etc.). Ce groupe est calcitique et aragonitique.

(3) Le groupe lamellaire-croisé (Fig. 5, 6 et 7) a une couche interne lamellaire-croisée complexe, c'est à dire dans laquelle des unités primaires plus ou moins prismatiques sont constituées de lamelles de 2ème ordre qui s'irradient à partir de l'axe de ces unités primaires et une couche externe lamellaire-croisée, c'est à dire une structure où il y a un assemblage de lamelles de degrés d'ordre différent (1er, 2ème et 3ème ordre) organisées de telle façon que 2 lamelles adjacentes de 1er ordre plongent dans 2 directions différentes en faisant un angle entre elles (*Cardiidés*, *Glycyméridés*, *Vénéridés*, etc.). Ce groupe a essentiellement une composition aragonitique.

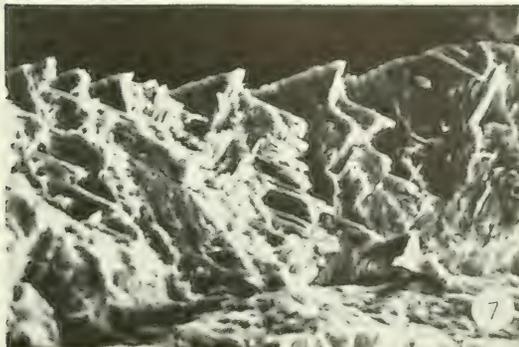
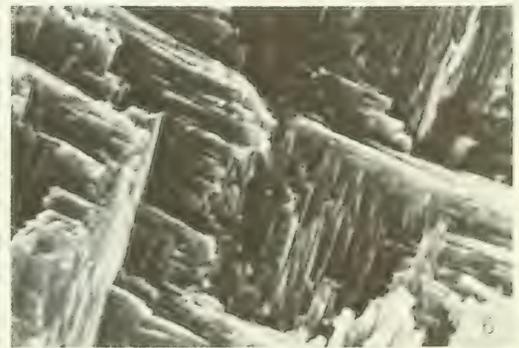
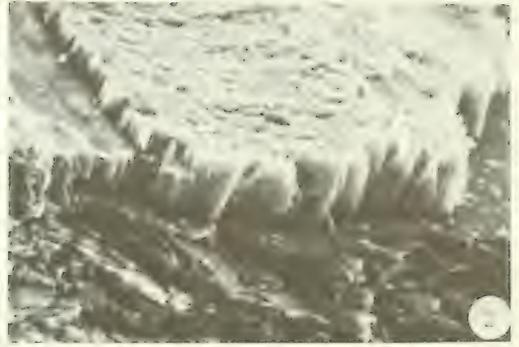
Certaines espèces présentent d'autre part des canaux ou "tubules" à l'intérieur de leur structure, la signification biologique de ces éléments restant imprécise (Fig. 8).

Une étape ultérieure d'étude conduira à l'examen des tendances évolutives qui peuvent exister dans les différentes familles de Lamellibranches tant fossiles qu'actuelles.

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FIG. 1. Couche prismatique, *Unio*, environ X2300. FIG. 2. Structure foliée; on y remarque une couche prismatique surmontant une couche lamellaire, *Ostrea* (Pliocène), environ X750. FIG. 3. Structure nacro-prismatique, détail des prismes de la couche nacrée chez *Mytilus*, environ X2300. FIG. 4. Structure foliée, *Chama* (Lutétien), environ X150. FIG. 5. Structure lamellaire-croisée avec lamelles de 1er ordre parallèles les unes aux autres chez *Cardium*, environ X150. FIG. 6. Détail de la structure lamellaire-croisée, *Dreissena*, environ X2300. FIG. 7. Couche externe lamellaire-croisée montrant les 2 directions d'empilement des lamelles de 1er ordre, *Lima*, environ X250. FIG. 8. Couche externe prismatique avec existence de tubules surmontant une couche lamellaire-croisée interne, *Spondylus*, environ X150.



SHELL MICROSTRUCTURE IN FOSSIL THECOSOME PTEROPODS

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In this preliminary study we have sought to discover whether the shell microstructure of living members of various families of the Thecosomata can be matched amongst fossil forms.

MATERIALS AND METHOD OF STUDY

Three species, each belonging to a different genus, have been studied.

—A spirally-coiled form, *Spiratella pygmaea* (Lamarck), occurring in the Paris and Hampshire basins (Middle Eocene).

—A form with a shell gently coiled in an open helix, *Camptoceratops priscum* (Godwin-Austen), known from the London and Aquitaine basins (Lower Eocene).

—A form with a straight, bilaterally symmetrical shell, *Vaginella depressa* Daudin, occurring in the Aquitaine basin (Lower Miocene).

The shell is broken along predetermined directions and the untreated fracture-faces so produced are metallised under vacuum with gold-palladium alloy and observed under a scanning electron microscope (J.S.M.P. 15, Université de Provence, Marseilles).

RESULTS

(1) *Spiratella pygmaea* (Lamarck) has a prismatic structure resembling that of certain living species of the same family (*S. inflata*, *S. trochiformis*). The prisms are like fine needles. They are mostly straight, but in some regions of the shell are slightly curved and, locally, arranged in a chevron pattern (Fig. 1a,b). Depending on their shape, the prisms lie approximately at right angles to the shell-surface or obliquely to it. The prismatic layer occupies almost the whole thickness of the shell and is normally covered on the outer surface of the shell with a thin layer of finely prismatic calcareous material.

Study of other fossil species referred to the Spiratellidae is in progress to verify whether these all have the structural characters described above or whether some possess the crossed-lamellar or lamellar/prismatic structure known in certain living species belonging to the family.

(2) *Camptoceratops priscum* (Godwin-Austen), whose shell is coiled in an open helix, has a spiral internal structure which foreshadows that of living straight-shelled forms. This remarkable structure is not known from any other group of the Mollusca (Rampal, 1972, 1975). The crystal units are in the form of long fibres which are strongly curved and which appear to follow a spiral path which encompasses almost the whole thickness of the shell (Fig. 1c,d). The outer surface is covered by a film of prismatic calcareous material of varying thickness. Near to the surface of the shell and over about a quarter of its thickness we have observed a pattern recalling that produced by the crossed-lamellar structure. Here the crystal elements lie in two directions inclined at about 130° and produce a latticed appearance (Fig. 1d). As this pattern is not found in all layers of the shell, it is unlikely that it represents a special structure; we suspect that it is a result of the intermeshing of adjoining spiral fibres.

(3) *Vaginella depressa* Daudin, a species with a straight shell, has an internal structure which resembles that of living symmetrical forms; that is, a characteristic spiral structure (Fig. 1e,f).

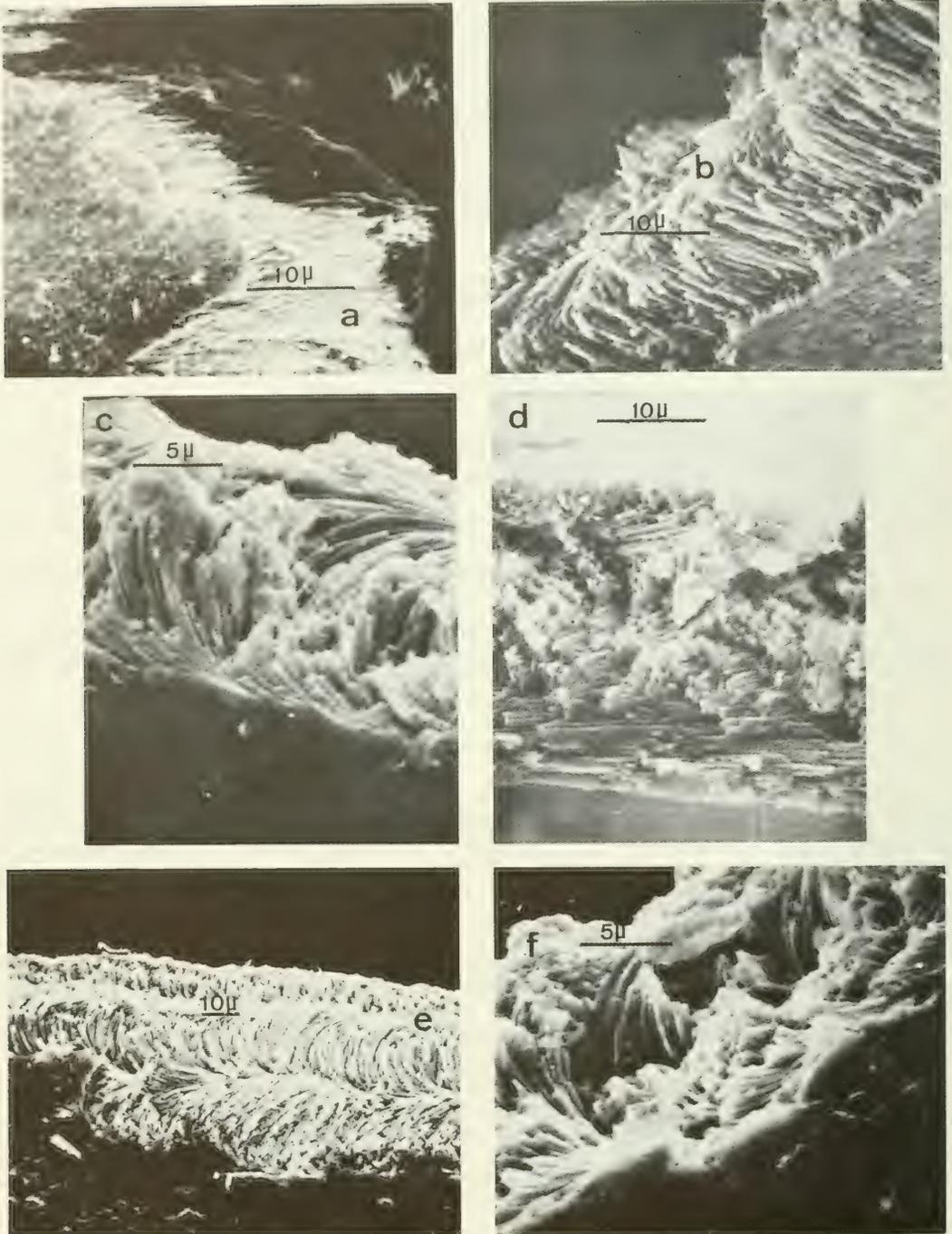


FIG. 1. Vertical sections of shells seen under a scanning electron microscope: a-b, *Spiratella pygmaea* (Lamarck); c-d, *Camptoceratops priscum* (Godwin-Austen); e-f, *Vaginella depressa* Daudin.

The long fibres arising at different levels are aligned diversely but in combination build up the total spiral pattern (Fig. 1f). The layer with spiral structure is overlain by a prismatic zone which varies in thickness but is always relatively thin. The above structural pattern has been observed also in another species of *Vaginella*.

CONCLUSION

The shells of living representatives of the different families of the Thecosomata are characterised by considerable structural diversity. Shells of spiral shape show a prismatic and/or crossed-lamellar structure, whilst straight shells have a spiral structure.

The fossil species with a spiral shell, *Spiratella pygmaea*, has a prismatic structure resembling that of some living shells of the same genus. However, it differs in that the constituent prisms are more or less strongly curved. Further study is required to explore whether this peculiarity is a specific one or whether it is found in other representatives of the Spiratellidae.

The 2 straight-shelled forms, *Camptoceratops priscum* and *Vaginella depressa*, display the spiral structure seen in living straight-shelled members of the Thecosomata. It seems that the evolution in that group from spiral to straight shells was reflected at a very early stage in the shell microstructure of the fossil forms.

The difficulty of deciding whether a particular spirally-coiled molluscan shell should be referred to the gastropods or the thecosomes is well-known. The principal diagnostic characters applied to fossil shells were listed by Curry (1965). However, it seems that a systematic study of shell microstructure may provide additional data for the resolution of this problem in cases of doubt.

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STROBILATION IN A PTEROPOD (GASTROPODA, OPISTHOBRANCHIA)

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ABSTRACT

The splitting of individuals of *Clio pyramidata* into 2 new specimens is a type of behaviour not expected to occur amongst Mollusca. After Van der Spoel (1973) was published, 2 questions were still left unanswered. The first one is "how does the primary specimen develop" and the 2nd is "is it real strobilation"? A detailed histological investigation of the primary animal after completion of the transversal fission revealed that there was no organ system or tissue in a stage of breaking down, while the gonads, accessory sexual glands, seminal groove and penis were in a stage of renewed development. Moreover the mantle gland, regarded as a shell secreting organ, was even more active than in juveniles with not yet fully developed shells, while the activity of neural cells in the pedal ganglia and commissures seems to be comparable with those of specimens in an early male phase. Consequently it seems acceptable to consider the primary specimen as again developing into a normal male specimen. Specimens were examined to find out whether the fission is perpendicular to the body axis of the animal and whether original liver tissue is present in the aberrant stage. These 2 facts are major characters of strobilation and both are found to be present in *Clio pyramidata* so that real strobilation seems to occur here.

INTRODUCTION

Mollusca are so typical that they share only few special characters with other invertebrate phyla. Most clear links with other groups consist of the worm-like trochophore larvae, the segmentation as found in *Neopilina*, the haemocyanine as in Arthropoda and probably the coelenterate-like strobilation. It is my opinion that a phenomenon of this last type is found in *Clio pyramidata* Linnaeus, 1767. In this cosmopolitan, oceanic plankton species, vegetative reproduction by division of the body into two parts occurs under natural conditions. No parasites or external damage have ever been found amongst the more than 100 specimens studied histologically or as intact soft parts. The phenomenon of vegetative reproduction has been found in all parts of the range of the species, though its frequency seems higher in hydrologically instable areas.

STROBILATION IN COELENTERATA

Strobilation in Coelenterata is transverse fission of a polyp. "In nature, . . . , polyps undergo a segmentation, or strobilation, process, i.e., transverse epidermal constrictions mark off a series of segments of the trunk, forming in sequence beginning at the distal end and eventually consuming all but the basal part of the polyp" (Berrill, 1971: 162). During this process there is no destruction of old tissues, but there occurs metamorphosis and regeneration. The strobilus (ephyra) is detached from the oral side, the fission crosses the primary body axis perpendicularly. It takes place in an area where inducers or inducer-transport, regulating development and differentiation, are in equilibrium. At both sides of the fission undifferentiated mesenchymous tissues are present. The apical pole of the polyp stays in position, the oral side develops a new "oral field." The posterior, detached part, the ephyra, develops a new anterior part.

If the opisthobranchous pteropod *Clio* shows real strobilation, exactly the same has to occur. In my 1973 paper I suggested that strobilation might be the case and a strobilating *Clio* was described, but proof for strobilation could not be given.

THE PRIMARY BODY AXIS

The study of Miss Pafort-van Iersel (in this volume) showed that the columellar muscle system in *Clio* is the same as a dorsoventral or septal muscle system, running from apical to oral. Here the terminology of Lemche (1971) is followed, being the only adequate one when comparing Mollusca with Coelenterata. The course of the columellar muscles thus marks the embryonic axis of the species. The fission between the two parts found in *Clio* is perpendicular to this body axis. The separation also crosses the four quadrants (A, B, C and D) which are in the upper part of the body, each characterized by a branch of the columellar muscle and by septal organs like penis, accessory sexual gland and by the incisions between the lobes of the hepatopancreas gland.

The section which in previous publications I called "aberrant, resting stage or secondary animal," thus the part below the split, is comparable to the polyp; the primary specimen or the part above the split is comparable to the ephyra or medusa.

THE SPLITTING

The incision between this "polyp" and "medusa" does not divide all organs into two parts. Only the skin (ectoderm), the liver (interstitial mesenchymous tissues), a genital tissue (meso-ectoderm) and the columellar muscle are split. The epidermal constriction occurs directly below the most caudal loop of the intestine so that no part of the endoderm is left in the lower part of the body (polyp) after division.

A new development in the area of epidermal constriction is the body of reserve food, which is found on top of the "polyp." Theoretically this mass of reserve tissue can be explained as follows. Fission occurs at a place where the concentration of inducers is in equilibrium, which permits regeneration and new development at both sides of the fission, differentiation in this area is thus ambivalent (Berrill, 1971). Strobilation in *Clio* is to all probability a slow process, because completion of the fission is preceded by development of undifferentiated reserve tissues. This reserve food is the only nutrition available to the "polyp" during its first development.

Apart from reserve tissue, parts of the liver are also located in the "polyp," which is very important as this type of undifferentiated tissue guarantees the possibility of development of new organ systems, which is a major criterion for strobilation.

THE SPLITTING OF THE COLUMELLAR MUSCLE

In an earlier paper (1973) I assumed that the anterior part or "polyp" realised a secondary fixing to the shell and that detachment preceded strobilation. If this is correct the soft parts, originally developed in the shell, detach and form a bud anteriorly. This bud then can realise this secondary attachment, and no strobilation has occurred.

However, all columellar muscles which lost contact with the shell or those which have recently become fixed secondarily, show twisting, or if this is not the case, the body is curled. The columellar muscle in the "medusa" or posterior part of the animal is always strongly twisted and the body is usually curled. In the anterior or "polyp" part twisting is never found. So the "polyp" has to all probability always been fixed to the shell, and the medusa is the only part which became detached. This evidently shows that no budding but real strobilation is found in these animals.

DEVELOPMENT OF THE ANTERIOR PART

The growth of the anterior part or polyp has been described before (Van der Spoel, 1963, 1967, 1973) but the typical characters of this development were not understood. However, a comparison with embryonic development is possible.

In the "polyp" a completely new gastric invagination takes place. The extremely thick

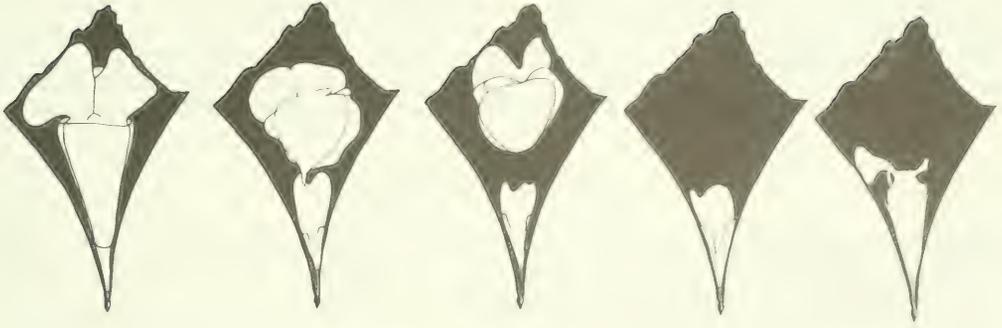


FIG. 1. Strobilation in *Clio pyramidata* (from left to right) (altered after Van der Spoel, 1973).

mucus cover of the "polyp" seems to have no function, as it is not concerned in feeding, unless one compares it with similar parts in invertebrate blastula and gastrula stages (cf. Berrill, 1971) where it contains the factors responsible for the regulation of differentiation and invagination.

The invagination results in a mouth, which is situated opposite to the attachment of the columellar muscle to the shell. This is thus comparable to normal larval invagination (the mouth is ontogenetically ventral, though with regard to the shell it is in dorsal position). The position of the wings in regard to the mouth in normal Thecosomata (not in Gymnosomata) suggests that the wings are a modified velum. If this is correct, the wings of the "polyp" also develop like a velum posterior to the mouth.

Another resemblance with embryos of Gastropoda is the development of the undifferentiated liver tissues. These interstitial tissues grow exactly as the mesoderm does in *Haliotis* embryos, as a left and a right band into the anterior body pole. Whether this is an analogy or homology I am not sure.

DEVELOPMENT OF THE POSTERIOR PART

The upper part or "medusa" attains full development after splitting from the "polyp." This could have been suggested already in my 1973 paper, but at that time it was impossible to trace gonad, gonoduct and accessory sexual glands. These organs have now been discovered as a band of interstitial cells present on the left side of the body between the neck and the place of fission. These cells occupy exactly the same place as the initial cells of the genital system in juveniles. As penial development and the occurrence of a seminal groove is also evident, it must be concluded, that the "medusa" develops again into a functional male specimen. However, it has lost its shell.

The shell secreting glands in the mantle edges on the other hand proved to be active, secretory cells being even more numerous than in juveniles. In all probability, a new shell is thus formed, but it is out of the question that an embryonic shell is formed by this stage. Thus there must exist specimens of *Clio pyramidata* with embryonic shells (direct development) and without embryonic shells (developed through strobilation). Both types of shells are usually present in larger samples, but absence of protoconch was usually attributed to damage. Probably, however, a number of these "damaged" shells belong to specimens born by strobilation and in that case the absence of a protoconch is normal.

CONCLUSION

The direction of cleavage and the development of the two separate parts when *Clio* reproduces vegetatively, unmistakably point to the occurrence of strobilation in this opisthobranch mollusc. This is a firm support for the theory that Mollusca are related to Coelenterata.

The conclusion that pteropods or opisthobranchs thus are a primitive group, however, does not hold. Explanation for strobilation in *Clio* can better be found in the fact that these euthecosomatous pteropods are neotenous animals. The structure of the muscle system (cf. Pafort-van Iersel, this volume) and the foot parts as well as the development of the shell (Van der Spoel, 1976) give strong evidence for the fact that the adult *Clio* is of a juvenile structure.

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THE COLUMELLAR MUSCLE SYSTEM IN *CLIO PYRAMIDATA* AND *CYMBULIA PERONI* (PTEROPODA, THECOSOMATA)

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ABSTRACT

The presence and structure of the columellar muscles in *Clio pyramidata* and *Cymbulia peroni* have been investigated. In *Clio* this muscle is attached to the shell aborally; above the diaphragm it splits into 2 branches, besides a branch ramifying to the penis. The 2 main columellar muscle branches split off 2 ventral ramifications each, while the remaining parts fan out into the wings. In *Cymbulia* the aboral part of the muscle has disappeared, the oral part shows the ramification to the penis, but is otherwise reduced. The phylogeny of the genera *Clio* and *Cymbulia* is discussed, and a Coelenterata-like ancestor is accepted for the whole group of Thecosomata, while it is postulated that *Clio* shows the primitive characters, on which the above conclusion is based, because of the fact that its adults, being neotenous, show a "larval stage" of development.

INTRODUCTION

The purpose of the present investigation is to explain the phylogenetic relation between *Clio pyramidata* Linnaeus, 1767, and *Cymbulia peroni* De Blainville, 1818, based on the structure of the columellar muscle system, and to trace the phylogenetic relations of the Thecosomata.

The specimens of *Clio pyramidata* have been taken from different samples of the Ocean Acre Project (Bermuda area) and those of *Cymbulia peroni* from different samples of the Dana Expeditions and the collection of the Institute of Taxonomic Zoology (North Western Atlantic). Of both species a series of specimens was sectioned (5 μ m), stained with H.E., Crossmon or Azan and used for histological study; other specimens were studied intact, either anatomically or after elucidation with transparent light.

The author is very much indebted to Dr. C. F. E. Roper and Dr. J. Knudsen for providing material.

MUSCLE SYSTEM IN *CLIO*

The mantle muscles and columellar muscle of *Clio pyramidata* are of the same histological structure and they belong to the same system. Aborally the columellar muscle is found dorsad and the mantle muscles ventrad. The origin of these muscles is near the embryonic shell. The mantle muscles are attached below the origin of the columellar muscle. In spiralized species this is a normal situation (cf. *Cylichna* in Lemche, 1956) and it points to spiralisation in the ancestors of *Clio*. At the aboral side near the apex, 8 mantle muscles occur, slightly shifted to the left side of the mantle. The 2 large ventral mantle muscles are fastened to the shell through connective tissue. The origin of the other 6 mantle muscles is not always clear; maybe some of them originate from the columellar muscle as is also known for other Opisthobranchia (Brace, 1977; Lemche, 1956). The muscles in the mantle probably have a retractor function while a more transversal course at some places is related to the protection of organs like heart and kidney in the mantle cavity. On the other hand it is possible that the mantle muscles promote the water current in the mantle cavity (Eales, 1949; Brace, 1977).

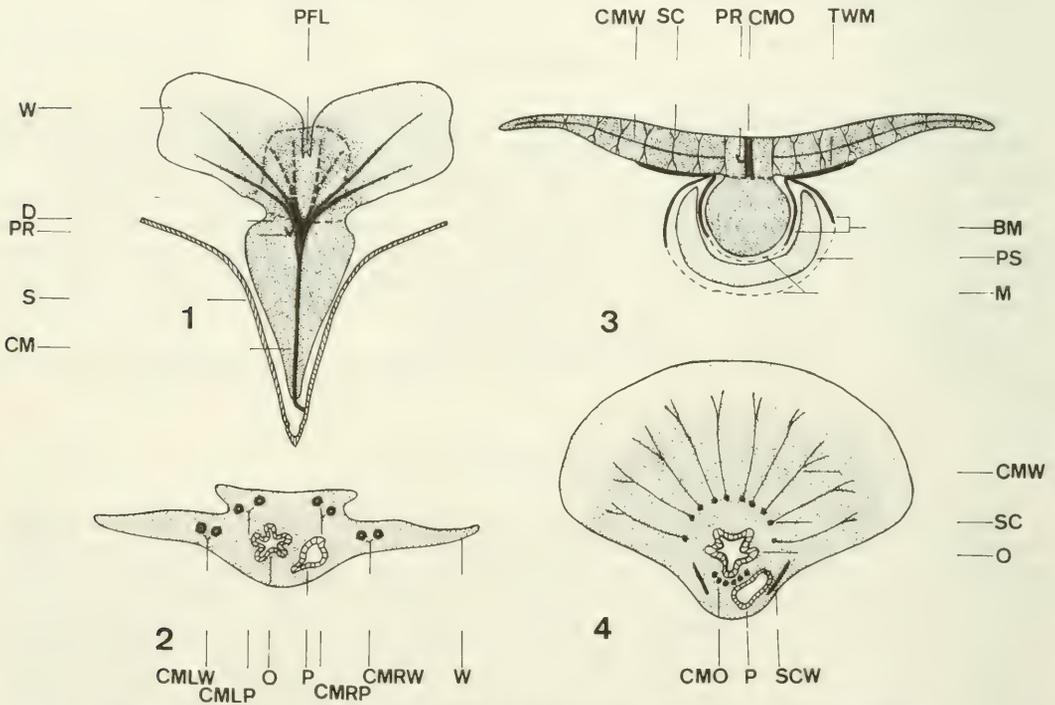
Below the diaphragm, the dorso-anterior part of the strongly developed columellar muscle has a retractor function. Due to the straight shell, the retractor part is symmetrically situated with regard to body and shell.

In the neck region the columellar muscle penetrates into the body, where it gives rise to the penis retractor and splits asymmetrically into 2 unequal bundles situated slightly to the right. There are few features in the anatomy which point to a spiralisised origin; the asymmetry of the columellar muscle-bundles in the neck is one of them. In the wings and head-parts no sign of asymmetry is found. This also corresponds with spiralisised species, where the asymmetrical organisation of the body does not influence the head-parts (Eales, 1949).

In the head a ramifying pattern of 8 columellar muscle bundles is found. Two ramifications fan out into each wing, while the other 4 bundles terminate in the posterior foot lobe near the mouth (Fig. 1, 2). This division of muscles very strongly resembles the division of muscles over 4 quadrants as found in coelenterates and worms.

Besides having a retractor function, the sections of the muscle in the wing also contribute to the swimming movements, while the bundles in the head and posterior foot lobe are concerned with food collecting. The subdivision into some smaller bundles in the head region increases the mobility in that part of the body (Brace, 1977), which has a positive effect on the collecting of food.

Along its course through the body, anchorage of the columellar muscle is ensured by thin muscle filaments, originating from the columellar muscle and terminating in mantle integument, body wall and wing wall. In other opisthobranchs like *Philine* (cf. Brace, 1977) and *Cylichna* (cf. Lemche, 1956) similar structures are found. The anchoring probably has a double purpose: all parts of the body become connected with the retractory system by these filaments, and the muscle is kept in position.



FIGS. 1-2. *Clio pyramidata*, 1. schematic dorsal view; 2. schematic cross-section near the mouth.

FIGS. 3-4. *Cymbulia peroni*, 3. schematic longitudinal section dorsad to the oesophagus; 4. schematic cross-section oral to the diaphragm.

Abbreviations: bm, body muscles; cm, columellar muscle; cmlp, columellar muscle branches in left part of posterior foot lobe; cmlw, columellar muscle branches in left wing; cmo, columellar muscle branches near the oesophagus; cmrp, columellar muscle branches in right part of posterior foot lobe; cmrw, columellar muscle branches in right wing; cmw, columellar muscle branch in wing; d, diaphragm; m, mantle; o, oesophagus; p, penis; pfl, posterior foot lobe; pr, penis retractor; ps, pseudoconch; s, shell; sc, supporting cell; scw, wall of supporting cells; twm, third aboral muscle layer of wing; w, wing.

The walls of the wings contain 2 layers of strongly developed striated muscles each. In opisthobranchs there is a stronger development of body wall musculature than in other gastropods, while the importance of the columellar muscle decreases. Especially in pteropods, considered to be the best swimming opisthobranchs, the striated musculature of the wing wall is very strongly developed (Thompson, 1976).

The muscles of stomach and oesophagus belong to a common system. The radula muscles are probably connected with the columellar muscle system, but the material studied gives no certainty.

One may conclude from the structure of the columellar system that *Clio* develops without metamorphosis. The adult still shows its larval stage. This is supported by the orientation of the mantle muscles somewhat to the left of the middle, and of the columellar muscle somewhat to the right of the middle in the neck region of the body, and by the place of the origin in the shell. The mantle muscles have to be considered as the original right retractor muscle in the veliger of Opisthobranchia (Brown, 1934), which is turned to the left in the Cavoliniidae by the 180° rotation of the body part (Meisenheimer, 1905; Tesch, 1913; Bonnevie, 1914). In the same way the columellar muscle of *Clio*, orientated slightly to the right, resembles the left retractor muscle in the veliger of Opisthobranchia, running to the velum and operculum (into the wings and posterior foot lobe respectively). The structure of the shell also points to a direct development from a larval stage and to absence of metamorphosis (Van der Spoel, 1976a). The fact, that the adult when full-grown is still in a larval stage may be considered a logical consequence of the identical pelagic behaviour of larva and adult.

The asymmetrical orientation of the 2 columellar muscle ramifications in the neck region, the origin of the columellar muscle above the fastening of the mantle muscles and the trace of spiralisation in the soft parts of the larvae (Van der Spoel, 1967), point to a spiralisation in the ancestors of *Clio*. Therefore, the external symmetry is an adaptation to the pelagic way of life and so of a secondary nature. Consequently, there is no reason to doubt the commonly accepted theory, that within the Euthecosomata the Limaciniidae are the ancestors of the Cavoliniidae (Pelseneer, 1892; Tesch, 1904; Meisenheimer, 1905; Bonnevie, 1914; Van der Spoel, 1967; Minichev, 1967).

MUSCLE SYSTEM IN *CYMBULIA*

According to Meisenheimer (1905) and Tesch (1913), the muscle plates or body muscles laterad to the body of *Cymbulia peroni*, would be identical with the columellar muscle. The present investigations, however, proved the lack of a columellar muscle aboral to the diaphragm. In *Cymbulia*, as is normal in Opisthobranchia, the loss of the shell is coupled with the loss of the retractory and attaching part of the columellar muscle (Lang, 1900). The forming of a secondary gelatinous shell did involve the forming of secondary attaching muscle systems, the muscle plates. These body muscles develop from an outer striated muscle layer of the wing wall. They are not attached to the pseudoconch, but terminate in mantle integument (Meisenheimer, 1905; Van der Spoel, 1976b). The muscle plates are doubly folded; the median parts are situated inside the pseudoconch, the lateral parts outside, in the external mantle integument (Fig. 3).

Oral to the diaphragm a weakly developed part of the columellar muscle system is still found. Some bundles run from the diaphragm dorsad along the oesophagus towards the area of the mouth as also found in the tectibranch *Philine* (Brown, 1934; Hurst, 1965; Brace, 1977). Like in *Clio* the penis retractor ramifies from this part of the columellar system. Obviously this part of the columellar muscle is functionally related to internal food transport. Connections of the columellar muscle with buccal organs have not been found.

Part of the columellar muscle runs in the wing lumen between the walls of the wing, where small muscle filaments radiate. These muscles contribute to the swimming locomotion of *Cymbulia*. The columellar muscle filaments in the wings are connected with numerous supporting cells, between the walls of the wings. The latter maintain the shape of the large wing disc (Fig. 3). Two little walls of supporting cells found lateral to the penis, besides having a supporting function, probably also have something to do with the evagination of the penis, because they have a larger quantity of muscle filaments than other supporting cells. These

supporting "walls" and the large supporting cells attach themselves ventrad and laterad on the diaphragm, forming a protecting envelope around oesophagus, ganglia and penis, and they separate the wing disc from the rest of the body (Fig. 4).

As in *Clio* the circular muscles of stomach and oesophagus form one single system. Only a few mantle muscle filaments could be recognised in the mantle; it seems that this system is not well developed.

The strongly developed striated muscles of the wall of the wing disc contain orally 2 layers and aborally 3 layers. The body muscles originate from the 3rd aboral layer.

In *Cymbulia* a metamorphosis occurs during which, among other changes, the larval shell is thrown off (Thiriot-Quévieux, 1970). There are no indications, that the reduced columellar muscle system did develop from a larval system, as supposed for *Clio*. But for *Cymbulia* too, there is no difference between the pelagic way of life of larva and adult.

Some features point in the direction of spiralised ancestors: the external symmetry is secondary. For example, on the right the mantle cavity penetrates further dorsad (Meisenheimer, 1905); the position of the body muscles is asymmetrical; one osphradium is present on the right side; the embryonic shell is ultra-dextral (Pelseneer, 1891). As to the present investigations, there is no cause to doubt the commonly accepted theory that the ancestor of *Cymbulia* is *Peraclis* (Meisenheimer, 1905; Bonnevie, 1914; Tesch, 1948; McGowan, 1968).

PHYLOGENY

The columellar muscle systems of *Clio pyramidata* and *Cymbulia peroni* are quite different. In *Clio* the columellar muscle is a strongly developed larval muscle system, whereas in *Cymbulia* the columellar muscle is reduced and many parts have even disappeared.

It seems that the adaptation to a pelagic behaviour of adults in Thecosomata resulted in *Clio* in the disappearance of a real metamorphosis (neoteny), while in the Cymbuliidae the calcareous shell is replaced by a gelatinous pseudoconch, thus increasing the floating capacity, while the columellar system is strongly reduced. *Clio* and *Cymbulia* belong to different lines of development of which the Pseudothecosomata seem to be more adapted to a pelagic life and therefore they are the most progressive group of the Thecosomata. The Euthecosomata and Pseudothecosomata are supposed to be related through an unknown ancestor which gave rise to *Limacina* and *Peraclis* respectively (Meisenheimer, 1905; Tesch, 1914, 1948; Van der Spoel, 1976b).

In *Clio pyramidata* 2 facts need special attention. First there is the strobilation as described by Van der Spoel (1967, 1973), a process comparable with the strobilation in Annelida and Coelenterata. Secondly we see in both mantle and head a pattern of 8 muscles of the columellar system. The posterior parts can be divided into 4 ontogenetical quadrants, each with 2 ramifications of the columellar muscle (right wing, left wing, right part posterior foot lobe, left part posterior foot lobe, see Fig. 2). This situation resembles that in *Cylichna* (Lemche, 1956) and suggests a phylogenetic relation with Annelida or Coelenterata. The 8 muscles in the head perhaps also resemble the 4 parts of larval retractor muscles in the Opisthobranchia. According to Lemche (1966), the 8 larval muscles show that the molluscs must be derived from a primitive tetracyclomeric type of organism like the Coelenterata and that 'from Molluscan-like ancestors Arthropods and Annelids have evolved independently.'

Summarizing, we may state that in the opisthobranch *Clio pyramidata* 2 primitive features appear, which affirm the possible relation to a Coelenterata-like ancestor. The fact that both these primitive features appeared in *Clio*, may be due to the 'larval stage' of the adult.

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NOTE ON VARIATION IN *DIACRIA* GRAY, 1847, WITH DESCRIPTIONS
OF A SPECIES NEW TO SCIENCE, *DIACRIA RAMPALI* NOV. SPEC.,
AND A FORMA NEW TO SCIENCE, *DIACRIA TRISPINOSA*
FORMA *ATLANTICA* NOV. FORMA

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ABSTRACT

Variation of shell shape and colour pattern in the *Diacria trispinosa* group consisting of 3 taxa, *D. rampali* n. sp., *D. trispinosa* f. *trispinosa* and *D. trispinosa* f. *atlantica* n.f., have been studied. *D. trispinosa* is distinct in colour pattern and has a shell shape somewhat different from that of *D. rampali*. The difference in shell shape consists chiefly of a higher position of the lateral spines; this position is indicated by the l/m ratio. Discriminant analysis has been used to separate *D. rampali* and *D. trispinosa*. Specimens from the Philippine area can be well discriminated but specimens from the Atlantic Ocean—can only be separated if one also uses differences in colour pattern. The colour patterns of *D. rampali* and *D. trispinosa* are distinct and their development is different. In the Atlantic north of 30° N *D. trispinosa* becomes larger with higher latitudes, due to adaptation in favour of the floating capacity. The teleoconch becomes fully coloured. These North Atlantic populations have been described as *D. trispinosa* f. *atlantica*. Specimens of *D. trispinosa* f. *trispinosa*, living at higher latitudes do not show enlargement of the teleoconch. *D. trispinosa* f. *atlantica* is restricted in distribution to the North Atlantic Ocean. *D. major* has been found in the North Atlantic and the Pacific Ocean. In the material studied, no *D. major* was found in the Indian, nor in the South Atlantic Ocean. *D. trispinosa* f. *trispinosa* has been collected in all oceans. *D. rampali* has been found in the Atlantic and the Pacific Oceans.

INTRODUCTION

The variability in *Diacria* has been discussed by various authors (Boas, 1886; Van der Spoel, 1970; Rampal, 1975; etc.). *Diacria trispinosa* (De Blainville, 1821) was considered a monotypic species, although its variation is great. The above-mentioned authors considered this variation as only ecophenotypic. In this paper it is shown that *D. trispinosa* can be split up into 3 taxonomic groups, mainly based on differences in colour pattern, correlated with differences in shell shape. One species and one forma, both new to science, has been distinguished besides *D. trispinosa* s.s.

MATERIAL AND METHODS

Alcohol-preserved material from the Atlantic Ocean and from West of New Guinea and Recent sediment material from the Atlantic and Indo-Pacific Oceans has been studied. Special attention was given to the Caribbean Sea, the North Atlantic Ocean and the Philippine area. This material has been collected mainly by the United States Bureau of Fisheries and the Dana expeditions. Eleven measurements of the shell have been taken, of which B, D, E, I, L, M and O (see Fig. 1) are accurate up to 0.07 mm and A, G, J and K are accurate up to 0.02 mm. Besides, attention was paid to the colour pattern. Material collected by the Dana expeditions in the South Atlantic, the Indian and the South Pacific Oceans has not been incorporated in the computer programs. Age discrimination was possible by means of histological examination of the developmental stage of soft parts. Discriminant analysis has been made to distinguish the groups according to size differences apart from colour pattern variation. This multiple discriminant analysis was performed using the SPSS subprogram DISCRIM (Nie et al., 1975).

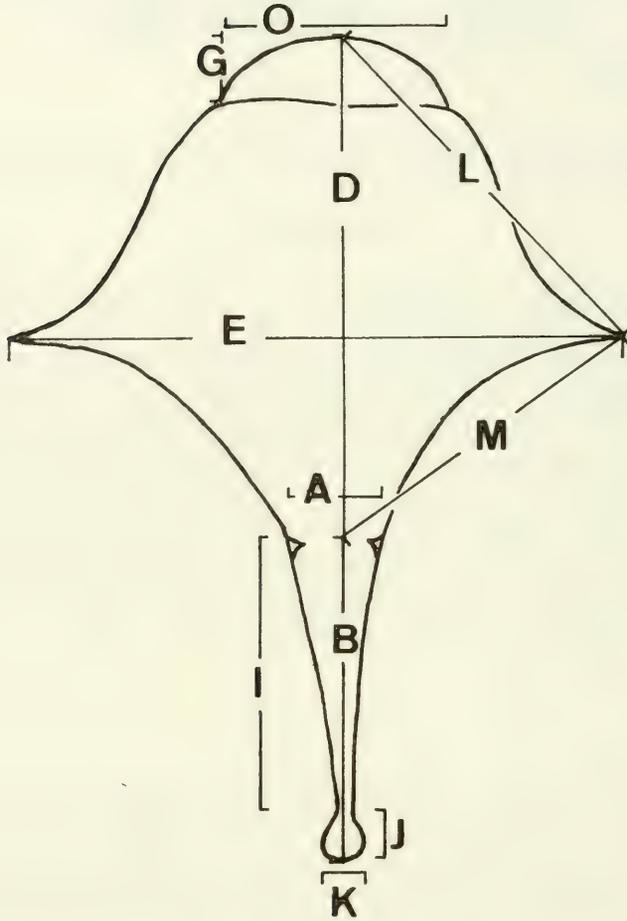


FIG. 1. Measurements taken from the shell:

A	width at the membrane (lowest part of the teleoconch)	(0.02)
B	length of the posterior spine	(0.07)
D	length of the teleoconch	(0.07)
E	maximal width (between lateral spines)	(0.07)
G	height of the shell aperture	(0.02)
I	length of protoconch II	(0.07)
J	length of protoconch I	(0.02)
K	width of protoconch I	(0.02)
L	length from the lateral spine to the dorsal rim of the shell aperture	(0.07)
M	length from the lateral spine to the membrane	(0.07)
O	width of the shell aperture	(0.07)

accuracy (in mm)

(0.02)
(0.07)
(0.07)
(0.07)
(0.02)
(0.07)
(0.02)
(0.02)
(0.07)
(0.07)
(0.07)

ACKNOWLEDGEMENTS

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RESULTS

In the present study variation of the colour pattern and size is compared. The colour pattern variation shows 2 types correlating with 2 types of shell shape. The development of the colour pattern has been studied in a series of specimens of different ages (see Fig. 2). The studied specimens, preserved in alcohol, have been collected by the Dana expedition 1922 at 16°06' N 76°02' W. The 2 types of colour pattern proved to be distinct throughout the developmental stages of the animals. In many samples, from plankton as well as from sediment, representatives of both types have been found. The 2 groups differing in colour pattern are sympatric in the Atlantic Ocean and the Philippine area. The colour patterns of the sympatric groups are distinct and therefore these groups will be considered different species: *D. rampali* nov. spec. and *D. trispinosa*.

Size variation is correlated with variation in coloration and distribution. A group of populations of *D. trispinosa* in the North Atlantic Ocean shows large, dark shells. Intermediates between these populations and more southern populations have been found. In the northern populations shell size increases with higher latitudes. Therefore the northern populations are considered to represent a forma new to science: *D. trispinosa* forma *atlantica* nov. forma.

***Diacria rampali* nov. spec. (Fig. 3)**

The species name is given in honour of Dr. J. Rampal, who was the first to pay full attention to the variation of *D. trispinosa* and its related forms.

Holotype: adult specimen in Universitetets Zoologisk Museum, Copenhagen.

Paratypes: 1 adult specimen, 2 transitionals and 2 minutes in Universitetets Zoologisk Museum, Copenhagen, and 2 adults and 6 transitionals in Zoological Museum, Amsterdam.

Type locality: 16°06' N 76°02' W; depth 300 meter wire, Dana expeditions, station 1215 IV; 27-1-1922.

This species may be identical with *Hyalaea aculeata* d'Orbigny, 1846: 687, pl. 7 fig. 1-5. The colour pattern of *Hyalaea aculeata* is the same as that of *D. rampali* but shell shapes are different.

Hyalaea trispinosa d'Orbigny, 1836: 106.

Diacria trispinosa Adams, 1853: 1, 52 pl. 6 fig. 2a; Adams, 1859: 45; (in part) Vayssière, 1915: 58, pl. 1 fig. 14.

Description. Rather small species; teleoconch slender posterior to the lateral spines, caudal spine long, shell aperture small, rim of shell-aperture and the middle of dorsal ribs brown. The colour of the ventral lip is connected with a spot on the teleoconch anterior to the lateral spines. Lateral spines are white and sometimes lateral ribs are very light brownish. When a specimen grows older, the colour becomes more intense. Minute stages and often transitional stages lack the brown spot on the teleoconch. In adults the anterior half of the teleoconch sometimes becomes fully coloured. Table 1 shows the measurements of the holotype.

TABLE 1. Measurements (in mm) of the holotype of *D. rampali*.

A	0.77	J	0.24
B	3.88	K	0.20
D	5.98	L	4.49
E	7.48	M	4.88
G	0.59	O	2.73
I	3.64	L/M	0.92

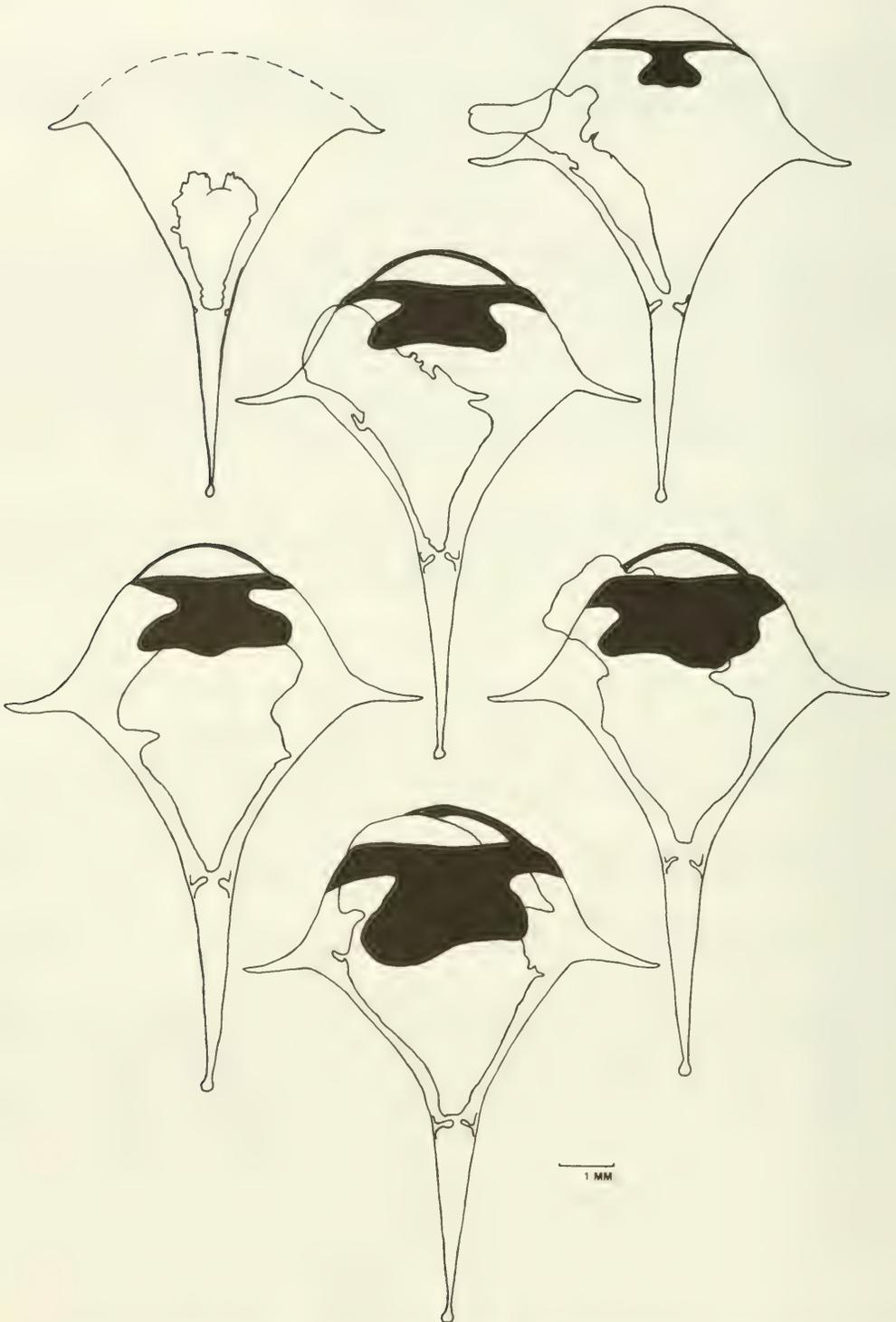
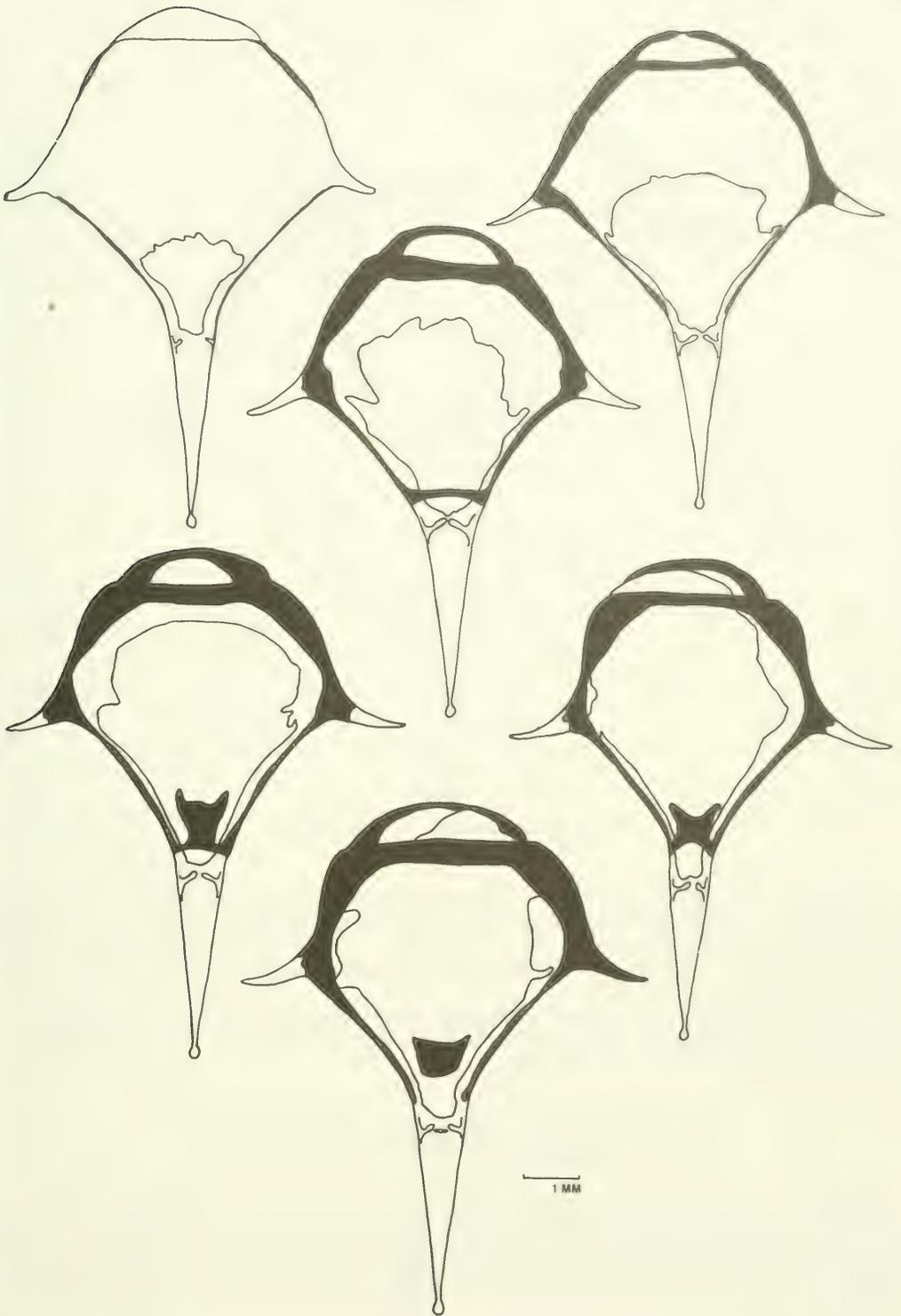


FIG. 2. Colour pattern development in *D. trispinosa* f. *trispinosa* (right) and in *D. rampali* (holotype and paratypes) (left).



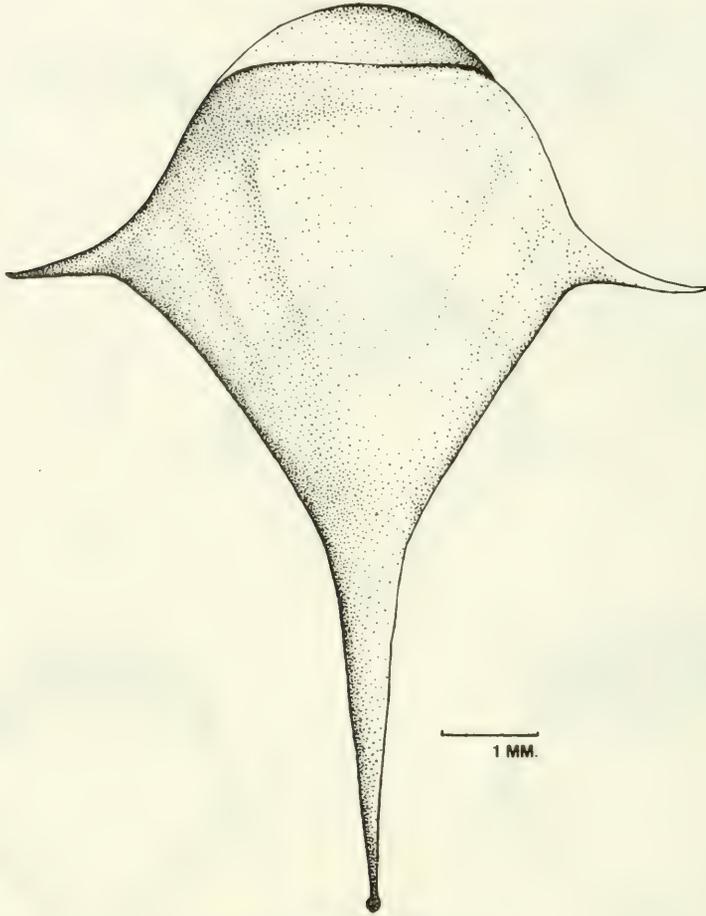


FIG. 3. Holotype of *D. rampali* nov. spec.

Compared to *D. trispinosa* the dorsal aperture rim is less curved, the caudal spine is longer, the vaulting of the teleoconch is slightly less, the lateral growth of the ventral aperture rim in the adult stage is less pronounced. The lateral ribs are white instead of brown as in *D. trispinosa*, and *D. rampali* lacks the light brown spot present in the adult stage of *D. trispinosa* near the caudal spine.

Compared to *Diacria major* (Boas, 1886) the teleoconch is smaller and slender, the lateral spines are less curved, the caudal spine is proportionally longer. The colour of *D. major* is restricted to the rims of the shell aperture.

***Diacria trispinosa* (ms Lesueur) (De Blainville, 1821)
forma atlantica nov. forma (Fig. 4)**

Holotype: adult specimen in Universitetets Zoologisk Museum, Copenhagen.

Paratype: 100 adult specimens in Universitetets Zoologisk Museum, Copenhagen, and 709 adults and 1 minute in Zoological Museum, Amsterdam.

Type locality: 39°21'N 21°51'W; depth 300 meter wire, Dana expeditions, station 1380 IV; 19-6-1922.

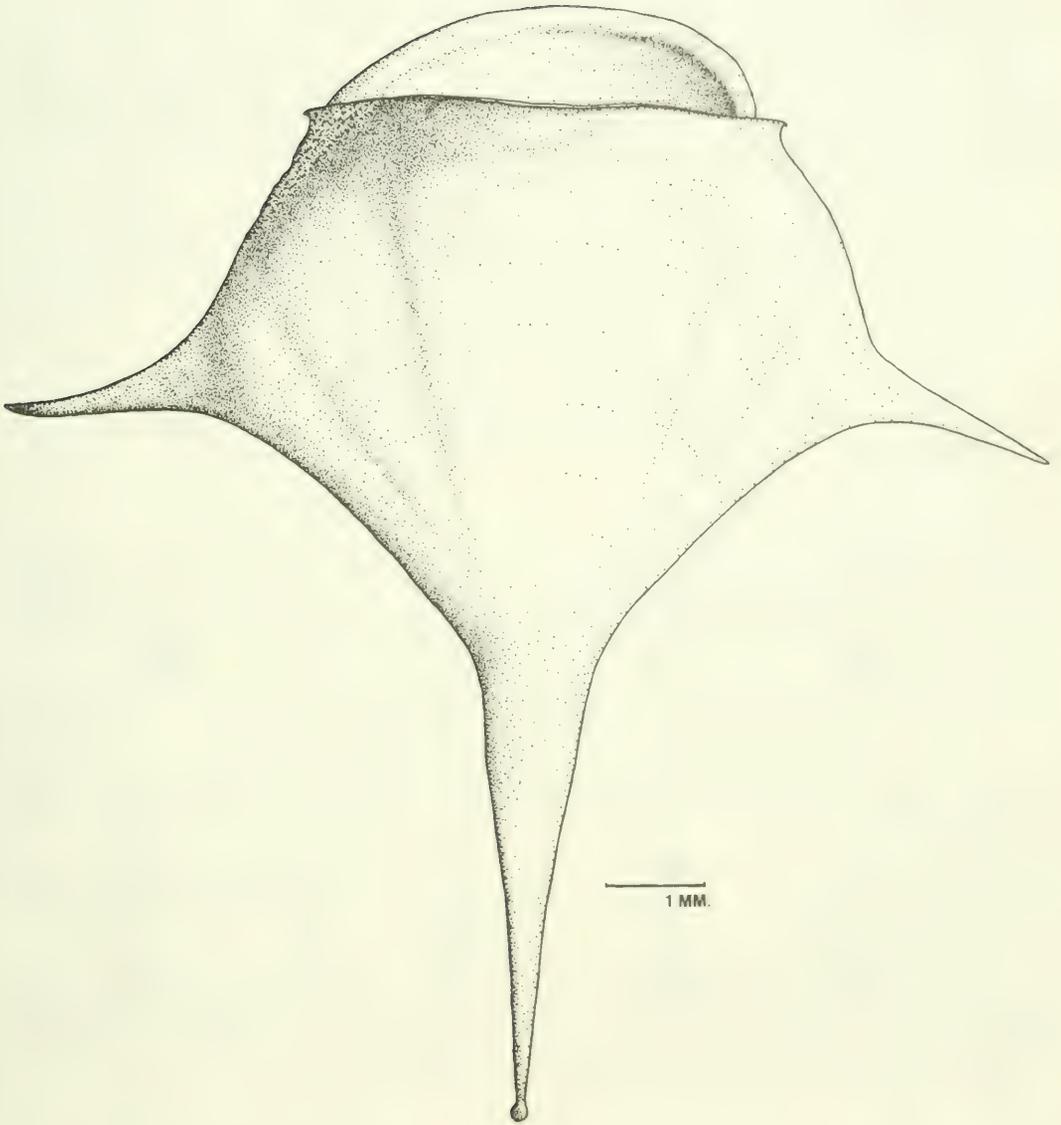


FIG. 4. Holotype of *D. trispinosa* f. *atlantica* nov. forma.

Hyalaea trispinosa. Quoy & Gaimard, 1832: 378, (1833) pl. 27 fig. 17-19; (in part) Souleyet, 1852a: 45; (in part) Souleyet, 1852b: 161.

Diacria trispinosa (in part). Gray, 1847: 203; (in part) Vayssière, 1915: 58, pl. 1 fig. 11; Tesch, 1907: 195.

Diacria depressa ? Gray, 1850: 11.

Diacria trispinosa var. *minor* (in part) Boas, 1886: 95, 210, pl. 1 fig. 3, pl. 2 fig. 14.

Description. Shell large, up to 9 mm teleoconch length. Lateral spines rather straight. Caudal spine proportionally short, shell aperture wide. The holotype is darkly coloured at the anterior part of the teleoconch; the posterior part is light brown and the spines are white. The position of the membrane is low in the caudal spine, therefore the teleoconch is long and the caudal spine short. Measurements of the holotype are given in Table 2.

TABLE 2. Measurements (in mm) of the holotype of *D. trispinosa* f. *atlantica*.

A	0.85	J	0.27
B	3.29	K	0.21
D	7.94	L	6.92
E	10.52	M	6.15
G	0.93	O	4.22
I	3.02	L/M	1.12

Shells of the northern populations of this taxon are darker and the teleoconch is completely brown with exception of the spines. In the southwestern populations only the ribs of the shell and the aperture rim are coloured. Intermediates between these colour patterns are abundant. The length of the teleoconch of this forma varies also with latitude.

Diacria trispinosa (ms Lesueur) (De Blainville, 1821)
forma *trispinosa* (ms Lesueur) (De Blainville, 1821)

Hyalaea trispinosa (ms Lesueur) (in part) De Blainville, 1821a: 82; De Blainville 1821b: 97; d'Orbigny, 1836: 106; (in part) Souleyet, 1852a: 45, pl. 3 fig. 1-7; (in part) Souleyet, 1852b: 161, pl. 6 fig. 1-6.

Hyalaea mucronata (non d'Orbigny, 1836). Quoy & Gaimard, 1827: 231, pl. 8b fig. 1-2.

Diacria trispinosa (in part). Gray, 1847: 203; (in part) Gray, 1850: 10; Chenu, 1859: 109, fig. 4, 65-466.

Diacria trispinosa var. *minor* (in part) Boas, 1886: 95, 210.

Description. Teleoconch length about 6 mm. The teleoconch length does not show variation with latitude. The colour pattern of *D. trispinosa* f. *trispinosa* is restricted to the ribs of the shell and the aperture rims, but sometimes adult specimens get a brown spot on the teleoconch posterior to the lateral spines, on the ventral side. The aperture is smaller and the posterior spine proportionally longer than in *D. trispinosa* f. *atlantica*.

REMARKS ON VARIATION

The measurements A, D, E, G, L and M (see Fig. 1) are used to study teleoconch variation. In the histograms (Fig. 5) the size of the different taxa may be compared. The ratio L/M (= distance from lateral spine to dorsal aperture rim/distance from lateral spine to membrane) is an indicator for the position and curving of the lateral spines. For *D. rampali* and *D. trispinosa* f. *trispinosa* from the Atlantic Ocean this ratio differs slightly. Most Atlantic and all Philippine specimens of *D. rampali* have a lower L/M ratio; consequently this means a higher position of lateral spines compared to *D. trispinosa* f. *trispinosa*. Atlantic specimens of *D. rampali* have

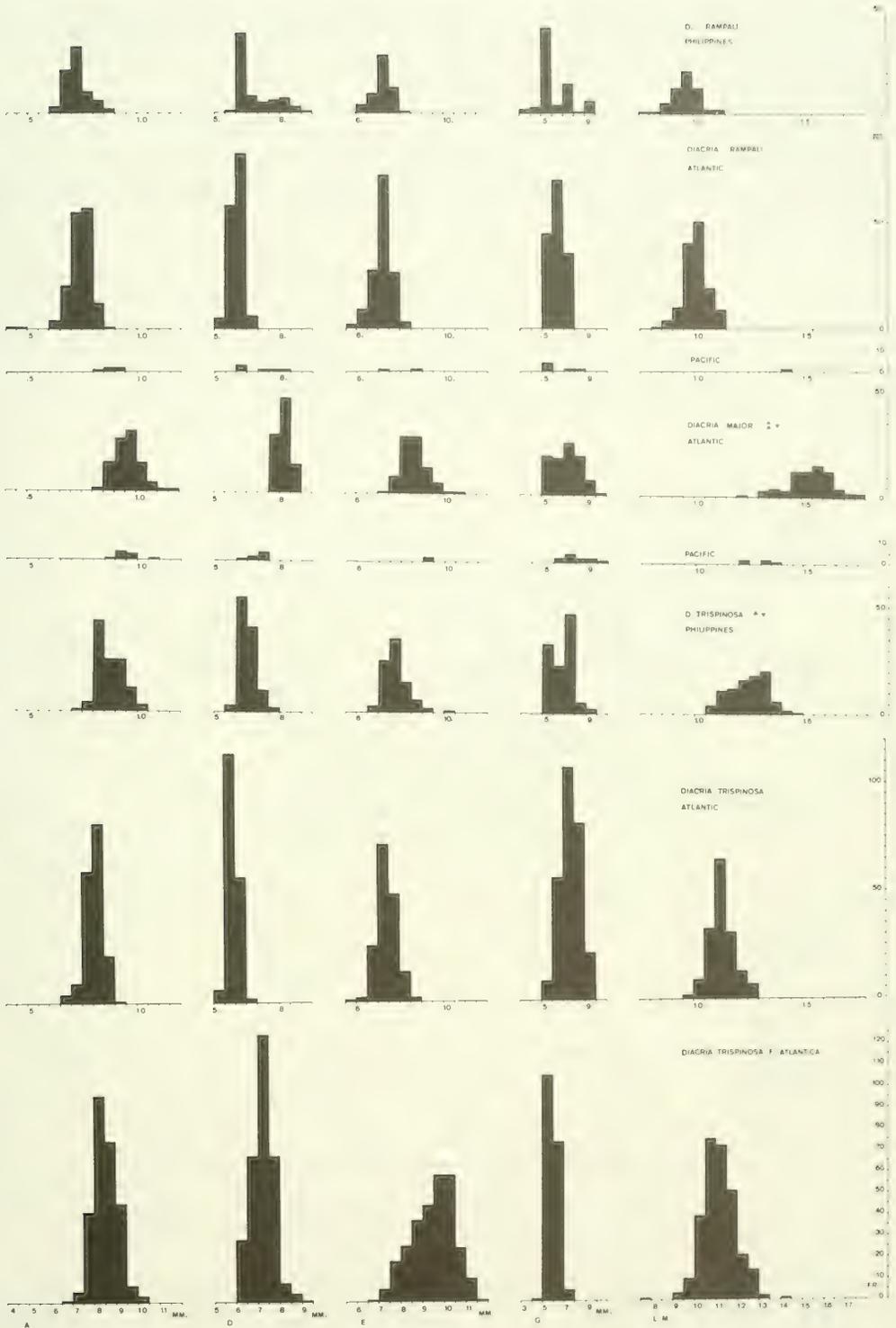


FIG. 5. Histograms of parameters of shell shape and size of different taxa in different oceans.

almost the same L/M ratio as *D. trispinosa* f. *atlantica*, but the latter is much bigger, as is shown by the teleoconch length (D) and the maximal width (E). The length of the teleoconch in Atlantic specimens of *D. trispinosa* f. *trispinosa* is slightly less than that in *D. rampali*; in the Philippine area the difference is just the reverse.

The length of the teleoconch of *D. trispinosa* f. *atlantica* becomes larger at higher latitudes. In Fig. 6 mean and standard deviation of data are plotted against latitude. According to Van der Spoel (1970a) this variation is due to adaptation to colder and less saline waters to enlarge the floating capacity. Only the mean of the samples from the Sargasso Sea, North of the Azores (arrows), does not fit. This is probably due to isolation of these water masses. In Fig. 6 the teleoconch length variation of *D. major* sampled in the Atlantic Ocean is also plotted, which shows a comparable phenomenon.

Discriminant analyses have been made for 4 groups, based on colour pattern: the taxa *D. rampali*, *D. major*, *D. trispinosa* f. *trispinosa*, *D. trispinosa* f. *atlantica*. Shell shape differences of these groups have been studied. The analyses were carried out with 3 parameters of the shell: A = width of the shell at the level of the membrane; L = distance of the lateral spine and the dorsal aperture rim; M = distance between the lateral spine and the membrane. These parameters have been chosen because they discriminate well and their correlation is limited (Table 3).

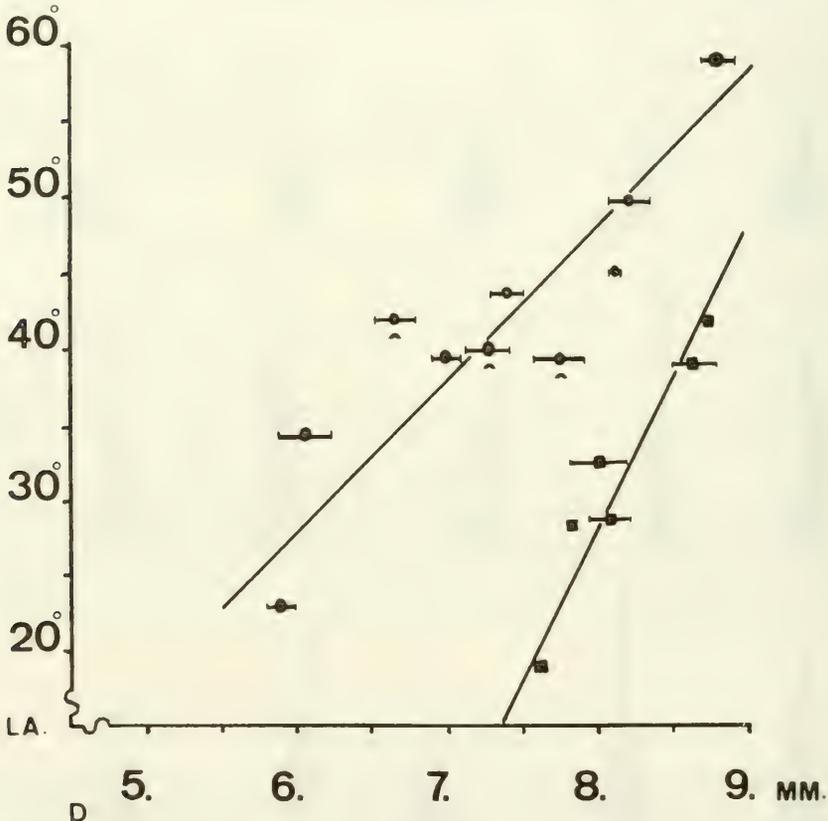


FIG. 6. Mean and standard deviation of samples of *D. trispinosa* f. *atlantica* (black dots) and of *D. major* (black squares), plotted against latitude.

TABLE 3. Correlation matrix of the parameters A, L and M in the taxa *D. rampali*, *D. major*, *D. trispinosa* f. *trispinosa*, *D. trispinosa* f. *atlantica*.

	<i>D. rampali</i>		<i>D. major</i>		<i>D. trispinosa</i> f. <i>trispinosa</i>		<i>D. trispinosa</i> f. <i>atlantica</i>	
	L	M	L	M	L	M	L	M
A	.19	.01	.37	.47	.01	.03	.24	.16
L	x	.48	x	.55	x	.44	x	.61

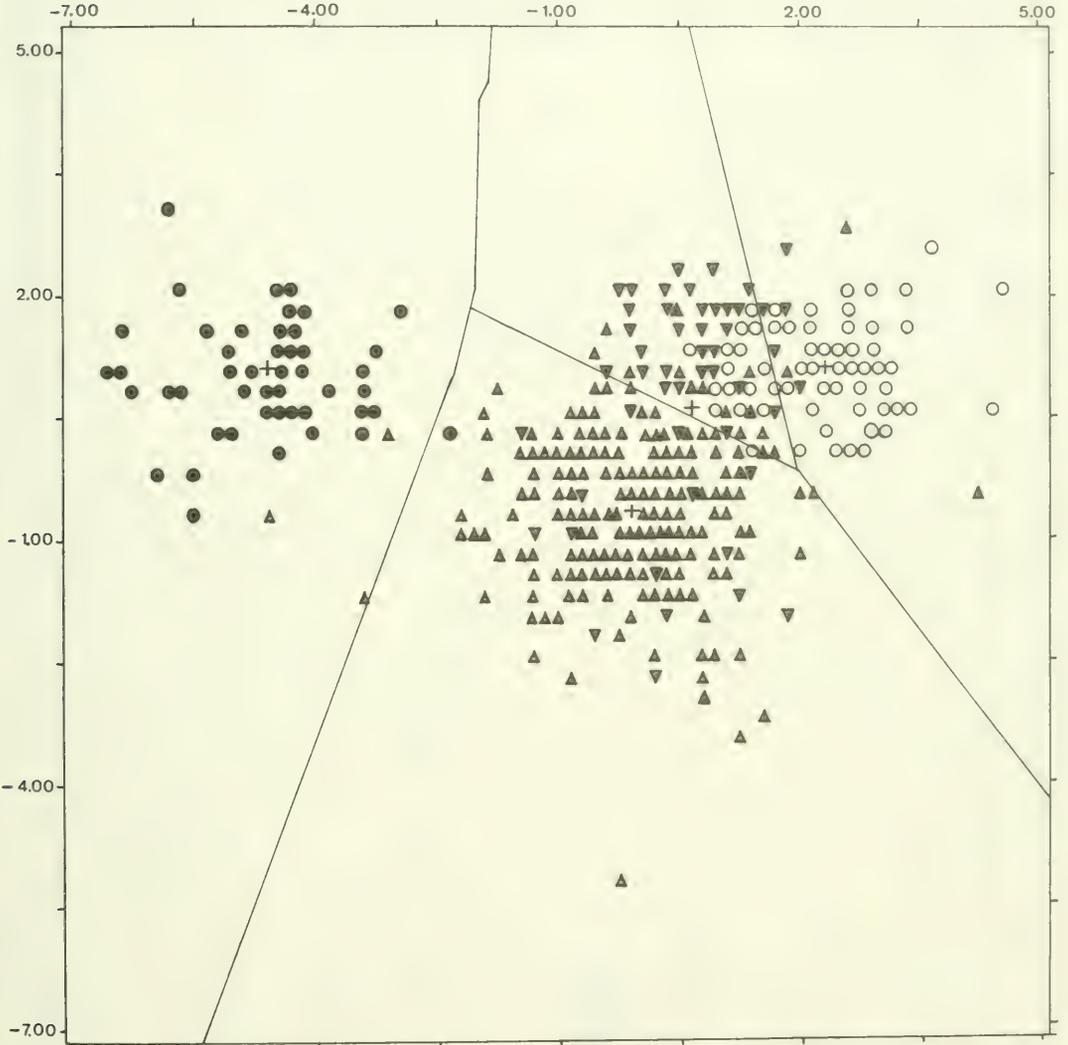


FIG. 7. 'Plot of single cases' of discriminant analysis for 4 groups: *D. rampali* (circles), *D. major* (black dots), *D. trispinosa* f. *trispinosa* (triangles downward), *D. trispinosa* f. *atlantica* (triangles upward), of which the cases or specimens were sampled in the Atlantic Ocean.

In Fig. 7 a discriminant analysis of specimens sampled from the Atlantic Ocean is given. Fig. 7 shows that *D. major* is easily recognised using the above mentioned parameters. The ranges of variability of *D. rampali* and *D. trispinosa* f. *trispinosa* overlap but the centroids are distinct. With this method it was possible to separate up to 81% of Atlantic specimens of *D. rampali* from *D. trispinosa* f. *trispinosa*. *D. trispinosa* f. *atlantica* and *D. rampali* do not overlap. Separation between *D. trispinosa* f. *trispinosa* and *D. trispinosa* f. *atlantica* is distinct for 89% of the specimens. In relation to these data one has to consider that the colour pattern of *D. rampali* is distinct from that of *D. trispinosa*, and that there are intermediates in colour pattern between *D. trispinosa* f. *trispinosa* and *D. trispinosa* f. *atlantica*. In Fig. 8 discriminant analysis of specimens sampled from the Philippine area is given. It shows that there is no overlapping of *D. rampali* and *D. trispinosa* f. *trispinosa* when parameters A, L and M are used for these specimens. Separation of *D. rampali* and *D. trispinosa* f. *trispinosa* only based on colour pattern gives the same groups as the separation based on shell shape.

Results drawn from the discriminant analyses are identical with the conclusions from the histograms.

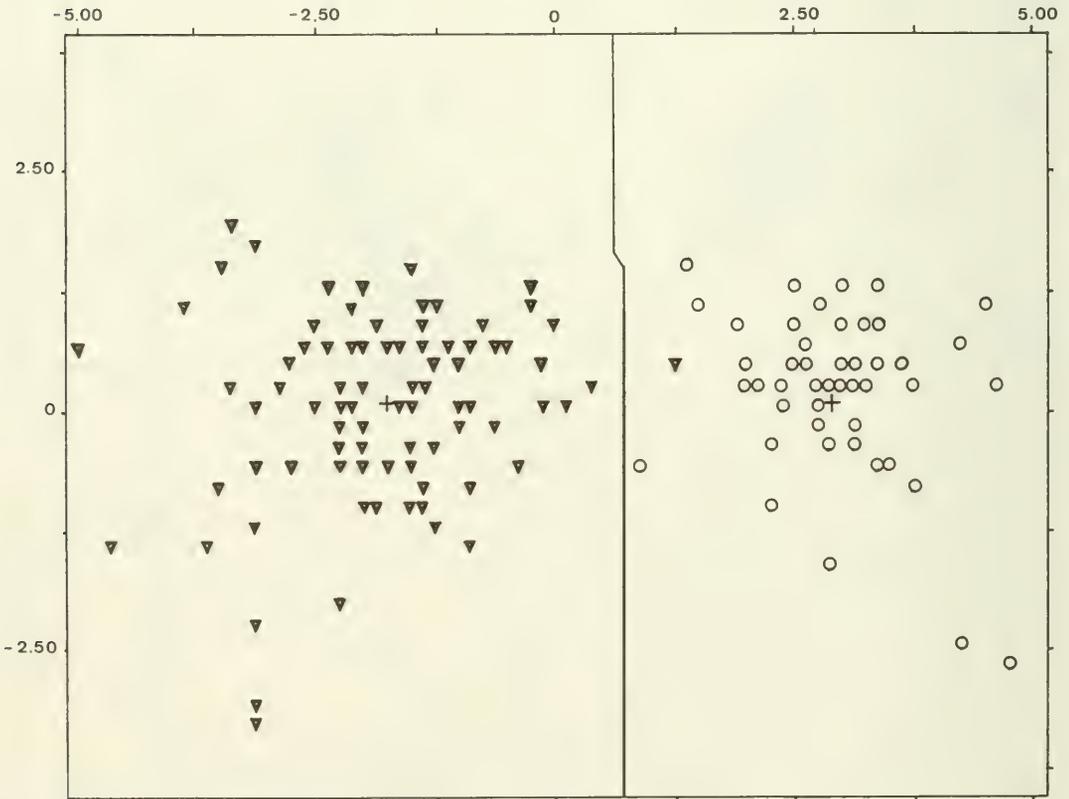


FIG. 8. 'Plot of single cases' of discriminant analysis for 2 groups: *D. rampali* (circles) and *D. trispinosa* f. *trispinosa* (triangles); cases or specimens were sampled in the Philippine area.



FIG. 9. Map of the distribution of *D. trispinosa* f. *trispinosa* (1) and of *D. trispinosa* f. *atlantica* (2). The boundary of the distribution of *D. trispinosa* is given with a single line. The arrow indicates the spot where *D. trispinosa* f. *trispinosa* has only been found in sediment and not as Recent material.

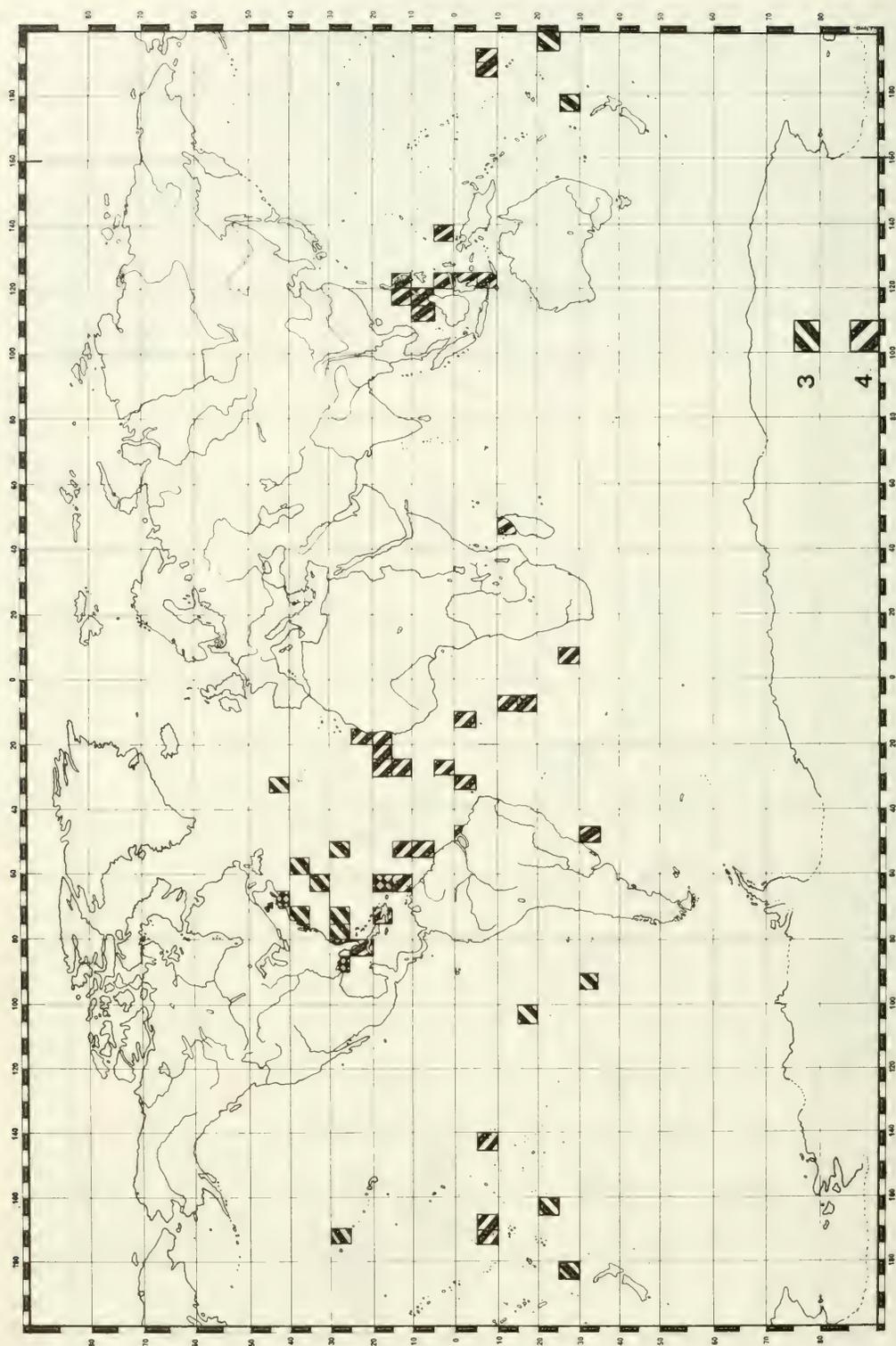


FIG. 10. Map of the distribution of *D. major* (3) and *D. rampali* (4). The arrow indicates the spot where *D. rampali* has only been found in sediment and not as Recent material.

DISTRIBUTION

The localities of the material studied, measured as well as unmeasured specimens, are represented on Figs. 9 and 10. Due to lack of material the North Pacific Ocean has hardly been investigated.

The distribution of *D. trispinosa* f. *atlantica* is restricted to the North Atlantic Ocean, between 60° N and 30-25° N. At the southern border intermediates with *D. trispinosa* f. *trispinosa* occur.

D. trispinosa f. *trispinosa* has been found in all oceans down to 35° S. It occurs up to 35° N in the Atlantic and, according to Van der Spoel (1967), up to 40° N in the Pacific Ocean. In most of the studied areas *D. trispinosa* f. *trispinosa* is sympatric with *D. rampali*.

D. rampali has been collected in the Atlantic Ocean between 30° N and 35° S. In the Pacific it has been sampled between 15° N (Philippine area) and 10° S. It is possible that the distribution of *D. rampali* in the Pacific Ocean is wider than given here.

D. major has been collected in the North Atlantic Ocean up to 30° N and in the Pacific Ocean down to 35° S. Among the material investigated no *D. major* has been found in the South Atlantic nor in the Indian Ocean.

Near the coast off Georgia, in sediment material, *D. trispinosa* f. *trispinosa* and *D. rampali* have been found. On the maps (Figs. 9 and 10) this is indicated by an arrow. Neither *D. rampali* nor *D. trispinosa* f. *trispinosa* have been collected recently in the same areas, though they have been well explored by the Deep Dumpsite Project. So either the 2 taxa are regressing in distribution or the waters near Georgia had a higher temperature in the recent geological past. The last hypothesis contradicts other paleontological and geological evidence. Therefore it is postulated here that *D. rampali* and *D. trispinosa* f. *trispinosa* have had a larger distribution in the Atlantic Ocean.

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VERBREITUNG DER FAMILIE ZONITIDAE

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ABSTRACT

The distribution of the Zonitidae is Holarctic. Of the about 550 species and subspecies roughly 1/3 live in the Nearctic and 2/3 in the West Palaearctic, only 1 species (*Zonitoides nitidus*) being fully Holarctic. Out of the almost 100 genera and subgenera only 2 (*Zonitoides* and *Nesovitrea*) are common to the Nearctic and all of the Palaearctic, while 2 Nearctic ones (*Pristiloma* and *Hawaiiia*) enter the East Palaearctic. In the Nearctic Region the Appalachians are the main centre of differentiation. Only 1 genus, *Pristiloma*, is exclusively western; in the south zonitids reach into Central America. In the West Palaearctic they live mainly in the Mediterranean region sensu lato, i.e., from the Azores to N.E. Iran. The southern limit is the desert belt of the Sahara and the Middle East, with only 1 genus and species crossing it, *Araboxychilus sabaesus* (Oxychilini), which is endemic in the mountains of the S.W. tip of Arabia. The Central Palaearctic is inhabited by only 2 wide-ranging species, which extend to the Far East, there meeting the few species of Nearctic origin. Northwards there is a rapid decrease in the number of species, with only very few widely distributed forms crossing latitude 50° in America and 52° in Europe.

Die Familie Zonitidae wird hier folgendermassen beschränkt und systematisch eingeteilt: Unterfamilie Gastrodontinae; Unterfamilie Zonitinae mit Triben Vitreini, Zonitini und Oxychilini; Unterfamilie Daudebardiinae. Viele Malakologen betrachten jedoch die Daudebardiinae und Schileyko (1972) auch die Gastrodontinae als besondere Familien. Die amerikanischen Autoren (H. B. Baker, Pilsbry) rechnen dagegen auch die Vitrinidae als eine Unterfamilie den Zonitidae zu. Die Euconulidae, die vorher zu den Zonitidae gezählt wurden, werden heute schon allgemein entweder als eine besondere Familie betrachtet oder den Helicarionidae zugerechnet. Als eine abgesonderte Familie nehme ich auch die Trochomorphidae an. Die hawaiische Gattung *Godwinia*, manchmal in eine Unterfamilie Godwiniinae ausgesondert, stelle ich gerade in die Zonitinae-Zonitini.

Die Zonitidae, in meiner Erfassung, sind eine fast ausschliesslich holarktische Gruppe. In der Orientalischen und Australischen Region (samt Ozeanien aber ohne Hawaii-Inseln) werden sie durch verwandte, aber abgesonderte Familien Helicarionidae, Euconulidae, Trochomorphidae, in der Äthiopischen Region durch schalentragende Urocyclidae ersetzt; in der Neotropischen Region fehlen die einheimischen Limacoidea wohl vollständig.

Die Zonitidae umfassen etwa 520 (-550) "gute" rezente Arten und Unterarten, die zu fast 100 Gattungen und Untergattungen gehören; aus dieser Zahl fallen etwa 350 Arten auf die (fast ausschliesslich westliche) Paläarktis und 170 auf die Nearktis samt Mittelamerika und den Hawaii-Inseln. Die Daudebardiinae (ca 30 Arten) und die Oxychilini (ca 160 Arten) leben endemisch in der West-Paläarktis. Die Gastrodontinae sind in der Nearktis zahlreicher und mehr differenziert; in der West-Paläarktis leben nur 4 *Zonitoides*-Arten, sowie die kleine endemische Gattung *Janulus* auf Madeira und den Kanaren. Möglicherweise zu Gastrodontinae gehören auch die Reliktgattungen *Spelaeopatula* (5 Arten in Süd-Jugoslawien und Albanien) und *Gastranodon* (eine Art in Nordost-Iran); ihre Anatomie und systematische Stellung bleiben aber bisher unbekannt. Im Tertiär waren die Gastrodontinae, wie es scheint, in Europa zahlreicher und mehr differenziert als heute. Die Vitreini und die Zonitini sind gleich reichlich, aber durch besondere Gattungen, in der Paläarktis und der Nearktis repräsentiert, wobei die Vitreini in der Nearktis und die Zonitini in der Paläarktis mehr differenziert sind.

Es gibt nur eine panholarktische Art der Zonitidae, *Zonitoides nitidus*, und nur 2 Gattungen sind für die ganze Paläarktis und die Nearktis gemeinsam, *Zonitoides* und *Nesovitrea* (N.B.: die

nearktische *Glyphyalinia* betrachte ich als besondere Gattung und nicht als Untergattung der paläarktischen *Retinella*). Überdies reichen 2 nearktische Gattungen, *Hawaiiia* und *Pristiloma*, in die östlichste Paläarktis (siehe unten).

VERBREITUNG IN DER NEARKTIS

Am häufigsten treten die Zonitidae im Osten der Vereinigten Staaten auf, ihr Zentrum der Differenzierung liegt im Gebiet der Appalachen (sensu lato). Hauptsächlich hier leben die artenreichen Gattungen *Mesomphix*, *Paravitrea*, *Glyphyalinia*, *Zonitoides* (samt *Ventridens*); zahlreiche Untergattungen sowie die Gattungen *Gastrodonta* und *Vitrinizonites* sind für das Gebiet endemisch. Im Westen der USA, der pazifischen Küste entlang, kommen nur die grosse Gattung *Pristiloma*, die zweite endemische Gattung *Ogaridiscus* mit 1-2 Arten, und wenige in Amerika weit verbreitete Arten vor.

Nach Norden (Kanada) nimmt die Anzahl der Zonitidae plötzlich ab, die endemischen Arten fehlen fast vollkommen (Ausnahme: manche *Pristiloma*-Arten im Nordwesten Nordamerikas). Nach Süden (Mexico) sinkt die Artenzahl langsamer und hier kommen endemische Arten oder sogar höhere Taxa vor: *Pycnogyra*, Untergattungen *Omphalinella*, *Patulopsis*, *Zonyalina* und *Moreletia* der Gattung *Mesomphix*. Die Zonitidae dringen im Süden in die Sonorische Übergangsregion: über Mexico nach Mittelamerika (Guatemala, Kostarika, Panama) vor.

Ausser dem nordamerikanischen Kontinent bewohnen die nearktischen Zonitidae die Bermuda- (endemische Gattung *Poecilozonites* der Gastrodontinae) und die Hawaii-Inseln (endemische Gattung *Godwinia*, einige endemische *Nesovitrea*- und *Striatura*-Arten, *Hawaiiia minuscula*). Die am weitesten verbreitete nearktische Art ist *Hawaiiia minuscula*, die jedoch auf manche Inseln des Pazifiks und des Karibischen Meeres sowie nach Südamerika sicher durch Menschen eingeschleppt worden ist.

Der Einfluss der nearktischen Fauna macht sich am östlichsten Rand Asiens geltend. Über Alaska und den Aläuten dringt dort die Gattung *Pristiloma* vor und wird auf Hokkaido, Sachalin, den Kurilen und im Süden Kamtschatkas durch eine endemische Art, *P. japonicum*, vertreten. Die einzige in der Ost-Paläarktis endemisch lebende, monotypische Gattung *Coreovitrea* (aus Nord-Korea) steht der nearktischen Gattung *Pristiloma* näher als irgendwelchen westpaläarktischen Zonitiden. Bis nach Primorskij Kraj und Korea reicht *Hawaiiia minuscula* (im natürlichen Bereich, nicht vom Menschen eingeschleppt!), bekannt auch aus dem Pleistozän Japans. Im Neogen musste die amerikanische Gattung *Hawaiiia* in paläarktischem Asien sehr weit verbreitet sein und von dort bis nach Südost-Europa reichen. Als Spuren ihres einstigen Areals gelten: die relikttä, endemische *Hawaiiia afghana* in Afghanistan und ein miozäner Fund der *Hawaiiia antiqua* im Kaukasus.

VERBREITUNG IN DER PALÄARKTIS

In der Paläarktis ist das Areal der einheimischen Zonitidae im Grunde auf den westlichen Teil der Region, hauptsächlich auf Europa beschränkt. Die Grenze dieses Areals markiert der Bereich der zahlreichsten Gattung, *Oxychilus* (ungefähr 150 Arten in über 20 meist eng verbreiteten Untergattungen), aus. Die Zonitidae bewohnen ganz Europa, nach Norden aber nimmt ihre Anzahl rasch, obwohl allmählich, ab. In Skandinavien kommen nur 9-10 Arten vor und von ihnen dringen nur 4-5 nach Island vor; dies sind in der Regel weit oder sehr weit verbreitete Arten. In Ost-Europa reichen die Zonitidae bis nach Estland, Lettland, Litauen, Weissrussland und der Ukraine und nur ganz wenige nach West-Russland. Östlich der Wolga, in den ausgedehnten Gebieten Sibiriens, der Mongolei und der zentralasiatischen Sowjetrepubliken treten nur die 2 am weitesten verbreiteten Arten auf: der holarktische *Zonitoides nitidus* und die paläarktische *Nesovitrea hammonis*, die bis nach Korea und Sachalin reicht. Erst am östlichen Rand Asiens erscheinen einige Arten nearktischer Herkunft. An den südöstlichen Rändern des Areals der Familie treffen die dort schon nicht mehr zahlreichen Zonitidae die Vertreter der verwandten, für die Orientalische Region charakteristischen Familien Helicarionidae und Euconulidae und kommen zusammen mit ihnen vor (z.B. in Afghanistan, Korea, auf Hokkaido).

Nach Westen reichen die Zonitidae bis auf den Azoren, Madeira und den Kanaren, wo sie recht reichlich und durch viele endemische Formen und Gruppen vertreten sind; z.B. auf den Azoren 15 Arten, darunter endemisch 9 Arten und 3 Untergattungen, auf Madeira und den Kanaren die endemische Gattung *Janulus*, auf den Kanaren die endemische Untergattung *Lyrodiscus*.

Die südliche Arealgrenze der Zonitidae in der West-Paläarktis ist sehr scharf, im Gegensatz zu der nördlichen und östlichen Grenze. Sie wird durch die Wüsten Afrikas und Vorderasiens oder durch die an ihnen vom Norden grenzenden extrem trockenen Gebirgen, z.B. Hoher und Saharischer Atlas, ausgemarkt. Die Zonitidae erreichen diese Grenze in einer merkbaren Anzahl hauptsächlich endemischer und stark differenzierter Arten. Die Artenzahl nimmt nach Süden nicht allmählich ab, sondern das Areal hört plötzlich auf. Den Rand des südlichen Bereiches bilden: West- und Nord-Marokko, Nord-Algerien samt Tell-Atlas, Nordwest-Tunesien, Kyrenaika in Libyen, Israel, West-Jordanien, Nordwest-Syrien, Süd-Türkei, irakischer Kurdistan und Nord-Iran.

Hauptgebiet des Vorkommens der paläarktischen Zonitidae sind die weit gefassten Mittelmeerländer, die sich den Breitenkreisen entlang von den Azoren an bis dem Chorassan und Kopet-dag-Gebirge an der Grenze zwischen Iran und der Turkmenischen SSR ziehen. In diesem breiten Gürtel zwischen dem 32° und 45° geographischer Breite befinden sich einige deutliche Entwicklungszentren der Zonitidae, die sich mit ihrer spezifischen Fauna aussondern. Unter anderen: das kabyllische Zentrum (in Nord-Algerien) mit endemischen Gruppen *Pseudopolita* und *Allogenes* und den verwandten *Oxychilus*-Formen; das dinarische (westbalkanische) Zentrum—hier leben die meisten Arten der Gattung *Aegopis*, *Paraegopis* s.s., die endemischen unterirdischen Gattungen *Spelaeopatula*, *Gyralina* und *Meledella*; das ägäische Zentrum, mit *Zonites* (mehrere Arten), *Eopolita* und *Lindbergia* s.s., Untergattungen *Hiramia*, *Helicophana* (ein einziger enger Endemit am östlichen Rand Kretas) und *Calloretinella* (eine endemische Art auf Zypern) der Gattung *Oxychilus*; das westkaukasische Zentrum, endemische Gattungen *Vitrinoxichilus* und *Discoxichilus* sowie Untergattungen *Conulopolita*, *Forcartiella*, *Retowskiella* und *Pontoxichilus* (der Gattung *Oxychilus*), Entwicklungszentrum weiter verbreiteter Untergattungen *Schistophallus* und *Longiphallus*.

Die nördlichsten Entwicklungszentren der Zonitidae bilden der West-Kaukasus, die Karpaten und die Alpen. Dabei sind die Zonitidae in den Karpaten und Alpen weit mehr in den südlichen als in nördlichen Teilen dieser Gebirgsmassiven differenziert. Besonders charakteristisch für die Karpaten sind: starke Differenzierung der Daubebardiinae und ihre endemische Gruppe *Cibinia*; Untergattung *Riedelius* und endemische, monotypische Untergattung *Cellariopsis* der Gattung *Oxychilus*; monotypische, endemische unterirdische Gattung *Troglovitrea*. In den Süd-Alpen liegt das Zentrum der Differenzierung der Gattung *Aegopinella*, ebendort und in benachbartem Toskanien das Entwicklungszentrum von *Oxychilus* s.s. und möglicherweise auch *Retinella* s.s.

In der Alten Welt ist nur eine Gattung und Art der Zonitidae bekannt, die ausserhalb der Grenzen der Paläarktis, in der Äthiopischen Region vorkommt. Dies ist *Araboxychilus sabaeus* der Tribus Oxychilini, der das Gebirge des südwestlichen Randes Arabiens endemisch bewohnt und durch eine weite Lücke vom Hauptareal der Familie getrennt wird. Analogisch zu der Verbreitung der Familie Vitrinidae zu urteilen, wäre die Entdeckung der Vertreter der Zonitidae auch in den Gebirgen Äthiopiens zu erwarten. *Araboxychilus sabaeus* in Arabien wie auch die Vitrinidae ebenda und in den Gebirgen Äthiopiens, sind Relikte aus den pluvialen Perioden des Spätneogens oder Pleistozäns, aus der Zeit als der Wüstengürtel, der heute keinen Kontakt und Austausch der mesophilen Malakofauna des mediterranen Raumes mit der Fauna der Äthiopischen Region erlaubt, noch nicht existierte.

In der vorliegenden Besprechung wurden die mehreren Fälle der Verschleppung einzelner Arten durch den Menschen und ihres Vorkommens ausserhalb des natürlichen Areals vollständig ausgelassen.

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SPECIES CONCEPT OF PROSOBRANCH FRESHWATER MOLLUSCS IN WESTERN EUROPE, I

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ABSTRACT

The species concept of prosobranch freshwater snails inhabiting caves and springs is discussed. There are basically 2 schools of thought, viz. authors who subscribe to a very narrow species concept (all geographically isolated taxa are species: high rate of endemism) and those who recognize fewer species with more extensive distribution. The isolation of many populations appears to be less strict than generally surmised. Waters may be connected underground and aerial dispersal is certainly possible. There are also transitions between spring and cave snails. This may be caused by underground water connections and is evidenced by reduction of eye pigment. There are 2 possibilities, viz. snail species of which the different populations each have a different type of eye pigmentation, and snail species of which individuals with and without eye pigment occur together in the various populations. Species of *Bythinella*, *Microna*, *Litthabittella* and *Horatia* are cited as examples of these phenomena.

With respect to the different taxa the evaluation of the specialists in many cases differs considerably. Therefore the confusion of other people coming in contact with this field must be even greater. For this reason, the species concept and criteria for distinguishing species used in this publication are to be clarified.

At present the situation is that according to one concept a certain number of species having a more or less wide distribution must be recognized. According to another concept, however, some of these species must be split into several species having much more limited distributions (endemism). For example, Binder (1957: 59) and in addition obviously Ant (1962: 71) combine the 10 *Horatia* species described by Bourguignat to one single species, whereas Schütt (1961: 69) combines them to 8 species, some of which are said to have separate distribution areas (cf. *klecakiana*, *praeclara* and *servaini* according to Schütt). Boeters (1970: 113) discusses a distribution of *Microna saxatilis* from central Portugal to Bosnia-Herzegovina. Radoman (1975: 29) reviews the genus for the Balkans; however, he does not mention *M. saxatilis* (Radoman has received from me alcohol material of this form from France). On the contrary, he elevates 4 subspecies to species rank (*M. croatica*, *M. fontinalis*, *M. kuesteri* and *M. lacheineri*, some of which have separate distribution areas, cf. Radoman, 1975: fig. 11).

BIOLOGICAL SPECIES CONCEPT

Although both concepts mentioned above lead to different results, it may be assumed that the authors who represent them, start from a common theoretical basis. This is, for example, obvious for Schütt (1961: 69) and his opponent Ant (1962: 71), since Schütt refers to Mayr (1942) from whose relevant theoretical basic concepts Ant also obviously starts.

Mayr (1975: 31) attributes the following 3 features to a species: (a) the individuals of a species form a reproductive community; (b) in addition, the species is an ecological unit; (c) finally, the species is a genetic unit of an intercommunicating gene pool. The species definition is as follows: species are groups of natural populations which cross with each other and which are, with respect to their reproduction, isolated from other such groups.

APPLICATION OF THE BIOLOGICAL SPECIES CONCEPT

An analysis (Tables 1-2) of the arbitrarily selected publication of Jaeckel, Klemm & Meise (1958) shows that endemism appears substantially in so-called spring snails and cave snails (crenobionts and troglobionts). This is the classical concept of obvious absence of any possibility for gene exchange between comparable neighbouring populations; these authors feel that their species concept is confirmed by morphological differences.

Therefore these 2 groups, spring snails and cave snails, and the variability of aquatic prosobranch molluscs will be considered in more detail below.

THE SPECIES CONCEPT OF SPRING SNAILS

In the present context snails whose populations are normally restricted to springs are designated spring snails. The reason for this restriction to springs consists in the fact that these snails reproduce themselves only in water of a relatively constant temperature (for *Bythinella* see Jungbluth, 1973: 1584, 1585; for *Microna* see Boeters, 1970: 120).

TABLE 1. Genera having up to 50% endemism (except Lake Ochrid and Lake Prespa; analysis of data of Jaeckel, Klemm & Meise, 1958). Genera in this table having no spring and cave species: 88%; genera having both spring and cave snails are only *Pseudamnicola* and *Sadleriana*.

Genus	Total number of species	'Endemic' species	
		number	%
<i>Cochlostoma</i>	20	7	35
<i>Pomatias</i>	2	0	0
<i>Acicula</i>	12	5	42
<i>Pupula</i>	1	0	0
<i>Renea</i>	2	1	50
<i>Viviparus</i>	4	1	25
<i>Valvata</i>	5	0	0
<i>Hydrobia</i>	6	0	0
<i>Marstoniopsis</i>	1	0	0
<i>Lithoglyphus</i>	5	2	40
<i>Emmericia</i>	1	0	0
<i>Truncatella</i>	1	0	0
<i>Bulimus</i>	4	1	25
<i>Pyrgula</i>	1	0	0
<i>Micromelania</i>	1	0	0
<i>Assimineae</i>	2	0	0
<i>Amphimelania</i>	1	0	0
<i>Fagotia</i>	2	0	0
<i>Pseudamnicola</i>	8	2	25
<i>Sadleriana</i>	3	1	33

TABLE 2. Genera having more than 50% endemism (except Lake Ochrid and Lake Prespa; analysis of data of Jaeckel, Klemm & Meise, 1958). Genera in this table having spring and cave snails: 91%.

Genus	Total number of species	'Endemic' species	
		number	%
<i>Paladilhia</i>	16	16	100
<i>Lartetia</i>	1	1	100
<i>Costellina</i>	1	1	100
<i>Plagigeyeria</i>	4	4	100
<i>Bythinella</i>	13	7	54
<i>Horatia</i>	3	3	100
<i>Hadziella</i>	1	1	100
<i>Microsalpinx</i>	1	1	100
<i>Lanzaja</i>	3	3	100
<i>Baglivia</i>	1	1	100
<i>Hydrocena</i>	1	1	100

If, exceptionally, a constant temperature is guaranteed to occur in waters other than springs, these snails are able to spread in these waters over large distances. For example, *Bythinella*, *Microna* and *Litthabitella* are known from subterranean waters (for *Bythinella* see Vire, 1902: 606, Locard, 1902: 608, Boettger, 1939: 19, and Boeters, 1968: 762, 764; for *Microna* see A. J. Wagner, 1914: 48, Kuscser, 1928: 50, and H. Wagner, 1935: 35; for *Litthabitella* see Bole, 1971: 15); in addition, *Bythinella* is known from the bottom of lakes (Brehm, 1909: 741, Mahler & Sperling, 1955: 3, Hadl, 1967: 167). The actual presence in lakes underlines Diluvial records according to which *Bythinella* was at that time accompanied by typical lake forms of the actual mollusc fauna (Ehrmann, 1914: 133).

During Quaternary climate changes the snails actively withdrew into the springs with increasing temperature (Jungbluth, 1971: 229). This led to splitting into separate populations; this would mean the beginning of a process of speciation if passive distribution could be ignored or excluded (Giusti & Pezzoli, 1977: 131).

POSSIBLE AERIAL DISPERSAL

For a long time aerial dispersal of small bivalves by insects has been assumed since these bivalves are able to occupy very small and isolated waters without any inlet or outlet. This has been supported by a number of observations (cf. Kuiper, 1976: 496). Although corresponding observations have been made for prosobranch land snails, the possibility of aerial dispersal of prosobranch spring snails has not been taken into account until now (for *Renea* cf. Rees, 1965: 271; for *Pomatias* cf. Rees, 1965: 271, and Boeters: in September 1975 a grasshopper, Orthoptera, was observed for about 15 minutes when transporting on one of its legs a live specimen of *Pomatias elegans* in France, dépt. Gard, Monteil at Orgnac-l'Aven). From the aerial dispersal of prosobranch land snails it is evident that (a) prosobranch molluscs are in principle able to close their shells so quickly that they may grab insect legs and, in addition that (b) they are able to hold on long enough to be transported. Therefore, it is possible to assume analogous conditions for both prosobranch spring snails as well as prosobranch land snails and small bivalves. This assumption is underlined by repeated records of *Bythinella* in wells (Blanchet, 1911: 356, Boeters, 1968: 762, Stock, 1961: 78).

For aerial dispersal of prosobranch spring snails the following insects may be taken into consideration. Lengerken (1924: 14), for example, mentions several beetles as inhabitants of springs (Dytiscidae, Hydrophilidae, Haliplidae) and Engelhardt (1955: 17) reports on various species of water beetles and water bugs (Hydrocorisae) which stop for short or longer periods in springs during overland flights in autumn.

To sum up, for the reasons given above the species characteristic of a communicating gene pool may be maintained over large distances even today.

TRANSITIONS BETWEEN SPRING AND CAVE SNAILS

It is not possible to distinguish sharply between spring snails and cave snails in every case; transitions do exist. However, regular records of cave snails in springs do not give evidence for such transitions because the animals may be transported by the water to the springs. Transitions exist (I) if species, which in certain places are restricted only to springs, have penetrated far into the subterranean area (cf. section on spring snails) or (II) if species (as evidenced below) show a reduction of their eye pigment. It is possible to distinguish 2 possibilities:

(1) Populations of one and the same species appear to have different eye pigmentation (4 examples):

- (a) *Bythinella cylindracea* in the département Aube (France) has normal eye pigmentation in springs and reduced eye pigmentation in interstitial waters (samples BOE 139 and 141 plus 146, respectively);
- (b) *Microna saxatilis* with eye pigmentation may be collected regularly in springs and, exceptionally, without any eye pigmentation (BOE 362, Fontaine in Arneguy, dépt. Basses-Pyrénées);
- (c) according to Bole (1971: 89) subterranean populations of *Litthabitella* are 'blind' in contrast to surface populations (presumably blind animals are merely albinos, cf. e.g. Richards, 1973: 49);

(d) *Horatia minuta* may be collected in neighbouring secondary springs of the Source-de-l'Ain with or without eye pigmentation (BOE 75 and 175).

(2) Different eye pigmentation appears in one and the same population, e.g. *Horatia minuta* in the subterranean waters of the Sour (dépt. Ariège, BOE 769) may be either with or without any eye pigmentation.

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NEW MALACOLOGICAL RECORDS FOR THE PROVINCE OF LEÓN (N.W. SPAIN) AND PERCENTAGES OF INFESTATION BY TREMATODA

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The province of León (Fig. 1) is situated in the northwestern part of Spain. According to the work "Mapas provinciales de suelos de León" (Provincial soil maps of León) carried out by the National Institute for Agrarian Research (Madrid, 1973), León is considered to be divided into 5 natural regions: Mountain, Transition, Central, El Bierzo, and La Cabrera. Roughly speaking, the first 3 correspond to the upper, mid and lower valleys of the rivers of the Douro basin respectively. The material discussed was collected in the period 1972-1975 at ca. 350 localities; the species forming the subject of this study were found generally in a few places only. A total of 761 molluscs was examined for parasites.

The following species of Helicidae (Gastropoda, Pulmonata), identified with the assistance of the Rijksmuseum van Natuurlijke Historie (Leiden, Holland), were recorded for the first time in the province.

Subfamily Helicellinae

Candidula intersecta (Poiret, 1801) (Fig. 2), found in 3 different parts of the Mountain-Transition border, in the Central region and in La Cabrera at heights of 819-978 m, the soil being mainly sandy and the vegetation of the Chenopodio-Scleranthea division; 4.2% of the 24 specimens studied had trematodes.

Candidula rocandioi (Ortiz de Zárate, 1950) (Fig. 3) was found in 16 localities within the Mountain region and on the Mountain-Transition border, 947-1199 m, mainly on sandy soils and in vegetation types principally of the Chenopodio-Scleranthea division. None of the 65 specimens studied had parasitic worms.

Helicella ordunensis (Kobelt, 1882) (Fig. 4) was found in 65 localities in Mountain, Mountain-Transition border and Central areas, at heights of 922-1260 m, mainly on alluvial terraces and sandy soils, the dominant vegetation type being the Chenopodio-Scleranthea division. Of the 316 specimens studied, 4.1% had trematodes in the kidney and 2.5% had trematodes in the hepatopancreas.

Helicella cf. madritensis (Rambur, 1868) (Fig. 5) was found in 15 localities in the Transition and Central regions at 736-909 m, mainly on alluvial terraces and clayey soils in the Chenopodio-Scleranthea division. Of 85 studied 2.3% had trematodes in the hepatopancreas.

Subfamily Monachinae

Monacha (Ashfordia) granulata (Alder, 1830) (Fig. 6) was found in 4 localities in the Mountain and Transition regions, at 857-1233 m, mainly on moist open soils, the dominant plant being *Phragmites communis*. Of 34 studied 2.9% had trematodes in the kidney.

Subfamily Hygromiinae

Hygromia inchoata (Morelet, 1845) (Fig. 7) was found in 6 localities in the Transition, Transition-Central border, Central, El Bierzo and La Cabrera areas, in the last named at heights of 380-877 m, chiefly on alluvial terraces and silty soils with an almost pure vegetation of the Chenopodio-Scleranthea division. Of 91 studied 38.5% had trematodes in the kidney and 1.1% in the hepatopancreas.

Hygromia (Pyrenaearia) cantabrica cantabrica (Hidalgo, 1873) (Fig. 8) was found in 3 localities within the Mountain region and on the Mountain-Transition border at 1019-1182 m, mainly on sandy soils with vegetation of the Festuco-Bromea division. The specimens studied were free of helminths.

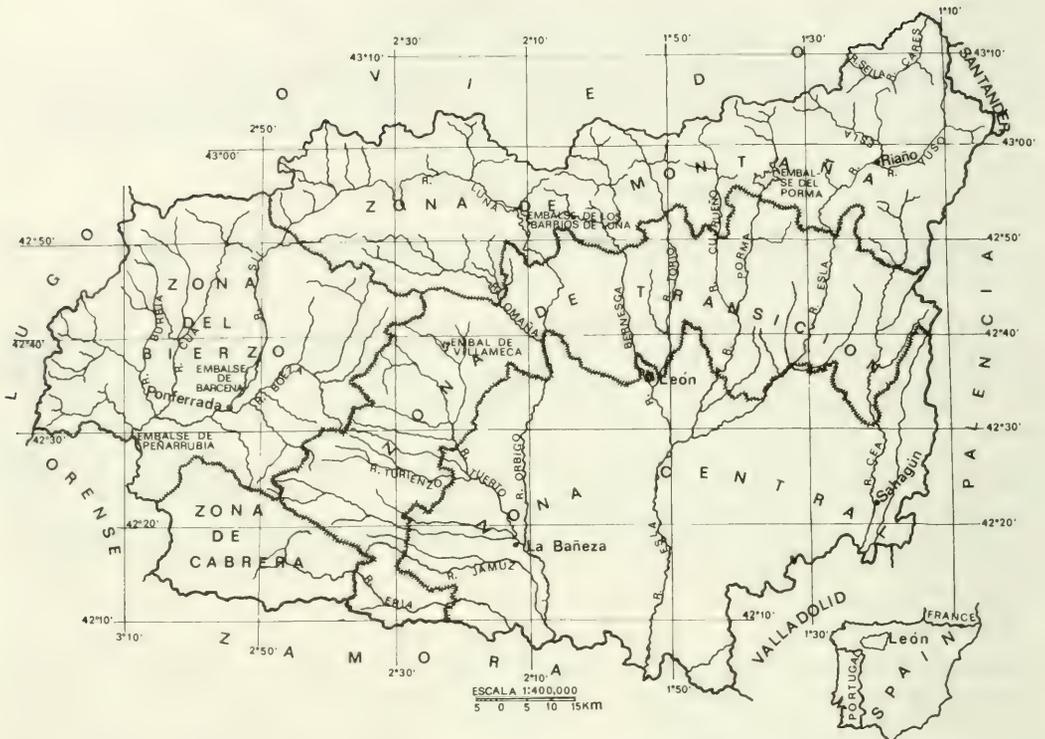


FIG. 1. Natural regions of the province of León, Spain; the following 5 regions may be distinguished—(1) Mountain region (Zona de Montaña), (2) Transitional region (Zona de Transición), (3) Central region (Zona Central), (4) El Bierzo (Zona de El Bierzo), (5) La Cabrera (Zona de Cabrera). Please note that longitudes refer to the meridian of Madrid and not Greenwich.

Hygromia (Pyrenaearia) cantabrica covadonga (Ortiz de Zárate, 1956) (Fig. 9) was found in 1 locality in the Mountain region (Cantabrian side) at 770 m on silty soils. None of the 3 specimens studied had trematodes.

Ponentina ponentina (Morelet, 1845) (Fig. 10) was found in 8 localities in the following regions: Mountain-Transition, El Bierzo and the Bierzo-Cabrera border at 380-877 m, on mainly silty soils with chiefly *Chenopodio-Scleranthea* vegetation; 16.2% of 37 specimens studied had trematodes in the kidney.

Euomphalia (Mengoana) brigantina (Da Silva Mengo, 1867) (Fig. 11) was found in 6 localities in the Mountain region at heights of 520 m (Cantabrian side) and 1337 m, on mainly open soils with *Chenopodio-Scleranthea* vegetation; 12.1% of the 66 specimens studied had trematodes in the kidney.

Subfamily Helicodontinae

Oestophora (Oestophora) barbula (Rossmässler, 1838) (Fig. 12) was found in 7 localities in El Bierzo, the Bierzo-Cabrera border and La Cabrera, at 380-900 m, mainly on silty soils with chiefly *Chenopodio-Scleranthea* vegetation. None of the 25 specimens studied had parasites.

Oestophorella buvinieri (Michaud, 1841) (Fig. 13) was found in 9 localities in the Mountain region and on the Mountain-Transition border, between 520 m (Cantabrian side) and 1278 m, mainly on sandy soils with a *Festuco-Bromea* vegetation. None of the 7 studied had trematodes.

ABBREVIATIONS IN THE FIGURES

a	—atrium genitale	ga	—albumen gland
ap	—appendix	gm	—glandulae mucosae
b	—bursa of receptaculum seminis	mrp	—penial retractor muscle
bd	—dart sac	ov	—oviduct
cb	—pedunculus of receptaculum seminis	p	—penis
cd	—vas deferens	pd	—distal part of penis
ch	—hermaphrodite duct	pr	—prostate
d	—dart	pro	—proximal part of penis
ep	—epiphallus	sod	—spermoviduct
f	—flagellum	v	—vagina
fd	—diaphragm		

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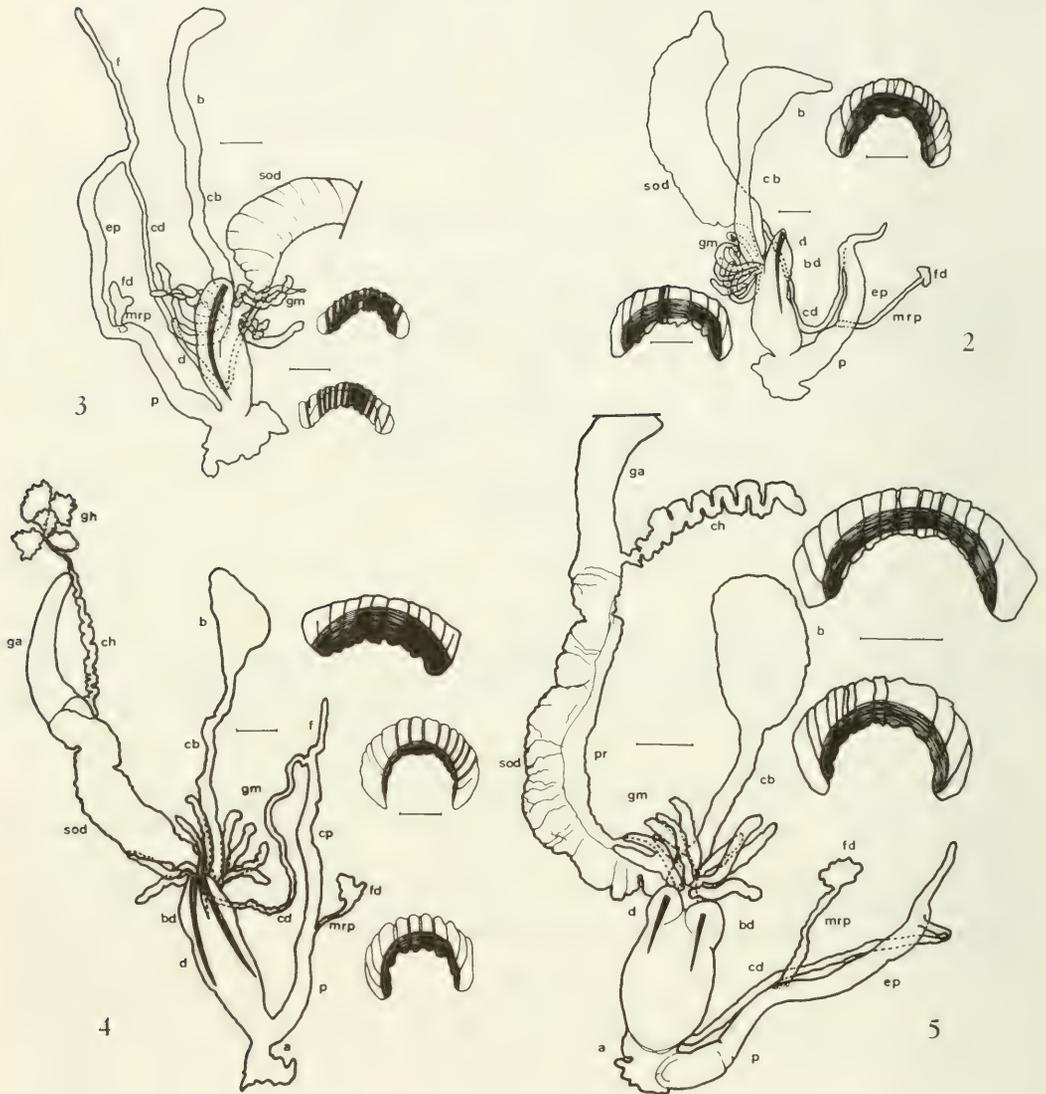


FIG. 2. *Candidula intersecta* (Poir.), genitalia (scale 1 mm) and 2 mandibulae (scale 0.2 mm). FIG. 3. *Candidula rocandioi* (Ortiz de Zár.), genitalia (scale 1 mm) and 2 mandibulae (scale 0.2 mm). FIG. 4. *Helicella ordunensis* (Kob.), genitalia (scale 1 mm) and 3 mandibulae (scale 0.2 mm). FIG. 5. *Helicella cf. madritensis* (Ramb.), genitalia (scale 1 mm) and 2 mandibulae (scale 0.2 mm).

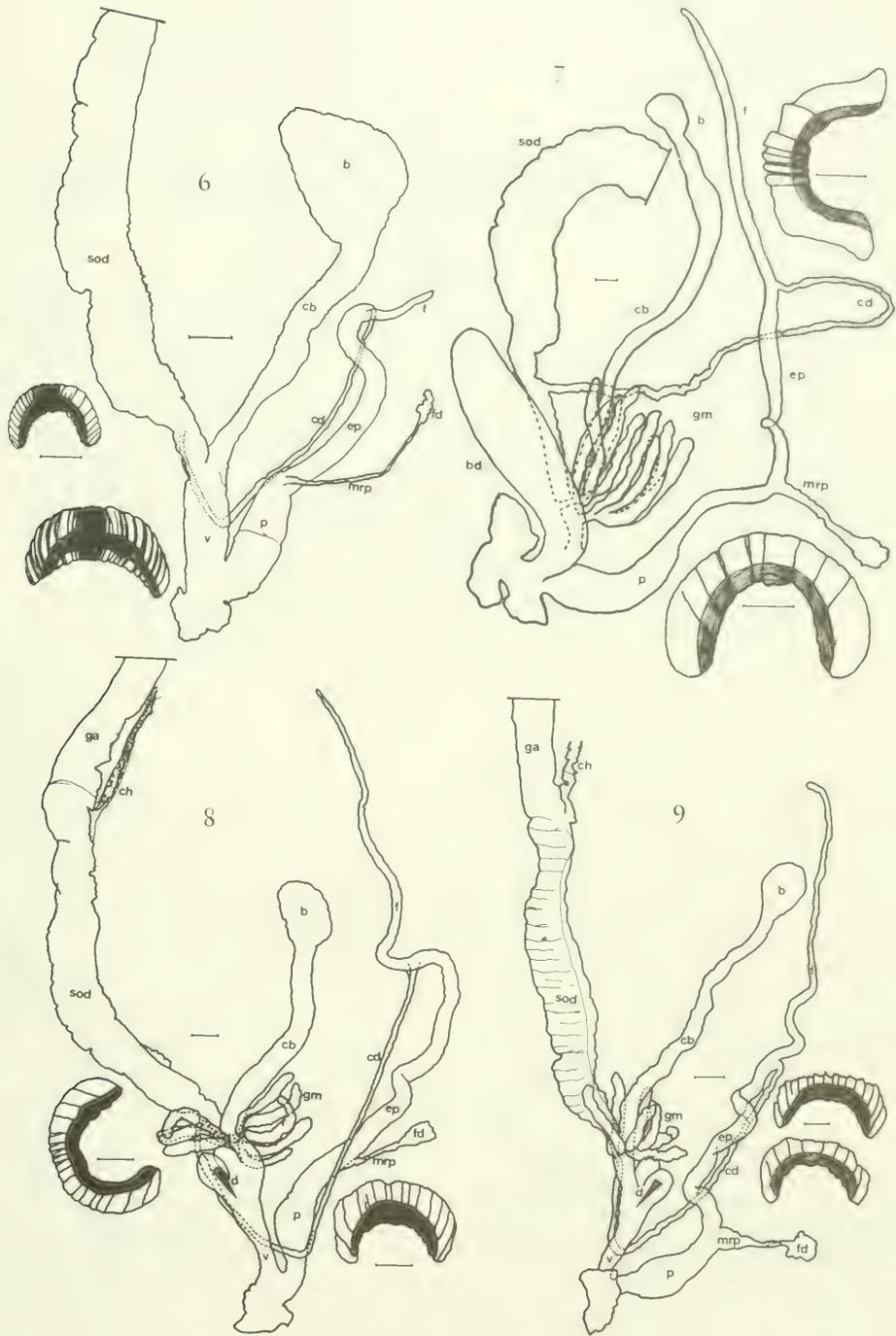


FIG. 6. *Monacha (Ashfordia) granulata* (Alder), genitalia (scale 1 mm) and 2 mandibulae (scale 0.2 mm). FIG. 7. *Hygromia inchoata* (Mor.), genitalia (scale 1 mm) and 2 mandibulae (scale 0.3 mm). FIG. 8. *Hygromia (Pyrenaea) c. cantabrica* (Hid.), genitalia (scale 1 mm) and 2 mandibulae (scale 0.3 mm). FIG. 9. *Hygromia (Pyrenaea) cantabrica covadongae* (Ortiz de Zár.), genitalia (scale 1 mm) and 2 mandibulae (scale 0.2 mm).

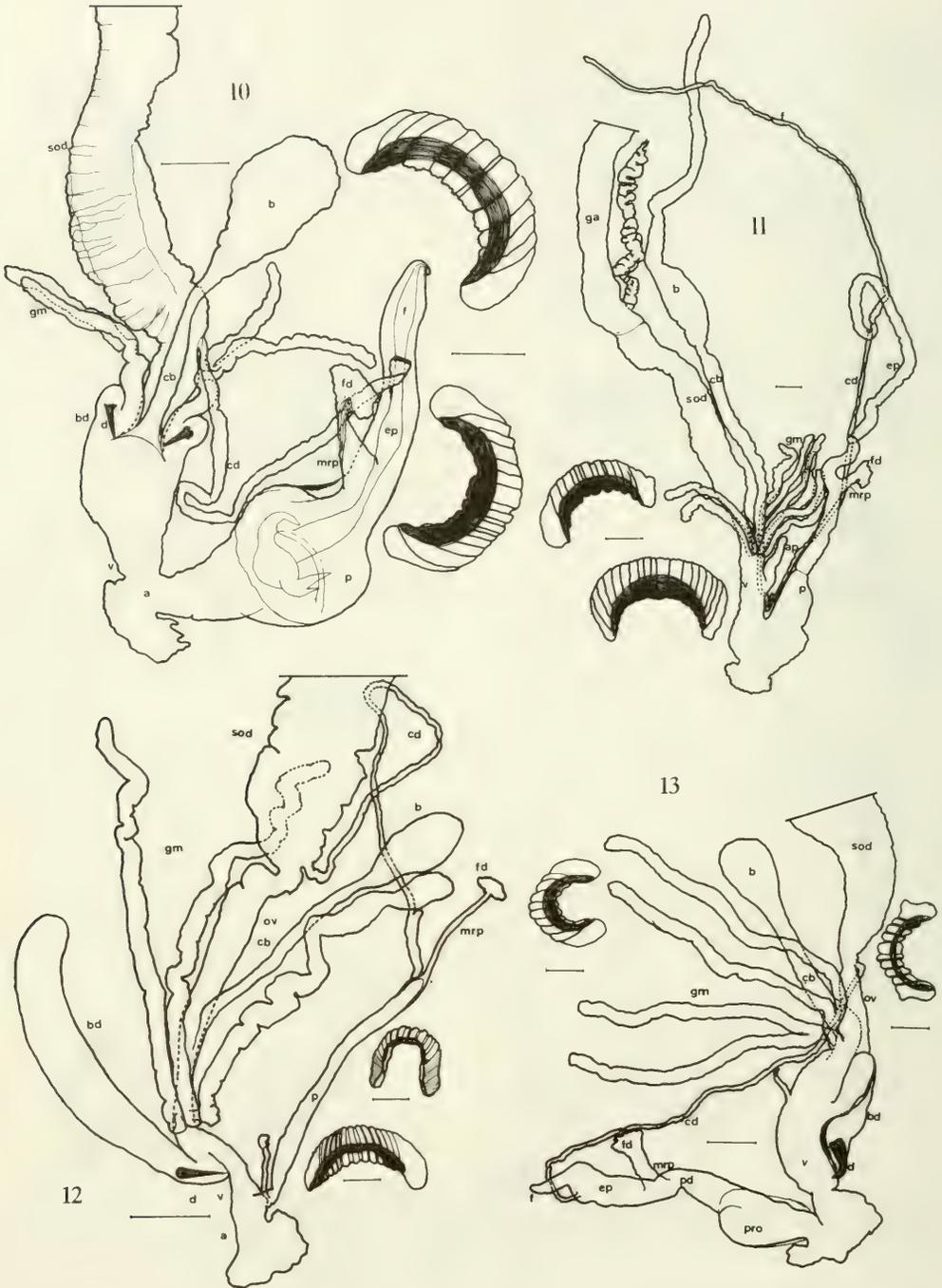


FIG. 10. *Ponentina ponentina* (Mor.), genitalia (scale 1 mm) and 2 mandibulae (scale 0.2 mm). FIG. 11. *Euomphalia (Mengoana) brigantina* (Da Silva Mengo), genitalia (scale 1 mm) and 2 mandibulae (scale 0.3 mm). FIG. 12. *Oestophora (Oestophora) barbula* (Rossm.), genitalia (scale 1 mm) and 2 mandibulae (scale 0.2 mm). FIG. 13. *Oestophorella buvinieri* (Mich.), genitalia (scale 1 mm) and 2 mandibulae (scale 0.2 mm).

THE PLANORBID GENUS *GYRAULUS* IN EURASIA

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ABSTRACT

Within the *Planorbis* tribe of the family Planorbidae the genus *Gyraulus* Charpentier is defined conchologically: a conspicuous gap in the range of shell variation serves to delimit it against *Anisus* and *Bathyomphalus*. Anatomical comparison indicates the existence of 6 subgenera: *Gyraulus* s.s. with a wide distribution in North America, Eurasia inclusive of N. Africa, and Indonesia, Australia, New Zealand, and the Pacific Islands; *Torquis* Dall in N. America and Europe; *Lamorbis* Starobogatov in N. and N.E. Europe; *Caillaudia* Bourguignat in Africa S. of the Sahara; *Carinogyraulus* Polinski in Lakes Ochrid and Prespa, Macedonia; *Choanomphalodes* Lindholm in Lake Biwa, Japan. Characters suitable for species discrimination are found mainly in the reproductive system, but mantle pigmentation and kidney shape have also proven to be more or less constant within a species or species group. While anatomical diversity is great in Europe, it is relatively low in Asia. From West Asia through the continent south of the high mountain chains to the Far East there is a group with characters grading into each other. This group is conceived to constitute a 'Rassenkreis' similar to that of *Radix auricularia*. Its oldest species name is *Gyraulus chinensis*; *euphraticus*, *convexusculus*, *spirillus*, and probably some other groups are regarded to be races of *G. chinensis*. The Malay Archipelago harbours a group that is anatomically quite different from the group of continental South Asia. The non-planispiral *Gyraulus* species in Lake Biwa and in the old Macedonian lakes appear to have a different origin within the genus, their conchological similarities being due to convergence.

The freshwater snails of the genus *Gyraulus* Charpentier, 1837, are distributed in all parts of the world except South America south of Venezuela. Several revisions in restricted parts of the distribution area have increased the number of named "species," because these were exclusively based on shell characters. A synoptic review of the genus over a wider area was hitherto not available. The parasitologists particularly want clarification as regards which species is or are the intermediate hosts of *Echinostoma ilocanum* and *E. lindoense*, intestinal flukes of man and animals in South East Asia. During the last few years I have therefore attempted to include anatomical data in taxonomical studies as far as material was obtainable. The main results are presented here.

The genus is characterized by a small shell with a rounded periphery, often more or less angulate or even keeled in its middle, and by the presence of a peculiar stylet on the tip of the penis. This latter feature it shares with the genera *Anisus*, *Bathyomphalus*, and *Armiger*, which are closely related and can be generically separated on conchological grounds only.

The European fauna outside that of the old Macedonian lakes consists of 5 species. These are conchologically rather dissimilar and easily separated since some corrections had been made after anatomical studies (cf. Meier-Brook, 1964). An anatomical comparison has revealed basic differences suggesting the separation of some species groups (Fig. 1). The main distinguishing characters are found in the male copulatory organ which usually has a club-shaped penis sheath. In 2 species, *riparius* and *rossmaessleri*, it is reduced in form and size. The penis itself generally has a terminal thickening comparable to the mammalian glans penis. The penis pore is situated in or near this thickening. This is the situation in the groups to which our species *albus*, *acronicus* and *laevis* as well as the species of the Ethiopian region belong. The narrow vas deferens is sharply set off against the penis sheath. The prostate gland has a number of diverticula which may be regularly and densely arranged as in *albus*, *acronicus*, *riparius*, and *rossmaessleri*, or irregularly and loosely arranged, as in *laevis*, or greatly reduced as in *costulatus*. The long tubular portion of the kidney may have conspicuously undulate margins

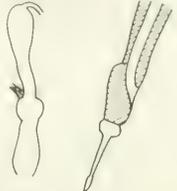
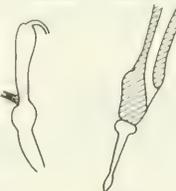
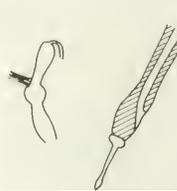
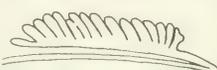
	<i>Gyraulus</i> s.str	<i>Torquis</i> Dall	<i>Lamorbis</i> Starobogatov	<i>Caillaudia</i> Bourg.
male copulat. organ				
prostate gland				
kidney (tub. port.)				
other charact.	shell often with spiral striae, angled periph. and fringe of periostr.	shell surface always smooth, neither angled nor with fringe of periostracum	ovotestis and seminal vesicle unusually big (the latter almost as large as digest.gland)	all reproduct. organs unusually small and delicate
distrib.	North America, North Africa, Europe, Asia, Pacific Islands, Australia	North America, Europe	North and East Europe	Africa south of the Sahara
examples of spp.	<i>albus</i> (O.F.Müller), <i>acronicus</i> (Fér.), <i>chinensis</i> (Dkr), <i>piscinarum</i> (Bourguignat)	<i>parvus</i> (Dall), <i>laevis</i> (Alder)	<i>riparius</i> (Westerlund), ? <i>rossmaessleri</i> (Auerwald)	<i>costulatus</i> (Krauss), <i>connollyi</i> Brown & van Eeden

FIG. 1. Main distinguishing characters of the widely distributed subgenera of *Gyraulus*. Kidney: tub. port. = tubular portion.

and large transverse septa, as in *laevis*, *riparius* and *rossmaessleri*, as far as the European fauna is concerned. Such a kidney is encountered in North American and European *Gyraulus* species but nowhere in Asia. Here and in Africa, Australia and in the majority of European snails of the genus the kidney has straight margins with only a few tiny septa. These and other constant differences suggest the existence of three subgenera in Europe: *Gyraulus* s.s., *Torquis*, and *Lamorbis*. A fourth subgenus, *Caillaudia*, is confined to Africa south of the Sahara. In the whole North Eurasiatic region from N. Scandinavia to Kamchatka *G. acronicus* is the only species according to the scarce material I could examine anatomically.

The Near East harbours at least 4 species which can be separated not only by anatomical characters. They all are members of the nominate subgenus.

In South and East Asia there is a great variety of shell forms which, during the last decades, usually were identified as *convexiusculus* (type locality in Afghanistan). Anatomical comparison revealed a uniformity which is quite surprising in view of the vast area stretching from Iran to Korea. The male copulatory organ is of the usual type (Fig. 2), and the penis pore is in the terminal thickening. Also variation in other features, such as prostate diverticula numbers (range 8-24, in contrast with 7-39 in Europe), is small, so that it is justified to unite all these forms in one "superspecies" or rather "Rassenkreis," similar to that of the lymnaeid, *Radix auricularia* (L.), as demonstrated by Hubendick (1951). The oldest name available for the South and East Asiatic *Gyraulus* is *chinensis* (Dunker). In only one case I had to decide to keep a species apart despite apparent anatomical resemblance, viz. *G. tokyoensis*. This extremely large form is conchologically very different and, moreover, is sympatric with the eastern race of *G. chinensis* in Japan and Okinawa, where Davis & Yamaguchi (1969) found "no gradation of *spirillus* into *G. tokyoensis*." They must, consequently, be reproductively isolated from each other.

The finding of great anatomical uniformity in S. and E. Asiatic *Gyraulus* snails might suggest that this vast area is inhabited only by the races of *G. chinensis* and its close relative, *G. tokyoensis*. But it must be borne in mind that from China a lot of *Gyraulus* forms have been named, and almost no material could be obtained for this study. The only available alcohol

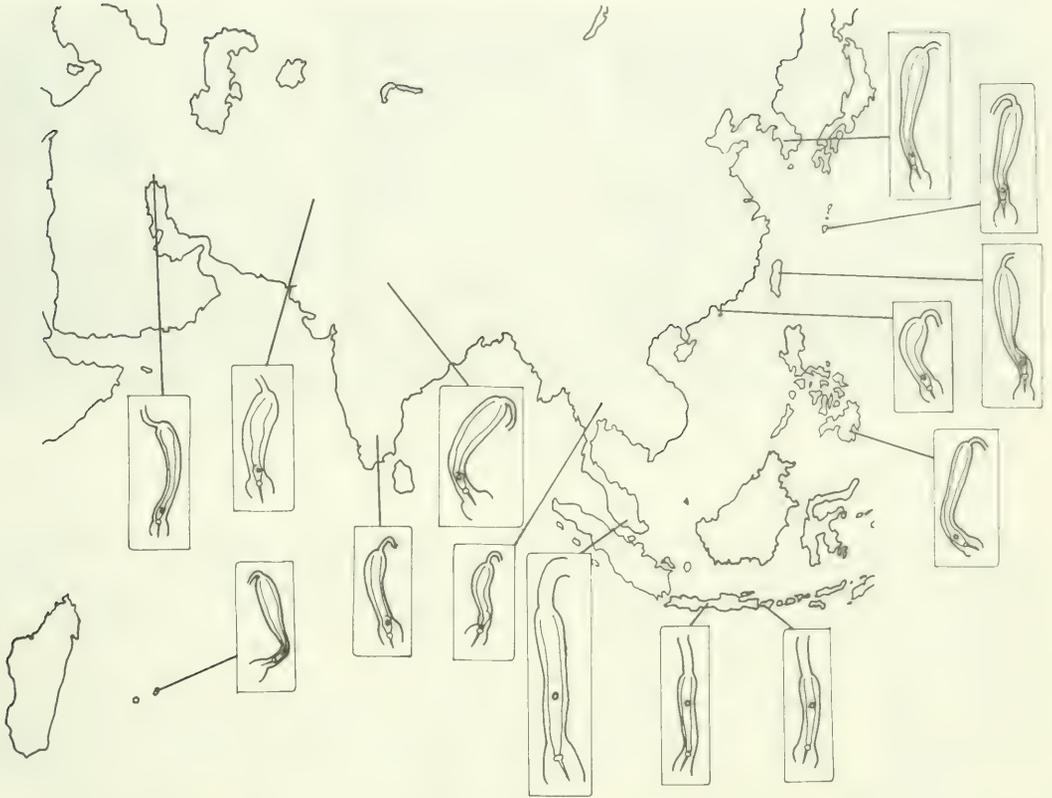


FIG. 2. Shape of the penis sheath and penis and positions of the penis pore in *Gyraulus* samples from South and East Asia and Indonesia. Equal magnification in all anatomical drawings.

preserved specimens, collected by the Sven Hedin Expedition to Inner Mongolia in 1927, actually represent a species hitherto unknown. Both anatomical and shell characters place it well outside the variability of the *chinensis*-group.

The widely distributed *Gyraulus* forms from Indonesia were so far identified as *convexusculus*, apart from a few species endemic to Sumatra. Shell characters in fact fall completely in the range of those of the *chinensis* races. Anatomically, however, the 2 lots examined from Central Java and Bali showed striking aberrations (Fig. 2). The vas deferens is much widened over its full length so that the site of its passing into the penis sheath is nearly indiscernible. The penis lacks the terminal thickening, and the penis pore is near the middle or even in the upper half of the penis. A penis pore so far from the usual site has been reported only once, namely by Hubendick & Radoman (1959) from a species in Lake Ochrid. All other specific characters, however, are so different that a convergent development of this one feature must be concluded. A 3rd lot showing characters very similar to those in the Indonesian forms is from Kuala Lumpur in Malaysia. Now the question arises whether or not the whole Malay Archipelago is inhabited exclusively by *Gyraulus* forms having these aberrant male copulatory organs. Any additional material will be greatly appreciated.

As far as can be judged from the few observations the Malay forms resemble the South Asiatic ones in other essential features, including a characteristic "patchy" pigmentation pattern on the roof of the mantle (Fig. 3). Such a pattern is never encountered in species indigenous to Europe. European species have a weak and diffuse mantle pigmentation; the only exception is *acronicus*, where a certain, but less distinct, pattern is visible. Whenever a conspicuous patchy pigmentation is found in Europe, a recent introduction from outside Europe must be suspected.



FIG. 3. Pigmentation of the mantle roof in 3 *Gyraulus* species.

G. chinensis has, e.g., been found at several places on this continent. A 2nd species introduced is *G. parvus* from North America, which has been collected at 2 German localities.

When we compare variation in *Gyraulus* in Europe and Asia we may say that it is high in the former and low in the latter. Europe harbours 3 subgenera: *Gyraulus* s.s., *Torquis*, and *Lamorbis*. In Asia the 2 latter are certainly absent. *Lamorbis* is apparently also absent from North America, as judged from Baker's studies (1945). There is also no evidence that in North America a further subgenus has evolved. This shows that Europe is the centre of differentiation within the genus. Moreover, the related genera, *Planorbis*, *Anisus*, *Bathyomphalus*, and *Armiger*, are also largely confined to Europe, only slightly extending into West Asia. Thus Europe may also be regarded to be the centre of differentiation within the whole *Planorbis* tribe. It is, therefore, probable that the genus *Gyraulus* had its origin in this part of the world as well.

Finally a brief account must be given of the species living in lakes of Tertiary origin. These are the Macedonian lakes Ochrid and Prespa and the Japanese Lake Biwa. Conchologically the shells are characterized by non-planispiral and multicarinate shells. These similarly directed deviations from the usual shell form have repeatedly caused speculations as to their possible common origin. Hubendick & Radoman (1959) were the first to dissect species from the Ochrid basin. In 4 of the 5 species they found a striking increase in numbers of prostate diverticula which are arranged in several rows (Fig. 4). In the species of the neighbouring Lake Prespa, as well as in Lake Biwa in Japan, this increase has not taken place. The radulae (Fig. 5, top and middle rows: radula forms of widely distributed taxa for comparison demonstrating great uniformity) show similar aberrations in both Macedonian lakes. Four of the 5 Ochridan species have elongated and unicuspid central and lateral teeth instead of bi- and tricuspid ones respectively. The species examined from Lake Prespa displays intermediary stages of cusp reduction with bi- and unicuspid central teeth and a general elongation of teeth together with a reduction of ectocones in the lateral teeth. The species from Lake Biwa, on the other hand, has adhered to the form usual throughout the genus. There is, thus, no evidence that the Japanese and Macedonian endemic species are more closely related within the genus. Most likely abandoning planispiral growth and development of carinae are the results of convergent evolution in the 2 parts of the world. On the other hand, similarities in radular structure, together with conchological affinities and the short distance between the two Macedonian lakes support the assumption of a common origin of the Prespan species and the 4 species of the Ochrid basin. These can be united in a separate subgenus, *Carinogyraulus* Polinski, while it seems equally justified to accept subgeneric rank for the species endemic to Lake Biwa, viz., *Choanomphalodes* Lindholm. Knowledge of anatomical characters described by Hubendick & Radoman (1959) and in the present study enable us to follow the possible course of evolution in the subgenus *Carinogyraulus*. After the non-planispiral and multicarinate shell had been developed, initially reduction of cusp numbers in central and lateral teeth took place. In the

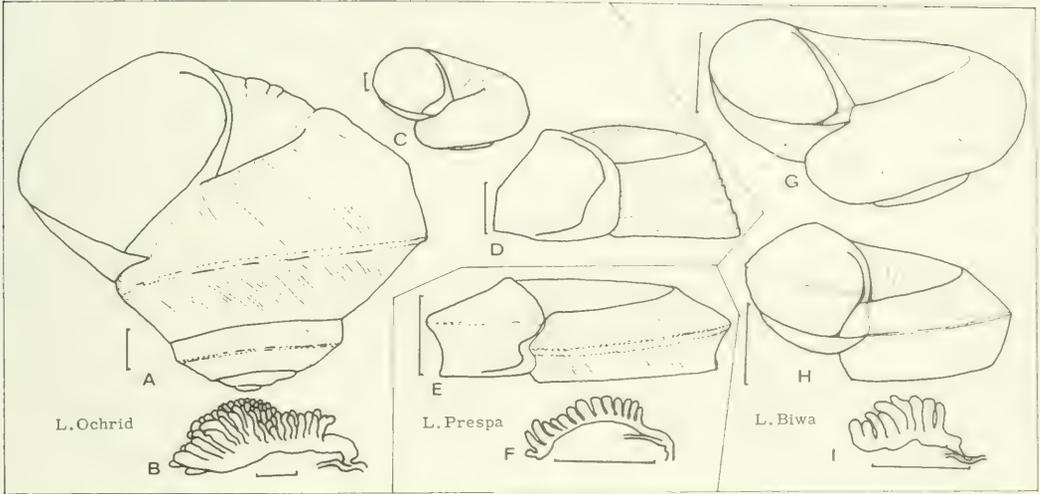


FIG. 4. Shell and prostate gland of *Gyraulus* species from old lakes. A & B, *G. lychnidicus* Hesse; C, *G. crenophilus* Hubendick & Radoman; G, *G. trapezoides* Polinski; E & F, *G. stankovici* Hadžišće; H, *G. amplificatus* (Mori) (?identical with *biwaensis*?); H & I, *G. biwaensis* (Preston). C & D after Hubendick & Radoman (1959); G original; all others after Meier-Brook (in press). Scales 1 mm).

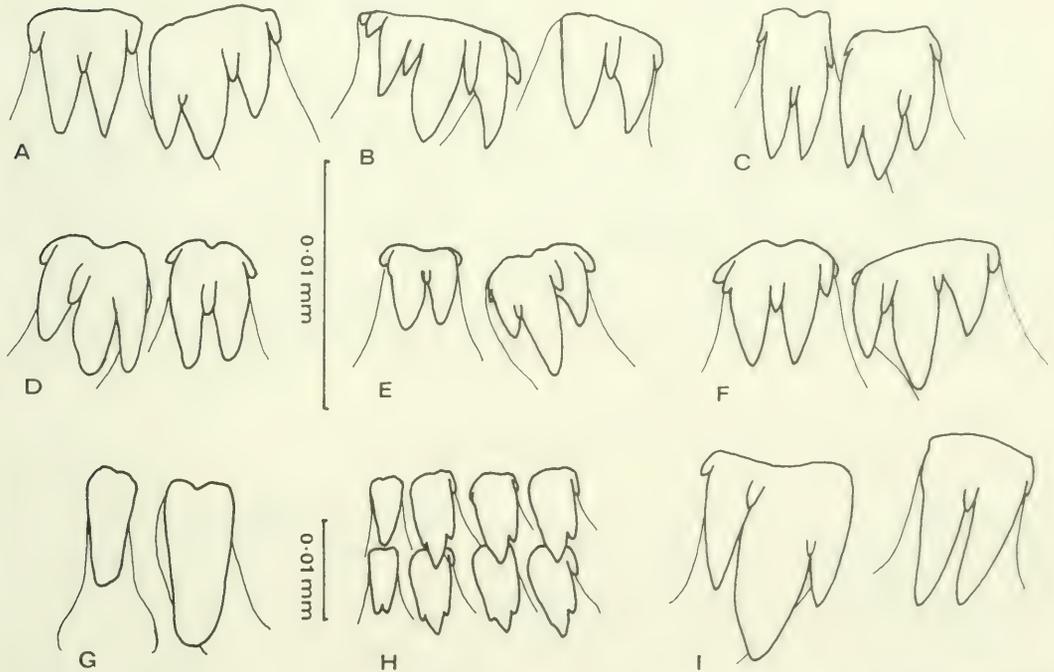


FIG. 5. Radulae of widely distributed *Gyraulus* species (A to F) compared with those of species endemic to old lakes (G to I). A, *G. albus* (O. F. Müller), Denmark; B, *G. parvus* (Dall), Iceland; C, *G. hebraicus* (Bourg.), Turkey; D, *G. chinensis convexiusculus* (Hutton), India; E & F, *G. ch. spirillus* (Gould), Taiwan and Korea; G, *G. lychnidicus*, Lake Ochrid; H, *G. stankovici*, Lake Prespa; I, *G. biwaensis*, Lake Biwa (short scale applies to G and H only).

Ochrid basin the 2nd step of evolution was done by increasing the number of prostates diverticula. A 3rd step of evolution including further reduction of cusp numbers in the lateral portions of the radula and some minor changes subsequently led to the evolution of the recent species.

I wish to express my gratitude to all the many colleagues and museum authorities who provided the material used in these studies, although it is impossible to mention them individually.

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NOTE ADDED IN PROOF. Continuing studies have revealed that (a) *Armiger* must be considered a further subgenus of *Gyraulus*, and that (b) *G. euphraticus* should be kept separate from the "Rassenkreis" of *G. chinensis* (cf. Meier-Brook, in press).

THE MALACOFAUNA OF MOUNT HERMON

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ABSTRACT

In this preliminary report of the first survey of the malacofauna of Mount Hermon (33°24' N 35°50' E), the outlines are presented of the geographical and altitudinal distribution of 33 land molluscan species. From these outlines, a few general patterns emerge.

INTRODUCTION

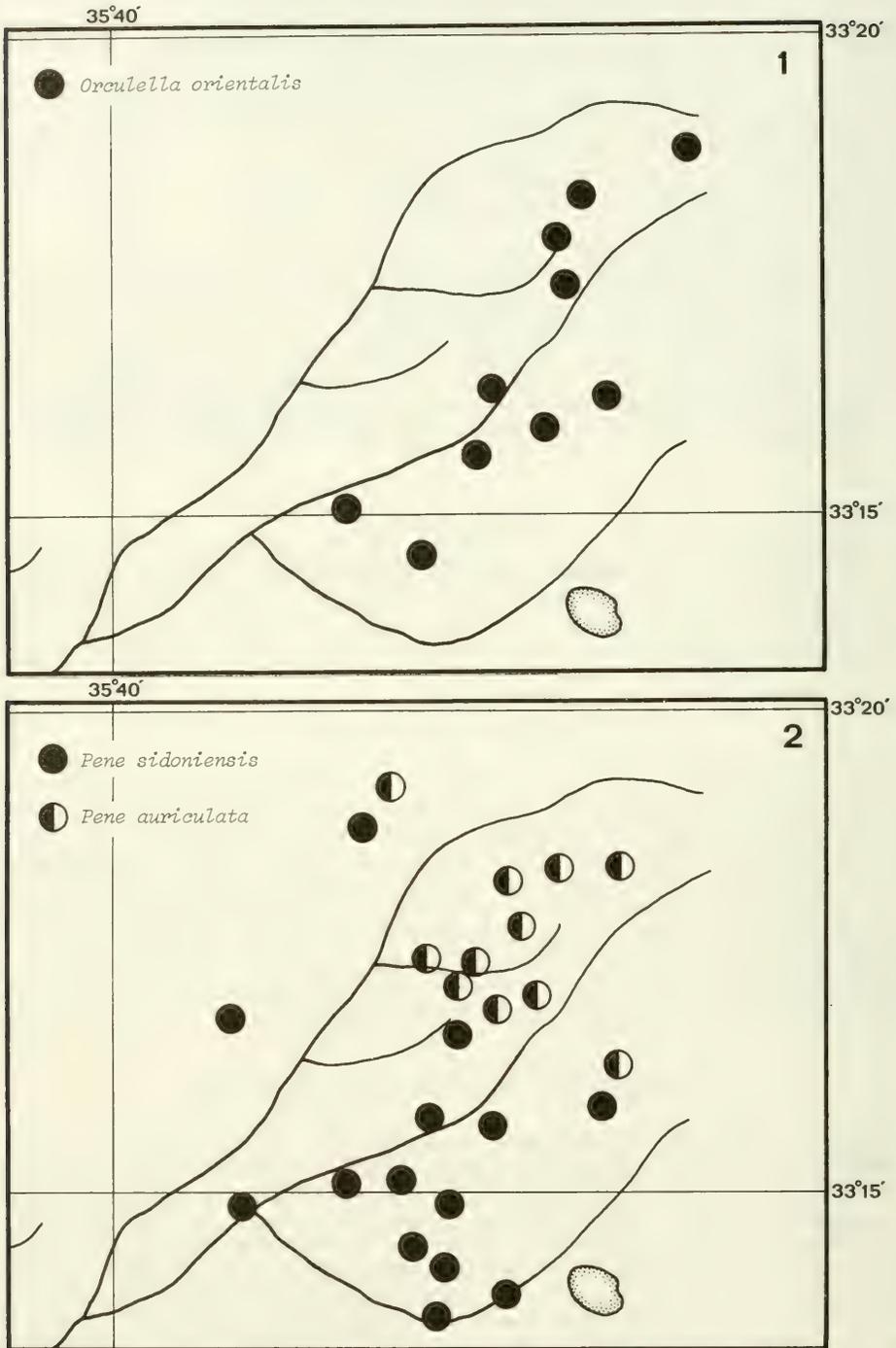
The malacofauna of Mount Hermon (33°24' N 35°50' E) has hardly been investigated before owing to its isolated position, political restrictions and severe climatic conditions which greatly limit the collection of live snails. Here are presented the preliminary results of a survey of all the known material collected on the western part of the mountain, Israeli-held since 1967.

PHYSICAL GEOGRAPHY

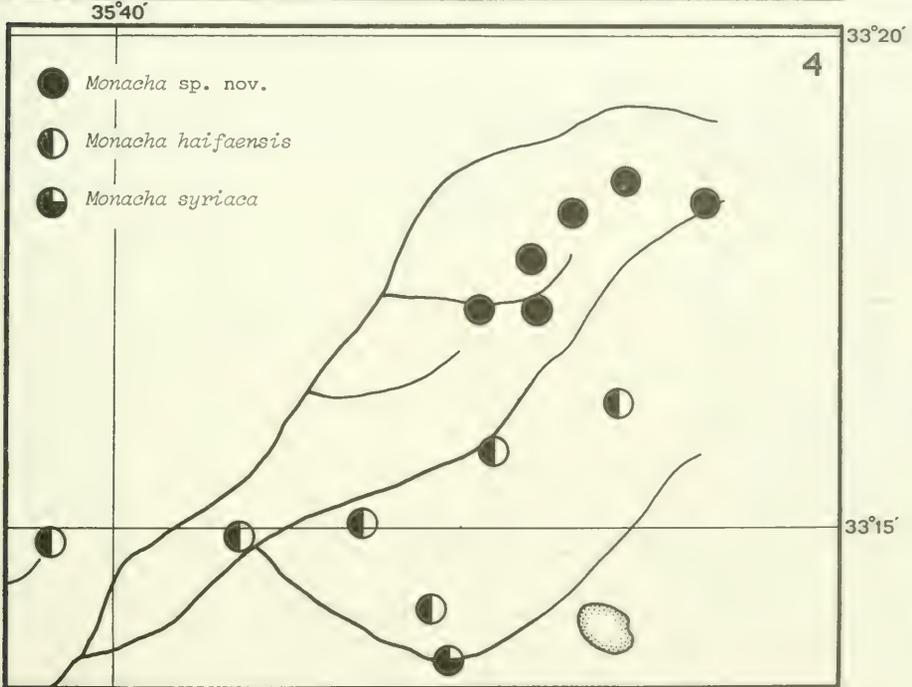
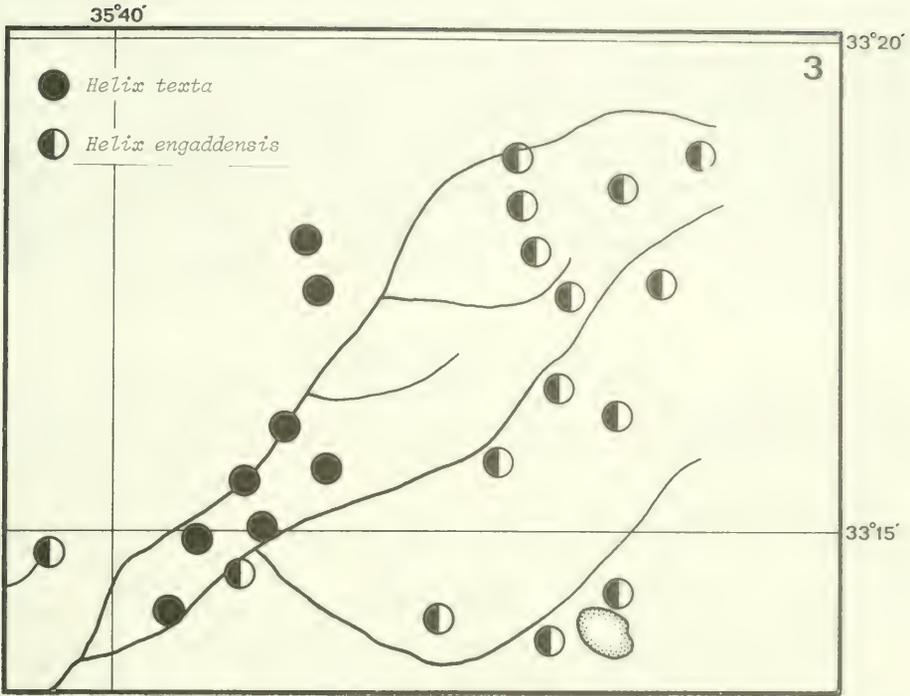
Mount Hermon is the southern continuation of the Anti-Lebanon whose anti-clinal axis runs NE-SW. In the east it borders on the Damascus basin, in the south on the basalt table land of the Golan, and in the west on the alluvial Hazbani Valley. The length of the ridge is some 45 km and its greatest width about 25 km. The summit reaches 2814 m. The main substrate is hard Jurassic limestone. Mean annual precipitation at the peaks is about 1700 mm, mainly in the form of snow and only on the eastern and western slopes as rain. Usually the snow remains on the ground for 3-5 months at altitudes above 1500 m. A substantial part of the rain and the melting snow quickly percolates through the porous rock, resulting in karstic erosion and an almost complete absence of top soil. In summer, when relative humidity is extremely low and there is no soil to contain water, dry steppe conditions prevail (Inbar, 1971; Orni, 1972).

THE SPECIES AND THEIR DISTRIBUTION

Sommerville (1869) did not only give the first, but so far also the most extensive, list of (7) land snails from an unidentified locality, probably situated at the foot of the western part of the mountain. Among the species mentioned in this list, *Buliminus labrosus* (Olivier, 1804), *Pene sidoniensis* (Férussac, 1821), *Helix texta* (Mousson, 1861), and *Sphincterochila cariosa* (Olivier, 1804) can be identified with certainty. The first slug from Mount Hermon was described by Pollonera (1908) as *Agriolimax libanoticus* (= *Deroceras* sp.). Recently several land snails from the Hermon area have been mentioned in monographic works: *Pleurodiscus erdelii* (Roth, 1839) (Bar, 1974), *Pene sidoniensis* and *P. auriculata* (Pallary, 1929) (Heller, 1974), *Buliminus labrosus* (Heller, 1975), *Sphincterochila cariosa* (Bar, 1975), *Xeropicta vestalis joppensis* (Schmidt, 1855) (Forcart, 1976), *Lauria cylindracea* (Da Costa, 1778) (Mienis, 1976) and *Monacha crispulata* (Mousson, 1861) (Bar, 1976). A complete list of the species found in this survey is presented in the table of altitudinal distribution.



FIGS. 1-2. Some typical distribution patterns of land snails on Mt. Hermon; the little lake on the right hand bottom corner of the maps is Birget Ram. H. Heijn del.



FIGS. 3-4. Some typical distribution patterns of land snails on Mt. Hermon; the little lake on the right hand bottom corner of the maps is Birket Ram. H. Heijn del.

these—notably very small species—may yet be found higher up. Some typical Mediterranean species enter the montane region. They all hide in cracks (*Pene*) or under stones (*Jaminia*, *Oxychilus*). *Helix engaddensis* is able to span the amazingly wide range of 0-2050 m. It aestivates while buried in the little soil that accumulates in a few cracks. In the surrounding Mediterranean and desert regions, land snails aestivate during the long, hot and dry summer, their activity being limited to the mild rainy winter and short spring. It seems likely that, at altitudes above 1300 m where snow covers the ground during several months, snails are being forced into two periods of dormancy, an unnatural condition for most species adapted to a Mediterranean climate.

Acknowledgments for assistance in completing this paper are due to Messrs. H. Heijn and Dr. A. C. van Bruggen of Leiden University, Holland.

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BIOGEOGRAPHICAL ASPECTS OF AFRICAN FRESHWATER GASTROPODS

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ABSTRACT

To Pilsbry & Bequaert (1927) the outstanding features of the freshwater molluscan fauna of the Ethiopian region were its taxonomic poverty and its uniformity over immense areas. This view can hardly be maintained having regard to taxonomic and distributional data accumulated in recent years largely due to interest in the transmission of human schistosomiasis. Various distribution patterns are evident at the levels of the species and of the genus, which are of considerable biogeographical interest. About 300 species of freshwater gastropods are living in Africa of which about one-fifth are endemic to certain of the great lakes. Most of the species which are not strictly lacustrine fall into two main groups:

(1) Species having their closest affinities with the fauna of Europe and/or South West Asia. These reach their southwestern limits in North Africa and some penetrate to Ethiopia.

(2) Species which are characteristic of Africa south of the Sahara and do not occur in North West Africa, though some are present in the lower Nile and a few extend into South West Asia.

Another, smaller, group of alien species can be distinguished of which some obviously have been introduced by man. Representative distribution maps are presented, with an analysis of species-diversity in relation to the major climatic regions to Africa.

INTRODUCTION

To Pilsbry & Bequaert (1927) the outstanding features of the freshwater molluscan fauna of the 'Ethiopian Region' were its taxonomic poverty and its uniformity over immense areas. This view can hardly be maintained having regard to taxonomic and distributional data accumulated in recent years largely through interest in the snail hosts concerned in the transmission of human schistosomiasis. Various distribution patterns of biogeographical interest are evident at the levels of both genus and species, and are discussed in the context of southern Africa by Brown (1978).

The present maps are based partly on unpublished records from the collections of the Experimental Taxonomy Unit, British Museum (Natural History), and the Danish Bilharziasis Laboratory. I am grateful to Dr. C. A. Wright and Dr. G. Mandahl-Barth for permission to include these data, and to the Department of Geography of the University of Chicago for permission to reproduce a map from the Goode Base Map Series in Figs. 1-9. The shaded areas approximate to the main areas of occurrence, but within these areas distribution may be discontinuous, and it is commonly disrupted by the scarcity of aquatic habitats in districts receiving comparatively low rainfall. However, a greater order of isolation is evident for some populations and these are indicated in the maps by a single spot, or open circles in the case of records based only on shells. More or less worn shells have been termed by authors 'sub-fossil' and their age is uncertain, though probably North African examples are of late Pleistocene to Recent age.

According to a revised synopsis of gastropods living in the fresh and brackish waters of Africa (Brown, in press), there is a total of about 314 species of gastropod (227 prosobranchs and 87 pulmonates) living in such waters on the African mainland. This number excludes three families which do not penetrate far from strong marine influence (Potamididae, Littorinidae and Ellobiidae), and the Hydrobiidae of North Africa which are in outstanding need of revision. Further, the ancyliid genera *Ferrissia* and *Burnupia* are included as single taxa because their nominal species are so poorly defined.

About one-quarter of the African freshwater gastropods are restricted to lakes and these will not be considered in the subsequent biogeographical account. This exclusively lacustrine group is made up mainly of about 25 species of Thiariidae endemic to Lake Tanganyika, and representatives of a number of widespread prosobranch genera, including *Bellamya*, *Gabbiella* and *Melanoides*. Amongst pulmonates, the genus *Ceratophallus* has the highest proportion of lacustrine species (most of them described originally as '*Gyraulus*').

The assemblage of about 235 species which are not exclusively lacustrine (though some may live at the edges of lakes) can be divided into 2 main groups based on biogeographical considerations, and a small 3rd group of introduced species.

(1) Palaearctic group. These species have their closest affinities with the fauna of Europe and/or South West Asia. These are characteristic of the northern part of Africa which is customarily included in the Palaearctic biogeographical region, but some extend into the highlands of Ethiopia, and 2 are widespread south of the Sahara (*Melanoides tuberculata* and *Lymnaea truncatula*).

(2) Afrotropical group. Most of the species included here are confined to Africa south of the Sahara or there occupy the main part of their range. They are thus characteristic of the traditional 'Ethiopian' biogeographical region, for which the name Afrotropical Region is preferable (Crosskey & White, 1977). Although the occurrence of shells shows that some of these species have been widespread in the Sahara, only *Bulinus truncatus* is known to live in North West Africa. Taxonomic problems make it difficult to decide exactly how many of the indigenous members of the Afrotropical group occur outside this region; these widespread species are comparatively few in number and belong to genera which are associated with brackish water or are confined to freshwater habitats near the coast (*Neritina*, *Septaria*, *Thiara*, and the Ellobiidae).

(3) Introduced species. *Physa acuta*, *Lymnaea columella* and *Helisoma* spp. are present in both the Afrotropical and the Palaearctic regions of Africa. Their occurrence appears to be closely associated with European settlement and probably these snails have been dispersed mainly or entirely during the recent historical period.

THE PALAEARCTIC GROUP

This group comprises about 20 species (Table 1) of which about 16 occur in North West Africa and 10 in Egypt and/or Ethiopia. Only 4 species are known from North West Africa and also Egypt. *Bithynia* and *Melanopsis* are remarkable in being present in North West Africa and in South West Asia, but neither occurs in Egypt (for Bithyniidae see Mandahl-Barth, 1968).

TABLE 1. The Palaearctic group of freshwater gastropods living in Africa; of these only *Melanoides tuberculata* and *Lymnaea truncatula* occur south of Ethiopia.

Species	Egypt (E) and/or Ethiopia (ET)	North West Africa	Both
<i>Theodoxus fluviatilis</i>	—	X	—
<i>T. niloticus</i>	E	—	—
<i>Valvata nilotica</i>	E,ET	—	—
<i>Bithynia</i> 2 spp.?	—	X	—
<i>Melanoides tuberculata</i>	E,ET	X	X
<i>Melanopsis praemorsa</i>	—	X	—
<i>Lymnaea truncatula</i>	E,ET	X	X
<i>L. palustris</i>	—	X	—
<i>L. peregra</i>	—	X	—
<i>L. stagnalis</i>	E	X	X
<i>Planorbis planorbis</i>	E	X	X
<i>Armiger crista</i>	ET	X	X
<i>Gyraulus ehrenbergi</i>	E	—	—
<i>G. sp.</i>	—	?	—
<i>Anisus</i> 2 spp.?	—	X	—
<i>Hippeutis</i> sp.	—	X	—
<i>Planorbarius metidjensis</i>	—	X	—
<i>Ancylus fluviatilis</i>	ET	X	X
<i>A. regularis</i>	ET	—	—

Isolation between the freshwater faunas of North West Africa and Egypt is demonstrated well by *Theodoxus* (Fig. 1), which does not penetrate south of the Sahara. This genus is represented in the northwest by forms related to *T. fluviatilis*, which belongs to *Theodoxus* s.s. In contrast, *T. nilotica* of Egypt belongs to the subgenus *Neritaea* and is closely related to *T. jordani* of South West Asia. The northern limit for the latter subgenus lies in Iraq and Iran.

Valvata (Fig. 2) presents a more extensive penetration into northeast Africa, being common in the highlands of Ethiopia and known as a 'subfossil' from Lake Rudolf. *Valvata tilhoi* is recorded from several localities in the Sahara desert, the most westerly being Fort Flatters in Algeria (Fischer-Piette, 1949). The very small shells from Algeria described as new species of *Valvata* by Hagenmüller (1884) seem unlikely to belong to this genus, which is otherwise unknown from North West Africa. The absence there of *V. piscinalis* is surprising but is perhaps related to lack of this species in southern Spain (Zilch & Jaeckel, 1962), where the southernmost localities appear to be near Valencia and Alicante (Gasull, 1971). The probable route of entry into northeast Africa is indicated by the presence of isolated living populations of *Valvata* in the Sinai peninsula (Tchernov, 1971).

The Lymnaeidae included in Table 1 are confined to North West Africa and/or Egypt with the exception of *L. truncatula* (Fig. 3), which is distributed discontinuously through eastern Africa to the Cape Province of South Africa. This snail is restricted to highland areas in the tropical region but inhabits a wider altitudinal zone in the southern temperate region; it is particularly abundant in Lesotho (Basutoland) (Prinsloo & Van Eeden, 1973). It seems correct to accept that *L. truncatula* has Palaearctic affinities, though its extensive range in Africa seems to be long established, as 'subfossil' shells are known from isolated localities which include the Ahaggar mountains in southern Algeria (Sparks & Grove, 1961). *L. glabra* is omitted from Table 1 although its presence in Algeria is indicated by Hubendick (1951, fig. 332) on the basis of shells in the collection of the British Museum (Natural History). I have examined two lots of shells from Algeria in this collection, originally identified as *L. glabra*, but they appear to be slender examples of *L. palustris*. Moreover, since *L. glabra* is unknown from the Iberian peninsula, it would be unlikely to occur in North West Africa (see note on p. 84).

The Planorbidae of North West Africa are almost unknown anatomically and consequently the correct taxonomic position of most of the nominal species recorded there is uncertain. *Planorbis planorbis* and *Planorbarius metidjensis* are widespread in this region. *P. planorbis* (Fig. 4) lives also in Egypt and is known from shells obtained at the 2nd Nile Cataract in northern Sudan (Martine, 1968), and in the Rift Valley of Ethiopia (Brown, 1965). *Armiger crista* is known in North West Africa and also lives in Ethiopia (Brown, 1967). It appears that the Palaearctic genera *Anisus* and *Hippeutis* are represented by one or more species in North West Africa, but anatomical study is necessary to establish the presence of any species of *Gyraulus*.

Ancylus occupies 2 widely separated areas in Africa (Fig. 5), one in North West Africa and the other in the Ethiopian highlands. A possible past connection between the Ethiopian populations and the main area of distribution in Europe is indicated by isolated localities for *A. fluviatilis* known in the Arabian peninsula and in Syria. This possible route of dispersal perhaps pre-dates the major earth movements which separated the highlands of South West Arabia from those in Ethiopia. Support for the view that *Ancylus* is long established in the Ethiopian highlands is provided by the occurrence there, in addition to *A. fluviatilis*, of 2 endemic species (Brown, 1965, 1973).

Melanoides tuberculata has a much greater range than the other species here considered in the Palaearctic group. It occurs not only in the southern part of the Palaearctic Region but also in the Indomalayan and Afrotropical Regions, and its geographical origins are not known. There is one record for Spain (Gasull, 1974), perhaps the result of introduction by man. In Africa *M. tuberculata* is commonest in the eastern part (Fig. 6) and it is known from few localities in West Africa. The presence of shells in many localities in the Sahara desert shows that this snail has been widespread in North Africa, and isolated living populations occur in Chad, Algeria and probably also Morocco. *M. tuberculata* is practically absent from the Zaire basin where there are numerous endemic species belonging to the genus.

AFROTROPICAL GROUP

The genera comprising this large assemblage can be divided according to their geographical range and the extent to which they have evolved species within Africa (Table 2). The most cosmopolitan genera, *Septaria* and *Thiara*, are represented by few species, which are widespread in the Indo-Pacific region. Secondly, there is a group of twelve widely distributed genera, each of which has some species endemic to Africa. Thirdly, a group of 28 genera can be regarded as strictly Afrotropical, although some species occur in the lower Nile, and some live on Madagascar and other islands in the Indian Ocean.

Considered in terms of geographical range, the most widespread of the Afrotropical genera is *Bulinus*. *B. truncatus* is present in Iberia and the Mediterranean region, and extends eastwards into Iran (Fig. 7). At first sight this large range seems inconsistent with an Afrotropical nature. However, since *B. truncatus* has a tetraploid number of chromosomes its distribution can be regarded as a secondary expansion from the area occupied by the ancestral diploid group within Africa (Fig. 8). Indeed the only diploid *Bulinus* known to occur north of Ethiopia belong to the *B. forskali* and *B. reticulatus* species groups, which are not closely related to *B. truncatus*. *B. reticulatus* has an extensive range in south and eastern Africa (Fig. 9), and the closely related *B. wrighti* occurs in Arabia penetrating eastwards into Oman.

Biomphalaria is also present in the eastern Mediterranean region and in South West Asia (Fig. 10), but is less widespread than *Bulinus*. However, *Biomphalaria* is also present in South

TABLE 2. Genera of freshwater gastropods living in Africa south of the Sahara (Afrotropical Region).

Widely distributed genera represented by widespread species:	Strictly Afrotropical genera (E—present in Egypt; M—present in Madagascar):
<i>Septaria</i>	Hydrobiidae
<i>Thiara</i>	<i>Lobogenes</i>
	<i>Soapitia</i>
	<i>Tomichia</i>
Widely distributed genera with endemic species in Africa:	Pilidae
<i>Neritilia</i>	<i>Afropomus</i>
<i>Neritina</i>	<i>Lanistes</i> (E,M)
<i>Bellamya</i>	<i>Saulea</i>
<i>Hydrobia</i>	Bithyniidae
<i>Potamopyrgus</i>	<i>Gabbiella</i> (E)
<i>Pila</i>	<i>Incertihydrobia</i>
<i>Assiminea</i> s.l.	<i>Jubaia</i>
<i>Melanoides</i>	<i>Congodoma</i>
<i>Lymnaea</i>	<i>Funduella</i>
<i>Gyraulus</i>	<i>Liminitesta</i>
<i>Biomphalaria</i>	<i>Sierraia</i>
<i>Ferrissia</i>	Assimineidae
	<i>Eussoia</i>
	<i>Pseudogibbula</i>
	<i>Septariellina</i>
	<i>Valvatorbis</i>
	Thiaridae
	<i>Potadoma</i>
	<i>Cleopatra</i> (E,M)
	<i>Pseudocleopatra</i>
	<i>Potadomoides</i>
	<i>Pachymelania</i>
	Planorbidae
	<i>Afroyrus</i> (E,M)
	<i>Ceratophallus</i>
	<i>Lentorbis</i>
	<i>Segmentorbis</i> (E,M)
	<i>Bulinus</i> (E,M)
	Ancylidae
	<i>Burnupia</i>

America and the Caribbean area, and the genus appears to be long established in the Neotropical Region. Surprisingly, these snails are absent from North West Africa although shells of extinct populations are found in numerous localities in the Sahara. *Biomphalaria* is absent from the coastal region of East Africa, perhaps because of an unfavourable high temperature, whereas low temperature is apparently a major factor restricting distribution in the southwestern part of the continent. In Fig. 10 the range is shown to extend continuously from Kenya to Ethiopia, but in fact there is a significant gap of about 400 km between the closest localities for *B. pfeifferi* known in Kenya (Marsabit) and Ethiopia (Lake Margherita). However, given suitable bodies of water there can be little doubt that this snail would occur in the intervening area. For some other taxa the isolation of the Ethiopian part of their range appears substantially greater, and this area is indicated separately in the maps (Figs. 13, 15, 17).

Bellamya (Fig. 11) also extends down the Nile into lower Egypt but is practically absent from South West Asia. However, this genus is present also in southern Asia. Effective isolation between the African and the Asian parts of this range is reflected in the lack of any species which lives in both areas. Although *Gabbiella* (Fig. 12) is also present in the lower Nile, it appears to be primarily an Afrotropical group comprising about 30 species mostly restricted to central Africa (Mandahl-Barth, 1968).

The Planorbidae provide many examples of taxa having extensive ranges but which are unknown north of the Sahara, including the *Bulinus natalensis/tropicus* complex (Fig. 8), the *B. africanus* group (Fig. 13), *Gyraulus costulatus* (Fig. 14) and *Ceratophallus* (of which many species were described originally as *Anisus*) (Fig. 15).

Potadoma (Thiaridae) (Fig. 16) has a remarkable distribution pattern comprising 2 separate areas, one in the west and another in central Africa. Their habitat, streams flowing through forest, is occupied in Madagascar by the closely related genus *Melanatria*.

There is one strictly Afrotropical genus of Ancyliidae, *Burnupia* (Fig. 17), reported most frequently from eastern Africa though probably widespread also in the streams of central Africa.

Many of the species living in tropical African freshwaters are absent from the southwestern part of the continent, which experiences a comparatively dry and cool climate. In the east, however, tropical species occur further south in a slender coastal zone termed the 'tropical corridor' which extends into the Natal province of South Africa. The general similarity in the southern limits for tropical species in this region (Figs. 10, 13, 14) appears to reflect the climatic transition between the tropical and the southern temperate climatic zones. *Gyraulus connollyi* is one of the few freshwater molluscs characteristic of the southern temperate zone (Brown & Van Eeden, 1969). This snail is not known to extend north of the Drakensberg ridge in eastern Transvaal province, where its lower altitudinal limit is about 1,500 m.

SPECIES DIVERSITY IN RELATION TO LATITUDE

An analysis limited to genera of freshwater pulmonates provided Hubendick (1962) with no evidence for greater faunal diversity in the warm tropics, and he concluded that "neither the Ethiopian nor the Neotropical fauna shows any clear increase of diversity from high to low southern latitudes." However, recent advances in taxonomy indicate a marked increase in diversity towards the equator in the freshwater gastropod fauna in southern Africa (Fig. 18). Excluded from the present analysis are species endemic to lakes, the genus *Tomichia* which does not seem truly to belong to the freshwater fauna, and the Ancyliidae because their taxonomy is so poorly known.

There is a decline of over 75% from the number of species (65) known in the equatorial zone between 0° and 5° South and the total (14) known in the coastal zone of South Africa lying between 30° and 35° South. Inclusion of *Tomichia* and Ancyliidae might considerably increase the number of species known in southern Cape Province, but almost certainly not to a level comparable with the totals for latitudinal zones north of 15°. In these zones major contributions to the fauna are made in eastern Zaire by *Potadoma* and *Melanoides*, in the lower Zaire river by endemic Hydrobiidae and Assimineidae, and in southeastern Zaire by a group of local species including the endemic hydrobiid genus *Lobogenes*.

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NOTE ADDED IN PROOF. *Lymnaea glabra* (p. 81) is reported to occur in the coastal region of northern Spain by SANCHEZ, J. A., 1965, *Boletín de la Real Sociedad Española de Historia Natural, Secc. Biologica*, 63: 9-14.

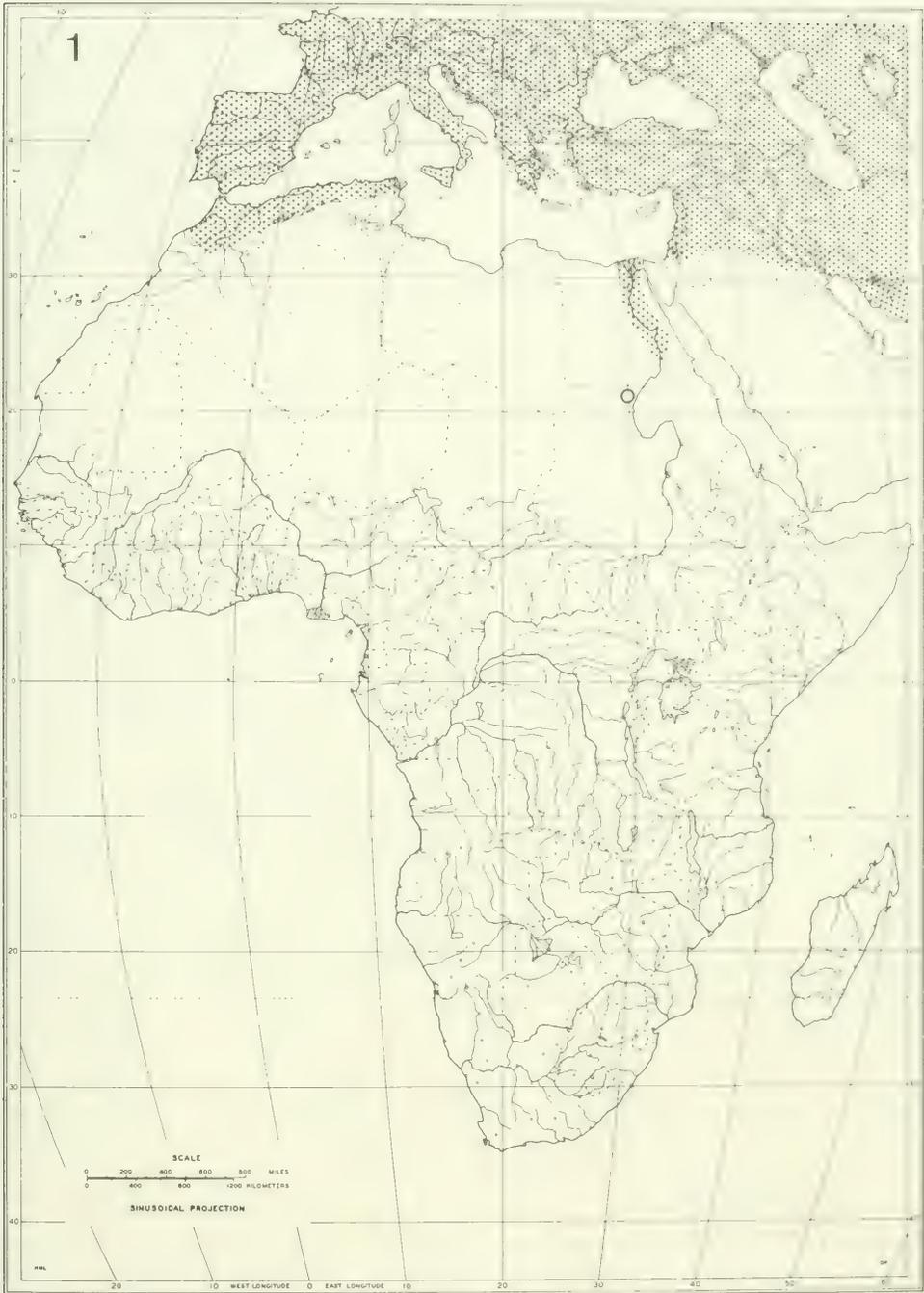


FIG. 1. The distribution of *Theodoxus* in Africa, southern Europe and South West Asia. An isolated locality is indicated for 'recent' shells from the 2nd Nile Cataract (Martine, 1968). Unconfirmed reports for Ethiopia (cited by Bacci, 1951) are omitted. *Theodoxus* s.s. is present in Europe and North West Africa, whereas the subgenus *Neritaea* occurs in Egypt and South West Asia. In this and subsequent maps no attempt is made to show distribution on all Mediterranean islands.

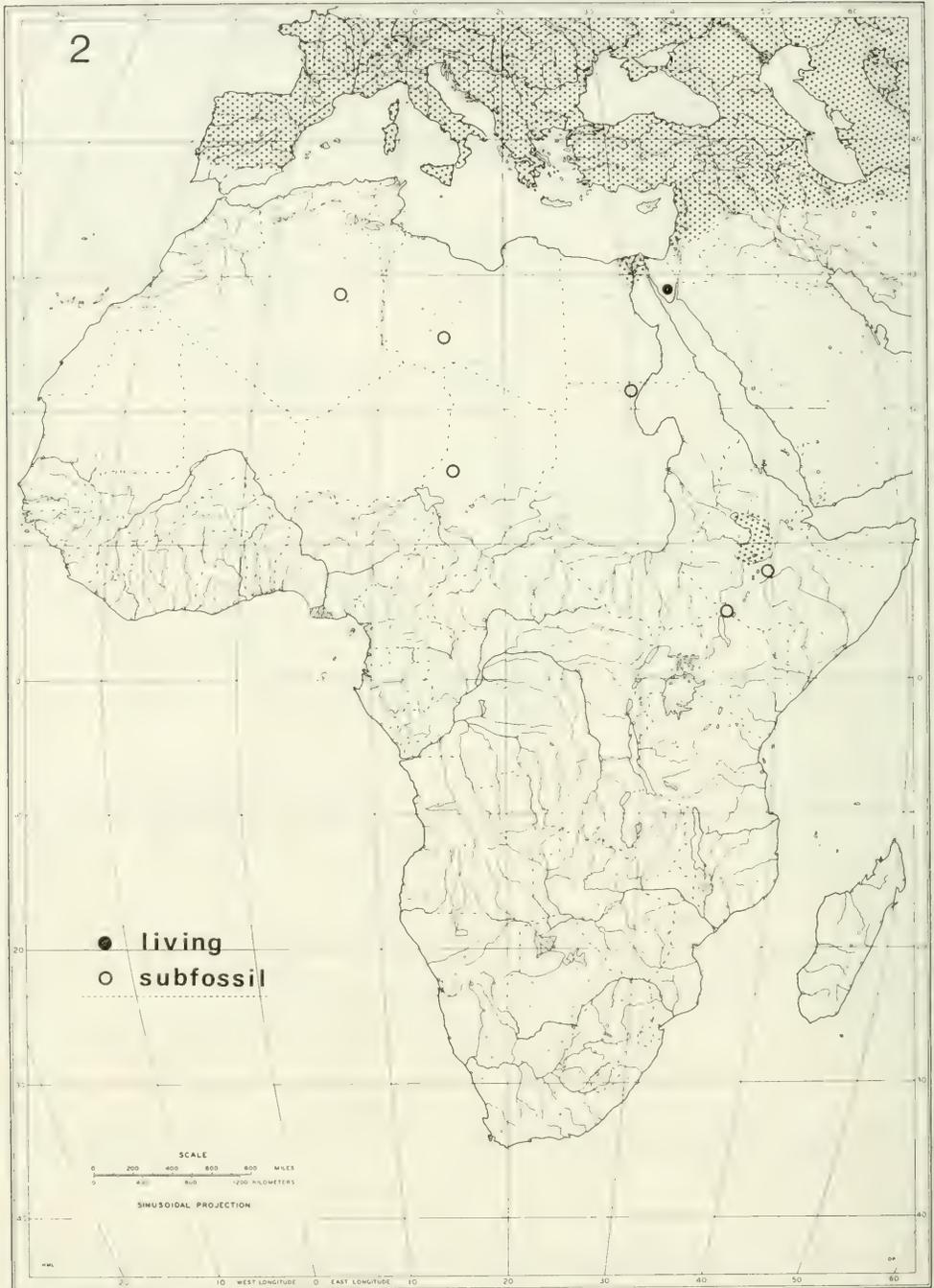


FIG. 2. The distribution of *Valvata* in Africa, southern Europe and South West Asia. This genus appears to be absent from the southern part of Iberia. Isolated localities are indicated for living populations in the Sinai peninsula, and for shells in Africa.

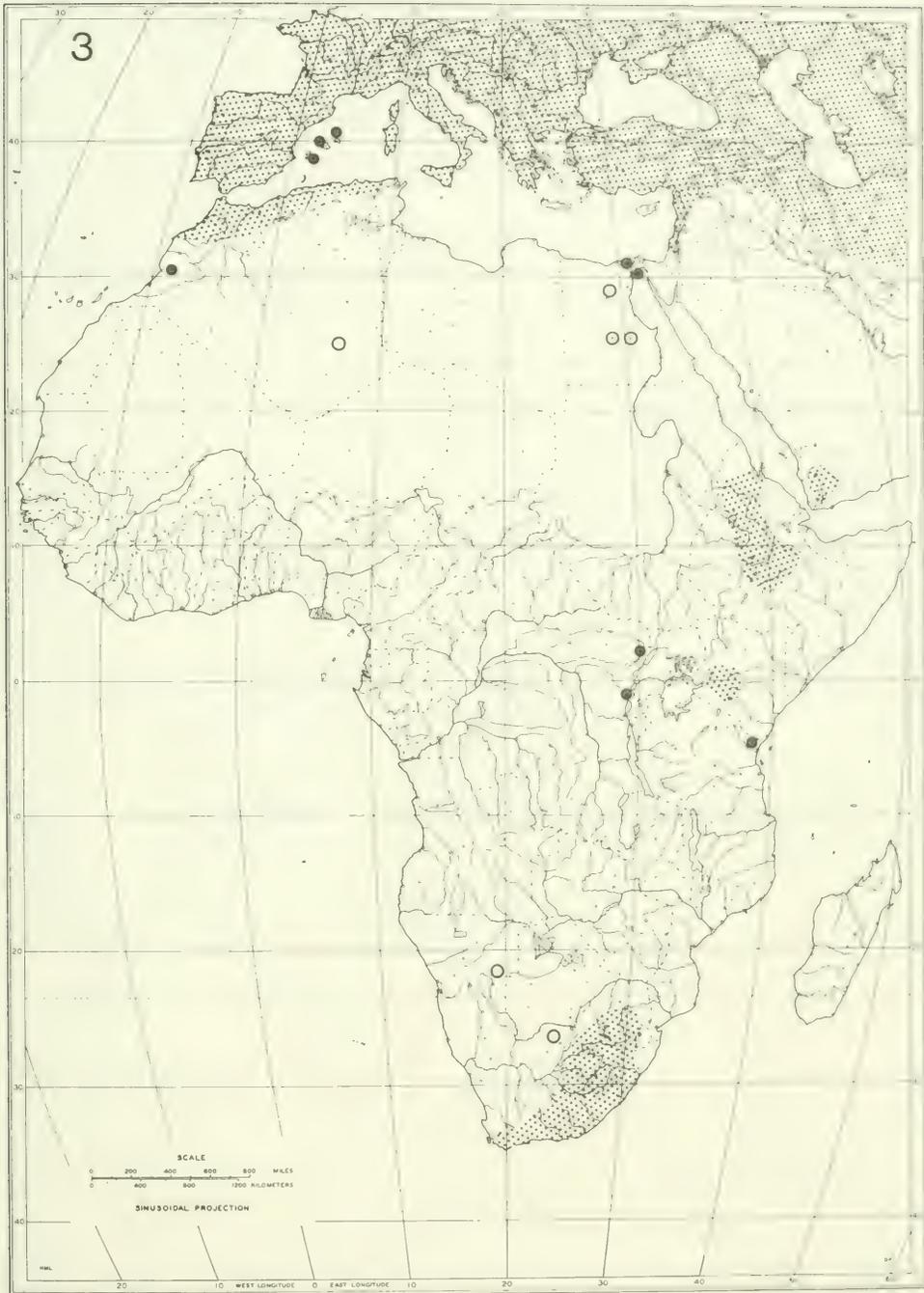


FIG. 3. The distribution of *Lymnaea truncatula* in Africa, southern Europe and South West Asia. Isolated localities are indicated for living populations and 'subfossil' shells.

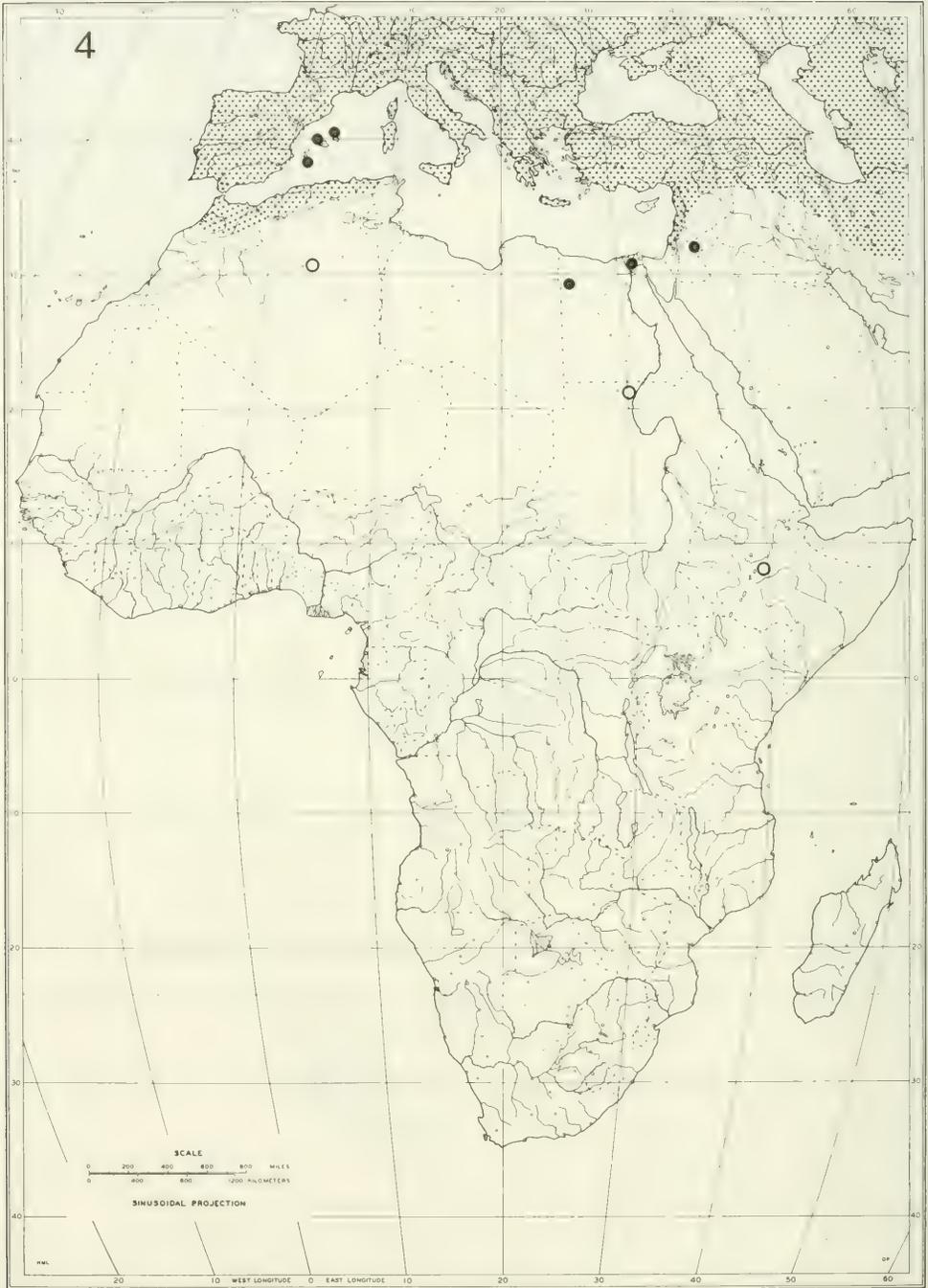


FIG. 4. The distribution of *Planorbis planorbis* in Africa, southern Europe and South West Asia. Isolated localities are indicated for living populations and 'subfossil' shells.

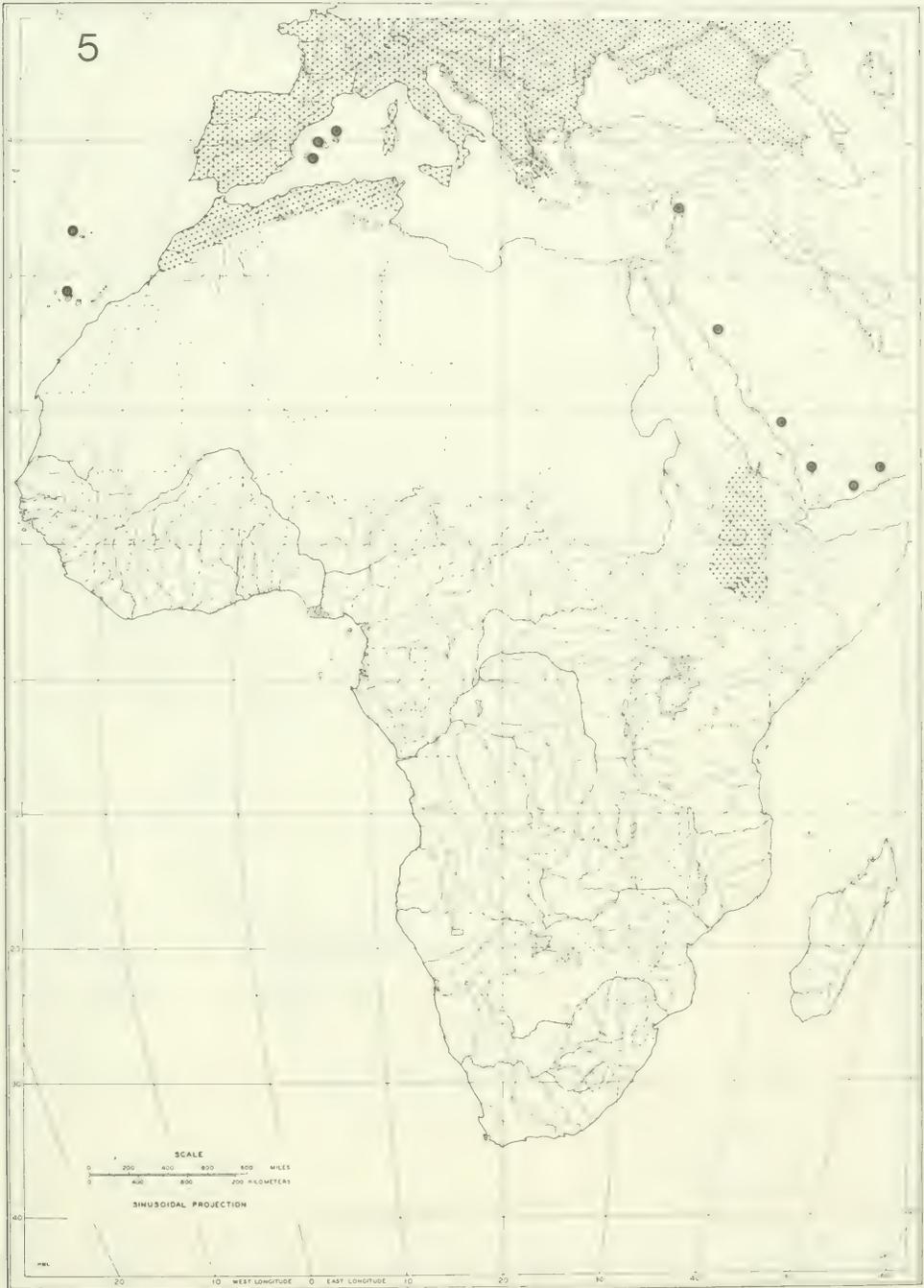


FIG. 5. The distribution of *Ancyclus* in Africa, southern Europe and South West Arabia. Isolated localities are indicated, including Madeira and Teneriffe for living populations or recently living specimens. There are also records for the Cape Verde islands. *A. fluviatilis* appears to be present almost throughout this range.

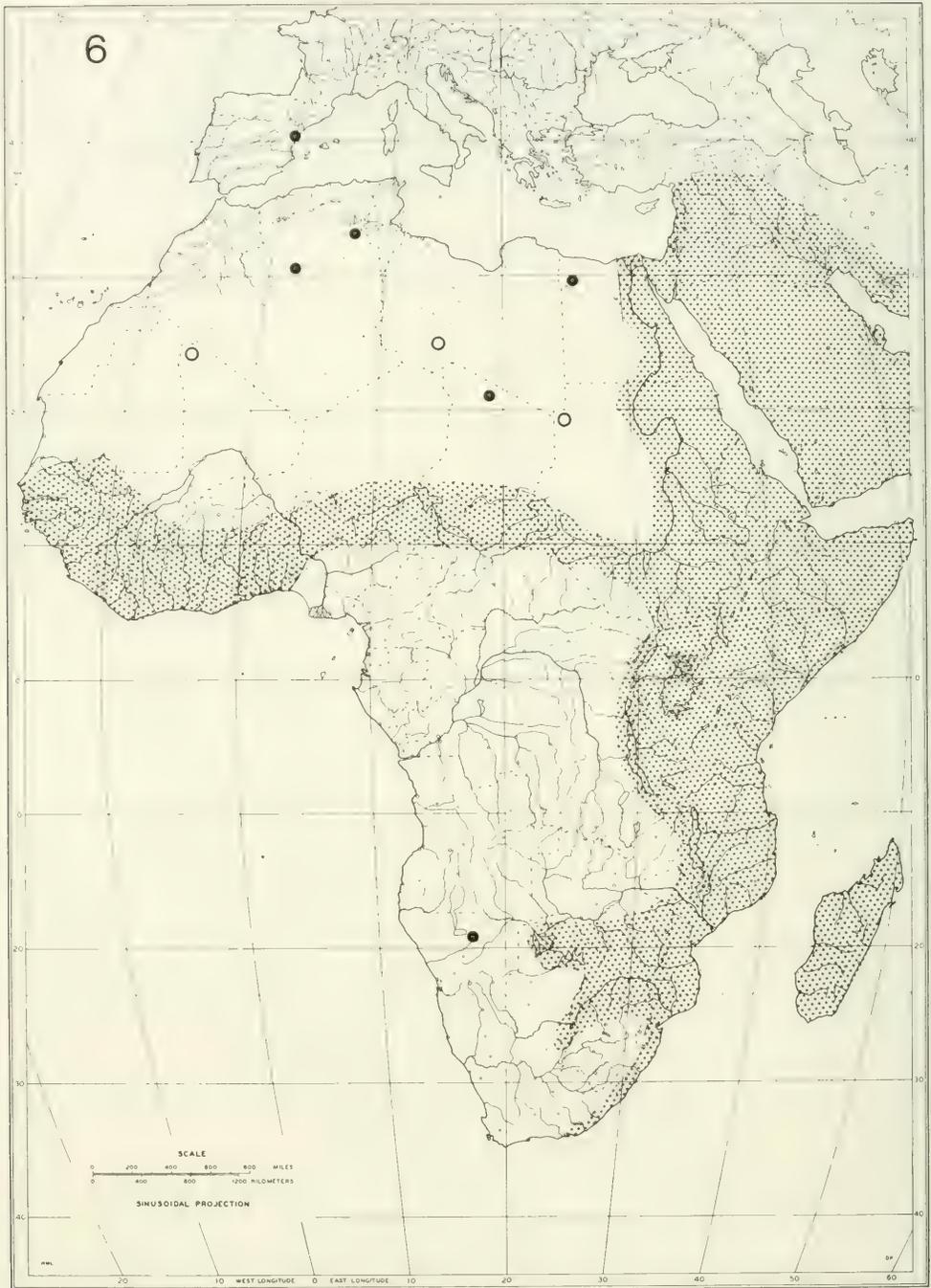


FIG. 6. The distribution of *Melanoides tuberculata* in Africa, southern Europe and South West Asia. The range extends eastwards through southern Asia to China, the Pacific islands and Australia. Isolated African localities are indicated for living populations and 'subfossil' shells. This snail is comparatively uncommon in West Africa.



FIG. 7. The distribution of *Bulinus truncatus* and other tetraploid populations of *Bulinus* (including records for '*Isidora contorta*'). Further cytological observations are necessary to establish more precisely the range in central Africa. Isolated localities are indicated for living populations and 'subfossil' shells presumed to belong to tetraploid snails.

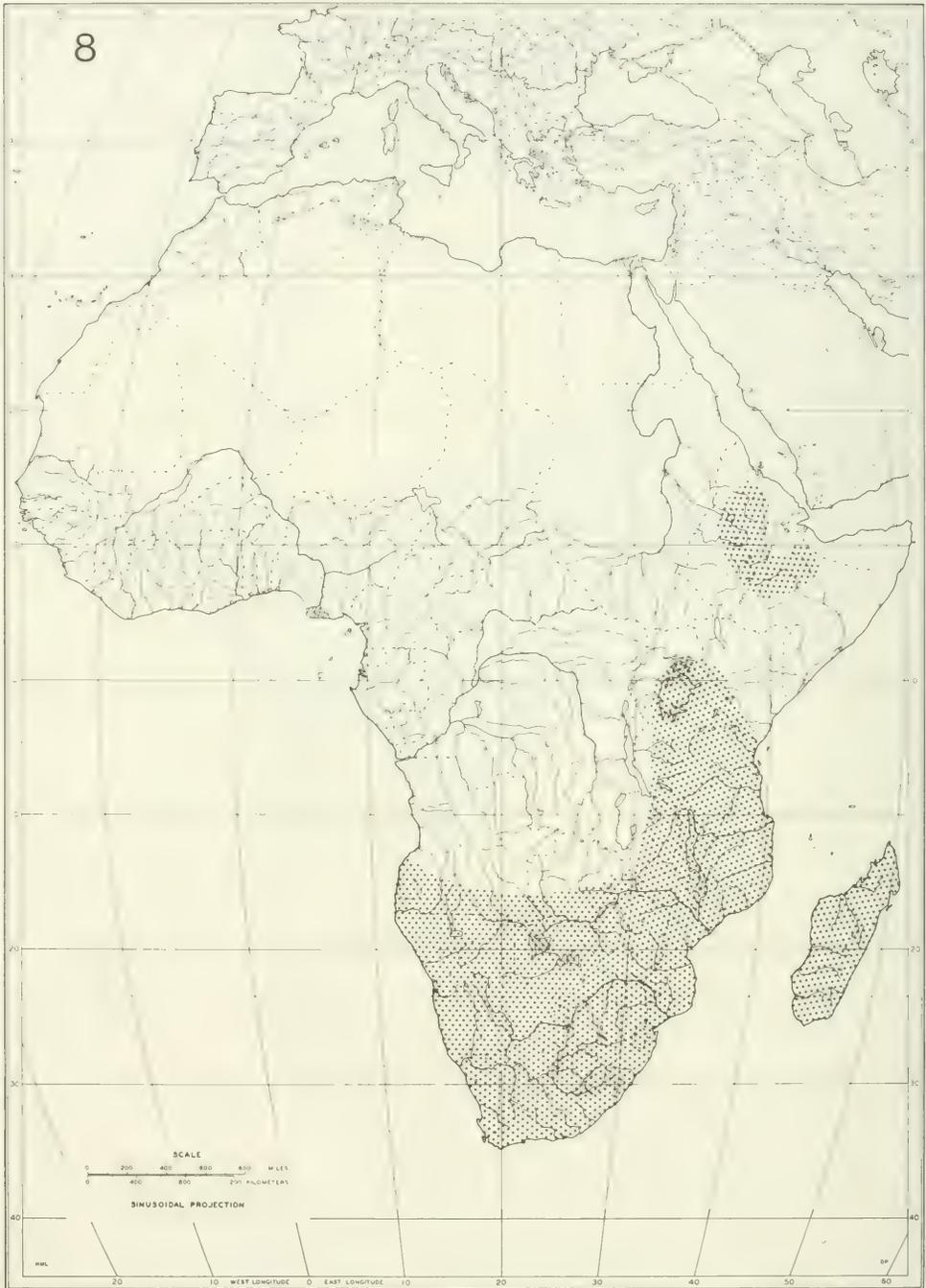


FIG. 8. The distribution of the diploid *Bulinus natalensis/tropicus* complex. Further cytological observations are necessary to establish more precisely the range in central Africa. Even so, there appears to be little overlap with the range of the related tetraploid populations (compare Fig. 7).

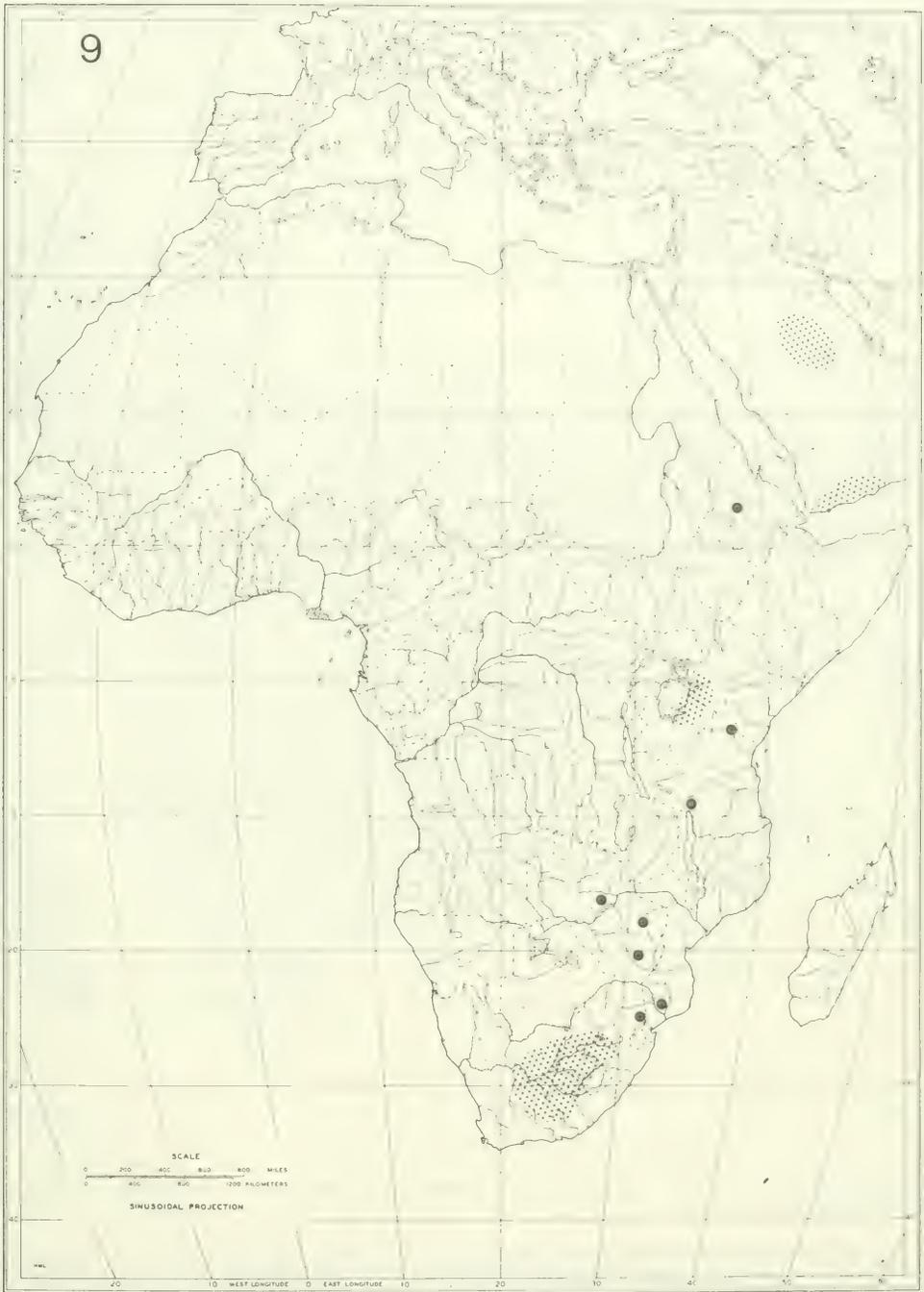
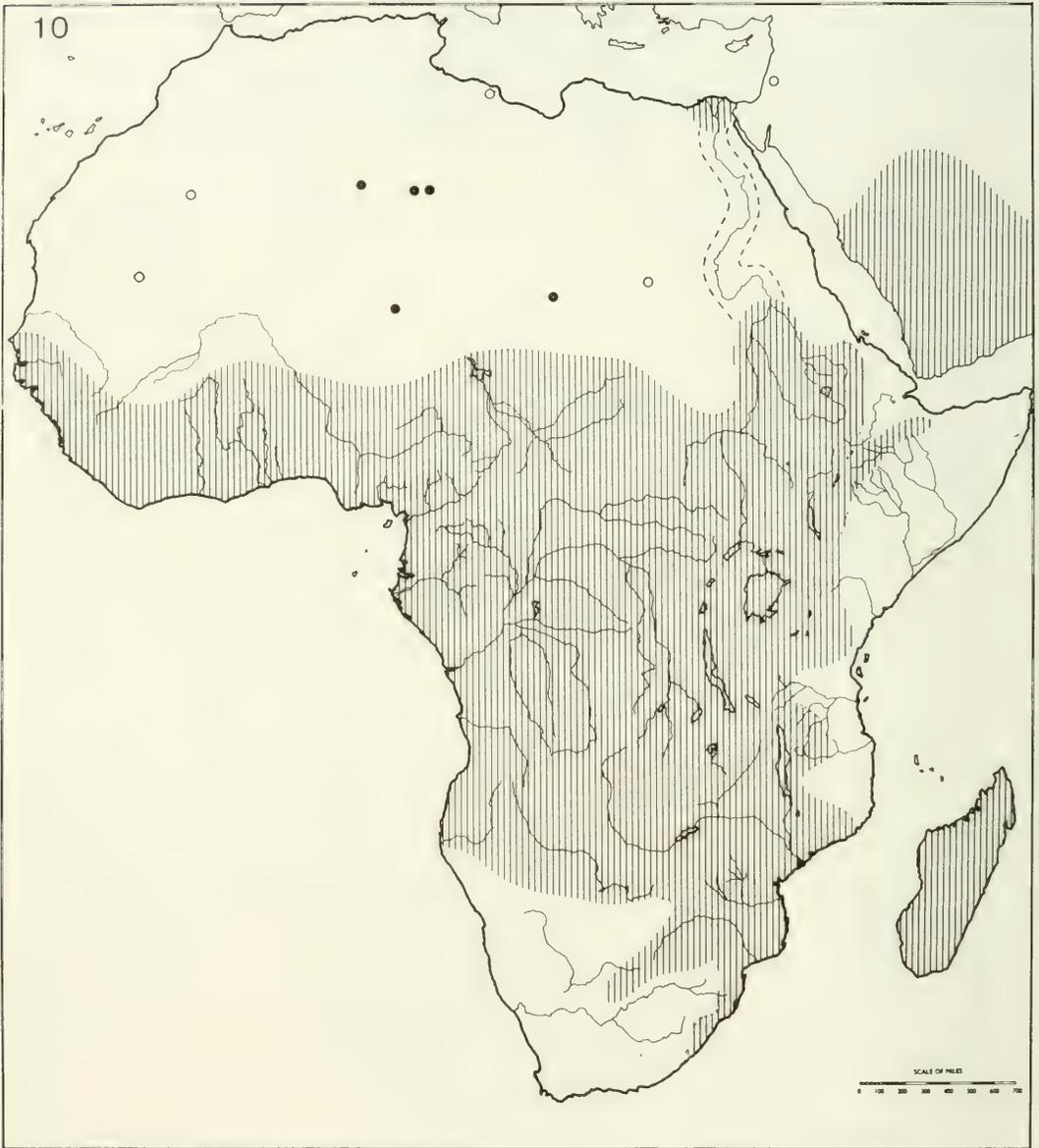


FIG. 9. The distribution of the *Bulinus reticulatus* species group. These snails occur in small seasonal waterbodies and are distributed discontinuously even within the shaded areas. *B. reticulatus* is present in Africa and *B. wrighti* in Arabia.



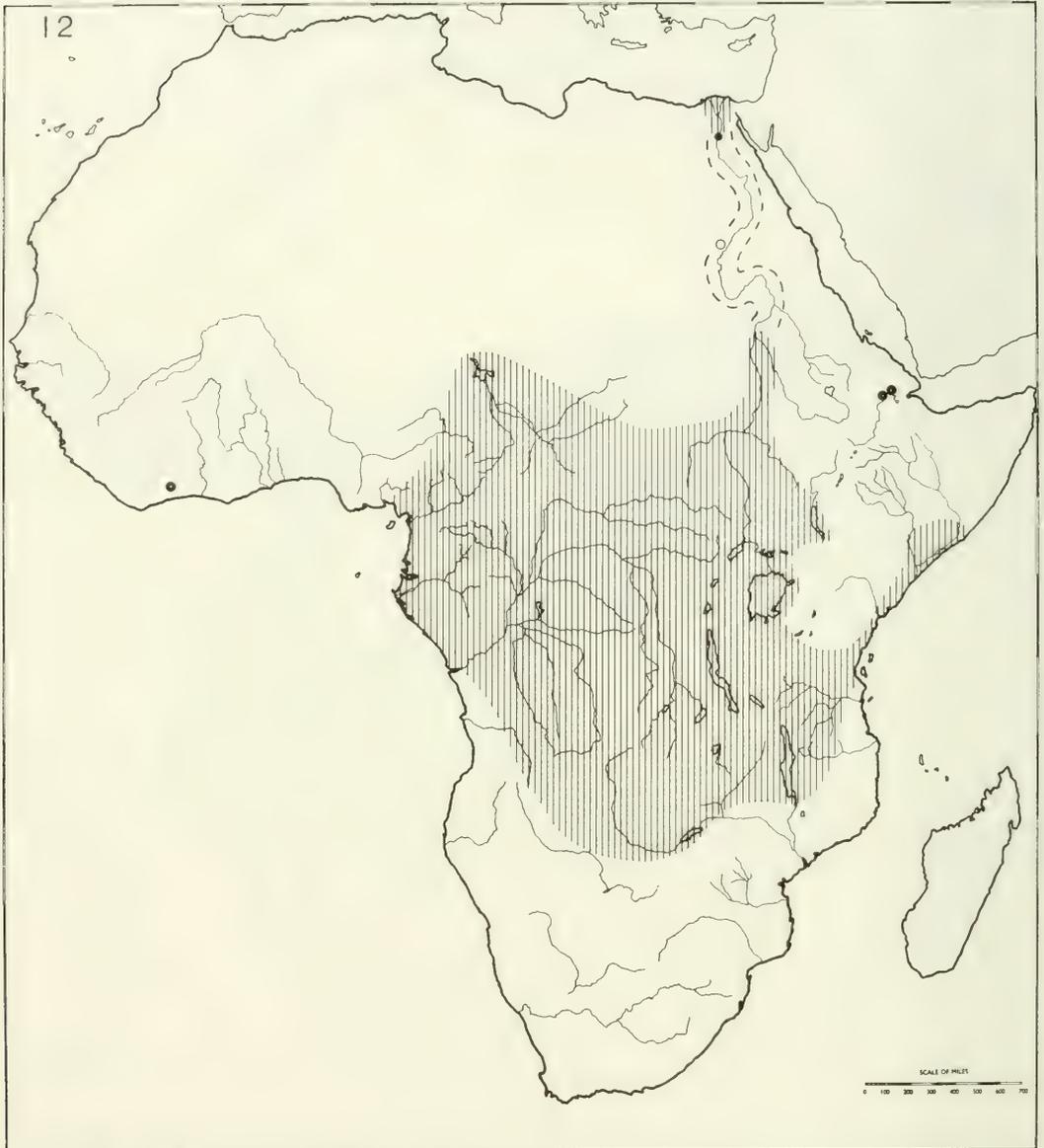
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FIG. 10. The distribution of *Biomphalaria* in Africa and Arabia. The genus is present also in the Neotropical region. Isolated localities are indicated for living populations and 'subfossil' shells. The locality on the Libyan coast (W. H. Wright, 1973) requires confirmation.



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FIG. 11. The distribution of *Bellamyia* in Africa. Isolated localities are indicated for living populations and 'subfossil' shells. Confirmation is required of records for Madagascar (Fischer-Piette & Vukadinovic, 1973), Yemen (Ayad, 1956) and in the Pleistocene fauna of the Jordan Valley (Tchernov, 1975).



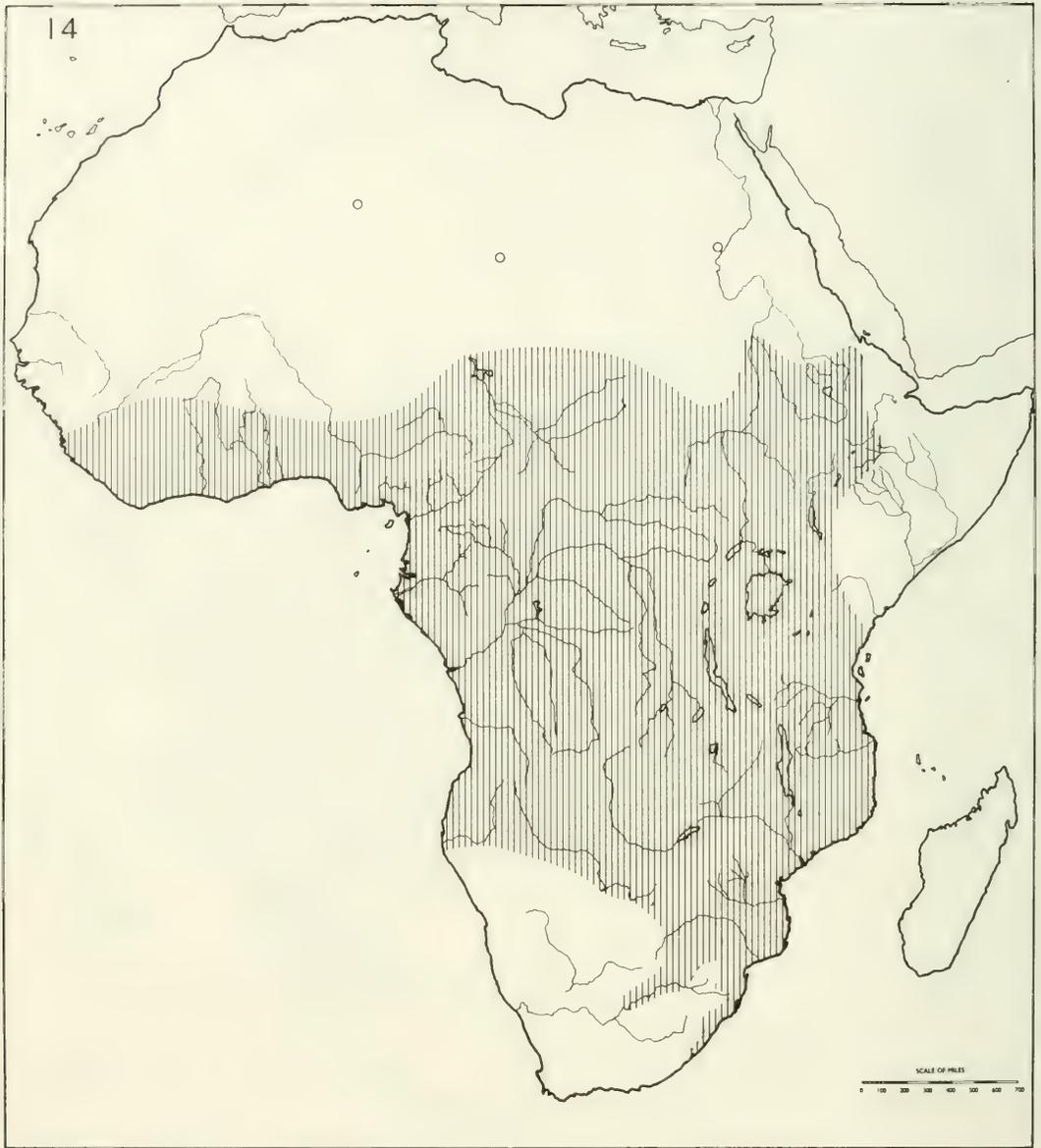
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FIG. 12. The distribution of *Gabbiella* and related genera (Bithyniidae). Most species are restricted to central Africa (Mandahl-Barth, 1968). However, *G. adspersa* occurs in isolated localities in eastern Ethiopia, and *G. senariensis* lives in Egypt. The isolated locality shown in Ivory Coast represents '*Bithynia*' *tournieri* Binder (1955).



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FIG.13. The distribution of the *Bulinus africanus* group. Isolated localities are indicated for living populations and 'subfossil' shells. There is no evidence that this group has penetrated further northwards, either in the Sahara or down the Nile. The area indicated in Madagascar represents *B. obtusispira* which shows some relationship with the *B. africanus* group (C. A. Wright, 1971).



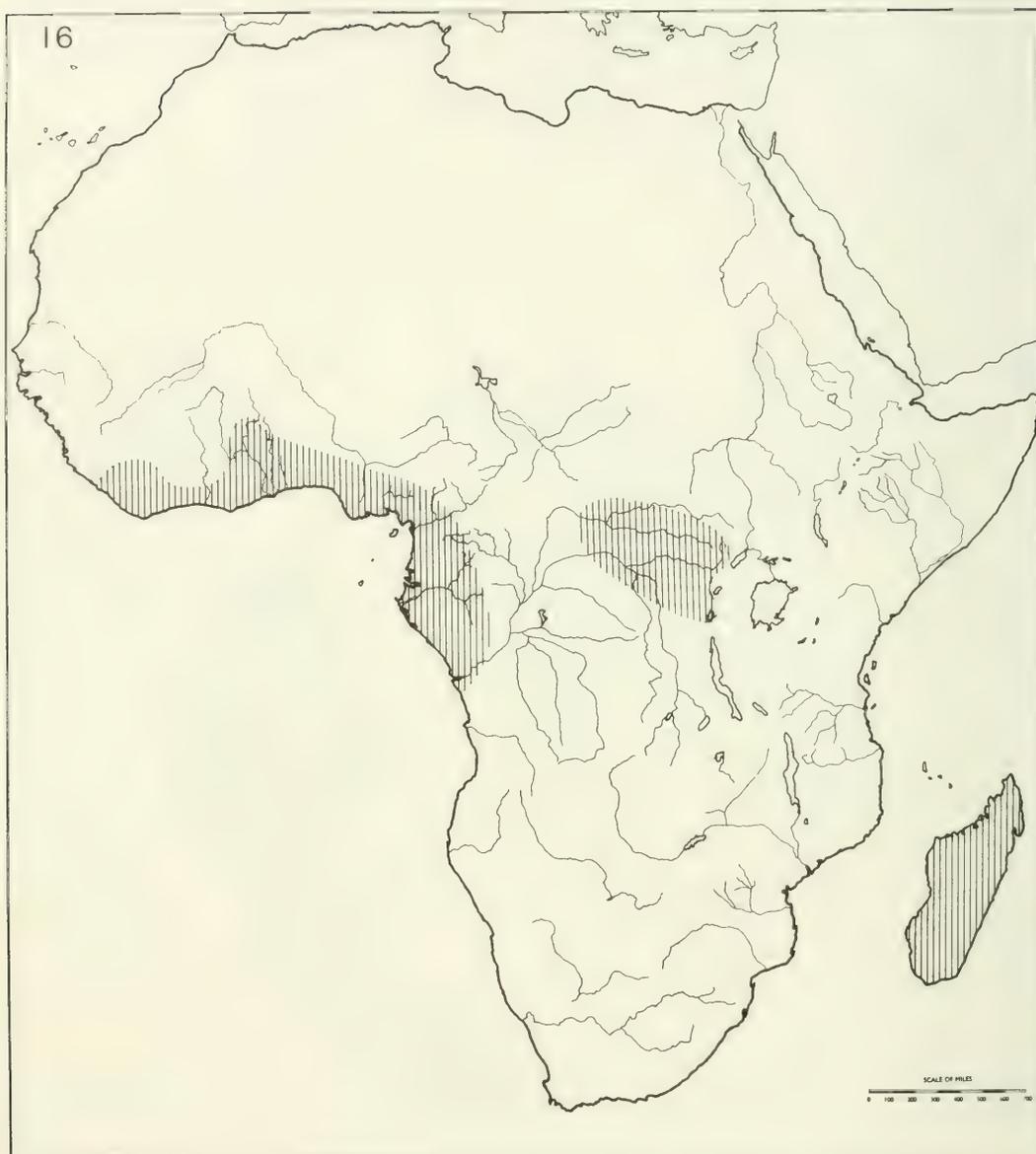
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FIG. 14. The distribution of *Gyraulus costulatus*. Isolated localities are indicated in the Sahara for 'subfossil' shells. The genus is represented in Egypt by a different species, *G. ehrenbergi*, which appears to be related to members of this genus living in South West Asia. *Gyraulus* is unknown in Madagascar, though present in the Mascarene islands.



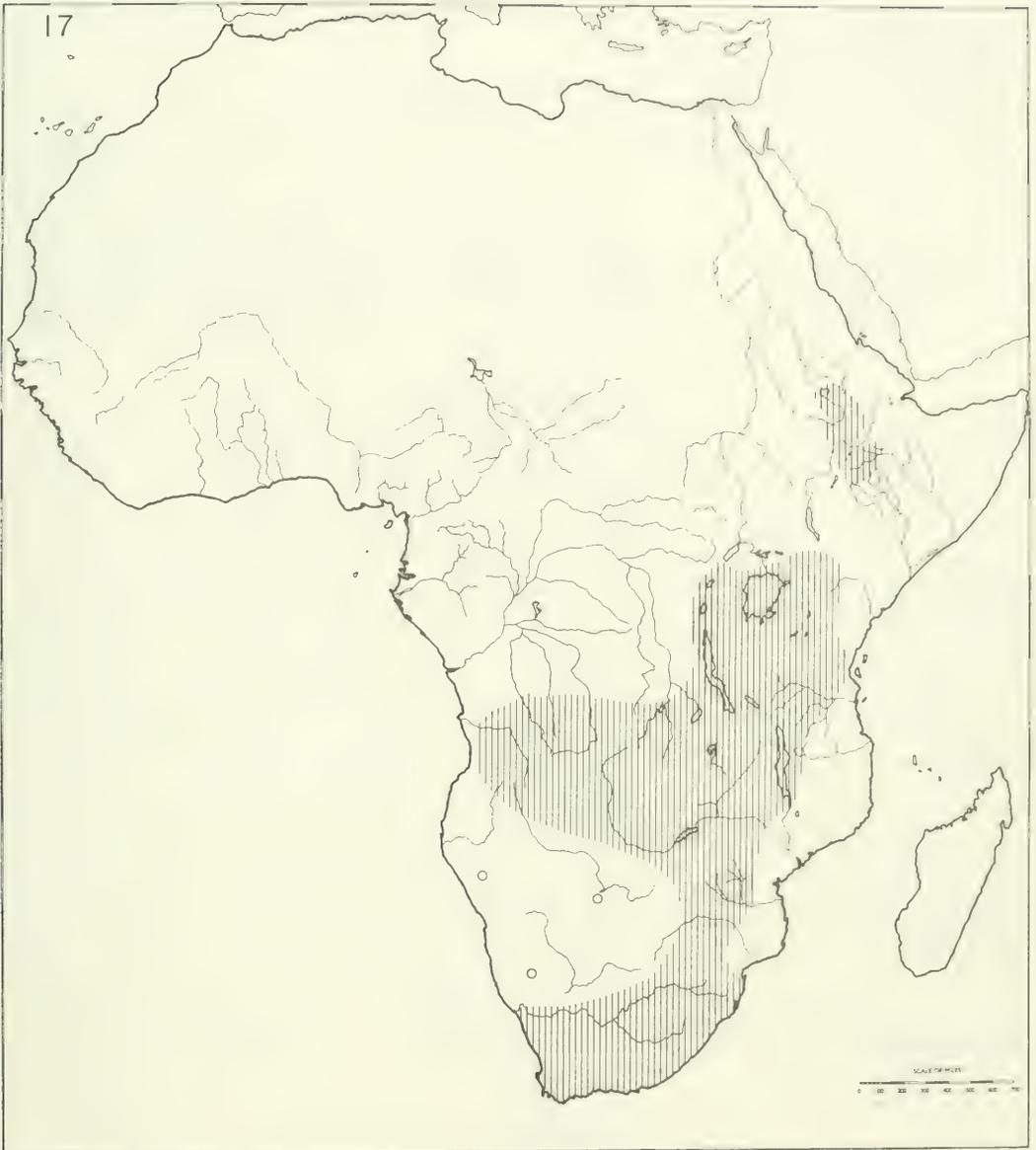
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FIG. 15. The distribution of *Ceratophallus natalensis* (recorded in earlier literature as '*Anisus*'). Isolated localities are shown for Lake Chad and in Zaire. Most other members of this genus are confined to lakes in East Africa.



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FIG. 16. The distribution of *Potadoma* and *Melanatria*. *Potadoma* lives in streams in two forested areas, one between Liberia and Lower Zaire and the other comprising part of northeastern Zaire and the adjacent Central African Republic. *Melanatria* is endemic to Madagascar, and related genera occur in southern Asia and on some Indonesian islands.



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FIG. 17. The distribution of *Burnupia*. These ancyliids are recorded most frequently from streams in the highlands of Ethiopia and in the temperate region of South Africa. Isolated localities for 'subfossil' shells are situated in the now semi-arid areas of Botswana and Namibia. Probably *Burnupia* is more widespread in central Africa than available records indicate.

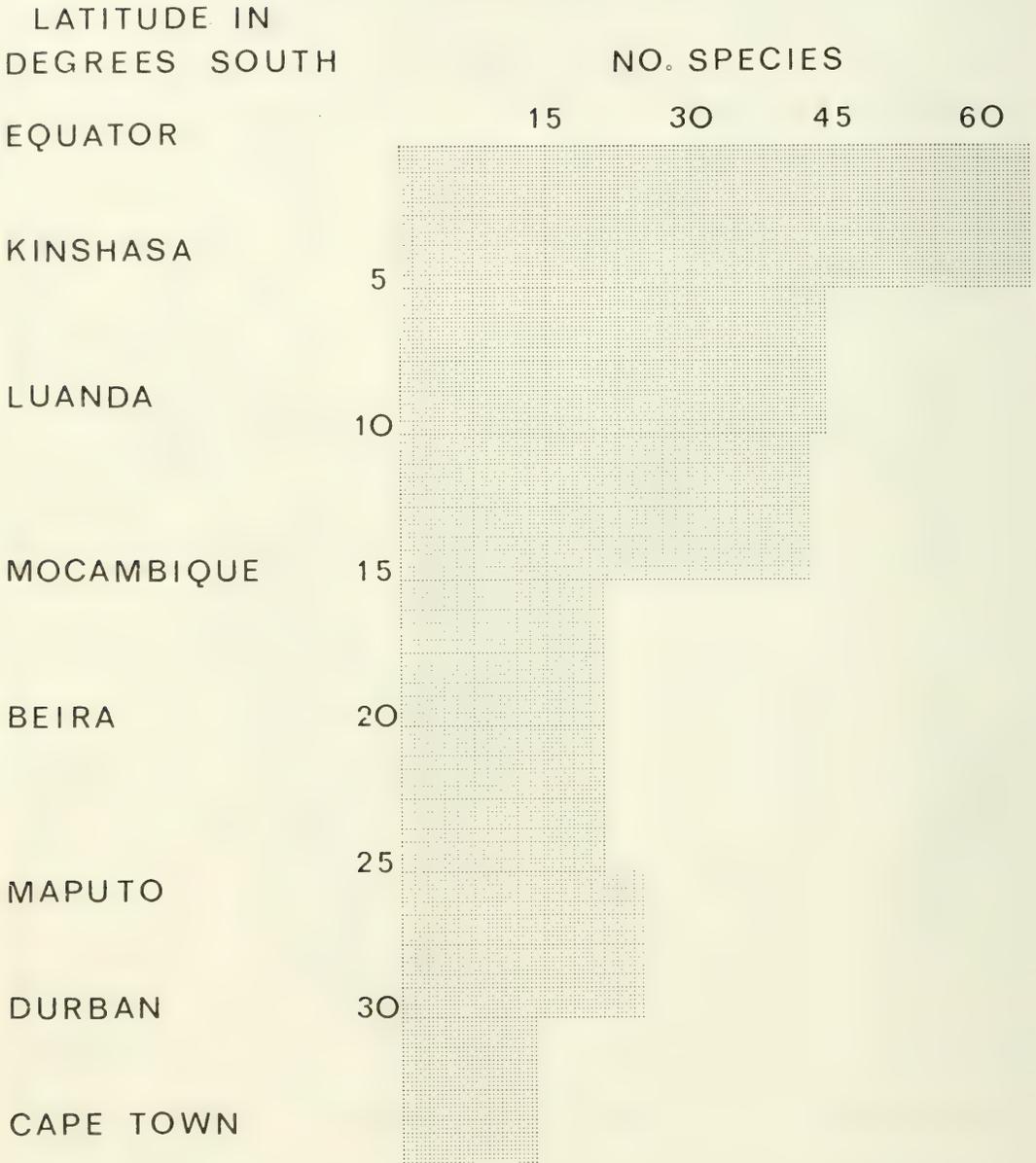


FIG. 18. Numbers of species of freshwater gastropods known in Africa south of the Equator, in latitudinal zones of 5 degrees. Excluded are species endemic to lakes, the genus *Tomichia* and the Ancyliidae. Based on Brown (1978 and in press).

SURVEY OF NON-MARINE MOLLUSCS OF SOUTH-EASTERN AUSTRALIA

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ABSTRACT

A survey is undertaken of the non-marine molluscs of south-eastern Australia in connection with future land use in this densely populated area with a concentration of heavy industry. The area harbours ca. 220 species in 39 families with a very high proportion of endemism.

INTRODUCTION

It has long been recognised that the flora and fauna of Australia shows distinct regionality (McMichael & Iredale, 1959) with a high incidence of regional endemism. The non-marine mollusc fauna can be divided into a number of regional faunal groups, each with its own characteristics and each dominated by one generic or family grouping. This dominance, both in terms of numbers of species and of individuals, takes the form of a radiation within one group.

The Euronotian Region, containing the Bassian sub-region, occupies the south-eastern part of Australia and consists of Victoria and Tasmania including the Bass Strait Islands and the southern parts of South Australia and New South Wales. This area is less than 20% of Australia's land surface but contains over 80% of the human population including the Federal and four State capitals and much of the heavy industry. The region is therefore the most man-modified of any of the faunal regions of Australia, with little completely natural bush or unchanged aquatic habitat. However, much of the native mollusc fauna remains, with remarkably little change, and new land use studies are producing demand-pressures for detailed taxonomic and distributional data of this fauna.

The present survey is being undertaken to acquire systematic distributional data of the non-marine mollusc fauna of south-eastern Australia. The area under study is bounded by the 33° South line of latitude between the Pacific Ocean and Spencer Gulf, South Australia. The area is divided into major grid areas of 1.5 degrees by 1 degree corresponding to the 1:250,000 standard map sheets. These areas are divided into 54 ten minute grid squares, there being over 2,500 grid squares in the survey area. It is intended to eventually produce an atlas of dot maps of the fauna based on this system.

Running concurrently with the distributional work is a series of taxonomic revisionary studies of various problem groups within the fauna based on the collections made for the survey. This is based on the early descriptive work of Iredale (1933 et seq.), Gabriel (1930) and many others.

HABITAT TYPES

The area is cool temperate to warm temperate, extending from 44°S to 33°S. Terrestrial habitats vary from the hot dry mallee regions in the north and west to the true alpine regions of the Great Dividing Range in Victoria and New South Wales and the Central Plateau in Tasmania. The southern slopes of the Great Divide and the western regions of Tasmania have large areas of temperate rain-forest with deep fern gullies and extensive litter and fungal growth. Large areas of dry sclerophyll forest occur on the more open slopes where the rainfall is lower. Coastal heathland comprises another major terrestrial habitat with extensive salt-marsh areas in sheltered embayments. Extensive land clearing has been carried out in all these habitats with the land being sown down with introduced pasture grasses or with monoculture of pines, wheat or other crops.

Many major rivers including the Murray, rise in the mountain areas of the region. Groups of isolated freshwater lakes are found in south-western Tasmania while a series of saline lakes occurs in western Victoria. However, most non-marine aquatic habitats are subject to regular severe drought cycles resulting in a consequent depauperate fauna. The aquatic habitats too have been extensively modified with storage and hydroelectric impoundments being constructed on many of the major rivers. Artificial connection of previously separate river systems has also occurred. Large areas in the northern part of the region are under irrigation with a consequent change in flow and salinity regimes.

FAUNA

The non-marine mollusc fauna of south-eastern Australia consists of about 220 species in 39 families (see Appendix). It is intended to produce a field guide to this fauna next year. Brief notes are given below to some of the more interesting aspects of the fauna.

About 30 introduced species have been recorded. Most of these originate in Europe and are classed as serious pests, and include 9 species of Helicidae and 9 species of slugs (Altena & Smith, 1975). A recent aquatic introduction is *Lymnaea columella*, an occurrence causing a great deal of concern because of its implication with sheep liver fluke. While many of the introduced species show wide distribution throughout the region, most of the native species have restricted distribution limits.

Tasmania has several unique faunal features due to its long period of isolation, though it is part of the south-eastern faunal region due to the intermittent Bass Strait land bridge. Transition zones occur between this region and the two adjacent regions, the central region and the east coast region. A high proportion of the fauna (over 60%) is endemic to the region with several species having a very restricted distribution.

The terrestrial fauna is dominated by the endodontoid snails. These are small to minute snails, most of which are 3 mm or less in diameter, belonging to the families Charopidae and Punctidae. The taxonomic status of many of the species in these families is unsure but it is estimated that the fauna contains about 54 species of charopids and 9 species of punctids. Most of these are endemic to the region with many of the charopids having very restricted distributions, being confined to one range of mountains (Smith, 1977) or area of forest. Contrasting with this dominance of the endodontoids are two families of snails, the Camaenidae and the Pupillidae, which, while having a widespread and even dominant role in the fauna of Australia as a whole, have a diminished role in this region. Nine or ten species of camaenids are described for the region but all except 1 or 2 have close affinities with the adjacent regions and are restricted to the northern part. No camaenids are found on Tasmania. The pupillids show a similar reduction in species numbers in a southerly direction, with only 1 or 2 of the 11 species known from the region found in Tasmania.

Another important element is the group of carnivorous snails belonging to the family Rhytididae. Thirteen species of this family, or almost half the Australian fauna, occur in this region, almost all being endemic including the endemic genus *Victaphanta* (Smith & Kershaw, 1971). The family Caryodidae contains the largest species of the region including two genera, *Caryodes* and *Anoglypta*, endemic to Tasmania. The genus *Bothriembryon*, belonging to the family Orthalicidae, shows a wide species radiation in the south-western Australian faunal region. However, two species occur in the southern and western regions of the south-eastern region including one species endemic to Tasmania. The final element of special interest in the terrestrial fauna is the group of native slugs belonging to the Cystopeltidae. These are common throughout the forest regions of Tasmania, Victoria and southern New South Wales and the family extends up as far as southern Queensland.

The aquatic non-marine fauna includes some supra-littoral marine and salt marsh species which can be found associated with terrestrial flora and fauna. It also includes estuarine and hyper-saline species as well as freshwater species inhabiting rivers, creeks, swamps and dams. The major family of aquatic snails is the Hydrobiidae, species of which are found in estuarine creeks, in hyper-saline lakes and in high alpine acid bogs. Of particular interest is the genus *Coxiella*, large populations of which are found in the salt lakes of western Victoria and the dune salt lakes of the Victorian, South Australian and Tasmanian coasts. Two species of the

aberrant hydrobiid genus *Glacidorbis* are found in acidic alpine bogs of Victoria and Tasmania (Meier-Brook & Smith, 1975).

Two operculate families, the Viviparidae and the Thiaridae, have species found only in the River Murray and its tributaries. The family Planorbidae is the most widely distributed aquatic group with respect to total area covered. The high-spired forms belonging to the genera *Bulinus*, *Physastra* and *Glyptophysa* are to be found in most freshwater habitats. The planispiral planorbids are less common but still make up a significant part of the fauna. The final group worthy of mention are the freshwater mussels belonging to the family Hyriidae. Ten species occur in the region including a few endemic species with very restricted ranges.

ACKNOWLEDGEMENTS

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APPENDIX

List of families of non-marine molluscs in south-eastern Australia, with approximate number of species in each family (I = contains introduced species; E = contains species endemic to the region).

Viviparidae	— 1	Rhytididae	— 13 (E)
Hydrobiidae	— 18 (E)	Caryodidae	— 4 (E)
Truncatellidae	— 2	Orthalicidae	— 2 (E)
Bithyniidae	— 1	Charopidae	— 54 (E)
Assimineidae	— 2	Punctidae	— 9 (E)
Thiaridae	— 1	Arionidae	— 3 (I)
Ellobiidae	— 8	Zonitidae	— 6 (I)
Amphibolidae	— 2	Limacidae	— 5 (I)
Physidae	— 1	Milacidae	— 1 (I)
Lymnaeidae	— 4 (I)	Cystopeltidae	— 2 (E)
Planorbidae	— 11 (I) (E)	Euconulidae	— 3 (I)
Ancylidae	— 2 (E)	Helicarionidae	— 6 (E)
Onchidiidae	— 5	Testacellidae	— 1 (I)
Succineidae	— 2	Camaenidae	— 9 (E)
Athoracophoridae	— 1	Bradybaenidae	— 1 (I)
Achatinellidae	— 1	Helicidae	— 9 (I)
Cionellidae	— 1 (I)	Hyriidae	— 10 (E)
Pupillidae	— 11 (E)	Corbiculidae	— 1
Valloniidae	— 1 (I)	Sphaeriidae	— 3
Ferrussaciidae	— 1 (I)		

TAXONOMICAL, ECOLOGICAL AND ZOOGEOGRAPHICAL RESEARCH ON BULIMULIDAE (GASTROPODA, PULMONATA)

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ABSTRACT

An introduction is given to the land snail family Bulimulidae and the purposes of a systematic research project are explained. A phylogenetic system of the family will be constructed with the aid of the theory of Hennig. Some preliminary results are given, viz. revisions of *Bulimulus* Leach, 1814 sensu Zilch (1960) and *Bothriembryon* Pilsbry, 1894. The most likely relationships of the latter genus are indicated. Furthermore, some ecological data are presented and the theoretical background of the zoogeographical analysis is indicated.

INTRODUCTION

The Bulimulidae form a relatively large family, mainly confined to the Neotropical Region. At present the family includes 144 genera and subgenera. The number of specific and subspecific names available is estimated at about 3,000. It is supposed that a critical revision might reduce these numbers to about 100 and 1,000 respectively.

The family is subdivided into five subfamilies, viz. Bulimulinae (the largest subfamily with ca. 90 genera and subgenera; mainly in South America, but also found in Central America, the West Indies and SW Australia), Amphibuliminae, Odontostominae (both confined to South America and the West Indies), Orthalicinae (South and Central America, West Indies) and Placostylinae (Melanesia and New Zealand).

The aim of the present research project is to carry out a revision of the (sub)genera of the Bulimulinae and to establish the phylogenetic relationships of these genera and of the five subfamilies. The resulting phylogeny and the ecological observations made during field research will form the basis for the zoogeographical analysis.

TAXONOMY

When constructing a phylogenetic system of this family the theory of Hennig will be used. This theory is characterized by the use of monophyletic groups, which are "groups of species that arose by species cleavage, ultimately from a common stem species that is the stem species only of those species included in the group in question" (Hennig, 1966). Within such a monophyletic group one should try to find out which characters belong to one and the same phylogenetic transformation series, i.e. which are homologous, and whether they are plesiomorphous ("primitive") or apomorphous ("derived"). Only the joint possession of apomorphous characters (synapomorphy) corroborates the assumption that the species in question belong to the same monophyletic group. As the cleavage of a species ultimately leads to the formation of (theoretically not more than) two daughter-species, which each form part of a monophyletic group, it follows that each monophyletic group will have one sister-group to which it is more closely related.

The present classification of the Bulimulidae is entirely based on morphological characters of the shell. The most important of these characters is the sculpture of the protoconch. Although this is certainly a very helpful criterion for classification it does not always lead to an

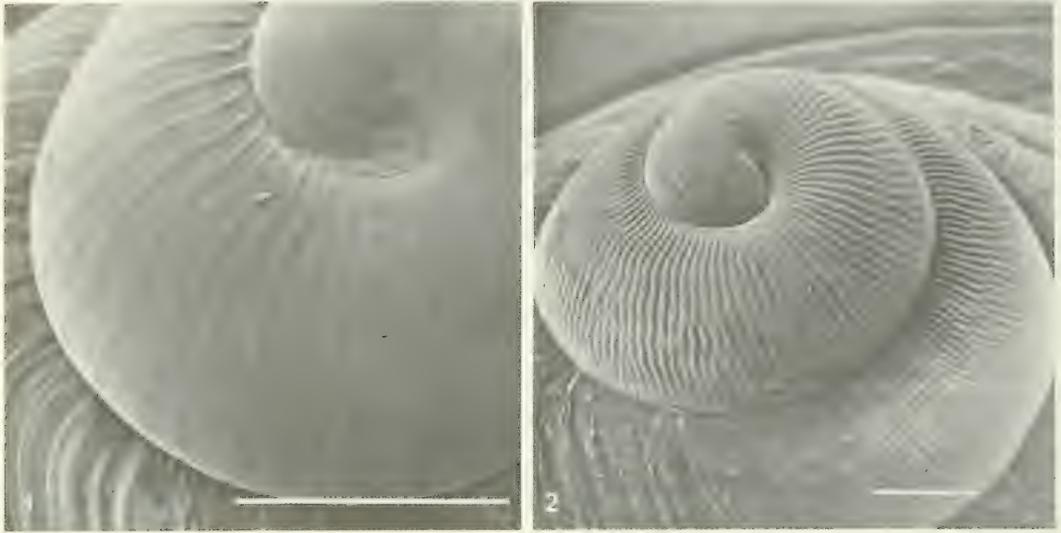


FIG. 1. Protoconch of *Bostryx ploegerorum* Breure.

FIG. 2. Protoconch of *Naesiotus wolffi* (Reibisch). Scales 0.5 mm.

unambiguous identification, which is shown, e.g., by the case of *Naesiotus (Naesiotellus) latecolumellaris* Weyrauch, 1967. This species, for which the monotypical subgenus *Naesiotellus* Weyrauch, 1967, was created, belongs instead to *Bostryx* Troschel, 1847; Weyrauch has probably been misled by the weak axial riblets of the protoconch sometimes to be found among *Bostryx* species. These riblets, however, are never as strong and regular as those of *Naesiotus* species (Figs. 1-2).

The following characters will now be used as a base for the phylogeny: (1) the morphology of the genitalia; (2) the internal structure of the genitalia, especially of the phallus complex; (3) the morphology of the radula and mandibula; (4) the structure of the protoconch; (5) the morphology of the pallial organs; (6) the morphology of the muscle system.

At present the results have not yet led to the recognition of a complete phylogenetic transformation series for any of the above-mentioned characters. But such characters as the morphology of the radula and the internal structure of the genitalia show already a certain pattern in which parts of a transformation series may be recognized.

Two examples of the preliminary results will now be given.

A. The genus *Bulimulus* Leach, 1814 sensu Zilch (1960) has been revised as follows: the nominate subgenus is widespread in the Neotropics and is characterized e.g. by (1) the structure of the protoconch (axial wrinkles, often anastomosing on lower part of whorl), (2) the morphology of the phallus complex (especially by the club-shaped penis) and (3) the internal structure of the phallus complex (penis with parallel tubes and epiphallus penetrating into penis; Fig. 3). *Rhinus* Albers, 1850, is retained as a subgenus of *Bulimulus*, differing mainly by (1) the structure of the protoconch (zigzag wrinkles) and (2) the presence of spiral series of epidermal hairs on the shell. The other taxa grouped by Zilch under *Bulimulus* are now treated as separate entities: *Rabdotus* Albers, 1850, is given generic status on account of, e.g., (1) the structure of the protoconch (straight axial riblets) and (2) the internal structure of the phallus complex (epiphallus not penetrating into penis; Fig. 4). *Leptobyrsus* Fischer & Crosse, 1875 (with its synonym *Puritania* Jacobson, 1958) and *Plicolumna* Cooper, 1895, are considered subgenera of *Rabdotus*. *Itaborahia* Maury, 1935, is also given generic status. The species of this genus, which are known from Miocene deposits in Brazil, also have a pattern of axial riblets on the protoconch (Breure, unpublished). *Dentaxis* Pilsbry, 1902, is transferred to *Bostryx* Troschel, 1847, because of the structure of the protoconch and of the anatomy. The anatomy of species

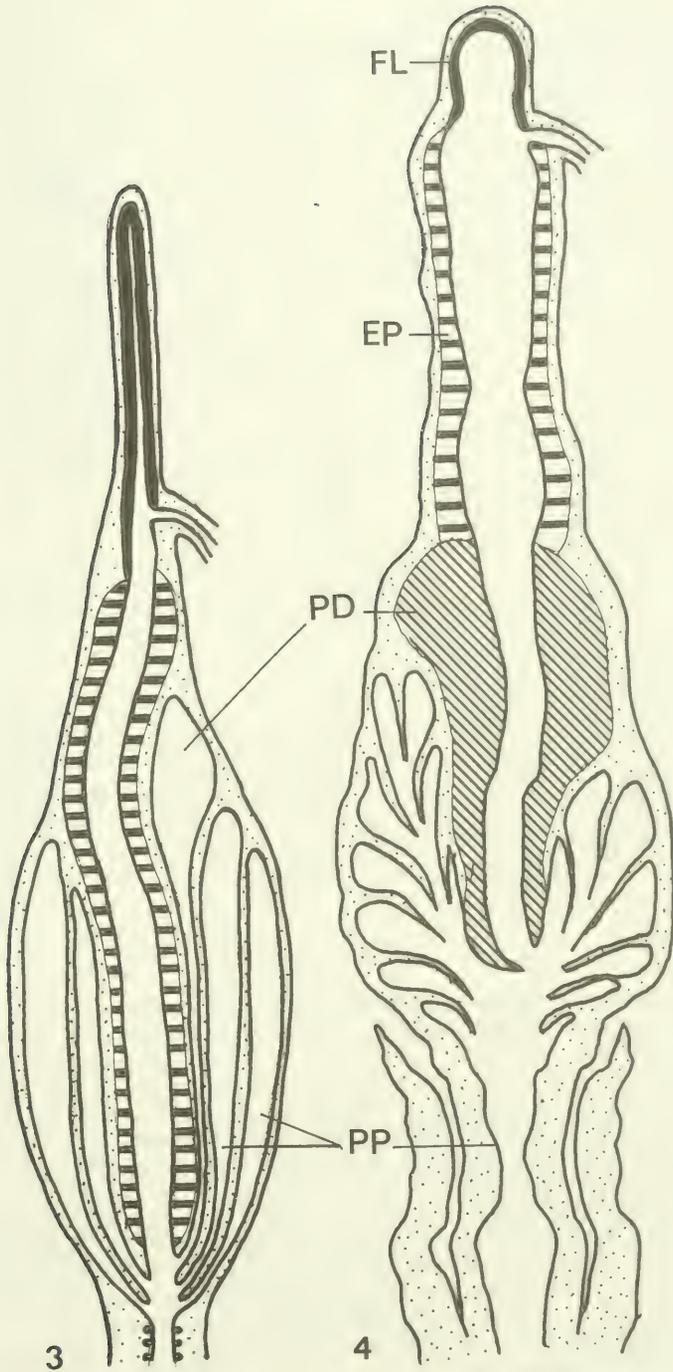


FIG. 3. Schematic reconstruction of the phallus complex in *Bulimulus (B.) guadalupensis* (Bruguière).
 FIG. 4. Do., *Rabdodus (R.) mooreanus* (W. G. Binney). EP = epiphallus; FL = flagellum; PD = distal part of penis; PP = proximal part of penis.

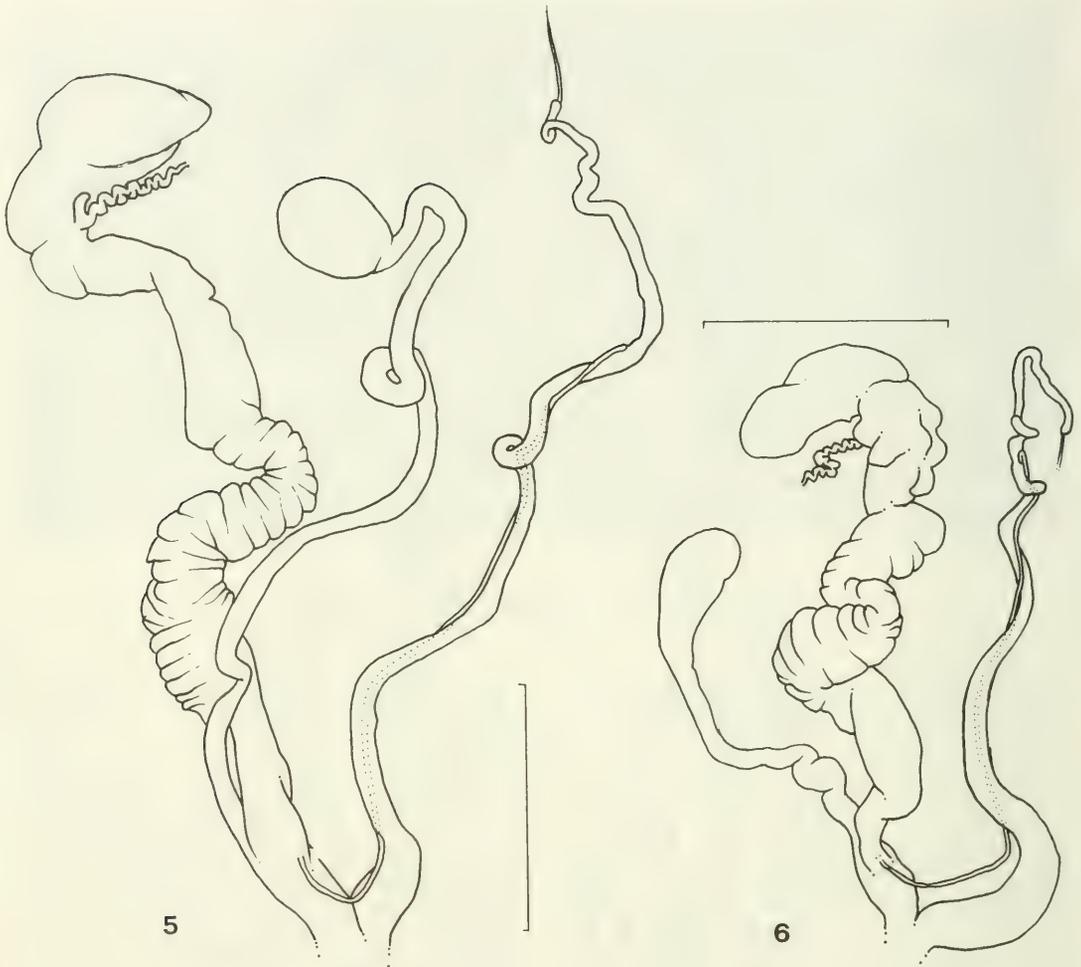


FIG. 5. Genitalia of *Bothriembryon (B.) indutus* (Menke). Scale 1 cm.

FIG. 6. Genitalia of *Bothriembryon (Tasmanembryon) gunnii* (Sowerby). Scale 1 cm.

of *Pseudoxychona* Pilsbry, 1930, demonstrates that this taxon has to be placed near *Leiostracus* Albers, 1850. Finally, all other subgenera mentioned by Zilch are treated as *Naesiotus* Albers, 1850 sensu lato (see Table 1).

B. The taxonomy of *Bothriembryon* Pilsbry, 1894, has been revised considerably. Nearly all subgenera created by Iredale (1933, 1939) could be synonymized with the nominate subgenus on account of the anatomy of their type species; only *Tasmanembryon* Iredale, 1933, proved to be different, viz. by (1) having the spermathecal duct reduced in length (Figs. 5-6) and (2) the different structure of the protoconch. Most probably the sister-group of *Bothriembryon* is *Plectostylus* Beck, 1837, which is found in Chile. The synapomorphic characters of these genera are (1) the presence of a single type of glandular tissue in the penis (Figs. 7-8); (2) the absence of a penis sheath; (3) a certain type of pallial organs, and (4) a certain type of radula (Figs. 9-12). This will be fully worked out in the final revision. Related genera are *Scutalus* Albers, 1850, and "*Peronaeus (Lissoacme)*" from Argentina.

TABLE 1. Classification of *Bulimulus* Leach, 1814.

Zilch, 1960	Breure, 1977
<i>Bulimulus</i> (<i>Bulimulus</i>)	<i>Bulimulus</i> (<i>Bulimulus</i>)
(<i>Rhinus</i>)	<i>B.</i> (<i>Rhinus</i>)
(<i>Dentaxis</i>)	<i>Botryx</i>
(? <i>Itaborahia</i>)	<i>Itaborahia</i>
(<i>Rabdotus</i>)	<i>Rabdotus</i> (<i>Rabdotus</i>)
(<i>Puritania</i>)	<i>R.</i> (<i>Leptobyrsus</i>)
(<i>Leptobyrsus</i>)	<i>R.</i> (<i>Plicolumna</i>)
(<i>Plicolumna</i>)	<i>Leiostracus</i> (<i>Pseudoxychona</i>) ?
(<i>Pseudoxychona</i>)	
(<i>Protoglyptus</i>)	
(<i>Maranhoniellus</i>)	
(<i>Naesiotes</i>)	<i>Naesiotes</i> s.l.
(<i>Raphiellus</i>)	
(<i>Granucis</i>)	
(<i>Nuciscus</i>)	
(<i>Reclasta</i>)	
(<i>Adenodia</i>)	
(<i>Stemmodiscus</i>)	
(<i>Olinodia</i>)	
(<i>Saeronia</i>)	
(<i>Ochsneria</i>)	
(<i>Granitza</i>)	
(<i>Granella</i>)	
(<i>Pleuropyrgus</i>)	
(<i>Pelecostoma</i>)	

ECOLOGY

Until now the ecology of the Bulimulidae was poorly known. Field research has led to the observation of the following generalized habitats: (1) species which are ground-dwellers and which are living off detritus found among leaf litter, etc.; (2) do., but only under or in the immediate vicinity of (large) stones; (3) species living on rock-faces, where they probably feed on mosses and algae; (4) species living on (low) shrubs; (5) species living on trees. As a rule species (of a certain genus) have been found only in one of these generalized habitats.

ZOOGEOGRAPHY

When the phylogenetic relationships have been worked out the analysis of the zoogeographical pattern of the Bulimulidae may be undertaken. At this stage of the research I only want to point at the theoretical background.

There are several theories to explain the intra- and intercontinental distribution. The most stimulating one is the theory worked out by Croizat; his concept of biogeography is one of a dynamic process in time and space, in which the idea of vicariant species (viz., closely related species which are geographically isolated) plays an important role. When plotting the distribution of a monophyletic group (which will include several vicariant species) one may draw a "track" which will connect the disjunct distribution areas. A number of congruent tracks will form a generalized track that estimates an ancestral biota that, because of changing geography, has become subdivided into descendant biotas.

ACKNOWLEDGEMENTS

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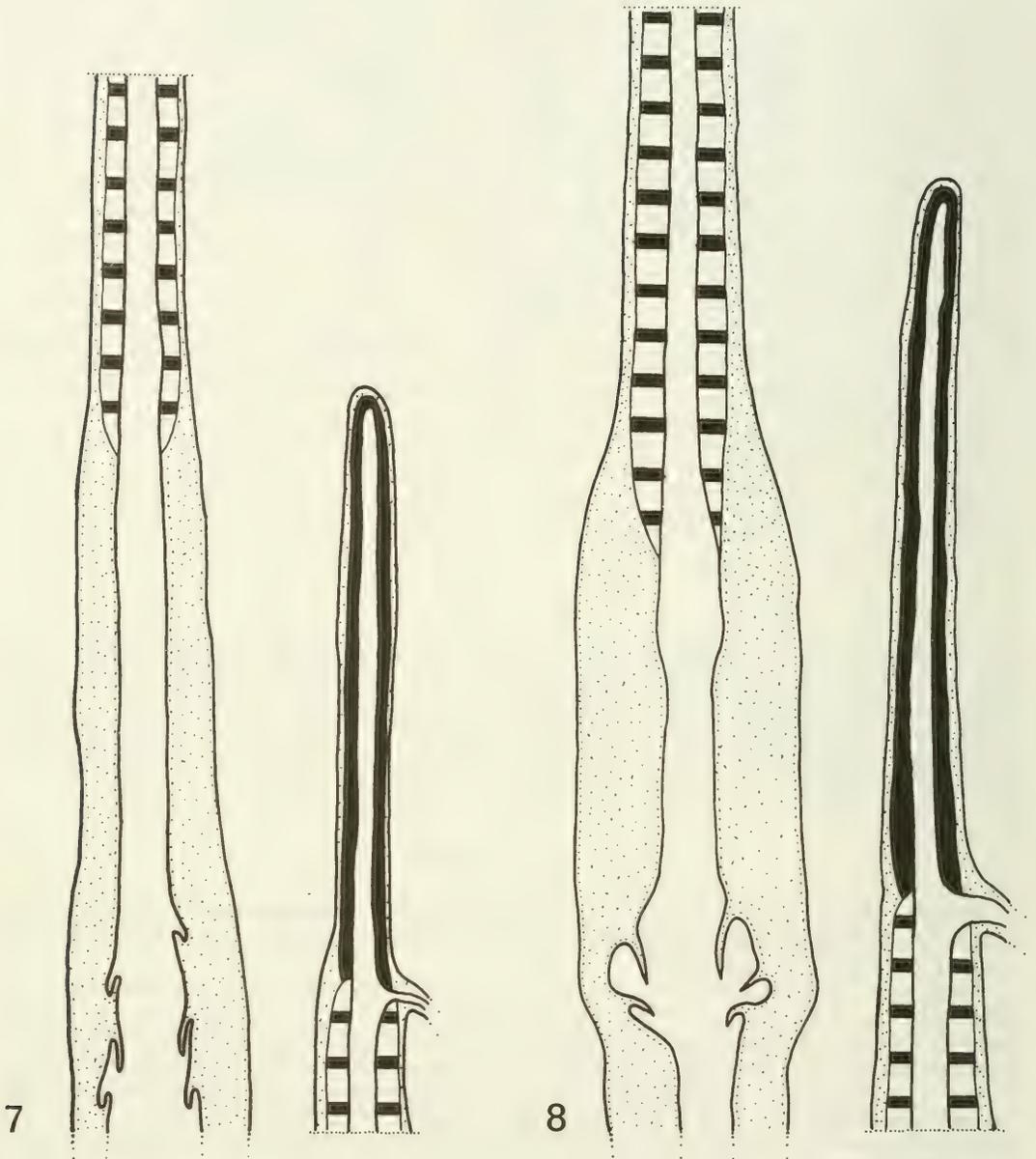


FIG. 7. Schematic reconstruction of the phallus complex in *Bothriembryon (B.) indutus* (Menke).
 FIG. 8. Do., *Plectostylus peruvianus* (Bruguère).

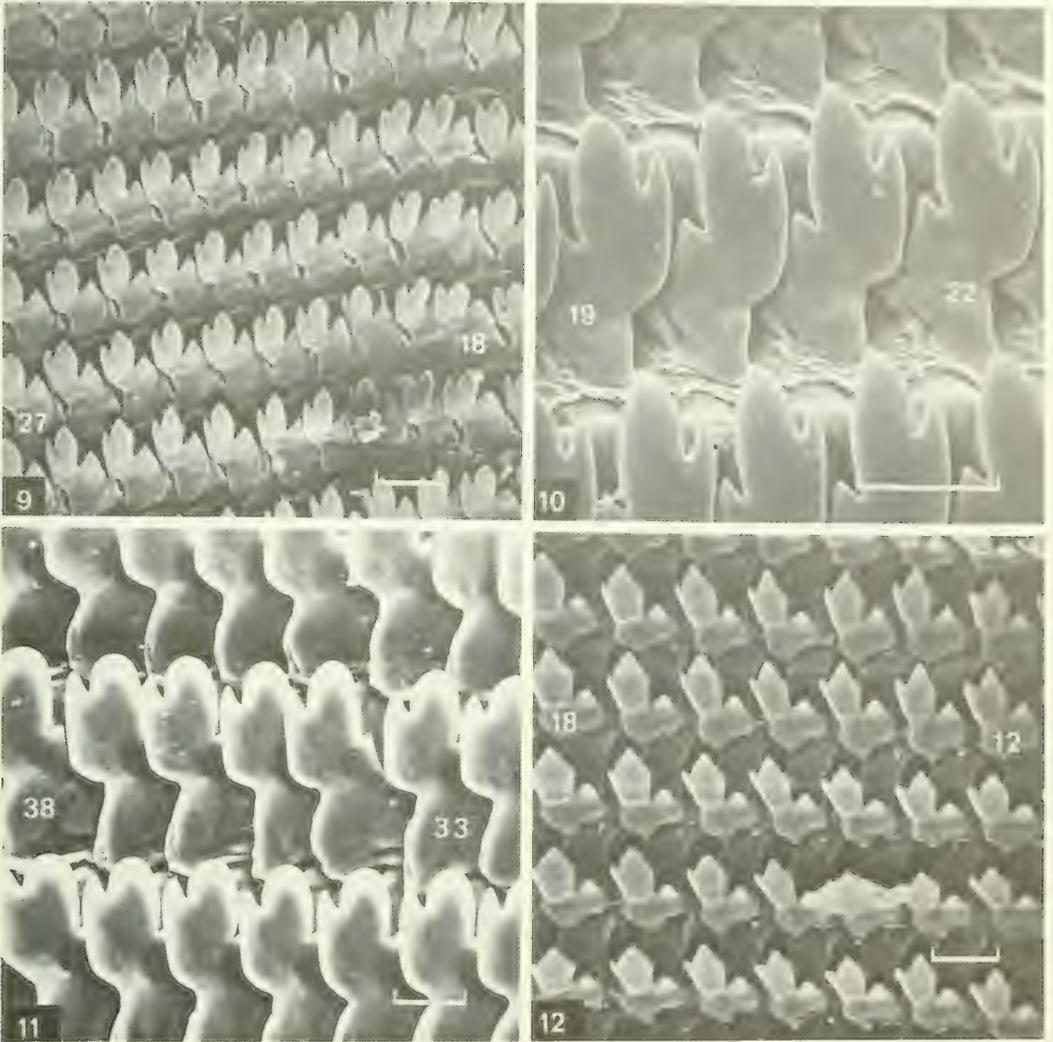


FIG. 9. Lateral teeth in *Bothriembryon (B.) melo* (Quoy & Gaimard). Scale 25 μ m.
 FIG. 10. Lateral teeth in *Bothriembryon (Tasmanembryon) gunnii* (Sowerby). Same scale.
 FIG. 11. Lateral teeth in *Plectostylus peruvianus* (Bruguière). Same scale.
 FIG. 12. Lateral teeth in "*Peronaeus (Lissoacme)*" *aguirrei* (Doering). Same scale.

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RESUMEN

Investigaciones taxonómicas, ecológicas y zoogeográficas
sobre Bulimulidae (Gastropoda, Pulmonata)

Se introduce la familia de caracoles terrestres Bulimulidae indicando los objetos de las investigaciones. Se construirá una sistema filogenética de la familia, usando la teoría de Hennig. Se presenta algunos resultados provisórios, o sea revisiones de *Bulimulus* Leach, 1814 sensu Zilch y *Bothriembryon* Pilsbry, 1894. Las relaciones más probables del último género estan indicadas. Además se presenta algunos datos ecológicos y se indica el fondo teórico del análisis zoogeográfico.

ANATOMY AND TAXONOMY OF *PROTOGLYPTUS QUITENSIS* (PFEIFFER)
(GASTROPODA, PULMONATA, BULIMULIDAE)

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ABSTRACT

The differences between *Naesiotus* Albers, 1850, and *Protoglyptus* Pilsbry, 1897, are given, based on characters of the shell and the anatomy. Further study is still needed to delimit these genera, both morphologically and geographically. After diagnosis of the 3 subgenera of *Protoglyptus*, the synonymy and a redescription of *Protoglyptus (Rimatula) quitensis* (Pfeiffer) are given. The radula, maxilla and genitalia of this species are figured. An examination of 1,700 specimens from different localities along the interandine plateau of Ecuador proved that recognition of subspecies is without taxonomical importance.

The species originally described as *Bulimus quitensis* Pfeiffer, 1847, was subsequently placed in different genera, or subgenera, of *Bulimulus*: *Scutalus* by Martens, 1855; *Thaumastus* by Cousin, 1887; *Lissoacme* by Pilsbry, first in 1835, and *Scutalus* in 1897. More recently (1940), attending to the embryonic sculpture, Rehder included it in *Naesiotus*; on this occasion Rehder also described several subspecies within its distributional area in Ecuador. This paper discusses the genitalia, radula and conchological characteristics of *B. quitensis*, on account of which it is now placed in the genus *Protoglyptus*.

Protoglyptus and *Naesiotus* have similar protoconch structures, consisting of regular and well-defined axial riblets, the spaces in between crowded with very fine spiral incisions in various degrees of development, sometimes more conspicuous in *Naesiotus* than in *Protoglyptus*. Such embryonic characteristics have important taxonomic value in Bulimulidae. Attending to these features only, it is very difficult to separate not just between the two genera, but also with allied groups belonging to the same ancestral stock, as *Neopetraeus* and *Rabdodus*. Similar difficulty is found in grouping species with smooth protoconchs as in *Bostryx* and *Peronaeus*.

To begin with the shell, *Naesiotus* has, generally, the surface spirally striated or strongly rugose; in many species the columella is twisted inside, with callosities or folds that even may develop into well formed teeth, and the last whorl is in many cases somewhat angulate. Such features are not found in *Protoglyptus*. Moreover, the features indicated for *Naesiotus* are shown conspicuously in species of the Galapagos Islands where the genus is endemic. Dall (1920) proposed a division for the genus into sections: *Granucis*, *Nuciscus*, *Reclasta*, *Adenoida*, *Stemmodiscus*, *Olinodia*, *Saeronia*, *Granitza*, *Granelia*. These sections were recognized by Thiele (1930), and Zilch (1960) included them as subgenera of *Bulimulus*. The shell differences among the types of such subgenera are considerable in relation to the type of *Naesiotus* s.s., *N. nux* Broderip. Some of the species, such as *Naesiotus (Nuciscus) tanneri* Dall, could be easily confused with a *Neopetraeus*. In the genus *Protoglyptus* on the other hand, shell modification is less important, and it appears to be a more natural group.

MAXILLA. In *Protoglyptus quitensis* it is characteristic of the genus. It is as strong as in *Neopetraeus* and *Scutalus* but without a large central plate; the central plates are narrower and closer together. The maxilla is 1 mm long and 0.25 mm wide, with ribs or plates cemented on to the upper portion (Fig. 4).

RADULA. Also typical of *Protoglyptus*, see Figs. 1-3. It has a blunt spine at the top of the rachidian tooth, while in *Naesiotus* (Fig. 5-7) the teeth resemble more those in *Neopetraeus*. *P. quitensis* has the central cusp shorter, without the additional cusps of *Naesiotus*, and the marginal teeth are not serrated.

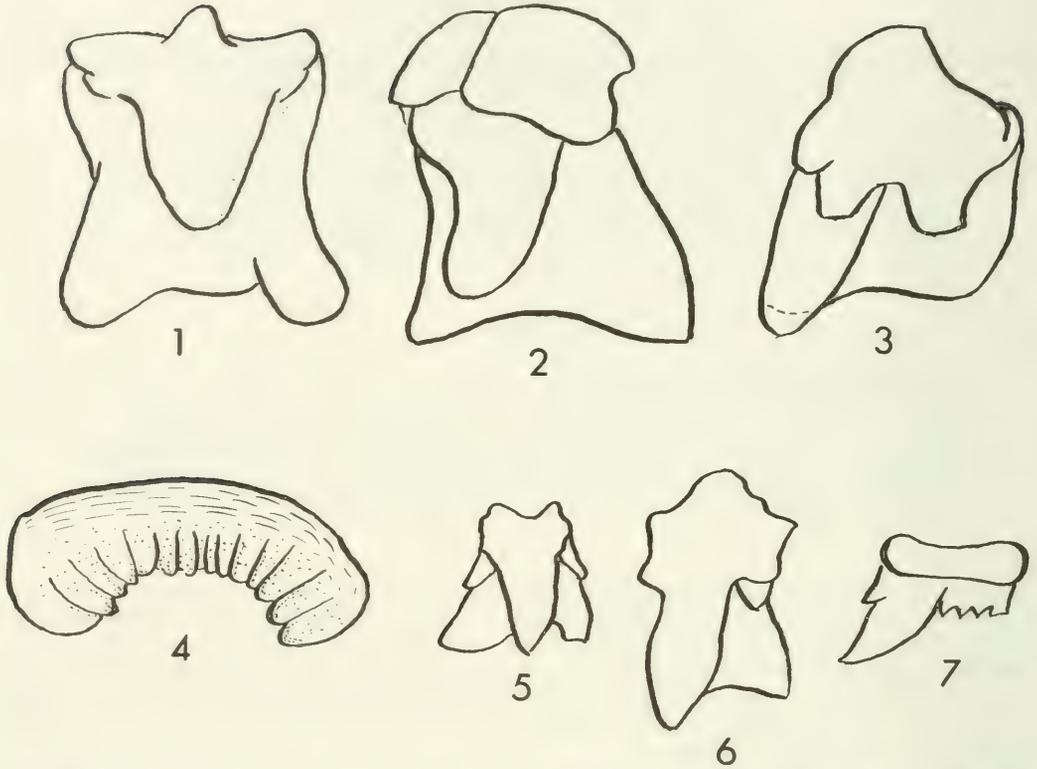


FIG. 1-4. *Protoglyptus quitensis*, radula: (1) rachidian, (2) lateral, (3) marginal; (4) maxilla (ca. 40X). FIG. 5-7. *Naesiotus nux* (after Dall), radula: (5) rachidian; (6) lateral; (7) marginal.

EXTERNAL BUCCAL ORGANS. In *Protoglyptus* these are of intermediate type with that of *Scutalus*, with "lips" rounded at the base, curved at the tips. In this it differs from other bulimulids.

GENITALIA. The differences in the genetal systems of *Protoglyptus* and *Naesiotus* have generic significance. In *Protoglyptus* (Fig. 8) the ovotestis or hermaphroditic gland, is more or less spirally enrolled as in *Bulimulus* and *Scutalus*, but not racemose as in *Neopetraeus*. The albumen gland, although large, does not show substantial differences as compared to that of other genera. The spermoviduct or uterus, is a sizeable organ, wider than in other bulimulids and not tortuous except sometimes at its end. The spermatheca or bursa copulatrix, is spheroid, united at the entrance of the vagina by a long duct which runs almost attached to the uterus wall. The vaginal sac is very short. Penis furnished with a long flagellum, which is broader and thicker at the base, into which the vas deferens running from the penis sheath is introduced; there is some similarity in this organ to that of *Scutalus*, but it is smaller and it differs from that in other bulimulids.

Considering the general features of the genitalia in their family affinities, the hermaphroditic gland of *Protoglyptus* is distinguished by not being racemose. The spermatheca is slender and the uterus ends, together with the spermatheca, nearer to the entrance of the vagina than in other genera.

Compared with the genitalia of the type-species of *Naesiotus*, *N. nux*, the differences are significant. Dall figured the genitalia of *N. nux* after Binney's drawings, calling attention to the incorrect nature of that figure, because the male and female openings appear separated, instead of being united at the atrium which is normal.

Neither Dall (1896, 1920), who studied most of the species of the Galapagos Islands, nor

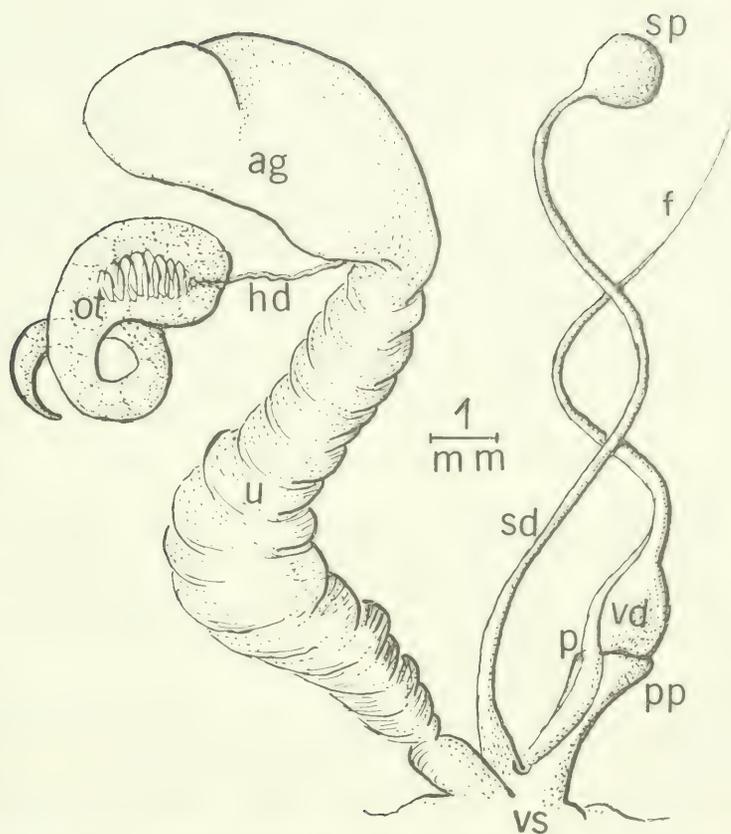


FIG. 8. Genitalia of *Protoglyptus quitensis* (Pfr.), Pillaro, Tungurahua, Ecuador. ag—albumen gland; ds—spermatic duct; f—flagellum; hd—hermaphroditic duct; ot—ovotestis; p—penis; pp—praeputium; sp—spermatheca; u—uterus; vd—vas deferens; vs—vaginal sac.

Pilsbry (1897) when he described the subgenus *Protoglyptus*, mentioned any anatomical differences. Pilsbry merely stated that "*Naesiotus* and *Orthotomium* [= *Rabdodus*?] are identical with *Protoglyptus* in apical sculpture" and that they, apparently, derived from the same (primitive) stock. This led subsequent authors to the description of species either as *Naesiotus* or *Protoglyptus*, without anatomical considerations; most of the species, of course, were described on the shell alone.

In both groups the shells are very variable in size and shape, but not to the extent which induced Weyrauch (1956) to describe *Naesiotus andivagus* and *N. pilsbryi* as belonging to the same genus. Subsequently, with the second species, Weyrauch (1958) created the subgenus *Maranhoniellus*, which Zilch categorized under *Bulimulus*. I agree with Weyrauch that it is advisable to treat *Naesiotus* as a genus, but so is *Protoglyptus*. On the other hand, the fact that *Naesiotus* is endemic in the Galapagos Islands, and *Protoglyptus* is better known in mainland South America, does not imply that all species of the Galapagos are *Naesiotus* or that *Protoglyptus* cannot be found on the islands. A better knowledge of most species is needed to establish the limits, morphological and geographical, of these groups.

Genus *Protoglyptus* Pilsbry, 1897

Manual of Conchology (2)11: 85 (as subgenus of *Bulimulus*).

Type-species: *Bulimulus pilosus* Guppy, from Trinidad, West Indies (near Venezuela) (American Journal of Conchology, 1871, 6: 310). Subsequent designation by Parodiz, 1946:

the first species included by Pilsbry under *Protoglyptus*. Zilch (1960) made, independently, the same designation, but instead of the type species, he illustrated *Protoglyptus durus* (Spix).

Pilsbry's brief diagnosis is applicable to a group of species which can be considered as *Protoglyptus* s.s. In 1946 the present author studied a number of species from the northwest and southern regions of South America, diagnosing three particular groups:

(1) *Protoglyptus* s.s.: *P. crepundia* (d'Orbigny), *P. munsteri* (d'Orbigny), and *P. punctustratus* Parodiz. Umbilicus wide and deep.

(2) Subgenus *Rimatula* Parodiz, 1946: 353. Type *P. deletangi* Parodiz from northern Argentina. It includes *P. oxylabris* (Doering), *P. montivagus* (d'Orbigny), and *P. pollonerae* (Ancey). This group is characterized by its very narrow umbilicus, partially covered by the columellar expansion.

(3) Subgenus *Obstrusus* Parodiz, 1946: 354. Type *Bulimus rocayanus* d'Orbigny; includes *P. chacoensis* (Ancey). Imperforate, with the columellar margin expanded over the umbilicus; base of columella with a torsion which projects the end of the aperture to the left; long spire, with more whorls than in other *Protoglyptus*. Such a subgeneric arrangement is for want of something better and may be proved to be untenable with increased knowledge of the species.

Protoglyptus (Rimatula) quitensis (Pfeiffer, 1848)

Bulimus quitensis Pfeiffer, 1848, *Proc. zool. Soc. Lond.*, 1847: 230. — Reeve, 1849, *Conch. icon.*, 5: fig. 317. — Hidalgo, 1870a, *Moluscos del Viaje al Pacifico*: 130, pl. 7, fig. 5-8. — Hidalgo, 1870b, *J. Conch. Paris*, 18: 63. — Pfeiffer, 1877, *Monogr. Helic. viv.*, 8: 157. — Pilsbry, 1895, *Man. Conch.* (2) 10: 158, pl. 51, fig. 16-18. [*Bulimulus (Bostryx-Lissoacme)*]. — Rehder, 1940, *Nautilus*, 53: 114, fig. 2, 4, 7, 9, 11, 13, 15, 16, 18, 20 [*Naesiotus*]. — Weyrauch, 1958, *Arch. Moll.* 87: 121 [*Naesiotus*].

Bulimus irregularis Pfeiffer, 1848, l.c.: 231. — Reeve, 1849, l.c.: fig. 454. — Martens, 1885, *Conch. Mitt.*, 2: 162 [*Bulimulus (Scutalus)*]. — Cousin, 1887, *Bull. Soc. zool. Fr.*, 12: 225 [*Thaumastus*]. — Pilsbry, 1897, *Man. Conch.* (2) 11: 34, pl. 34, fig. 71 [*Bulimulus (Scutalus)*].

Bulimus striatus King sensu Reeve, 1848, l.c.: fig. 139.

Bulimus caliginosus Reeve, 1849, l.c.: fig. 609. — Hidalgo, 1870a, l.c.: 59; Martens, 1885, l.c.: 161. — Cousin, 1887, l.c.: 223 [*Thaumastus*]. — Pilsbry, 1897, l.c.: 33, pl. 4, fig. 43-45 [*Bulimulus (Scutalus)*]. — Germain, 1910, *Mission Serv. géogr. Amer. Sud*, 9: C.31 [*Bulimulus (Scutalus)*].

Bulimus catlowiae Pfeiffer, 1853, *Monogr. Helic. viv.* 3: 427. — Pfeiffer, 1854, *Proc. zool. Soc. Lond.*, 1852: 154. — Hidalgo, 1870a, l.c.: 128, pl. 7, fig. 9-10. — Hidalgo, 1870b, l.c.: 63. — Pfeiffer, 1877, l.c.: 154. Miller, 1878, *Malak. Bl.*, 25: 194 [*Scutalus*]. — Pilsbry, 1897, l.c.: 34, pl. 5, fig. 69-70 [*Bulimulus (Scutalus)*].

Bulimus anthisanensis Pfeiffer, 1853, l.c.: 406 [in part]. — Pfeiffer, 1854, l.c.: 155. — Albers, 1860, *Die Heliceen*, 2e Aug. 217. — Pilsbry, 1897, l.c.: 32, pl. 4, fig. 41-42 [*Bulimulus (Scutalus)*].

Bulimulus (Scutalus) quitensis rufescens Germain, 1910, l.c.: C.35, pl. 4, fig. 1-2.

Naesiotus quitensis jacksoni Rehder, 1940, l.c.: 116, fig. 1, 5.

Naesiotus quitensis orinus Rehder, 1940, l.c.: 116, fig. 6, 10.

Naesiotus quitensis vermiculatus Rehder, 1940, l.c.: 117, fig. 17, 19.

Naesiotus quitensis ambatensis Rehder, 1940, l.c.: 117, fig. 12, 14.

Naesiotus quitensis antisana Rehder, 1942, *Nautilus*, 55: 103.

Type locality: Quito, Ecuador.

Pilsbry in his monograph of the Bulimulidae (1895: 159) declared not having seen this species, and being not aware of its apical sculpture, placed it in the combination *Bostryx-Lissoacme*, but adding that it may prove to be a *Scutalus*.

Description—Shell of regular bulimoid type with large body whorl and short spire (ovate-conical), with 6-7 whorls, including the 1½ of the protoconch which is axially and obliquely ribbed and the spaces between ribs filled with numerous, very fine, spiral striae; the ribs become finer and closer near the middle of the second whorl, then disappear altogether; the following whorls with irregular creamy-white axial costulae of different width, the spaces between of chestnut color, giving the surface a rough appearance of clear and dark streaks. Spire with slightly convex whorls; body whorl globose. Aperture ovate-elongate; inside chestnut, and sufficiently translucent to make the external streaks visible. The columella is dark and straight, the basal end forming an angle with the lip. Peristome thin and not reflexed. Average size 30 mm long and 13-15 mm wide.

The variations in size, color and shape have led to the description of several species and subspecies.

The examination of 1700 specimens from different localities along the interandine plateau of Ecuador (Fig. 9) proved that separation of geographically limited subspecies is not only

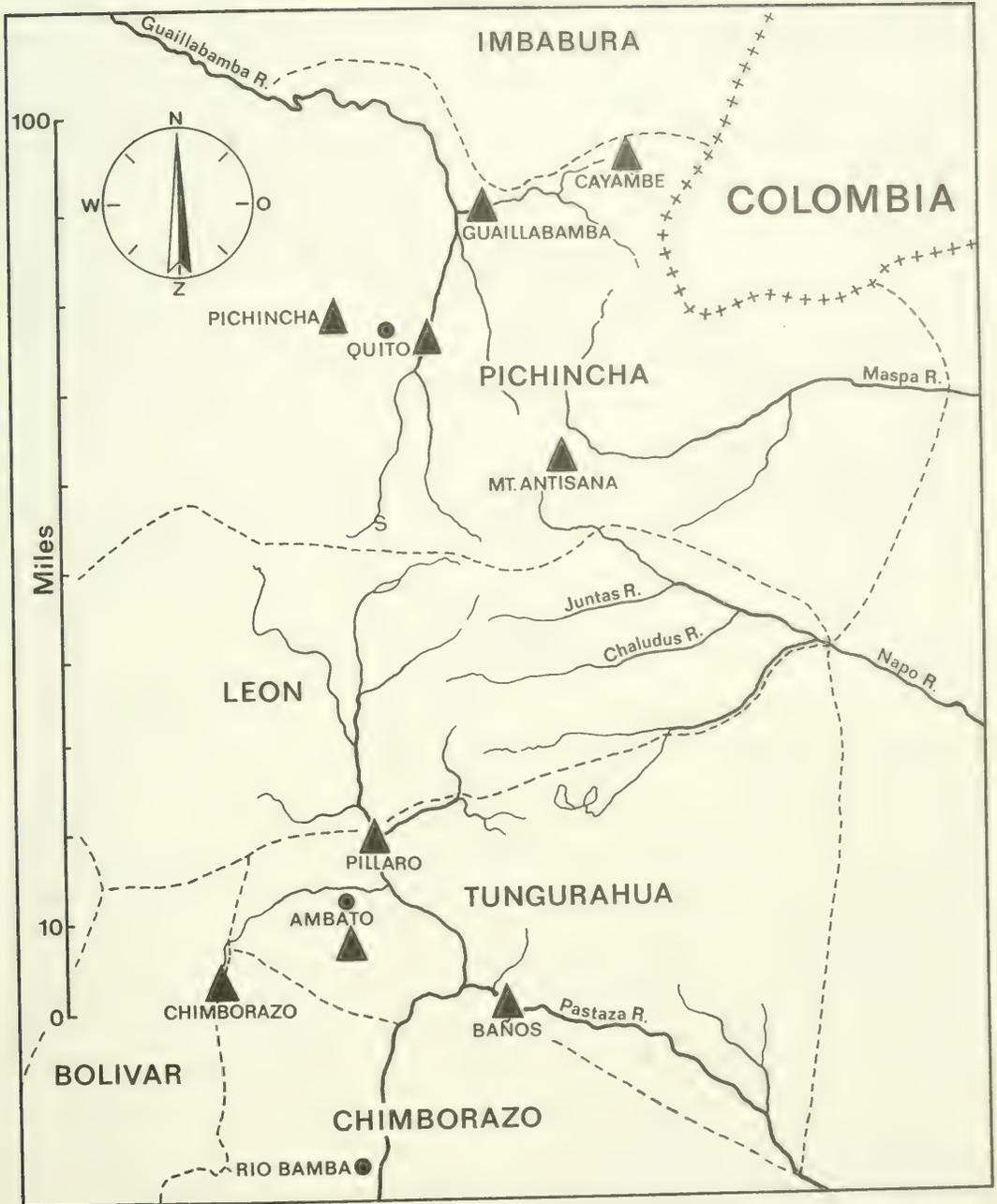


FIG. 9. Localities of the studied populations of *Protoglyptus quitensis* (Pfeiffer). H. Heijn del.

extremely difficult but without taxonomic importance. A northern sample, from Cayambe, including 171 specimens, shows different types of interpopulation variations: 36 small *vermiculatus* type; 15 small with surface less rugose than in *vermiculatus* Rehder; 50 larger, with distinct brown streaks but with grayish ground color; 12 of medium size with wider straw-yellow streaks and chestnut background, as in *anthisanensis* Pfeiffer or *antisana* Rehder; 35 with wide clear and dark areas; 18 larger creamy-white with some chestnut bands as in *jacksoni* Rehder; 5 uniformly brown-colored without streaks; some albino as in *orinus* Rehder. The proportional measurements of these specimens are also variable, ranging from narrow (25 X 11 mm) to very globose shells (24 X 15 mm).

More diverse, but not exclusively distinct, is a sample of 67 specimens from Pichincha, with paler colors: 23 pure white to straw-yellow with pale brown bands, some with pinkish apices, interior of the aperture white, showing clearly the external bands, surface wrinkled; 8 smoother with umbilicus diversely opened; 14 larger, fleshy-colored and with strong wrinkles, but interior of aperture darker; 11 with a combination of the above characteristics. The shells are 18-28 mm long and 10-14.5 mm wide. This seems to be a population closer to the typical *quitensis*, but other lots of the same locality, Pichincha and around Quito, include many specimens of the darker *anthisanensis* type, or as rugose as in *vermiculatus*.

In the following key to color patterns and size within the species, the "forms" are designated according to the specific or subspecific names given by the authors, with their type localities.

- | | |
|---|---|
| 1 — Pale colored, with less contrasting streaks; medium to large size | 2 |
| — Darker; small to medium size | 3 |
| 2 — Variable populations with yellowish to pale brown streaks | <i>P. quitensis quitensis</i> ,
including forms such as
<i>irregularis</i> Pfeiffer,
<i>rufescens</i> Germain,
<i>orinus</i> Rehder
(Hidalgo's var. b). Quito. |
| — Very pale with sometimes flesh-colored streaks | 4 |
| 3 — Shell wider (most common form), streaked with very contrasted chestnut
and pale brown axial bands; various sizes | <i>vermiculatus</i> Rehder.
Tungurahua. |
| 4 — Longer and slender, very pale, without or with very pale streaks | <i>jacksoni</i> Rehder.
Guailabamba. |
| — Flesh-colored, sometimes purplish, with irregular lighter streaks; small | <i>catlowiae</i> Pfeiffer.
<i>ambatensis</i> Rehder.
Ambato. |

Southern samples from Chimborazo included variation between the typical *quitensis* and *vermiculatus* but with predominance of the *anthisanensis* form. In a large sample from Mount Antisana, Chimborazo and Pillaro there is a mixture of all "forms" from 1 to 4.

When collecting has been unbiased, it is impossible to find any particular form restricted to a single locality or zone. All forms are sympatric.

Protoglyptus quitensis is broken up, not into subspecies or even races, but is a polymorphic species with transitional and overlapping forms, which can be summarized in 6 series of basic patterns of color and surface structure.

Series A1 to A6, from yellowish-white and light brown to darker specimens, with increasing rugosity of the surface:

- A1—Very dark, with a few fine yellow lines and dark apex;
- A2—With more yellow and brown lines;
- A3—Yellow-brown lines very abundant;
- A4—With many grayish-white streaks, aperture chestnut;
- A5—With 2 or 3 of the bands darker, aperture paler;
- A6—Predominance of dark streaks, aperture colored at margin but uncolored inside.

Series B1 to B5 with progressive darkening of background color and gradual intensification of the streaks, including the larger *vermiculatus* type:

- B1—Resembling A1 but with the yellow-brown zones wider;
- B2—Intermediate between A2 and A3;

- B3—Clearer background color and more banded;
 B4—Same as B3 but with diffuse streaks;
 B5—Strong dark streaks well separated by white zones (as fig. 609 in Reeve's *caliginosus*).

Series C1 to C4 with progressively paler colors, more diffusely streaked:

- C1—Intermediate between B1 and B2;
 C2—Resembling B3 but darker;
 C3—With many diffuse streaks;
 C4—Background very pale brown to whitish with very diffuse streaks.

Series D1 to D3 from paler many-streaked specimens to some with only 2 or a single streak:

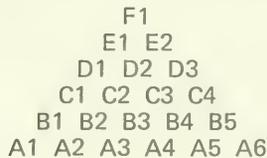
- D1—Light brown and white zones more evenly divided;
 D2—White with irregular brown streaks;
 D3—White with 1 or 2 conspicuous streaks (as fig. 317 in Reeve).

Series E1 and E2, from rugosely streaked to almost white:

- E1—Many fine brown lines with rugose surface (as in fig. 454 in Reeve);
 E2—Ashy white or almost white with the streaks reduced to the end of the last whorl;
 streaks on the upper whorls, if present, very weak.

F1—Completely white without streaks.

When these series are compared, all kinds of transitional and individual variations are found; thus, D2 can be considered as transitional between C2 and C3, etc. The following diagram shows the relationship of any of the intrapopulation variants with others closely associated:



DISTRIBUTIONAL FACTORS

Protoglyptus quitensis is distributed along the interandine plateau of Ecuador, from Cayambe to Riobamba. It is abundant at both sides of the watershed of the three main rivers of the area, Guallabamba, Napo and Pastaza.

The headwaters of the Guallabamba River, which runs northwest to the Pacific, are separated from the headwaters of the Pastaza River (a tributary of the Marañón) by elevations of over 18,000 feet. The high plateau on the west side of the Cordillera Oriental is divided into 2 sections. The type localities for *P. quitensis quitensis* and *P. quitensis jacksoni* are in the northern section, in the Pichincha province; to the south, Chimborazo is the type locality of *P. quitensis orinus*, and the type localities of *P. quitensis ambatensis* and *P. quitensis vermiculatus* are in the Tungurahua province. *P. quitensis quitensis* is also found in Tungurahua, thus, the central elevation of Leon province is not a barrier factor for isolation. With regard to *P. quitensis anthisanensis* (= *antisana*), its type locality is on the highest eastern part of the plateau, on the headwaters of the Napo River (another tributary of the Marañón), which forms the 3rd division, but that form is also found elsewhere in the other sections.

In northern specimens from Cayambe, near the type locality of *P. quitensis jacksoni*, all other intermediate types of variation occur, as well as in Pichincha (north) and Chimborazo (south). There is no allopatry for any of the variations which were described as subspecies. It is possible to find a certain degree of differentiation among typical *P. q. orinus* and *P. q. vermiculatus*, which vanishes when transitional forms are considered. Furthermore, most of the collecting was done in the provinces of Pichincha, Tungurahua and Chimborazo, but almost nothing is reported from Leon where, in all probability, all types will be found highly mixed.

At certain periods of the expansion of the species, different populations of *P. quitensis* probably became isolated geographically, resulting in a rapid development of ecophenotypes, but the isolation never was completed in such a manner as to result in allopatric subspecies, and

a continuous exchange of genetic material of the populations has resulted in a mosaic of a polymorphic species, with gradual and uninterrupted gradient characteristics. Although not evident at present, the species may have been, formerly, of a clinal nature.

Localities of the material examined: (1) Province of Pichincha—Cayambe, Guallabamba, Volcano Pichincha, Quito, Mount Antisana; (2) Province of Tungurahua—Pillaro, Ambato, Agoyan on Pastaza River, Agoyan at Baños de Tungurahua; (3) Province of Chimborazo—Mount Chimborazo.

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PHYLOGENETISCHE PROBLEME BEI NACKTSCHNECKEN AUS DEN
FAMILIEN LIMACIDAE UND MILACIDAE
(GASTROPODA, PULMONATA)

Andrzej Wiktor¹ und Ilia M. Likharev²

ABSTRACT

As we do not know how to interpret the scant fossil remnants of slugs, the authors had to have their phylogenetic considerations based on Recent animals only. Their conclusions are based on an analysis of external characters, anatomy of the alimentary canal, pallial complex, and genitalia, as well as geographical distribution and biology. Milacidae and Limacidae are undoubtedly of different origin. It seems necessary to separate the Boettgeriidae from the Milacidae, and the Agriolimacidae from the Limacidae; these should be considered to represent four different families.

Die Feststellung der verwandschaftlichen Beziehungen ist bei den Nacktschnecken besonders schwierig. Bis jetzt sind wir noch nicht im Stande die verkümmerte Schale dieser Tiere für systematische Zwecke zu verwenden. Deshalb ist es auch noch nicht möglich fossile Reste zu deuten, was für phylogenetische Studien von grosser Bedeutung wäre. Daher können wir uns bei Forschungen zur Ermittlung der verwandschaftlichen Beziehungen in dieser Gruppe nur auf morphologische Merkmale rezenter Formen stützen, teilweise auch auf Angaben über die Bionomie, Ökologie und Verbreitung. Untersuchungen in dieser Richtung haben wir im Zusammenhang mit einer monographischen Bearbeitung der Nacktschnecken der UdSSR und der angrenzenden Gebiete unternommen, d.h. an Material von einem grossen Teil der Paläarktis.

Mit der Phylogenese der hier behandelten Familien beschäftigte sich in der letzten Jahrhundertwende insbesondere Heinrich Simroth (1901)—der sachverständigste Nacktschnecken-spezialist der damaligen Zeit. Spätere Aussagen zu diesem Thema, darunter auch jene von Hesse (1926) und Wagner (1934, 1935, 1936) sind eigentlich nur als Vervollständigungen oder als geringe Modifikationen der bisherigen Erkenntnisse anzusehen. In den letzten 70 Jahren nach Simroth's Tätigkeit sammelten sich zahlreiche Informationen an, welche uns neben unseren eigenen Forschungsergebnissen zu einer Aktualisierung der Meinung über dieses Thema angeregt haben.

Wir konnten uns überzeugen, dass für die systematische Gruppierung der Familien- und Gattungsrangen folgende Merkmale verwendbar sind:

- (a) der Habitus;
- (b) der Bau des Verdauungssystems;
- (c) der Bau des Palialkomplexes;
- (d) der Bau der Schale;
- (e) das Bauschema der Genitalien.

Diese Merkmale korrespondieren eindeutig mit den entsprechenden Angaben über die Bionomie und Verbreitung der Arten. Unter dem Begriff "Nacktschnecken" ist eine heterogene Gruppe zu verstehen, deren einziges gemeinsames Merkmal in der verkümmerten Schale besteht. Letztere ist gewöhnlich unter dem Mantel verborgen. Die Reduktion der Schale bedingt bei den Nacktschnecken einerseits den Verlust einer Schutzvorrichtung, andererseits gelangten diese Tiere dadurch zu ganz anderen Bewegungsmöglichkeiten. Die Schale verwandelte sich allmählich in ein abgeflachtes Gebilde, wodurch es zu wesentlichen Änderungen im Habitus und zu Verschiebungen der inneren Organe, speziell des Eingeweidesackes, kam.

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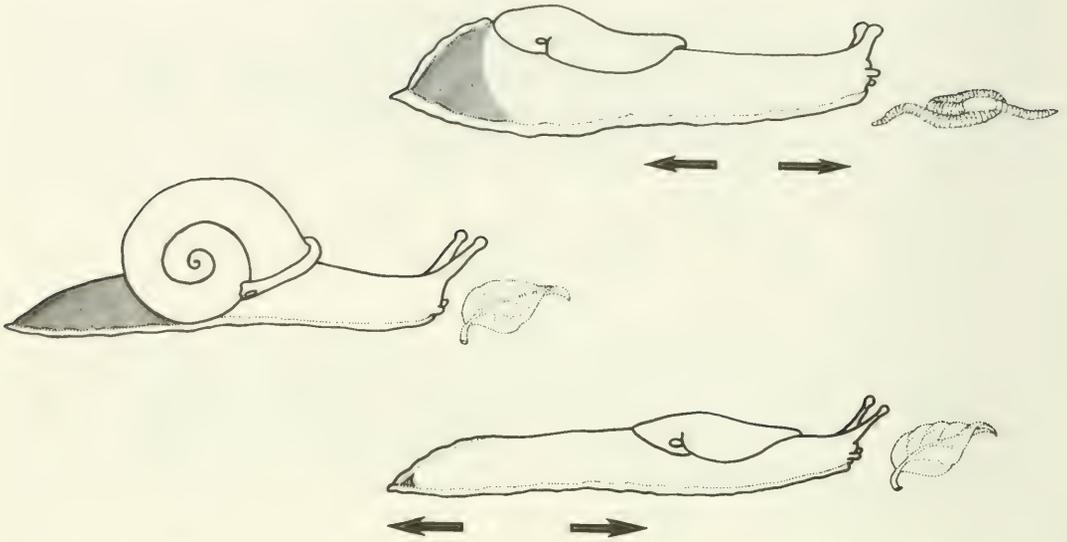


FIG. 1. Das Entstehen der 2 verschiedenen Land-Nacktschnecken-Gruppen: Raubnacktschnecken und phytophage Nacktschnecken (die Pfeilen zeigen die Entwicklung des Cephalopodiums).

Unter den Land-Nacktschnecken lassen sich zwei verschiedene biomorphologische Gruppen unterscheiden.

(1) Bei der ersten Gruppe ist der vordere Körperabschnitt stark entwickelt d.h. der Schlund-Abschnitt und der vordere Teil des Fusses. Gleichzeitig wurde der Mantel mit dem ganzen Pallialkomplex nach hinten verschoben und abgeplattet. Der hinter dem Mantel befindliche Körperabschnitt ist kurz. Hierher gehören verschiedene Raubschnecken, wie z.B. die Vertreter der Familien Trigonochlamydidae, Testacellidae, Daudebardiidae usw. (Fig. 1).

(2) Bei der zweiten Gruppe sind der mittlere und der hintere Teil des Fusses stark entwickelt. Der Eingeweidesack dringt sich in das Cephalopodium hinein und reicht bis zu seinem Ende. Der Mantel und der Pallialkomplex, oder zumindest der Letzte, befinden sich auf der vorderen Körperhälfte. Der hinter dem Mantel liegende Körperabschnitt ist stark verlängert und birgt den grössten Teil der inneren Organe. Hierher gehören ursprünglich phytophage Schnecken, wie z.B. die Vertreter der Familien Limacidae, Milacidae, Arionidae, Philomycidae usw. (Fig. 1).

Aus unseren Untersuchungen geht hervor, dass sowohl die Milaciden wie auch die Limaciden nach der bisherigen Auffassung als uneinheitliche Gruppen anzusehen sind, wobei von der ersten, wie Van Goethem (1972) es bereits suggerierte, die Familie Boettgerillidae abgetrennt werden sollte, während von der zweiten die Familie Agriolimacidae abzuspalten wäre. Einen ähnlichen Vorschlag in Bezug auf die letzte Gruppe finden wir in den Veröffentlichungen von Wagner (1935). Unsere Untersuchungen bestätigen, dass die schon zeitiger bemerkten Eigentümlichkeiten mancher Merkmale eine neue Einteilung der Nacktschnecken gerechtfertigen. Deshalb haben wir die erwähnten Gruppen als selbstständige Familien aufgestellt.

Alle oben genannten Gruppen unterscheiden sich deutlich im äusserem Habitus. Dies betrifft das allgemeine Gepräge und den Muskelbau der Fusssohle. Sowohl die Milaciden wie auch die Boettgerilliden besitzen auffällige Mantelfurchen, während den Vertretern der beiden übrigen Familien solche Furchen fehlen. Sie unterscheiden sich dagegen untereinander durch das vorhandene bzw. fehlende Ring rund dem Pneumostom (Fig. 2).

Das Verdauungssystem entwickelte sich ebenfalls in verschiedenen Richtungen. Als primär dürfte die Anordnung des Darmes mit zwei Schlingen anzusehen sein, denn einen solchen Bau weisen fast alle Gehäuseschnecken und die meisten Nacktschnecken auf. Die Ausbildung einer dritten Schlinge im Zusammenhang mit der Verlängerung des Darmes, sowie die Entwicklung eines Blinddarmes ist als Resultat einer späteren Spezialisierung anzusehen, welche unter den

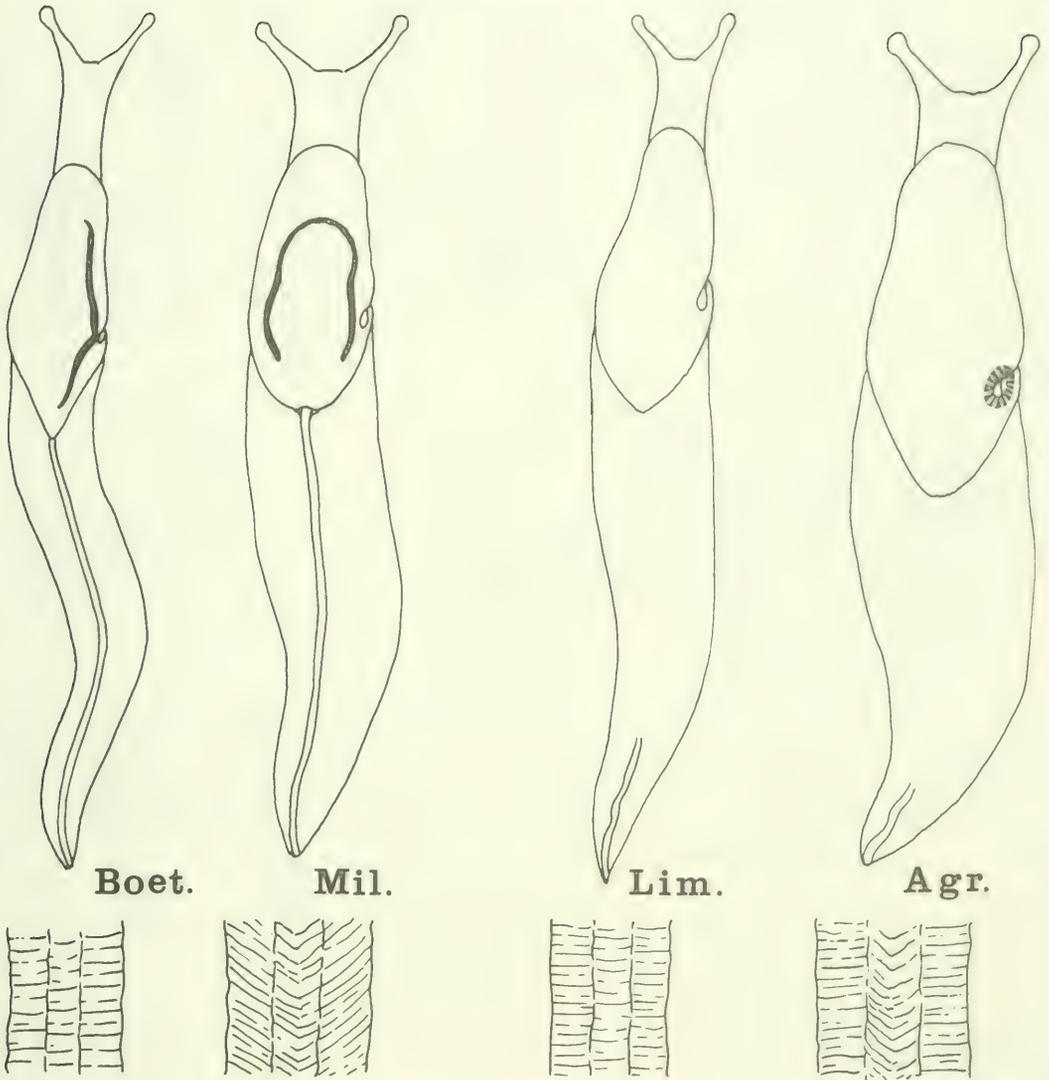


FIG. 2. Der äussere Habitus. Unten: die Fusssohleoberfläche. Boet.—Boettgerillidae, Mil.—Milacidae, Lim.—Limacidae, Agr.—Agriolimacidae.

Gastropoden nur bei Limaciden vorkommt. Von wesentlicher Bedeutung scheint u.a. das Verhältnis der Schlingenlängen im Darm zu sein, weiter die Position der Schlingen und der Abzweigung des Blinddarmes. Bei allen Limaciden reicht die erste Schlinge am weitesten nach hinten und alle ausser der Gattung *Eumilax* besitzen 3 Darmschlingen. Die übrigen 3 Familien haben nur 2 Darmschlingen, wobei die erste Schlinge weiter vorn liegt als die zweite. Ein Blinddarm ist nur bei manchen Limaciden und Agriolimaciden vorhanden. Im ersten Fall bildet der Blinddarm eine Verlängerung der dritten Schlinge, wobei dieser lang ist, dagegen ist dieses Organ bei den Agriolimaciden klein, liegt ziemlich entfernt vom Mantel und bildet eine seitliche Tasche des Rectum (Fig. 3).

Der bei systematischen Untersuchungen bisher wenig beachtete Pallialkomplex erwies sich als recht brauchbar für die Bestätigung der Selbständigkeit der einzelnen Gruppen. Im Pallialkomplex sind besonders die Niere, ferner der Ureter und die hiermit verbundenen Strukturen

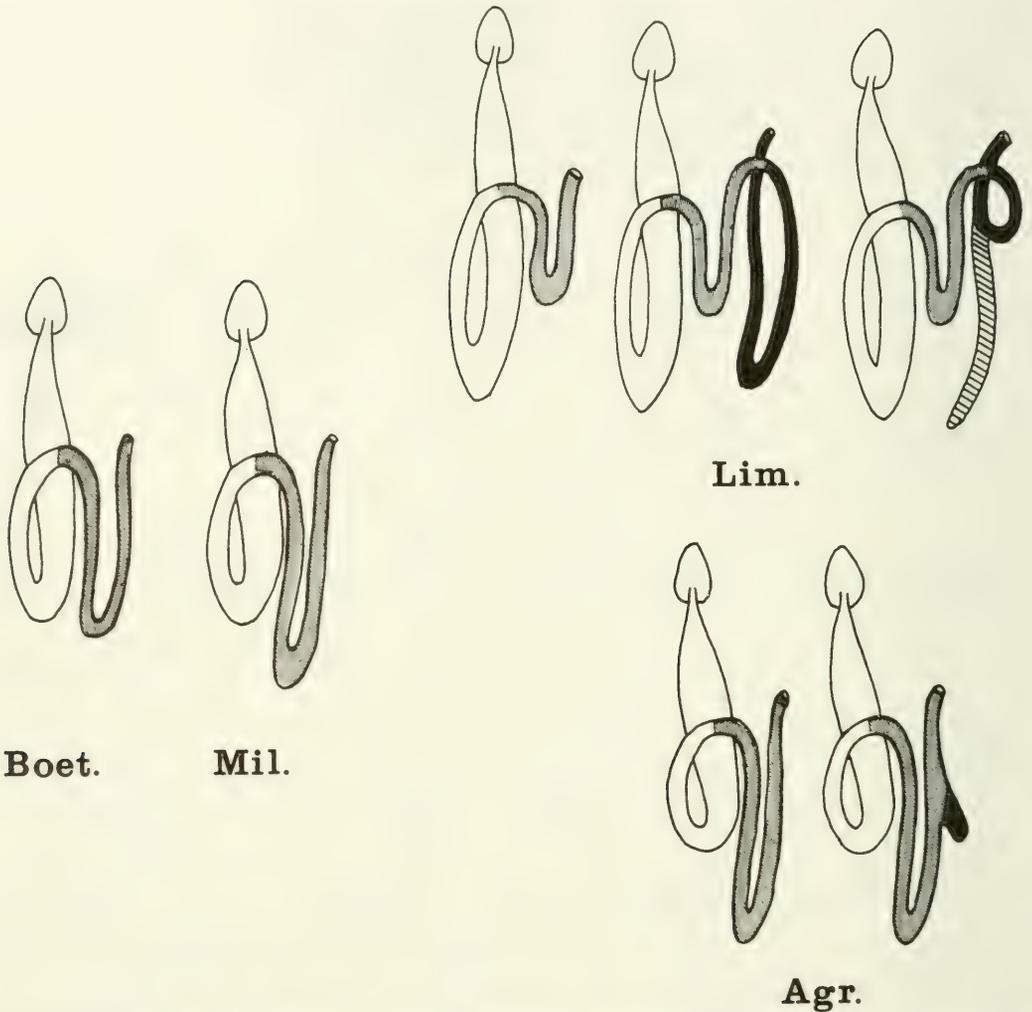


FIG. 3. Die Verdauungssystemen. Boet.—Boettgeriidae, Mil.—Milacidae, Lim.—Limacidae, Agr.—Agriolimacidae.

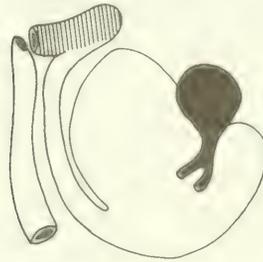
eigenartig. Bei den Boettgeriiden ist der Bau der Ausscheidungsorgane besonders charakteristisch. Bei den Agriolimaciden und Milaciden ist ein "Nierenlappen" (Lobus) vorhanden, während bei den Limaciden dieses Merkmal fehlt. Bei den Limaciden und Agriolimaciden gibt es ferner eine Harnblase, welche wiederum bei den Milaciden nicht vorkommt (Fig. 4).

Die Schale ist so stark reduziert, dass danach nicht einmal Gattungen gedeutet werden können. Trotzdem ist es möglich nach diesem Merkmal die verschiedene Herkunft der Milaciden einerseits, sowie der Limaciden und Agriolimaciden andererseits zu erkennen. Bei den Milaciden liegt der embryonale Teil der Schale auf der symmetrischen Achse und daraus ist zu schliessen, dass der Prototyp dieser Schale ähnlich wie die Schalen der rezenten Daudebardiidae gebaut sein konnte. Die asymmetrische Schale der beiden übrigen Familien dagegen musste aus einer anderen Schalenform entstanden sein. Bei der Gattung *Boettgerilla* ist die Schale symmetrisch wie bei den Milaciden, aber die hier extrem fortgeschrittene Verkümmerng ist so stark, dass es schwierig ist hierfür einen Prototyp ausfindig zu machen (Fig. 5).

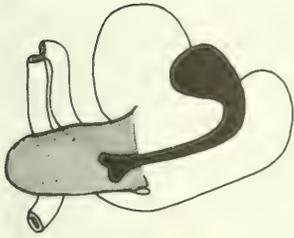
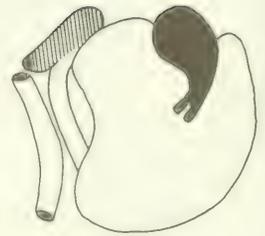
Der Bau der Genitalien ist recht gut brauchbar für die systematische Gliederung in Gattungen und Arten. Für die Gruppierung in höhere systematischen Einheiten ist besonders die Anwesenheit oder das Fehlen von verschiedenen akzessorischen Organen massgebend und zwar



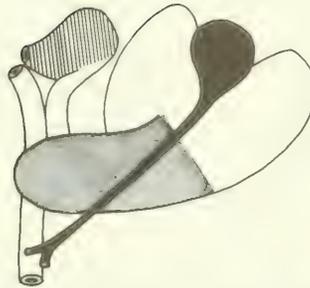
Boet.



Lim.



Mil.



Agr.

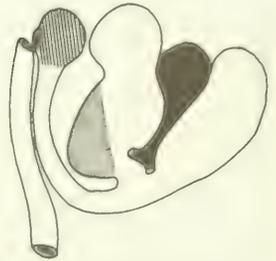
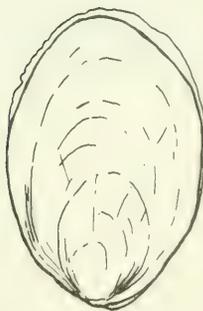


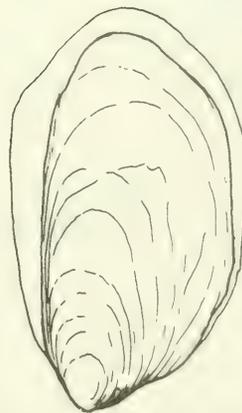
FIG. 4. Der Pallialkomplex. Boet.—Boettgerillidae, Mil.—Milacidae, Lim.—Limacidae, Agr.—Agriolimacidae.



Boet.



Mil.



Lim.



Agr.

FIG. 5. Die Schalen. Boet.—Boettgerillidae, Mil.—Milacidae, Lim.—Limacidae, Agr.—Agriolimacidae.

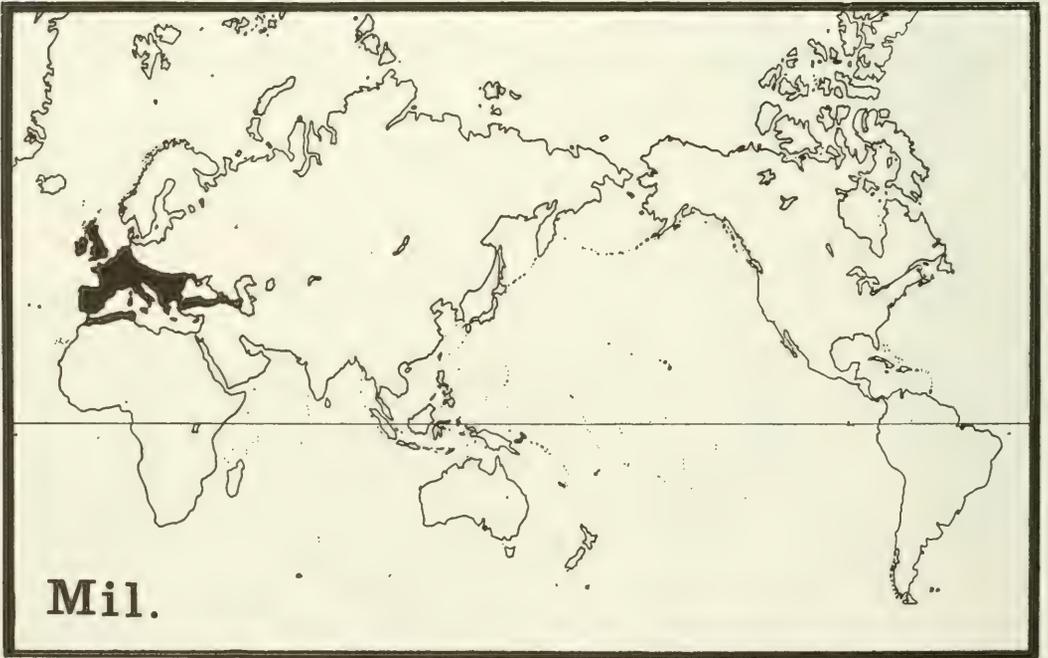
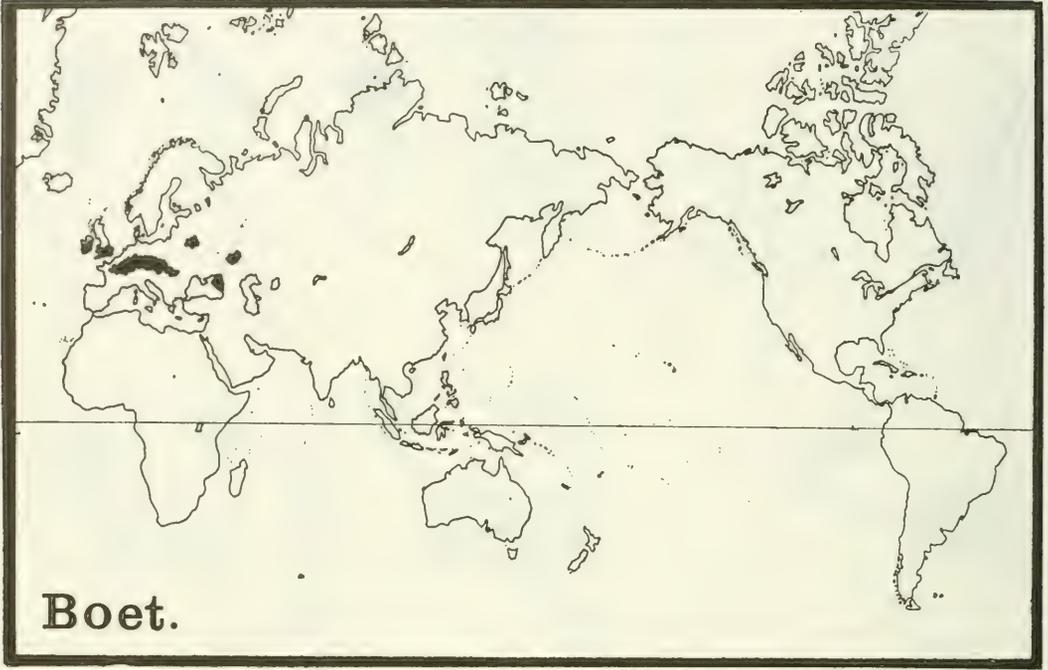


FIG. 6a. Die Verbreitung. Boet.—Boettgerillidae (Achtung ! endemisch für Kaukasus, hier zusammen mit Verschleppungsareal), Mil.—Milacidae.

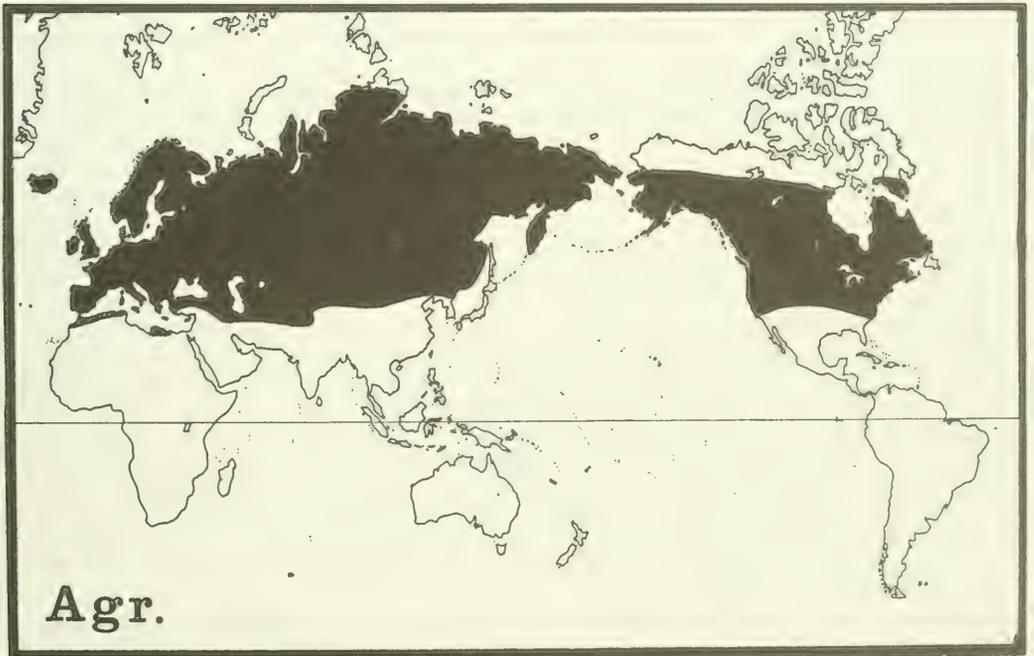
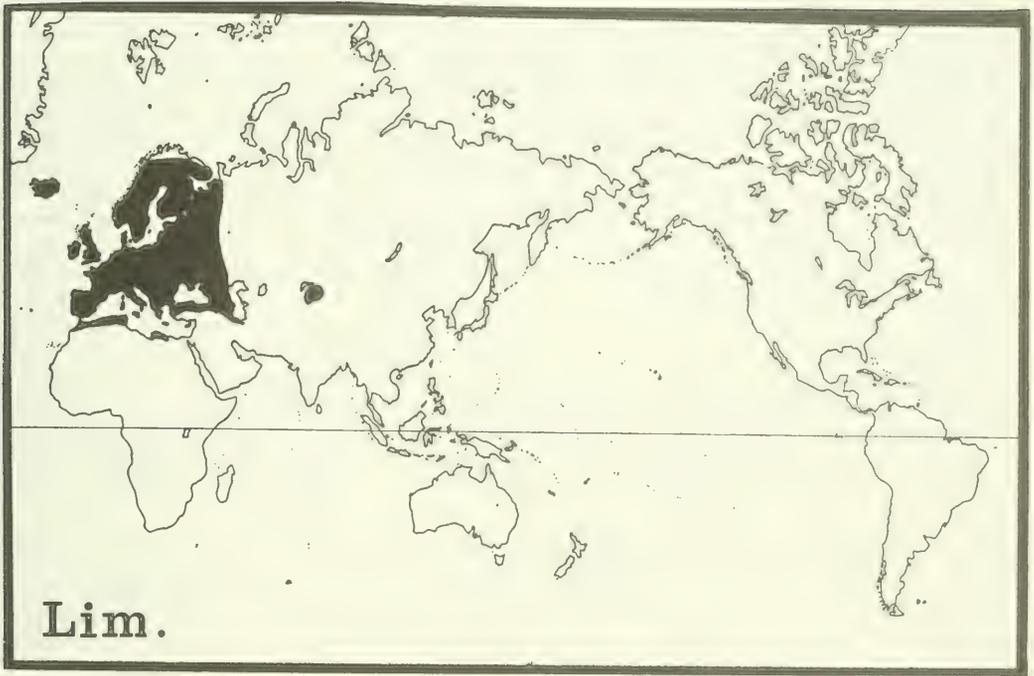


FIG. 6b. Die Verbreitung. Lim.—Limacidae, Agr.—Agriolimacidae.

sowohl im männlichen wie auch im weiblichen Teil des Geschlechtssystems. Wichtig ist auch die Verbindung der Bursa copulatrix mit den übrigen Organen. Charakteristisch für die Milaciden ist das Vorhandensein eines Epiphallus und der Anhangdrüsen in der Nähe der weiblichen Kopulationsorgane oder des Atriums. Die Boettgerilliden zeichnen sich unter den paläarktischen Schnecken durch das Spindelorgan (Corpus fusiforme) aus. Die Limaciden und Agriolimaciden besitzen keine deutlichen Trennungsmerkmale, doch fehlen hier gewisse Organe, welche bei den vorher erwähnten Familien vorkommen. Die Unterschiede zwischen diesen Familien sind nicht so deutlich ausgeprägt und bestehen hauptsächlich in den Ausmassen der Vertreter. Schliesslich ist noch zu erwähnen, dass bei den Milaciden und Boettgerilliden die Bursa copulatrix mit dem weiblichen Kanal, dagegen bei den Agriolimaciden und Limaciden mit dem Penis, verbunden ist.

Die hier vorgeschlagene Gruppierung der Nacktschnecken nach morphologischen Merkmalen findet auch eine deutliche Unterstützung durch zoogeographische Argumente. Obwohl wir über die primären Verbreitungszentren dieser Schnecken wenig wissen, ist bekannt, dass sich die Verbreitung der rezenten Vertreter dieser Familien recht unterschiedlich gestaltet. Die Boettgerilliden sind endemischen Formen des Kaukasus, später anthropogen weit in Europa und Asien verschleppt. Die Milaciden besiedeln Gebiete mit mildem Meeresklima, d.h. den Mittelmeerraum in weiteren Sinne. Die Limaciden besitzen ein grösseres Verbreitungsareal, welches auf die westliche Paläarktis und Tian-Schan Bergsystem begrenzt ist. Die Agriolimaciden bewohnen die ganze Holarktis, doch kommt die Mehrzahl der Arten in der Paläarktis vor (Fig. 6).

Die Verschiedenheit der aufgezählten Familien wird auch durch viele bionomische Merkmale bestätigt. So sind beispielweise die Boettgerilliden sehr beweglich und leben im Boden, d.h. unterirdisch. Die Milaciden sind wärmeliebend, widerstandsfähig gegen Trockenheit und wenig beweglich, besiedeln die Bodenoberfläche. Die Limaciden sind mesophil und sehr beweglich. Die Agriolimaciden sind am stärksten eurytop und besiedeln offenes Gelände mit starken Klimaschwankungen. An solche Verhältnisse haben sich diese Tiere mit kurzen Lebens- und Entwicklungszeiten angepasst, während die übrigen Nacktschnecken bedeutend länger leben.

Es zeigt sich also nochmals, dass das einzige gemeinsame Merkmal dieser vier Gruppen in deren "Nacktheit" besteht, wobei die Körperproportionen charakteristisch für die phytophagen Nacktschnecken geblieben sind.

Zur Vervollständigung unserer Kenntnisse lohnt es sich noch die verwandtschaftlichen Beziehungen zwischen den Gattungen der behandelten Gruppen zu analysieren. Die Familie Boettgerillidae umfasst nur eine Gattung mit 2 hoch spezialisierten Arten. Die Familie Milacidae umfasst rund 50 Arten, welche leicht in 3 gut unterscheidbare Gattungen aufgeteilt werden können. Als Trennungsmerkmal ist hier die Lage der Anhangdrüsen und des Stimulators massgebend (Fig. 7). Die Familie Limacidae ist sehr stark differenziert und umfasst 10 Gattungen mit insgesamt 150 Arten. Ausser Genitalien sind zur generischen Gruppierung besondere Einzelheiten im Bau des Verdauungssystems verwendbar, wie z.B. die Grösse der dritten Darmschlinge und des Blinddarmes.

Die grössten Schwierigkeiten bereiten die Agriolimaciden. Hierher gehören 5 Gattungen mit etwa 110 Arten. Die grösste Gruppe dazwischen bildet die Gattung *Deroceas*. Eine Einteilung in kleinere Gruppen ist hier schwer durchzuführen und kann nur unter Berücksichtigung von mehreren morphologischen Merkmalen erfolgen. Es handelt sich hier zumeist um Merkmale, die nur bei einer Gattung vorhanden sind und bei den übrigen fehlen. Als Beispiel kann hier das Vorhandensein oder Fehlen des Blinddarmes, des Stimulators, des Kieles auf dem Rücken oder der Längsfurchen am Fuss erwähnt werden. Die hier behandelte Gruppe bleibt weiterhin am wenigsten durchforscht und nach der Ansammlung von weiteren neuen Erkenntnissen dürften über diese Artengruppe zukünftig noch rege Diskussionen zu erwarten sein. Es hat den Anschein, dass wir hier mit der phylogenetisch jüngsten Gruppe zu tun haben, worauf ihre starke Differenzierung hinweist.

Zusammenfassend weisen wir darauf hin, dass wir die bisher angenommene Meinung, nach welcher alle 4 Gruppen gemeinsamer Herkunft sein sollen, nach den dargestellten Überlegungen nicht vertreten. Die Milaciden sind zweifellos von anderen Vorfahren abzuleiten als die Limaciden, Agriolimaciden und Boettgerilliden. Darauf deutet der Bau der Schale, des Mantels, des Fusses usw. Die Limaciden und Agriolimaciden wie auch Boettgerilliden scheinen miteinander näher verwandt zu sein und es ist möglich, dass diese Familien von einem gemeinsamen Zweig unter den Gehäuseschnecken abstammen. Die Boettgerilliden scheinen eine hochspezialisierte, unterirdische, in dem Boden lebende Gruppe zu sein.

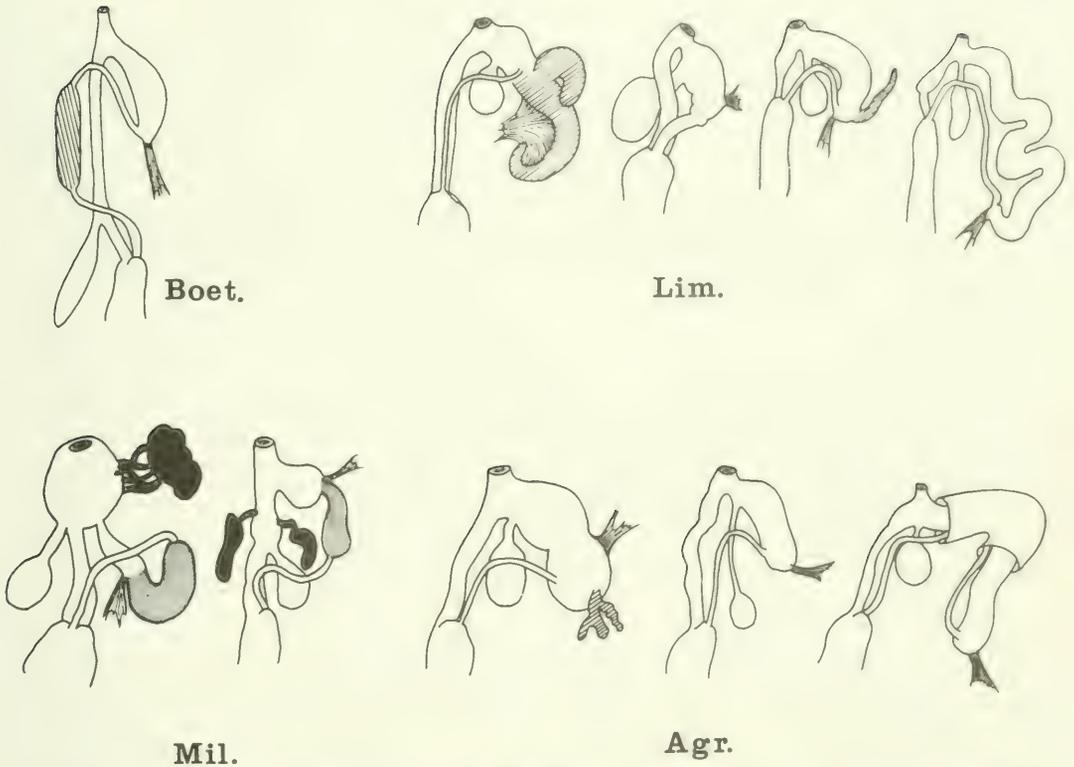


FIG. 7. Die Genitalien. Boet.—Boettgerillidae, Mil.—Milacidae, Lim.—Limacidae, Agr.—Agriolimacidae.

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ANATOMICAL STUDIES IN THE AFRICAN ACHATINIDAE—A
PRELIMINARY REPORT

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ABSTRACT

Basic anatomical patterns of the reproductive system in the Achatinidae are reported. Both macro- and microphallate species are found in genus *Archachatina* and subgenera *Achatina* and *Lissachatina* in genus *Achatina*, an interpretation for which is still elusive. Good characters are found at the generic, subgeneric and usually specific levels. In some closely related species complexes, the anatomies may be very similar. Subspecific differences seem to rest entirely in conchological features. Both soft anatomy and shell characters are necessary for a better understanding of the phylogeny in this group. Great emphasis is placed on the importance of an adequate series of specimens in good condition and of variable age. Different methods of expanding, killing, preserving and examining are evaluated.

In 1944 and 1945, my early anatomical studies of the Achatinidae in, then, the Gold Coast (Ghana) and Nigeria established the fact that at least some of the species in this family are distinct from each other in the anatomy of the genital system. This, in turn, raised the hope that a comparative anatomical study would contribute substantially to a better understanding of the phylogeny in this popular, taxonomically confused group. With the indispensable conchological support of Dr. Joseph C. Bequaert (1950), such a study was initiated in 1948 at the Museum of Comparative Zoology at Harvard under the sponsorship and support of the National Research Council-National Academy of Science and the Office of Naval Research (Mead, 1950). The principal thrust of that study was to establish through comparative anatomy the identity of the "parent" continental African population of the pestiferous *Achatina fulica* Bowdich, which had spread widely through the Indo-Pacific region (Mead, 1961), and to distinguish it from its related achatinid species. Such a study was judged to be a necessary prelude to an exploration for the "natural enemies" of this species in its native hearth as possible biological control agents (Williams, 1951).

Achatina hamillei Petit and *A. fulica* Bowdich proved to be indistinguishable in the basal genital structures but tangibly distinguishable in their conchological features. Bequaert therefore placed *A. hamillei* as a subspecies of *A. fulica* and assigned the populations that were not on the African mainland, or on Zanzibar and other African coastal islets, to the nominate subspecies (1950: 86). This early established the parameter of minimal consistent anatomical differences of the soft anatomy at the level of species and the parameter of minimal conchological differences at the level of subspecies. In contrast, *A. immaculata* Lamarck [= *panthera* (Fer.)], the only other achatinid to become established outside continental Africa, and *A. fulica* are often so strikingly similar conchologically, particularly in old or worn specimens, that they are misidentified in many collections. Anatomically these 2 species manifested extreme dissimilarity (Figs. 1, 2), leaving no doubt about their validity as distinct species. In further contrast, the only 2 grossly reticulate achatinid species, *A. reticulata* Pfeiffer and *A. albopicta* E. A. Smith, were found to be conchologically so similar, and therefore frequently confused in collections, that Bequaert initially decided to place *A. albopicta* as a subspecies of *A. reticulata*. However, the anatomy of the basal genital structures proved to be so different (Figs. 3, 4) that the 2 taxa were maintained as separate but closely related species. As a matter of fact, the basal genital structures of *A. fulica* and *A. albopicta* seem superficially more similar to each other than they are to *A. immaculata* and *A. reticulata*, respectively, because they both retain what is

believed to be the prototypic, diminutive, completely ensheathed penis and the heavily muscular basal vagina. All are in Bequaert's subgenus *Lissachatina* (Bequaert, 1950: 49). It was clear at this point that anatomical and conchological features had to be considered in concert to understand and interpret better the phylogenetic relationships and to establish a more accurate taxonomy in this group.

These early, encouraging results at Harvard set the stage for more extensive and intensive studies of the Achatinidae during the periods October 1974 to June 1975 (Mead, 1976) and July to September 1977, principally at the Musée Royal de l'Afrique Centrale in Tervuren, Belgium, but also at the following institutions: Institut Royal des Sciences Naturelles in Brussels, Rijksmuseum van Natuurlijke Historie in Leiden, Muséum National d'Histoire Naturelle in Paris, British Museum (Natural History) in London, Zoölogisch Museum in Amsterdam, and the Naturhistoriska Museet in Göteborg, Sweden. Whereas the earlier project included mainly East African forms, the more recent studies have included to a greater extent the West African and South African forms. The present report is intended to be only preliminary, with anatomical details and new taxa being fully described elsewhere.

The species of subgenus *Achatina*, as in subgenus *Lissachatina*, were found to fall into 2 anatomically distinct groups, *viz.* those with enlarged penes, usually well extended beyond the penial sheath (Fig. 5), and those with diminutive, tripartite penes usually entirely embraced by the penial sheath (Fig. 6). But the differences are proportionately greater in subgenus *Achatina*. Fig. 5, of which *Achatina achatina* (L.) is typical, shows a large, thick-walled penis; a long, bipartite, thick-walled vagina; a short free oviduct; and a spermatheca adjacent to the uterine portion of the spermooviduct. In direct contrast, many other species in this group (Fig. 6) have a small, tripartite penis entirely enclosed in the penial sheath; a slender, short, thin-walled vagina; a long free oviduct; and a spermathecal duct so short that the spermatheca does not reach the uterine portion of the spermooviduct. The penes of the macrophallate forms in *Lissachatina* (Fig. 2) are relatively thin-walled and, as in the microphallate forms in that group (Fig. 1), there is a thin-walled prepuce (PC) basal to the penial sheath (PS) and apical to the genital atrium (GA). A prepuce could not be distinguished in subgenus *Achatina*. The tripartite penis of *Lissachatina* (Fig. 1) has a thick-walled muscular apical penis (AP); an extremely slender, tube-like medial penis (MP); and a thin-walled basal penis (BP) that is set off from the unensheathed prepuce (PC) by a constriction. The tripartite penis of subgenus *Achatina* (Fig. 6), however, has a thick-walled arcuate apical penis; a broad medial penis usually containing a pilaster or pilaster-like thickening; and a thin-walled basal penis that is confluent with the genital atrium. In contrast to *Lissachatina*, the tripartite penis of subgenus *Achatina* is attached throughout its length, and in all directions, to the inner wall of the penial sheath by a thick webbing of filamentous muscle strands—so much so in some species that the penis literally has to be excavated after the penial sheath has been cut. Heavier muscle strands, apparently originating on the outer surface of the basal penial sheath, connect broadly with the basal vagina, genital atrial wall, and adjacent portions of the body wall, sometimes considerably obscuring these structures.

The basal genital structures of the 7 examined species in subgenera *Archachatina*, *Calachatina* and *Megachatinopsis* of genus *Archachatina* show a remarkable resemblance (Fig. 7) to those of the macrophallate species of subgenus *Achatina* (Fig. 5). The vagina in the macrophallate *Achatina*, however, is bipartite and contrastingly very thick-walled. In both groups there is a thick but conspicuous band of muscle fibers, running from the basal vagina to the region of the juncture of the free oviduct and the spermathecal duct, originating in the wall of the basal female conduit and attaching narrowly to the right body wall along the juncture of the lung diaphragm, mantle, and neck. This band probably functions as a vaginal retentor (VR). It is tempting to assume a correlation between this and the macrophallate condition; but the absence of it in the near-macrophallate *Lissachatina* (Fig. 4) and the presence of it in 5 examined microphallate species of subgenus *Tholachatina* of *Archachatina* (Fig. 8) caution against this assumption. At least 3 of the 5 South African species of *Tholachatina* have a longitudinally grooved or doubly folded penis (Fig. 8), the expanded condition of which during copulation probably produces a formidable intromittent organ (Van Bruggen & Appleton, 1977). This would seem to justify the existence or persistence of the retentor muscle. The examination of other *Tholachatina* species is beginning to bridge the gap between the macro- and microphallate

species of *Archachatina*. In addition, the congeneric or consubgeneric status of some of the species currently assigned to *Tholachatina* is coming into question.

On the basis of earlier work (Mead, 1950), the species of *Limicolaria* and the closely related *Limicolariaopsis* remain distinctive in that a verge or penis papilla is present. Collaboration between this author and Dr. A. C. van Bruggen of the Rijksmuseum van Natuurlijke Historie in Leiden has shown that some species in *Callistoplepa* (= *Callistopepla*) are not congeneric. A co-authored taxonomic revision of this genus is currently in progress. Other genera in the Achatinidae have revealed so far only tantalizing bits of anatomical information and have yet to take a firm place in the taxonomic scheme.

The question is often asked, "Just how valuable and dependable is the reproductive anatomy in determining relationships in the Achatinidae?" At the generic and subgeneric levels it is good, although the known anatomies in each of genus *Archachatina* and subgenera *Achatina* and *Lissachatina* (and apparently *Tholachatina*), fall into 2 rather distinct groups, viz. macro- and microphallate. An explanation for these parallel anatomical dichotomies is slow in emerging. At the level of species, the soft anatomy is generally providing good criteria, although in certain groups of closely related species, such as the *schweinfurthi-stuhlmanni-tincta-weynsi* complex in subgenus *Achatina*, the anatomies understandably tend to be very similar. Differences at the subspecific level appear to rest entirely in conchological features.

What is really needed to establish the true nature of the soft anatomy, and its relative taxonomic value, is an adequate series of specimens in *good* condition and of variable age. Through the careful examination of such a series, artifacts of preservation, anomalies, and juvenile features can be put into perspective. Regrettably, all too often specimens in wet collections are found to be improperly preserved. Dropping alive directly into 70% ethanol produces a badly contracted and distorted specimen that usually is so dehydrated that protracted soaking in water or, better, 2% trisodium phosphate is required. Even then, the inner layers of the body wall may remain so hard that the genital system literally has to be excavated. The basal genital structures in such specimens are not infrequently extremely attenuated, contracted, or distorted. Putting the specimen directly into unneutralized formalin is actually worse because the corrosive action etches and lifts off the periostracum, erodes the nepionic whorls beyond recognition, obscures the diagnostic sculpturing of the shell, fuses portions of the mantle with the chemically changed nacreous layer, alters the colors (e.g. yellows to browns), and causes the shell to become so thin, brittle and chalky that the soft parts cannot be removed without shattering it.

Ideally, the specimen should be narcotized for several hours in a tepid solution of any one of several quite good agents (berthanol chloride, propylene phenoxetol, nembutal, chloral hydrate, chloretone, etc.) to relax and extend it. In the absence of such agents, either the specimen can be drowned for several hours in boiled water or the shell of the live animal can be crushed and the specimen examined directly. Protracted drowning, especially where the temperature is elevated, often causes a prolapse of the genital atrium and the basal portions of the male and female conduits. It is virtually impossible to reconstruct the normal condition of the genitalia in such specimens. If the shell is to be saved, the live specimen can be dropped in near-boiling water and removed from its shell as soon as the columella muscle will give way (3-5 minutes). Large series of *Achatina fulica* heat killed and dissected in this manner showed remarkably little anatomical distortion as compared to most alcohol or formalin preserved material. Live dissected material served as a control.

In the field, when there is no time for dissecting, and where it is important to keep shell and soft parts together, the snails should be drowned in boiled water (at room temperature—usually warm) overnight (ca. 8 hours) to extend them. Either of the following 2 methods can then be used with usually excellent results: The snails are injected and submerged in 4% neutralized formalin (no higher percentage!) for no more than 48 hours. They are then removed, washed thoroughly in water and preserved in 70% ethanol, usually with another change of solution. Or, the snails are put directly into 70% ethanol for 2-3 days, removed, injected with and immersed in a new solution of 70% ethanol. I have had relatively little trouble removing all or nearly all of the soft parts of such specimens without damaging the shell, and the soft parts remain firm and pliable. Where the specimen is small, the soft parts can be removed from the shell with strong rat-tooth forceps. In large specimens, a heavy metal probe can be forced through the

All illustrations are diagrammatic and not drawn to scale. The penial retractor, all of the apical vas deferens, most of the basal vas deferens, the spermoviduct, and the spermatheca (in species where it is attached to the uterine portion of the spermoviduct) have been omitted for simplicity and to emphasize relevant structures.

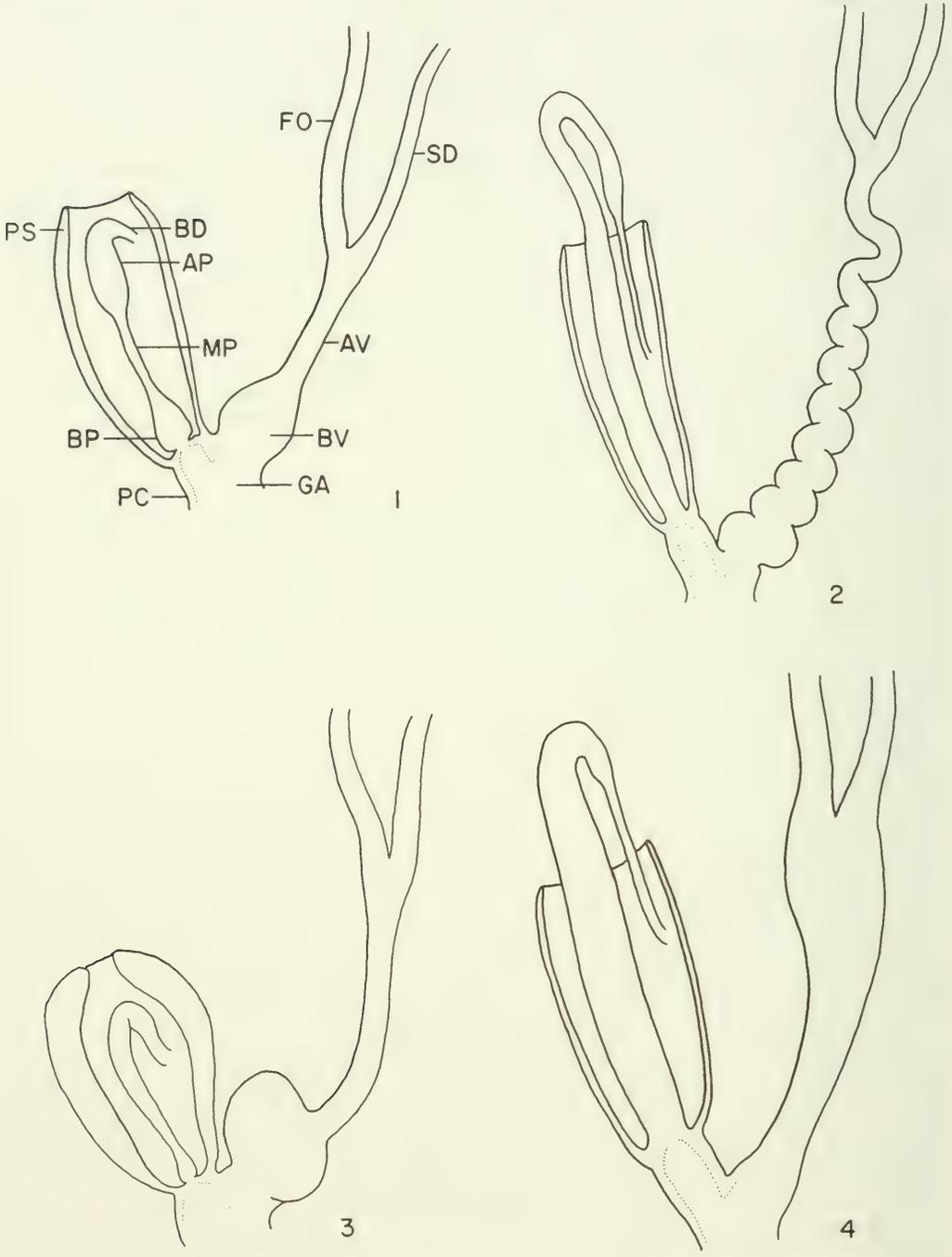


FIG. 1, *Achatina fulica* Bowdich; FIG. 2, *A. immaculata* Lamarck; FIG. 3, *A. albopicta* E. A. Smith; FIG. 4, *A. reticulata* Pfeiffer.

AP—apical penis, AV—apical vagina, BD—basal vas deferens, BP—basal penis, BV—basal vagina, FO—free oviduct, GA—genital atrium, MP—medial penis, PC—penial prepuce, PS—penial sheath, SD—spermathecal duct, VR—vaginal retentor.

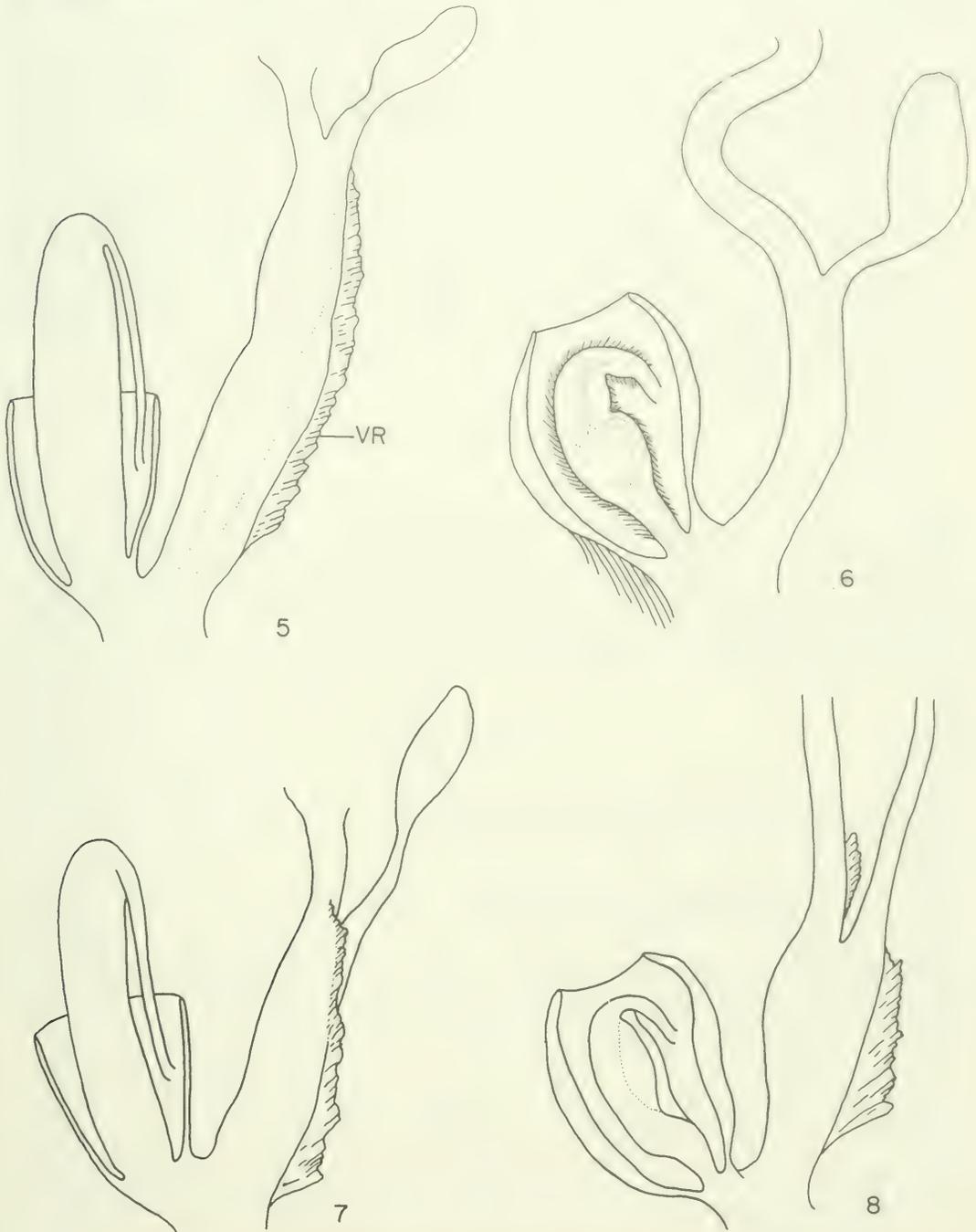


FIG. 5, *A. achatina* (L.); FIG. 6, microphallate type, subgenus *Achatina*; FIG. 7, macrophallate type, genus *Archachatina*; FIG. 8, microphallate type, some species of subgenus *Tholachatina*.

center of the foot and the soft parts can literally be unwound from the shell with the aid of a jet of water directed into the shell behind the body mass. The upper part of the digestive gland, gonads, hermaphroditic duct, talon, and possibly the albumen gland with adjacent portions of the spermiduct may be lost, but the diagnostic basal genital structures usually remain intact. Of course, if the series is large enough, the shell may be sacrificed in selected specimens.

The greatest impediment in the present study is the paucity of preserved anatomical material. The collection in the Musée Royal de l'Afrique Centrale is outstanding and is doubtless without equal any place in the world. Collections elsewhere so far have proven to be considerably more modest in series or in represented species, or both. With conditions being what they are today in many parts of Africa, particularly in Angola where there are a number of little known, confused, smaller species of *Achatina*, the outlook is not at all optimistic. Even when preserved specimens are available in fair series, conclusions frustratingly may have to remain tentative because of the lack of ecological data and the presence of questionable characters that could be explained as artifacts of preservation, immaturity of specimens, or natural variability. One thing remains clear. As announced earlier (Mead, 1950: 287), both conchological and anatomical data are needed as complements. To these emphatically must be added ecological data wherever possible. Ultimately, genetics, physiology and histology will contribute their part to a better understanding of the phylogenetic relationships and therefore taxonomy of the zoogeographically important family Achatinidae.

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ON *ELONA* (PULMONATA, ELONIDAE FAM. NOV.)

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ABSTRACT

The genus *Elona* Adams & Adams, 1855, is represented by 2 recent species, *E. quimperiana* (Férussac) and *E. pyrenaica* (Draparnaud), both living in SW Europe. However, while studying the genitalia of these species it became evident that they are clearly different from what is usual in the Helicidae. The mucous glands, inserting on the upper part of the vagina, are irregularly bulbous. There is a double-walled penis without papilla; the inner tube shows longitudinal ridges or papillae in the lumen. *Elona* obviously does not fit well in any of the known subfamilies or families. Therefore, a new family, Elonidae, is proposed.

E. quimperiana and *E. pyrenaica* differ in many characters of shell and genitalia. Evidently, however, they are more closely related to each other than to any other Recent pulmonate snail species, which should be recognized in the nomenclature. The relation to *Tropidomphalus* Pilsbry, 1895, known from the European Oligocene-Pliocene, remains unclear.

The name *Elona* has been introduced by Adams & Adams (1855: 211) as a nomen novum for *Sterna* Albers, 1850, non Linnaeus, 1758, a name proposed for a single pulmonate snail species, *E. quimperiana* (Férussac, 1821), known from Brittany and, separated from there by a 500 km gap, also from the northeastern Atlantic coastal area of Spain as far east as the extreme SW of France (Fig. 1). *E. quimperiana* differs conspicuously from all other western Palaeartic gastropods in shell shape (Figs. 2-5), somewhat resembling certain tropical Camaenidae, especially of the genus *Chloritis* Beck, 1837. The shell is thin and transparent, and has a strongly inflated last whorl and an immersed apex. The first ca. 1½ whorls show a regular pattern of spirally arranged elongated papillae, formed by the calcareous part of the shell and accentuated by periostracal, erect (usually deciduous) scales. On the following about 1½ whorls comparatively big and widely spaced round calcareous papillae are developed, forming the bases of ca. 0.15 mm long, thick periostracal hairs; additionally, many very fine periostracal papillae are found on this part of the shell. On the last whorls only an irregular radial sculpture is seen, with very fine, more or less obsolete spiral striae.

As early as 1855-1856, the brilliant French malacologist Moquin-Tandon published anatomical data on *E. quimperiana* as well as on many other gastropod species represented in France. He had discovered that *E. quimperiana* was not only aberrant in shell shape, but also in having club-shaped mucous glands instead of glands of the normal finger-like type. He also had found a 2nd species with similar mucous glands, known as *Helix pyrenaica* Draparnaud, 1805. This species, which is restricted to a small area in the eastern Pyrenees in France, Andorra and Spain (Fig. 1), strongly resembles certain representatives of the European Campylaeinae in shell shape (Figs. 4, 5). The shell is less thin than in *E. quimperiana*, the body-whorl is not strongly inflated and the apex is not immersed. The first ca. ¼ whorls show an irregular pattern of papillae and wrinkles. On the following ¼-½ whorls, irregular radial riblets become more obvious and vague, spirally elongated papillae are developed, most clearly on the part of the whorl adjoining the outer suture; on the opposite part, near the inner suture, the pattern of roundish papillae is continued, in some specimens as far as the aperture of the shell. Irregular radial riblets and very fine spiral striae are seen on the younger whorls; the striae may be obsolete or completely reduced on the body whorl. As all specimens studied were well "cleaned", additional periostracal structures have not been observed although these might be present.

Moquin-Tandon (1855-1856: 126) assigned *E. quimperiana* and *E. pyrenaica* to a *Helix*

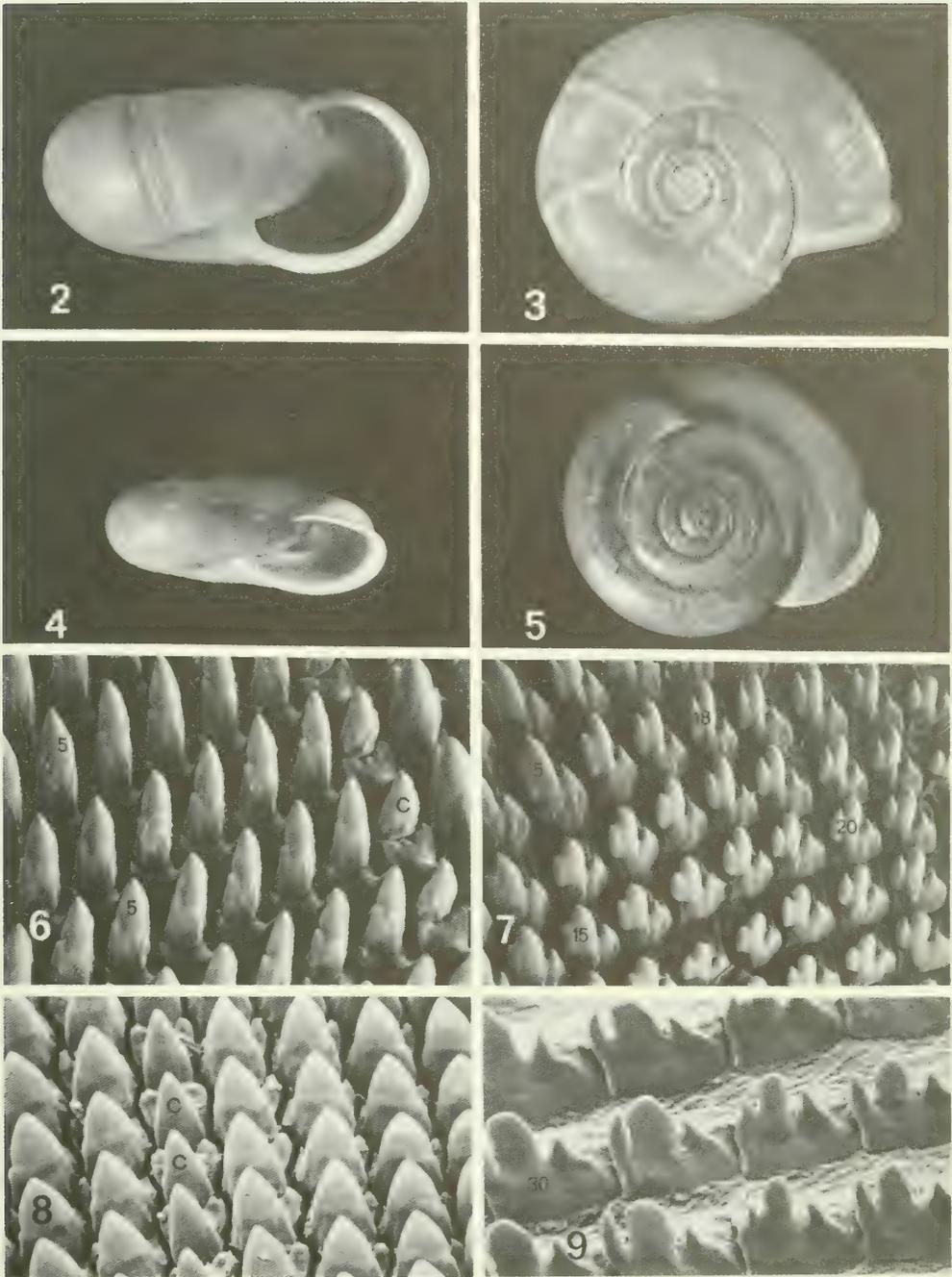


FIG. 1. Distribution of *Elona quimperiana* and *E. pyrenaica*, after Germain (1930: 229-230) and material present in the Rijksmuseum van Natuurlijke Historie, Leiden, Netherlands, and in the Senckenberg Museum, Frankfurt am Main, Germany.

subgenus of their own, characterized by the club-shaped mucous glands. He used the name *Corneola* Held, 1837, for this taxon, apparently overlooking that Gray (1847: 172) selected "*Helix cornea*" as type-species of *Corneola*, in conformity with the ICZN, as Held (1837: 912) listed "*cornea* Drap." under *Corneola*. *H. cornea* is assigned to *Chilostoma* Fitzinger, 1833, by Moquin-Tandon (1855-1856: 134). Later on Hesse (1885) restudied the genitalia of *E. quimperiana* and Ortiz de Zárate (1946: 337-340) did the same for *E. pyrenaica*. Hesse (1885: 4) emphasized the isolated position of *E. quimperiana*, stating that the species most certainly does not belong to *Campylaea* ("alles Andere . . . als eine *Campylaea*"); however, he could not suggest any alternative classification. Ortiz de Zárate (1946) confirmed the observations published by Moquin-Tandon (1855-1856) and gave some additional information. Zilch (1960: 700) classified *Elona* among the Campylaeinae, Helicidae, as had been done by Germain (1930: 228), which author only used a different name, Helicigoninae, for the subfamily; Pilsbry (1895: 307) considered *Elona* even a subgenus of *Helicigona* Férussac, 1821.

The present paper deals with two main questions: (1) should *E. quimperiana* and *E. pyrenaica* be considered congeneric or not, (2) are these 2 species only aberrant amidst the many representatives of the Helicidae by the shape of the mucous glands, or are they both different in other characters as well.

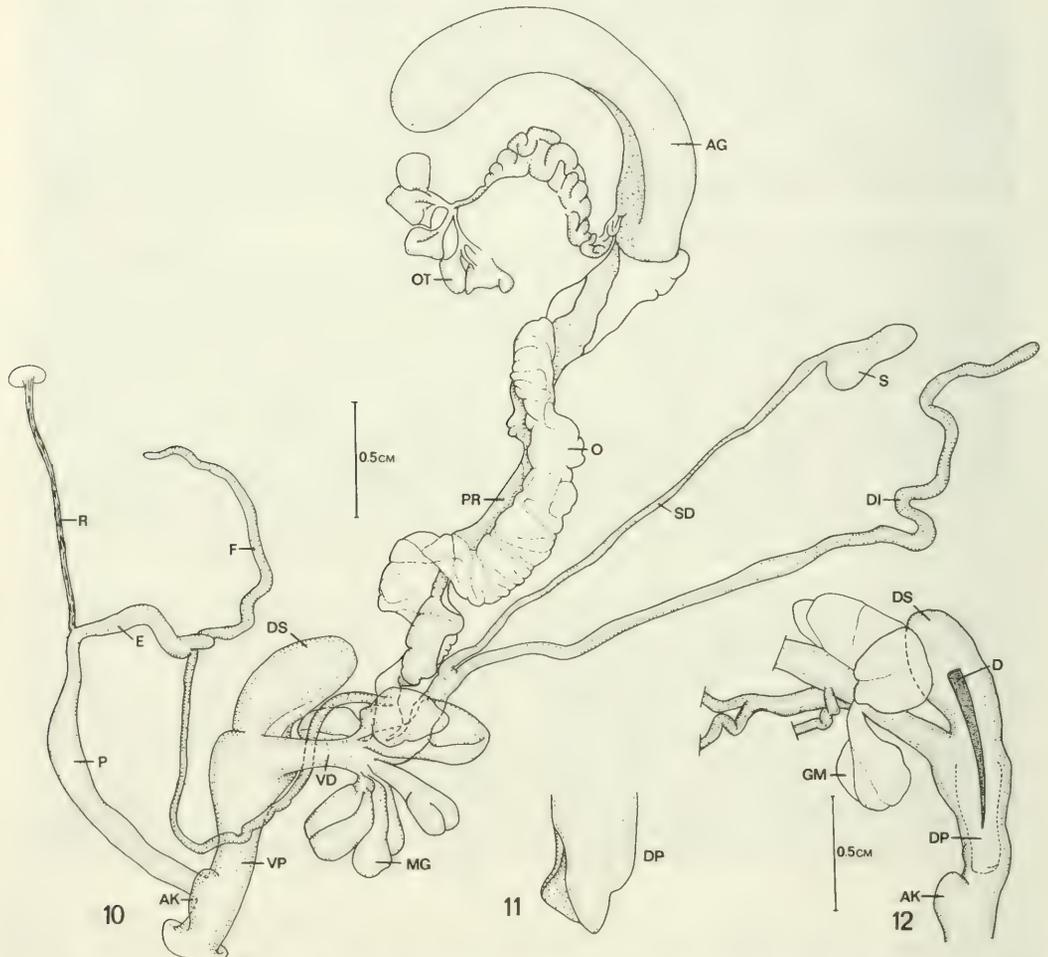
The conspicuous differences in shell shape and structure have been mentioned above. The genitalia of *E. quimperiana* and *E. pyrenaica* are clearly different as well. In *E. quimperiana* (Figs. 10-13) the genital atrium has a thick muscular knob (AK). The vagina consists of a broad proximal part (VP) bearing a large dart sac (DS) with a long and slender dart (D) inside, and a more slender distal part (VD) around which the about six club-shaped mucous glands (MG) insert. In the thick proximal vagina there is a conspicuous dart papilla (DP). The receptaculum seminis has a diverticulum (DI) which is longer than the spermatheca (S) and its duct (SD)



FIGS. 2, 3. *Elona quimperiana*, Spain, Santander, Ramales, Español leg.; width 26.8 mm (RMNH, Leiden). FIGS. 4, 5. *Elona pyrenaica*, Spain, Gerona, Rialp, Queralps, C. Altimira leg.; width 21.1 mm (RMNH, Leiden). FIGS. 6, 7. *Elona quimperiana*, details of the radula (numbers of teeth indicated), France, Finistère, between Berrien and Scrignac (SE of Morlaix), J. P. M. Clerx leg.; 6, X550; 7, X525. FIGS. 8, 9. *Elona pyrenaica*, details of the radula (numbers of the teeth indicated), France, Pyrénées-Orientales, Villefranche-de-Conflent, D. Aten leg.; 8, X600; 9, X1275.

together. The penis (P) is slightly longer than the flagellum (F) and more than twice as long as the epiphallus (E). The flagellum is widest at its base. From its insertion on the genital atrium about 4/5 of the penis is double-walled. The inner tube is subdivided from proximal to distal in 3 parts according to the surface structure of its lumen, which consists of a few irregular longitudinal ridges, many fine papillae and a few longitudinal ridges, respectively. There is no penis papilla. On Fig. 10 the albumen gland (AG), the oviduct (O), the ovotestis (OT), the prostate (PR) and the retractor muscle (R) of the penis, which do not show special characters, are indicated as well.

In *E. pyrenaica* (Figs. 14, 15) the genital atrium (A) has no knob. The distal part of the vagina (VD) has a very thick wall and is, therefore, as thick as the proximal part (VP). The club-shaped mucous glands (MG) have shorter stalks (ducts). A dart papilla is absent and the small dart sac (DS) contains a very short dart (D) with a broad base. Externally the male part of the genitalia differs from that of *E. quimperiana* in the very long flagellum (F), which is more than twice as long as the penis (P), and the epiphallus (E) which equals the penis in length. The flagellum is broadest at about the middle of its distal half. The double-walled part of the penis, comprising 4/5 of its total length, ends at the insertion of the penis retractor. Therefore, in contrast to what is found in *E. quimperiana*, the most proximal part of the penis



FIGS. 10-12. *Elona quimperiana*, France, Finistère, between Berrien and Scignac (SE of Morlaix), J. P. M. Clerx leg. (RMNH, Leiden); 10, genitalia; 11, detail of the dart papilla; 12, detail showing the position of dart papilla and dart.

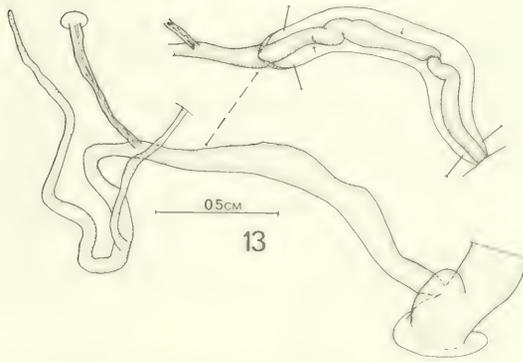


FIG. 13. *Elona quimperiana*, specimen of Figs. 10-12, detail illustrating the position of the inner tube of the penis, with arrows indicating where its 3 parts end (see also the text).

is simple. The inner tube can be subdivided in only 2 parts, the most proximal one with papillae, which are less small than in *E. quimperiana*, and the distal part with some longitudinal ridges. A penis papilla is also absent in *E. pyrenaica*. There are no conspicuous differences in the shape of the receptaculum seminis; the diverticulum may be swollen at the end (Fig. 14).

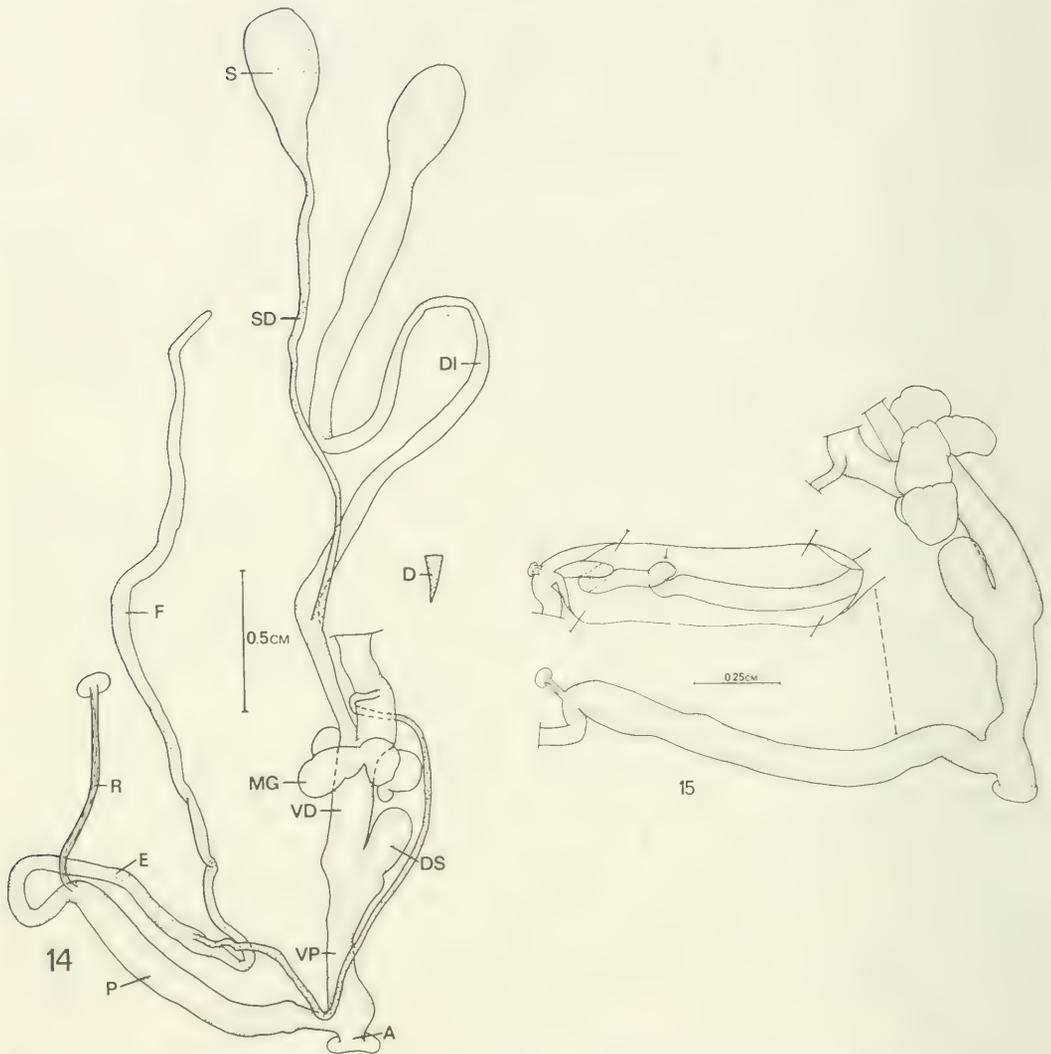
In both *E. quimperiana* and *E. pyrenaica* the right eye retractor muscle passes between penis and vagina. The foot-sole is not subdivided in the first species; this character could not be investigated in the other taxon. In *E. quimperiana* the mantle shows irregular dark spots, visible through the transparent shell, and, therefore, causing a kind of mimicry as seen independently in various groups of gastropods (cf. e.g. Van Bruggen, 1978: 900, Fig. 10). On the mantle of *E. pyrenaica*, which has an opaque shell, no dark spots were observed.

Both species have an odontognathous mandibula. The radulae (Figs. 6-9) are similar. The same type of teeth are seen in *E. quimperiana* and *E. pyrenaica*, in which the formulae C + 50 (after one specimen) and C + 45-47 (after 2 specimens) were found respectively.

Summarizing we may say that *E. quimperiana* and *E. pyrenaica* not only differ clearly in shell shape and microsculpture, but also in the morphology of the genitalia. The relative measurements of many parts are very different in both species. Some structures are found in only one of the 2 (atrial knob, dart papilla), others show obvious differences in shape (e.g. flagellum, dart, inner surface of the inner penial tube). However, *E. quimperiana* and *E. pyrenaica* are more closely related to each other than to any other western Palearctic gastropod species known at present. I prefer to demonstrate this relationship in nomenclature, rather than to emphasize the many structural differences by introducing a new genus or subgenus name.

Obviously, *Elona* cannot be assigned to the Campylaeinae, which are characterized by a completely different type of genitalia (e.g. Knipper, 1939). A double-walled penis as seen in certain Helminthoglyptidae (e.g. Pilsbry, 1939: 67), and mucous glands as in Bradybaenidae (e.g. Pilsbry, 1895: xxxvi), inserting, however, on the vagina as in Helicidae, make the classification of *Elona* difficult. Therefore, a new family, Elonidae, is proposed, with some hesitation, as much research on the higher pulmonate taxa still has to be done.

One could pose the question whether fossil representatives of the Elonidae are known. Unfortunately, as we have seen before, these might not be clearly distinguishable from the Campylaeinae, as only shell characters will be available. Schlickum & Strauch (1972: 79, fig. 4) described *Elona kowalczyki* from the German Upper Pliocene based on a single damaged shell, without giving details on the microsculpture. Judging from the description and figure only, it remains obscure to me why these authors suppose that this species does not belong to *Tropidomphalus* Pilsbry, 1895. In fact, it would be most interesting to know how the representatives of this genus, known from the Oligocene to the Pliocene in Europe, differ from the *Elona* species. Judging from Zilch (1960: 699-700, figs. 2434, 2435) the *Tropidomphalus* species bridge the gap between *E. quimperiana* and *E. pyrenaica*, at least in shell shape. Here we find another argument against creating a separate genus or subgenus for *E. pyrenaica* at this moment.



FIGS. 14, 15. *Elona pyrenaica*, France, Pyrénées-Orientales, Villefranche-de-Conflent, D. Aten leg. (RMNH, Leiden); 14, genitalia and dart; 15, detail illustrating the position of the inner tube of the penis, with an arrow indicating the transition between its 2 parts (see also the text).

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TAXONOMICAL STUDIES ON *BULINUS* USING ISOENZYME ELECTROPHORESIS WITH SPECIAL REFERENCE TO THE *AFRICANUS* GROUP ON KANO PLAIN, KENYA

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ABSTRACT

Isoenzyme electrophoresis is shown to be an important aid in species discrimination in taxonomically difficult groups such as *Bulinus*. *B. africanus* and *B. nasutus* do not show any phosphoglucose isomerase allozyme variation in the Nyanza Province, Kenya, and the mobilities of phosphoglucose isomerase are sufficiently different for identification using this particular character.

Species of the planorbid genus *Bulinus* act as intermediate hosts for a number of *Schistosoma* species causing bilharzia in man and his livestock, while other species are of no importance in this context. Due to the great variability of morphological and anatomical characters, species of this genus are very difficult to tell apart. Thus at the Danish Bilharziasis Laboratory we have initiated a long term programme involving the study of by now up to 20 enzymes in natural populations of *Bulinus* in order to improve the species determination.

On the Kano Plain, Kenya, the distinction between the different species belonging to the *africanus* group is a very difficult matter (Brown, 1975; Southgate & Knowles, 1975, 1977). On a morphological basis the latter authors suggest the occurrence of hybrid populations between *B. africanus* and *B. nasutus*.

In Fig. 1 the area covered by the samples is shown. In Fig. 2 a representative phosphoglucose isomerase zymogram of *africanus* group species is shown. In the table the mobilities and gene frequencies observed in the different samples are given.

It is seen that the 2 species *B. ugandae* and *B. globosus* in Nyanza Province have the same mobilities in the phosphoglucose isomerase isoenzymes (Pgi-1.00, Pgi-1.25 and Pgi-1.36). However, a significant difference exists in the frequency of the alleles Pgi-1.00 being rare in *B. ugandae* ($p = 0.03$) and common in *B. globosus* ($p = 0.33$). None of the alleles found in these 2 species have been observed in either *B. africanus* or *B. nasutus* from Kenya. *B. africanus* and *B. nasutus* do not show any phosphoglucose isomerase allozyme variation in the Nyanza Province samples and the mobilities of phosphoglucose isomerase observed in the 2 species are so different that an identification using this character is easily made.

The samples which have been available from other areas of Kenya and Tanzania do not show any overlap in phosphoglucose isomerase mobilities, thus strongly indicating the usefulness of the phosphoglucose isomerase character. However, more live material is needed to clarify this. The data on the phosphoglucose isomerase isoenzymes presented here are further supported by data on other isoenzymes.

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FIG. 1. Map of Kenya and Tanzania giving location of towns. 1 Kisumu, 2 Nairobi, 3 Mombasa, 4 Tanga, 5 Mwanza, and 6 Iringa.

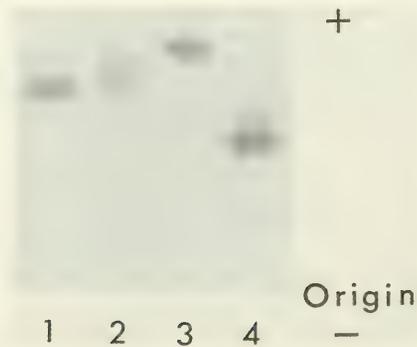


FIG. 2. Starch gel phosphoglucose isomerase isoenzyme pattern of whole animal extracts. 1 *Bulinus ugandae* Pgi-1.25, 2 *B. ugandae* Pgi-1.25/1.36, 3 *B. africanus* Pgi-1.44, and 4 *B. nasutus* Pgi-1.04.

TABLE 1. Phosphoglucose isomerase allele frequencies in populations of the *Bulinus africanus* group from Kano Plain, Kenya, the Kenyan coast and Tanzania. N = number of wild snails tested. + = allele present in an old laboratory stock.

Species and locality	Pgi alleles					N
	1.00	1.04	1.25	1.36	1.44	
<i>B. ugandae</i>						
Kenya, Nyanza Province, Kisumu 1	0.08		0.89	0.03		18
Kenya, Nyanza Province, Dunga	0.09		0.85	0.07		23
Kenya, Nyanza Province, Kaloka	0.03		0.95	0.02		39
Kenya, Nyanza Province, Aram Market	0.03		0.93	0.04		38
<i>B. globosus</i>						
Kenya, Nyanza Province, Kisumu 1	0.33		0.63	0.04		12
<i>B. africanus</i>						
Kenya, Nyanza Province, Kisumu 2					1.00	2
Kenya, Nyanza Province, Kisumu 3					1.00	10
Kenya, Nyanza Province, Kisumu 4					1.00	2
Kenya, Nyanza Province, Kisumu 5					1.00	5
Kenya, Eastern Province, Kinui					1.00	3
Kenya, Eastern Province, Machakos					+	
Tanzania, Kalenga at Iringa						
Tanzania, Misungwi at Mwanza					+	
<i>B. nasutus</i>						
Kenya, Nyanza Province, Kisumu 2		1.00				12
Kenya, Nyanza Province, Kisumu 3		1.00				14
Kenya, Nyanza Province, Kisumu 4		1.00				2
Kenya, Coast Province, Kinango		1.00				14
Kenya, Coast Province, Tiwi		1.00				14
Tanzania, Tanga Area, Muheza			1.00			3
Tanzania, Tanga Area, Magila		0.83	0.17			3
Tanzania, Tanga Area, Amani			+			
Tanzania, Misungwi at Mwanza		1.00				4

DIFFERENCIATION DES GENRES *HOMALOCANTHA*, *JATON* ET
MAXWELLIA (GASTEROPODES, FAMILLE MURICIDAE) AU MOYEN
DE LA STRUCTURE MICROSCOPIQUE DE LEUR COQUILLE

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ABSTRACT

Results of studies on the microscopical structure and mineralogical nature of the shells belonging to the muricid genera *Homalocantha*, *Jaton* and *Maxwellia* lead to taxonomic conclusions. The observed structures are consistent on the generic level (primary character: presence or absence of a calcitic cortex) or on the subgeneric level (secondary characters). Quantitative variation in the relative thickness of the layers, caused by ecological factors, does not influence the taxonomical conclusions. The genus *Homalocantha* is easily distinguished from *Jaton* and *Maxwellia* in the shells being entirely aragonitic, while *Jaton* and *Maxwellia* have a calcitic cortex. On the other hand, there appears to be no difference between the species of the latter two genera as regards secondary characters (form of the connecting zone cortex-ostracum, relative thickness of the cortex, orientation of the leaflets of the first ostracal layer). One may conclude that other morphological characters are required to justify separating *Jaton* and *Maxwellia*.

La systématique des Muricidae est encore source de discussion et de problèmes, malgré les nombreux travaux qui lui ont été consacrés. Certains genres semblent bien connus, mais, en réalité, les espèces qui les constituent changent souvent de position taxonomique avec les auteurs.

Un exemple en est fourni par le groupe d'espèces dont la suture présente à son contact avec les varices une série de "puits" assez profonds. Ces espèces sont actuellement réparties entre trois genres: *Homalocantha* Mörch, 1852, *Jaton* Pusch, 1837 et *Maxwellia* J. L. Baily jr., 1950, mais les avis des auteurs sur cette répartition sont loin d'être concordants. Il apparaît parmi ces espèces 2 types morphologiques assez distincts à l'oeil: certaines ont des varices foliacées et digitées comme *Murex scorpio* Linné, d'autres des varices arrondies comme *Murex gemma* Sowerby. Entre ces 2 types extrêmes, on trouve des espèces polymorphes comme *Murex gibbosus* Lamarck, polymorphisme qui peut sans doute être dû à des conditions écologiques (profondeur, agitation des eaux), comme cela a déjà été observé chez d'autres espèces.

Nous avons appliqué à ces espèces les critères de différenciation que nous avons mis en évidence dans une précédente étude (Petitjean, 1965): il s'agit de la structure microscopique et de la nature minéralogique de la coquille.

Le premier travail important sur ce sujet est dû à Carpenter (1845, 1848) qui a prouvé (1) que l'examen, fut-ce d'un petit fragment, est suffisant pour déterminer la structure de la coquille; (2) que la structure est qualitativement la même chez tous les individus d'une espèce; (3) que les structures sont indicatrices, à un degré élevé, des affinités zoologiques entre les espèces et, même, entre les genres.

Ces conclusions ont été admises et vérifiées par tous les auteurs qui ont ensuite fait des études détaillées de la structure des coquilles, en particulier Bøggild (1930) et Kessel (1933, 1936). Notre étude de plusieurs centaines d'espèces appartenant à la famille des Muricidae aboutissait aux mêmes résultats, à savoir: ou bien la coquille est entièrement aragonitique, ou elle est formée d'une couche interne d'aragonite (l'ostracum) et d'une couche externe de calcite (le cortex). Toutes les espèces d'un même genre sont du même type. Cette alternative constitue ce que nous avons appelé: "caractère primaire."

Or Lowenstam (1954a, 1954b) prit le contre-pied des opinions admises jusque là. Il estimait que la nature minéralogique des coquilles, loin d'être fixée génétiquement, était un caractère

somatique fonction des seuls facteurs écologiques, surtout la température. Il admettait en particulier que l'aragonite pouvait exister seule chez les individus des mers tropicales et pouvait, au contraire, disparaître totalement, au bénéfice de la calcite, chez les individus des mers boréales. Nous avons déjà critiqué ce point de vue (Petitjean, *loc. cit.*) mais nous allons le refaire ici brièvement.

Lowenstam n'a fait que des études par diagrammes de Debye et Scherrer *in toto*, sans jamais faire de lame mince de ses échantillons, donc sans *voir* leur structure microscopique. Il a utilisé une méthode "quantitative," mise au point simultanément par Sabatier (1953) et par Chave (1954), mais dont les insuffisances ont été mises en évidence ensuite par Davies & Hooper (1963).

Si la théorie de Lowenstam était exacte, les pourcentages d'aragonite devraient varier au sein d'une espèce entre 0% et 100%. Si, au contraire, la structure se conserve qualitativement, et que les facteurs écologiques fassent seulement varier plus ou moins l'épaisseur relative du cortex et de l'ostracum, les valeurs des analyses doivent se placer entre des limites finies. Et c'est bien ce qu'on observe si on considère les propres résultats de Lowenstam, dans les cas où le nombre d'analyses est suffisant pour être significatif. De plus au sein de certaines espèces comme *Mytilus edulis*, Lowenstam lui-même donne des teneurs en aragonite *plus faibles* pour les individus de stations *plus chaudes*, ce qui est contraire à sa théorie.

Autre critique: Lowenstam trouve peu d'aragonite dans les espèces boréales de certains "genres" et 100% d'aragonite dans les espèces tropicales. Mais il a adopté comme ouvrage de systématique celui de Thiele, dont les "genres" sont considérés aujourd'hui par tous les taxonomistes comme des super-genres ou des tribus. Si l'on en revient à la conception actuelle du genre, l'objection de Lowenstam ne tient plus, notamment en ce qui concerne l'argument majeur qu'il tirait de l'étude des Littorinidae: les espèces boréales, appartenant au genre *Littorina*, auraient un cortex calcitique, les espèces tropicales aragonitiques appartiennent à un tout autre genre: *Melaraphe*.

Ces objections levées, nous pouvons donc appliquer nos critères d'étude dans le cas des espèces de Muricidae rapportées aux genres *Homalocantha*, *Jaton* et *Maxwellia*.

Mörch (1852) a créé le genre *Homalocantha* pour le *Murex scorpio* Linné, qu'il prit pour espèce-type, mais il ne donna aucune description du genre.

Pusch (1837) a créé le genre *Jaton*, en discutant l'hétérogénéité du genre *Aquila* Montfort, qu'il proposait d'écarter de la nomenclature. Il a pris comme espèce-type *Murex decussatus* Gmelin, synonyme de *Murex gibbosus* Lamarck et de "*Le Jatou*" d'Adanson. Il lui ajoutait des espèces sans affinités, comme *Murex miliaris* Gmelin qui est un *Vitularia*. Dans sa description du genre, il indique seulement que les espèces possèdent "des côtes transversales avec, entre elles, des sillons transverses profonds et un peristome très plissé."

J. L. Baily jr. (1950) a créé le genre *Maxwellia* pour *Murex gemma* Sowerby, *Murex santarosana* Dall, *Murex fimbriatus* A. Adams et *Murex (erinaceoides?) indentatus* Carpenter. Il refusait l'association de ces espèces avec *Murex festivus* Hinds, comme le faisaient Dall (1921) et Grant & Gale (1931). Effectivement cette espèce n'a pas de "puits" suturaux. Mais il niait aussi leur parenté avec *Murex decussatus* et le genre *Jaton*, qui en possèdent, sans en donner de raisons.

Les auteurs de monographies des Muricidae, postérieurs à Mörch ont adopté des répartitions des espèces qui ne coïncident pas entre elles, ni avec celles des auteurs précédents. G. B. Sowerby jr. met dans sa section IV: *Murex festivus*; V: *Murex linguavervecina* Chemnitz = *M. lingua* Dillwyn avec des espèces aujourd'hui placées dans le genre *Cerostoma* Conrad; VI: *M. scorpio* Linné, *M. rota* Sowerby, *M. secundus* Lamarck, *M. varicosus* Sowerby, *M. digitatus* Sowerby, *M. fenestratus* Chemnitz, *M. gemma* Sowerby et *M. fimbriatus* A. Adams (coquilles pyriformes avec des "puits" à la suture).

Tryon (1880) reconnaissait le genre *Homalocantha*. Il y plaçait les mêmes espèces que Sowerby sauf *M. gemma* et *M. fimbriatus*, mais faisait de cette dernière espèce un *Phyllonotus*, et de *M. gemma* un membre d'une section d'*Ocenebra*, où il l'unissait à *M. tetragonus* Broderip. Il plaçait *M. gibbosus* dans une section de *Pterynotus*. Enfin, pour lui *M. indentatus* était synonyme de *M. californicus* Carpenter et de *M. trialatus* Sowerby, donc un *Cerostoma*.

Enfin, dans sa monographie des Muricidae, Maxwell Smith (1953) adopte la répartition suivante: dans *Homalocantha*, les mêmes espèces que Tryon; dans *Pterynotus*: *M. lingua* et *M. santarosana*; dans "*Muricidea*"(?): *Maxwellia gemma*; dans *Cerostoma*, *M. festivus*. Enfin *M. fimbriatus* devenait un *Typhis*!

L'étude de la littérature montre donc une très grande confusion. Seul des 3 genres, *Maxwellia* a fait l'objet d'une description détaillée. La question reste donc entière, quant à la validité zoologique de ces genres; y en a-t-il 1, 2 ou 3? et, quelle est la bonne répartition des espèces entre les genres?

Nous avons donc abordé le problème par notre méthode habituelle: dans chaque coquille, nous avons découpé des fragments de 5-8 mm de long, les uns parallèles aux stries d'accroissement, les autres perpendiculaires à elles; ces fragments ont été meulés pour en faire des lames minces que l'on a observées au microscope polarisant. En raison de la taille des cristaux, la calcite se distingue d'emblée de l'aragonite. Elle montre de plages d'extinction franche du blanc au noir, alors que l'aragonite présente des couleurs de polarisation et la structure bien connue en couches prismatiques et entrecroisées. La vérification de la nature minéralogique des couches séparées les unes des autres a été faite par des diagrammes de Debye et Scherrer, qui ont toujours confirmé l'observation optique.

Nous n'avons pu, faute d'échantillons, étudier toutes les espèces supposées appartenir à ces 3 genres. Nous avons examiné *M. scorpio*, *M. rota*, *M. digitatus*, *M. fenestratus*, *M. heptagonatus pauli* Tournoué (qui avait été placée de façon erronée dans le genre *Favartia* par Cossmann & Peyrot, 1924), *M. gibbosus*, *M. lingua*, *M. gemma*, *M. santarosana*, *M. festivus*, *M. californicus*. Ces échantillons provenaient des doubles des collections du Museum National d'Histoire Naturelle de Paris, comme la collection Jousseume. Leur lieu de récolte est donc moins précis que ce à quoi l'on est habitué pour les récoltes actuelles. *M. scorpio* provient des Philippines, *M. rota* de la Mer Rouge, *M. gibbosus* et *M. lingua* du Sénégal, *M. festivus*, *M. gemma*, *M. santarosana* et *M. californicus* de Californie, *M. heptagonatus pauli* du Tertiaire d'Aquitaine.

Résultats: les espèces rattachées habituellement au genre *Homalocantha* (*M. scorpio*, *M. rota*, *M. heptagonatus pauli*, *M. digitatus*, *M. fenestratus*) sont entièrement aragonitiques (Fig. 1).

Par opposition avec ce groupe d'espèces, toutes les autres possèdent un cortex calcitique (Fig. 2). Le critère primaire (présence ou absence d'un cortex calcitique) nous conduit donc à une différenciation entre: d'une part *Homalocantha*, aragonitique; d'autre part *Jaton* et *Maxwellia*, à cortex calcitique.

À côté du caractère primaire, nous avons observé que des distinctions étaient parfois possibles, dans les genres à cortex, entre genres différents ou sous-genres, sur la base des caractères secondaires: forme de la zone de jonction entre cortex et ostracum, importance relative du cortex par rapport à l'ostracum, orientation des lames de la couche externe de l'ostracum, etc. Nous avons donc étudié chez les espèces de *Jaton* et de *Maxwellia* ces caractères secondaires, pour y détecter les différences éventuelles.

La zone de jonction entre le cortex et l'ostracum est souvent bien visible car elle est marquée en général par un dépôt de conchioline. Chez *M. gibbosus* (Fig. 3) et *M. lingua*, elle suit exactement les ornements de la surface externe de la coquille. Il en est de même chez *M. festivus*. Chez *M. gemma*, la zone de jonction est plus régulière, plus aplatie, et suit de plus loin les ornements externes. Chez *M. santarosana* (Fig. 4), la zone de jonction présente des ondulations dont la "longueur d'onde" et l'amplitude sont une sorte de "négatif" de la surface externe: l'amplitude diminue et la "longueur d'onde" augmente au niveau des ornements en relief; c'est le contraire dans les zones plus lisses de la coquille. Ceci a pour effet de donner au cortex une épaisseur irrégulière: il est plus épais au niveau des tubercules ornementaux; il est plus mince et plus "tourmenté" entre ceux-ci. Cependant, s'il existe bien certaines différences entre les espèces étudiées, il ne semble pas qu'elles soient assez marquées pour justifier une distinction générique.

L'importance du cortex est à peu près la même dans les 5 espèces. Il forme le 1/5 ou le 1/6 de l'épaisseur totale de la coquille.

L'ostracum est formé de 3 couches (4 chez un *M. gibbosus*, mais nous savons qu'au delà de 3 couches, ceci peut-être un phénomène d'épaississement individuel). Les feuilletts formant la couche la plus externe sont dans les 5 espèces perpendiculaires aux stries d'accroissement.

Donc, aucun des caractères structuraux secondaires ne permet de justifier la séparation des *Jaton* et des *Maxwellia* en genres distincts. Il faudrait, pour le faire, se référer à d'autres critères morphologiques. L'opercule, figuré par Vokes (1964) pour *Maxwellia* a un nucleus sub-basal qui paraît peu différent de certains opercules purpuroïdes, où la latéralité du nucleus est peu marqué. L'opercule des espèces de *Jaton* n'a jamais été figuré.

Les différences entre les deux genres reposent actuellement sur leur répartition géographique

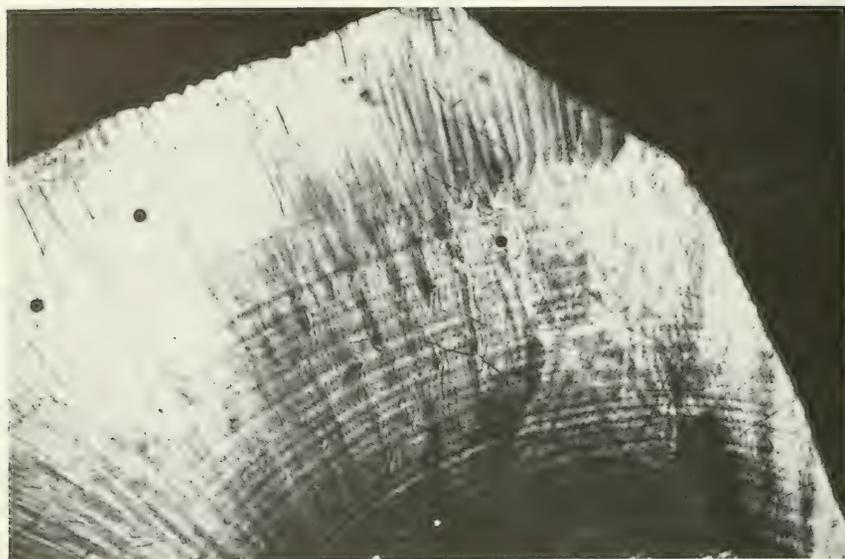


FIG. 1. Structure de la coquille dans le genre *Homalocantha*: *H. heptagonatus pauli*. Coupe parallèle aux stries d'accroissement, X50.

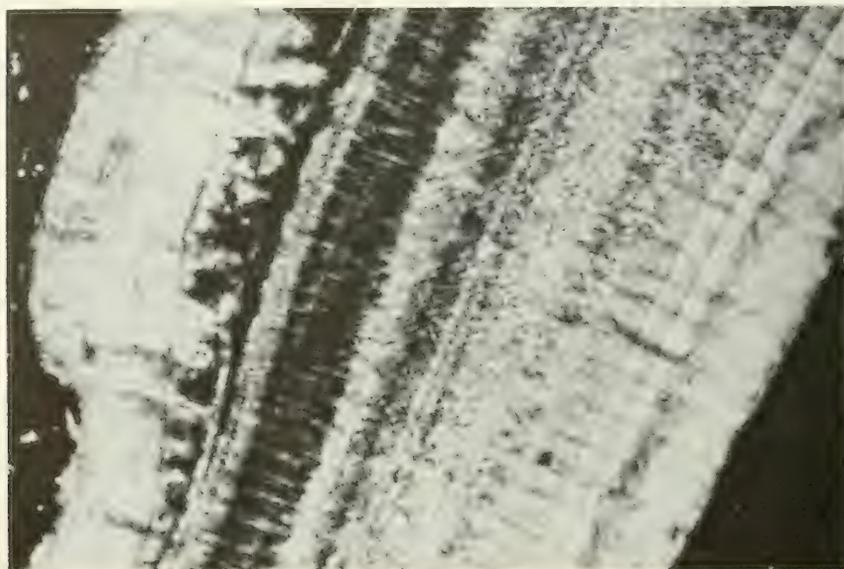


FIG. 2. Structure de la coquille dans le 'genre' *Maxwellia*: *M. gemma*. Coupe parallèle aux stries d'accroissement, X375.

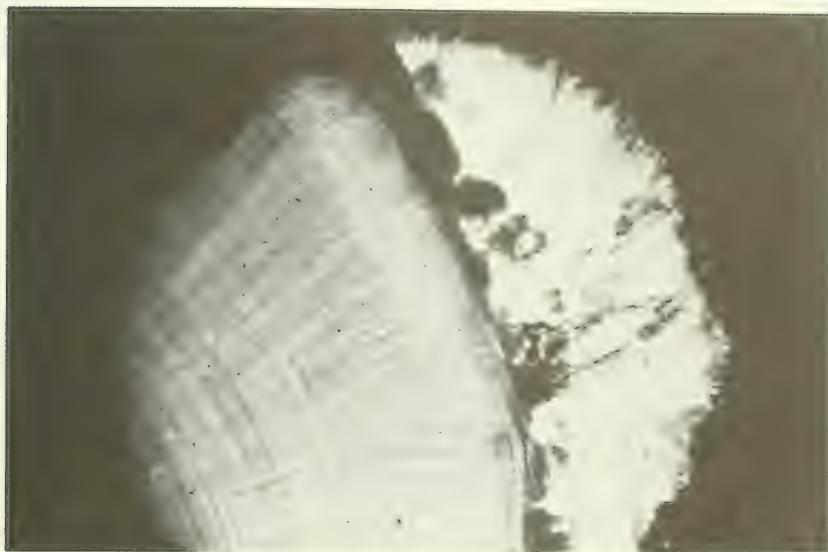


FIG. 3. Structure de la coquille dans le 'genre' *Jaton*: *J. gibbosus*. Coupe perpendiculaire aux stries d'accroissement, X50.



FIG. 4. Structure de la coquille dans le 'genre' *Maxwellia*: *M. santarosana*. Coupe perpendiculaire aux stries d'accroissement, X100.

(*Jaton*, en Afrique, *Maxwellia* dans le Pacifique américain) et le nombre des varices assez nombreuses chez les *Maxwellia*, alors qu'il n'y en a que 3 chez *Jaton*.

On séparera *M. festivus* de ces 2 genres, si on considère que les "puits" de la suture en sont une caractéristique. Cette espèce pourrait alors, soit être rattachée au genre *Ocenebra* (bien que les espèces américaines pacifiques du genre aient en général une orientation des feuillettes de la première couche de l'ostracum, parallèles aux stries d'accroissement), soit laissée *incertae sedis* pour le moment, conclusion qui paraît moins hasardeuse.

Conclusions: En tenant compte, à la fois, des travaux des autres auteurs et de nos propres études sur la structure microscopique et la nature minéralogique de la coquille des Muricidae, nous arrivons à la répartition suivante des espèces:

- dans le genre *Homalocantha* (entièrement aragonitique): *M. scorpio*, *M. rota*, *M. digitatus*, *M. fenestratus*, *M. heptagonatus pauli*;
- dans le genre (?) *Jaton* (à cortex calcitique): *M. gibbosus*, *M. lingua*;
- dans le genre (?) *Maxwellia* (à cortex calcitique): *M. gemma*, *M. santarosana*;
- incertae sedis*: *M. festivus*.

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ALBINISM IN THE GENUS *ANCILLA* (GASTROPODA, OLIVIDAE)

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ABSTRACT

In the shell of molluscs a great variability in colour forms can often be observed. Two forms have received special names: black is called melanism, white (= the lack of any colour) is called albinism. Partial albinism is also known. In the Cypraeidae melanism is known from a number of species. Albinism is rare, but seems scattered throughout all groups of the gastropods. However, albinism seems to occur more often in *Ancilla*. The genus *Ancilla* (fam. Olividae) contains about one hundred recent species; more than ten subgenera can be recognized. They are living in marine tropical and subtropical waters. The shell is mostly coloured yellow-orange-brown. From the collection of the Zoological Museum in Amsterdam and from the literature at least ten species are known to have (partial) albinistic forms. Some of these albinos were originally described as distinct species, like *Ancilla candida* (Lamarck) and *A. nivea* (Swainson).

INTRODUCTION

Colour variation of molluscan shells has always attracted the attention of malacologists. In many species these colour forms received names. In older literature they were described as varieties, like var. *alba*, *aurea*, *nigra*, *rubra*, *viridis*, etc. In modern literature they are considered to be colour forms, *forma rubra*, etc.

General terms are being used for two colour forms: black colouration is called melanism, white is known as albinism. These 2 terms are not only used for shells, they are valid in all groups of animals. Within the gastropods melanism is known from several species of Cypraeidae (Cernohorsky, 1963). The reference list in Old (1964) comprises the literature on melanistic Cypraeidae. Black specimens of *Cypraea* are known in particular from New Caledonia, like *Cypraea arabica niger*, *C. stolidia crossei*, *C. caurica thema*, and *C. eglantina*. Some metals, found on New Caledonia and in the waters around this island, are thought to have caused these melanistic shells. It is therefore considered an ecological factor.

Albinism is the lack of any colour. It is known from most classes of the animal kingdom. When both parents are albino, their offspring is albinistic as well. Therefore albinism is hereditary, and caused by mutation. In "partial albinism" the animal is only partially white.

Not all white shells should be regarded as albinos. Sometimes the natural colour of the shell is white, as in many *Epitonium* species and in the Lucinidae. In collections the original colour of the shells may have faded through the influence of daylight. Shells found on the beach are often bleached by sunlight. Many fossil shells have become white in time.

Albinos are sometimes described as formae of the normal shell, usually with the indication *alba* (= white), *candida* (= shiny white), *nivea* (= snow white), or *virginea* (= virgin-like).

Albinism is rare; in gastropods it is known from a very limited number of species only. Mrs. Waverley H. Harmon of New York has a special interest in albino shells, and has collected them for about 15 years. Her albino collection now contains a little over 100 species, both gastropods and bivalves, from a number of families, including land, marine and freshwater molluscs. It is therefore obvious that albinism is rare, and scattered throughout all taxa of the gastropods. However, from literature and the collection of the Zoological Museum, Amsterdam (= ZMA), it appears that albinism occurs more often in shells of the genus *Ancilla*.

ALBINISM IN *ANCILLA*

The genus *Ancilla* Lamarck, 1799 (syn. *Ancillaria* Lamarck, 1811) belongs to the family Olividae of the Prosobranchia. About 100 Recent species are known, which are placed in ten subgenera. The species of *Ancilla* are living in (sub)tropical marine waters. Most of them are found in the Indopacific area, some subgenera having a limited distribution, such as *Sparella* from the Red Sea to the Persian Gulf, and *Anolacia* around Madagascar and Mauritius. The subgenus *Eburna* occurs from the southern Caribbean Sea to the coast of Brazil. Furthermore species of *Ancilla* are found around South Africa, South Australia, and New Zealand. Recent species of *Ancilla* are not known from the tropical eastern Pacific, nor from the Mediterranean Sea and West Africa. However, fossil *Ancilla* are known from Europe and North America.

The shell of *Ancilla* is elongate to fusiform, and solid; length 1-10 cm; the surface is smooth and glossy; spire high and conical; aperture rather wide, often with parietal callus, columella twisted and grooved; colour yellow, orange, brown, and white; operculum small.

Albinistic specimens are known from the following species.

Subgenus *Ancilla* s.s.

Ancilla ampla (Gmelin, 1791) from the Indian Ocean is yellow coloured (Fig. 1a). One albino shell from Ceylon (Fig. 1b) is present in the collection of ZMA. Evidently albinism is not rare within this species, as the albino form was described as *Ancillaria candida* Lamarck, 1811. Albinistic specimens were also figured by Sowerby II (1859, pl. 212, fig. 29) and Reeve (1864, pl. 8, fig. 27a).

Subgenus *Ancillus* Montfort, 1810

Ancilla muscae Pilsbry, 1926. This is a new name for *Ancillaria elongata* Gray, 1847, non Deshayes, 1830. The species is living in Australia, the shell is white. Normally the upper part of the spire is covered with a brown periostracum (Fig. 2a). When the periostracum is removed, a white shell (Fig. 2b) remains, giving the impression of an albino. This is an example of pseudo-albinism. Sowerby II (1859, pl. 213, figs. 52-53) also figured a white specimen next to one with the brown periostracum.

Subgenus *Sparella* Gray, 1857

Ancilla fulva (Swainson, 1825) from the Red Sea is cream to light brown (Fig. 3a). One albino (Fig. 3b) is in the collection of ZMA. Sowerby II (1859, pl. 214, fig. 75) also figured an albino.

Ancilla cinnamomea Lamarck, 1801, is from the Red Sea and Persian Gulf area. The shell is brown, the spire has brown and white bands (Fig. 4a). The ZMA collection contains some partial albino shells (Fig. 4b), which have the spire banded, but the last whorl is completely white. *Ancilla tronsoni* (Sowerby II, 1859) is considered a synonym of *A. cinnamomea* by Burch & Burch (1960). As *A. tronsoni* is pure white (Sowerby II, 1859: 58, pl. 212, figs. 20-21) it must be the albino form of *cinnamomea*.

Ancilla castanea (Sowerby I, 1830) is a chestnut coloured species from the Red Sea. An albino is figured by Sowerby II (1859, pl. 214, fig. 76).

Subgenus *Anolacia* Gray, 1857

Ancilla mauritiana (Sowerby I, 1830), syn. *A. torosa* (Sowerby II, 1859), is found around Madagascar and Mauritius. It is the only species in this subgenus. Next to the brown coloured shells (Fig. 5a) albinos are often seen in collections (Fig. 5b). The albinistic form is also known in literature (Sowerby II, 1859, pl. 212, fig. 31). The coloured juveniles of this species were named *Ancilla aperta* (Sowerby I, 1825), and juvenile albinos were described and figured by Sowerby II (1859: 58, pl. 212, figs. 37-38) as *Ancillaria scaphella*.

Subgenus *Eburna* Lamarck, 1801

Ancilla glabrata (Linné, 1758) has a very limited range in the southern Caribbean. The island of Aruba seems to be the centre of its distribution. It is one of the largest species of the genus *Ancilla*; the ZMA collection contains a specimen of 75 mm length. The shell is yellow (Fig. 6a).

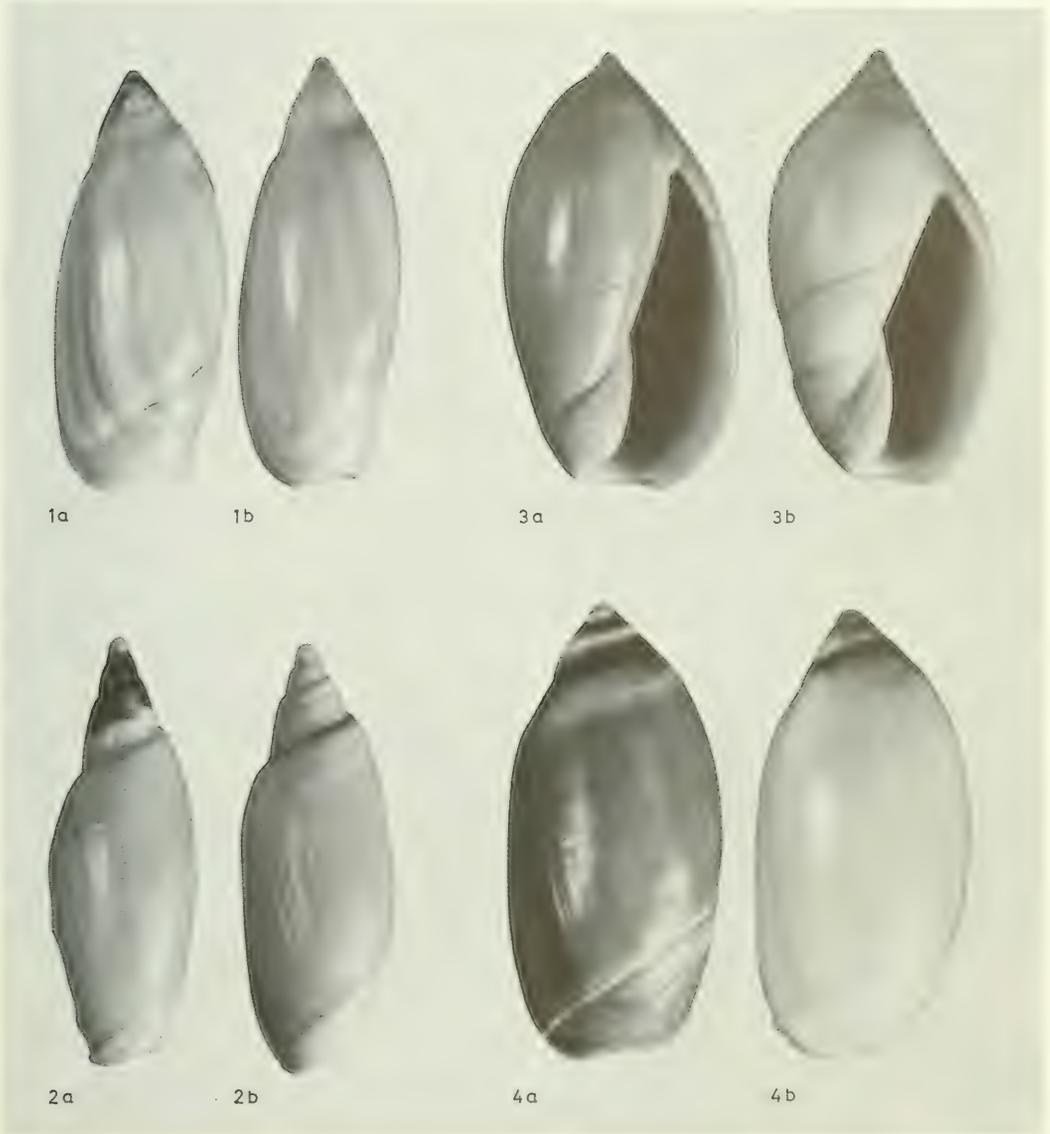


FIG. 1. *Ancilla (Ancilla) ampla* (Gmelin). a. Yellow, length 23.1 mm, Ceylon. b. Albino = *A. candida* (Lamarck), length 29.4 mm, Ceylon.

FIG. 2. *Ancilla (Ancillus) muscae* Pilsbry. a. White with brown spire, length 43.5 mm, W. Australia, Exmouth Gulf. b. Pseudo-albino, length 36.7 mm, Australia, Torres Strait.

FIG. 3. *Ancilla (Sparella) fulva* (Swainson). a. Cream coloured, length 31.6 mm, Red Sea. b. Albino, length 29.8 mm, Saudi Arabia, Jubail.

FIG. 4. *Ancilla (Sparella) cinnamomea* Lamarck. a. Brown, length 26.1 mm Persian Gulf. b. Partial albino, white with brown band around the suture, length 27.4 mm, Persian Gulf.

(Specimens in collection Zoological Museum, Amsterdam, photographs by L. A. van der Laan).

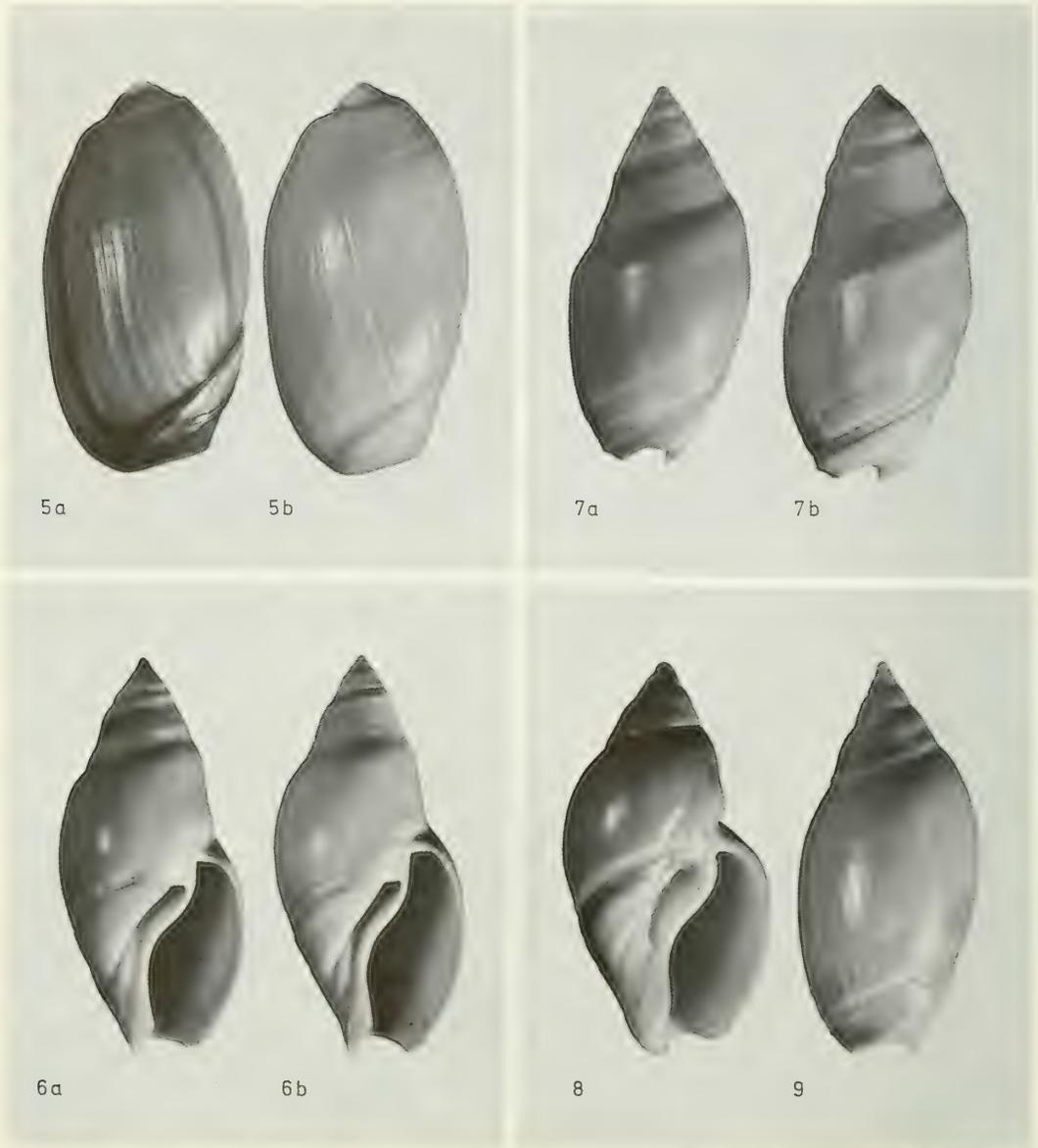


FIG. 5. *Ancilla (Anolacia) mauritiana* (Sowerby I) a. Brown, length 41.7 mm, Madagascar. b. Albino, length 41.8 mm, Madagascar.

FIG. 6. *Ancilla (Eburna) glabrata* (Linné). a. Yellow, length 71.8 mm, Aruba. b. Albino, length 71.9 mm, Aruba.

FIG. 7. *Ancilla (Eburna) balteata* (Swainson). a. Yellow, length 39.5 mm, West Indies. b. Albino = *A. nivea* (Swainson), length 50.3 mm, Antilles.

FIG. 8. *Ancilla (Eburna) lienardi* (Bernardi). Yellowish-brown, length 31.2 mm, Brazil, Acaraù, Cearà.

FIG. 9. *Ancilla (Eburna) tankervillei* (Swainson). Yellow, length 56.5 mm. Venezuela, Isl. Margarita.

(Specimens in collection Zoological Museum, Amsterdam, photographs by L. A. van der Laan).

The figured albino (Fig. 6b) is very large too. Albino specimens are also known from the literature (Sowerby II, 1859, pl. 213, fig. 63).

Ancilla balteata (Swainson, 1825) is known from the same area as the former species. However, in literature and in old collections it is mentioned from Ceylon. This species is closely related to *A. glabrata*, but smaller and with a shouldered shell (Fig. 7a). The colour is orange-yellow. Albino specimens (Fig. 7b) were described as *Ancillaria nivea* Swainson, 1825. Sowerby II (1859: 66) considered *A. balteata* and *A. nivea* to be distinct species, both from "Ceylon." Reeve (1864, spec. 49) already recognized the synonymy, he mentioned the relation with *A. glabrata*, and gave *A. balteata* a West Indian distribution, i.e. "probably Gulf of Mexico."

Ancilla lienardi (Bernardi, 1858) from the coast of Brazil has a yellowish-brown to reddish-orange shell (Fig. 8). We have not seen any albino of this species; however, Reeve (1864, spec. 50) figured an albino (pl. 12, figs. 50c-d) next to a coloured specimen (figs. 50a-b).

Ancilla tankervillei (Swainson, 1825) is known from Venezuela (Isl. Margarita) and the north coast of Brazil. The shell is coloured yellow (Fig. 9). The collection of ZMA does not have any albino, but a white specimen is figured by Sowerby II (1859, pl. 211, fig. 5).

DISCUSSION

Although albinism is very rare in the Gastropoda, it is remarkable that in the genus *Ancilla*, with about 100 species, albinos are known from at least 9 species. In most of these 9 species, albino specimens are not rare at all, so we may conclude that albinism is rather common in *Ancilla*. Albinism in *Ancilla* occurs in a number of subgenera, and is not connected with any zoogeographical province. Albino *Ancilla* species are known from the Indian Ocean, Red Sea, and the West Indies.

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ALEXANDER CROSBIE AND THE "CHALLENGER" *TEREDO*

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ABSTRACT

Alexander Crosbie was staff-surgeon aboard HMS "Challenger" during her circum-global expedition of 1873-76. His private collection of shells in the Royal Scottish Museum, Edinburgh, contains 4 shells labelled "*Xylophaga dorsalis* from fruit of screw pine (palm) [*Pandanus*]. 1400 fms. 29/8/74" ["Challenger" station 184, off Cape York, Queensland]. No *Xylophaga* from this or any other station appears in the "Challenger" report on the Lamellibranchiata (Smith, 1885), but a juvenile specimen of an unnamed species of *Teredo* is recorded from this station. Moseley (1895), quoted in the summary of results of the expedition, writes: "The trawl brought up half a dozen large palm fruits... the husks were bored by the young of a *Teredo*-like bivalve." Examination of the "Challenger" material at the British Museum (Natural History) showed that there are actually 2 specimens, preserved in spirit, both very damaged and very small but undoubtedly *Xylophaga* and conspecific with Crosbie's specimens. The wide-spread occurrence of deep-water species of *Xylophaga* was not known until Knudsen (1961) described 17 species taken from depths greater than 900 m by the "Galathea" expedition of 1950-52. Comparison of Crosbie's specimens and Knudsen's descriptions and figures identifies them as *X. wolffi*, previously known only from the 2 "Galathea" specimens taken from 5050 m in the Sulu Sea.

In the "Challenger" Report on the Lamellibranchiata (Smith, 1885: 27) the first species, under the heading *Teredo* sp., is described as follows: "A single very small specimen, all that was obtained, may possibly be the young state of the *Teredo* mentioned in the Report of the collections made during the Voyage of HMS "Alert" in Torres Strait. The striae on the anterior part of the valves are, however, rather coarser. Although from Station 184, to which a depth of 1400 fathoms is assigned, it seems probable that this shell, which contained the animal, got into the trawl near the surface, during the process of hauling in. This, however, is not certain, for water-logged wood might be found at that depth into which it might bore."

Smith's uncertainty as to the provenance of this species was resolved by Moseley (in Murray, 1895: 681) who observed that at Station 184, off Cape York, Queensland: "The trawl brought up half a dozen large palm fruits... the husks were bored by the young of a *Teredo*-like bivalve." No mention of this material was made by Turner (1966: 55-56) in her account of the occurrence of Teredinidae in deep water, and the identity of the "Challenger" *Teredo* might have remained conjectural had it not been for new evidence from an unexpected quarter.

Late in 1971, in preparation for an exhibition at the Royal Scottish Museum to mark the "Challenger" centenary, the Crosbie collection of shells was examined as a possible source of specimens for display. Alexander Crosbie (Fig. 1) was staff-surgeon aboard HMS "Challenger" during her circumglobal expedition of 1873-76. His interest in natural history was considerable, and he took the opportunity afforded by the voyage to add to his collection of shells, which was presented to the Royal Scottish Museum in 1927. Amongst his "Challenger" material was a box labelled: "*Xylophaga dorsalis* from fruit of screw pine (palm). 1400 fms. 29/8/74." The date of collection and the depth identified the locality of Station 184, but the identity of the specimens was obviously incorrect, as *X. dorsalis* (Turton, 1819) is a coastal species of the NE Atlantic and Mediterranean.

Within the box, apart from 4 specimens of a *Xylophaga*, were 6 specimens of *Myrina coppingeri* Smith, 1885, a mytilacean described from Station 184 as a new species as yet unreported from any other locality, and three specimens of a small, unidentified and possibly undescribed gastropod. Of the 4 specimens of *Xylophaga* (RSMNH 1927.120.128) 3 are



FIG. 1. Alexander Crosbie, Surgeon, R.N.

complete; the valves are attached, being held together by the dried remains of mantle tissue still adhering to the shell, and the accessory plates are in place although some distortion of their position has occurred in the smallest specimen. In the 4th specimen the valves have separated and the accessory plates have been lost.

Comparison of these specimens with the descriptions and illustrations of bathyal and abyssal *Xylophaga* collected by the "Galathea" expedition and reported by Knudsen (1961) established their identity with *X. wolffi* Knudsen, 1961, known only from 2 "Galathea" specimens with adhering juveniles taken from 5050 m in the Sulu Sea. The dimensions (in mm) of the largest of the RSM specimens are: length 12.2, height 11.2, breadth 12.5; the umbonal area is eroded as in the "Galathea" specimens (Knudsen, 1961: 187, fig. 29). The figured specimen (Figs. 2-3) is free from erosion; its dimensions are 11.2, 11.0, 11.7 mm. For comparison, the dimensions of Knudsen's type are 8.5, 8.9, 8.9 mm.

Although the Crosbie collection provided evidence that the "Challenger" had obtained specimens of *X. wolffi* from off Cape York it remained to discover whether the specimen described by Smith was the same. Turner (1966: 56) mentioned the occurrence of living *Teredothyra smithi* (Bartsch) from the same "Galathea" station as yielded *X. wolffi*, so the co-existence of a *Xylophaga* and a teredinid in deep water was a possibility. Investigation of the "Challenger" material at the British Museum (Natural History) revealed that there are in fact 2 specimens, preserved in alcohol (BMNH 1889.11.11.153), both very small and very damaged.

The larger specimen (Figs. 4, 5, 8, 9) is 6.9 mm long. The right valve is fragmentary, and only the disc and part of the anterior slope of the left valve remain. Fortunately the contracted incurrent siphon and the aperture of the excurrent siphon with 6-7 small tentacles on each side and a larger tentacle posterior to the aperture can be distinguished. The form of the siphons is an important taxonomic character in the Xylophaginae, and there is a close correspondence



2



3

FIGS. 2-3. *Xylophaga wolffi* Knudsen, RSMNH 1927.120.128.

between the siphons visible in this specimen and those of *X. wolffi* figured by Knudsen (1961: 188, fig. 30b).

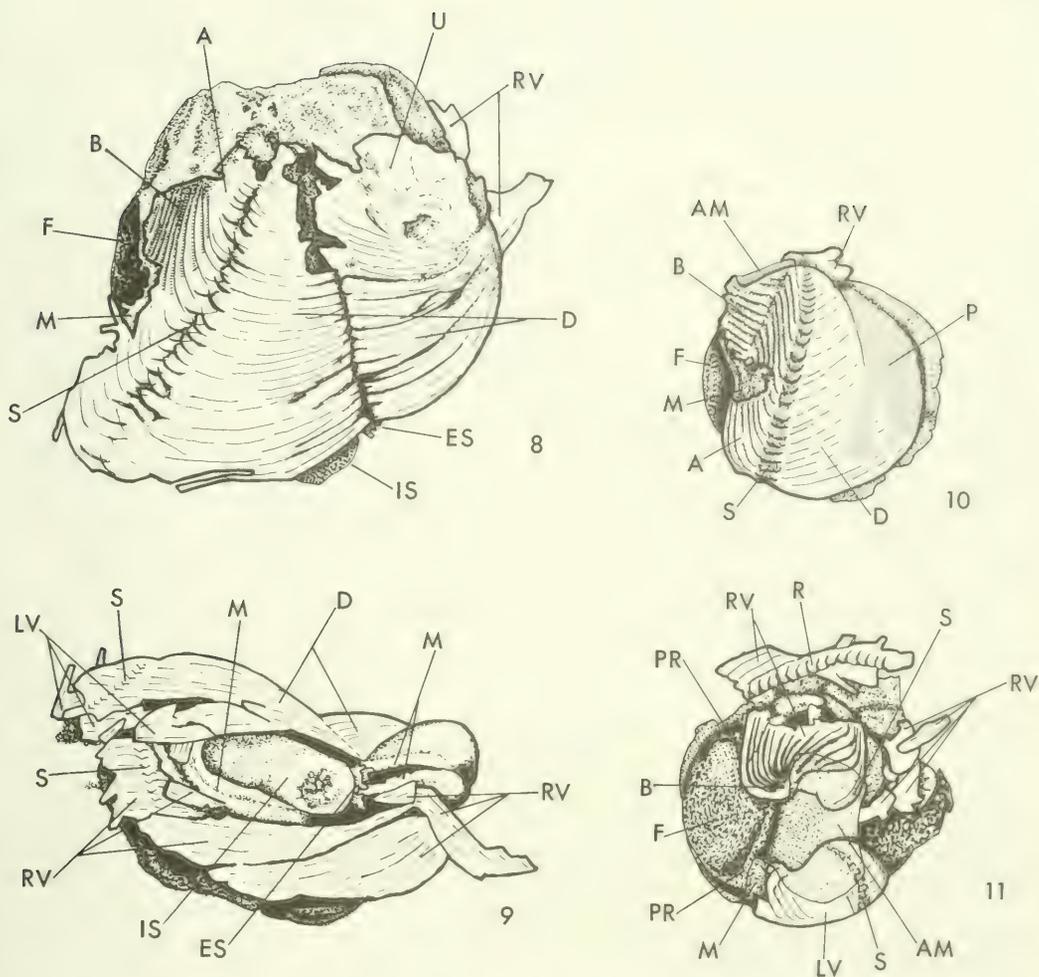
The smaller specimen (Figs. 6, 7, 10, 11) is 2.5 mm long. The right valve is fragmentary but the left valve is more or less intact. The siphons are not visible but sufficient characters remain to establish that it is a *Xylophaga* and not a terebrinid: the adhesive surface of the foot is surrounded by a pedal ridge, the anterior fold of the mantle (exposed by the missing mesoplax) is visible in the anterior incision overlaying the anterior adductor muscles, and the umbonal-



FIGS. 4-7. "*Teredo*" sp., BMNH 1889.11.11.153. Damage to the shells has resulted in the mantle and visceral mass being squeezed out around the broken edges of the valves.

ventral ridge of the right valve (corresponding on the inner surface of the shell to the umbonal-ventral sulcus of the outer surface) is visible where a broken fragment of that valve has been displaced away from the mantle. The sculpture on the anterior slope and beak of the left valve corresponds closely to that on the Crosbie specimens. Diagrams have been provided (Figs. 8-11) to aid in the interpretation of the damaged specimens (Figs. 4-7); the terminology used in the diagrams and elsewhere in this paper is based on that established by Purchon (1941) and Turner (1955).

It was concluded from the above investigation that the "*Teredo* sp." of the "Challenger" Report is *Xylophaga wolffi* Knudsen, 1961. The "Alert" *Teredo* from Torres Strait was also examined (BMNH 1881.11.10.174-5). Only a single right valve was present in the box. The presence of a apophysis and the lack of an umbonal-ventral ridge indicated that this specimen is a teredinid and not a *Xylophaga*.



FIGS. 8-11. Diagrams of the specimens illustrated in Figs. 4-7. A = anterior slope; AM = anterior mantle fold; B = beak; D = disc; ES = excurrent siphon; F = foot; IS = incurrent siphon; LV = left valve; M = mantle edge; P = stained area of periostracum; PR = pedal ridge; R = umbonal-ventral ridge; RV = right valve; S = umbonal-ventral sulcus; U = eroded area adjacent to umbo.

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PRIMARY SUCCESSION OF LAND MOLLUSCS IN AN
UPLIFT ARCHIPELAGO OF THE BALTIC

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ABSTRACT

In the archipelago of Quarken, Gulf of Bothnia, primary succession is controlled by land uplift. The successional changes of both vegetation and land mollusc malacocenosis is best demonstrated on islets of less than 10 ha. The age of the islands can be calculated according to the altitude above sea level, but besides age, also isolation, area and ecological factors have a considerable effect on the mollusc fauna. The number of land mollusc species found on the islands reflects the succession: islands of the first stage are inhabited by 1-6 species, the 2nd stage by 7-12 species, and the last stage by more than 12 species. On the outer uplift archipelago there are 5 typical pioneer species, viz. *Vallonia pulchella*, *Deroceras agreste*, *Oxyloma pfeifferi*, *Deroceras laeve*, and *Vertigo alpestris*. Moreover, there are some species which can be considered as species of a late successional stage and many species from in between these stages. Whether studying primary succession on whole islands or in different habitats the results will be very similar as regards pioneer species and also species of later successional stages. The species diversity derived from Shannon's measure of diversity H' and the evenness J' will increase with succession.

INTRODUCTION

Primary succession is a dynamic phenomenon. In the area studied it is controlled by land uplift. The sequence of biocenoses goes from somewhat grassy shores (open meadows), thickets to forests. Primary succession is usually connected with time-consuming changes in the composition of the malacocenosis. In the extreme conditions pioneer communities may persist for a fairly long time.

Of the many aspects which are more or less significant for primary succession, e.g. biomass, production, diversity, uniformity and stability, I shall deal with the factors controlling speed of the primary succession, changes within species composition, pioneer species and successional order of species in different biotopes. Moreover, I will deal with species diversity, since variations in the diversity are positively correlated with stability of various biotic and abiotic components of ecosystems (Leigh, 1965; Margalef, 1968; Slobodkin & Sanders, 1969; Whittaker, 1975).

THE STUDY AREA AND THE AGE OF ISLANDS

The study area, the Quarken archipelago, is at the centre of the land uplift area in the northern Baltic (63°N, 22°E). The earth's crust is rising at a rate of 0.8 m per century. Within the last 9000 years the Quarken region has risen about 250 m (Kääriäinen, 1953). The age of an island of known height has been calculated according to the formula (see Okko, 1967; Kukkamäki, 1971)

$$T = \frac{\ln \left(1 + \frac{PH}{V} \right)}{\ln (1 + P)}$$

T = the age of the island

H = height of island (m)

V = recent relative uplift (0.8 m/century)

P = magnitude of retardation (about 1.6%/century)

Because the land is flat, especially on the Finnish side of the Quarcken archipelago, new islets continuously appear and the area of the existing islands enlarges. Thus, the area is extremely suitable for studying primary succession. I have studied 95 islets younger than 430 years (height ≤ 350 m), 88 islands older than that (3.51-25 m), and 36 "islands" on the mainland.

HEIGHT CLASSES (m) AND MAX. AGE (YEARS)

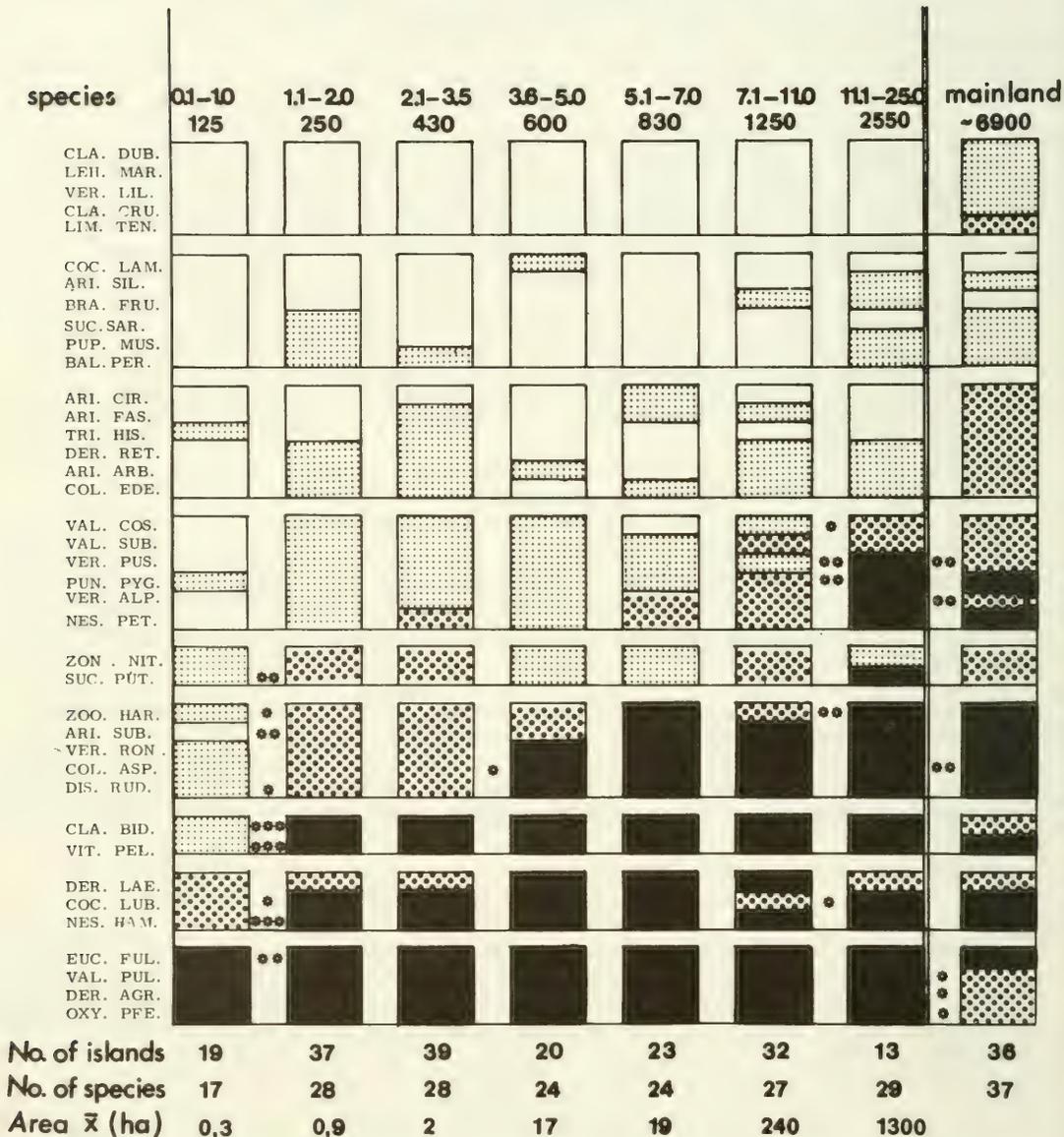


FIG. 1. Constancies of the species recorded from islands of 7 height/age classes. Constant species = black (const. $\geq 50\%$); accessory species = coarse dots (const. less than 50%, but statistically different from 0); accidental species = fine dots (const. does not differ statistically from 0). Asterisks denote the statistical significance between classes at the 0.05 (*), 0.01 (**) and 0.001 (***) probability level.

AGE OF ISLANDS AS A CRITERION OF SUCCESSION

Of the 39 indigenous species found in the study area, 34 also live on the archipelago. The number of species can be considered as some kind of criterion for the succession of this uplift area. Fig. 1 shows the species recorded from islands of 7 age classes and from the mainland. In the classes where islands are older than 125 years the number of species (24-29) does not increase with time. On the young and small islets there are 17 species and 37 species can be found on the mainland, which is the species pool.

It is typical for an uplift archipelago that once a type of biotope has appeared, it exists on each island throughout the succession, but its location changes according to altitude from the sea level. However, the proportional area of these pioneer biotopes will diminish greatly on the islands of the sub-climax stage. This is why the species of young islets also occur on the older ones.

The large number of species in the classes of islets of 125-250 and 250-430 years old show that the effects of age, height and area are not the only factors involved, but the effectiveness of isolation and certain ecological factors should also be considered.

Moreover, the stage of primary succession does not depend on the age of the island alone. Especially on small islets the area is important. Waves and movements of ice considerably affect accumulation of soil and primary succession may thus progress very slowly. On the other hand, 2 islands of very different size (even though the one is a 100 times as large as the other) may be at the same stage of land mollusc succession, if the larger is effectively isolated, but the smaller one is not.

On islands of equal age and size differences in the numbers of species between isolated and non-isolated islands are greater on smaller islets, and will diminish towards larger ones (Fig. 2). For instance, increase of size from 0.1 to 10 ha will increase the number of species from 9 to 13 in non-isolated islands (grouping effect greater than 75%) but from 2 to 10 in isolated islands (grouping effect less than 75%) (Fig. 2).

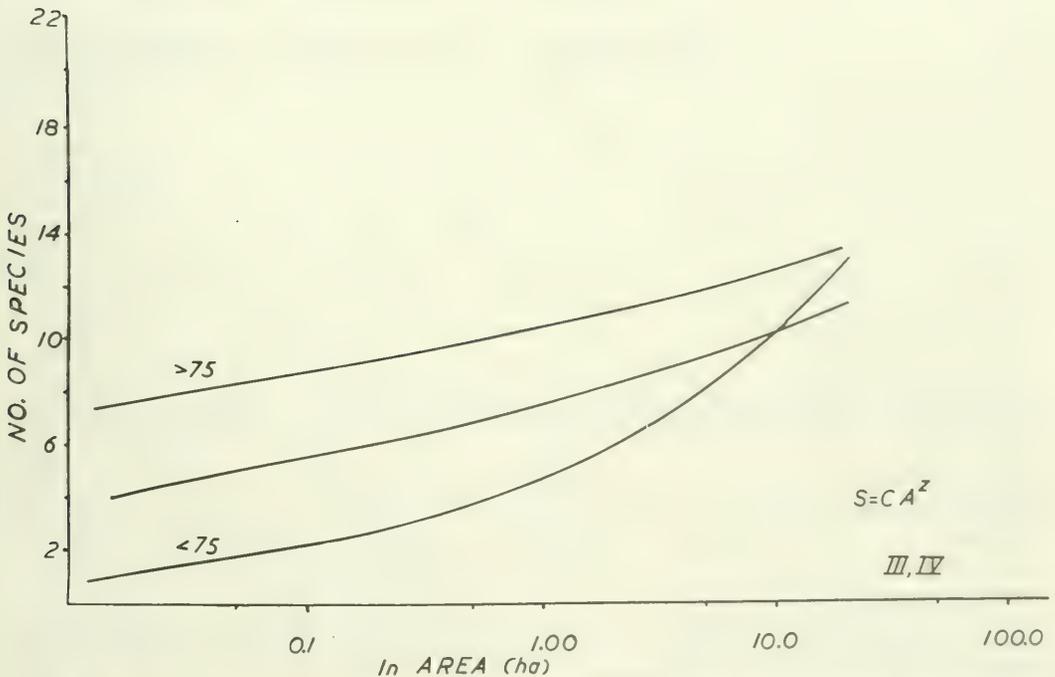


FIG. 2. Number of land mollusc species on isolated (grouping effect $\geq 75\%$) and non-isolated (grouping effect over 75%) islands.

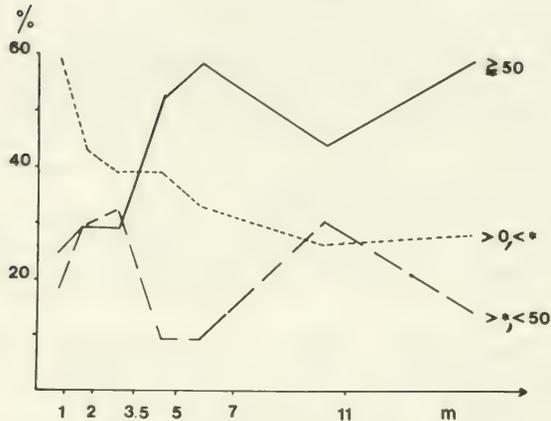


FIG. 3. The amount of constant ($\geq 50\%$), accessory ($> *, < 50\%$) and accidental ($> 0, < *$) species as a function of the height/age of islands.

However, age alone can give only a rough successional trend of land molluscs. The islands will reach stability with constant, accessory and accidental species after ca. 600 years (Fig. 3). The proportion of constant species (constancy $\geq 50\%$) will increase from 20 to 60%, the accidental species (constancy value does not differ statistically from 0, according to Fisher's exact probability test) will decrease from 60 to 30% and the accessory species between these 2 groups, will decrease from 20 to 10% (Figs. 1 and 3).

PIONEER SPECIES AND THE SUCCESSIONAL ORDER OF THE SPECIES

It is very difficult to compare islands of different age, area, isolation and vegetation. Therefore I have grouped together all islands with 1-6, 7-12, and 13-21 species in order to trace typical pioneer species of the outer archipelagoes and to find the order in which the species will occupy the islands (Fig. 4).

Islands with 1-6 species are usually treeless, grassy skerries with sparse thickets. According to the vegetation they are at the early successional stages. The constancy values of 5 species, viz. *Vallonia pulchella* (O.F. Müller) (10), *Deroceras agreste* (L.) (38), *Oxyloma pfeifferi* (Rossm.) (14), *Deroceras laeve* (O. F. Müller) (36), and *Vertigo alpestris* Alder (7) do not differ statistically from the corresponding constancies on islands with 7-12 or 13-21 species (cf. Valovirta, 1977). These pioneer species can be considered so-called r-strategists (MacArthur & Wilson, 1967; Horn, 1974; Pianka, 1974). They are easily dispersed and colonize nearly all the archipelagoes in the study area. Pioneers are also prolific and not particular about their habitat on low islets.

Islands with 7-12 species are at an intermediate stage of succession. The biotopes are variable and there are already trees of moderate size. Five common species, *Euconulus fulvus* (O. F. Müller) (40), *Nesovitrea hammonis* (Ström) (24), *Vitrina pellucida* (O. F. Müller) (23), *Clausilia bidentata* (Ström) (42), and *Succinea putris* (L.) (13) reach their maximum constancies at this stage. However, the constancies do not differ statistically from those on islands with 13-21 species. The other species of this stage, viz. *Cochlicopa lubrica* (O. F. Müller) (1), *Columella aspera* Waldén (3), *Discus ruderatus* (Férussac) (17), *Vertigo ronneybyensis* (Westerlund) (8), *Arion subfuscus* (Draparnaud) (20), and *Zoogenetes harpa* (Say) (12) are more constant on islands with 13-21 species, and can be considered as species of later successional stages (Fig. 4).

Islands with 13-21 species are large, less isolated and the forests are usually in the sub-climax stage. Typical species of the late successional stage are *Nesovitrea petronella* (L. Pfeiffer) (25), *Punctum pygmaeum* (Draparnaud) (16), *Vertigo pusilla* O. F. Müller (4), and *Columella edentula* (Draparnaud) (2). These species are the last ones to occupy islands on the outer archipelagoes of the study area (Fig. 4).

Study of the primary succession in different biotopes gives very similar results as when studying the succession on islands as a whole, both as regards pioneer species and species of

OUTER ARCHIPELAGOES

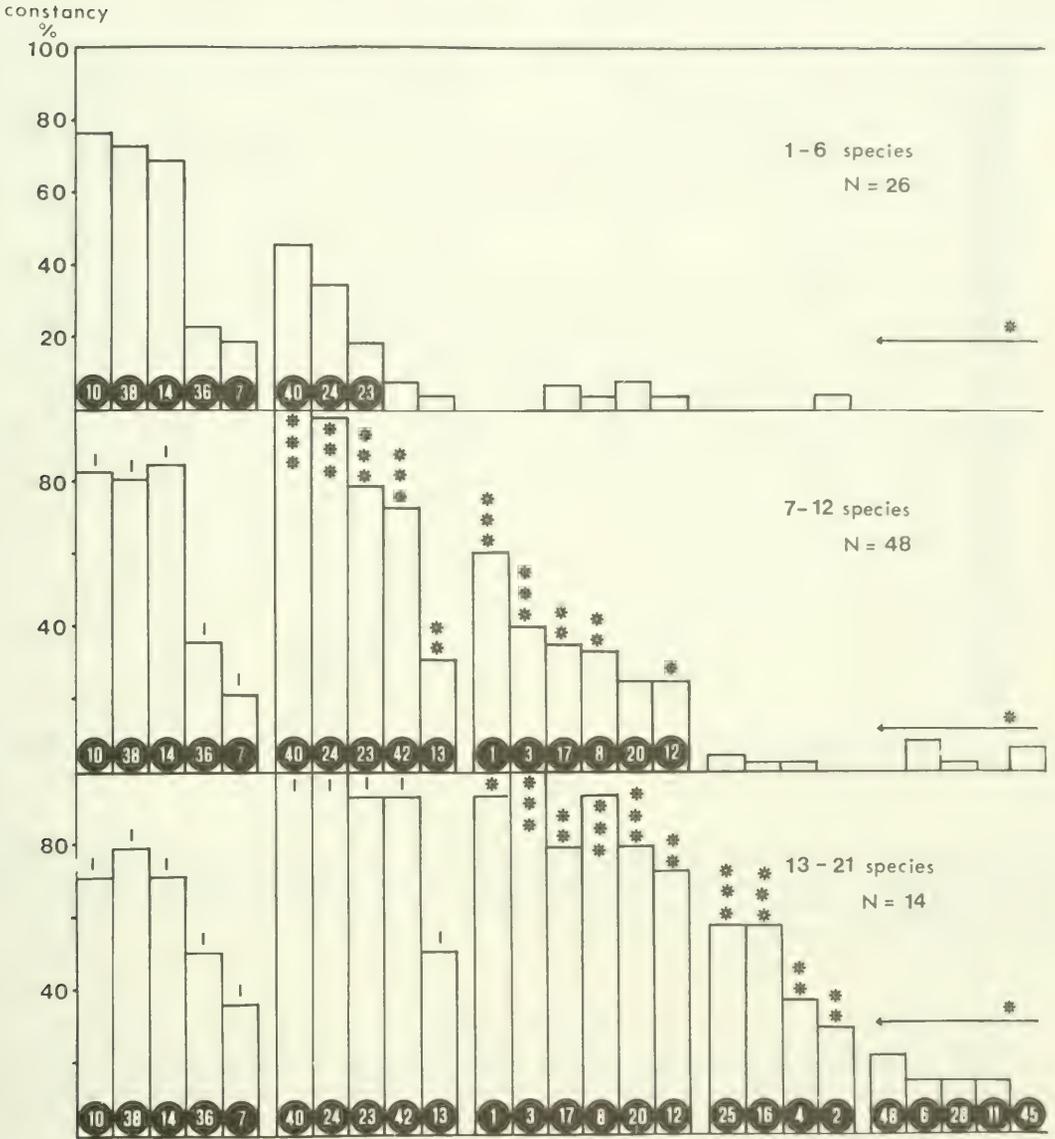


FIG. 4. The number of land mollusc species found on the islands of the outer archipelagoes reflecting the succession. Pioneer species are constant on islets with 1-6 species. The species of an intermediate stage of succession reach their maximum constancies on the islands with 7-12 species; the species of later successional stages occur mainly on islands with 13-21 species (cf. text). Numbers inside the black circles are the codes of the species. Asterisks denote the statistical significance as in Fig. 1.

later successional stages. Fig. 5 shows a successional series of biotopes from open beach meadows to shore thickets and to forests in the middle of islands. The intermediate biotopes have also been included. According to the constancy values the order of the first 10 species of the open grassy skerries has been marked on the left side of the diagram. The first 3 species are the same as in Fig. 4. However, during the successional series of biotopes these 3 pioneer species will disappear from

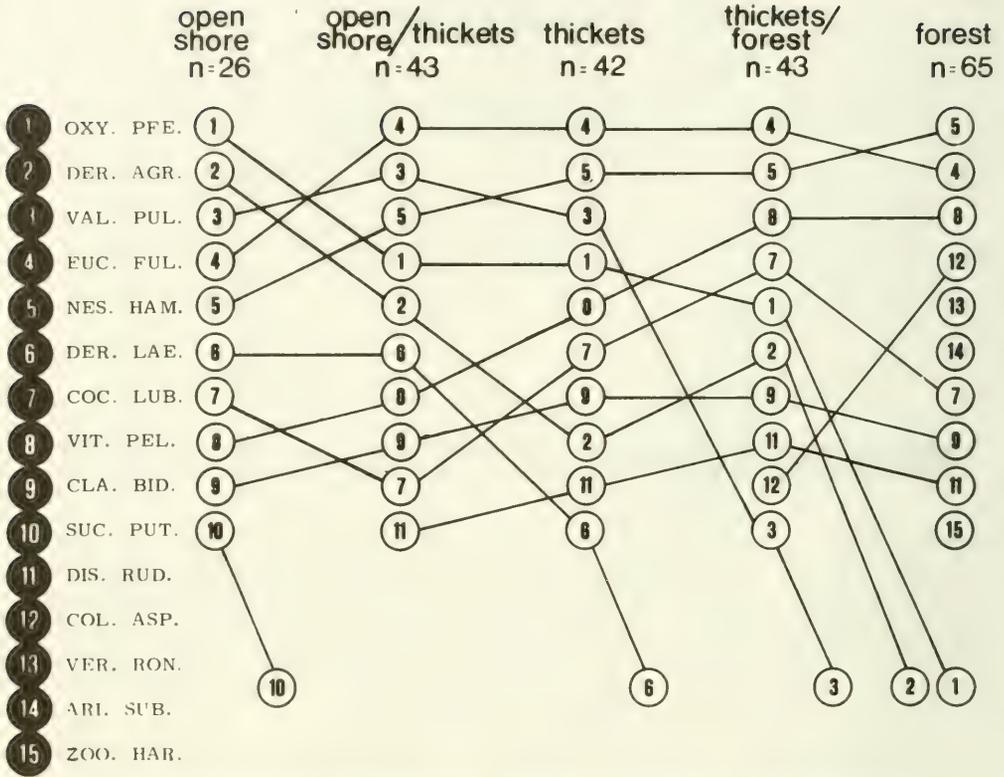


FIG. 5. The order (numbers) of land mollusc species on the successional series of biotopes from open shore meadows (grassy skerries) to forest, including the intermediate biotopes.

the group of the 10 first species before the sub-climax stage. Species which belong to the group of the 10 species which occur only in forests or in mixed biotopes of forest and thicket are *Columella aspera*, *Vertigo ronnebyensis*, *Arion subfuscus*, and *Zoogenetes harpa*. These are also the species of later successional stages in Fig. 4.

SPECIES DIVERSITY

Since the variations in species diversity are positively correlated with the stability of various biotic and abiotic components of ecosystems, diversity is significant in the study of succession (Whittaker, 1975). In Fig. 6 the species diversity has been derived from Shannon's measure of diversity (H') corrected according to Hutcheson (1970),

$$H' = -\sum p_i \ln p_i,$$

where p_i is the proportion of the total number of individuals (H'_{ind}) or the abundance frequency (individuals/litre) of the i th species (H'_{abu}) in the studied biotope or successional stage.

In Fig. 6 the successional stages of the islands in the outer archipelagoes have been arranged according to vegetation, and the biotopes represent the oldest stage of maturity on every island. The average ages of the first successional stages do not differ greatly because of the effective compensation of the other island factors (see above). However, on the uplift islands the diversity will increase with island maturity (cf. Väisänen & Järvinen, 1977).

Differences in diversity (H'_{ind}) from 1.65 to 2.45, imply that at the early stages of succession of the grassy skerries (1) the number of "equal common species" $\exp(H'_{ind})$ will be

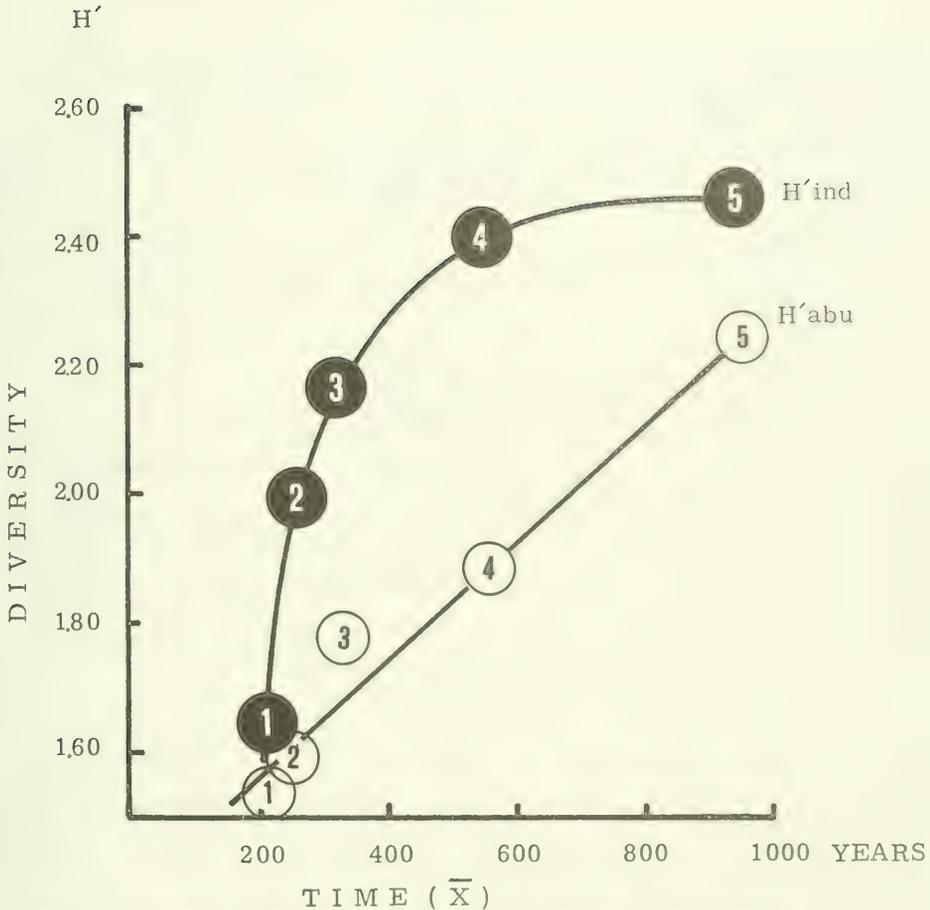


FIG. 6. Diversity of land mollusc species as a function of average age of islands grouped in 5 successional stages according to vegetation. H'_{ind} = individual-based diversity, H'_{abu} = abundance-based diversity, 1 = shore meadows, 2 = shore meadows/shore thickets, 3 = shore thickets, 4 = shore thickets/forests, 5 = forests.

5.2 and that it will increase up to 8.8 in thickets (3) and 11.6 in forests of the late sub-climax stage (5).

Tramer (1969, 1975) has suggested that H' values for taxa in unstable environments should vary as a function of evenness (cf. Pielou, 1966). Therefore, the evenness has been measured according to the formula $J' = H'_{ind}/\ln S$, where S is the number of species at the stage of succession, in this material 10, 14, 17, 17, and 21 respectively. Evenness will increase first from 0.72 (1) to 0.85 (4). After that it differs from the function of H'_{ind} and decreases to 0.81 (5). Correlation between H'_{ind} and J' is 0.913 and the coefficient of determination (R^2) will be 83.3% (see Järvinen & Sammalisto, 1973).

Fig. 6 shows that the 500-600 years old islands which are at the thicket/forest stage (4), have reached some kind of stability according to the diversity (H'_{ind}) values, which is nearly the same as on the islands of sub-climax forests (cf. Fig. 3). By using abundance-based diversity (H'_{abu}) the diversity will increase continuously from 1.51 (1) to the value of 2.24 of the sub-climax stage.

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REPRODUCTION OF TWO SPECIES OF LAND SNAILS IN
RELATION TO CALCIUM SALTS IN THE FOERNA LAYER

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ABSTRACT

In culture experiments with *Cochlicopa lubrica* (Müll.) and *Discus rotundatus* (Müll.) addition of calcium citrate and calcium oxalate to the substrate were demonstrated to have a positive influence on reproduction. The number of young produced in the culture boxes could also be increased by sodium carbonate additions, from which one may infer that both the calcium content and the pH-increasing action of the calcium compounds in the foerna are important. Ca citrate was significantly better than Ca oxalate. To facilitate comparisons with conditions in nature a small biometrical study of *C. lubrica* was made. Length of the reproductive season, young production and yearling growth were studied. Leaves of oak and beech are rich in oxalate-bound calcium, while in ash, lime, maple and elm more soluble Ca compounds (e.g. citrate) are dominating. This may be related to mollusc fauna differences between different types of deciduous woods on non-calcareous bedrock.

INTRODUCTION

In 1966-76 snails of two species, *Cochlicopa lubrica* (Müll.) and *Discus rotundatus* (Müll.), were cultured in plexiglass boxes under semi-natural outdoor conditions (cf. Wäreborn, 1970: 290, the few experiments with *D. rotundatus* are described in that paper). In this study the effect of calcium citrate and calcium oxalate additions to the substrate on the reproduction of *C. lubrica* is discussed. A calcium-deficient beach litter was used as substrate in all boxes. Besides this, there was one series, where pH was increased without Ca additions. Sodium carbonate was used instead. In order to kill predators, the litter was boiled for 5 minutes in distilled water (or in sodium carbonate solution) before it was placed in the boxes. In the 2 Ca salt series, the salt additions correspond to an increase of calcium of 1.5% of the dry weight of the substrate. Before addition it contained 1.0% Ca. The boxes were moistened with distilled water or in the sodium carbonate series with a dilute soda solution during dry weather periods. The experiments were started in the middle of June and lasted for about 17 weeks. During the 10 years when the experiments were conducted, there was a normal variation between dry and rainy summers.

There were different pH changes in the different box series. When no calcium salt was added, pH increased about one unit during the culture period (from about 5.0 to 6.2). Ca citrate had an immediate increasing effect (from 5.0 to about 7.3). Ca oxalate addition gave a rise about a month after the start of the experiments (from 5.3 to about 7.5). With sodium carbonate the pH was kept between 7.0 and 7.8 during the whole culture period.

Differences in effect on molluscs of different calcium salts naturally occurring in plants have not been presented by other authors. Voelker (1959) describes growth experiments with *Achatina fulica* Bowd. including pH increase with sodium carbonate. There was, however, no positive effect of the soda additions.

As a complement to the study, sift collections of *C. lubrica* from 9 localities in southern Sweden were biometrically studied in order to get an understanding of the life history of the species. Thereby it was revealed, that more or less distinct "winter lines" may be distinguished in the shells.

TABLE 1. Reproduction of *Cochlicopa lubrica* (Müll.): culture results. Culture experiments with different substrates: A, beech litter without calcium salts added; B, as A, but watered with sodium carbonate solution; C, beech litter mixed with Ca citrate; D, beech litter with Ca oxalate. Significance has been calculated on the mean differences between young numbers in paired samples of culture boxes.

Substrate category	Culture results: number of young						Md = mean of differences Significance	
C (with citrate)	15	38	28	38	29	36		
A (no Ca added)	0	6	10	17	7	9	Md = 22.50	t = 8.96
Differences	15	32	18	21	22	27	p < 0.001	
D (with oxalate)	13	21	19	17	25	28		
A (no Ca added)	6	10	7	1	17	7	Md = 12.50	t = 5.84
Differences	7	11	12	16	8	21	p < 0.005	
C (with citrate)	38	17	13	28	38	29		
D (with oxalate)	13	9	6	21	28	25	Md = 10.17	t = 3.31
Differences	25	8	7	7	10	4	p < 0.03	
B (soda watered)	7	15	13	9	9	8		
A (no Ca added)	4	9	10	7	1	4	Md = 4.33	t = 4.71
Differences	3	6	3	2	8	4	p < 0.01	

RESULTS OF THE CULTURE EXPERIMENTS

The culture results with *C. lubrica* are presented in Table 1. Eight culture experiments with *Discus rotundatus* have also been made (Wäreborn, 1970); the results are in accordance with the above. The significance tests are based upon pairing of the experiments (Snedecor & Cochran, 1967: 93). Thereby pairs were formed of experiments from the same year. If more than one pair in a series could be formed per year, boxes placed close to or near each other were designed to form the pairs. It would have been desirable to have more pairs in every series; this kind of work is, however, very time-consuming.

BIOMETRICAL STUDIES

In a small series of complete sift collections of *C. lubrica* the following measurements were taken: length and width of the shell and height of the aperture. The number of whorls were counted (Ehrmann, 1956: 21). The collections (belonging to the Natural History Museum of Gothenburg) were from the end of the months of May to October. Three samples from October and one sample from each of the other months were measured. A total of 177 adults and 851 juveniles was studied biometrically. The latter were sorted into different year classes, mainly on the basis of winter lines. These are distinct in young specimens and may still be discernible in about two thirds of the adult shells. Drought may produce lines in the shells and so do various types of repaired damage; the latter cannot, however, be mistaken for winter lines. With increasing experience most of the drought lines can also be sorted out. As the lines penetrate to the surface of the shell, they cannot be caused by inside epiphragms. *C. lubrica* has a long reproduction season from (May) June to October (November) and a rather rapid elimination of juveniles. It does not seem possible to discern year classes only on the basis of length measurements or whorl counting. Results from this small biometric study are presented in Table 2. The collections are from rather different wood habitats. There are great variations in the rate of elimination of the juveniles.

CONCLUSIONS

From the biometrical study and from the study of winter lines in the shells one may infer that young production in the culture boxes, where calcium salts were added, was within the natural variation of reproduction in good *Cochlicopa lubrica* localities in the area. Yearling

TABLE 2. Biometrical results and results from a study of winter lines in the shells of *Cochlicopa lubrica*. Locality type No. 1 = oligotrophic, 2 = mesotrophic, 3 = eutrophic. The distribution of juveniles in year classes is mainly based on winter lines.

Collection date	Type of locality		Maximum length of shell in year-lings mm	Maximum growth of year-lings (whorls)	Number of adults	Number of juv. year No.				Number of juv. per 10 adults	
	No.	pH				year-lings	II	III	IV	2nd year	year-lings
21.5.1967	3	6.5	0.84	0.1	38	1	58	12	11	15.3	
26.6.1971	2+	6.5	0.87	0.2	9	4	41	12	1	45.6	
28.7.1971	3	6.0	1.19	0.6	13	11	18	1	—	13.8	
19.8.1965	3	7.0	1.37	0.8	14	31	1	1	3	22.1	
29.9.1963	2	5.7	2.02	1.6	14	16	36	15	2	11.4	
25.10.1973	3	7.5	2.42	1.9	21	283	18	17	2	135.0	
31.10.1973	2	6.0	1.72	1.2	23	33	8	9	4	14.4	
31.10.1970	3-	7.7	2.39	2.0	45	200	3	5	—	44.4	

growth in these boxes has also been within natural variation (0.0-0.8 whorls in boxes with Ca citrate and 0.0-0.9 with Ca oxalate). Both young production and growth was less in boxes with no Ca salt additions (growth in boxes moistened with distilled water and in soda moistened boxes was 0.0-0.5 whorls). The juveniles may need 3-4 years to reach adult size.

Reproduction is increased by additions of calcium citrate and calcium oxalate. The former is significantly better than the latter. This is interesting, because in nature leaves of ash, lime, maple and elm are rich in rather soluble calcium compounds (e.g. Ca citrate), while in leaves of oak and beech calcium occurs mainly as Ca oxalate (Mattson & Koutler-Andersson, 1946). Litter from the 2 latter tree species contains more tannic acid and other acid substances (Edwards & Heath, 1975) than that from the citrate-rich trees. Calcium oxalate needs months in the foena to be disintegrated, and until then it has no neutralizing effect upon acids. The mollusc faunas of woods on soils without calcium carbonate (e.g. on bedrock of granite, gneiss or greenstones) seem to depend upon citrate-rich trees, and where such trees occur the faunas are as a rule much richer both in individuals and in number of species (Wäreborn, 1969). It is probable that the effect of the calcium salts depends *both* on their Ca content and their pH-increasing action (cf. the soda experiment series, Table 1). In the surface of the soils of oak and beech woods on non-calcareous bedrock pH is about one unit lower than in woods with ash, lime, maple or elm upon the same type of bedrock substratum (Wäreborn, 1969).

ACKNOWLEDGEMENTS

Prof. Per Brinck, Department of Animal Ecology, Lund University, has taken a continuous interest during more than a decade in this work and has given valuable advice. The same can be stated for Dr. Henrik Waldén, Natural History Museum of Gothenburg, who also kindly has contributed material to the biometric study from collections made by himself and belonging to the museum. Thanks are also due to Dr. B. Hubendick, head of the Gothenburg Museum, who agreed to lending the material. Assistance in the work has in various ways been given by mesdames G. and A. Wäreborn.

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NOTE ADDED IN PROOF. A recent work dealing with winter lines in snail shells should be included in the list of references:

- POLLARD, E., COOKE, A. S. & WELCH, J. M., 1977, The use of shell features in age determination of juvenile and adult Roman snails *Helix pomatia*. *Journal of Zoology*, 183: 269-280.

POPULATION DYNAMICS OF SOME LAND GASTROPODS IN
A FOREST HABITAT IN POLAND

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ABSTRACT

In the Hel peninsula (Poland) litter and the surface of the soil of an old *Alnus* forest were square-frame sampled 4 times a year. All the 10 snail species were numerous in spring and autumn. In July and August a dramatic drop in numbers was noted. Presumably the snails escape the summer drought, digging themselves into the soil. *Vitrina pellucida* has a one-year life cycle; they hatch in the spring, with a shell diameter of 0.8 mm, reach full size and maturity in late autumn, then lay eggs and die. Only a few survive until next April. *Cochlicopa lubrica* needs approximately 22 months to attain maturity; adults live over 1 year. *Euconulus fulvus* is probably also slow-growing, long-lived, and the young lead a largely subterranean life.

In order to understand the place of gastropods in the structure and function of terrestrial ecosystems, more information is needed on their life cycles and population dynamics.

STUDY AREA, METHODS, MATERIAL

The study area was in the Hel peninsula (Baltic coast), 1 km NW of the summer resort Kuźnica. It is an old *Alnus* forest, covering a sand flat among dunes, 2 m above sea level. The lower layer was formed by *Sorbus aucuparia* and *Sambucus nigra*, the still lower stratum by *Rubus (Eubatus)* sp. with some *Ribes* sp. The litter and top 2 cm of soil were hand-sampled 4 times a year in 1970-1972 and again in 1976, using a square-frame of 1/16 m². Each sample consisted in principle of 16 frames. It is assumed, that the disturbance by sampling itself was negligible. The collected material comprised 1399 live specimens and 2261 shells of: *Vitrina pellucida* (Müller) (47.5%), *Euconulus fulvus* (Müller) (28.0%), *Cochlicopa lubrica* (Müller) (12.3%), *Punctum pygmaeum* (Draparnaud), *Nesovitrea hammonis* (Ström), *Vertigo pusilla* Müller, *Cepaea hortensis* (Müller), *Helicigona arbustorum* (L.), *Vallonia pulchella* (Müller), and *Arion subfuscus* (Draparnaud).

DENSITY

All 10 species show at least one common regularity—in July and August their density is extremely low. Most are fairly numerous in spring and autumn. Data on the 3 dominant species are summarized in Fig. 1. In *Vitrina pellucida* these changes in density are vividly pronounced—in July 1972 and 1976 not a single living individual was found—but the other species clearly follow the same pattern. This summer disappearance is not due to mortality, because the animals found in autumn unquestionably represent the same age class which was found in May or June, as will be shown later. Migration is obviously out of the question. The snails must dig themselves into the sandy soil, escaping the summer drought. Hence what is shown here is not the actual population density, but density of animals obtained by the procedure adopted.

¹Dr. Umiński has been unable to attend the Amsterdam congress; as an exception his paper is published in these Proceedings.

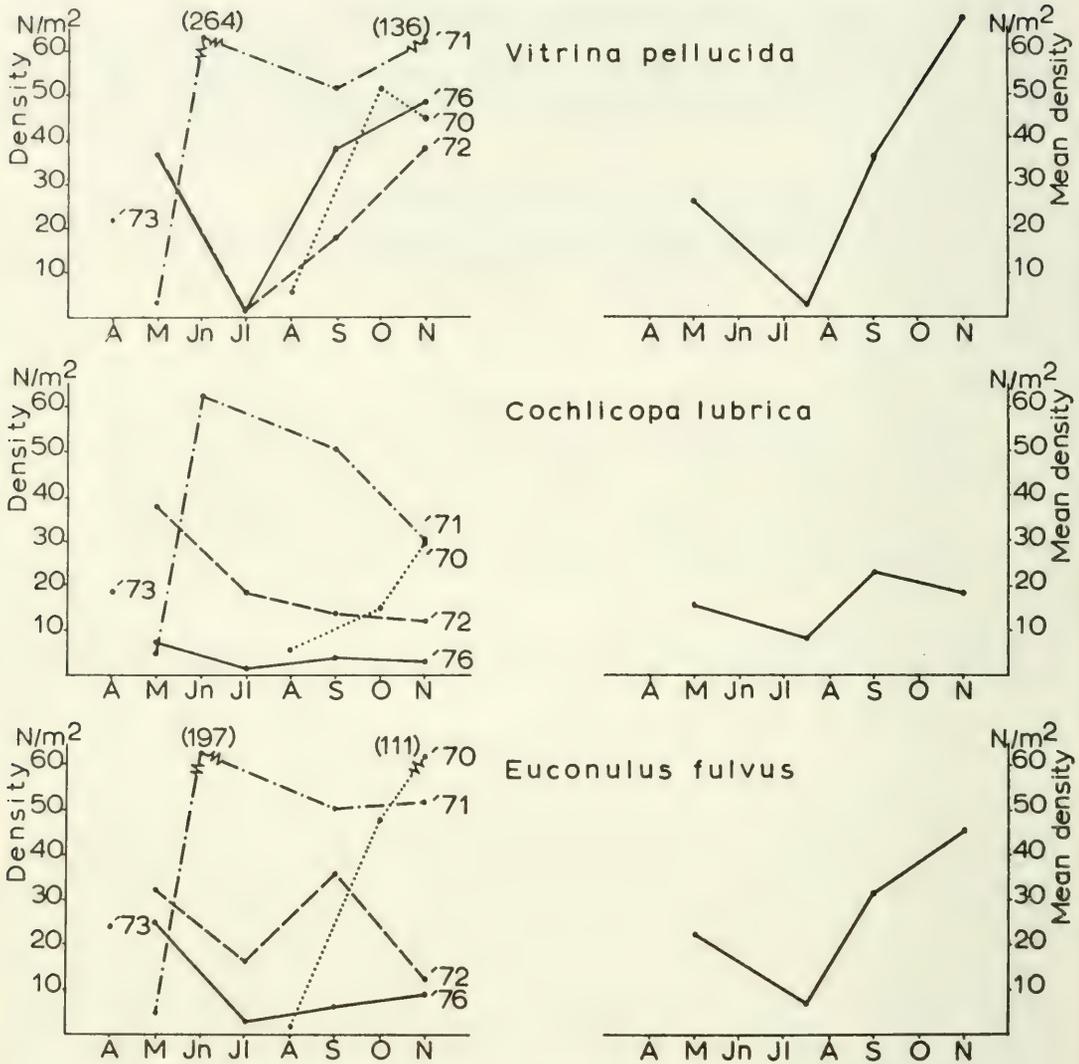


FIG. 1. Yearly changes in density. Mean density calculated where at least 3 values were available. July and August data combined.

SIZE DISTRIBUTION

Vitrina pellucida (Müller)

Yearly changes in size distribution as shown in Fig. 2 indicate a clear-cut one-year life cycle. In spring and early summer there are only very small individuals, bigger ones in September and really big ones in November. We conclude that they hatch about April with a shell diameter of 0.8 mm, reach their final size in November, then lay eggs and die. Occasionally some may hatch in autumn, as witnessed by single, very small individuals, collected in November 1970 and September and November 1972. These may hibernate, as undoubtedly did the 2 specimens of May 1971; these had shell diameters of 2.5 and 2.9 mm and were both at the stage juvenile I (Umiński, 1975a), their genital systems barely visible strands of tissue. Snails of 2.9 mm shell

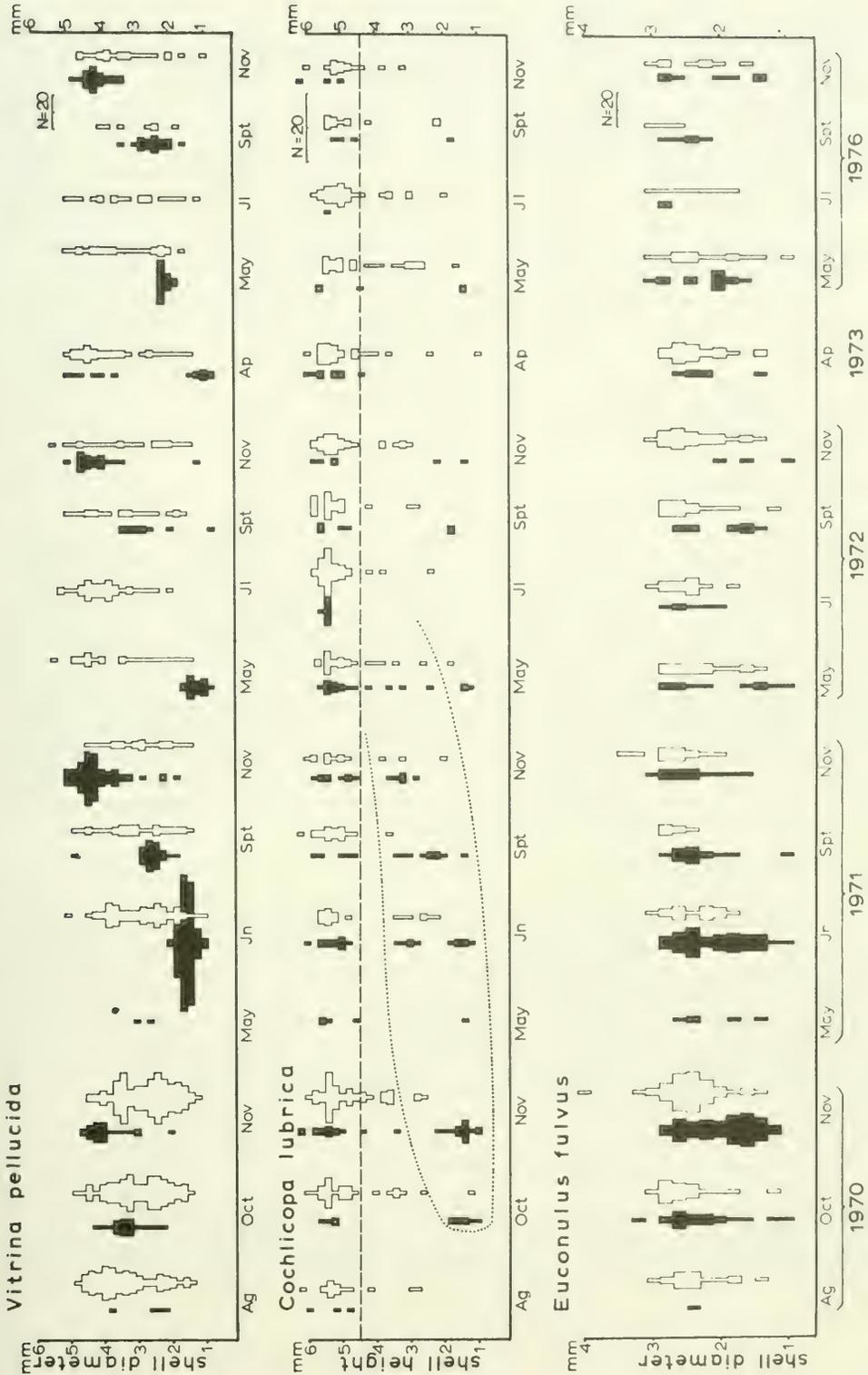


FIG. 2. Yearly changes in size distribution. Graphs depict absolute numbers at 0.2 mm intervals. Solid black—live animals, contour—shells, broken line—"maturity line," dotted line—presumed outline of one age class. The shells of May 1971 were lost by accident.

diameter, taken in November 1970 and 1971 were mature II, i.e. after copulation, ready to lay eggs, their genital systems occupying something like $\frac{1}{4}$ of the whole specimen's bulk. This relative independence of size and maturity is in keeping with data on populations of the Tatra Mountains, with a 2-year life cycle (Umiński, 1975b). Hibernation of full-sized adults was noted in April 1973. Thus the postembryonic life span here would be 8-12 months. It is most striking, that growth is largely arrested during summer. Over the period June-September in 1971 and May-September in 1972 and 1976 mean shell diameter of this population increased by 0.4, 0.45 and 0.1 mm per month respectively. In sharp contrast, this increase during October-November 1970 and September-November 1971, 1972 and 1976 amounted to 0.8, 0.8, 0.7 and 0.8 mm respectively. No such thing was ever found in the Tatra populations. Drought is the probable cause. It seems, that the snails, when in hiding, are not active, possibly close to anabiosis. Data on empty shells here are not as instructive as they were in the Tatras. All samples of July, August and even of September comprise a fair proportion of shells of animals which, judging from their size, must have died not later than April. In other words, *Vitrina* shells persist here for at least 3-5 months of the snow-free season, while in the Tatras they disintegrate completely in less than 2 months, serving as a precise measure of mortality in the preceding month. Still, comparison of number and size of animals and shells, collected simultaneously suggest that mortality is about the same for all age size groups, quite low until late autumn.

Cochlicopa lubrica (Müller)

As in this population 98.3% of animals and shells with an incassate labial margin are larger than 4.5 mm (shell length) this value was taken as a "maturity line" (Fig. 2). In all samples, regardless of season, there is always a well-defined group of adults. The young are usually few and, with the exception of those of May 1972, differ from mature ones in size by being 1-3 mm shorter. The group, which can be reasonably presumed to represent one age class, was first noted in October 1970, with a shell length of 0.9-1.8 mm. It could be followed (dotted line) in all subsequent samples until May 1972, when it began to merge with the grown-ups. The very small specimens of May, September and November 1972 could represent the next age class. Hence *C. lubrica* needs 21-24 months to attain final size and maturity. Adults must live at least for 1 year to account for their constant presence. The remarkably low number of young as compared to that of adults would imply even higher longevity of the latter, as well as a balance based on low natality and low mortality. It seems that breeding is possible at any time; the most successful hatching must have been in September.

Euconulus fulvus (Müller)

Shell size distribution (Fig. 2) resembles that of *C. lubrica* in that a relatively numerous group of big individuals is present almost constantly, indicating longevity of adults. The young were fairly numerous, but their occurrence did not show any orderly pattern. Not a single age class could be traced the way it was with *C. lubrica*. Very small young of under 1 mm diameter were remarkably scarce, particularly so, as similarly small specimens of other species, including *Punctum pygmaeum*, were more numerous. This whole picture could be accounted for if young *E. fulvus*: (a) were similarly slow-growing as *C. lubrica*, (b) led a largely subterranean life, (c) emerged onto the soil surface only during temporary favourable weather conditions.

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E.I.S.—BEITRÄGE AUS DER BUNDESREPUBLIK DEUTSCHLAND

H. Ant¹ und J. H. Jungbluth²

ABSTRACT

This is a summary of European Invertebrate Survey (E.I.S.) contributions in the Federal Republic of Germany (BRD) with examples of local distribution of selected species (Figs. 1-6) and graphical presentation (Figs. 8-9). Fig. 7 shows the areas covered with the respective numbers of species.

VORBEMERKUNG

Auf die noch ausstehende, zeitgemässe malako-faunistische Erforschung der BRD hat Waldén (1963) in einem Aufsatz hingewiesen, auf den Ant (1963a) geantwortet hat. Bis zu diesem Zeitpunkt lagen nur lokale oder höchstens regionale Teilbearbeitungen von Molluskenfaunen vor. Für die Untersuchungsräume wurden Faunenlisten erstellt und die Fundorte benannt; die kartographische Darstellung in der Form von Punktkarten war eine Ausnahme. Den ersten umfassenderen Kartenbeitrag legte Ant (1963b) vor, wenn von den Najaden-Bearbeitungen von H. Modell einmal abgesehen wird, die sich jeweils mit einem Flusssystem befassten.

Die Organisationsstrukturen für eine bundesdeutsche Beteiligung am E.I.S.-Programm wurden erst nach dem Symposium 1972 in Saarbrücken geschaffen. Am dortigen Schwerpunkt für Biogeographie wurde schliesslich das nationale Kartierungszentrum (Prof. Dr. P. Müller) geschaffen. Die Einrichtung und finanzielle Sicherung zogen sich jedoch über mehrere Jahre hin, so dass bis heute erst ein Verbreitungsatlas für Lepidopteren publiziert werden konnte. Nach dessen Erscheinen im Jahre 1976 sind weitere, abgeschlossene Kartierungs-Beiträge im Druck und werden wahrscheinlich noch 1977 erscheinen, so auch der erste Molluskenatlas (Jungbluth, 1978).

PROBLEME

Der Fortschritt der Kartierung wird im wesentlichen von zwei Faktoren bedingt:

(a) die Ausstattung der Mitarbeiter mit dem erforderlichen Grundkartenmaterial. Die U.T.M.-Karten sind nicht in jedem gewünschtem Masstab frei im Handel erhältlich,

(b) der rasche Druck der abgeschlossenen Kartierungsbeiträge. In Saarbrücken wurden inzwischen die UTM-Gitternetz-Karten für die Bundesländer und auch für kleinere Gebiete erstellt, so dass dieses Problem zumindest teilweise gelöst ist. Weiter wurde ein Computer-System zur Erstellung von Fundortkarten für die BRD codiert (Klomdat, s. Klomann & Müller, 1975).

Abschliessend ist noch auf das Problem von Zeitaufwand und Mitarbeitern einzugehen. Die Erfahrungen der eigenen Arbeitsgruppen haben gezeigt, dass die Verwendung der Einzelbeleg-Karten für kleine Teams entschieden zu zeitaufwendig ist und nicht bewältigt werden kann, dies auch unter dem Gesichtspunkt, dass z.Z. eine sofortige Einspeisung der Daten in den Computer noch nicht möglich ist. Wir erfassen daher die Daten in Karteien auf Fundortsammelkarten bzw. in Registern mit Fundortsammelblättern, auf denen jeweils grosse Anzahlen von Daten je Art gesammelt werden können.

Vom Zeitaufwand her ist die blosse Anfertigung von Grid-Verbreitungskarten nicht vertretbar, so dass hier weitere Daten in das Kartenbild eingehen müssen. In der BRD wird versucht, über die reinen Artverbreitungskarten hinaus Organismen-Kataster für kleinere und grössere Räume zu erstellen (Klomann & Müller, 1975).

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KARTIERUNGSBEITRÄGE

Erste Erfahrungen wurden in der BRD bei der Kartierung der Mollusken des Vogelsberges nach der E.I.S.-Methode gesammelt (Jungbluth, 1975a). Für die eigenen Arbeitsgruppen hat sich gezeigt, dass entweder kleine Gebiete (s. Fig. 2) insgesamt bearbeitet werden können oder bei grossräumigen Kartierungen eine geringe Artenzahl ausgewählt werden muss. Für die Beurteilung von Standort- und Raumqualitäten durch Zeigerarten oder -gesellschaften muss ein kleines Grid-Raster gewählt werden (z.B. 1 X 1 km).

Bislang wurden folgende Kartierungen abgeschlossen:

1. Vogelsberg, 131 Arten; 2,5 X 2,5 km (Jungbluth, 1975a)
2. Odenwald, 170 Arten; 1 X 1 km (Ritter, 1974)
3. Heidelberg und Umgebung, 151 Arten; 1 X 1 km (Kirchesch, 1976)
4. Hessen, 204 Arten; 10 X 10 km (Jungbluth, 1978)
5. Nordrhein-Westfalen, 54 Arten; 10 X 10 km (Ant)

Darüber hinaus liegen die Verbreitungskarten von *Bythinella bavarica* (H. Boeters),¹ *Bythinella dunkeri* (J. H. Jungbluth)¹ und *Margaritifera margaritifera* (J. H. Jungbluth) für die BRD vor.

AUSBLICK

Für die weiteren Kartierungen liegen die Kartierungsanweisungen (Ant, 1973) vor. Die Anfertigung weiterer U.T.M.-Gitternetz-Karten ist auf der Basis des vorhandenen Kartenmaterials (auch im Masstab 1:50.000) möglich. Die Mitglieder der Deutschen Malakozoologischen Gesellschaft wurden zur Mitarbeit aufgerufen (Jungbluth, 1975b) und ein Überblick über die bisherigen Kartierungsbeiträge an anderer Stelle gegeben (Jungbluth, 1976).

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¹(Jungbluth & Boeters, 1977)

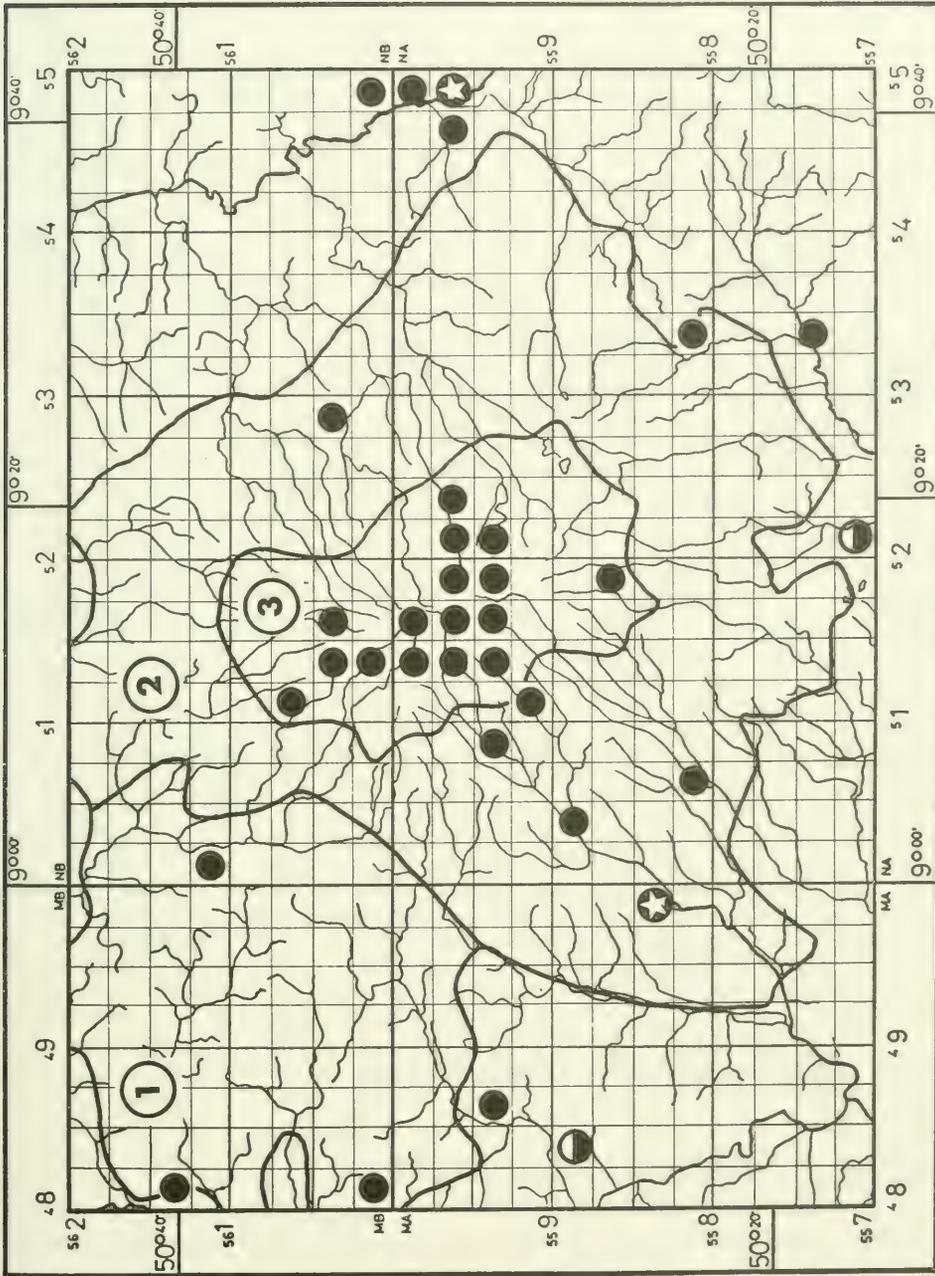


FIG. 1. *Discus rotundatus* (O. F. Müller, 1774) im Vogelsberg/Oberhessen. In die Gitternetzkarde sind die naturräumlichen Einheiten eingezeichnet: 1 = Vorderer Vogelsberg; 2 = Unterer Vogelsberg; 3 = Hoher Vogelsberg. Die Waldart zeigt eine konzentrierte Verbreitung im Hohen Vogelsberg, der einen relativ geschlossenen Waldkomplex darstellt. (2.5 x 2.5 km; Punkt: Funde nach 1960; halber Punkt: Funde vor 1960; Stern: Literaturangaben).

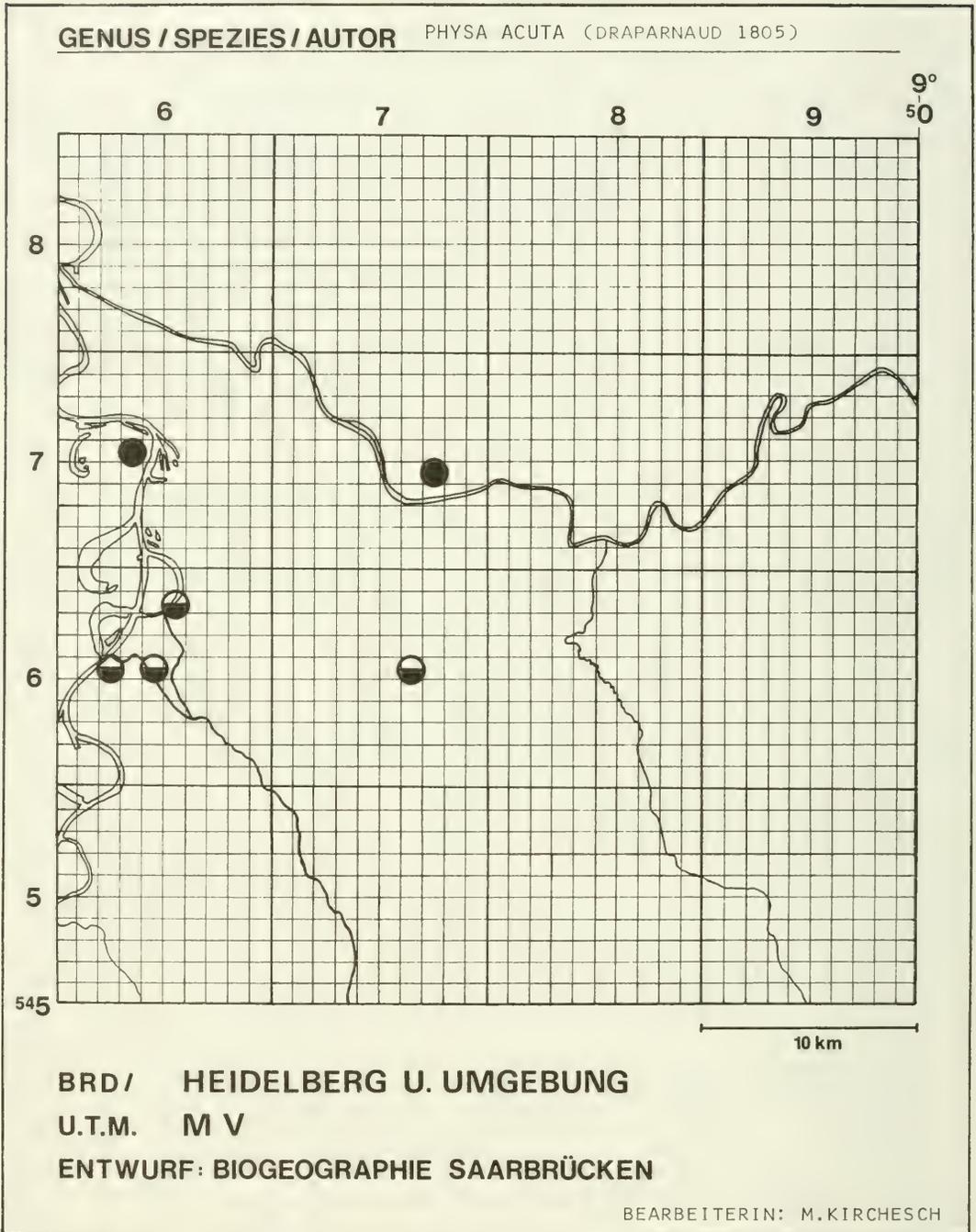


FIG. 2. *Physa acuta* (Draparnaud, 1805) in der Umgebung von Heidelberg. Die Art gilt bei uns als eingeschleppt und hat sich im Rheingebiet verbreitet. Isolierte Fundorte gehen wahrscheinlich auf separate Aussetzungen von Aquarianern zurück. (1 × 1 km; Punkt: Funde nach 1960; halber Punkt: Funde vor 1960; Bearbeiterin: Monika Kirchesch).

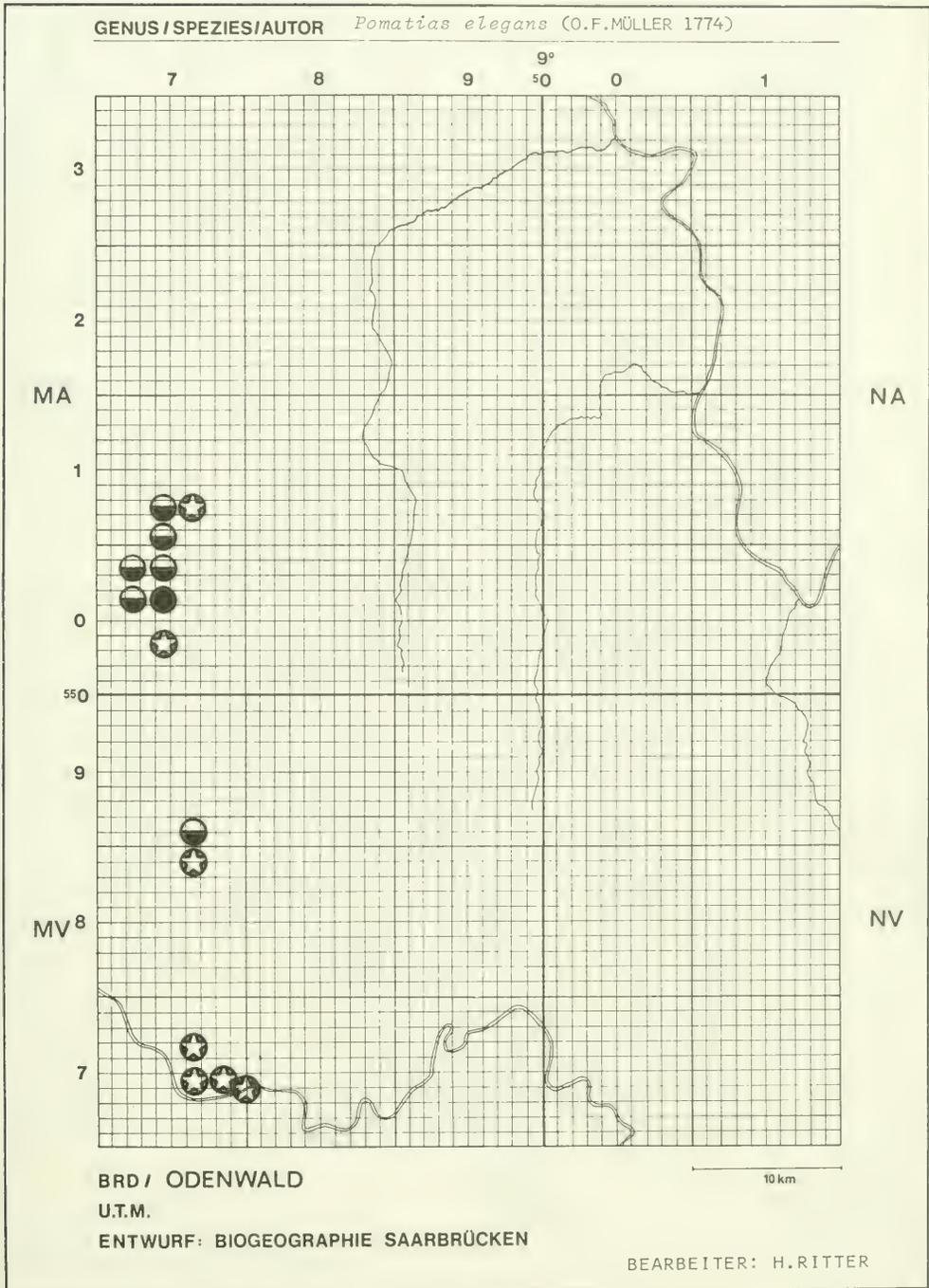


FIG. 3. *Pomatias elegans* (O. F. Müller, 1774) am westlichen Odenwaldabhang. Diese substratgebundene Art ist entlang der naturräumlichen Einheit Bergstrasse verbreitet. Sie besiedelt hier das westlich an den Odenwald anschließende Gebiet, das durch Lössauflagen einen entsprechenden Mindestkalkgehalt im Boden aufweist. (1 X 1 km; Punkt: Funde nach 1960; halber Punkt: Funde vor 1960; Stern: Literaturangaben; Bearbeiter: Helmut Ritter).

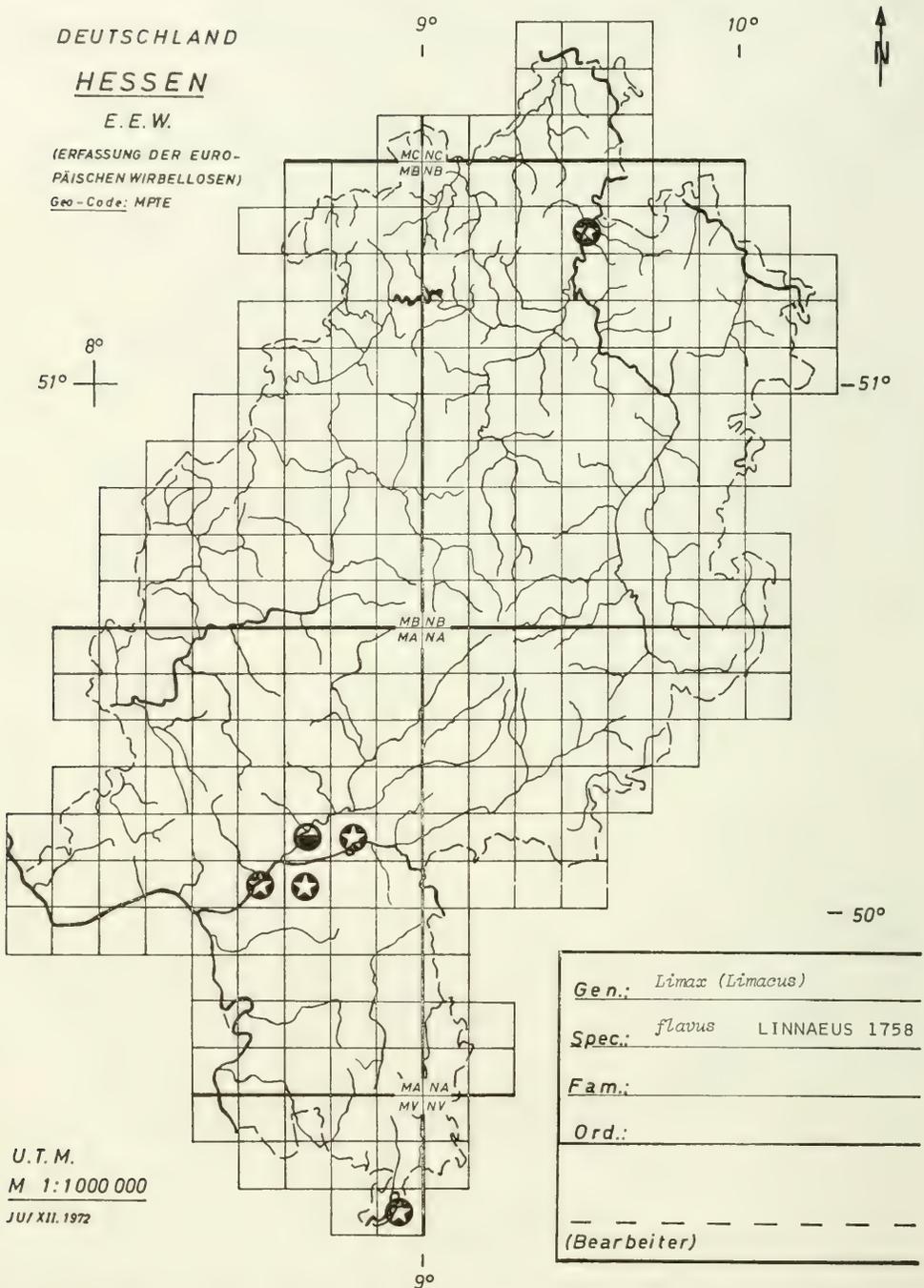


FIG. 4. *Limax flavus* Linnaeus, 1758, in Hessen. Das Kartenbild zeigt, dass neuere Fundnachweise fehlen. Dies ist offensichtlich auf die für die Art veränderte ökologische Situation in Mitteleuropa zurückzuführen. Hier kam *L. flavus* früher in den feuchten, mit Lehmböden ausgestatteten Kellern vor, die heute nur noch ausnahmsweise vorhanden sind. (10 × 10 km; halber Punkt: Funde vor 1960; Stern: Literaturangaben).

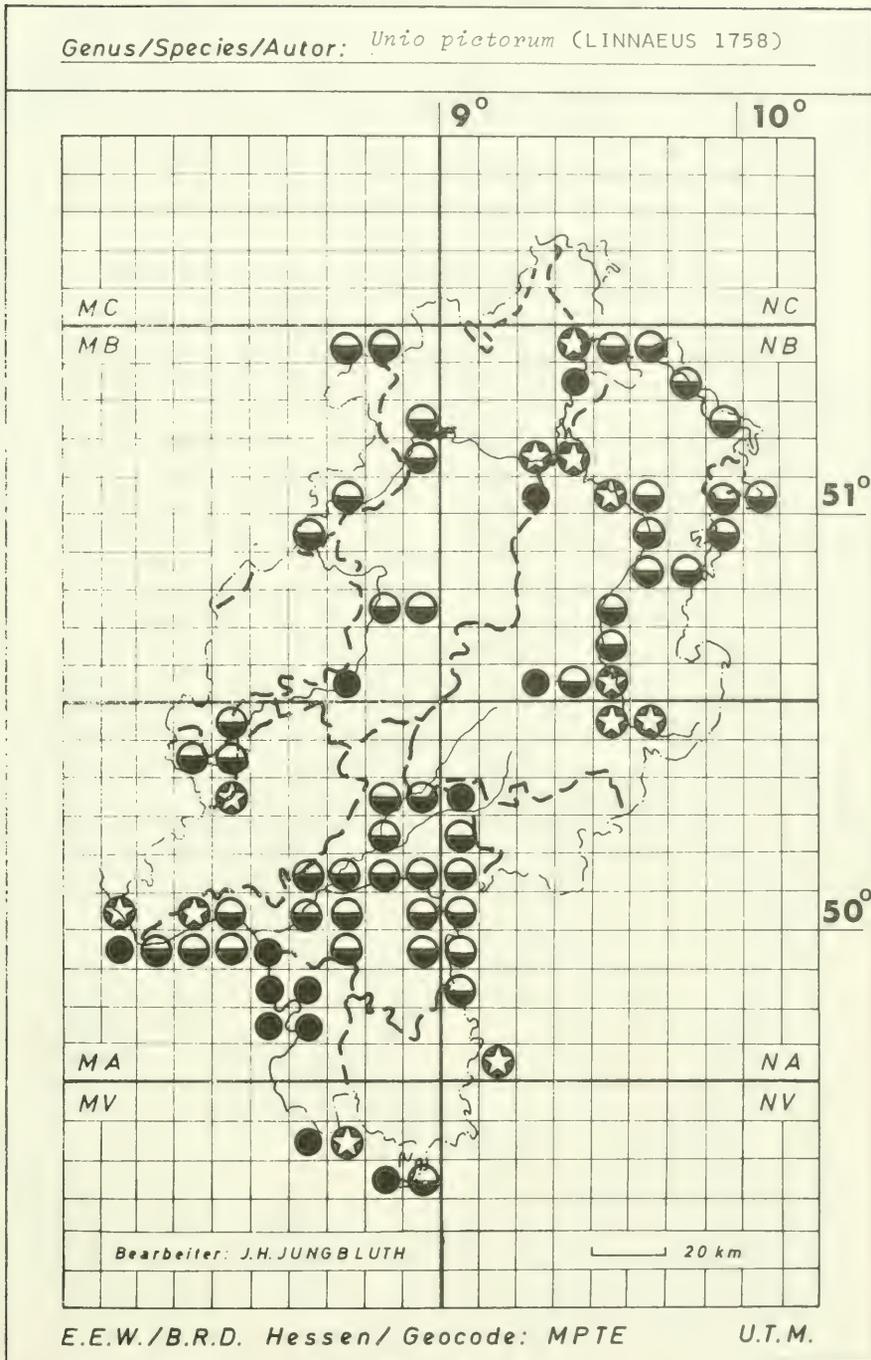
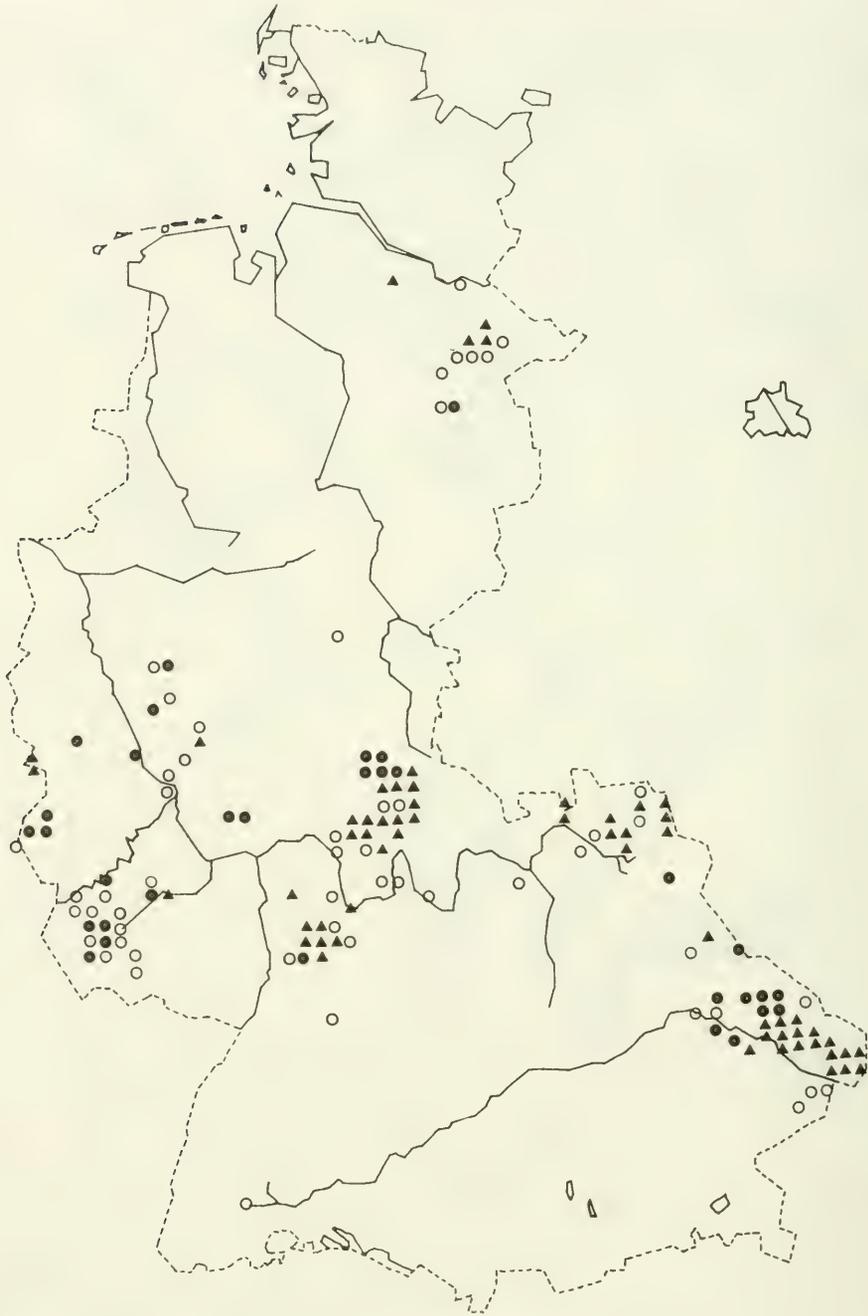


FIG. 5. *Unio pictorum* (Linnaeus, 1758) in Hessen. Die Art ist nicht in dem Masse an grosse Fließgewässer sowie deren Altwässer wie *U. tumidus* gebunden und weist so eine weitere Verbreitung auf. (10 X 10 km; die naturräumlichen Einheiten sind eingezeichnet; Punkt: Funde nach 1960; halber Punkt: Funde vor 1960; Stern: Literaturangaben).



Margaritifera margaritifera (LINNAEUS 1758)

FIG. 6. *Margaritifera margaritifera* (Linnaeus, 1758) in der BRD. Die Flussperlmuschel hat in den letzten Jahrzehnten erhebliche Teile ihres Arealen in Mitteleuropa verloren und ist heute meist nur noch in schwachen Populationen vertreten. (Computer-Karte der BRD, jede Signatur entspricht einem 10 X 10 km Quadrat; Punkte: Funde aus dem Zeitraum 1950-1975; Kreise: Funde vor 1950; Dreieck: Literaturangaben).

GENUS/SPECIES/AUTOR

ERFASSUNG DER EUROPÄISCHEN WIRBELLOSEN (E.E.W.)

BUNDESREPUBLIK DEUTSCHLAND

U.T.M.



ME	NF	RF		
LE	ME	NE	PE	
LD	MD	ND	PD	UU
KC	LC	MC	NC	PC
KB	LB	MB	NB	
KA	LA	MA	NA	PA
LV	MV	NV	PV	QV
LU	MU	NU	PU	QU
LT	MT	NT	PT	
			QA	TR
			TO	UR
			TQ	VQ
			TP	VP
			TN	UN

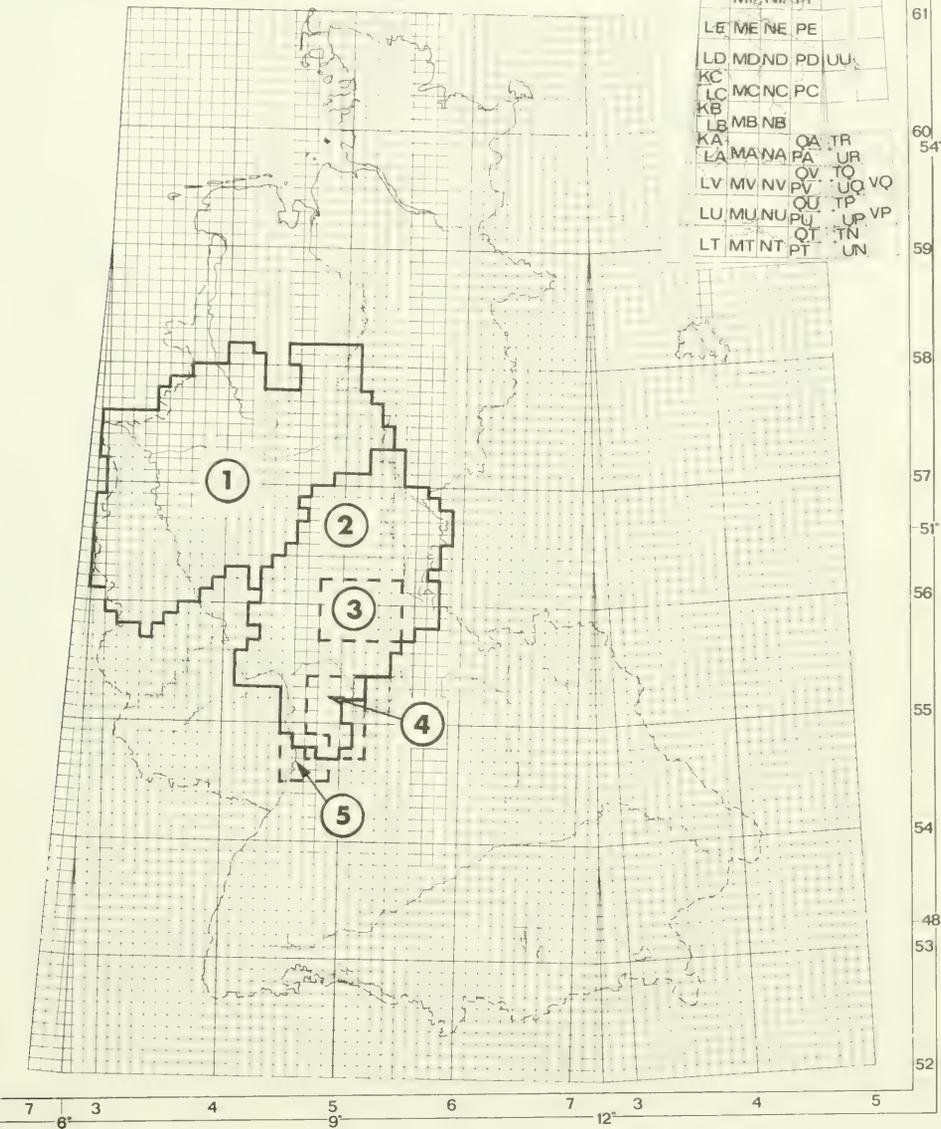


FIG. 7. Karte der BRD mit malakozologisch bearbeiteten Gebieten. In die Gitternetz Karte der BRD sind die bearbeiteten Gebieten eingezeichnet: 1 = Nordrheinwestfalen; H. Ant; 60 Arten, 1977; 2 = Hessen; J. H. Jungbluth; 204 Arten, 1978; 3 = Vogelsberg; J. H. Jungbluth; 131 Arten, 1975 (publiziert, s. Literatur); 4 = Odenwald; H. Ritter; 170 Arten, 1974; 5 = Heidelberg; M. Kirchesch; 151 Arten, 1976.

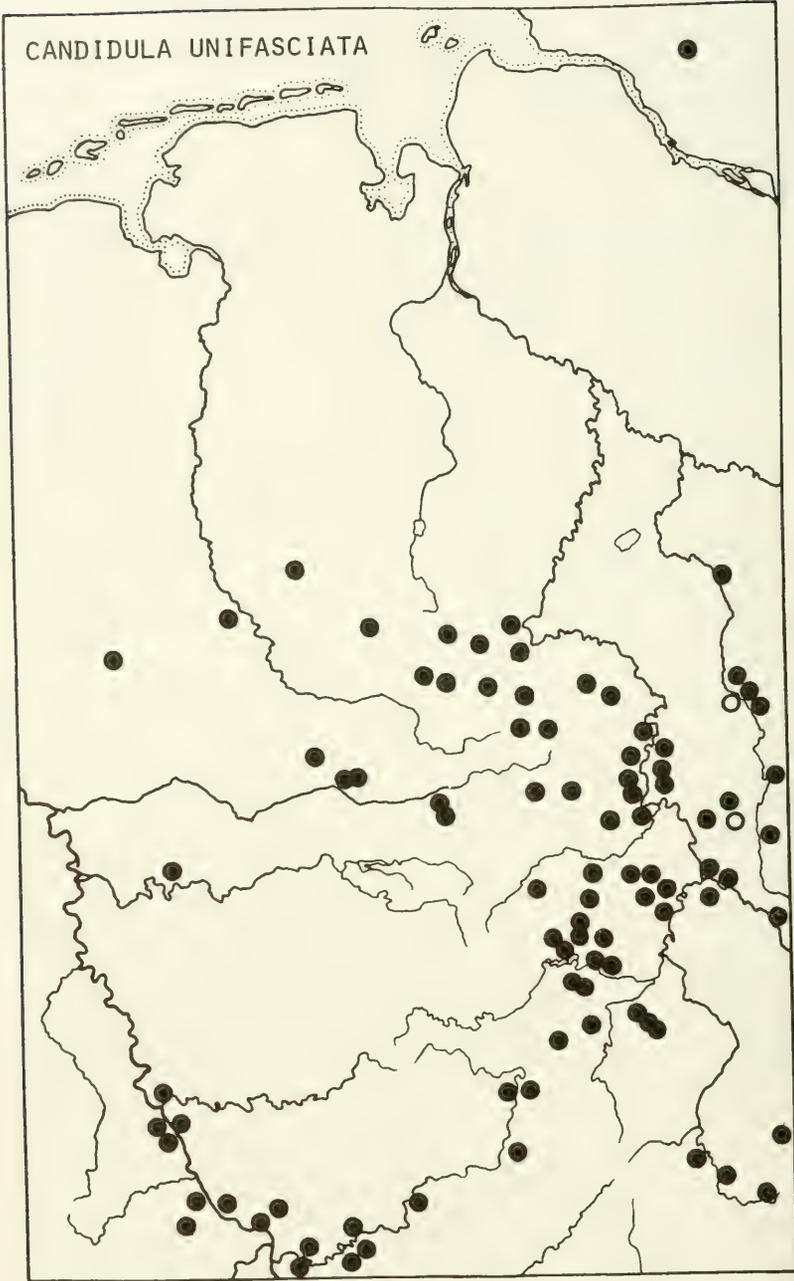


FIG. 8. Verbreitung von *Candidula unifasciata* (Poiret, 1801) in Nordwestdeutschland, dargestellt als Punktverbreitungskarte ohne Gitternetz (aus Ant, 1963b). Diese Art der Darstellung ist heute überholt und sollte nicht mehr verwendet werden.

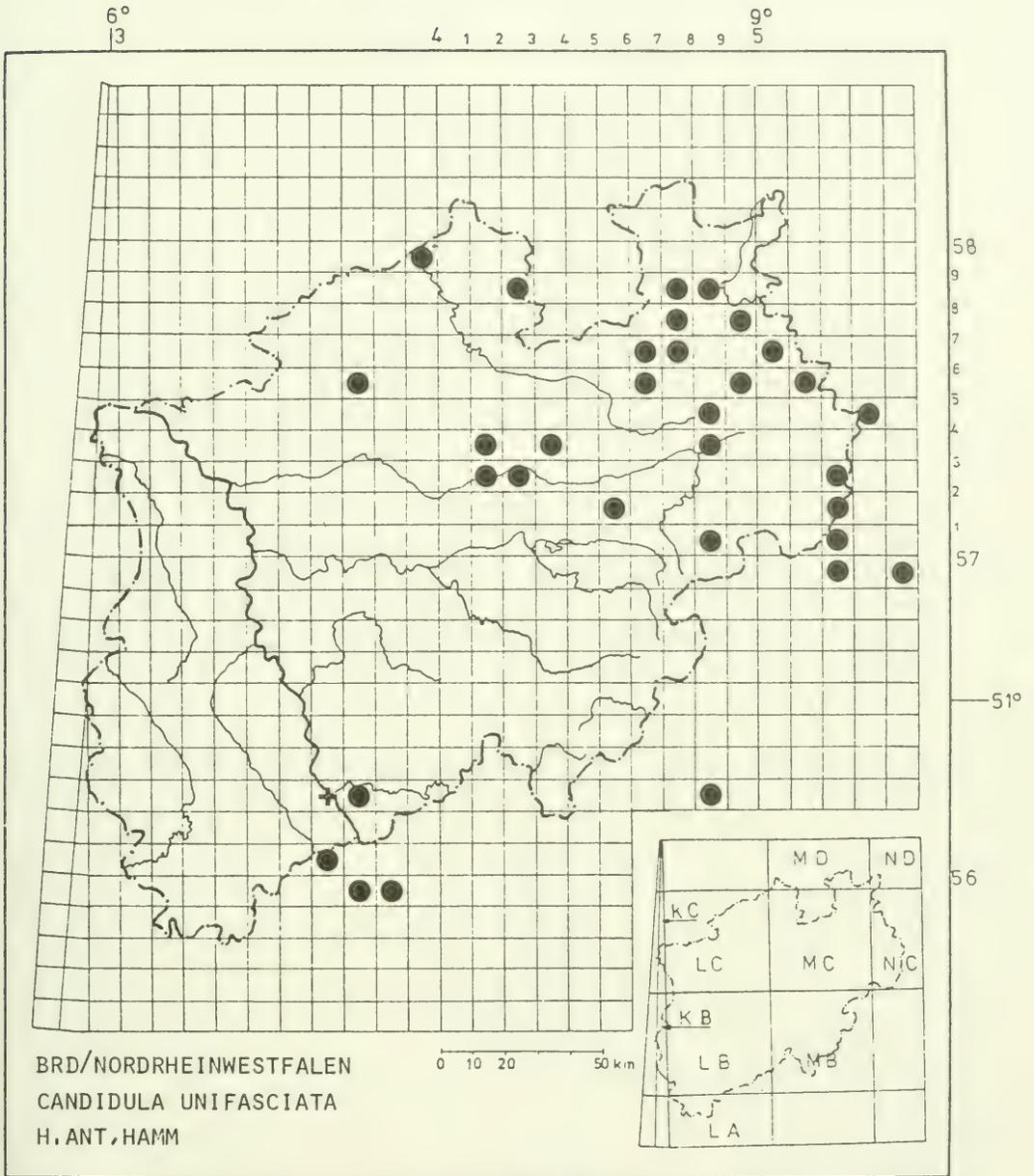


FIG. 9. Verbreitung von *Candidula unifasciata* (Poiret, 1801) in Nordrheinwestfalen. (10 × 10 km; Punkt: rezentes Vorkommen, Kreuz: Genistfund).

ZUR INTEGRATION CHOROLOGISCHER UND ÖKOLOGISCHER BEFUNDE
DER MALAKOZOLOGIE IN DIE ÖKOLOGISCHE
LANDSCHAFTSFORSCHUNG

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ABSTRACT

Until recently ecological studies of the landscape have only been carried out by geographers; zoological data have hardly been taken into consideration. The following remarks, based on zoogeographical and ecological results from malacological work in Hessen (West Germany), partially using the UTM grid, are the first zoological contributions to this branch of science. An ecological study of the landscape should try to record all facts of importance, both biotic and abiotic, in order to characterize a certain type of landscape. This may be done by an elementary analysis, deductive (cf. the division of natural regions in West Germany) as well as inductive (cf. the nature-conditioned landscapes in East Germany). According to the size of the areas under discussion, it is possible to use chorological results for the larger ones, and ecological results for smaller areas, the so-called topes in a topological dimension. Freshwater molluscs characterize their biotopes (habitats) by special coenoses and in addition they indicate the quality of the water. Therefore they may be used to classify the natural region where such waters occur. The ecology of land gastropods shows that the species belong to certain coenoses and biotopes (habitats). The most important factors here are geological formation, soil, humidity, temperature, etc. With regard to the above it is possible to find the same species and coenoses in topes of equal abiotic and biotic arrangement in a topological dimension.

Die ökologische Landschaftsforschung hat sich die Erfassung der Landschaft in ihrer Gesamtheit (Alexander von Humboldt: "Charakter einer Erdgegend," vgl. Schmithüsen, 1976) zum Ziel gesetzt. Zur Verwirklichung dieses Anspruches in ganzheitlicher Sicht bedient sie sich der Elementaranalyse und der Komplexanalyse (Neef, 1965). Die unterschiedlichen Wege, dieses Ziel zu erreichen, fanden in der "naturräumlichen Gliederung" der BRD (Schmithüsen, 1953 u.a.) als deduktivem und in den "naturbedingten Landschaften" der DDR (Schultze, 1955 u.a.) als induktivem Ergebnis ihren Niederschlag, um nur zwei Beispiele zu nennen. Beide Konzeptionen sind als methodisch einander ergänzend und nicht als konträr anzusehen. In der topologischen Dimension finden sie heute mit verfeinerten Feld- und Laboranalysen in den Untersuchungen zur "naturräumlichen Ordnung" (Haase, 1964; Richter, 1967) ihre Fortsetzung. In dieser räumlichen Dimension werden die Daten zur Charakterisierung und Bilanzierung der kleinsten Einheiten, der Tope, erhoben. Nach ihren stabilen bzw. labilen Merkmalen oder ihrer "ökologischen Varianz" abgegrenzt, werden diese Räume nach dem sie prägenden Geofaktor bezeichnet. Wir sprechen von Morpho-, Pedo-, Klima- und Hydrotopen bzw. Phyto- und Zootopen (Fig. 1). Der Physiotope (site) umfasst alle Daten der abiotischen Kategorie und gilt als Zentralbegriff der komplexen physischen Geographie (Neef, Schmidt & Lauckner, 1961). Seine biotische Entsprechung sind Flora und Fauna (cover) als die Summe der Geofaktoren der vitalen/biotischen Kategorie. Die Integration der Biota (Phytotop + Zootop) und Abiota (d.h. der zum Physiotope vereinigten abiotischen Tope) spiegelt über den Ökotope hinaus auch das Ökosystem wieder.

Trotz der engen Verknüpfung der Biota mit der ökologischen Landschaftsforschung seit Alexander von Humboldt und ihrer bereits früh erkannten Zeigereigenschaften wurde zumeist nur die Flora berücksichtigt, was zweifellos auf methodische Probleme zurückzuführen ist. Klink (1966) und Haase (1967) haben auf die vordringliche Berücksichtigung und Untersuchung

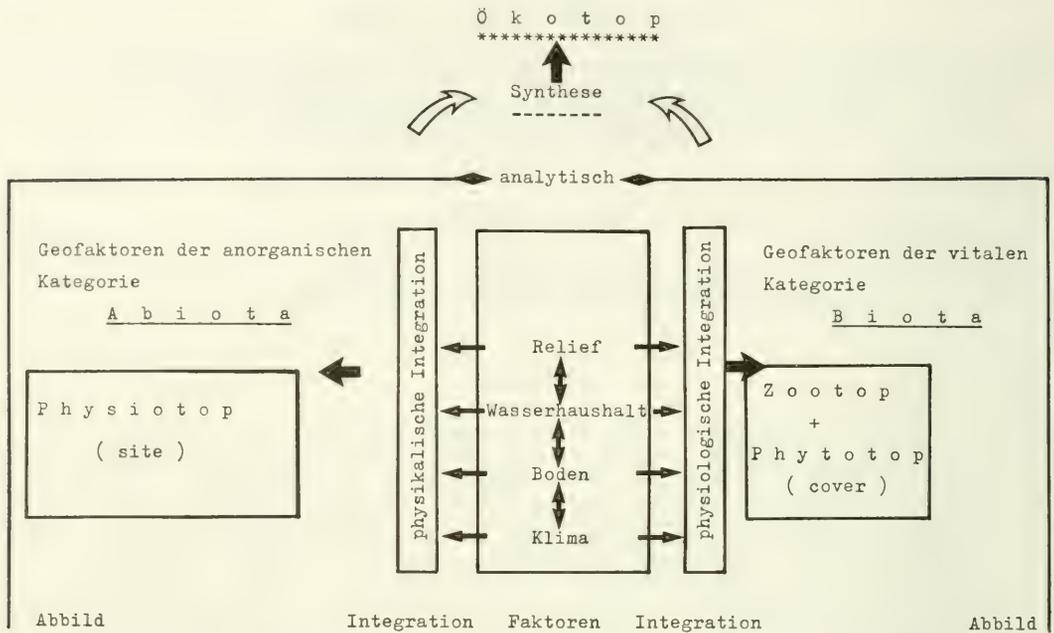


FIG. 1. Verknüpfungen und Betrachtungsweisen innerhalb der topologischen Dimension (in Anlehnung an Neef und Schultze).

der Tierwelt (und hier insbesondere auf die der ortsteten Kleinlebewesen) hingewiesen. Durch in der topologischen Dimension erhobene Befunde wird eine Beurteilung enger Korrelationen zwischen Umwelt/Organismus und Umwelt/Coenose möglich, die zur Erhellung kausaler Zusammenhänge innerhalb des Geokomplexes (funktionell: Ökosystem) beiträgt. Für eine entsprechende Berücksichtigung der Fauna in der ökologischen Landschaftsforschung erscheint ein multipler Ansatz notwendig:

(I) zur Integration in die bereits vorliegenden Landschaftsbearbeitungen der naturräumlichen Gliederung in der BRD bieten sich besonders chorologische (biogeographische) Methoden und damit grossräumig gewonnene Ergebnisse an;

(II) in den Rahmen der Gesamtbilanzierung und -charakterisierung kleinster Räume (Tope) in der topologischen Dimension scheinen sich mit tierökologischen Methoden erzielte Daten am ehesten einzufügen.

Eine Synthese beider Ansätze wird über die Eigenschaft der Biota als Zeigerarten und -gesellschaften,—d.h. als Bioindikatoren—, zur Beurteilung von Raum- bzw. Standortqualitäten führen. Auf diesem Wege wäre über die Integration der Daten aller Tope der anorganischen (abiotischen) und vitalen (biotischen) Kategorien eine Charakterisierung und Bilanzierung und damit die Erfassung der Landschaft in Sinne Alexander von Humboldt's möglich.

Direkte Beiträge zur ökologischen Landschaftsforschung liegen im Augenblick nur ansatzweise vor, da zumeist das zoologische Objekt im Vordergrund stand. Am ehesten ist hier noch die Arbeit von Mörzer Bruijns, Van Regteren Altena & Butot (1959) zu nennen oder auch die von Koepcke (1961). Weitere Untersuchungen beschäftigen sich mit verschiedenen Tiergruppen in landschaftsökologisch abgegrenzten Räumen, jedoch mehr aus chorologischer Sicht.

MATERIAL UND METHODE

Für die Prüfung einer möglichen Integration chorologischer Daten aus dem Bereich der Malakozologie wurden verschiedene Gebiete in der BRD (Vogelsberg, Odenwald, Hessen) nach

der Methode des European Invertebrate Survey auf UTM-Gitternetz-Karten mit unterschiedlichen Quadratgrößen (1 × 1 km; 2,5 × 2,5 km; 10 × 10 km) bearbeitet. Dabei wurden alle erreichbaren Sammlungsdaten und Literaturangaben für Land- und Wassermollusken erfasst; die Sammlungsdaten wurden nach Zeiträumen (vor und nach 1960) aufgeschlüsselt in den Karten markiert. Für den Vogelsberg wurden z.B. für 131 Molluskenarten insgesamt 1.281 Angaben kartiert (2,5 × 2,5 km) und für Hessen von 204 Arten nahezu 19.000 (10 × 10 km). Obwohl nur für einige Arten flächendeckende Kartierungen vorliegen, ermöglichen die gesammelten Daten erste Aussagen über das malakozoologische Inventar verschiedener Naturräume.

Unter ganz anderem Aspekt sind in dieser Beziehung die ökologischen Ergebnisse zu sehen und zu werten. Diese wurden nach den herkömmlichen Methoden (siehe z.B. Jungbluth, 1976) kleinräumig für einzelne Arten (autökologisch) oder für einzelne Gesellschaften (synökologisch) ermittelt. Eine Integration in die ökologische Landschaftsforschung scheint hier in enger Beziehung mit pflanzensoziologischen Befunden am sinnvollsten möglich zu sein; allerdings fehlen hierzu noch weitere Daten bzw. eine entsprechende Kooperation. Am Rande ist darauf hinzuweisen, dass für den Bereich der Zoologie bislang kaum synökologische Kartierungen vorliegen. Ökologische Untersuchungen wurden an Land- und Wassermollusken im Vogelsberg und im Odenwald durchgeführt.

ERGEBNISSE

I. Tiergeographische Ergebnisse

Bei der Auswertung der Verbreitungsmuster und deren Bedeutung für eine landschaftsökologische Berücksichtigung müssen die Wasser- und Landmollusken getrennt betrachtet werden, da die Ausbreitung und die Arealie der zuerst genannten Gruppe durch das Gewässernetz vorgegeben sind. Die Wassermollusken sind über ihre autozoische Dimension Bestandteil der Naturräume, in denen die entsprechenden Wohngewässer vorhanden sind. Trotzdem können sie mit ihrem Areal Naturräume charakterisieren und von angrenzenden, in denen die von der Art bevorzugten Gewässertypen fehlen, abheben. Für *Bythinella dunkeri compressa* haben wir dies an anderer Stelle bereits dargelegt (Jungbluth, 1976). *Bithynia leachii* gilt als palaearktisch sehr lückenhaft verbreitet und bewohnt gewöhnlich pflanzenreiche Tümpel und Gräben. In unserem Untersuchungsgebiet ist ihr Vorkommen auf die Naturräume des Rhein-Main-Tieflandes (Fig. 2) begrenzt und strahlt lediglich noch in das Rheintal ein. *Galba glabra*, eine Art mit sehr ähnlichen Biotopansprüchen, weist eine vergleichbare Bindung an die genannten Naturräume auf und erreicht hier in flächenhafter Verbreitung ihre südliche Arealgrenze in Deutschland (Fig. 2). Bei den Landschnecken gelten Feuchtigkeit, Temperatur und Substrateigenschaften als verbreitungslimitierende Faktoren. Diese können in den naturräumlichen Einheiten punkthaft ein Milieu bedingen, das die Voraussetzungen für das Vorkommen einzelner Arten ist, ohne dass hier ein Bezug zum grösseren Naturraum evident wird. An dieser Stelle führen die kleinräumig ermittelten ökologischen Daten zur weiteren Klärung: so konnte für *Pomatias elegans*, einer calciphilen Art, in der Verbreitung eine gute Übereinstimmung mit entsprechenden Pflanzengesellschaften und über diese mit den zugehörigen Bodentypen (Pedotopen) gefunden werden. Im Untersuchungsgebiet fällt die Hauptverbreitung dieser Art mit der naturräumlichen Einheit Bergstrasse zusammen, die durch Lössauflagen am westlichen Odenwaldhang und das Gunstklima des nördlichen Oberrheintieflandes gekennzeichnet ist und so grossräumig die Bedingungen für das Auftreten dieser substratgebundenen Art schafft. Die chorologischen Ergebnisse für hygrophile Arten oder aber auch südliche und östliche Arten lassen ähnliche Übereinstimmungen mit naturräumlichen Einheiten im Untersuchungsgebiet erkennen (Jungbluth, 1976).

II. Tierökologische Ergebnisse

Die Landschnecken erscheinen von der Anlehnung ihrer Coenosen an die Vegetationsformationen her gut geeignet, um als zoologische Komponente in der ökologischen Landschaftsforschung Berücksichtigung finden zu können; wie bereits erwähnt wurden jedoch noch keine speziellen Untersuchungen unter diesem Aspekt durchgeführt. In der topologischen Dimension ergeben sich

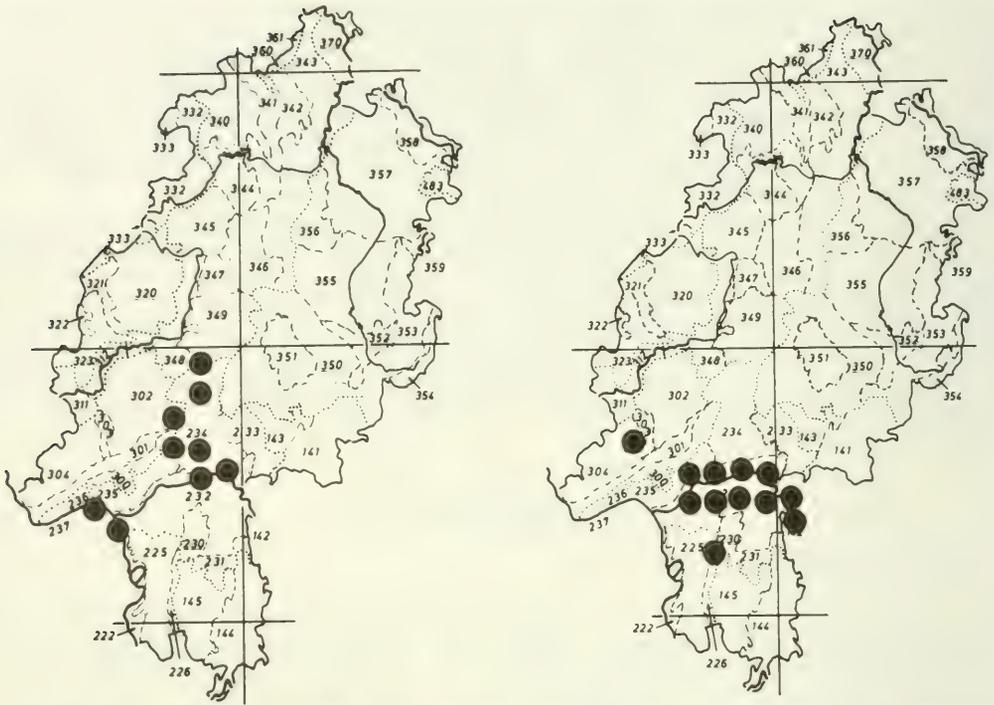


FIG. 2. Die Verbreitung von *Bythinia leachii* (links) und *Galba glabra* (rechts) in den Naturräumen (nach Ordnungsstufen nummeriert) von Hessen.

hier gute Übereinstimmungen zwischen Tier- und Pflanzengesellschaft sowie dem Physiotope in der Ausdehnung. Die Untersuchung der *Helicella itala-Zebrina detrita*-Coenose am Weinberg, einem Kalkhang im südlichen Vogelsberg, sei hierfür beispielhaft genannt (Fischer, 1972). Der Hang bei Kressenbach kann wegen seiner gleichartigen morphographischen Eigenschaften als Morphotop aufgefasst werden, der zwei verschiedene Pflanzenassoziationen aufweist. Die Zerteilung der Phytocoenose spiegelt sich auch in den Schneckengesellschaften wieder. Der grösste Teil des südexponierten Hanges ist von Mesobrometum bestanden, seitlich schliesst sich ein an Rosengewächsen und Hasel reich bestandenes Gebüsch an. In diesem waren vier Waldschnecken nachzuweisen, während sonst subthermophile und thermophile Arten den Hang besiedelten. Die Waldarten *Cochlodina laminata* und *Laciniaria biplicata* waren auch an schattigen Stellen der sonst offenen Fläche des Mesobrometums zu finden während die Leitarten der Coenose *Helicella itala* und *Zebrina* nicht in den Bereich der Hecke eindrangen. Schneckengesellschaft und Vegetationsformation wiesen in ihrer Bindung an den Morphotop des südexponierten Kalkhanges gute Übereinstimmung auf. Ähnliche Beobachtungen konnten auch bei Untersuchungen einer Schneckengesellschaft des Arrhenaterions an einem südlichen Hang des West-Odenwaldes bzw. eines feucht-kühlen Bruchwaldes auf Pseudogley im nördlichen Hohen Vogelsberg gemacht werden.

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DIE SUKZESSION DER SCHNECKENZÖNOSEN IN DEN WÄLDERN DES ALFÖLD UND DIE METHODEN ZUM STUDIUM DER SUKZESSION

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ABSTRACT

The role of river-transported land snails in primary stocking of flooded shores was studied in the lowlands of Hungary. Eight Recent and fossil samples studied (Table 1) show differences on the basis of the order of succession of the species carried (Table 2) and on the basis of accidental elements. The composition of the floating fauna carried is determined, as already expected, by the climatic potentialities of the country adjoining the rivers. The difference in composition of the Recent and Pleistocene alluvial samples may be attributed to different climatic conditions. The composition of the alluvial fauna may therefore also be used in climate reconstruction analysis (Table 3). The frequency distribution of the snails of the Tisza Salicetum is close to the order of succession of the river-carried fauna of the Tisza river. The living snails carried to the banks of the river downstream may become adapted to local conditions and through organogenic succession may become naturalized elements or accidental elements in other types of vegetation (Fig. 1) in accordance with local environmental conditions.

EINLEITUNG

Im Laufe meiner sich auf rund 20 Jahre erstreckenden Studien der Schnecken des Ungarischen Alföld (Tiefebene) habe ich in den Waldassoziationen des Alföld das Ineinanderübergehen der Schneckenzönosen, d.h. den Prozess ihrer Sukzession, untersucht und dabei die Sukzession der Schneckenbestände parallel mit der Vegetationsukzession, vornehmlich in Naturschutzgebieten bzw. in den noch vorhandenen Wäldern mit natürlicher Erneuerung, studiert. In der vorliegenden Studie wird untersucht, welche Rolle den vom Flusswasser getragenen Schnecken in der primären Besiedlung der Wälder entlang den Flussläufen zukommt.

UNTERSUCHUNGSMETHODIK, ZIELSETZUNG

Anhand von Probenentnahmen nach der Quadrat-Methode habe ich annähernd 400, insgesamt 13 Waldtypen angehörende, Waldassoziationen untersucht (Bába, 1977). Die untersuchten Waldtypen gehören 3 Vegetationsukzessionsreihen an. Studiert habe ich die Verteilung der Zahl der festgestellten Arten in den Wäldern der 3 Vegetationsukzessionsreihen.

Um zu einer Entscheidung hinsichtlich der faunentransportierenden Rolle des Flusswassers zu kommen, habe ich 24 den Forderungen der zufälligen Probenentnahme entsprechende Flussgeschiebepben analysiert. Neben den Flüssen des Alföld habe ich vergleichsweise auch Geschiebepben von einem Bach im Ungarischen Mittelgebirge, sowie auch aus dem Oberen und Unteren Pleistozän stammende Flussgeschiebepben mituntersucht. In den rezenten und den Pleistozän-Proben kamen 36,290 Individuen von insgesamt 108 Arten vor (Tabelle 1). Weitere 9, für die Geschiebefauna neue Arten kamen im Laufe der Einsammlungen von Bába (Vásárhelyi, 1962 in Bába et al., 1962) entlang der Theiss zum Vorschein. Diese sind: *Acme similis* Reinhardt, *Cecilioides petitiiana* (Benoit) (Horváth, 1962), *Arion subfuscus* (Drap.) (Bába), *Oxychilus glaber striarius* (West.), *O. orientalis* (Cless.) (Vásárhelyi, 1958 in Bereczk et al., 1958), *Deroceras agreste* (L.), *D. laeve* (O. F. Müller) (Bába), *Trichia villosula* (Rm.), *T. unidentata* (Drap.), *Hygromia transsylvanica* (West.) (Vásárhelyi, 1958 in Bereczk et al., 1958), *Helix lutescens* Rm. (Bába).

Dies bedeutet einen Anstieg der aus Geschiebepben zum Vorschein gekommenen Arten bis auf 117. Die aus dem Flussbett herausgespülten fossilen Schalen und ausgeblichenen

subfossilen Schalen wurden bei der Analyse der rezenten Flussgeschiebeprouben unberücksichtigt gelassen. Unter Zugrundelegung der Dominanzwerte der in den einzelnen Proben vorkommenden Arten habe ich eine Rangordnungsskala betreffs der Tragehäufigkeit der Arten aufgestellt. Eine Rangliste habe ich auch aufgrund der Schneckenarten der das erste Stadium der organogenen Sukzession bildenden (Salicetum) Weidenbestände angefertigt. Die Geschiebe- bzw. Tragungsausdehnung der von den verschiedenen Flüssen transportierten gleichen Arten habe ich aufgrund der Häufigkeitskonfidenzintervall-Berechnung verglichen.

Bei der Analyse der Flussgeschiebeprouben strebte ich nicht eine faunistische Analyse an, wie die früheren Autoren (Czógler & Rotarides, 1938), sondern trachtete zu klären, ob die Flusswasser-getragene Fauna gemeinsame qualitative und quantitative Züge aufweist, ob hinsichtlich der einzelnen Flussabschnitte und ihres Charakters Abweichungen im Transport bestehen und ob zeitliche Gesichtspunkte des Transports beantwortet werden können. Die Möglichkeit hierzu war gegeben, da die Proben aus den Jahren 1922, 1958 und 1975 stammen.

Die Fundorte der Geschiebeprouben waren: 1, Bükk-Gebirge, Szalajka-Bach, VII. 1963, leg. A. Horváth; 2-3, Dorogpuszta, Dorogháza-Ujtelep, VI. 1976; 4-6, Zagyva, Pásztó, III-IV. 1975, IV. 1976, leg. A. Varga; 7-12, Theiss (von Norden nach Süden): Tiszavid, VII. 1966, Vásárosnamény-Bagiszeg, X. 1958, Kisköre, VII. 1975, Hódmezővásárhely-Szeged, 1922; 11-12, Szeged, 1938, 1975, leg. G. Kolosváry, A. Horváth, K. Bába, M. Rotarides, K. Czógler, und K. Bába; 13, Marosch, 2 km oberhalb der Flussmündung, VII. 1975, leg. K. Bába; 14-19, Donau: Esztergomsziget, Pilismarót, Esztergom: Wasserwerk-Ferenceskert-Kenyérmező, V-VII. 1965, leg. L. Pintér; 20, Szabadhidvég: Unteres Pleistozän; 21-23, Kőröshegy: Obere Phase des Unteren Pleistozän; 24, Hódmezővásárhely: Oberes Pleistozän, leg. E. Krolopp.

Den Herren Kollegen Krolopp, Pintér und Varga entbiete ich für die Überlassung der Proben auf diesem Wege meinen Dank.

Die Artenliste der Geschiebeprouben enthält Tabelle 1, vereinfacht auf Fundorte bezogen. Bei den Proben ist nur das Vorkommen vermerkt. Die Proben der gleichen Flüsse sind durch Addieren vereinfacht; die Ziffern bedeuten die Häufigkeit des Vorkommens.

DIE VERTEILUNG DER IN DEN WALDSUKZESSIONEN GEFUNDENEN ARTEN

49% der im Alföld gefundenen 72 Arten (Bába, 1977) sind waldbewohnende, feuchtigkeitsliebende Elemente.

Solche mit höheren Feuchtigkeitsansprüchen sind: *Acicula polita*, *Pomatias rivulare*, *Aegopinella pura*, *Oxychilus glaber*, *O. inopinatus*, *Arion circumscriptus*, *Limax cinereoniger*, *Cochlodina laminata*, *Clausilia pumila*, *Laciniaria plicata*, *L. biplicata*, *Ruthenica filigrana*, *Discus rotundatus*, *Perforatella bidentata*, *P. dibothrion*, *P. incarnata*, *P. vicina*, *Helicigona banatica*, *H. arbustorum*, *Trichia hispida* und *T. unidentata*.

Über niedrige Feuchtigkeitsansprüche verfügen: *Columella edentula*, *Punctum pygmaeum*, *Arion subfuscus*, *Vitrea crystallina*, *Nesovitrea hammonis*, *Limax nyctelius*, *Aegopinella minor*, *Bradybaena fruticum*, *Hygromia kovacsii* und *Euomphalia strigella*.

Auffallend ist, dass diese Arten in den mit fließenden Wässern reichlicher versehenen, waldigen Anteilen anzutreffen sind. Die Zahl der Arten mit höhern Feuchtigkeitsansprüchen wird mit zunehmender Entfernung von den Flüssen geringer. Auch die Gesamtartenzahl lässt nach, und zwar je nach den Vegetationssukzessionsreihen in unterschiedlicher Weise. Die Verteilung der Gesamtartenzahl in den 3 Vegetationssukzessionsreihen veranschaulicht Fig. 1. Es ist festzustellen, dass die grössten Artenzahlen nahe den fließenden Gewässern in der organogenen Sukzessionsreihe erscheinen.

Die Mehrzahl der Arten mit höheren Feuchtigkeitsansprüchen sind in niedriger Individuenzahl vorkommende, akzidentelle Elemente (Vorkommenshäufigkeit 0-10%); andere werden in den Inundationswäldern zu konstant-dominanten Elementen. Die Erklärung der Erscheinung: der Reichtum der Schneckenfauna des Ungarischen Alföld an waldbewohnenden Arten ist das Resultat der ständigen faunentransportierenden Wirkung der fließenden Gewässer, was für eine Bevölkerung der Malakofauna des Alföld von den Gebirgsgegenden her spricht. Dies beweist der Umstand, dass, mit der Entfernung von den Flüssen, in den Waldtypen der Waldsukzessionsreihen Waldbewohner selten sind. Ebenfalls gering ist die Zahl der waldbewohnenden Schnecken in dem an Wasserläufen armen südlichen Alföld und im nördlichen Teil des Alföld

petitiانا, *Trichia sericea*, *Helicigona faustina*, *H. banatica* (aus der Theiss und auch aus der Marosch kamen *Aghardia parreysi*, *A. bielzi* und *A. truncatella* zum Vorschein).

Donau: *Vertigo alpestris*, *Trichia striolata danubialis*, *T. unidentata*, *T. hispida*, *Helicigona arbustorum* und *Cepaea hortensis* (die beiden *Orcula*- und die *Zebrina*-Art werden ausser von der Donau auch von der Zaggyva und der Marosch transportiert).

Die Zahl der akzidentellen Arten (30%) der Theiss liegt höher als die in der Donau anzutreffende (21%), was auf den Unterschied zwischen den Wassersammelgebieten hindeutet (Tabelle 2).

In quantitativer Hinsicht noch interessanter ist es, wenn man die in den gesamten Proben vorkommenden Arten untersucht. Es waren 23 Arten auffindbar, die in den Geschiebeproben der Theiss, der Donau und des Oberen Pleistozän gleichermaßen vorkommen (Tabelle 3).

Von den Werten der Trageausdehnung der Theiss und der Donau gleichermaßen verschieden sind jene des Tragebereichs im Pleistozän. Von den Arten waren die *Granaria*-, 2 *Vallonia*-, die *Succinea*-, *Chondrula*- und *Trichia*-Arten nach der Ansicht von Ložek die Schnecken der Lös-Steppen. All dies deutet darauf hin, dass die quantitative Zusammensetzung der Geschiebefauna sich dem durchschnittlichen Klimazustand der Epoche anzupassen scheint. Dies bedeutet auch, dass die Flüsse auf ihren Flussabschnitt im Alföld *ab ovo* in einem solchen prozentuellen Verhältnis Schneckenarten tragen inklusive auch die selteneren Waldbewohner, in welchem prozentuellen Verhältnis sie vom Wasser aus den Bergen heruntergespült werden. Bei den klimempfindlichen Schnecken hängt dieses Verhältnis vom Klimazustand der Wassersammelgebiete ab.

Das in Mittel-Europa herrschende, teils kontinentale, Klima schafft auf weiten Gebieten gleiche Bedingungen. Daher stehen das Tragungsausmass der von der Theiss und der Donau transportierten Schnecken einander näher. Die Wassersammelgebiete der einzelnen Flüsse sind aber meso- und mikroklimatisch verschieden, daher kommt es, dass die einzelnen Flüsse sich in der Transporthäufigkeit der transportierten Arten, d.h. in ihrer Rangordnung, voneinander unterscheiden. An der Rangordnung-Skala an Tabelle 2 sind die ersten 10 Arten eingetragen. Die Ziffern in den einzelnen Spalten geben auf den Fluss bezogen die Rangordnungsreihenfolge des Tragens an. Der Unterschied zwischen den Flüssen betrifft die Trage-Rangordnung der Schnecken entspricht den mesoklimatischen Unterschieden der Wassersammelgebiete. Die an der Rangordnung-Skala der Geschiebeschnecken der Donau eingetragenen Arten zeigen aufgrund ihrer klimatischen Ansprüche den kühleren, feuchteren Charakter der Alpen, während die in der Rangordnung-Skala der Theiss verzeichneten Arten einen warmen, feuchten Charakter vertreten. Ebenso differieren auch die Pleistozän-Proben. So tragen von den Proben aus dem Unteren Pleistozän die ersten einen glazialen, die zweiten und die aus dem Oberen Pleistozän stammenden aber interglazialen Charakter zur Schau. Aufgrund der Rangordnung-Skala wird eine Rekonstruktion der Klimaabweichungen möglich.

Die letzte Spalte in Tabelle 2 zeigt die Rangordnung-Skala der aus den Weidenbeständen entlang der Theiss zum Vorschein gekommenen konstant-dominanten Arten. Von beweisendem Wert ist der Vergleich mit der Rangordnung-Skala der Geschiebefauna der Theiss. Die Gegenüberstellung der Rangliste der vom Flusse getragenen Arten und der Arten aus den Wäldern entlang den Flussufern beweist hinsichtlich der ersten 6 Stellen die aktive Faunentransportierende Rolle des Flusses.

AUSWERTUNG DER BEFUNDE

Die Bevölkering der organogenen Vegetationssukzessionsreihen mit Schnecken geschieht folgendermassen. Das Wasser trägt die Schnecken lebend herunter. In den niedrig gelegenen, alljährlich mehrmals von Überschwemmungen heimgesuchten Weidenbeständen vermögen nur wenige Arten zu leben und sich zu vermehren, wie z.B. die *Arianta arbustorum* im Donau-Tal. In die höher gelegenen Orte, z.B. Auwälder, gelangt, gehen nur jene Arten nicht zugrunde, deren Toleranz die gegebene Umwelt entspricht. In den Auwäldern lassen sich diejenigen Arten dauerhaft nieder, die gemäss dem Charakter der lichtreichen Wälder auch mässig oligotherm sind. Aus den Auwäldern gelangen sie infolge der Vegetationssukzession in andere, von den Flüssen entfernt gelegene Waldtypen. Von den Buschweiden an ändern sich in den einzelnen Waldtypen auf immer höher gelegenen Terrains die Zusammensetzung und die quantitativen Verhältnisse der Schneckenfauna. Entsprechend den veränderten Umweltverhältnissen kommt es bereits in den Weidenbeständen (*Salicetum*) zur Adaptation der auf dem Wasserwege eintreffenden Schneckenarten. Daher stimmt die Rangordnungsliste der Weiden-

bestände nicht vollkommen mit jener der eintreffenden Geschiebefauna überein. Die Wahrscheinlichkeit der Ansiedlung und des Nachschubes sichern die zeitlich konstant wirksamen erneuten Überschwemmungen. Die im Flusslauf aufscheinenden regionalen quantitativ-qualitativen Abweichungen sind den regional verschiedenen Trage- bzw. Geschiebeausdehnungen zu verdanken. Aus diesem Umstand und den Transportunterschieden der Nebenflüsse erklärt sich zoogeographisch der in den verschiedenen Landschaften des Alföld zu beobachtende Unterschied in den Schneckenzöosen. Die Realität des Prozesses unterstützt auch, dass in den Auwäldern die meisten Waldbewohner akzessorische Elemente mit Konstanzwerten von 10-20% sind. In den verschiedenen Landschaftseinheiten können die Waldbewohner abweichende sein. In den Hainbuchen- und Maiglöckchen-Eichenwäldern, die von den Flüssen schon lange abgeschlossen sind, kommen wenig waldbewohnende Elemente vor.

Bemerkt sei, dass auch im Falle eines kontinuierlichen Zusammenhanges von Alföld- und Gebirgswäldern die Verbreitung einiger montaner Arten im Alföld zu beobachten ist, so z.B. die *Perforatella dibothrion* im Nordöstlichen Alföld. Heute ist diese Verbreitungsweise wegen des Aufhörens der zusammenhängenden Waldungen selten.

TABELLE 1. Die Artenliste der Geschiebeprobe bezüglich der einzelnen rezenten und Pleistozän-Flüsse (die Proben der Gleichen Flüsse durch Addition vereinfacht).

No.	Arten	Szalajka-Q	Zagyva-F.	Theiss-F.	Maros-F.	Donau-F.	Szabad-hídvég	Köröshegy	Hódmezővá-sárhely
1.	<i>Pomatias rivulare</i> Eichw.	-	-	1	-	-	-	-	-
2.	<i>Paladilhia oshanovae</i> Pintér	-	-	-	-	1	-	-	-
3.	<i>Acicula banatica</i> Rossm.	-	-	1	-	-	-	-	-
4.	<i>Carychium minimum</i> O. F. Müll.	-	4	3	+	5	+	2	+
5.	<i>Carychium tridentatum</i> Risso	+	5	3	+	2	-	-	-
6.	<i>Cochlicopa lubrica</i> O. F. Müll.	-	5	6	+	6	+	2	+
7.	<i>Cochlicopa lubricella</i> Porro	-	4	3	+	5	-	-	-
8.	<i>Columella edentula</i> Drap.	+	-	3	-	-	-	-	+
9.	<i>Truncatellina cylindrica</i> Fér.	-	5	3	+	5	+	1	+
10.	<i>Vertigo angustior</i> Jeffr.	-	-	2	+	3	+	2	+
11.	<i>Vertigo parcedentata</i> A. Braun	-	-	-	-	-	-	-	+
12.	<i>Vertigo genesii</i> Gredl.	-	-	-	-	-	-	-	+
13.	<i>Vertigo pusilla</i> O. F. Müll.	-	3	2	-	-	-	-	-
14.	<i>Vertigo antivertigo</i> Drap.	-	4	3	-	4	+	3	+
15.	<i>Vertigo moulinsiana</i> Dupuy	-	-	-	+	1	+	-	-
16.	<i>Vertigo pygmaea</i> Drap.	-	5	5	+	5	+	1	+
17.	<i>Vertigo alpestris</i> Alder	-	-	-	-	2	-	-	-
18.	<i>Vertigo substriata</i> Jeffr.	-	-	-	+	-	-	-	+
19.	<i>Argna bielzi</i> Rossm.	-	-	2	-	-	-	-	-
20.	<i>Argna parreyssi</i> Pfr.	-	-	3	-	-	-	-	-
21.	<i>Orcula jetschini</i> Kim.	-	-	2	-	-	-	-	-
22.	<i>Orcula doliolum</i> Brug.	-	2	2	-	1	-	-	-
23.	<i>Orcula doliu</i> Drap.	-	-	-	-	1	-	-	-
24.	<i>Granaria frumentum</i> Drap.	-	2	4	+	5	+	3	+
25.	<i>Gastrocopta moravica</i> Petrbo	-	-	-	-	-	+	-	-
26.	<i>Pupilla loessica</i> Ložek	-	-	2	-	-	-	-	-
27.	<i>Pupilla sterri</i> Voith	-	-	-	+	-	-	-	+
28.	<i>Pupilla muscorum densegyrate</i> Ložek	-	-	-	+	-	-	-	-
29.	<i>Pupilla muscorum</i> L.	+	5	5	+	6	+	3	+
30.	<i>Pupilla triplicata</i> Stud.	-	-	4	-	-	-	2	+
31.	<i>Vallonia pulchella</i> O. F. Müll.	+	5	5	+	6	+	3	+
32.	<i>Vallonia costata</i> O. F. Müll.	-	5	4	+	4	+	3	+
33.	<i>Vallonia tenuilabris</i> A. Braun	-	-	-	-	-	-	2	+
34.	<i>Acanthinula aculeata</i> O. F. Müll.	-	3	-	-	1	-	-	-
35.	<i>Chondrula tridens</i> O. F. Müll.	-	4	6	+	6	+	3	+
36.	<i>Chondrula tridens albolimbata</i> Pfr.	-	-	4	+	-	-	-	-
37.	<i>Mastus venerabilis</i> L. Pfr.	-	-	-	-	-	-	-	+
38.	<i>Ena obscura</i> O. F. Müll.	-	1	1	-	-	-	-	-
39.	<i>Zebrina detrita</i> O. F. Müll.	-	-	-	+	1	-	-	-
40.	<i>Cochlodina orthostoma</i> Menke	-	-	2	-	-	-	-	-
41.	<i>Cochlodina cerata</i> Rossm.	-	-	2	-	-	-	-	-
42.	<i>Cochlodina laminata</i> Montagu	+	1	4	-	1	-	-	-
43.	<i>Cochlodina commutata</i> Rossm.	-	-	1	-	-	-	-	-

TABELLE 1. (fortgesetzt)

No.	Arten	Szalajka-Q	Zagyva-F.	Theiss-F.	Maros-F.	Donau-F.	Szabad- hidvég	Kőröshegy	Hódmezővá- sárhely
44.	<i>Ruthenica filograna</i> Rossm.	-	-	2	-	-	-	3	-
45.	<i>Iphigena ventricosa</i> Drap.	-	2	3	-	-	-	-	-
46.	<i>Iphigena latestriata</i> A. Schm.	-	-	3	-	-	-	-	-
47.	<i>Iphigena tumida</i> Rossm.	-	-	1	-	-	-	-	-
48.	<i>Clausilia dubia</i> Drap.	-	-	2	-	-	-	-	-
49.	<i>Clausilia pumila</i> C. Pfr.	-	1	4	-	4	+	1	-
50.	<i>Laciniaria plicata</i> Drap.	-	3	5	+	-	-	1	-
51.	<i>Laciniaria biplicata</i> Montagu	-	2	2	-	1	-	-	-
52.	<i>Laciniaria vetusta</i> Rossm.	-	-	1	-	-	-	-	-
53.	<i>Pseudalinda gulo</i> E. A. Bielz	-	-	1	-	-	-	-	-
54.	<i>Alopiá bielzi</i> Pfr.	-	-	1	-	-	-	-	-
55.	<i>Clausilia</i> sp.	-	-	-	-	-	-	3	+
56.	<i>Succinea oblonga</i> Drap.	+	4	3	-	5	+	3	+
57.	<i>Succinea elegans</i> Risso	-	2	2	+	3	-	2	-
58.	<i>Succinea putris</i> L.	-	3	3	-	3	-	-	-
59.	<i>Cecilioides acicula</i> O. F. Müll.	-	5	1	+	6	-	-	-
60.	<i>Punctum pygmaeum</i> Drap.	+	4	1	+	4	+	3	+
61.	<i>Helicodiscus singleyanus</i> Pilsb.	-	2	-	-	-	-	-	-
62.	<i>Discus ruderatus</i> Hartm.	-	-	-	-	-	-	1	+
63.	<i>Discus rotundatus</i> O. F. Müll.	-	-	-	-	-	-	1	-
64.	<i>Discus perspectivus</i> Mühlf.	-	1	1	-	-	-	-	-
65.	<i>Vitrea pellucida</i> O. F. Müll.	-	4	-	-	1	-	-	-
66.	<i>Zonitoides nitidus</i> O. F. Müll.	+	5	6	+	5	-	-	+
67.	<i>Vitrea diaphana</i> Studer	-	2	2	-	-	-	-	-
68.	<i>Vitrea subrimata</i> Reinh.	-	-	-	-	-	-	-	-
69.	<i>Vitrea crystallina</i> O. F. Müll.	-	-	5	+	4	-	2	+
70.	<i>Vitrea contracta</i> West.	-	5	-	-	1	-	-	-
71.	<i>Vitrea transsylvanica</i> Cless.	-	-	3	-	-	-	-	-
72.	<i>Aegopinella pura</i> Alder	-	-	-	+	-	-	-	-
73.	<i>Aegopinella minor</i> Stabile	-	2	1	-	2	-	-	-
74.	<i>Nesovitrea hammonis</i> Ström	+	1	1	+	-	-	2	+
75.	<i>Oxychilus cellarius</i> A. J. Wagner	-	-	1	-	-	-	-	-
76.	<i>Oxychilus draparnaudi</i> Beck	-	-	1	-	-	-	-	-
77.	<i>Oxychilus glaber striarius</i> West.	-	1	-	-	-	-	-	-
78.	<i>Oxychilus inopinatus</i> Ulicný	-	5	2	-	-	-	-	-
79.	<i>Daudebardia rufa</i> Drap.	-	1	-	-	-	-	-	-
80.	<i>Limax</i> sp.	-	-	-	-	-	+	2	-
81.	<i>Deroceras laeve</i> O. F. Müll.	-	-	-	-	1	-	-	-
82.	<i>Deroceras agreste</i> L.	-	-	2	-	-	-	-	-
83.	<i>Euconulus fulvus</i> O. F. Müll.	-	2	1	+	3	-	2	+
84.	<i>Bradybaena fruticum</i> O. F. Müll.	-	2	4	-	4	+	2	-
85.	<i>Helicella obvia</i> Hartm.	-	2	3	-	6	-	-	-
86.	<i>Helicopsis striata</i> O. F. Müll.	-	-	-	-	1	+	3	+
87.	<i>Helicopsis cereoflava</i> Rossm.	-	-	2	+	-	-	-	-
88.	<i>Monacha cartusiana</i> O. F. Müll.	-	5	4	+	5	-	-	-
89.	<i>Perforatella bidentata</i> Gm.	-	-	5	-	-	+	3	+
90.	<i>Perforatella dibothrion</i> M. Kim.	-	-	1	-	-	-	-	-
91.	<i>Perforatella rubiginosa</i> A. Schmidt	+	5	6	+	5	-	-	+
92.	<i>Perforatella incarnata</i> O. F. Müll.	-	3	2	-	4	-	-	-
93.	<i>Perforatella vicina</i> Rossm.	-	-	4	-	-	-	-	-
94.	<i>Trichia bakowskii</i> Polinski	-	-	1	-	-	-	-	-
95.	<i>Trichia unidentata</i> Drap.	-	-	-	-	2	-	-	-
96.	<i>Trichia striolata danubialis</i> Clessin	-	-	-	-	1	-	-	-
97.	<i>Trichia hispida</i> L.	+	5	2	+	6	-	3	+
98.	<i>Trichia sericea</i> Drap.	-	-	2	+	-	-	-	-
99.	<i>Trichia bielzi euconulus</i> Polinski	-	-	2	-	-	+	-	-
100.	<i>Trichia</i> sp.	-	-	-	-	-	+	-	-
101.	<i>Euomphalia strigella</i> Drap.	-	5	4	-	-	-	1	-
102.	<i>Helicigona banatica</i> Rossm.	-	-	3	-	-	-	-	-
103.	<i>Helicigona Faustina</i> Rossm.	-	-	2	-	-	-	-	-
104.	<i>Helicigona arbustorum</i> L.	-	-	3	+	3	-	-	+
105.	<i>Isognomostoma isognomostoma</i> Schröter	-	-	1	-	-	-	-	-
106.	<i>Cepaea vindobonensis</i> Fér.	-	2	4	-	4	+	3	-
107.	<i>Cepaea hortensis</i> O. F. Müll.	-	-	-	-	4	-	-	-
108.	<i>Helix pomatia</i> L.	-	5	2	-	3	-	1	-

TABELLE 3. Häufigkeits-Verhältniskonfidenz Intervalle (Geschiebe-Ausdehnungen).

No.	Arten	Recenten-Flüsse		F. der. O- Pleistocäne
		Theiss	Donau	Körösgegy
1.	<i>Carychium minimum</i>	- 0,33- 0,41	- 3,03- 4,19	- 2,74- 7,46
2.	<i>Cochlicopa lubrica</i>	- 8,37- 9,16	- 4,82- 6,23	- 0,73- 1,99
3.	<i>Cochlicopa lubricella</i>	- 1,05- 1,36	- 0,64- 1,24	-
4.	<i>Truncatellina cylindrica</i>	- 0,29- 0,47	- 2,46- 3,52	0,08- 2,02
5.	<i>Vertigo antivertigo</i>	- 0,31- 0,49	- 0,07- 0,36	- 0,36- 3,21
6.	<i>Vertigo pygmaea</i>	- 1,71- 2,09	- 4,09- 5,42	- 0,02- 2,35
7.	<i>Granaria frumentum</i>	- 1,65- 2,03	- 0,73- 1,38	-31,21-41,55
8.	<i>Pupilla muscorum</i>	- 4,77- 5,39	-13,11-15,29	- 0,17- 1,71
9.	<i>Vallonia pulchella</i>	-17,82-18,90	-38,92-41,98	-10,76-18,33
10.	<i>Vallonia costata</i>	- 3,13- 3,63	- 1,79- 4,18	-10,82-18,42
11.	<i>Chondrula tridens</i>	- 4,98- 8,42	- 1,46- 2,31	- 2,04- 6,34
12.	<i>Succinea oblonga</i>	- 0,20- 0,35	- 0,54- 1,08	- 1,73- 6,18
13.	<i>Punctum pygmaeum</i>	- 0,08- 0,19	- 0,61- 1,19	- 0,62- 1,22
14.	<i>Zonitoides nitidus</i>	- 6,71- 7,42	- 1,55- 2,41	- 1,47- 5,39
15.	<i>Vitrea crystallina</i>	- 2,39- 2,83	- 4,18- 5,52	0,21- 1,51
16.	<i>Bradybaena fruticum</i>	- 1,64- 2,01	- 0,07- 0,35	0,24- 1,42
17.	<i>Helicella obvia</i>	- 0,17- 0,30	- 1,62- 2,51	-
18.	<i>Perforatella bidentata</i>	- 1,62- 1,99	-	- 0,57- 3,66
19.	<i>Perforatella rubiginosa</i>	- 9,15- 9,97	- 3,04- 4,21	-
20.	<i>Perforatella incarnata</i>	- 0,41- 0,61	0,00- 0,19	-
21.	<i>Trichia hispida</i>	- 1,67- 2,05	- 0,86- 1,53	0,27- 1,19
22.	<i>Cepaea vindobonensis</i>	- 2,52- 2,98	- 0,19- 0,56	- 1,43- 5,30
23.	<i>Helix pomatia</i>	- 0,04- 0,11	0,02- 0,14	0,29- 0,93

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DISTRIBUTION OF ENVIRONMENTAL FACTORS AND FRESH-WATER SNAILS (GASTROPODA) IN NORWAY: USE OF EUROPEAN INVERTEBRATE SURVEY PRINCIPLES

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ABSTRACT

This article is a preliminary report on some of the new results arrived at in a study comprising the distribution, ecology, and morphology of the fresh-water snails of Norway, including aspects of regional limnology. Emphasis has been on subjects related to the European Invertebrate Survey (EIS). The base map for Norway comprises 189 modified 50 km squares. This map is used for both national and international projects. EIS-principles have been used for mapping environmental factors and distribution of species and population densities.

Mapping environmental factors. Maps have been made showing (1) elevation above sea level, (2) geology, (3) vegetation in the surroundings of habitats, (4) macrovegetation along the shores of lakes, (5) substratum, (6) wave exposure in lakes, (7) hydrogen-ion concentration (pH), (8) calcium concentration, (9) water colour, and (10) water temperature. A selection of maps is presented. Primary data: 1,498 fresh-water habitats in Norway (mostly lakes) investigated 1953-1973 during 819 days of field work.

Mapping of the 27 Norwegian species of fresh-water Gastropoda. Maps have been made showing (1) geographical distribution of each species, (2) total number of species in each square, (3) average number of species per lake, (4) number of lakes with given number of species, (5) number of individuals collected per half-hour (time-catch abundance) for each species, and total of all species. A selection of maps is presented. Primary data: 73,000 individuals collected in habitats with ecological data (cf. above), some 34,000 specimens in museums etc., and literature records.

Correlation studies. Comments are given on single and multiple factor analyses.

A preliminary report of some of the results arrived at in a study comprising the distribution, ecology, and morphology of the fresh-water snails of Norway, including aspects of regional limnology, was given at the 3rd European Malacological Congress in Vienna in 1968 (Ökland, 1969). Since then the study has proceeded. The present communication will give a short outline of some of the new results which still have a preliminary character.

Emphasis will be put on subjects related to the European Invertebrate Survey (EIS), particularly with regard to mapping. For the mapping projects a base map of Norway has been constructed (Fig. 1A). The location of the national squares in relation to the remaining parts of Europe, with special reference to the joint EIS dot map surveys, is shown in Fig. 1B.

Construction of maps for both environmental factors and distribution of species is only a part of the study which basically has an ecological approach. The major aim is to elucidate the importance of various environmental factors for the geographical distribution of the species.

SOME REMARKS ON THE TOPOGRAPHY OF NORWAY

Norway forms the north-western border of the European continent. It covers more degrees east-west and is longer and narrower than any other European country. It borders Sweden, Finland, and the Soviet Union.

The number of lakes is estimated at 250,000 and their surface area at about 15,000 km², i.e. more than the arable land in Norway. Among the lakes are those located at the highest latitude on the European continent (Fig. 2A), that is about 71°N, in the North Cape area. The lakes

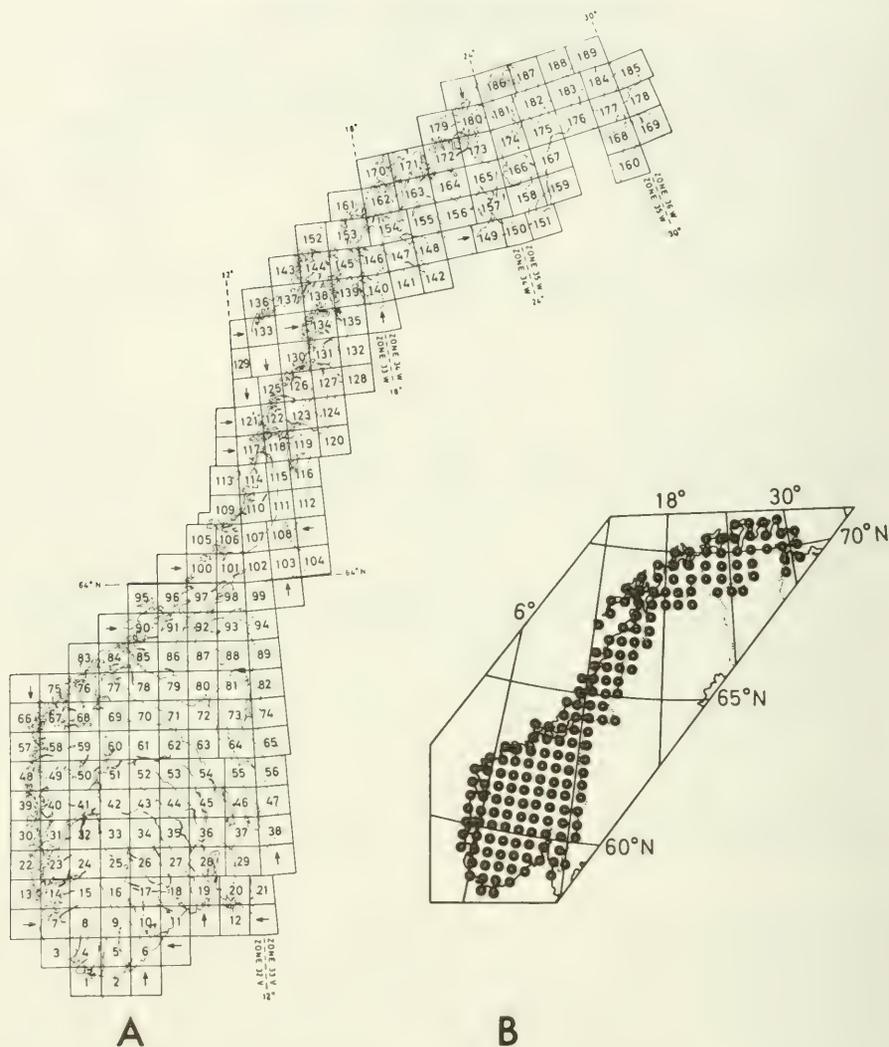


FIG. 1A. Base map of Norway, consisting of modified 50 km squares for use in European Invertebrate Survey. Total: 189 squares. B. From a base map of Europe at 1:10 million. Dots represent 189 modified 50 km squares completely or partly located in Norway. From Ökland, 1976.

with the highest elevation in North Europe are also located in Norway (Fig. 2B), as well as the 4 deepest lakes in Europe (Fig. 2C shows the deepest one).

Owing to great variation in climate and geology Norway is well suited for regional ecological studies. In south-eastern Norway the gradient from lowland districts to high mountain areas, up to more than 2,000 m above sea level, is of great ecological significance. In the lowland part of south-eastern Norway we find the region which geologically is called the Oslo Region. This fairly small geographical area has an unusually wide variety of bedrock and Quaternary deposits which greatly influence hydrochemical and biological factors in its numerous water bodies. Both rich eutrophic lakes (Fig. 2D), dystrophic lakes with bordering *Sphagnum* mires (Fig. 2E) and poor oligotrophic lakes (Fig. 2F) are present in great numbers within a restricted area.

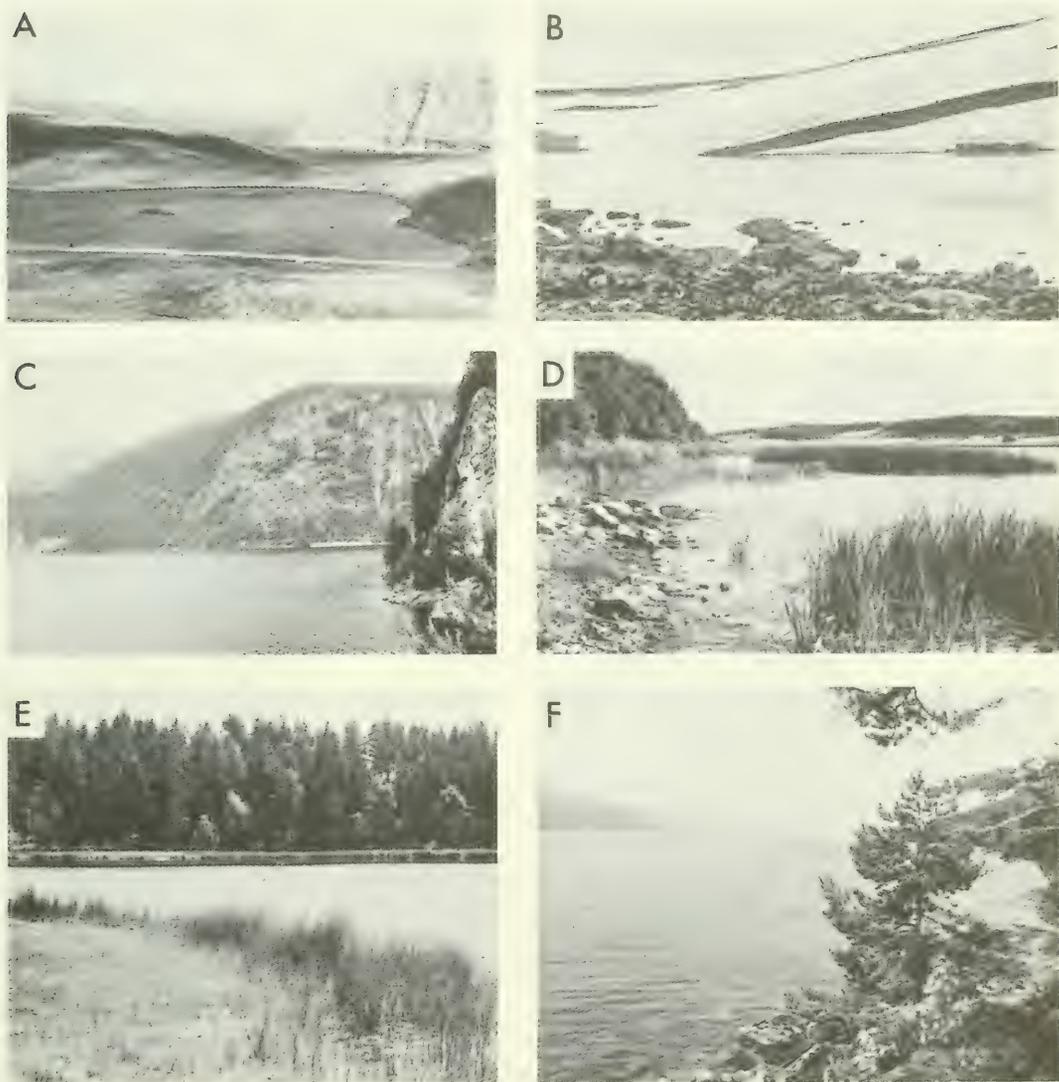


FIG. 2. A selection of photographs of the variety of fresh-water habitats in Norway. A. The lake located at the highest latitude on the European continent is nameless, and lies near North Cape (shown in the background). B. The lake with highest elevation in North Europe (Lake Gjuvvatn, 1,837 m above sea level); the glacier descending into the lake is shown in the background. C. The deepest lake in Europe (Lake Hornindalsvatn, 514 m deep). In south-eastern Norway a wide variety of lake types is present within a small geographical area, such as D. A rich eutrophic lake (Lake Borrevatn), E. A dystrophic lake surrounded by a *Sphagnum* bog (Lake Åsentjern), and F. A poor oligotrophic lake (Lake Eikeren). The latter 3 lakes are located west of Oslo Fjord, in the county of Vestfold.

GASTROPODA—ENVIRONMENT AND SPECIES

A field study of the 27 species of fresh-water gastropods in Norway was undertaken in 1953-1973. A total of 1,498 habitats—among them about 1,000 lakes—was investigated. Fig. 3A shows the number of investigated habitats in each square. A total of 819 days was used for

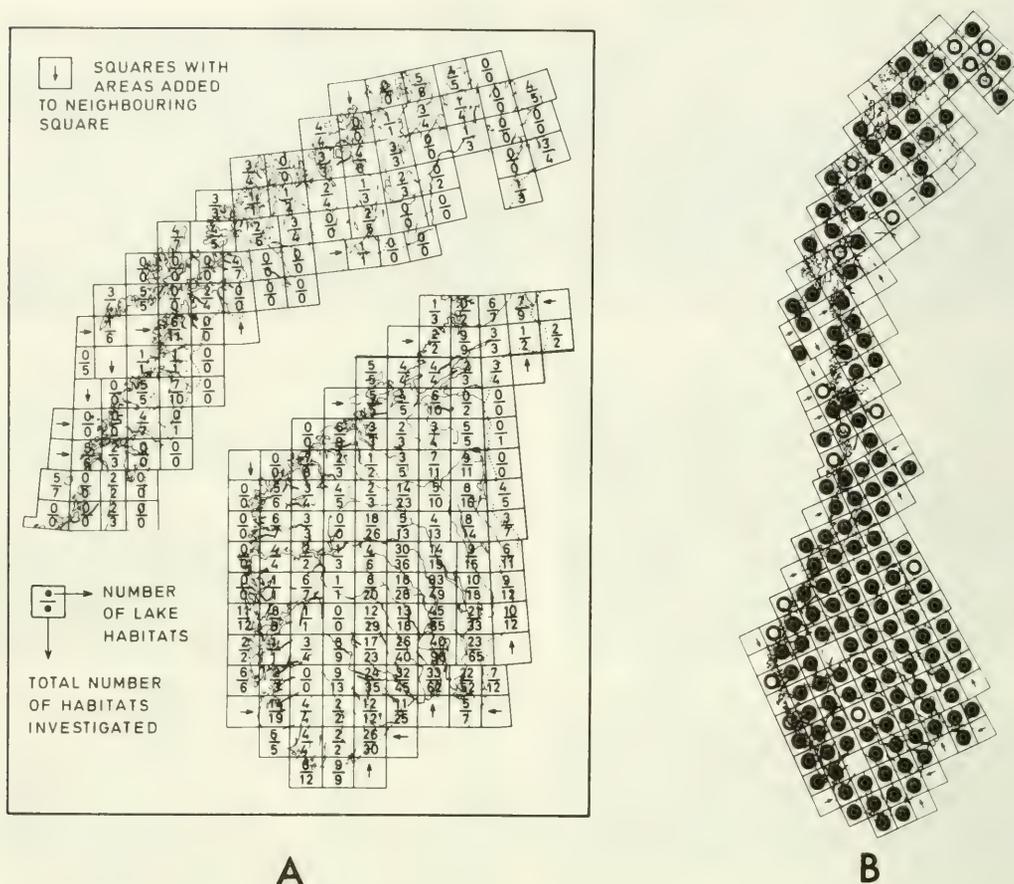


FIG. 3A. Division of North and South Norway according to modified 50 km squares. The map shows number of investigated fresh-water habitats with data on environment. Total: 1,498 habitats investigated 1953-1973 during 819 days of field work. B. ● = Squares with data on both environment and Gastropoda, ○ = Squares with data on Gastropoda only.

field work. In all habitats environmental factors were recorded and gastropods searched for, using a time-catch method for estimating population density.

In Fig. 3B the dots show squares with data on both environment and Gastropoda. Rings mark the squares from which only data on Gastropoda (from other sources) were available.

The habitat was the smallest unit investigated in the field. Usually only one habitat was investigated in each lake or river. The habitat may be defined as a place where gastropods were obtained and certain ecological factors measured and classified. In lakes and rivers the habitat consists of a certain stretch of shores—usually about 200 m—defined by special ecological characteristics. The average investigation time per habitat in lakes and rivers was 1 hour. For the smaller water bodies like ponds and puddles, the entire water body was investigated and considered as one habitat.

Mapping environmental factors. Using the EIS-system, maps have been made showing 10 different sets of environmental parameters: (1) elevation above sea level, (2) geology, (3) vegetation in the surroundings of habitats, (4) macrovegetation along the shore of lakes, (5) substratum, (6) wave exposure in lakes, (7) hydrogen-ion concentration (pH), (8) calcium concentration, (9) water colour, and (10) water temperature.

In this communication only a few examples of such maps will be given. First we shall consider 4 maps based on *average values within squares*.

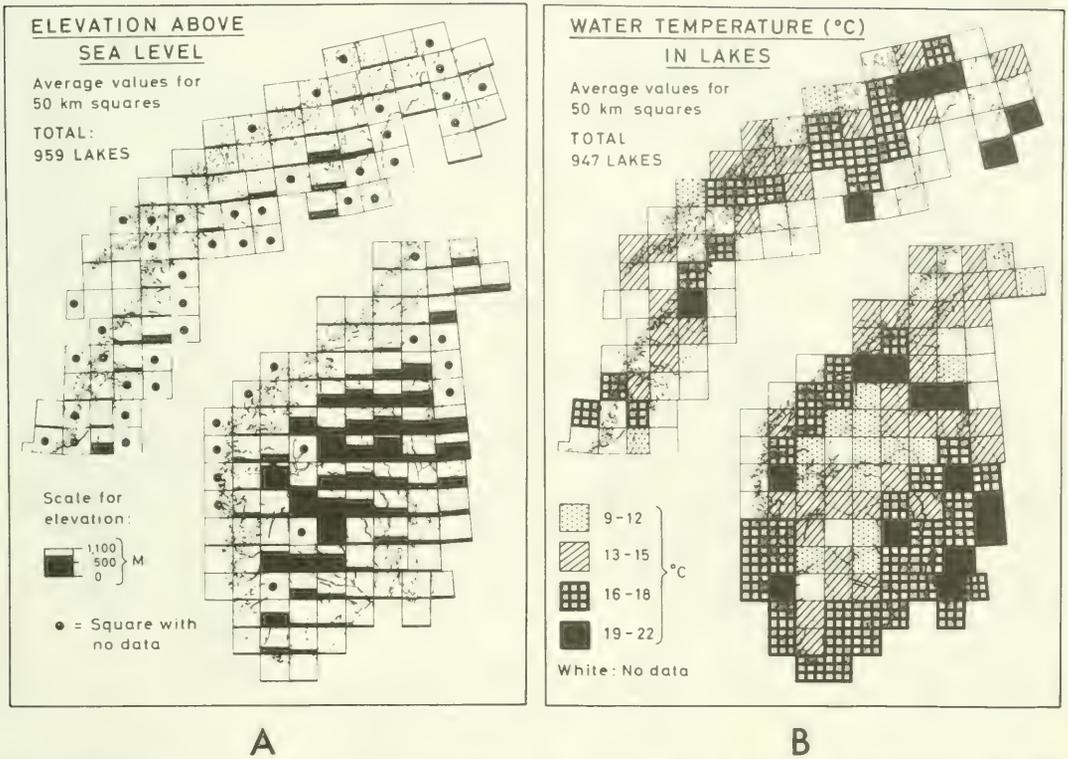


FIG. 4A. Elevation above sea level for investigated lakes. B. Observed surface water temperature in summer in investigated lakes.

Fig. 4A shows elevation above sea level for investigated lakes. The highest average values are found in the central part of South Norway.

Fig. 4B shows water temperature in lakes at the time of investigation, i.e. during summer. Two points are worth mentioning: (1) low temperatures are prominent in the central part of South Norway, and (2) high temperatures are found in coastal districts in South Norway as well as in many parts of North Norway.

Fig. 5A shows calcium concentration (as total hardness) in investigated lakes, expressed as average values for summer surface water. We note that large areas in the western part of South Norway are very poor in lime salts. Since there is generally a correlation between calcium concentration and the buffer capacity of the water, we understand that the lakes in large parts of South Norway are but poorly suited to neutralize acid substances from precipitation or from the terrestrial environment surrounding the lakes.

Finally, Fig. 5B shows hydrogen-ion concentration (pH) in investigated lakes, expressed as average values for summer surface water. Areas with acid lakes are more widely distributed in South Norway as compared with North Norway. In South Norway areas with acid lakes are found especially in the southern and western parts as well as in districts along the border with Sweden.

A more detailed way of illustrating environmental parameters within squares is to indicate *per cent of habitats belonging to a certain category*. Maps of this type show that within a given square there are usually many types of habitat.

Fig. 6 shows such a map. The area is south-eastern Norway, built up of a total of 40 modified 50 km squares. This area has been especially closely studied. The map indicates geology in the surroundings of investigated lakes, expressed as percentage of lakes belonging to one of 4 possible categories. In south-eastern Norway lakes influenced by geological categories

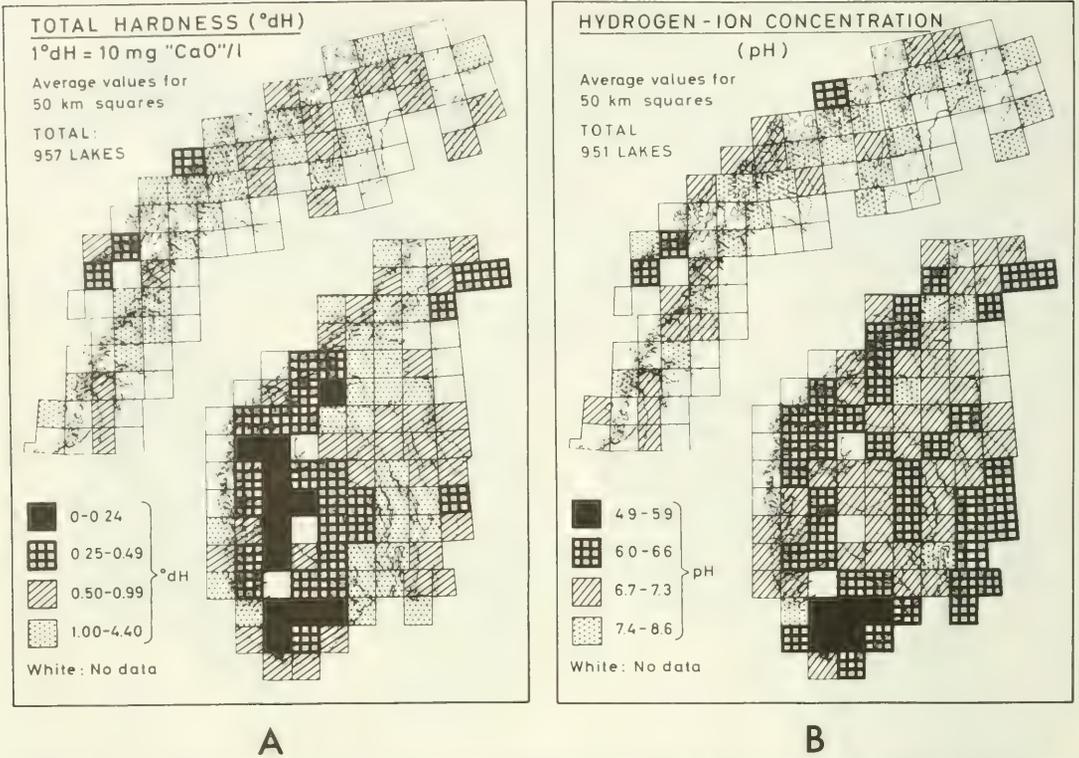


FIG. 5A. Calcium concentration (as total hardness) in investigated lakes. Average values for summer surface water. B. Hydrogen-ion concentration (pH) in investigated lakes. Average values for summer surface water.

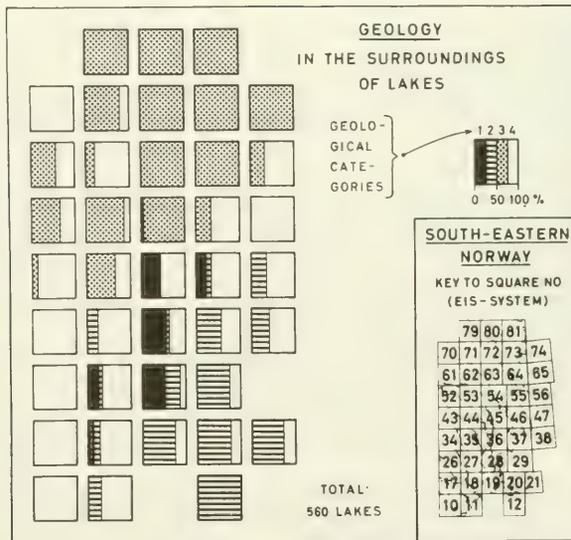


FIG. 6. South-eastern Norway is represented by 40 modified 50 km squares. For each of these squares this map shows geology in the surroundings of investigated lakes, expressed as percentage of lakes belonging to one of 4 possible categories: (1) unaltered Cambro-Silurian rocks, rich in lime, (2) marine clay, (3) strongly altered Cambro-Silurian rocks, Eocambrian rocks, etc., (4) Pre-Eocambrian rocks, and Permian plutonic and effusive rocks of the Oslo Region.

Nos. 1 (bed rock rich in lime) and 2 (marine clay) are especially favourable for fresh-water gastropods.

Mapping distribution and population density of fresh-water Gastropoda. The material for this mapping consists of 3 parts: (1) 73,000 individuals from 1,498 habitats with ecological data, (2) 34,000 individuals in museums, etc., and (3) records from literature.

Using the EIS-system, maps have been made showing (1) geographical distribution of each species, (2) total number of species in each square, (3) average number of species per lake, (4) number of lakes with given number of species, (5) number of individuals collected per half-hour (time-catch abundance) for each species, and total for all species.

A few examples of such maps will be shown. The 4 maps in Fig. 7 show distribution patterns for 4 of the 27 species of fresh-water gastropods in Norway, i.e. (A) *Lymnaea peregra* (Müll.), a widely distributed species with great ecological amplitude, (B) *Acroloxus lacustris* (L.), mainly restricted to more eutrophic habitats in the southern part of South Norway, (C) *Potamopyrgus jenkinsi* (Smith), a recent immigrant to Norway, restricted to fresh and brackish water localities in the southern part of South Norway, and (D) *Valvata sibirica* Middendorff, a species with a northern range.

Fig. 8A shows the total number of species in each square. This map accentuates a common feature for many groups of fresh-water invertebrates in Norway: the highest number of species occurs in the south-eastern part of South Norway. The communities may also be fairly rich in species in other parts of South Norway and in the interior eastern part of North Norway.

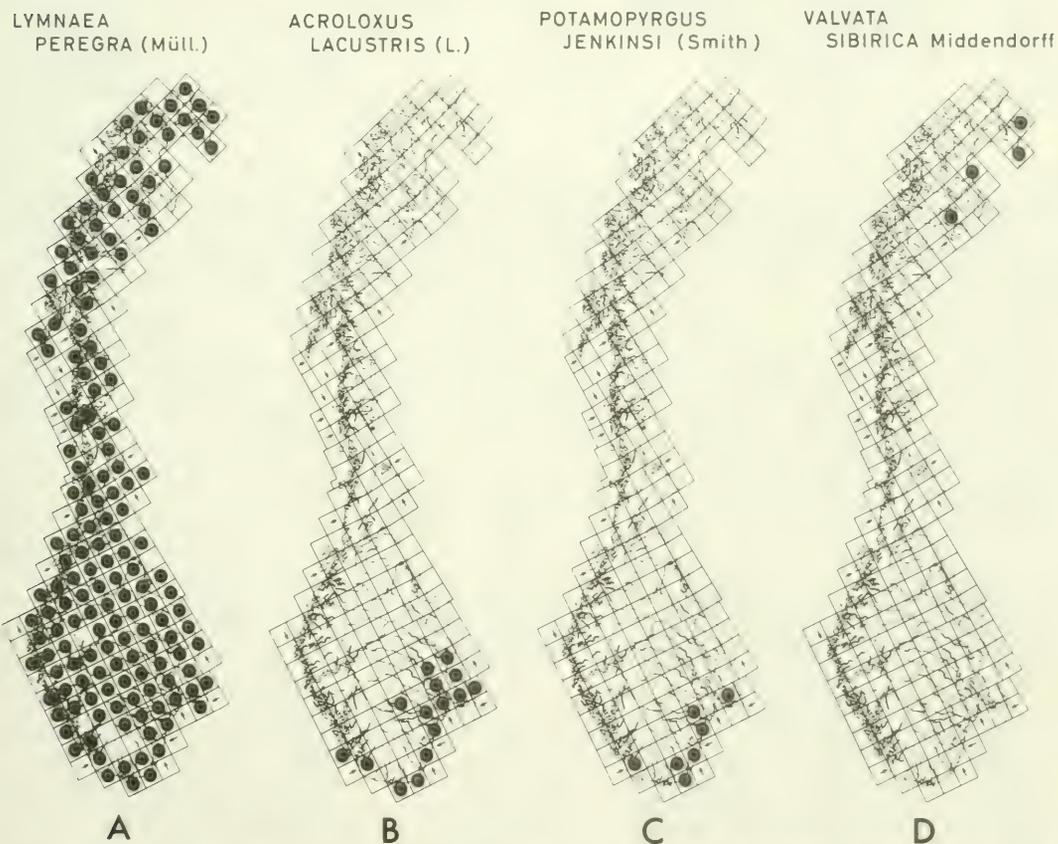
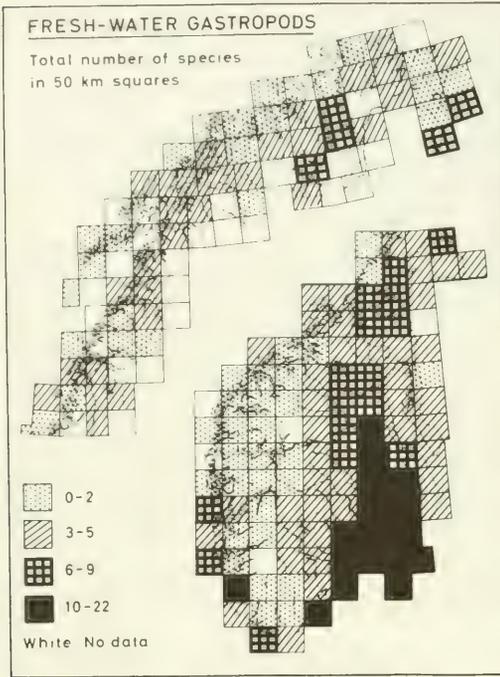
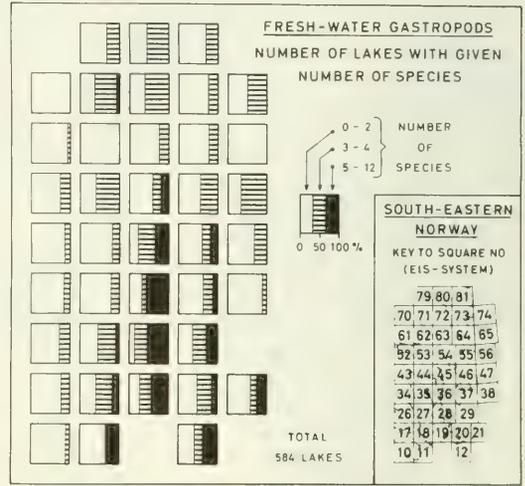


FIG. 7. Geographical distribution of some fresh-water snails in Norway. A. A widely distributed species. B. A species restricted to the southern part of South Norway. C. A recent immigrant to Norway (first record: 1954), also restricted to the southern part of South Norway. This species also occurs in brackish water. D. A northern species.

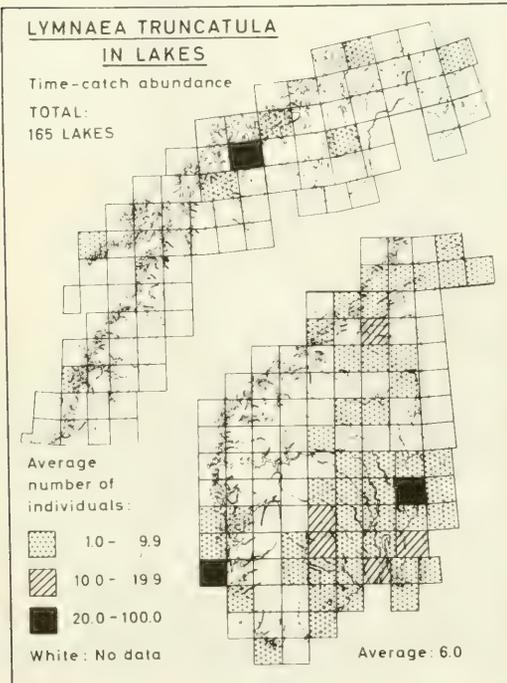


A

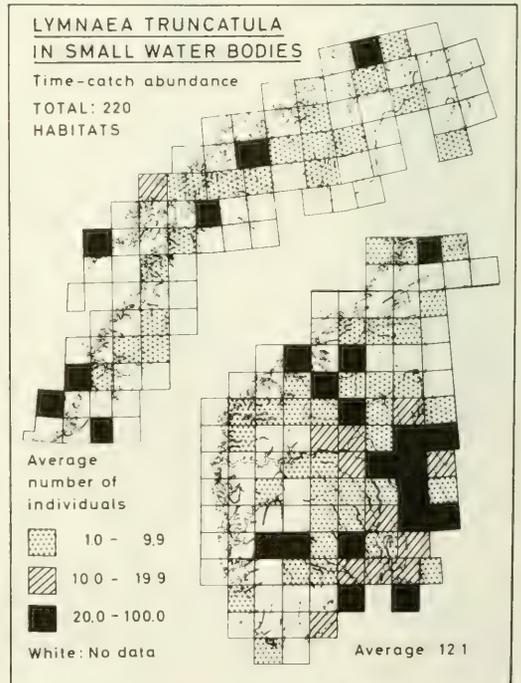


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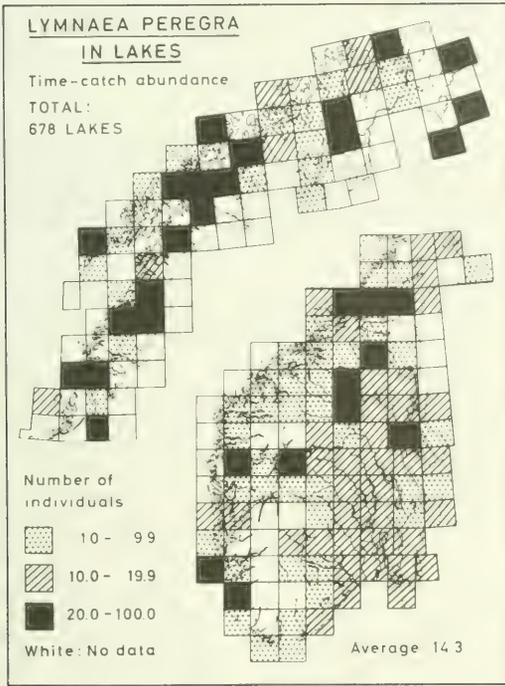
FIG. 8A. Total number of species of fresh-water gastropods in the various 50 km squares in Norway. B. Percentage of lakes with given number of species in 50 km squares in south-eastern Norway.



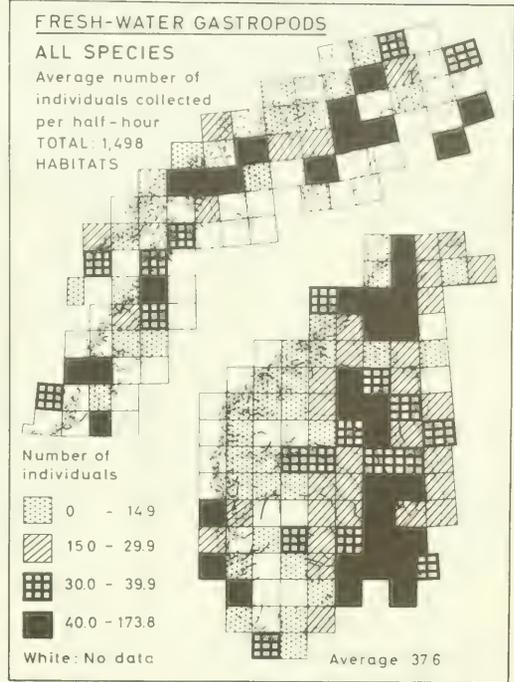
A



B



A



B

FIG. 10A. Time-catch abundance for *Lymnaea peregrina* (Müll.) indicates that in the south-eastern part of Norway none of the squares has the highest category of abundance. This is the area with the highest number of species of fresh-water gastropods in Norway (cf. Fig. 7A). The reduced population density in this area is possibly due to competition with more specialized species. B. Number of individuals collected per half-hour (all 27 species treated together).

Fig. 8B shows the number of lakes with a given number of species for south-eastern Norway. We note that in a large proportion of the investigated lakes only 0-2 species were detected. Especially in the southern and central part of South Norway a fair proportion of the investigated lakes contained 5-12 species.

Let us now consider 4 maps (Figs. 9-10) dealing with population density, expressed as time-catch abundance (based on number of individuals collected per half-hour). Only major trends of population density can be shown by such a rough method, and the results obtained should be used with caution.

Fig. 9 shows results for *Lymnaea truncatula* (Müll.). Since this species is known to prefer small water bodies like ponds and ditches (Boycott, 1936; Hubendick, 1947; Ökland, 1964), it is not surprising that average population density in small water bodies is higher (12 individuals collected per half-hour, cf. Fig. 9B) as compared with the density in lake habitats (6 individuals collected per half-hour, cf. Fig. 9A).

Fig. 10A shows results for *Lymnaea peregrina* in lakes. This species has a fairly low density in the south-eastern part of Norway, which is very rich in species (cf. Fig. 8A). This may be explained by competition with more specialized species which are better adapted for a life in south-eastern Norway.

Average time-catch abundance for *Lymnaea peregrina* in lakes was 14 individuals collected per half-hour (Fig. 10A), i.e. a higher value than observed for *L. truncatula* in lake habitats (cf. Fig. 9A).

FIG. 9. A time-catch method was used to obtain a rough estimate of population density for the various species of fresh-water gastropods. These maps show densities for *Lymnaea truncatula* (Müll.), A for lake habitats (average 6 specimens collected per half-hour), B representing small water bodies such as ponds and ditches (average 12 specimens collected per half-hour).

Treating all species of fresh-water gastropods together, Fig. 10B summarizes population density in Norway. Although many parts of North Norway have rather few species (cf. Fig. 8A), the total number of individuals belonging to the gastropod fauna may be fairly large. Since gastropods are important fish-food organisms, this implies that biomass of fish-food may be large although number of species is small.

Correlation studies. In order to point out some general trends in the correlation between major factors of environment and the occurrence of fresh-water gastropods in Norway, single factor analyses of 10 environmental parameters have been performed. An example is given in Fig. 11 which indicates how the frequency of the 14 commonest species in south-eastern

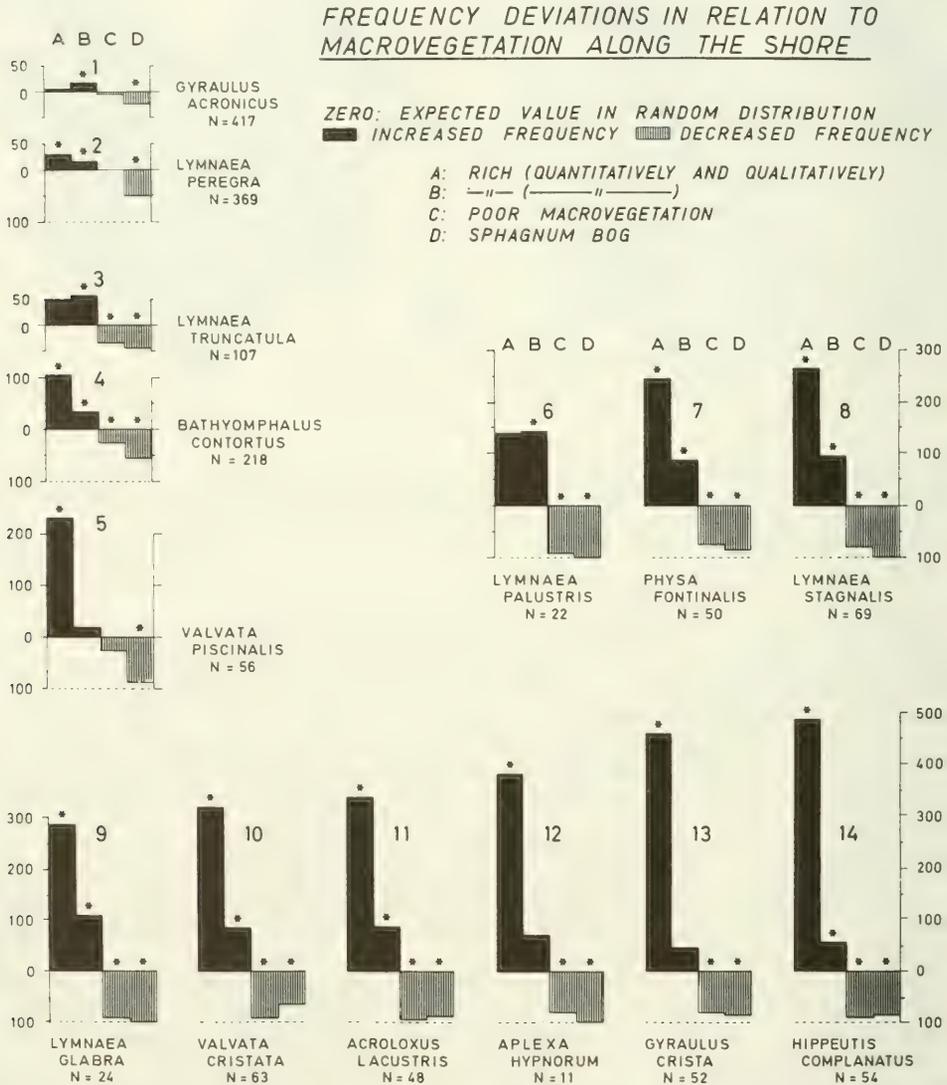


FIG. 11. Occurrence of 14 species of fresh-water gastropods in south-eastern Norway in lakes with different types of macrovegetation along the shore. Material: 541 lakes investigated 1953-57. For each species is indicated the frequency in 4 different groups of macrovegetation (A-D). Increased frequency is indicated by black bars, decreased frequency by shaded ones. The zero level represents expected frequency in random distribution. Stars indicate deviations significantly different from zero.

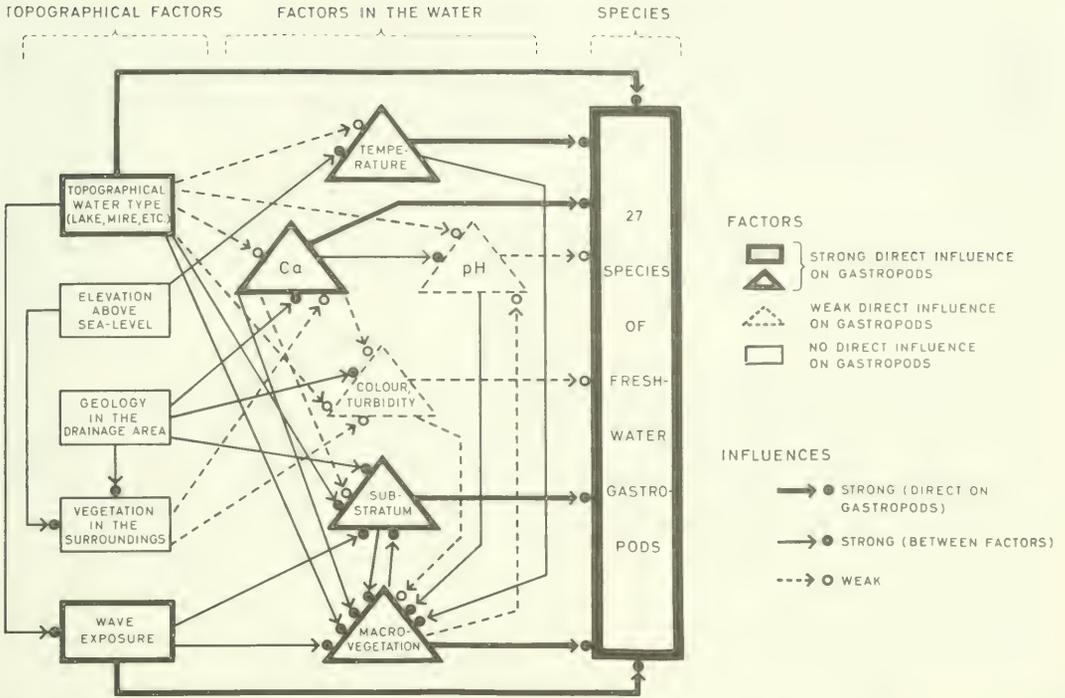


FIG. 12. Main relations between environmental factors and fresh-water gastropods. The effect of a given factor is considered as direct when the action does not necessarily involve a step through any of the other factors. Note how the factor "macrovegetation" is hit by many "ecological cannon balls," i.e. this factor reflects a multitude of environmental parameters and has a strong direct influence on the gastropod fauna. The effect of a given factor varies according to the development of other factors. The relative importance of each factor is to be elucidated by multiple regression analyses.

Norway is influenced by quantity and quality of macrovegetation along the shore of lakes. For each species frequency deviations are indicated in relation to a zero value obtained in random distribution. Most species have a significantly increased frequency in lakes where the macrovegetation belongs to category A or B, i.e. rich vegetation either both quantitatively and qualitatively, or only quantitatively. Generally the species have a decreased frequency where macrovegetation is poorly developed (category C) or consists of a *Sphagnum* bog (category D).

The relative importance of each environmental factor is to be elucidated by multiple regression analyses. Fig. 12 shows the main relations between environmental factors and fresh-water gastropods. The effect of an environmental factor is considered as direct when the action does not necessarily involve a step through any of the other factors here mentioned.

The picture is more complicated than shown on this figure. We know, for instance, that the effect of a given factor varies according to the development of other factors. For example: the effect of the hydrogen-ion concentration (pH) of the water increases with decreasing calcium concentration (total hardness).

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SPHAERIIDAE OF NORWAY: A PROJECT FOR STUDYING ECOLOGICAL REQUIREMENTS AND GEOGRAPHICAL DISTRIBUTION

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ABSTRACT

The purpose of the project is to study the pH tolerance in the field of the 20 species of Sphaeriidae in Norway and to elucidate whether they live in low-buffered lakes with pH values near their lower tolerance limit, in areas threatened by acidification. Using the European Invertebrate Survey base map of Norway, preliminary distribution maps for *Pisidium casertanum*, *P. conventus*, *Sphaerium corneum*, and *S. nitidum* are presented. The small Bivalvia are important fish food organisms and the project is part of the Norwegian interdisciplinary research project "Acid Precipitation—Effects on Forest and Fish"—the SNSF-project. A few results of the SNSF-project are outlined, especially those concerning fish kill due to acidification.

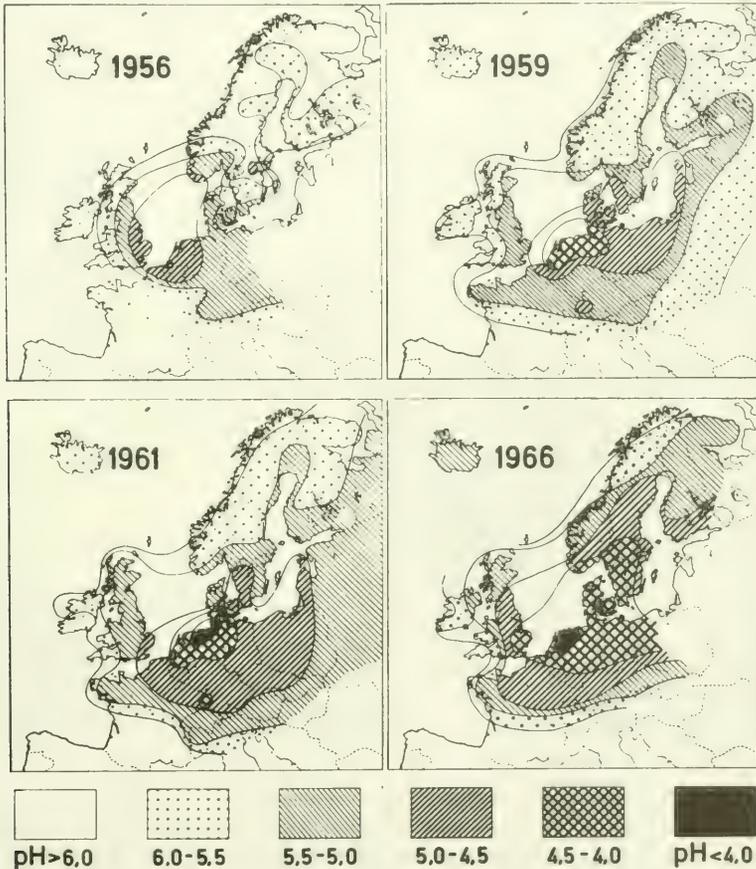


FIG. 1. Maps showing areal distribution of pH values in yearly precipitation over Europe (Odén, 1971).

In the 1960s measurements indicated that the precipitation in Europe was becoming increasingly acid. A central area with highly acid precipitation was extending from year to year, as illustrated in Fig. 1. Recent results are given by Tollan (1977) in a map illustrating mean pH values, based on daily measurements of precipitation in Europe between July 1972 and March 1975. This is constructed from data collected by the Norwegian Institute for Air Research (Schaug, 1975, 1976). It was suspected that increasing emission of sulphur dioxide from the combustion of fossil fuels is the main cause of this acidification.

In Norway acid precipitation was seen as the possible cause of increasing acidity of the watercourses in the southern part of the country and of the gradual disappearance of trout and salmon from many lakes and rivers. It was also feared that the input of acid might reduce

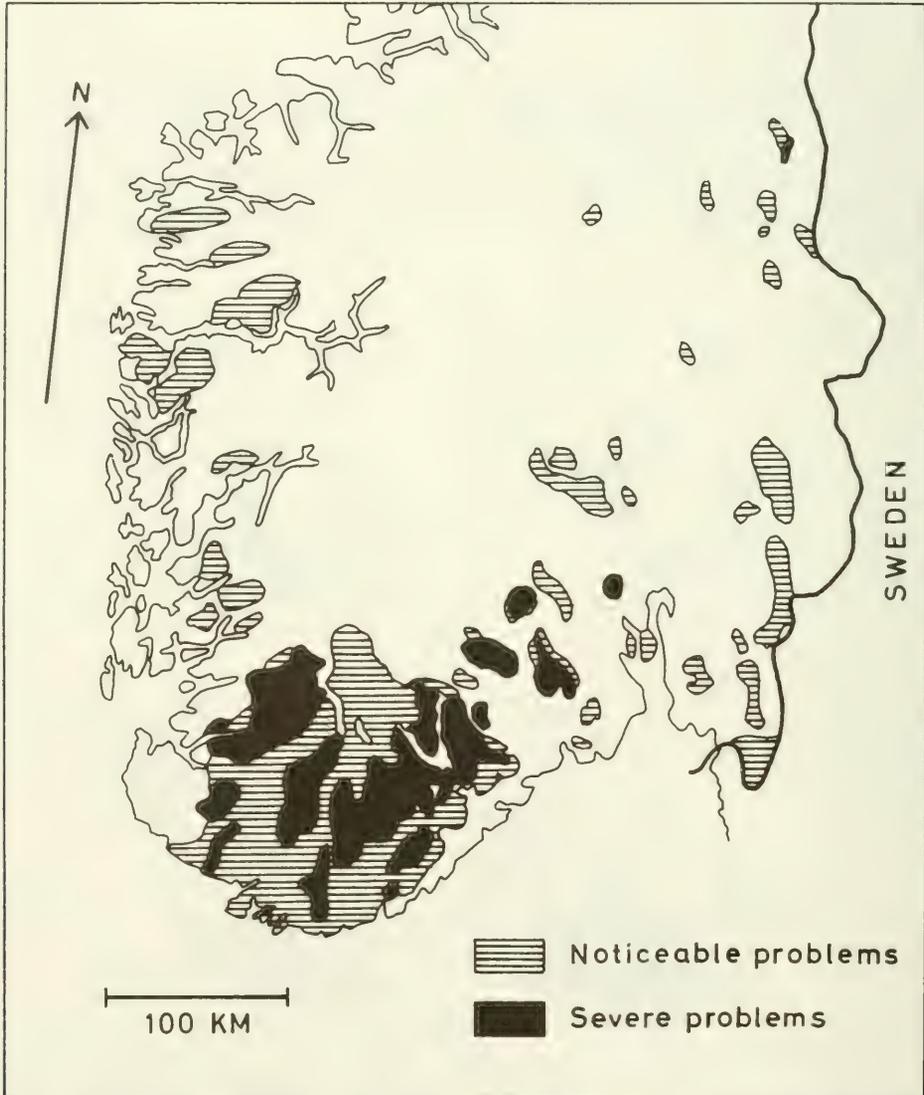


FIG. 2. Fish status in lakes of Norway south of 63°. The map is based on data from more than 2,000 lakes. "Severely affected" areas are those where a majority of lakes have lost their fish populations. In "noticeably affected" areas at least some of the lakes have had reduced population density in recent years (modified from Leivestad et al., 1976).

forest growth through increased leaching of nutrient elements from the soil. Widespread concern about these matters led to the organization of the interdisciplinary research project "Acid Precipitation—Effects on Forest and Fish"—(SNSF-project) early in 1972 (Braekke, 1976).

Information on past and present status of the trout populations in more than 2,000 lakes has been collected and the "problem" areas have been mapped by the SNSF-project (Fig. 2). Lakes which have lost their trout populations are clearly more frequent in those parts of the country where the influx of acid pollution is high.

First affected are small, high altitude lakes with low buffer capacity in the catchment area, the bedrock being highly resistant, mainly granitic. Originally, many of these lakes were densely populated, harbouring stocks of relatively small, slow-growing fish. The first symptom of acid stress is usually a decrease in population density, with the remaining fish showing faster growth. This is a result of recruitment failure and test-fishing shows a shortage of the younger age classes (Jensen & Snekvik, 1972; Leivestad et al., 1976).

The disappearance of fish is easily observed, but the distribution of the small invertebrates which serve as food for the fish is mostly so poorly known in Norway that a possible disappearance is hardly noticed. Therefore, within the SNSF-project a sub-project has been launched on selected invertebrate groups. This sub-project started in January 1977 and will last for 3 years.

In a preliminary survey on gastropod ecology in Norway ecological data from about 1,500 freshwater habitats are presented (J. Ökland, 1979). The same data will be used for studying ecological requirements of other groups of animals that were collected or observed together with the gastropods. The 20 species of small Bivalvia were chosen for 2 reasons: they are important as food for fish, and there are reasons to believe that low pH values in the waters are important limiting factors for their distribution in Norway.



FIG. 3. Preliminary maps of the distribution of 4 species of Sphaeriidae in Norway. A. *Pisidium casertanum*, B. *P. conventus*, C. *Sphaerium corneum*, D. *S. nitidum*.

The first step is to find the pH tolerance of each of the species in natural habitats, as previously done for the freshwater shrimp *Gammarus lacustris* Sars (K. A. Ökland, 1969). The next step is to find out if some of the species live in low-buffered lakes, with pH values near their lower tolerance limit, in areas threatened by acidification.

Fig. 3 shows preliminary distribution maps of 4 species of Sphaeriidae, constructed on the European Invertebrate Survey base map of Norway (J. Ökland, 1979). *Pisidium casertanum* (Poli) is probably the species found in most localities in Norway. *P. conventus* Clessin is a cold water species found in many lakes in the north. In the south it mainly occurs in deeper water or in high altitude lakes. *Sphaerium corneum* (L.) is found in many parts of the country, but most records are from the south-east. *S. nitidum* Clessin is a more northern species, in the south found mostly in high mountains.

The work on the Sphaeriidae is made possible by the valuable contribution of Mr. J. G. J. Kuiper, Paris. He has kindly identified our samples and is also responsible for the revision of the Norwegian museum collections.

This is SNSF-contribution FA 24/78.

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TEMPERATURE AND REPRODUCTIVE CYCLE RELATIONS IN *RADIX PEREGRA* O.F. MÜLLER

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ABSTRACT

Oviposition as a consequence of reproductive activity of *Radix peregra* O.F. Müller was researched both under laboratory conditions and outdoors. An earlier supposition (Meštrov & Krkač, 1970, Krkač & Meštrov, 1970) that oviposition in *Radix peregra* living in habitats with distinct seasonal temperature variations occurs after a rapid rise of water temperature in the spring, was corroborated by research in the spring region in Bijela Rijeka (Jugoslavia). The population in the basin of non-captive water of Tuheljske Toplice (thermal springs) lays eggs continuously throughout the year regardless of seasons. Here the temperature of the water varies from 29-32.1°C. When migrating to the compact layer of algae spread over the surface of the warm water the animals are exposed to a sudden change of temperature. The air temperature near the surface of the algal layer is considerably lower than that of the water, regardless of season. So, the temperature gradient produces a permanent incentive to egg-laying throughout the year. Laboratory experiments support the outdoor data. Animals with no possibility of changing their temperature zone do not lay eggs. The frequency of egg-laying depends on an average (breeding) temperature and its fluctuation.

INTRODUCTION

Temperature has been shown by many authors to be one of the factors regulating the reproductive cycle of Gastropoda (Duncan, 1959; Michelson, 1961; Smith, 1967; Van der Steen, 1967; Timmermans, 1959; Wolda, 1967).

The eurythermic species *Radix peregra* inhabits a very large area including biotopes with very different thermal conditions. In Jugoslavia it was found in Tuheljske Toplice (thermal springs) at a temperature of 32.4°C and in Stubičke Toplice at 42.2°C (Matoničkin, 1957), as well as in the subterranean cave waters of Rakov Škocijan (Bole, 1966). Subterranean cave waters characteristically have a relatively low and almost constant temperature throughout the year.

Outdoor research (Krkač & Meštrov, 1970) suggested that a thermic jump (an abrupt temperature rise in the spring) was followed by egg-laying. A similar reaction was caused by a sudden change of temperature zone (Krkač, 1973). Special attention was paid to the relation between the temperature regime of the habitat and the reproductive cycle in the context of research on the population dynamics of *Radix peregra* (Krkač, 1975). Outdoor data were supplemented by laboratory experiments.

MATERIAL AND METHODS

The phenomenon of egg-laying was taken as a parameter of relationship between the temperature of the natural or artificial habitat and the reproductive cycle.

Outdoors, oviposition was studied in the spring region of Bijela Rijeka, where temperature variations are distinctive, as well as in the non-captive basin of Tuheljske Toplice which has high and almost unvarying temperatures throughout the year. Outdoor studies were performed in both localities every month from October 1970 to November 1971 and in Tuheljske Toplice in 1972, 1973 and 1974. Certain physico-chemical factors such as temperature of the water, quantity of dissolved oxygen, acidity (pH), were registered at the same time, always before noon.

TABLE 1. Oviposition and physico-chemical factors in periods of research in the spring area of Bijela Rijeka. Egg-mass: ++, plenty; +, present.

Date of sampling	Egg-mass	Water temp. °C	pH	Alkalinity mval/l	Hardness °d	CO ₂ mg/l	O ₂ mg/l	O ₂ % of saturation
27.X.1970		7.5	6.8	5.20	14.57	9.84	10.6	88.11
24.XI.1970		7.0	6.6	5.30	14.51	8.74	11.8	100.34
17.XII.1970		7.5	6.8	5.30	14.85	9.84	10.3	88.71
26.I.1971		7.0	7.0	5.91	16.56	10.09	10.5	89.28
24.II.1971		8.0	6.8	5.70	15.96	—	10.6	92.41
25.III.1971		6.0	7.0	5.30	14.84	4.4	11.3	93.69
27.IV.1971		8.0	—	7.20	20.16	22.0	10.9	95.03
26.V.1971	++	15.5	7.1	5.30	14.92	29.39	12.5	129.39
24.VI.1971	++	17.0	7.1	5.33	14.93	—	12.2	130.20
3.VIII.1971	+	18.0	7.1	6.17	17.73	10.87	7.5	81.69
5.IX.1971		14.0	—	5.01	14.05	3.20	10.2	102.20
12.X.1971		10.0	6.7	5.12	14.34	0.44	10.6	97.06

TABLE 2. Oviposition and physico-chemical factors in period of research in the non-captive basin of Tuheljske Toplice. Egg-mass: +, present; ++, plenty; +++, abundant.

Date of sampling	Egg-mass	Water temp. °C	pH	Alkalinity mval/l	Hardness °d	CO ₂ mg/l	O ₂ mg/l	O ₂ % of saturation
29.X.1970	+	31.0	7.0	6.52	18.27	22.96	3.3	44.47
26.XI.1970	+	29.0	7.0	7.03	19.70	38.26	1.6	20.94
21.XII.1970	+	29.5	7.5	7.5	21.42	31.70	2.7	35.62
28.I.1971	+	30.0	7.1	6.63	18.56	33.84	2.1	27.88
23.II.1971	+++	30.1	6.4	7.10	19.88	30.84	1.4	18.61
30.III.1971	+++	30.2	7.2	6.70	18.76	30.84	1.5	19.97
24.IV.1971	++	31.0	6.7	7.10	19.88	26.4	1.1	14.82
27.V.1971	+	31.0	7.3	6.48	18.14	29.92	1.0	13.47
30.VI.1971	+	31.2	7.2	6.69	18.73	31.71	1.6	21.62
5.VIII.1971	+	32.1	7.1	6.43	18.00	31.16	1.0	13.67
8.IX.1971	+	31.0	—	6.37	17.85	24.73	3.4	45.82
4.X.1971	+	30.9	7.0	6.19	17.27	19.26	3.2	43.06
4.III.1972	+++	31.2	7.0	6.10	17.08	39.16	1.4	18.91
2.IV.1972	+++	—	—	—	—	—	—	—
9.VIII.1972	+	—	—	—	—	—	—	—
12.IX.1972	+	31.2	—	—	—	—	—	—
5.XII.1972	+	30.9	—	—	—	—	—	—
26.II.1973	+++	31.1	—	—	—	—	—	—
24.V.1973	+	—	—	—	—	—	—	—
5.VIII.1973	+	32.1	—	—	—	—	—	—
29.X.1973	+	31.5	—	—	—	—	—	—
25.I.1974	+	30.5	—	—	—	—	—	—
23.IV.1974	+++	31.0	—	—	—	—	—	—

The material was collected with a benthos net from all parts of the water, i.e. the bottom, from among rooted aquatic plants, from leaves on the bottom and from the layer of algae on the surface. Considering the specific water regime of Bijela Rijeka, sampling was not performed in a quantitative sense. Therefore, the presence of egg-masses was defined in both localities by relative frequency in a sample. When the egg-masses were found all over aquatic plants, leaves on the bottom, bits of wood submerged for a long time, the sample was indicated as "abundant" and marked by 3 symbols (+++) (Tables 1 and 2); when egg-masses were found easily in the same places, sampling was defined as "plenty" (++) and when possible only by use of the benthos net, sampling was called "present" (+).

Studies of the first oviposition and the frequency of egg-laying were performed with animals of 2nd and 3rd generations raised in the laboratory from the population of Tuheljske Toplice. Since many authorities claim that parthenogenetic reproduction is common in the Lymnaeidae (Crabb, 1928; Colton & Pennypacker, 1934; Hubendick, 1951; Cain, 1956), the animals were maintained separate in laboratory vessels filled with 200 cc of water from the natural habitat.

Water was changed every 14 days and the vessels cleaned. When required, the animals were fed with lettuce. The emergence of egg-masses and the frequency of egg-laying were registered daily in 3 or 4 temperature zones. The animals of the 1st group (Table 3) were maintained in water of varying temperature (28-31°C), i.e. within the limits of the temperature fluctuations of the original population's habitat. The vessels with the animals were submerged in water constantly warmed by a heater connected with a thermoregulator. The animals contacted the layer of air above the water when emerging and taking in fresh air, being always in the same temperature zone.

The 2nd group of animals (II, Table 3) was kept at a room temperature varying from 17-25°C. This group was a control since all experimental animals were raised at the same temperature.

The 3rd group (III, Table 3) was maintained at an average experimental temperature of 13.9°C (9-22°C). The temperature of the water in the experimental containers of the 2nd and 3rd groups was gradually changing, following the temperature variations of the surrounding air. Daily fluctuations of up to 5°C were recorded.

Series of experiments, indicated as A in Table 3, were repeated under the same conditions with animals of the 3rd generation (B, Table 3), raised in the laboratory with a slightly different temperature regime. Another temperature zone (group IV, Table 3) with a smaller interval of variation (13-17.5°C) and a higher average temperature (less than 1°C higher than the average temperature in the 3rd group) was added to the experiments already mentioned.

TABLE 3. Quantity of egg-laying at experimental temperatures defined by average number of eggs per day and per animal. Significance of the size differences among the animals at first oviposition was checked with the F-test.

Experiment	Shell-length at first oviposition (mm)	P value	Quantity of egg-laying in 30 day periods from first oviposition					Average exper. temp. in °C	Temperature variations in °C during 120 days after first oviposition	Temperature variations in °C during experiment
			0-30	31-60	61-90	91-120	121-240			
I A	—		—	—	—	—	—	29.5	—	28-31
I B	—		—	—	—	—	—	29.8	—	28-31
II A	10.9±0.9	>0.05	1.1	1.3	1.2	1.4	0.7	20.5	19-25	17-25
II B	11.1±0.8	>0.05	1.4	1.3	1.1	0.9	0.4	20.3	18-22	18-22
III A	10.5±0.9	>0.05	1.6	2.6	1.2	0.1	0.1	13.9	15-22	9-22
III B	10.8±0.7	>0.05	0.5	0.3	0.5	0.4	0.4	14.1	15-20	12-22
IV	13.9±0.9	<0.05	0.2	0.1	0.01	0.2	0.1	15.0	14.5-17	13-17.5

RESULTS AND DISCUSSION

The population from the spring region of Bijela Rijeka commences oviposition late in the spring (Table 1) when the difference of the water temperature between two successive recordings attains 7.5°C. At the beginning of the reproductive season egg-laying is intensive but it gradually diminishes towards the end of the season. The period of oviposition is limited to 2-3 months, as is in accordance with our earlier studies on *Radix peregra* in a small basin not far from the centre of Zagreb (Krkač & Meštrov, 1970). Egg-laying in that locality with distinct seasonal temperature fluctuations was also recorded after the thermic jump in the spring. Egg-laying continues for the next 2-3 months at a higher and unvarying temperature. The onset of oviposition depends on the temperature conditions of a certain year.

The population living in warm, non-captive springs of Tuheljske Toplice with temperatures from 29-32.1°C, lays eggs continuously regardless of the season. However, oviposition is more intensive in February, March and April (Table 2). Literature data point out (Wesenberg-Lund,

1939; Hubendick, 1951; Clench, 1959) that Lymnaeidae lay eggs in spring or summer. Considering such recordings the population of Tuheljske Toplice is quite specific in its reproductive characteristics. It appears that the supposed effect of a thermal jump, supported by research in the spring region of Bijela Rijeka, could not be applied to the present population. Particular behaviour of these animals, i.e. emerging and staying for a considerable time on the surface of a compact layer of algae spread over the basin of the thermal spring, was an incentive to study that phenomenon (Krkač, 1973).

The experiment shows that the migration to the surface layer of algae is not regular with regard to the time of the day. When migrating, the animals are subjected to various temperature shocks due to the time of day or season. The abrupt temperature change produces a permanent stimulus to oviposition.

In fact, these results are in accordance with Van der Steen (1967) indicating that the beginning of breeding of *Lymnaea stagnalis* is a consequence of certain natural temperature conditions. So a sudden rise of temperature stimulates the egg-laying, though not at the very beginning, while a decrease in temperature inhibits it. The stimulative effect of the temperature rise from 20°C to 25-28°C was demonstrated in an experiment of Timmermans (1959), in which 50% of *Planorbis corneus* specimens were laying eggs almost simultaneously in the course of 3 hours.

Studies on the influence of temperature and its variations on first oviposition and the frequency of egg-laying were performed in the laboratory.

The quantity of the egg-mass was defined as the average number of eggs per animal per day (Table 3). It should be noted that the reproductive potential of the experimental animals raised in the laboratory was deficient when compared to that of the animals collected and transferred to the laboratory and maintained in groups in vessels. The animals of the 1st experimental group (IA and IB, Table 3) kept under a temperature regime of 28-31°C and deprived of changing the temperature zone, did not produce a single egg-mass.

Like a rise in temperature, a change in the amount of oxygen also stimulates oviposition in *Lymnaea stagnalis* (Lever, 1946; Timmermans, 1959; Joosse, 1972). Outdoor observations on emerging and staying out of the water of specimens of the Tuhelj population suggest the same effect in *Radix peregra*. Considering the same period, the saturation of oxygen in the spring region of Bijela Rijeka recorded was never less than 81.69% compared to 45.82% in Tuheljske Toplice. Emergence of animals out of the water in the spring area of Bijela Rijeka was never noticed. The experiment in which animals were separately maintained at a temperature from 28-31°C refuted such a possibility for *Radix peregra*. The animals in the experiment were often leaving the water just like those from Tuheljske Toplice. By emerging, the animals contacted an environment rich in oxygen but of the same temperature, since the water in which the containers with the animals were submerged, warmed the layer of air over the vessels. Although the animals could reach an environment abundant in oxygen, they never produced a single egg-mass. The experiment serves as a contribution to the conclusion that a change of the temperature experienced when the snails emerge out of the water, proves to be a significant factor in oviposition.

Group IIA maintained at temperatures from 19-25°C laid eggs in greatest quantities in a period of 240 days but only a small number of egg-masses was normally shaped. The term 'egg-mass' is used indiscriminately whether the empty gel masses were laid with the size of a normal egg-mass (see a, Fig. 1), or shaped like a long irregular ribbon (b and c) or whether an egg-mass contained a normal (d), excessive (e) or small (f) number of eggs. The quantity of egg-laying in experiment IIB was somewhat lower but shape and size of the egg-mass was the same as in experiment A. Average temperatures at which the animals were maintained during experiments A and B in group II differed only 0.2°C, while variations in temperature during the process of egg-laying in group A were 6°C and in experiment B 4°C.

Considerable differences in amount of egg-laying in group III appeared in the 1st (A) and 2nd (B) experiments. The animals of experiment A laid a larger number of eggs in the initial period of 120 days after laying the 1st egg-mass; the amount of egg-laying in experiment B was 1/3 of that in experiment A regardless of whether recordings of egg-laying continued for 120 or 240 days of experiment. The temperature fluctuations in experiment A were 7°C and in B 5°C.

The quantity of egg-laying in the 4th group was only 0.1 in the 1st and 2nd periods of 120 days, i.e. less than in other groups. A number of empty gel masses, shaped like ribbons or



FIG. 1. Egg-masses of *Radix peregra*, group IIA (see text); a, empty gel mass with normal size; b, empty gel mass shaped like a long, irregular ribbon; c, another abnormal empty gel mass; d, egg-mass with normal number of eggs; e, egg-mass with excessive number of eggs; f, egg-mass with small number of eggs (approximately natural size, scale 1 cm).

sometimes like small balls exceeded egg-masses normal in shape and number of eggs. Some of the animals of the 4th group produced egg-masses containing more than the usual number of eggs. The temperature variations for these according to outdoor observations, supposed to act as a stimulus for egg-laying, are here almost half (2.5°C) the smallest variation recorded in experiment II B (4°C).

An analysis of the results of the experiments suggests that the quantity of egg-laying depends on both the amplitude of temperature variation and on the average ambient temperature.

The animals of the 2nd and 3rd group are not particularly distinctive considering the shell-length at the beginning of oviposition. The animals of the 4th group laid eggs considerably later, after having attained greater shell-length than those of the 2nd and 3rd groups.

CONCLUSION

Onset of oviposition in habitats with distinct seasonal temperature fluctuations is caused by a raise of the water temperature in the spring.

Permanent egg-laying of the population of the non-captive basin of Tuheljske Toplice is conditioned by the stimulative effect of the change in temperature, to which the members of the population are exposed all the year round when migrating to the surface layer of algae.

Quantity of egg-laying in an experiment depends on the amplitude of the temperature variations and on the average temperature conditions.

Specimens raised at high temperatures (29.5°C and 29.8°C), and deprived of possibilities to change the temperature zone, laid no eggs at all.

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COMPETITION ENTRE *MELANOPSIS* (GASTROPODA: PROSOBRANCHIA)
ET BASOMMATOPHORES EN ALGERIE: L'ELIMINATION DE
BULINUS TRUNCATUS TRUNCATUS

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ABSTRACT

A 3-year study in Algeria has revealed that there is marked competition between species of the genus *Melanopsis* and Basommatophora, particularly *Bulinus t. truncatus*. *Melanopsis praemorsa* appears to be generally more resistant and prolific than *Planorbarius metidjensis* and *Physa acuta* and tends to replace these in most habitats of both the low-lying and higher plateaus of Tell. It even starts to invade the semi-arid zone of the tablelands of the Atlas Mts. In areas of oases *Melanopsis dufouri* progressively eliminates *Bulinus t. truncatus* in irrigation canals. A reduction in the number of foci of schistosomiasis in the region coincides with this phenomenon. *Melanopsis praemorsa* s.l. has a very wide circummediterranean distribution (North Africa, Asia Minor, etc.) and is therefore obviously well adapted to arid conditions. This species may be of potential value in combatting schistosomiasis and experiments in this line are certainly called for.

INTRODUCTION

En Algérie, l'existence de la bilharziose et de son vecteur privilégié, *Bulinus truncatus truncatus* (Audouin), a été signalée à diverses reprises par Sergent (1939), Pallary (1939), Mandoul & Jacquemin (1953) et Simonet (1959). Sur ce territoire, *Planorbarius metidjensis* (Forbes) peut être aussi considéré comme hôte potentiel de *Schistosoma haematobium* (cf. Mandahl-Barth, 1958).

Cependant, en 1965, Davadie & Metge font état de la régression d'un foyer de bilharziose au Sahara sans, toutefois, en préciser l'origine exacte.

Or, dans la nature, des agents biologiques multiples, étroitement impliqués dans l'équilibre des biocénoses, peuvent agir comme facteurs de régulation démographique des Gastéropodes vecteurs de la bilharziose (Michelson, 1957; Wright, 1966); mais leur efficacité reste limitée dans la majorité des cas. Toutefois, la compétition entre des Gastéropodes vecteurs et non vecteurs, ayant simultanément des exigences écologiques identiques, peut être très sélective.

En Algérie, précisément, un Prosobranchie Thiaridae du genre *Melanopsis* Férussac, non vecteur, tend à éliminer ses concurrents vecteurs, *Bulinus t. truncatus* ou *Planorbarius metidjensis*, de leurs biotopes sahariens ou oranais.

BASOMMATOPHORES ET *MELANOPSIS* EN ORANIE

La faune des Basommatophores en Oranie est représentée par 5 espèces paléarctiques: *Galba truncatula* (Müller), *Physa acuta* Draparnaud, *Planorbarius metidjensis* (Forbes), *Anisus vortex* (L.) et *Ancylus fluviatilis* (Müller).

Cet inventaire diffère nettement de celui de Pallary¹ recensant une trentaine de Basommatophores en Algérie non saharienne (1901-1939). Depuis le Pliocène, il ne semble pas que sa composition ait sensiblement évolué (Flamand, 1911; Pallary, 1901, 1926), les conditions paléoclimatiques elles-mêmes n'ayant guère fluctué depuis le début du quaternaire, dans le Tell et l'Atlas. Les Basommatophores fossiles et subfossiles, par contre, avaient une distribution beaucoup

¹De nombreuses espèces admises par Pallary n'ont pas été reconnues valides ultérieurement.

plus vaste que de nos jours et leur aire de dispersion couvrait les basses et hautes plaines telliennes ainsi que certains secteurs du Sahara septentrional (Chevallier, 1969).

Actuellement, ils végètent dans les Monts de Tlemcen et sur les hauts plateaux de Seb dou (Fig. 1), à une altitude comprise entre 700 et 1000 mètres, à l'exception, cependant, des Ancyles qui fréquentent aussi les oueds des basses et hautes plaines telliennes. Ils occupent une aire beaucoup plus restreinte qu'au début du quaternaire, située entre les isothermes 0°C et 3°C, à climat sub-humide ou semi-aride (Ouled Mimoun, Seb dou), la pluviosité estivale moyenne étant comprise entre 25 et 35 mm ou bien 25 et 31 mm, selon Alcaraz (1969). Pour les Basommatophores oranais et plus spécialement *Planorbarius metidjensis*, ce milieu montagnard, au sens large, est un milieu refuge en limite d'aire, ayant surtout l'inconvénient d'amoin drir leur potentiel évolutif. Leur capacité de reproduction, comparativement à celle de *Melanopsis praemorsa* (L.), reste faible et ils s'isolent en groupements relictuels, composés d'écotypes non différenciés, dans des points d'eau à pH nettement acide (6.5, 6.6) moins favorables à leur croissance que les eaux alcalines (7.1-8.4) des plaines telliennes.

Cet isolement écologique peut s'expliquer par l'action combinée de 3 facteurs:

(1) en premier lieu, par l'action des facteurs anthropozoogènes sur les conditions édaphiques. En effet, il faut tenir compte de l'intensification de la viticulture depuis plus d'un siècle dans les basses et hautes plaines telliennes, de la déforestation abusive, de la pratique de l'incendie et du surpâturage;

(2) en second lieu, par l'influence d'un facteur biologique intrinsèque en rapport avec la moindre résistance des Basommatophores à la sécheresse estivale étalée sur une période de 5 mois;

(3) enfin et surtout, par l'action du facteur de compétition malacologique en rapport avec la similitude des exigences écologiques des Basommatophores et de *Melanopsis praemorsa*.

Melanopsis praemorsa est une espèce euryèce, prolifique, adaptée à tous les degrés d'aridité relative, aux variations thermiques de grande amplitude, et capable de résister à l'inanition. Toutefois, sa tolérance thermique ne lui permet pas de supporter une minimale moyenne inférieure à 0°C et sa pénétration géographique ne paraît pas dépasser l'isotherme 0°C vers le sud. Son aire de dispersion est beaucoup plus étendue que celle des Basommatophores et couvre tous les étages bioclimatiques entre Oran et Seb dou (Fig. 1). Leur fécondité est comparable d'un étage bioclimatique à un autre et leur reproduction n'est interrompue que pendant les 2 mois les plus chauds de l'année (juillet, août). En outre, la castration parasitaire par les Digènes est toujours nettement circonscrite et elle n'affecte pas la progression démographique globale de cette espèce, au demeurant très polymorphe.

Moeurs amphibies, résistance prolongée à la déshydratation des sols, solidité du test operculé, très forte fécondité et très forte densité de population (25/100 cm²), réversibilité des régimes alimentaires ont favorisé la conquête des niches écologiques de *Planorbarius metidjensis* et des autres principaux Basommatophores par *Melanopsis praemorsa*, en Oranie.

BULINS ET *MELANOPSIS* DANS LA VALLEE DE LA SAOURA

La régression d'un foyer de bilharziose dans la vallée de la Saoura a été signalée en 1965 par Davadie & Metge, alors que, selon Simonet, différents foyers de la maladie étaient encore très actifs en 1959.

Or, l'enquête effectuée dans les oasis d'El Aouatta, Hanefid et Agdal, dépendant du secteur de Béni Abbès, de 1972 à 1974, a révélé qu'il n'y avait jamais eu de traitement des puits infectés par un molluscicide ni de contrôle thérapeutique systématique des populations locales.

L'enquête malacologique, par contre, a montré que l'espèce concurrente de *Bulinus t. truncatus*, *Melanopsis dufouri* Férussac s.l., a colonisé tous les biotopes où se trouvait le Bulin, il y a encore une dizaine d'années. L'élimination du Bulin de la majorité des points d'eau de la vallée de la Saoura, dans le secteur de Béni Abbès, a fortement contribué à faire régresser l'endémie.

CONCLUSIONS

L'aire géographique de *Melanopsis praemorsa* et de ses formes vicariantes couvre le bloc hispano-mauresque (Pérès, 1939, 1943-1945), la Palestine, la Syrie (Germain, 1921), la Grèce, les

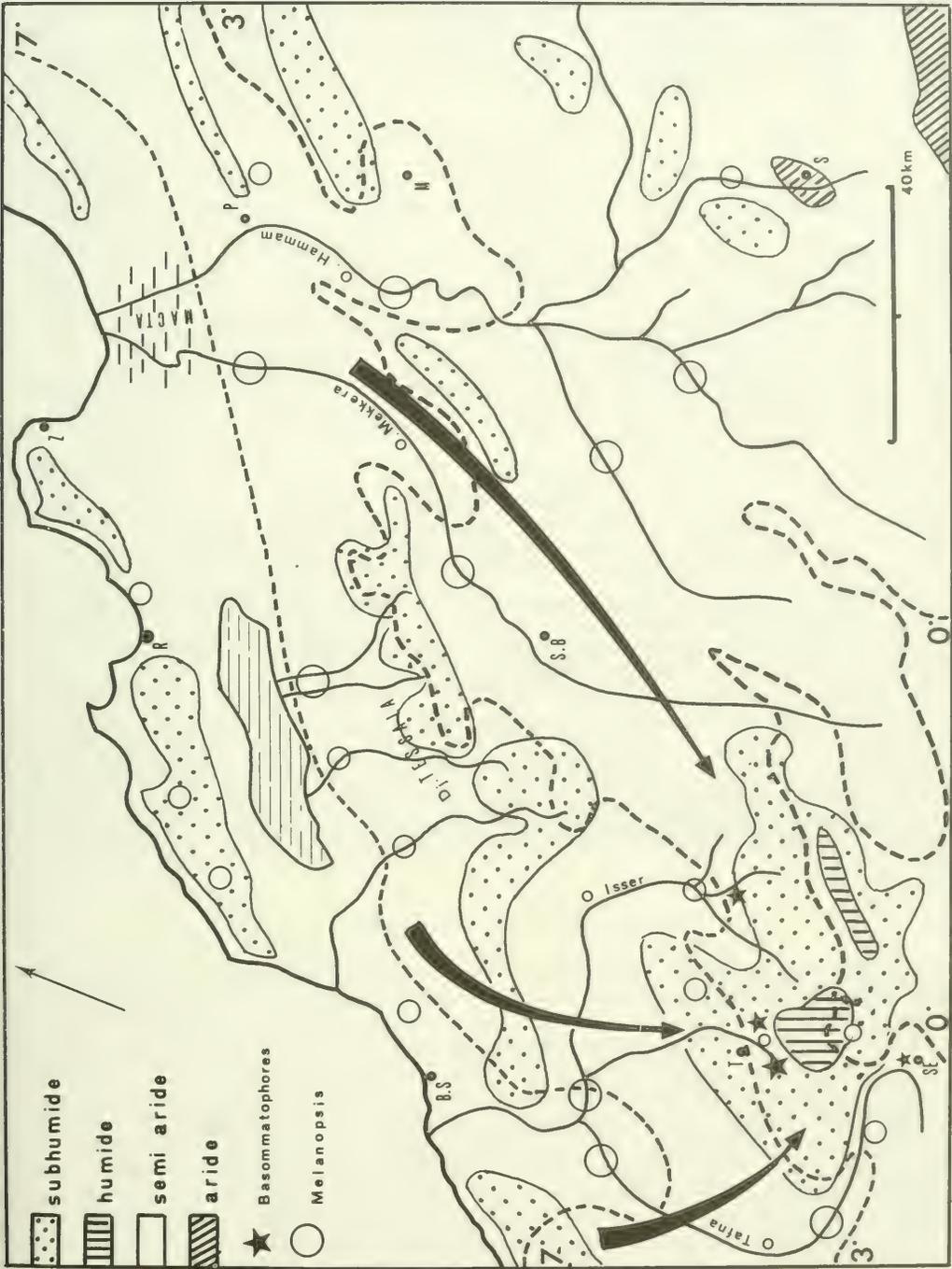


FIG. 1. Répartition des Basomatophores et des *Melanopsis* en Oranie en fonction des étages bioclimatiques (nomenclature d'Alcaraz, 1969). Le relict nord-sud des Basomatophores en direction des monts de Tlemcen (T) est représenté par 3 flèches. B.S: Béni Sat; M: Mascara; P: Mohammadia; R: Oran; S: Saïda; S.B: Sidi Bel Abbès; SE: Sebdou; Z: Arzew.

régions méridionales de la Russie (Zhadin, 1952) et se morcelle au niveau du Sahara. Son extension déborde, au nord-est de l'Asie Mineure, l'aire de dispersion de *Bulinus t. truncatus*, hôte intermédiaire de la bilharziose hématurique, comme *Planorbarius metidjensis*.

L'élimination de *Bulinus t. truncatus* dans le bassin de la Saoura et la régression des niches à *Planorbarius metidjensis* en Oranie, incitent à l'utilisation du taxon *Melanopsis* dans la lutte biologique contre les Basommatophores responsables de la transmission de la bilharziose hématurique dans la zone circumméditerranéenne.

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THE POPULATION DYNAMICS OF THE PULMONATE SNAIL
BULINUS (PHYSOPSIS) AFRICANUS (KRAUSS)

The influence of temperature on mass increase and survival

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ABSTRACT

Cohorts of the freshwater snail *Bulinus (Physopsis) africanus*, an intermediate host for human schistosomes, were reared in a series of aquaria at different constant temperatures. Records of their increase in mass and their mortality were kept. Mathematical functions reflecting the mass increase and survival at any age at different constant temperatures, were developed. From these a model, expressing the biomass increase at any temperature regime, was formulated.

INTRODUCTION

In South Africa *Bulinus (Physopsis) africanus* acts as an intermediate host of the flukes *Schistosoma haematobium* (Bilharz) and *S. mattheei* Veglia & Roux, both of which parasitise man (Wright, 1971). This snail species is distributed mainly in the temperate to tropical climatic regions of South Africa in a strip along the eastern seaboard and bordering through the Lowveld and the mid-Transvaal highveld regions (Van Eeden et al., 1965). The health hazard associated with its presence makes it imperative that its ecology be studied in detail.

One of our principal objectives in the study on the dynamics of this snail species is to understand how various environmental factors determine and regulate the size and age composition of the population. It is hoped that, eventually, it might be possible to predict accurately changes in the population variables, using information on changes in environmental factors. The present paper concerns only a part of this broader objective and a mathematical model is formulated whereby the biomass of a cohort of snails under any given temperature regime can be estimated.

MATERIALS AND METHODS

Thirty-five eggs laid overnight by snails collected from a natural habitat were kept at a constant temperature in an experimental aquarium, where they hatched and lived for the duration of their natural life. Six such cohorts of snails were maintained simultaneously under similar environmental conditions except that the temperatures were rigidly controlled at different levels viz. 17, 20, 23, 26, 29 and 32°C. The details of the aquaria and the daily feeding and maintenance procedures are given by De Kock (1973). Apart from the daily recording of the age and mortality of the snails we measured the mass increase of the cohort, or what remained alive of it, every 14 days by means of a procedure described by De Kock (1973). In order to reduce the physical disturbance of the snails to a minimum it was decided not to weigh the snails individually. The total mass was divided by the number of snails still alive at each weighing to obtain a mean mass value for the individuals of the cohort at the successive ages. The mean mass values obtained towards the end of the experiment were disregarded in this analysis as these were based on too few snails and are therefore unreliable.

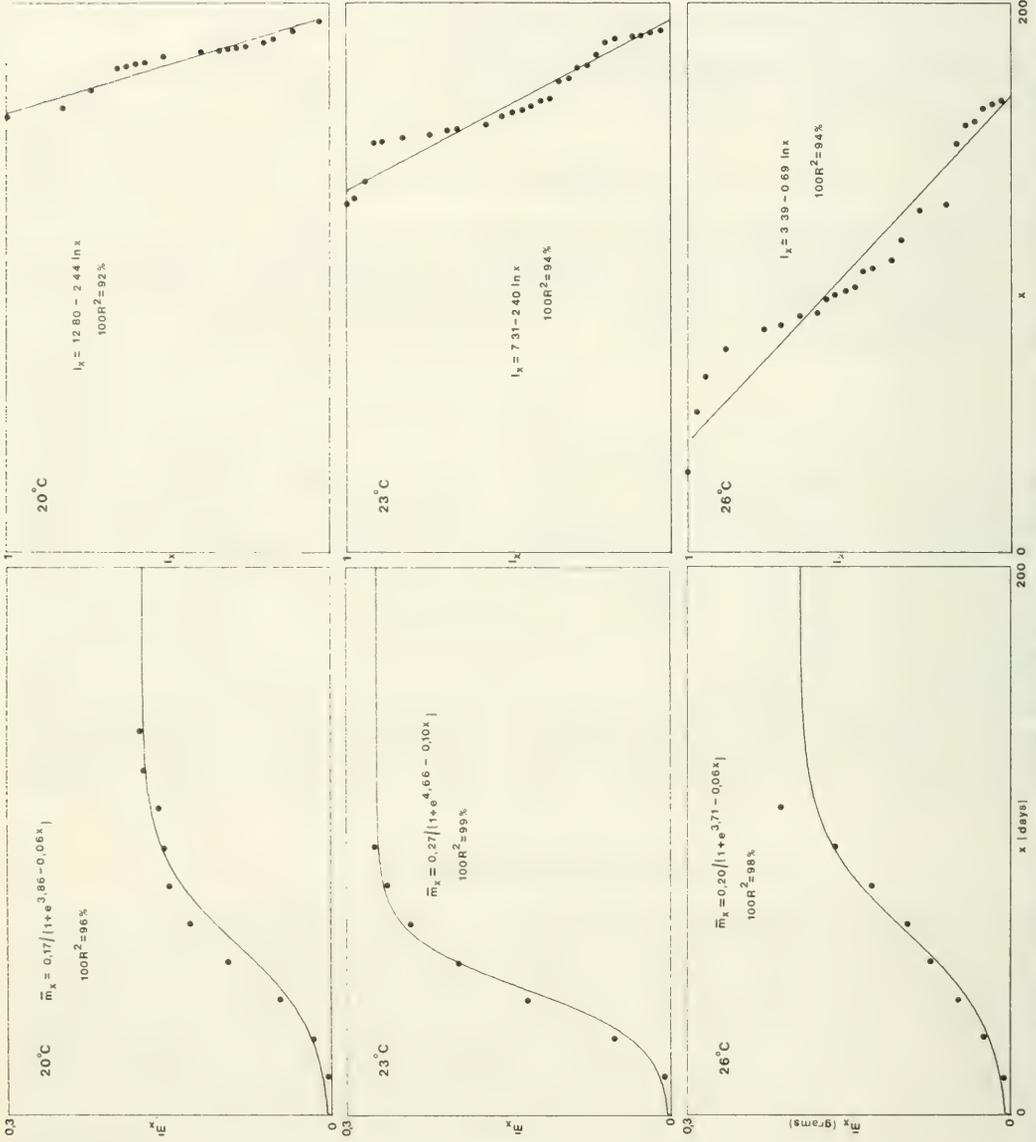


FIG. 1. The mean mass (\bar{m}_x) at fortnightly intervals and daily survival (I_x) of *Bulinus (Physopsis) africanus* at three different constant temperatures (T). Curves calculated by means of the given equations are superimposed on the data. The abscissa of the survival data is in natural logarithms.

RESULTS

At only 3 of the temperatures (20, 23 and 26°C) the snails hatched and grew satisfactorily. At the other temperatures they either failed to hatch or the few that did, failed to grow at all and died within a short period after hatching. Obviously, these temperatures (17, 29 and 32°C) were outside their tolerance range both for hatching and growth. In Fig. 1 the mean mass is plotted against their relevant ages for the temperatures at which measurable mass increases occurred. In the same figure the proportion surviving (l_x = snails alive/35 at age x days) is similarly given.

THE MATHEMATICAL MODEL AT CONSTANT TEMPERATURES

The data in Fig. 1 reveal that the increase in mass roughly follows the sigmoid pattern found for most animals. This is particularly evident for the snails reared at 23°C. In an attempt, therefore, to find an empirical description for our results, it was decided to fit to the data a logistic equation of the form

$$\bar{m}_x = K/(1 + \exp. (b - ax)) \tag{1}$$

in which m_x = the mean mass per specimen of the snails at any given age (x), K = the upper asymptote of mass and a and b represent coefficients of the curve (Fig. 1). The relation between mean mass and temperature seems to be such that as the temperature increases, it initially promotes growth up to a maximum after which further temperature increases inhibits growth. Such a relation might be described by a downward opening parabola. A parabolic function between temperature (T) as the independent and the coefficients K , a and b of the growth curve as the dependent variables yielded the following results:

$$\begin{aligned} K(T) &= -0,0096 T^2 + 0,4456 T - 4,8928 \\ a(T) &= -0,0046 T^2 + 0,2112 T - 2,3168 \\ b(T) &= -0,0969 T^2 + 4,4299 T - 45,9878 \end{aligned}$$

If, in equation (1), the coefficients be replaced by these functions of temperature, then an empirical description of the influence of a life-long constant temperature on the mean mass increase is obtained,

$$\bar{m}_x = K(T)/(1 + \exp. (b(T) - a(T)x)). \tag{2}$$

The survival (l_x) of the snails can be described empirically by a model of the form

$$l_x = q - p \ln x. \tag{3}$$

The coefficients q and p of this equation were estimated from the data and are given in Fig. 1. Once again, because of the trend of change of the coefficients from the lower to the higher temperatures, parabolic functions between the coefficients and temperature were calculated:

$$\begin{aligned} q(T) &= 89,7092 - 5,5970 T + 0,0876 T^2 \\ p(T) &= -17,9578 + 1,1486 T - 0,0186 T^2 \end{aligned}$$

Substituting these coefficients as functions of temperature into equation (3) the following survival model dependent on temperature is obtained:

$$l_x = q(T) - p(T) \ln x. \tag{4}$$

The mean mass will increase indefinitely as no provision is made for the eventual mortality of the snails in equation (3). To account for this eventually equations (2) and (4) must be combined in some way. To do this, consider the equation for the increase in numbers of a population with a stable age distribution in a limitless environment (Birch, 1948).

$$N_x = N_0 \exp. (rx) \tag{5}$$

where N_x = the number of animals with age x , and r = intrinsic rate of natural increase.

Separating the birth and survival components of this equation and converting it to biomass by multiplying the number of animals by their mean mass (\bar{m}_x), an expression is obtained for the biomass (B_x) of the population,

$$B_x = \bar{m}_x N_o \exp. (bx) \exp. (-dx). \quad (6)$$

This equation evidently proportions the number of animals (N_o) between births (exp. (bx)) and survival (exp. (-dx)) in terms of mass. If a fixed number of animals, say 100, of the same age is surmised for the population and we postulate that there are no births (exp. (bx) = 1) and substituting our symbol l_x for the proportion surviving, the equation becomes a means whereby the biomass changes in a cohort of animals may be traced as they become progressively older

$$B_x = 100 \bar{m}_x l_x. \quad (7)$$

By substituting our formulations for mass increase (equation (2)) and survival (equation (4)) into equation (7) the combination we set out to do is completed,

$$B_x = 100(K(T)/(1 + \exp. (b(T)-a(T)x))) (q(T)-p(T)l_x). \quad (8)$$

The function $K(T)$ determines the upper asymptote for mass increase and represents, so to speak, the maximum mass to which any individual snail can grow at the particular temperature. It is obvious that negative values for $K(T)$ have no meaning in that an animal cannot grow smaller. The mass increase factor of equation (8) is, therefore, only applicable when $K(T)$ is positive. In addition, the proportion survival can only lie between 0 and 1. As formulated in equation (8) it can extend beyond these values. The survival factor of equation (8) must, therefore, be limited to these values which occur at the ages

$$\begin{aligned} q(T) - p(T)l_x &= 1 \\ l_x &= (q(T)-1)/p(T) \\ x &= \exp. ((q(T)-1)/p(T)) \end{aligned} \quad (9)$$

$$\begin{aligned} \text{and } q(T) - p(T)l_x &= 0 \\ l_x &= q(T)/p(T) \\ x &= \exp. (q(T)/p(T)). \end{aligned} \quad (10)$$

With all these constraints the model finally becomes

$$\begin{aligned} K(T) &\leq 0 \\ (\text{no mass increases}) \end{aligned}$$

$$B_{x+1} = \begin{cases} B_x \text{ iff } x < \exp. ((q(T)-1)/p(T)) \\ B_x l_x \text{ iff } \exp. ((q(T)-1)/p(T)) \leq x < \exp. (q(T)/p(T)) \\ 0 \text{ iff } x \geq \exp. (q(T)/p(T)) \end{cases}$$

$$\begin{aligned} K(T) &> 0 \\ (\text{mass increases}) \end{aligned}$$

$$B_{x+1} = \begin{cases} N_o \bar{m}_x \text{ iff } x < \exp. ((q(T)-1)/p(T)) \\ N_o \bar{m}_x l_x \text{ iff } \exp. ((q(T)-1)/p(T)) \leq x < \exp. (q(T)/p(T)) \\ 0 \text{ iff } x \geq \exp. (q(T)/p(T)). \end{cases} \quad (11)$$

With this model the biomass of a cohort of snails reared life-long at a constant temperature can be calculated at any age provided that temperature is the only governing factor (Fig. 2). Some insight can be gained into the effect of temperature on survival and mass increase of *B. (P.) africanus* if the restraints of the model are plotted as in Fig. 3. In this figure the shaded area represents the zone in which mortalities may be expected to occur in accordance with the equation given in the shaded area. To the left of the shaded area there should be no mortalities ($l_x = 1$) and to the right of the shaded area there should be no survivors ($l_x = 0$). In addition a zone is demarcated in which the prevailing temperatures (in this case 18 to 28°C) will permit mass increases to take place in accordance with the equations given on the right hand side of the figure. The most striking feature of this figure, however, is the fact that the longest lived cohort, before any deaths occur, is to be expected towards the lower temperatures whereas the longest lived individuals of a cohort should be found at approximately halfway between 18 and 28°C. It, therefore, seems that the temperature optimally suited for the survival of an entire cohort is lower than the temperature optimally suited for growing to a larger size by an individual specimen in a cohort.

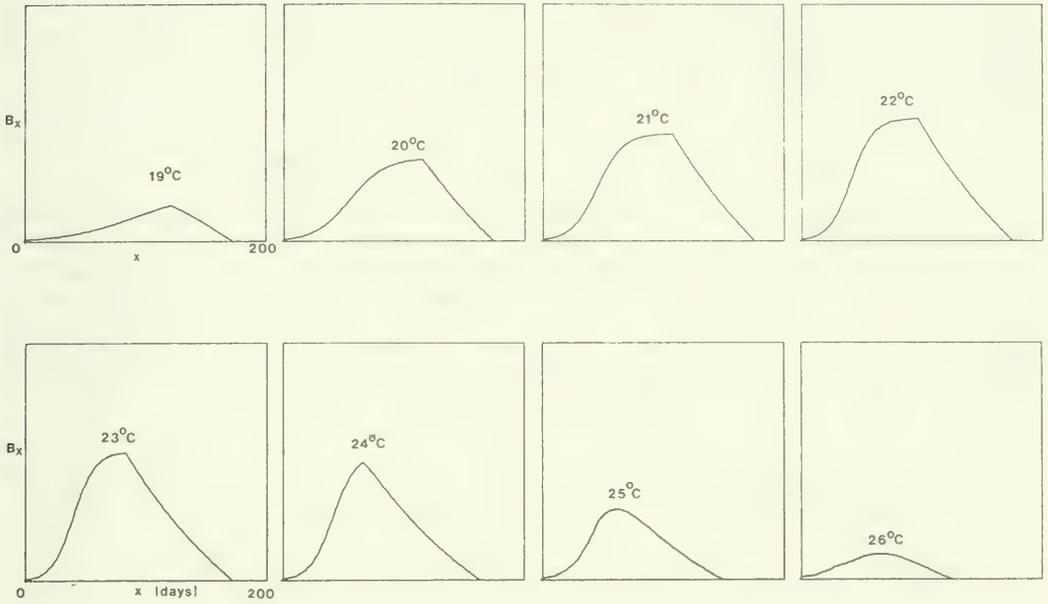


FIG. 2. The biomass (B_x) of a cohort of a 100 snails generated by the model given in the text in one day age (x) intervals.

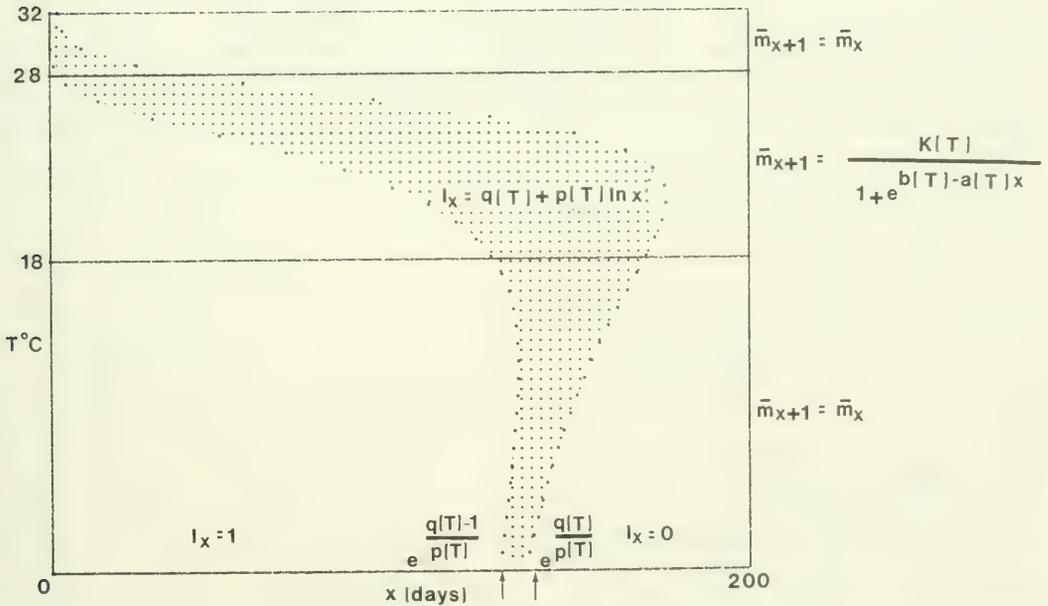


FIG. 3. The relation between survival (I_x), mean mass (\bar{m}_x), age in days (x) of the snails and various constant temperatures (T).

The model presented and described so far was constructed from results obtained at only 3 temperatures viz. 20, 23 and 26°C and it is particularly significant that it predicts that no growth is to be expected at constant temperatures of 17, 29 and 32°C. These predictions are in full agreement with our experimental findings at the latter temperatures. This circumstance might be taken to testify to the reliability of the model.

A MODEL AT CONSTANTLY CHANGING TEMPERATURES

The model developed so far is, of course, only valid for those instances where the temperatures are kept constant for the duration of the animal's life. In nature, however, the snails are subjected to constantly changing temperatures not only diurnally but also seasonally.

One is, consequently, confronted with the problem of having to use the information obtained at constant temperatures to predict what would happen if the temperatures were constantly changing. To do this we can, fortunately, resort to a well established mathematical procedure in which the biomass at the beginning of any time period together with the slope of the biomass curve during that period is used to calculate the biomass at the end of the period as follows:

$$B_{x + \Delta x} = B_x + (\partial B_x / \partial x) \Delta x. \quad (12)$$

To find the slope of the biomass curve ($\partial B_x / \partial x$) one may proceed as follows:

$$\begin{aligned} B_{x + \Delta x} &= B_x + (\partial B_x / \partial x) \Delta x \\ &= B_x + n_o (\partial \bar{m}_x l_x / \partial x) \Delta x \\ &= B_x + N_o (\bar{m}_x \partial l_x / \partial x + l_x \partial \bar{m}_x / \partial x) \Delta x \\ &= B_x + N_o (\bar{m}_x (-p(T)/x) + l_x \bar{m}_x a(T) (1 - \bar{m}_x / K(T))) \Delta x. \end{aligned} \quad (13)$$

An important assumption in this formulation is that the temperature remained constant during the period selected for the change in age (Δx). Obviously, the shorter the period Δx , the more reliable is the assumption that the temperature remained constant during this time. For instance, the biomass estimate would be more reliable on the basis of an hourly change ($\Delta x = 1/24$) than on the basis of a daily change ($\Delta x = 1$) because the hourly change of water temperature in a natural habitat is negligible compared to the diurnal change.

Again, certain parts of the model operate only between certain limits as explained for equation (11). In addition it may happen that at any given time the biomass exceeds the value which the model predicts ($K(T) \leq \bar{m}_x$) for the temperature prevailing at that time. This suggests a decrease of biomass which, of course, is impossible except, amongst others, through the effect of mortality, egg production and erosion of the shell.

A model dependent on the age of the snail (x), the change in their age (Δx) and any temperature (T) regime can, therefore, formally be written as:

1. $K(T) \leq 0$ and/or $K(T) \leq \bar{m}_x$
 - 1.1 $B_{x + \Delta x} = B_x$
iff $x < \exp. ((q(T) - 1)/p(T))$
 - 1.2 $B_{x + \Delta x} = B_x + B_x (\partial l_x / \partial x) \Delta x$
 $= B_x + B_x (-p(T)/x) \Delta x$
iff $\exp. ((a(T) - 1)p(T)) \leq x < \exp. (q(T)/p(T))$
 - 1.3 $B_{x + \Delta x} = 0$
iff $x > \exp. (q(T)/p(T))$
2. $K(T) > 0$ and $K(T) > \bar{m}_x$
 - 2.1 $B_{x + \Delta x} = B_x + (\partial \bar{m}_x / \partial x) \Delta x$
 $= B_x + N_o \bar{m}_x a(T) (1 - \bar{m}_x / K(T)) \Delta x$
iff $x < \exp. ((q(T) - 1)/p(T))$
 - 2.2 $B_{x + \Delta x} = B_x + N_o (\bar{m}_x (-p(T)/x) + l_x \bar{m}_x a(T) (1 - \bar{m}_x / K(T))) \Delta x$
iff $\exp. ((a(T) - 1)/p(T)) \leq x < \exp. (q(T)/p(T))$
 - 2.3 $B_{x + \Delta x} = 0$
iff $x > \exp. (q(T)/p(T))$.

(14)

The model is only valid for snails of the same age and predicts the average trend only. In a natural habitat, however, snails with a whole range of different ages can be found at any time. Obviously, therefore, births as a function of temperature must also be incorporated into a model of this kind. If this could be done the restriction that the model can be applied only to a single cohort of snails would fall away. The model could be made still more powerful if a measurement of the variability of the mass in snails of the same age as reported by Prinsloo & Van Eeden (1973) could be incorporated into it.

ACKNOWLEDGEMENTS

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DISTRIBUTION OF FRESHWATER MOLLUSCS IN
MOUNTAIN STREAMS OF TROPICAL INDO-PACIFIC ISLANDS
(MADAGASCAR, CEYLON, NEW CALEDONIA)

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ABSTRACT

The distribution of freshwater molluscs between the head-waters and the mouths of mountain streams of the tropical continental islands of Madagascar, Ceylon and New Caledonia is discussed. The occurrence of the species of typical freshwater families in the different parts of running waters of the different Indo-Pacific islands is compared.

During several hydrobiological missions to tropical, high-elevated continental islands in the Indo-Pacific (Starmühlner, 1962, 1968, 1969, 1970, 1973, 1977; Costa & Starmühlner, 1972) the distribution of freshwater molluscs between the head-waters and mouths of mountain streams was studied. Collections were made qualitatively and quantitatively ($1/16 \text{ m}^2$ - 1 m^2) at selected points of the streams. In connection with the collections ecological factors of the habitat, such as velocity of the current, temperature of the water, bottom material (mud, sand, gravel, boulders, rocks), aquatic vegetation, and chemistry of the water [hardness, pH, SBV(alkalinity), electrolytic conductivity, etc.] were studied (Weninger, 1968, 1972).

MADAGASCAR (Fig. 1)

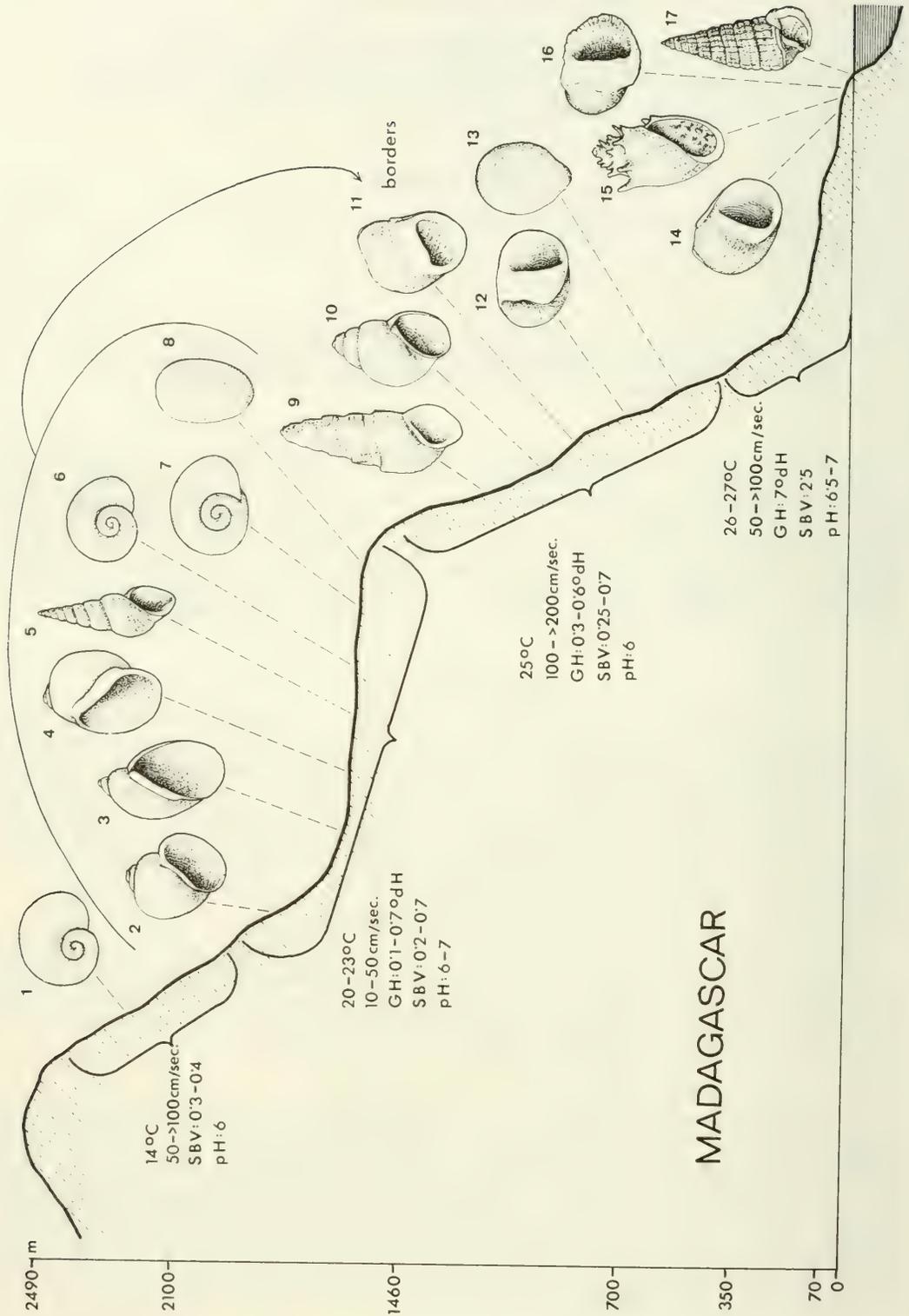
The old continental island of Madagascar consists of a Precambrian granitic socle in the centre, interrupted by later volcanic eruptions. On the east coast the central highland slopes in a steep gradient to the Indian Ocean. This part is covered with the last remnant of primary rain forest. On the west coast adjacent to the Mozambique Channel, the slope is less steep and the coast consists of sediments originating from the Triassic to the Tertiary or Quaternary. The mountains in the Precambrian centre reach up to 2656 m, the high plateau is about 1000 to 1500 m high and today shows a steppe-like landscape. The former subtropical woods were destroyed by burning and clearing over the last centuries.

(A) Head-waters in the central mountains (2500-1500 m).

Torrents originate in mountain forests, partly flowing through open landscapes with mountain shrubs and bushes. The bottom contains rocks and boulders, near the margins and pools between cascades it contains sand, mud and vegetable debris; water temperature between 11° and 16°C ; current 50 cm/sec-more than 1-2 m/sec; chemistry, SBV 0.3-0.4, pH 6. Species found: *Afrogyrus* nov.sp. (below flat stones near the margin).

(B) Highland streams (1500-1000 m).

Flowing through highland steppes, partly cultivated land with paddy fields. Bottom: muddy stones, sand, mud, vegetable debris; water temperature between 20° and 23°C ; current 10-50 cm/sec; chemistry, hardness 0.14°dH to 0.7°dH , SBV 0.20-0.70, pH 6-7. Species found: *Pila cecillei*, *Melanoides tuberculata* (only in waters with a hardness of more than 1°dH), *Lymnaea (Radix) natalensis hovarum*, *Bulinus liratus*, *Afrogyrus crassilabrum*, *Biomphalaria madagascariensis*, *Ferrissia (Pettancylus) modestus*. All species found also occur in still waters; *Ferrissia (Pettancylus) modestus* is found exclusively on the lower surface of floating water-plants, wood etc. in a slow current of 30 cm/sec. In the mountain and highland streams no forms typical of running waters were found.



(C) Torrents and cascade streams of the steep slope of the N.W. and E. coast (1000-100 m).

Flowing in waterfalls and cascades through primary rainforest; between the steplike cascades, occasional deep pools. Bottom: rocks, boulders; near the margins and in the pools: sand with vegetable debris; water temperature between 23° and 25°C; current: 1 m/sec-more than 2 m/sec (near the margin: 30-50 cm/sec, in pools: 0-20 cm/sec); chemistry, hardness 0.3-0.6°dH, SBV 0.25-0.7, pH 6. Species found: (a) on rocks and boulders (1-2 m/sec), *Melanatria fluminea*, *Cleopatra* spp. (such as *madagascariensis*, *colbeaui*, *grandidieri*), mostly behind and below the boulders, protected against the strong current; (b) near the margins and in the pools between cascade zones (10-30 cm/sec), *Pila cecillei*, *Melanoides tuberculata* (if the hardness is more than 1°dH), *Lymnaea (Radix) natalensis hovarum*, *Bulinus liratus*, *Afrogyrus crassilabrum*, *Biomphalaria madagascariensis*.

(D) Lower courses of streams near the N.W. and E. coast (100-1 m).

Flowing through primary and secondary forests, partly through cultivated land. Bottom: boulders, gravel, near the margins and in the pools between cascade zones: sand, mud, vegetable debris; water temperature 26-27°C; current 50 cm-1 m/sec; chemistry, hardness up to 7-8°dH, SBV up to 2.5, pH 6.5-7. Species found: on the surface of the boulders, *Septaria borbonica*; on the sides of and below the boulders, *Neritina (Vittina) gagates*, *Neritina pulligera knorri*.

(E) Transition between lower courses and the mouths of the streams, partly under influence of brackish water during high tide (10-0 m).

Flowing through cultivated areas and near the coast in connection with the mangrove zone. Bottom: rarely boulders, gravel, sandy-mud, sometimes near the coast dead coral blocks; water temperature 27-28°C; current 50 cm/sec, near the margins 0-20 cm/sec; chemistry, during high tide slightly brackish, during low tide the same as in the lower courses of the streams. Species found: *Clithon spiniperda*, *Clithon coronata (= longispina)*, *Neritina (Neripteron) auriculata* (below stones), *Thiara amarula*.

(F) Transition region of the mouths in the littoral zone of the sea (0 m).

Ecological factors as in (E), except that brackish water also occurs during low tide; many marine animals occur here. Species found: *Cerithidea decollata*.

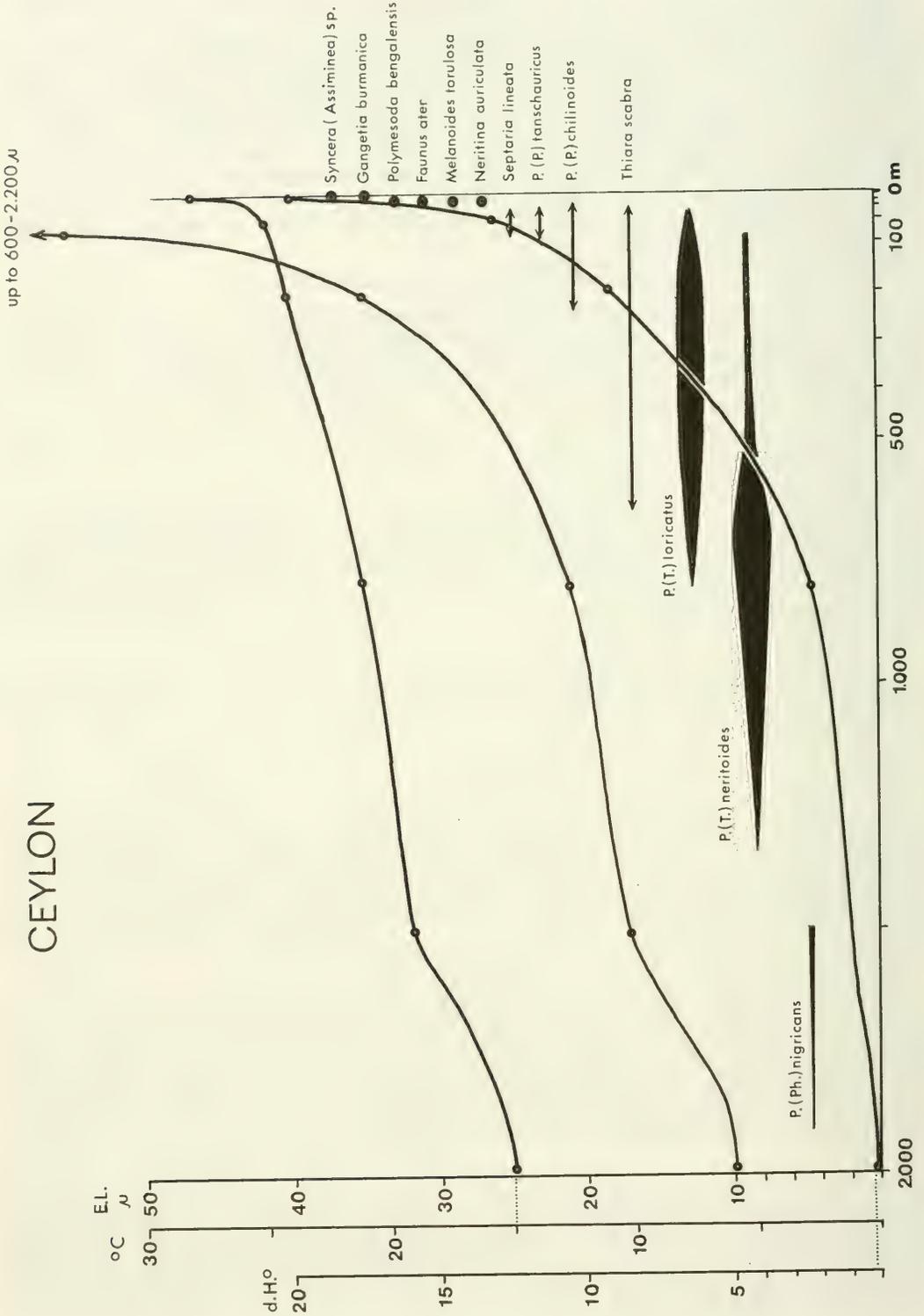
CEYLON (Fig. 2)

The island is a detached part of the continental Deccan plateau of ancient Precambrian crystalline rocks. The southern mountains with a general elevation of 1400-1800 m, are surrounded by 2 peneplains, the upland from 700/500 m to about 150 m, and the lowland from 150/100 m to the coastal zone. Due to their relatively short courses the running waters of the highlands have steep falls. These cause high current velocities of between 1 to more than 2 m/sec.

(A) Head-waters in the central mountains (2000-1500 m).

Head-water torrents flowing through foggy mountain forests, very much shaded. Bottom: granitic rocks, boulders, gravel, near the margins and in the pools between the cascades: sandy with vegetable debris; water temperature 15-19°C; current 1 m/sec-more than 2 m/sec, except near the margins and in the pools between cascades below 50 cm/sec; chemistry, conductivity 8-17 μ Siemens, hardness 0.6-2.35°dH, pH 6.5-6.8 (if hardness below 0.1°dH molluscs are absent). Species found: *Paludomus (Philopotamis) nigricans*.

FIG. 1. Cross section of the east coast of Madagascar with distribution of the freshwater gastropods: (1) *Afrogyrus* n.sp., (2) *Pila cecillei*, (3) *Lymnaea (Radix) natalensis hovarum*, (4) *Bulinus liratus*, (5) *Melanoides tuberculata*, (6) *Afrogyrus crassilabrum*, (7) *Biomphalaria madagascariensis*, (8) *Ferrissia (Pettancyclus) modestus*, (9) *Melanatria fluminea*, (10) *Cleopatra madagascariensis*, (11) *Neritina (Vittina) gagates*, (12) *Neritina pulligera knorri*, (13) *Septaria borbonica*, (14) *Clithon spiniperda*, (15) *Thiara amarula*, (16) *Neritina (Neripteron) auriculata*, (17) *Cerithidea decollata*.



(B) Upper courses of the mountain streams (1500-800 m).

Flowing through primary or secondary rain forests, which are partly cleared with tea estates covering the slopes. In such cases the direct sunshine is reflected by higher water temperatures. Bottom: granitic rocks, boulders, gravel; near the margins sandy banks, also in the pools between the cascade zones; in forest areas thick layers of vegetable debris; water temperature 19-21°C; current 1 m/sec-more than 2 m/sec, near the margins and in the pools between the step-like cascades below 50 cm/sec; chemistry, conductivity 17-21 μ Siemens, hardness 2.3-9.2°dH, pH 6.5-7. Species found *Paludomus (Tanalia) neritoides*, and rarely *Paludomus (Philopotamis) sulcatus*, *Paludomus (Philopotamis) regalis*, *Tricula montana*.

(C) Middle courses of the mountain streams (800-200/150 m).

The valleys and hills of the upland are covered with secondary rain forest and cultivated areas (tea estates in the higher parts, rubber plantations in the lower parts, paddy fields in the valleys—influence of fertilizer and sewage from the densely populated villages and towns). Bottom: sometimes granitic rocks, but mostly boulders and gravel, near the margins sandy and muddy with vegetable debris, near settlements laundry places and polluted areas; water temperature 21-24°C; current 50 cm-1 m/sec, in longer sections and near the margins the current is slower than 50 cm/sec; chemistry, conductivity 35-300 μ Siemens, hardness 9-13°dH, pH 6.5-7. Species found: (a) parts with fast currents, *Paludomus (Tanalia) loricatus*; (b) near the margins and in parts with a slight current, *Thiara (Plotia) scabra*, *Melanooides tuberculata* and stillwater forms, such as *Bithynia (=Bulimus) stenothyroides* and *Indoplanorbis exustus*.

(D) Lower courses of the mountain streams (200/150-50/10 m).

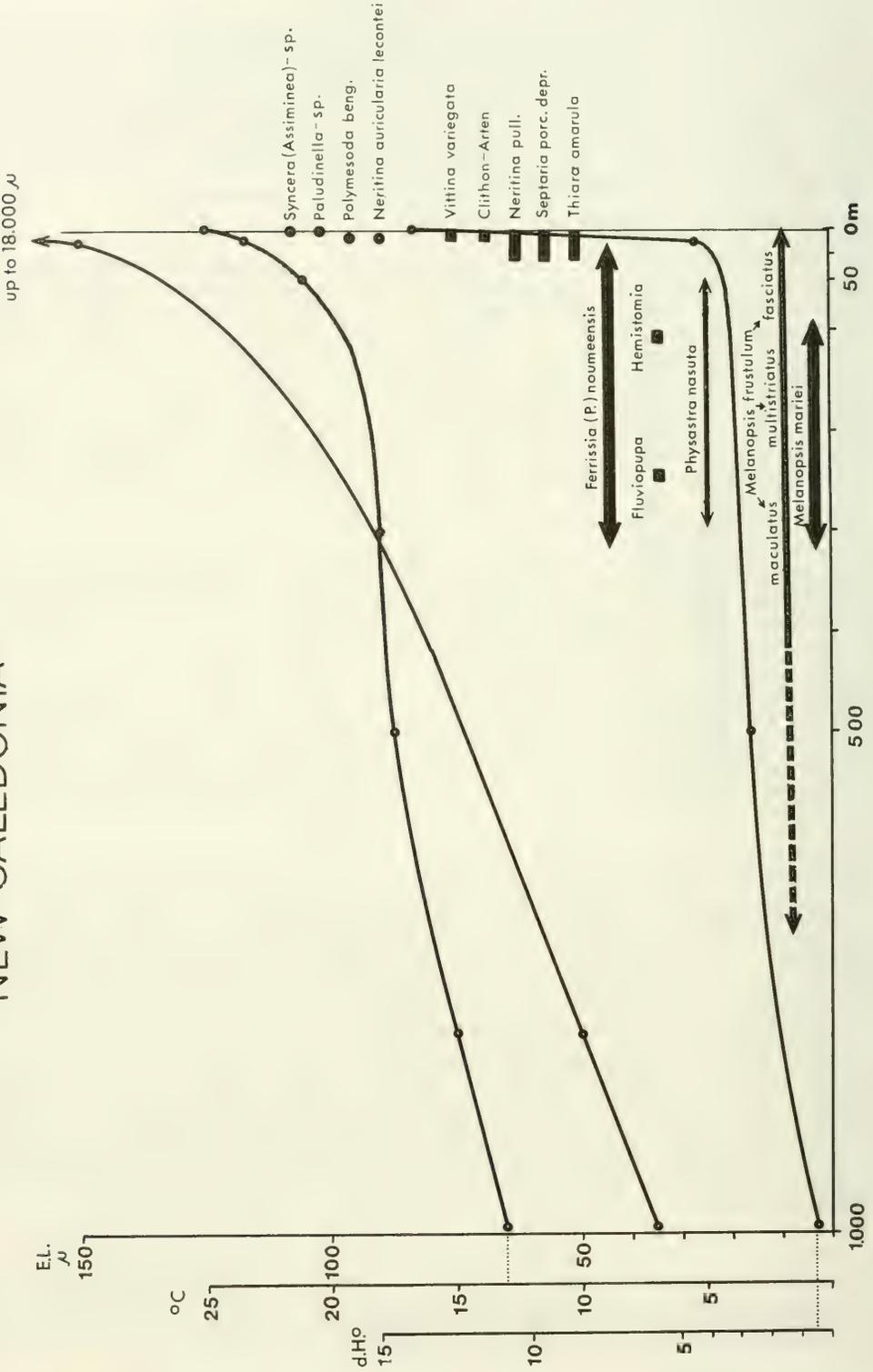
In the low country the running waters cross partly secondary forest, plantations and paddy fields. Longer stretches of the streams are sometimes highly polluted as a consequence of running through cultivated areas with a high population density. Bottom: rarely boulders, more gravel and sand, near the margins sometimes thick layers of organic mud, dense vegetation of submerged water plants; water temperature 25-26°C; current 30-50 cm/sec, near the margins and longer stretches in the middle of the stream 10-20 cm/sec; chemistry, conductivity 300-600 μ Siemens, hardness 13-20°dH, pH 7. Species found: (a) parts with moderate current, *Paludomus (Tanalia) loricatus*; (b) parts with a slow current and near the margins, *Paludomus (Paludomus)* species, such as *P. chilinooides*, rarely *P. bicinctus*, *P. decussatus*, *P. inflatus* (in N. Ceylon also *P. tanschauricus* s.s. and subspecies *nasutus*), *Thiara (Plotia) scabra*, *Melanooides tuberculata* and stillwater forms such as *Bithynia (=Bulimus) inconspicua* and *Indoplanorbis exustus*.

(E) Transition of lower courses to the mouths of the lowland streams, upstream of the limit of brackish water during high tide (50/10-0 m).

The coastal region of the lowlands is very densely populated in southwestern Ceylon, but only sparsely in the north and east. The villages are surrounded by paddy fields, plantations of tropical fruits and particularly coconut palms. The streams carry muddy sediments and near the settlements they are polluted with organic material. The margins have a dense vegetation of submerged plants. Bottom: gravel, but mostly sandy-muddy, in creeks thick layers of vegetable debris are deposited; water temperature 26-28°C; current 10-30 cm/sec, almost no current near the margins with the creeks; chemistry, conductivity 600 μ Siemens (and more), hardness 13-15°dH, pH 7-7.5. Species found: (a) moderate current, *Septaria lineata*, *Paludomus (Paludomus)* species, such as *P. chilinooides* and in N. Ceylon *P. tanschauricus*, and the mussels *Lamellidens lamellatus*, *L. testudinarius* and *Parreysia corrugata*; (b) slow current or stagnant water in creeks near the margins, *Thiara (Plotia) scabra*, *Melanooides tuberculata* and stillwater forms such as *Bithynia (=Bulimus) inconspicua* and *Indoplanorbis exustus*, sometimes also mussels as listed above.

FIG. 2. Diagram showing temperature, hardness and conductivity of the water correlated with the distribution of the gastropods and bivalves between the head-waters and mouths of the mountain streams of southern Ceylon (temperature in °C; hardness in °dH; conductivity, $E_{l,0}$, in μ Siemens).

NEW CALEDONIA



(F) Mouth region with influence of brackish water during high tide (0 m).

During high tide the back flow of brackish water reaches the mangrove areas in the mouth region of the streams and the inhabitants of this habitat are covered with brackish water during this period. At low tide fresh water is running down to the sea. In these areas there are many immigrants from the sea such as marine bivalves and snails, hermit crabs, shrimps, *Periophthalmus*, etc. Bottom: gravel and sand, mostly stones mixed with dead coral and empty shells, rich vegetation of filamentous algae adapted to brackish water; water temperature 28°C; current during low tide 30-50 cm/sec, near the margins 0-10 cm/sec, back current of 10-20 cm/sec during high tide; chemistry, conductivity during high tide under the influence of brackish water up to more than 2000-3000 μ Siemens, hardness up to 20° dH and more, pH 7-8. Species found: (a) moderate current, *Neritina (Neripteron) auriculata*, under stones; (b) slow current, *Melanoides (Stenomelania) torulosa* (with free living veliger larvae), *Faunus ater*, in N. Ceylon also *Gangetia burmanica*, *Syncera (=Assimineae)* species; (c) in muddy sand of bigger streams, *Polymesoda bengalensis (=ceylanica)*.

NEW CALEDONIA (Figs. 3, 4)

This continental island between eastern Australia, New Guinea and New Zealand is an old continental socle superimposed by sediment deposits, metamorphic rocks and volcanic effusion material, such as peridotite, basalt and serpentine. The island is about 400 km long, but has a maximum width of only ca. 50 km. The central mountain range along the spine of the island reaches up to 1600 m. The eastern slopes are very steep, whereas on the western slopes the gradient is less marked. The remnants of primary forest are only recently protected in the higher ranges of the central mountains. The major part of these forests was cleared for nickel mining and in the valleys for plantations and pasture.

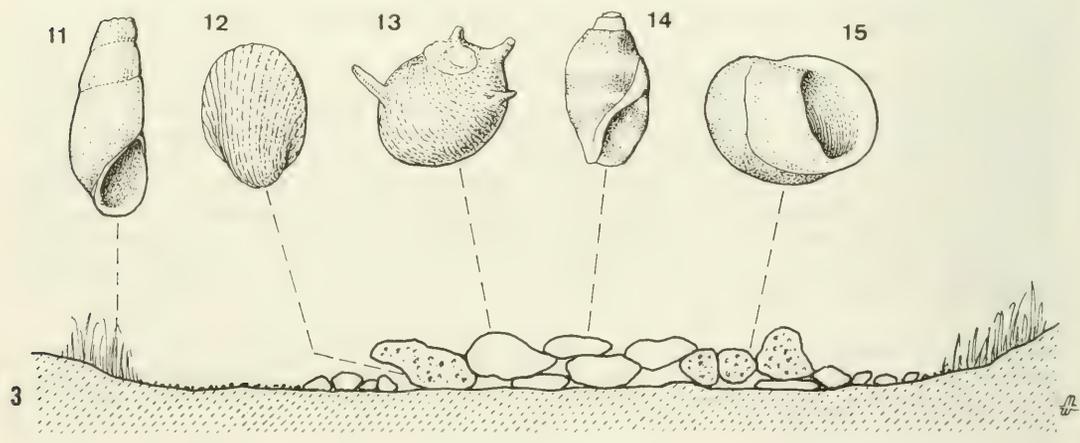
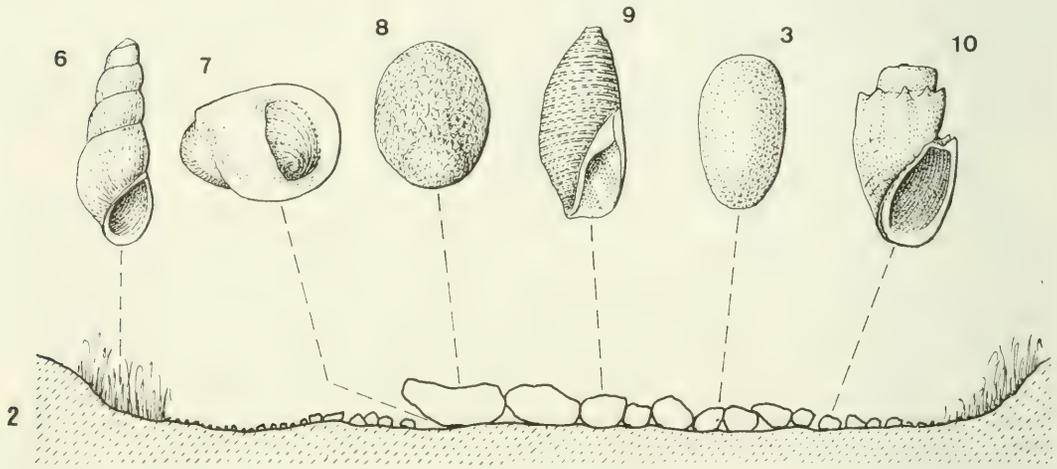
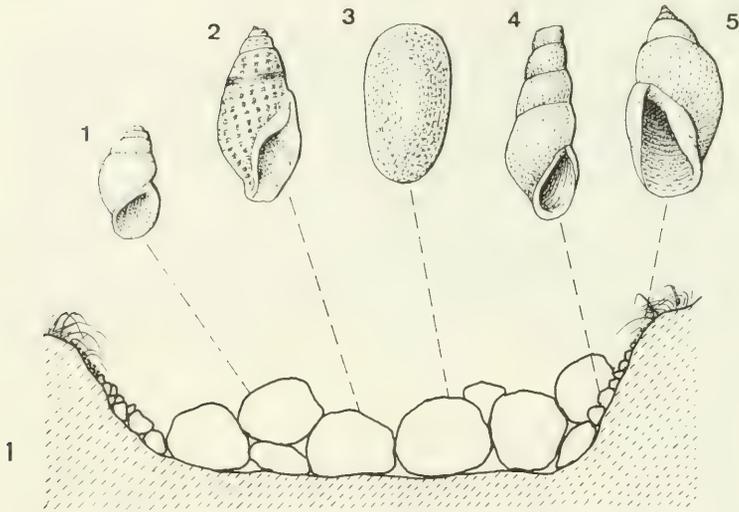
(A) Head-waters and upper courses in the central mountains (1000-500 m).

The head-waters have their sources in the remnants of the primary forest or in the bush steppe of the cleared slopes in the areas of nickel mining. In the shady parts of the forest the temperature of the running water is lower than in the open landscape of the secondary bush steppe and savanna of Niaouli. Bottom: rocks, boulders, gravel, near the margins and in the pools between the cascades, sand and vegetable debris; water temperature from 13°C (sources, in shady forests of 800-1000 m) to 15°C (upper reaches in shady forests, 500-800 m) and 17.5°C (upper reaches in open landscape, sunny, 500-800 m); current 50 cm/sec to more than 1 m/sec, except near the banks and in the pools where it is less than 30 cm/sec; chemistry, conductivity 34-50 μ Siemens (depends on the bottom, such as peridotite, basalt, grauwacken, schists and, only rarely, limestone), hardness 0.5-2.7° dH, pH 5.3-7 (if the hardness is slower than 0.5° dH and the pH lower than 6 there are no gastropods). Species found: *Melanopsis frustulum f. maculatus*.

(B) Transition from the upper to the middle courses of mountain streams (500-100 m, Fig. 4-1).

In part the streams cross secondary forest, plantations, pastures and the Niaouli savanna. Bottom: boulders, gravel, near the margins and in the pools between the cascades, sandy and muddy, vegetable debris; water temperature 17-20°C; current 50 cm/sec to more than 1 m/sec, near the banks and in the pools 0-30 cm/sec; chemistry, conductivity 50-150 μ Siemens (depends on the bottom, see above), hardness 3-4.5° dH, pH ca. 7. Species found: (a) parts with moderate current, *Melanopsis frustulum f. maculatus* (in the serpentine macchia of the south: *M. mariei*), *Fluviopupa* sp. (and *Hemistomia cf. caledonica*); (b) under floating water plants, stones, *Ferrissia (Pettancyllus) noumeensis*; (c) near the banks and in pools, *Melanoides tuberculata*, *Physastra nasuta*.

FIG. 3. Diagram showing temperature, hardness and conductivity of the water correlated with the distribution of the gastropods and bivalves between the head-waters and mouths of the mountain streams of New Caledonia (for details see Fig. 2).



(C) Transition of the middle courses to the lower courses of the mountain streams (100-10 m, Fig. 4-2).

Flowing through plantations, pastures and Niaouli savanna, open landscape without much shade. Bottom: gravel, sand, near the banks partly muddy, dense vegetation of submerged water plants; water temperature 21-23°C; current 50-75 cm/sec, longer sections with 30-50 cm/sec, near the banks and in pools 0-20 cm/sec; chemistry, conductivity 150 μ Siemens, hardness 4-5°dH, pH 7. Species found: (a) parts with moderate current, *Septaria porcellana depressa* (found up to 1 m/sec over short distances such as small cascades), *Melanopsis frustulum* f. *multistriatus*, *Neritina pulligera*; (b) under floating water plants, stones, *Ferrissia (Pettancyllus) noumeensis*; (c) near the banks and in pools, *Melanooides tuberculata*, *Thiara amarula* (and *Physastra nasuta*).

(D) Lower courses near the back flow of brackish water during high tide (10-0 m, Fig. 4-3).

Flowing through plantations (mostly coconut) and pastures, banks bordered by *Pandanus* species. Bottom: gravel, sand, banks with muddy sand and dense vegetation of submerged water plants; water temperature 23-25°C; current 30-50 cm/sec, near the banks 0-10 cm/sec; chemistry, back flow of brackish water during high tide (see sub E), low tide conditions like C. Species found: (a) parts with moderate current, on top of stones, *Melanopsis frustulum* f. *fasciatus*, *Clithon nucleolus*, *Neritina (Vittina) variegata*; below the stones, *Neritina (Neripteron) auriculata* s.s. and f. *lecontei*; (b) near the banks, *Melanooides (Stenomelania) arthurii*; (c) in the sand, *Polymesoda bengalensis sublobata*.

(E) Mouth region under the influence of the brackish water (0 m).

Bordered upstream by *Pandanus* and transition to the typical mangrove zone on level parts of the coast; many marine animals occur here. Bottom: gravel, sand, dead coral, empty shells of marine molluscs; water temperature 25°C; current 30-50 cm/sec, near the banks 0-10 cm/sec; chemistry, conductivity up to 18,000 μ Siemens, hardness up to 14°dH, pH 7.5-8. Species found: (a) on stones and dead coral, *Syncera (=Assimineae) savesi*, *Paludinella hidalgoi*, *Melanopsis frustulum* f. *fasciatus* and f. *fuscus*; (b) attached to stones and dead coral, *Modiolus bourailensis*, *Brachyodontes cf. ramosus*; (c) near the banks, *Truncatella cerea*, *Cassidula intuscarinata*.

CONCLUSIONS

A comparison of the distribution of the freshwater molluscs in mountain streams of the 3 continental islands of the Indo-Pacific leads to the following conclusions.

In the isolated parts of the mountains where the head-waters and upper and middle course of the running waters show a strong or moderate current (rocky-stony bottom) old faunistic elements of the family Thiaridae (=Melaniidae) dominate with endemic genera, subgenera and species (in New Caledonia also species of the family Hydrobiidae, respectively ?Rissoidae):

MADAGASCAR	CEYLON	NEW CALEDONIA
<i>Melanatria</i> species	<i>Paludomus (Tanalia)</i> species	<i>Melanopsis</i> species
<i>Cleopatra</i> species	<i>Paludomus (Philopotamis)</i> species	(<i>Fluviopupa</i> sp., <i>Hemistomia</i>)

Only in New Caledonia does *Melanopsis* (a genus with brackish water affinity) also occur in the lower parts and the region of the mouth of the stream, which contains brackish water during high tide (back flow). The lower courses of the mountain streams upstream of the back flow of brackish water and with a strong to moderate current are characterized by species of the families Neritidae and Thiaridae (=Melaniidae):

FIG. 4. Distribution of freshwater gastropods in the various parts of the mountain rivers of New Caledonia, viz., (1) head-waters to upper and middle course of the rivers, (2) middle course, (3) lower course to the mouth. Gastropod species: (1) *Fluviopupa* sp., (2) *Melanopsis frustulum maculatus*, (3) *Ferrissia (Pettancyllus) noumeensis*, (4) *Melanooides tuberculata*, (5) *Physastra nasuta*, (6) *Melanooides tuberculata*, (7) *Neritina pulligera*, (8) *Septaria porcellana depressa*, (9) *Melanopsis frustulum multistriatus*, (10) *Thiara amarula*, (11) *Melanooides (Stenomelania) arthurii*, (12) *Neritina (Neripteron) auriculata lecontei*, (13) *Clithon nucleolus*, (14) *Melanopsis frustulum fasciatus*, (15) *Neritina (Vittina) variegata*.

MADAGASCAR	CEYLON	NEW CALEDONIA
<i>Septaria borbonica</i>	<i>Septaria lineata</i>	<i>Septaria porcellana depressa</i>
<i>Neritina (Vittina) gagates</i>		<i>Neritina (Vittina) variegata</i>
<i>Neritina pulligera knorri</i>		<i>Neritina pulligera</i>
	<i>Paludomus (Paludomus) species</i>	
	<i>Lamellidens lamellatus</i>	
	<i>Lamellidens testudinarius</i>	
	<i>Parreysia corrugata</i>	

In the region of the mouths of the streams in the area where there is a temporary influence of the brackish water because of the back flow of sea water during high tide and where there is a strong to moderate current, the genus *Clithon* and the subgenus *Neripteron* of the genus *Neritina* (both Neritidae) and furthermore some Thiaridae (=Melaniidae) and the bivalve *Polymesoda bengalensis* occur typically:

MADAGASCAR	CEYLON	NEW CALEDONIA
<i>Clithon spiniperda</i>		<i>Clithon nucleolus, C. bicolor,</i>
<i>Clithon coronata (=</i>		<i>C. corona</i>
<i>longispina)</i>		
<i>Neritina (Neripteron)</i>	<i>Neritina (Neripteron)</i>	<i>Neritina (Neripteron) auricu-</i>
<i>auriculata</i>	<i>auriculata</i>	<i>culata s.s. and lecontei</i>
<i>Thiara amarula</i>		<i>Thiara amarula</i>
	<i>Melanooides (Stenomelania)</i>	<i>Melanooides (Stenomelania)</i>
	<i>torulosa</i>	<i>arthurii</i>
	<i>Faunus ater</i>	<i>Melanopsis frustulum f. fasci-</i>
		<i>atus</i>
	<i>Polymesoda bengalensis s.s.</i>	<i>Polymesoda bengalensis f.</i>
		<i>sublobata</i>

It should be noted that species of the subgenus *Stenomelania* of the viviparous genus *Melanooides*, living exclusively in the mouth region with temporary influence of brackish water during high tide, possess free living veliger larvae. All other species of *Melanooides* (subgenus *Melanooides* s.s.) live in purely fresh water and pass the veliger stage in the brood pouch of the female; young crawling snails leave the opening of the brood pouch later on.

The outer part of the mouth of the streams in transition to the mangrove or the littoral marine zones of the coast is also under the influence of brackish water during low tide. These brackish water species are mixed with typically marine forms such as Mytilidae, Ostreidae or species of the genus *Nerita*:

MADAGASCAR	CEYLON	NEW CALEDONIA
<i>Cerithidea decollata</i>	<i>Cerithidea cingulata</i>	
<i>Syncera (=Assimineae) species</i>	<i>Syncera (=Assimineae) species</i>	<i>Syncera (=Assimineae) savesi</i>
	<i>Gangetia burmanica</i>	<i>Paludinella hidalgoi</i>
	<i>Faunus ater</i>	<i>Melanopsis frustulum f.</i>
		<i>fasciatus</i> and <i>f. fuscus</i>
		Mangrove species:
		<i>Truncatella cerea, Cassi-</i>
		<i>dula intuscarinata,</i>
		<i>Modiolus bourailensis,</i>
		<i>Brachyodontes cf.</i>
		<i>ramosus</i>

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THERMOREGULATION IN THE FRESHWATER LAMELLIBRANCH *PARREYSIA CORRUGATA*

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ABSTRACT

Thermal relations of the freshwater lamellibranch *Parreysia corrugata* were studied. The mussels, maintained at a laboratory temperature of 26°-28°C, could not survive for 24 hours at 37°C and 8°C. Their 24 hour median heat tolerance limit was 36.2°C and 13.5°C at the upper and lower ranges respectively. In warm acclimated mussels (33.0°C) the 24 hour median tolerance limit was raised from 36.2°C to 39.5°C. In cold acclimated mussels (16.0°C) the 24 hour median tolerance limit fell from 36.2°C to 35.5°C. Younger mussels could withstand more heat than the adults. The protein and fat levels increased with the increase in temperature but the glycogen level decreased when the temperature was raised. A sudden rise of temperature (10.0°C) maintained for an hour resulted in an emptying of the neurosecretory cells of the cerebral ganglion while a fall of temperature is followed by a significant increase in the secretion products of the same cells.

INTRODUCTION

Temperature is one of the most important environmental factors which limits the distribution of animals and determines their rate of activity. Experiments in which animals were maintained at constant temperatures for a period of time provided a more accurate determination of lethal temperatures as well as allowed comparison among the species. In such experiments either time to death or % survival at intervals was noted (Spoor, 1955; Brett, 1956; Fry, 1967; McWhinnie, 1967).

On the basis of laboratory acclimation, season, microgeography and latitude, acclimation to low and high temperatures has been demonstrated in many animals. Acclimation has been demonstrated by a higher rate of function at any intermediate temperature when low temperature acclimated animals are compared with high acclimated ones. In the periwinkle *Nodilittorina granularis* Oshawa & Tsukuda (1956) found seasonal acclimation in response to temperature. Acclimation to higher temperature in the winter snails is established more rapidly than the acclimation to lower temperature in the summer snails. Microgeographical acclimation has been demonstrated in the limpet *Acmaea limatula*; Segal (1956) found that samples taken from lower intertidal levels had a higher rate of heart beat than those from higher levels.

Acclimation has its effect on the temperature tolerance of a species. Warm acclimation is known to raise the thermal resistance while cold acclimation decreases the tolerance to high temperature. A few investigators have studied the influence of warm and cold acclimation on median heat tolerance limit of molluscs (Read, 1967; Nagabhusanam & Kulkarni, 1970; Kennedy & Mihursky, 1971; Mantale, 1971; Waugh & Garside, 1971; Waugh, 1972).

Effect of size on the temperature tolerance of the animals varies with the species. Belehradek (1955) stated that resistance to heat diminished as the size increased. Contrary evidence has been reported by Mcleese (1956) and Diaz (1973).

Biochemical changes in molluscs following acclimation to high and low temperatures seem to have received little attention. Rao & Ramchandra (1961) in *Lamellidens* and Mantale (1971) in *Cryptozona* have made some useful contributions. Effect of thermal stress on neurosecretory activity of molluscs has been studied only by a few workers (Nagabhusanam, 1955; Khatib, 1975).

Relatively little work has been done on the temperature relations of *Parreysia corrugata*. The present investigation was therefore undertaken to study in detail the thermal relations of

Parreysia. The investigation provides information on the thermal relations of *Parreysia* in connection with the heat tolerance limit, size of the mussel, biochemical changes and neurosecretory changes.

MATERIAL AND METHODS

The freshwater mussels, *Parreysia corrugata*, were collected from the Kham river near Aurangabad. At the time of collection, the river temperature varied from 26-30°C. The mussels were maintained in the laboratory in shallow glass aquaria containing tap water with a temperature range of between 26-28°C. Water in the aquaria was changed every day and it was aerated to keep the animals in good health. Groups of 30-50 animals were taken at random from stock aquaria and maintained in well aerated glass aquaria at acclimation temperatures of 33° ± 0.5°C and 16° ± 0.5°C. The mussels remaining in the stock aquaria were used as controls.

The heat tolerance of control and experimental animals was studied by testing their survival for 24 hours at different temperatures. The warming period lasted for 1-2 hours according to the difference between acclimation and test temperatures. The mussels were brought from the acclimation temperature to the test temperature slowly rather than abruptly.

To find out the effect of size on heat tolerance, 2 groups of mussels were taken. One group of mussels ranging in length from 2 to 2.5 cm and the other group from 6 to 6.5 cm were tested for their heat tolerance.

To study the biochemical changes associated with heat tolerance the mussels were acclimated to temperatures of 16°C, 28°C and 33°C. The water percentage was calculated by drying up the mussel tissue at 100°C. Glycogen was estimated by the method of Kemp et al. (1954). Total nitrogen was estimated by Microkjeldahl method (Hawk et al., 1954). The amount of protein was calculated by multiplying the nitrogen value by the factor 6.25. Fat was extracted from the tissue powder in Soxhlet apparatus.

For studying the influence of temperature on neurosecretory activity, the mussels were kept at 2 different temperatures, a low temperature of 16°C and a high temperature of 36°C for one hour. The ganglia were then preserved in Bouin's fluid for observation of the neurosecretory cells.

RESULTS

Heat tolerance of control animals

The mussels were acclimated to the laboratory temperature for one week and their 24 hour survival at different temperatures was recorded. In no experiment could the mussels tolerate temperatures of 8°C and 37°C and 100% mortality was observed at these temperatures. Fig. 1 shows that the 24 hour median heat tolerance limit at the upper and lower ranges of temperature where 50% mortality was recorded lies at 36.2°C and 13.5°C respectively.

Effect of warm and cold acclimation on heat tolerance limit

Groups of mussels were acclimated for a week to a high temperature of 33°C ± 0.5°C and a low temperature of 16°C ± 0.5°C with control animals maintained at 28°C laboratory temperature. These mussels were then tested for survival at the higher temperature of 34°, 35°, 36°, 37°, 38° and 39°C (Fig. 2). At the lowest temperature of 34°C there was 100% survival of the warm acclimated and control mussels, whereas the survival of cold acclimated mussels at this temperature was 72%. At a temperature of 37°C, the survival of warm acclimated, control and cold acclimated mussels was 72, 10 and 0% respectively. The warm acclimated mussels could tolerate still higher temperatures. In these mussels there was 50% survival at a temperature of 39.5°C and almost 0% survival at 40.0°C. It is interesting to note that the percent survival of the mussels increased with the increasing temperature of acclimation resulting in the increase of a median tolerance limit from 36.2°C to 39.5°C in warm acclimated mussels, but there is a decrease in the median tolerance limit from 36.2°C to 35.5°C in cold

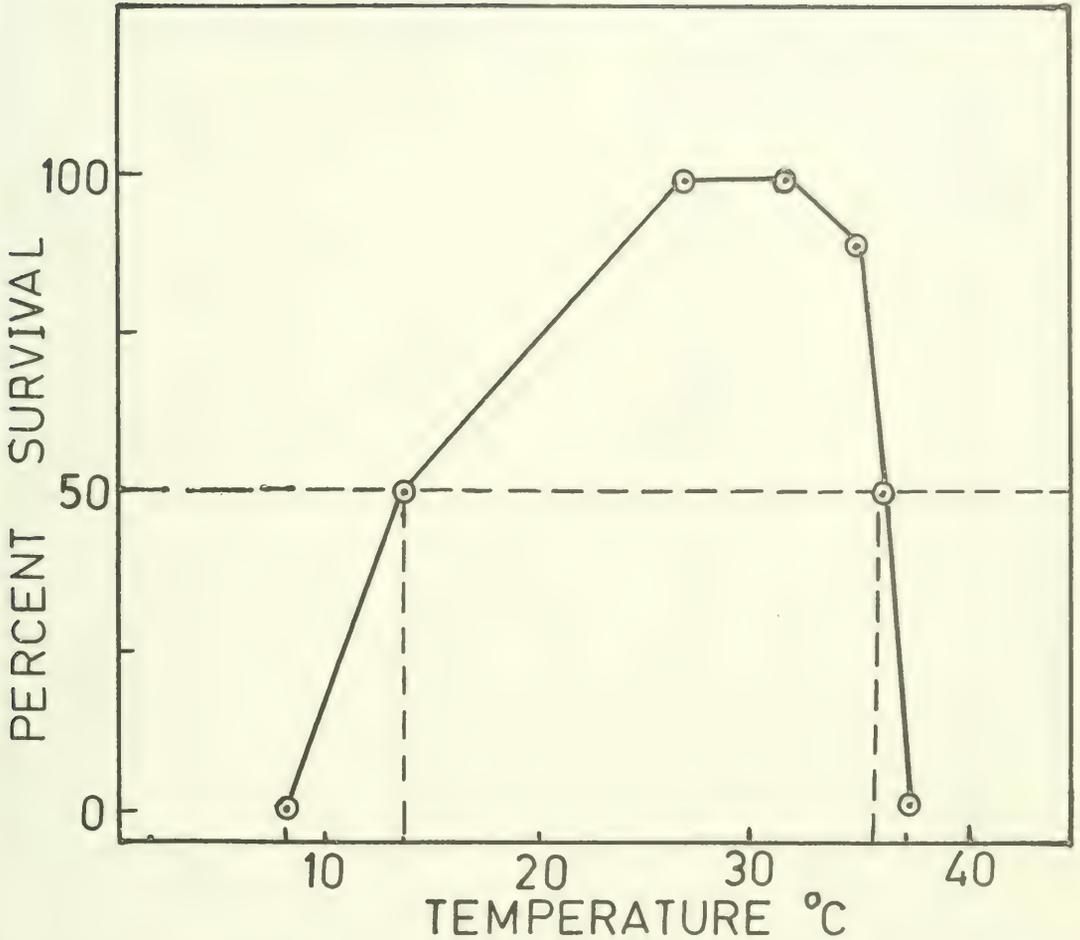


FIG. 1. Percent survival of *Parreysia corrugata* at different temperatures.

acclimated mussels. Thus the increase in the acclimation temperature from 28° to 33°C, i.e. 5°C, has resulted in the increase of the median tolerance limit by 3.3°C, while a decrease in the acclimation temperature from 28°C to 16°C has resulted in a decrease by 1.3°C. These results indicate that there is some gain in the heat tolerance limit when the mussels are acclimated to higher temperatures and much less when acclimated to lower temperatures.

Effect of size on the temperature tolerance

Mussels of 2 different size groups were selected to test the effect of size on the temperature tolerance. The results are shown in Fig. 3. The mussels of 2-2.5 cm size group have shown a median tolerance limit at 37.5°C and the mussels of 6-6.5 cm size group have shown a median tolerance limit at 36.0°C. Thus the younger mussels are more heat tolerant than the adults.

Biochemical changes associated with thermal acclimation

To find out the changes in biochemical composition due to thermal acclimation the mussels were acclimated at temperatures of 16°C, 28°C and 33°C for 7 days. The results are shown in Table 1.

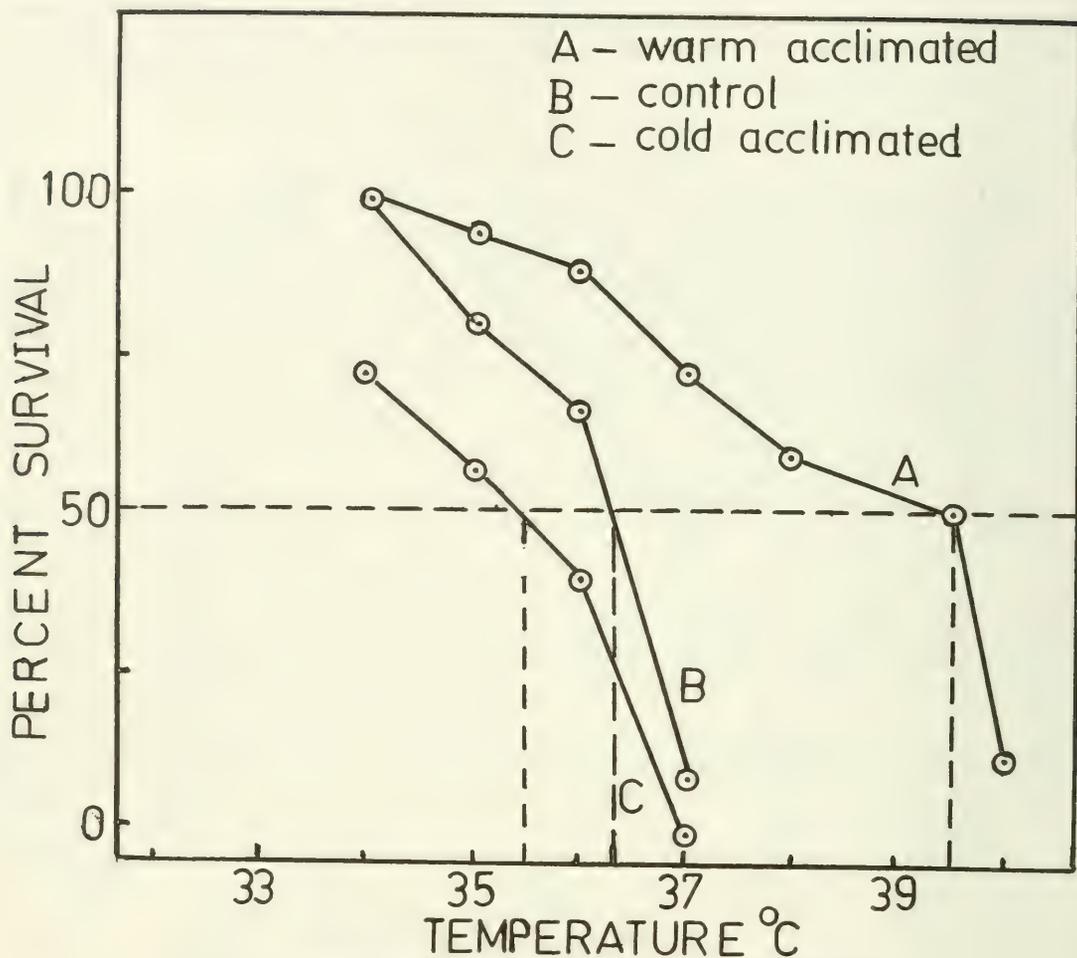


FIG. 2. Heat tolerance of warm and cold acclimated *Parreysia corrugata*.

TABLE 1. Biochemical changes associated with thermal acclimation in *Parreysia corrugata*.

Biochemical constituent	Control 28°C.	Warm acclimation 33°C	Cold acclimation 16°C
Water	70.90 ±0.018	75.45 ±0.007	71.10 ±0.001
Protein	38.74 ±0.007	41.30 ±0.090	37.13 ±0.018
Glycogen	5.25 ±0.003	3.90 ±0.020	5.92 ±0.013
Fat	3.10 ±0.010	4.70 ±0.015	2.90 ±0.310

Water content of the mussels increased when they were subjected to higher temperatures. Percentage of water increased to 75.45% at 33°C and it dropped to 71.10% at 16°C. Protein and fat contents increased at higher temperature whereas glycogen content decreased when the temperature was raised.

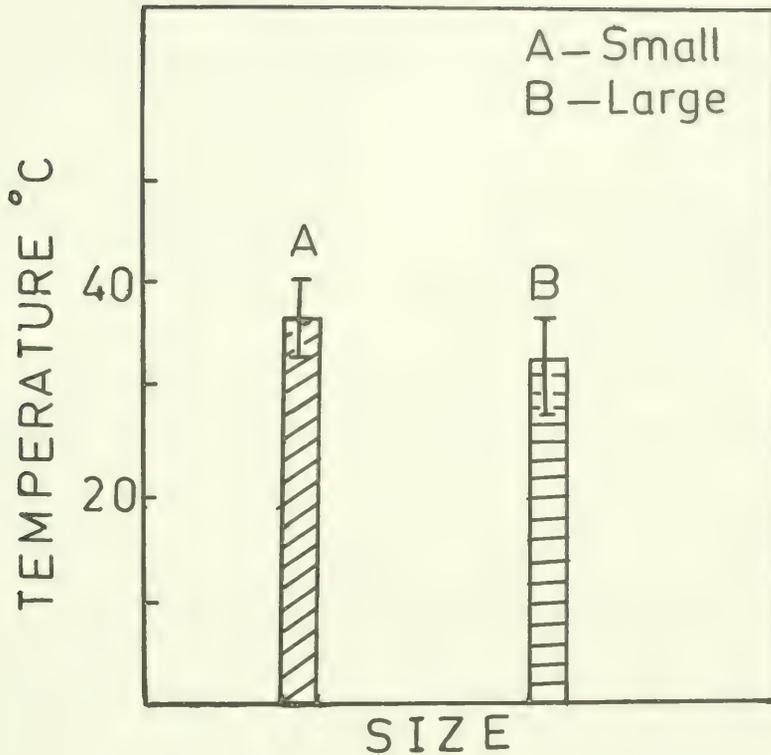


FIG. 3. Effect of size on temperature tolerance of *Parreysia corrugata*.

Effect of thermal stress on neurosecretion

The effect of a sudden rise in temperature on the neurosecretory activity of *Parreysia* was studied. The mussels were subjected to a sudden rise of temperature (10°C) for an hour and the cerebral ganglia of the mussels were observed for stainable neurosecretory cells. It was found on observation that there was an emptying of the neurosecretory products in the stressed animals. When the mussels were subjected to a low temperature it was observed that there was a significant increase in the secretion product of the cells (Fig. 4).

DISCUSSION

Physiological adaptation to environmental stress is one of the recurring themes in the biological literature (Bullock, 1955; Prosser, 1955). The literature on thermal tolerance of poikilotherms is sufficiently convincing to prove that median heat tolerance is dependent on the acclimation temperature. The lethal effect has been worked out in more detail in poikilothermic vertebrates (Fry, 1967) than in invertebrates. However, some data are available for Mollusca.

Parreysia corrugata could not survive well beyond the thermal range of 8°C - 37°C . The median heat tolerance limit was found to be at 36.2°C when the laboratory temperature ranged between 26° - 28°C . The median tolerance limit of *Lamellidens corrianus* was 37.4°C when the laboratory temperature was $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ (Lohagaonkar, 1974) and the tolerance limit of *Corbicula regularis* was 37.2°C when the laboratory temperature was $27^{\circ} \pm 2^{\circ}\text{C}$ (Mudkhede, 1974). In *Indonaia caeruleus* the median tolerance limit was 36.5°C when the temperature

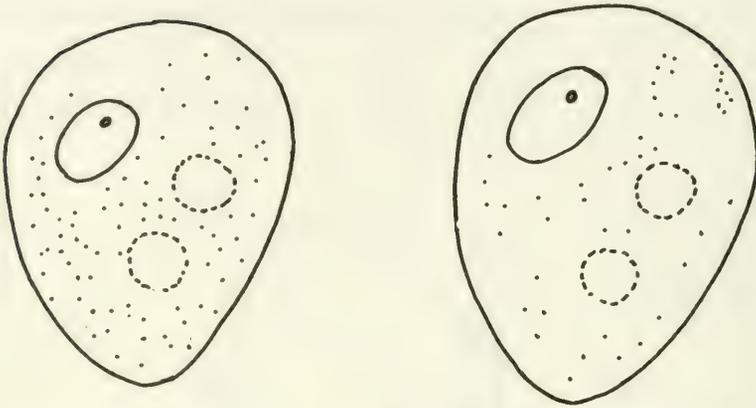


FIG. 4. Effect of thermal stress on neurosecretion of *Parreysia corrugata*. Experiment on the left, control on the right.

range in the laboratory was 27° - 29°C (Khatib, 1975). Heat tolerance limit varies according to variation in the environmental temperature (Brett, 1956).

In *Parreysia* it was found that the upper median tolerance limit was increased due to an increase in acclimation temperature and vice versa. When acclimated at the warm temperature of $33^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ the mussels have elevated their upper tolerance limit to 39.5°C and when acclimated at $16^{\circ} \pm 0.5^{\circ}\text{C}$ the heat tolerance limit was dropped to 35.5°C . Fry et al. (1942) stated that for every 3°C rise in temperature the upper tolerance limit was increased by 1°C and the lower limit by 2°C in the fish *Carassius auratus*. In a bivalve, *Indonaia caeruleus*, the heat tolerance limit was raised to 37.1°C from 36.5°C when acclimated at 32°C and it dropped to 34.6°C from 36.5°C when acclimated at 18°C (Khatib, 1975). In warm acclimated (32°C) *Melanooides tuberculatus* the tolerance limit was elevated from 36.4° to 39.0°C and in cold acclimated snails it fell from 36.4°C to 33.0°C (Khot, 1977).

Young *Parreysia* were more heat resistant than the adults. Kennedy & Mihursky (1971) stated that at all acclimation temperatures smaller clams were more heat resistant and adults were more sensitive to high temperatures. Young snails of *Melanooides tuberculatus* could tolerate more heat than the adults (Khot, 1977).

McWhinnie (1967) stated that the biochemical changes provide a molecular basis for thermogenesis essential to account for activity and synthesis at suboptimum temperature as well as survival and homeostasis at superoptimum temperature.

In *Parreysia corrugata* protein level was increased at high temperature. In *Lymnaea* protein level was increased as the temperature was raised (Azmatunnissa, 1974). Khot (1977) also reported an increase in protein level in *Melanooides tuberculatus*. Decrease in glycogen content was reported in warm acclimated *Indonaia* (Khatib, 1975). Similar results were obtained in *Parreysia corrugata*. The fat content was found to be increased slightly in warm acclimated mussels. In *Melanooides* there was also a slight increase in fat. Water percentage was related to the acclimation temperature.

Increased water and decreased glycogen content in the warm acclimated mussels probably suggest the increased oxidation of carbohydrates consequent to the increased metabolic activities at this higher temperature. During cold acclimation, fat content decreased and glycogen increased. This suggests retardation of physiological activities due to cold stress.

Influence of temperature on the neurosecretory activity in *Parreysia* indicates that at high temperatures there was depletion of the secretory material while at low temperatures there was accumulation of the secretory material. Nagabhushanam (1964) found that there was emptying of the secretory product when the animals were subjected to thermal stress. Khatib (1975) reported that there was depletion of the neurosecretory material at high temperatures, while the neurosecretory material was accumulated at low temperatures.

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SUR LES MOLLUSQUES DE LA LIMITE ENTRE LE PLIOCÈNE SUPÉRIEUR ET LE PLEISTOCÈNE INFÉRIEUR EN ROUMANIE¹

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ABSTRACT

The border line between Pliocene and Pleistocene deposits in Rumania has traditionally been based on data derived from fossil mammals. However, recent research has shown that a large number of Pliocene molluscs, accompanied by mammals considered to be of Pleistocene age, penetrates into the Lower Pleistocene. Therefore the border line between the Upper Pliocene and the Lower Pleistocene in Rumania cannot be based on molluscan data. This is fully discussed in the present paper.

Sur la base de la faune de mammifères fossiles, nous pouvons fixer, pour la Roumanie, la limite entre le Pliocène et le Pléistocène au moment où apparaît le genre *Elephas* (ses formes primitives), comme c'est le cas à Tulucești (distr. Galați) et à Cernătești (à Dealul Calului), au NO. de Craiova (distr. Dolj). Ainsi, à Tulucești, *Elephas planifrons* Falc. (Athanasiu, 1915) apparaît à côté de *Anancus arvernensis* Cr. & Job., *Cervus (Elaphus) issidorensis* Cr. & Job. et une molaire de *Mastodon borsoni* Hays. À cette liste Ghenea & Rădulescu (1964) ajoutent une mandibule de *Camelus alutinensis* Stef. et un métacarpien de *Hippotigris stenorhis* Cocchi.

Très proche de la faune de Tulucești est celle de Dealul Calului de Cernătești (Dolj), d'où Schoverth et al. (1963) décrivent des couches moyennes sablonneuses de "Cîndești" (Stefănescu, 1897) à Unionides, des molières de *Anancus arvernensis* Cr. & Job., *Elephas (Archidiskodon) meridionalis* Nesti (très probablement, d'après nous, *E. planifrons* Falc.); *Rhinoceros* cf. *etruscus* Falc., *Equus* sp. et une molaire de *Mastodon borsoni* Hays.

Il est très important que cette faune de mammifères, incontestablement du pléistocène inférieur, se trouve dans les dépôts moyens de sables et graviers à Unionides, nommés "Couches de Cîndești" (Stefănescu, 1897), de l'Olténie et de l'ouest de la Munténie, connus de Bucovăț (à l'Ouest de Craiova), Cernătești, Amărăști, Urdade-Jos, Valea Muerei et autres localités de l'Olténie (Fig. 1). Cette faune est considérée par Stefănescu (1897) comme "levantine." À présent, à cause des mammifères cités plus haut, elle est attribuée au Pléistocène inférieur, ne restant comme Pliocène supérieur que l'horizon inférieur pélimitique (marno-argileux) des "Couches de Cîndești," qui contiennent: *Unio lenticularis* Sabba, *Psilunio recurvus* Sabba, *Vivipara dezmaniana* var. *altercarinata* Brus., *V. bifarcinata* var. *stricturata* Neum., *Melanopsis pterochila* var. *breastensis* Brus. *Valvata sibirica* Neum., et qui constitue le seul horizon appartenant au "Levantin" (Bandrabur, 1971).

Au-dessus suivent les couches moyennes de "Cîndești" à Unionides, formées par sables et graviers, d'où Schoverth et al. (1963) cite la faune de mammifères mentionnée plus haut, de même qu'une faune de mollusques de ces couches à Bucovăț et les autres localités mentionnées (Cernătești, Amărăști, etc.).

D'après Stefănescu (1897), Schoverth et al. (1963), Bandrabur (1971) et d'autres auteurs antérieurs, cette faune est constituée surtout des espèces suivantes:

¹Prof. Macarovici has been unable to attend the Amsterdam congress; nevertheless, by way of exception, his paper is published in the Proceedings.

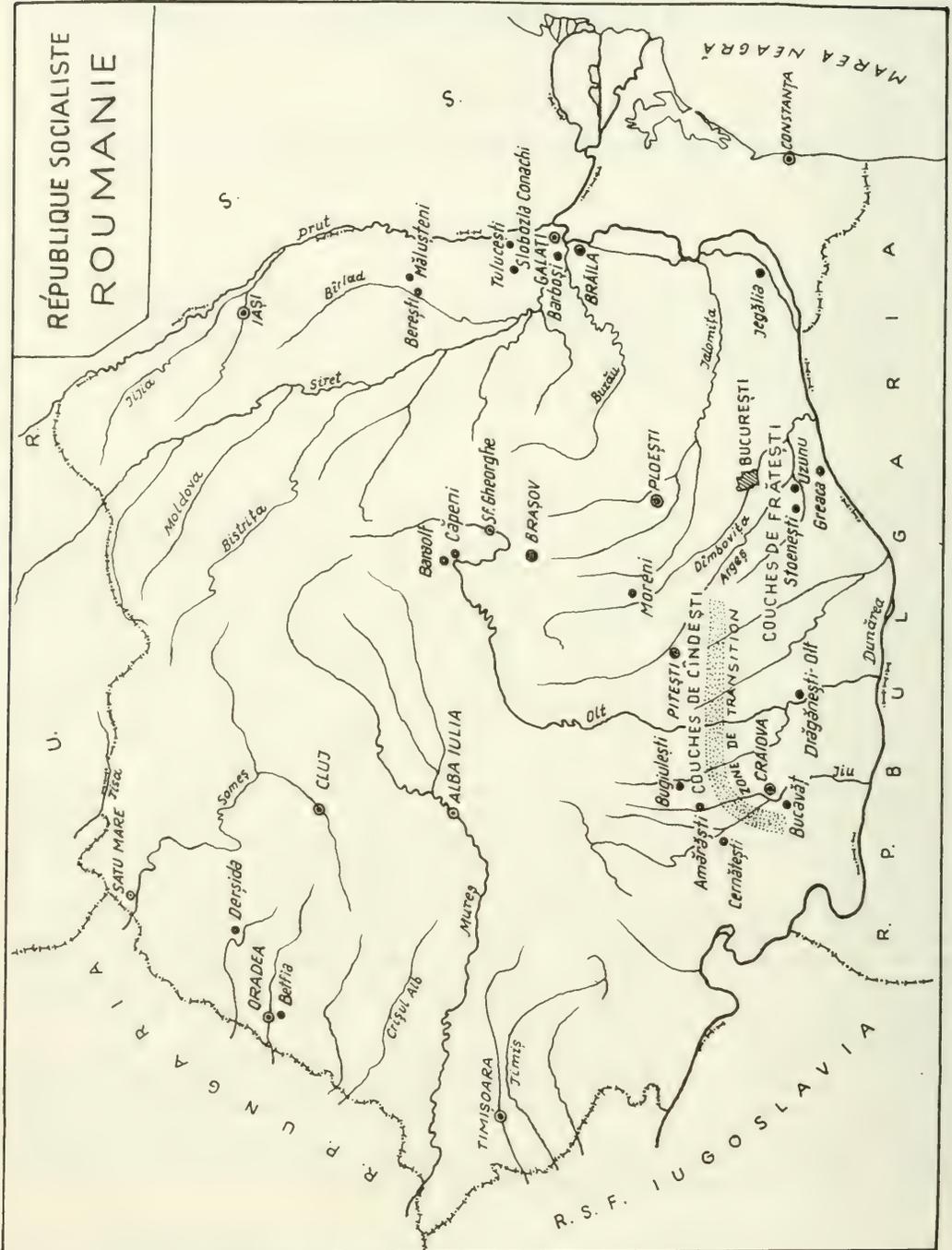


FIG. 1. Carte de la République Socialiste Roumanie. Les Couches de Cîndești se trouvent au sud du pays.

<i>Unio (pristinus) Bielz</i>	<i>Viviparus (turgidus) pilari</i> Brus.
<i>Unio procumbens</i> Fuchs	<i>Viviparus (turgidus) turgidus</i> Bielz
<i>Unio davilai</i> Por.	<i>Melanopsis rumana</i> Tourn.
<i>Unio porumbarui</i> Tourn.	<i>Melanopsis narzolina</i> Siesm.
<i>Unio (Scalenaria) bielzi</i> Czek.	<i>Melanopsis onusta</i> Sabba
<i>Psilunio sculptus</i> Brus.	<i>Melanopsis (Canthidomus) soubcirani</i> Por.
<i>Psilunio berbestiensis</i> Font.	<i>Melanopsis (Canthidomus) porumbarui</i> Brus.
<i>Psilunio condai</i> Por.	<i>Melanopsis (Canthidomus) hybostoma</i> Neum.
<i>Psilunio brandzae</i> Sabba	<i>Theodoxus quadrifasciatus</i> Bielz
<i>Psilunio doljensis</i> Sabba	<i>Theodoxus licherdopuli</i> Sabba
<i>Viviparus craiovensis</i> Tourn.	<i>Theodoxus scriptus</i> Sabba
<i>Viviparus mammatus</i> Sabba	<i>Valvata (Cincinnati) piscinalis</i> Müll.
<i>Viviparus bifarcinatus</i> Bielz	<i>Emmericia rumana</i> Tourn.
<i>Viviparus (rudis) rudis</i> Neum.	<i>Emmericia candida</i> Neum, etc.
<i>Viviparus (rudis) strossmayerianus</i> Brus.	<i>Bulimus vukotinovici</i> Brus.

Il faut remarquer (Schoverth et al., 1963) que les valves des Unionides sculptés et des Vivipares ornementés de cette liste sont très érodées, ce qui indiquerait qu'une grande partie de cette faune a été remaniée, son dépôt ayant, d'après Schoverth et al. (1963), le caractère "torrentiel."

Mais Schoverth et al. (1963), Macarovici (1965), Liteanu et al. (1957, 1960, 1961, 1967) et Bandrabur (1971) attribuent les "couches moyennes de Cîndești" au Villafranchien s.str., donc à la base du Pléistocène. Elles sont connues également de la Dépression Gétique et de la Plaine Roumaine. Une faune semblable à celle des couches moyennes de Cîndești a été décrite par Botez (1916), de Moreni (Dîmbovița) et par Barbu & Barbu (1953) de la rive nord du lac de Greaca (au S. de Bucarest); ces dépôts se trouvent donc aussi dans la Plaine Roumaine.

Au-dessus de l'horizon moyen des couches de Cîndești on trouve l'horizon supérieur de ces couches dans lequel les restes de *Mastodon* deviennent de plus en plus rares, tandis qu'on rencontre fréquemment celles de *Elephas (Archidiskodon) meridionalis* Nesti, signalées aussi par Ștefănescu (1897).

Les couches (marno-argileuses) supérieures de Cîndești contiennent une faune de mollusques dont la liste est, en général, constituée par les espèces suivantes:

<i>Unio sculptus</i> Brus.	<i>Viviparus mammatus</i> Sabba
<i>Unio iconomianus</i> Tourn.	<i>Viviparus (rudis) rudis</i> Neum.
<i>Unio stefanescui</i> Tourn.	<i>Viviparus plicatus</i> Sabba
<i>Unio porumbarui</i> Tourn.	<i>Bythinia vukotinovici</i> Brus.
<i>Unio herjeui</i> Porumb.	<i>Theodoxus semiplicata</i> Neum.
<i>Scalenaria bielzi</i> Czek.	<i>Theodoxus scripta</i> Sabba
<i>Psilunio craiovensis</i> Tourn.	<i>Melanopsis narzolina</i> Siesm., etc.

Au-dessus suivent les couches de Frătești qui constituent la fin du Pleistocène inférieur de l'Olténie et de la Plaine Roumaine.

Ce que nous avons exposé jusqu'ici sur les mollusques des couches de Cîndești, ne nous permet pas de préciser la limite entre le Pliocène et le Pléistocène dans la Dépression Gétique (en Olténie). La cause en est (d'après Bandrabur, 1971: 53) que, au-dessous de l'horizon inférieur marneux à *Unio lenticularis* Sabba, suit une autre succession de sables et graviers, alternant avec des intercalations d'argiles, caractérisées du point de vue paléontologique par des Unionides sculptés et Vivipares ornementés, présentant des espèces qui se rencontrent également dans les couches moyennes et supérieures de Cîndești. Mais l'auteur ne nous donne pas la liste de la faune rencontrée plus bas que l'horizon à *U. lenticularis* Sabba ce qui abaisserait la limite entre le Pliocène et le Pléistocène au-dessous de la cote de 0 m (de beaucoup au-dessous du niveau d'érosion du Jiul). Ainsi la limite entre le Pliocène et le Pléistocène doit être établie, toujours d'après les mammifères, dans les limites des couches moyennes de Cîndești.

Au-dessus des couches supérieures de Cîndești suivent (comme nous l'avons déjà dit), dans la Plaine Roumaine entre le Jiu et l'Argeș, les couches de Frătești, formées par des sables avec des graviers et des blocs vers la base.

Les couches de Frătești ne sont que rarement fossilifères; d'après Bandrabur (1971), à

Drăgănești-Olt elles contiennent les mollusques suivants: *Planorbis umbilicatus* Müll., *P. planorbis* L., *Valvata sulekiana* Müll., *V. piscinalis* Müll., *Bulimus vukotinovici* Brus., *Pisidium amnicum* Müll., *Sphaerium rivicola* Leach et des exemplaires de *Unio* sp. roulés.

À côté de ces mollusques (qui ne donnent pas des précisions sur l'âge) on trouve, dans les couches de Frătești, très souvent des molaires et parfois aussi des défenses de *Archidiskodon meridionalis* Nesti, pas associées aux restes de *Mastodon*. La continuité de sédimentation qui existe entre les couches de Cîndești et celles de Frătești (Bandrabur, 1971) montrerait que les couches de Frătești achèvent la Pléistocène inférieur de la Plaine Roumaine.

Dans le sud de la Moldavie, à Tulucești (Galați), dans le ravin Rîpa Bălăiei (sous les couches qui contiennent les mammifères que nous avons attribués au Pléistocène inférieur), on trouve un dépôt sableux comprenant une faune d'Unionides, citée par Grigorovici-Berezovski (1915) et par Macarovici (1960) et formée par les espèces: *Unio sandbergeri* Neum., *U. sibirica* Pen., *U. wetzleri flabelliformis* Mikh. Ces sables s'étendent tant au nord (sur la vallée de la Horincea) que à l'ouest de Tulucești, où (par exemple à Slobozia Conachi) ils contiennent: *Unio wetzleri flabelliformis* Mikh., *U. aff. stoliczkai* Neum., *U. zelebori* Hörn., *U. sandbergeri* Neum., *Viviparus* aff. *turgidus* Bielz.

Ces dépôts à Unionides s'étendent aussi vers l'est, sur le versant gauche de la vallée du Prut (dans la R. S. S. Moldave), où Grigorovici Berezovski (1915) a distingué 2 horizons de faune, à savoir: (1) Un horizon inférieur, présent aux villages Brînza, Slobozia Mare et Cișlița, contenant les espèces: *Unio stoliczkai* Neum., *U. beyrichi* Neum., *U. moldaviensis* Hörn., *U. cf. zelebori* Hörn., *U. cf. nicolaianus* Brus., *U. sibirica* Pen., *U. sandbergeri* Neum., *U. bogatschevi* Mikh., *U. wetzleri flabelliformis* Mikh., *U. lenticularis* Sabba. Grigorovici-Berezovski compare cet horizon avec la partie supérieure des dépôts moyens à *Paludina* de Slavonie et avec l'horizon inférieur "des dépôts levantins de Craiova" (c'est-à-dire avec l'horizon inférieur des couches de "Cîndești").

(2) Le second horizon de la faune du versant gauche du Prut, établi par Grigorovici-Berezovski, est celui de Giurgiulești-Reni, caractérisé par: *Unio procumbens* Fuchs, *U. davilai* Por., *U. porumbarui* Tourn., *U. doljiensis* Sabba, *U. bielzi* Czek., *Vivipara bifarcinata* Bielz, *V. rudis* Neum., etc. L'auteur parallélise cet horizon avec la partie inférieure des couches supérieures à *Paludina* de Slavonie (l'horizon à *Paludina sturi* Neum. et *P. hoernesii* Neum.), de même qu'avec les couches moyennes ("levantines") de Cîndești de Bucovaș-Craiova.

Ces dépôts à Unionides, de la côté gauche de Prut, ont été attribués par Macarovici (1940) au "Levantins supérieur" (Pliocène supérieur). Il faut pourtant observer que ces dépôts pliocènes supérieurs ne sont, en fait, représentés que partiellement au confluent Prut-Danube, puisque tant Grigorovici-Berezovski (1915) que Macarovici (1940) signalent, sur la rive orientale du lac de Cahul, des valves remaniées de *Unio sturi* Hörn., dans les sables contenant la faune pleistocène de Babele. Donc, les dépôts qui les contenaient ont été érodés. Pourtant, Bogatchev (d'après Eberzin, 1957) figure des valves intactes provenant des dépôts pliocènes de la vallée du Sal, de la région du Don. Le même auteur signale *Unio sturi* dans les dépôts apšeroniens (fin du Pliocène) de la Transcaucasie de l'est. Eberzin (1957) cite (d'après Bogatchev) des exemplaires de *U. sturi* du Pliocène supérieur de la rivière Kutchurgan (dans le Sud de l'Ukraine). Bogatchev figure aussi une valve de *U. sturi* de la 5-ème terrasse (fin du Pliocène) du Dniestre à Boșerînița (près de Rezina).

Donc, à la fin du Pliocène, *U. sturi* a eu une grande extension sur le territoire de l'Union Soviétique, depuis l'embouchure du Prut jusqu'en Transcaucasie. Dépôts à *Unio sturi* sont connus aussi en Roumanie (valves intactes) à Uzunu et Stroești (au S. de Bucarest), les dépôts étant d'âge Pléistocène inférieur (Macarovici & Coteș, 1962). Nous admettons cet âge parce que les couches à *Unio sturi* de Uzunu (S. de Bucarest) gisent, à ce qu'il paraît (mais sans certitude), au-dessus des "couches de Frătești" (comme le montre les 2 auteurs, 1962). Pour le moment, nous ne pouvons pas leur attribuer une position sûre pour la Roumanie.

Mais Eberzin (1957) affirme que les couches à *U. sturi* seraient les couches par lesquelles se termine le "Levantins supérieur" (le Pliocène supérieur) en Union Soviétique; mais ces couches ont été très souvent érodées.

Eberzin (1959) toujours, ayant en vue la présence de certaines espèces d'Unionides (*U. flabelliformis*) qui se trouvent dans les dépôts du Pliocène supérieur du confluent du Prut avec le Danube, de même que dans le Sud de la Moldavie (mais sont absentes dans les dépôts de Bucovaș-Craiova), utilise le terme stratigraphique de Poratien inférieur et supérieur pour les 2

horizons à Unionides du Prut. Mais par l'utilisation du nom de "Poratien," la limite inférieure du Pléistocène continue de rester indéfinie. Sur la base des mollusques, Eberzin (1959) équivaut le Poratien (sur lequel il place les couches à *U. sturi*) à l'Apşeronien, sous-étage par lequel se termine le Pliocène supérieur dans l'échelle stratigraphique de l'Union Soviétique.

Pour la Roumanie, nous le répétons, nous ne pouvons pas préciser, sur la base de la faune de mollusques, la limite entre le Pliocène et le Pléistocène. Cette limite peut être tracée, d'une manière approximative, seulement après l'apparition de la faune à *Elephas*, comme nous l'avons déjà dit.

Même la faune de mollusques décrite par Jekelius (1932) dans les dépôts pliocènes du bassin de Braşov, ne peut pas nous offrir une indication sûre quant à la limite entre le Pliocène supérieur et le Pléistocène inférieur. Des 85 espèces décrites par Jekelius (1932) 64 ont été décrites par cet auteur pour la première fois dans la littérature comme provenant du bassin de Braşov; 12 formes seulement sont connues aussi dans d'autres dépôts pliocènes, où elles constituent des éléments ordinaires; 6 autres formes sont récentes et 3 formes ne sont décrites que génériquement.

Jekelius dit que cette faune considérée en entier "fait l'impression d'être endémique." Elle commence, probablement, au Dacien, puisque à Căpeni on a trouvé, dans le lignite, des molaires de *Mastodon borsoni* Hays et de *Mastodon arvernensis* Cr. & Job., à côté des valves de *Limnocardium fuchsii* Neum. La partie supérieure des dépôts pliocènes du bassin de Braşov est attribué par Jekelius aux Levantin et Quaternaire, sans pouvoir nous donner, sur la base de mollusques, la limite entre ces 2 formations. Cette limite a été tracée par Rădulescu & Samson (1969) au moment où apparaît, dans la faune de mammifères, *Archidiskodon meridionalis* Nesti, dans l'horizon faunique III de Baraolt.

En conclusion, d'après ce que nous avons exposé plus haut, nous pouvons dire que la faune de mollusques pliocènes connue en Roumanie ne nous offre d'indications sûres pour tracer la limite entre le Pliocène supérieur et le Pléistocène inférieur. Cette limite ne peut être mise en liaison qu'avec l'apparition de la faune de mammifères à *Elephas*, qui indique le Pléistocène inférieur—sans pourtant pouvoir associer, avec précision, le commencement de celui-ci avec un certain horizon.

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PRELIMINARY INVESTIGATION INTO SOME FACTORS AFFECTING
THE SETTLEMENT OF THE LARVAE OF THE MANGROVE OYSTER
CRASSOSTREA GASAR (ADANSON) IN THE LAGOS LAGOON

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ABSTRACT

The settlement of the larvae of *Crassostrea gasar* on different types of collectors (asbestos, hardwood and oyster shells) at varying water depths, in light or shade was investigated. Results indicate that shaded collectors, particularly the hardwood, had the best spat concentration. There was no statistical difference in spat settlement between upper and lower surfaces of the various collectors. Optimum settlement occurred on collectors placed between 70 and 120 cm below the water surface. There was marked difference in spatfall on stationary and floating collectors, stationary collectors being preferred.

INTRODUCTION

The mangrove oyster, *Crassostrea gasar*, is one of the commercially important bivalves used extensively as food by the people along the mangrove swamps in the coastal areas of West Africa (Nicklès, 1950). It is commonly found in clusters attached to stilt roots and the lower branches of the mangrove tree *Rhizophora racemosa* which lines the edges of the creeks, estuaries and lagoons of Nigeria. The per caput consumption of animal protein in Nigeria is 23 kg of which 11 kg is fish. Fish produced from all sources is ca. 663,000 metric tons while the actual fish demand is 869,000 tons leaving a deficit of 206,000 tons. With a growth of 2.5% per annum, this deficit is estimated to be more by 1985. The fish demand by 1985 is estimated at 1.8 million tons. This deficit is unlikely to be met by capture fishery alone, hence the importance of shellfish culture (with special emphasis on oyster culture) can not be over-emphasized. The present investigation is geared towards bridging this gap.

A review of the literature shows that very little has been written on the biology of *C. gasar* in the Lagos lagoon system. Sandison & Hill (1966) gave an account of its distribution in relation to salinity in Lagos harbour and adjacent creeks. They indicated that in Kuramo Water and Badagri Creek (Fig. 1) spatfall was at its peak during February and early April and again during the short break in the rains in August and September. Sandison (1966) gave an account of the effect of salinity fluctuations on the species.

Although much work has been carried out on the American oyster, *Crassostrea virginica*, the Pacific oyster, *Crassostrea gigas*, and the European oyster, *Ostrea edulis*, little has been published on the settling behaviour and culture techniques of the spat of *C. gasar*. A marketable size of about 10.0 cm is attainable by the European oyster *O. edulis* in 4-5 years whereas the mangrove oyster *C. gasar* is found to attain 6.4 cm in about 6 months. This study is therefore aimed at establishing a method of spat collection which might be useful for culturing the species. Factors investigated in the study include: (1) Settlement on various types of collectors (asbestos, hardwood, oyster shell); (2) Larval preference for under-surface or top-surface of collectors; (3) Larval preference for collectors in shade or light for settlement; (4) Depth preference for larval settlement; (5) Preference for floating and stationary collectors for larval settlement.

The present investigation was carried out at Kuramo Water (Fig. 1) which is a shallow (maximum depth 3.7 m) sheltered lagoon where the spat occur throughout the year (Sandison & Hill, 1966).

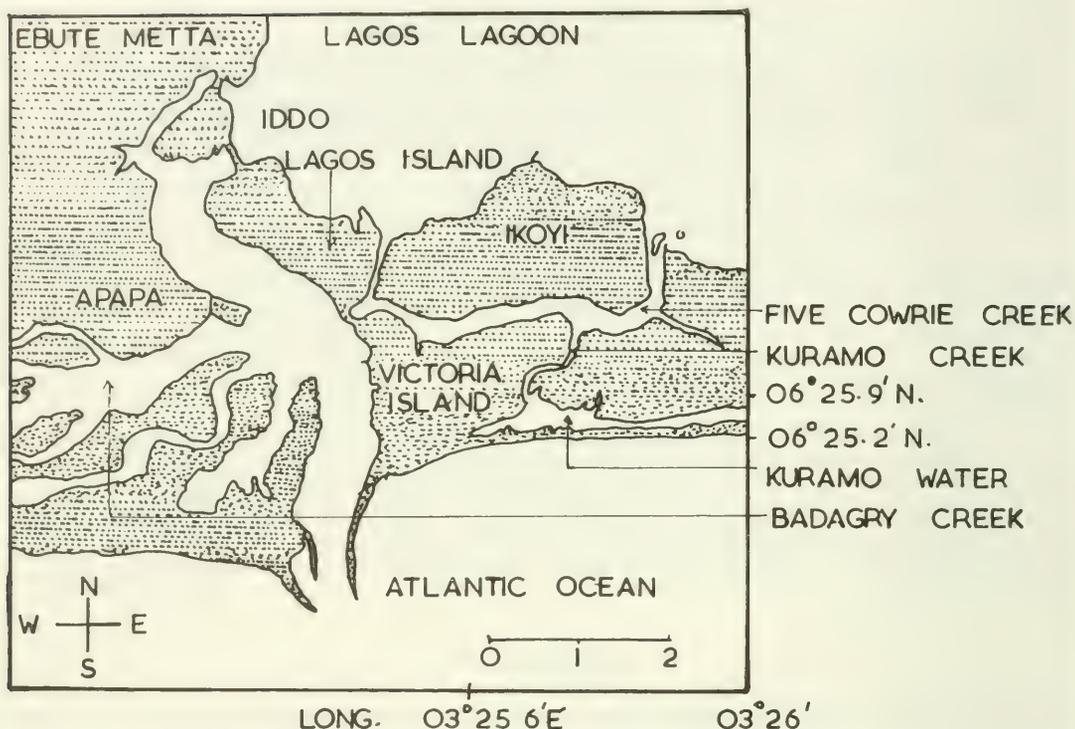


FIG. 1. Map of Lagos Lagoon (Nigeria) showing the position of Kuramo Water.

Other animals found alongside *C. gasar* on the stilt roots are *Chthamalus aestuarii*, *Balanus pallidus*, *Mercierella enigmatica*, *Hydroides uncinata* and various unidentified sea anemones, hydroids and sponges. *Mercierella enigmatica* and *Balanus* compete with *C. gasar* for food and space. They also settle on the shells.

MATERIALS AND METHODS

(a) The collectors. Although the spat of *C. gasar* settle naturally on mangrove stems and roots, these are unsuitable for culture purposes as they cannot resist decay and are liable to rot before the spat reach maturity. Studies were therefore initiated to find alternative collectors. The following substrates were used: (1) asbestos sheets, (2) hardwood (rectangular mahogany planks), and (3) old oyster shells. The choice of collectors was based on availability and cost.

(b) Stringing of collectors. Ten of the rectangular collectors (asbestos sheets 17.0 × 10.2 × 0.4 cm and hardwood 17 × 10.2 × 1.8 cm) had holes bored through them on opposite ends and were strung onto 2 nylon cords (Fig. 2). Paired knots along each cord kept the rectangular collectors about 15 cm from one another.

In the case of the dead oyster shells, a hole was drilled in the middle and these shells were strung in batches of ten with 15 cm spacing. Heavy pieces of discarded metal were used as anchors for each string of collectors to prevent floating and drifting.

Strings of various types of collectors were tied to the underside of bamboo rafts (Fig. 3) held in place by means of 4 stakes in such a way that the raft may move up and down on the stakes with the tide. Two sets of such rectangular rafts were set up 21 m apart; one in the light and the other was shaded with palm tree fronds. Strings of the various types of collectors were also tied to fixed horizontal beams on the stakes so that the top collectors on each string were exposed at low tide.

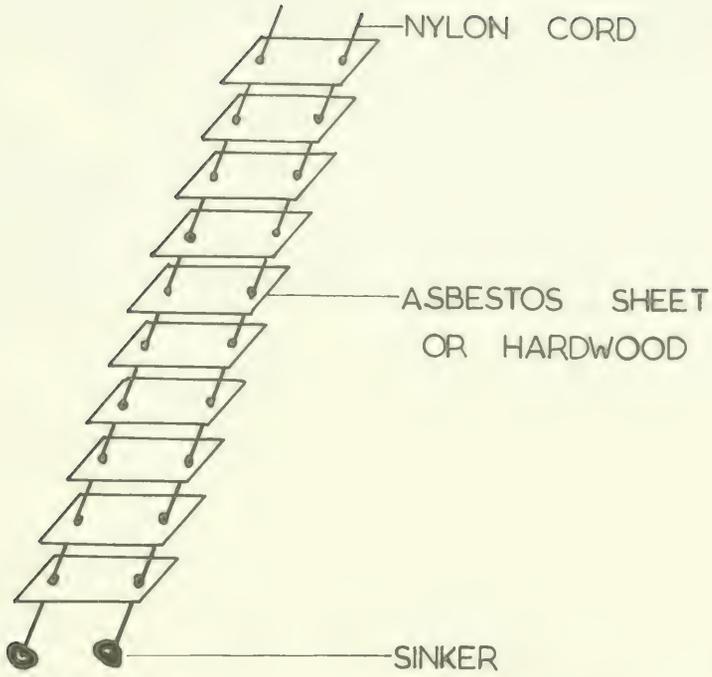


FIG. 2. Experimental strung collector.

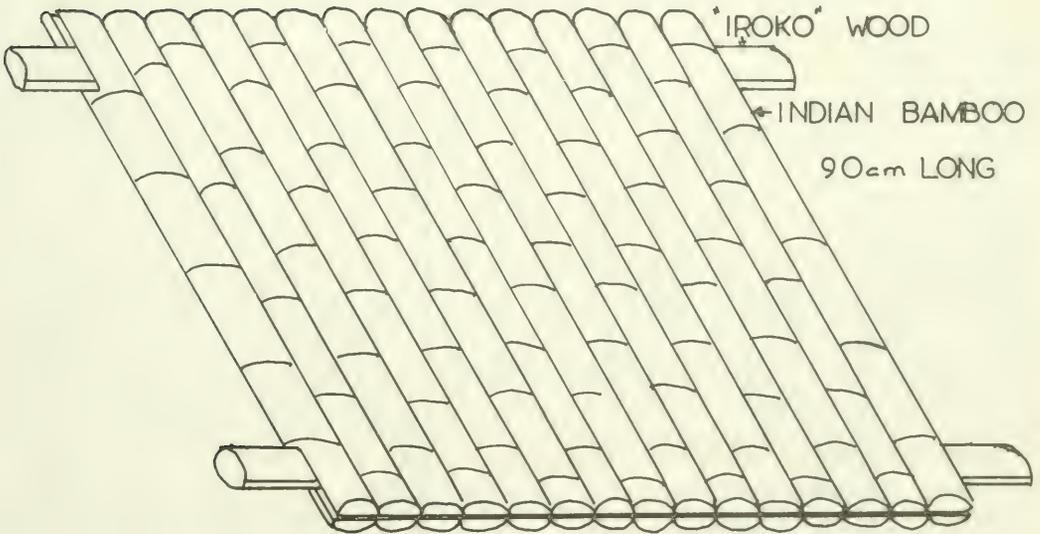


FIG. 3. Experimental raft used in the study.

At weekly intervals each string of collectors was taken out of the water and the total number of both dead and living spat on each surface was counted and recorded. Remains of dead spat were scraped off the collectors at each visit.

RESULTS AND DISCUSSION

The number of spat settling on the various types of collectors and under different conditions was expressed as number of spat per 500 sq. cm of surface area of collector. Figs. 4a-d show a summary of the results obtained during this study.

(a) Collector preference. The results of the present study indicate that the larvae of *C. gasar* prefer hardwood for settlement. There was, however, no appreciable difference in the number of spat settling on the two other types of collectors used. Apart from the relatively rougher

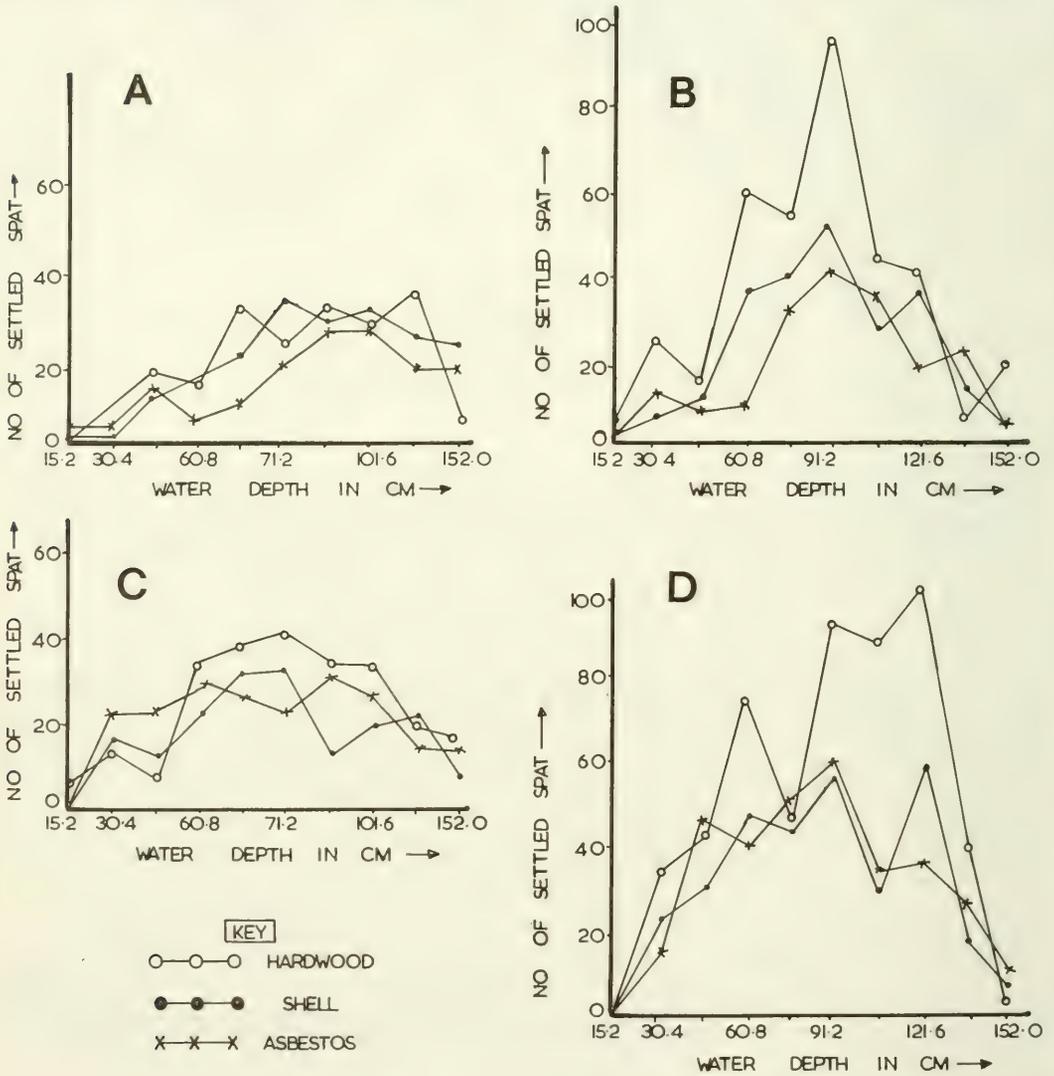


FIG. 4. Settlement of oyster spat on various collectors. A. Floating unshaded collectors. B. Floating shaded collectors. C. Stationary unshaded collectors. D. Stationary shaded collectors.

nature of the two surfaces of the hardwood, which could stimulate the settling process whereby the larvae fix themselves by an adhesive secretion to the surface, no other explanation can be advanced for the preference for hardwood. Perhaps as suggested by Cole & Knight-Jones (1939), Knight-Jones & Stephenson (1950), and Knight-Jones (1953 a,b) for *O. edulis*, the planktonic larvae of *C. gasar* exhibit certain preferences for settlement surfaces. Although the hardwood proved most successful of the 3 collectors tested, however, in commercial practice the use of hardwood is not feasible. An alternative material from which spat can be easily flicked off is being tried.

The present study offered an opportunity for comparing the mortality of spat on the different collector types. More deaths were recorded on the shells and asbestos than on the hardwood. This in itself can be responsible for the higher density of spat observed on the hardwood.

(b) Spat settlement under light/shade. Differences in settlement of larvae under shade and unshaded environments were more striking. Figs. 4B-D show that the shaded environment seems more conducive to settlement. As has been shown for many sessile marine animals, the concentration of settlement of the larvae of *C. gasar* on the lower rather than top surfaces of collectors may be due to their avoidance of light. Nelson (1921) found that the "eyed" larvae of *C. virginica* are stimulated by light and continue to move until they reach a shaded place where they become quiescent. It therefore seems that, as in the case of *C. gigas* and *O. edulis* (Walne & Helm, 1974), light is an important factor affecting settlement of the larvae of *C. gasar*.

(c) Settlement in relation to depth. The present investigations show that settlement was intense in the middle column of the water but less dense on the collectors at the top and bottom of the water column. Less settlement on the top collectors can be attributed to the avoidance of direct sunlight while the presence of debris at the bottom may be responsible for the scanty spatfall on the collectors there. Davis (1960) claimed that silt is inimical to the larvae of *C. virginica*.

(d) Settlement in relation to stationary and floating collectors. A comparison of Figs. 4A-B and 4C-D shows that there were more larvae settling on the various types of stationary collectors than on the floating collectors, especially on the collectors in the central region of the water column. The low settlement recorded on the stationary collectors placed 15 cm below the surface may be correlated with the fact that they were exposed at low tides. The few larvae settling on these collectors were found dead and therefore not recorded.

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TIDALLY DEPOSITED GROWTH BANDS IN THE SHELL OF THE COMMON COCKLE, *CERASTODERMA EDULE* (L.)

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ABSTRACT

Specimens of the intertidal bivalve *Cerastoderma edule* were marked by treatment with a cold shock and allowed to grow under different environmental conditions. In animals kept in an intertidal environment the number of growth bands deposited coincided with the number of tidal emersions. Similarly, in the laboratory, individuals grown in a simulated semi-diurnal tidal regime of 8 hours high tide and 4 hours low tide under 3 different artificial light regimes, 12 hours light:12 hours dark, continuous dark and continuous light deposited a number of growth bands which coincided with the number of tidal emersions. However, the number of bands deposited was independent of any change in the light regimes. Individuals continuously immersed in the laboratory and in the field showed ill defined growth bands, which however approximate to a semidiurnal frequency. It is suggested that the bands are formed during emersion, and are thicker when conditions cause the animals to close for longer periods. A lunar cycle with periodic alternation of thin and thick bands is thus produced. There is a possibility that an endogenous rhythm exists which is entrained in intertidal animals by periods of emersion, but which persists when the animals are kept continuously immersed.

INTRODUCTION

The calcareous skeletons of many living marine animals show evidence of periodic deposition in the form of regular external growth ridges or internal banding. Corals (Wells, 1963), some limpets (Kenny, 1977) and pectinids (Clark, 1968, 1975; Wheeler et al., 1975) exemplify the former, the bivalves *Cerastoderma edule* (cf. House & Farrow, 1968), *Clinocardium nuttalli* (cf. Evans, 1972, 1975) and *Mercenaria mercenaria* (cf. Pannella, 1975) and the barnacles *Balanus balanoides* (cf. Bourget & Crisp, 1975 a,b) and *Elminius modestus* (cf. Crisp & Richardson, 1975) exemplify the latter.

The periodicity of deposition is of great interest since it has been put forward as the basis for the interpretation of similar ridges and bands in fossil shells and skeletons, and thereby to formulate the daily and lunar components of the primaeval calendar (Runcorn, 1964). Much of the geological literature attempts to analyse the periodicities observed into tidal, daily or lunar components (House & Farrow, 1968; Dolman, 1975; Whyte, 1975) by reference to regularities in the relative distinctness of the bands and spacings within the patterns themselves. A necessary check on the supposed correlation of these periodic patterns with astronomical events is through direct experiments on living animals. Essential to this approach is the exact dating of 2 or more bands or ridges.

Bourget & Crisp (1975b) and Crisp & Richardson (1975) dated the internal growth bands in the shells of 2 species of intertidal barnacle by allowing them to grow for short periods in calcium enhanced sea water, thereby forming an abnormally thick increment. They were thus able to show that normally each shell increment corresponds to a single, semidiurnal period of tidal immersion. Fewer but thicker increments were laid down in simulated tidal regimes of greater than normal duration.

Experiments on living bivalves have not as yet allowed any consistent interpretation, nor have they concurred with the once prevalent view amongst geologists and others that the bands are laid down once daily (Davenport, 1938; Petersen, 1958; Barker, 1964; House & Farrow,

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1968). Dolman (1975), on the basis of 24 hour experiments, concluded that the bands were tidal, but Whyte (1975), from 3-hourly sampling during neap tides, appeared to conclude that the increment is laid down only at night and therefore formed once per 24 hours. Neither experiment, as reported, carries conviction. Evans (1972, 1975) working on another cockle from east Pacific shores, where the tidal regime has a strong diurnal component, offers good evidence that each growth increment correlates with the period of immersion, and the intervening bands with times of low water.

A similar confusion exists regarding other bivalves. Pannella & MacClintock (1968) marked shells of *Mercenaria mercenaria* and withdrew them 2 years later. They found 1 growth band per day, though they also stated that daily increments were further divided into 2 parts. Later, Rhoads & Pannella (1970) found more strongly marked bands in intertidal transplants of the same species, which Bourget & Crisp (1975b) suggested would be more consistent with tidally produced bands. Pannella (1975) has now reached the same conclusion, but from animals in Puerto Rico where there is a strong diurnal tide. Clark (1968) described daily bands in juvenile *Pecten diegensis* growing continuously submerged.

It can thus be seen that in intertidal bivalves at least, the view that growth bands are laid down daily is giving way to the view that they are produced tidally. The series of experiments described below were designed to clinch this matter for the European cockle *Cerastoderma edule*.

MATERIALS AND METHODS

Shells of experimentally grown cockles were briefly cleaned in 10% sodium hypochlorite solution to remove organic debris. The right valve of the shell was dried and embedded in resin (Metaserv s.w. resin 137/12742) and radial sections were cut in a plane from the umbo to the growing point. Sections were ground on successively finer grit, wet and dry "Trimite" paper, polished with a cloth soaked in Brasso for 30 sec and etched for a period of 20-25 min with cold 0.01 normal HCl. Acetate peel replicas of the polished and etched surfaces were prepared by allowing small strips of replicating material (Polaron Equipment Ltd.) to become almost molten after 30 sec in ethyl acetate. The strips were applied to the etched shell surface and after 5 min when the ethyl acetate had evaporated the peel could be removed and attached to a glass slide for microscopical examination.

Attempts were made to mark the shell internally at a given date. Various concentrations of the fluorescent dye tetracycline hydrochloride were not successful, and though Alizarin Red S could be incorporated into the shells it was considered unsatisfactory since it adversely affected the deposition of the shell. Finally it was found possible during the summer to mark the shells by maintaining them for 3 days in moist air at a temperature of 4°C. Analysis of replicas of shells returned to the natural environment showed that the shells were always notched by the 3 day shock. Fig. 1 illustrates the appearance of the peel in the region of the cold shock. A depression, sometimes shallow but frequently in the form of a deep cleft, (D) can be seen which is sometimes associated with the development of a spine (Sp) in the immediate post-shock region of the shell. The first few growth bands formed after the cold shock are relatively thin, but the subsequent acceleration of growth can be seen to the right of the figure from the increasing width of the growth bands.

Three types of experiments were undertaken to obtain peels of animals maintained for known periods under different conditions.

(1). Intertidal experiments.

Cockles were given an identification mark and treatment by cold shock before being set out at defined levels on the mud flats of the south shore of the Menai Straits at Gorad y Gyt at the eastern end (May, 1976) and in sandy substrata at Traeth Melynog, Abermenai Point at the western end of the Straits (July, 1976, April, 1977). Animals were recovered after various intervals of time and some in the first experiment were marked on the 2nd occasion by cold shock treatment and returned to the shore. These experiments were designed to investigate the effect of tidal level on shell growth band pattern. Due to losses, not all levels could be investigated in each experiment.

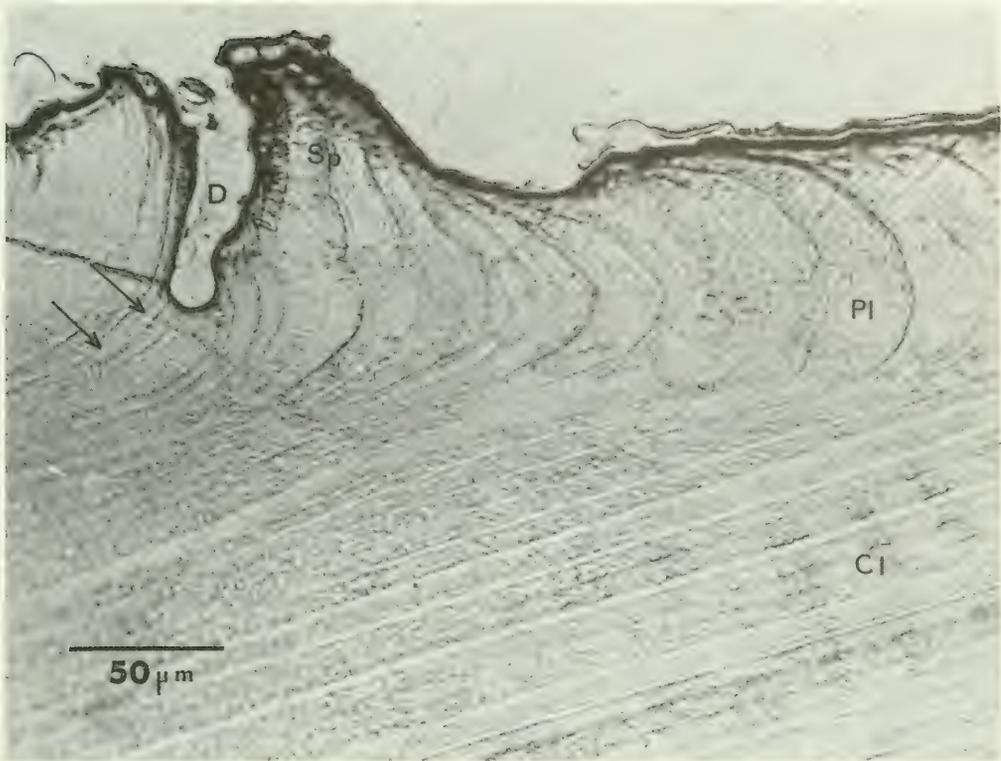


FIG. 1. Acetate peel showing the cleft (D) in the peripheral layer (PI) of *Cerastoderma edule* produced by the discontinuity of growth after a 3-day cold shock treatment. The associated spine (Sp) can be seen to the right of the cleft. Arrows indicate the band at which growth is assumed to have restarted after the cold shock. Note that for several days after treatment growth bands can be seen to be closer than they are to the right of the figure at the time that normal growth has presumably restarted. This animal was kept under simulated tidal conditions in the laboratory during April 1976. Cl—crosslamellar layer.

(2). Raft experiments.

Cockles, similarly prepared, were placed in a continuously submerged box on a raft in the Menai Straits. One half of the box, the "dark box," consisted of a light tight chamber, while the other half, the light box," was identical in every respect except that a perspex lid and sides replaced the opaque panelling. The passage of water was regulated and light kept out by a series of baffles at the entrance and the exit. This experiment was designed to test the effect of continuous immersion, natural photoperiod and continuous darkness on shell growth band pattern.

(3). Laboratory experiments.

Cockles were kept successfully in the laboratory without natural substrata, at a temperature of $16 \pm 1^\circ\text{C}$, in light controlled tanks with a facility for simple tidal fluctuations, in which running sea water was supplied to a depth of 10.5 cm for 8 hours of the simulated high tide, and the tanks drained for 4 hours of simulated low tide. Alternatively, the cockles could be kept continuously submerged. As there was a possibility that the temperature might rise at simulated low tide with no water protecting the animals from the lamps, the tank lids were specially designed with baffles to allow heat but not light to escape. Both illuminated and non-illuminated tanks were provided with similar lids and lights but the lids of the former had perspex panels to allow the light to pass, while those of the latter had opaque panels to exclude

light. In this way both tanks would have been similarly heated during the "lights on" period. The cockles did not put on appreciable shell growth unless well fed. Seven to 8 litres of algal suspension at 2×10^9 cells l^{-1} were added to the header tank supplying the 4 experimental tanks at every high tide. The initial concentration achieved was approximately 10^8 cells l^{-1} . Appreciable growth would not take place during winter, even if the animals were fed, hence the majority of experiments were restricted to the season of natural growth between April and September.

The laboratory experiments were designed to investigate the effect on shell growth band pattern of tidal and non-tidal conditions combined with equinoctial photoperiod, continuous darkness and continuous light.

At the end of each of the above experiments, as many cockles as could be recovered were sectioned and peels prepared for examination in a projection microscope. Photographs were taken of typical peels. The number of growth bands was counted and the average thickness of the increments estimated from the total shell growth as measured by a calibrated eyepiece micrometer. Three counts were made of the numbers of bands, both in the peripheral layer (Fig. 2 PI) and in the crosslamellar layer (Fig. 2 CI) of each shell between the position of one cold shock cleft and the next, and/or to the growing end of the shell. The total number of bands for each shell layer was averaged over all shells and standard errors calculated. To quantify differences seen in the relative distinctiveness of the bands each band was assessed comparatively in each shell layer, and assigned to 1 of the 3 categories "strong," "intermediate," or "weak" and the number of each class averaged over all shells. Finally the presence of alternate strong and weak bands and the regularity of the increments was noted but not quantified.

RESULTS

(1). Intertidal experiments.

Fig. 2 illustrates the appearance of an acetate peel taken from a radial section of a cockle grown at low water neap tide level. The cleft D marks the beginning of the experiment period from which 54 growth bands can be counted up to the growing edge of the shell on the right. The peel replicates 3 layers; the outermost is a thin, uncalcified periostracum showing as a thin dark line extending over the upper surface including the spines, Sp. Next is the peripheral layer, PI, of the calcified shell in which the growth banding runs approximately parallel to the truncated region at the growing point of the shell. The innermost is the crosslamellar layer, CI, with the bands running approximately parallel to the inner surface of the shell. The crosslamellar bands join the peripheral bands at a sharp change in angle, corresponding to the acutely pointed growing tip.

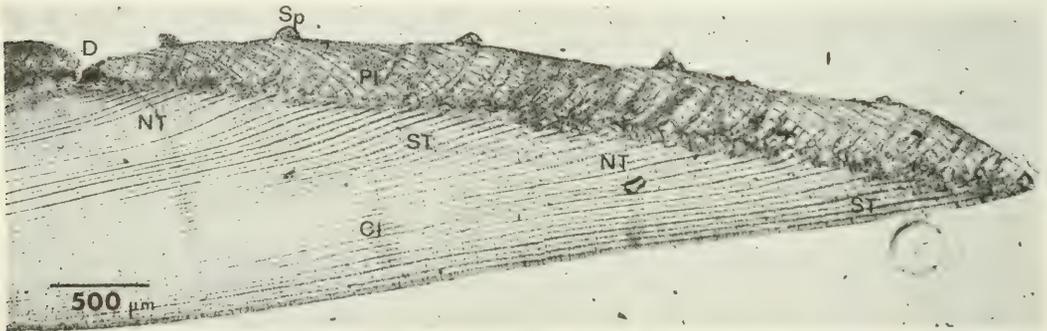


FIG. 2. Acetate peel from *Cerastoderma edule* grown at low water neap tide level at Abermenai Point, marked by cold shock on 15 July 1976 and removed from the environment at 19.00 on 12 August 1976. Note the cleft at D from which 54 bands can be counted. From a knowledge of the time of removal, the last visible band must have been laid down at approximately 19.00 on 12 August 1976 and the last increment took place during the ensuing period of daylight. ST—maximum spring tide; NT—maximum neap tide; PI—peripheral layer; CI—crosslamellar layer; Sp—spines.

The banding appears as thin dark lines with thicker, more transparent, regions between. The former will be referred to as growth bands, and the distance separating the centres of the growth bands as growth increments. It is evident that the growth increment is not constant but varies with position along the growth band and with the angle at which it is measured. Since the growth increments relate to growth rates rather than to the origin of the pattern, they will be dealt with in a separate paper. The growth bands in the peripheral layer are further apart but those in the crosslamellar layer are generally more distinct. Each band can usually be identified, but sometimes very faint bands are difficult to make out and may be restricted to one shell layer. Therefore counts of peripheral and crosslamellar bands were separately assessed and averaged; the conformity in the counts of the 2 layers being a good criterion of reliability. Occasionally bands were obscured by defects in grinding. Occasionally, late in the season or in shells from high water, little growth had taken place, making counting very difficult. Where considerable uncertainty existed the counts were excluded from analysis.

Fig. 2 shows an alternation in the thickness of the bands which appears periodically. From the dating of this shell it can be seen that strongly marked alternations coincided with or slightly preceded spring tide maxima, while the fainter banding with less evident alternations, corresponded with the neap tide period. Furthermore, the last band deposited was a thin one, and the penultimate a thick one. Since the shell was collected on the evening low tide, the last, fainter line, assuming tidal periodicity, must have been laid down during the morning, and the earlier thick line during the previous evening and so on. Other shells sampled from the same collection show consistency in the pattern of alternation of the bands indicating that the pattern relates to the environment rather than to a particular individual.

Table 1 records the band counts made from some 70 animals grown in 2 habitats at various shore levels in which the exposure of every individual was accurately known in relation to dated bands. Therefore the band frequency could be related to the number of light and dark periods and to the number of tidal cycles of immersion and emersion. Columns 5 and 6 show clearly that the number of bands tend to coincide with the number of tidal cycles, and not the number of days. In 4 of the 6 experiments (rows 1, 2, 4 and 6) the agreement is within 2% of expectation, and entirely within the 5% confidence limits (approximately $2 \times$ Standard Error) for rows 1, 2 and 4. The counts are significantly below expectation for the high water neap and mean tide level samples at Traeth Melynog. In the higher shore samples the growth increments are smaller and the closer banding makes them harder to read, especially in the crosslamellar layer where the bands approach most closely. An underestimate of band frequencies is therefore more likely at these tidal levels.

It will also be noted from Table 1 that very few of the bands were weak, nearly all being classified as strong or intermediate. This feature is evident from a cursory inspection of the peels, the striped light and dark pattern in all intertidal exposures being very clear.

(2). Raft experiments.

When the peels of cockles which had been transferred from an intertidal habitat to the raft were examined, a sudden change in the appearance of the pattern of banding could be seen, starting at a point corresponding to the cleft induced in the shell surface by cold shock treatment (Fig. 3). In place of the clearly marked darker and lighter stripes of the intertidal pattern were fainter more widely spaced and less regular bands. The fainter bands produced while continuously submerged were slightly less obscure in the peripheral layer than in the crosslamellar layer of the shell. Furthermore many of the bands were very faint indeed. Immediately after being placed under continuous submersion, there was, in many shells, a zone of almost continuous deposition without any bands being clearly identifiable. Later, a series of bands were re-established. These never fully resembled the intertidal banding pattern however.

Table 2 records the number of bands counted in the region of the shell corresponding to the 45 days and 87 tidal periods of this experiment. The table indicates 2 of the features mentioned above, the high proportion of weak growth bands observed and the larger number of bands seen in the prismatic layer. The difficulty in reading these peels, or possibly a real variation in band number, leads to a much larger standard error as well as a significant difference between the 2 shell layers. Nevertheless when the results are averaged out the number of bands fits better to the number of tidal cycles than to the number of daily periods of light and dark. Moreover, having regard to the large standard errors, there is no significant

TABLE 1. Predicted and observed number of growth bands in shells of *Cerastoderma edule* kept for known periods in the intertidal zone.

Conditions of exposure	Shore level	No. of shells examined for peripheral (P) & cross-lamellar (C) layers	Duration of experiment (days)	No. of emersions	Total no. of bands (mean)	Standard error		Mean no. of bands	
						strong	intermediate	strong	intermediate
Mud flats									
Gorad y Gyt marked by cold shock on 6th & 22nd May 1976	MTL	(P) 14	14.5	27	(P) 27.0	±0.3	13.9	11.4	2.0
		(C) 17			(C) 27.1				
Between marks 1 & 2 (6-20 May)	MTL	(P) 13	8.5	15	(P) 14.4	±0.3	6.9	6.2	1.3
		(C) 18			(C) 14.7				
Between mark 2 & end of experiment (22-30 May)	MTL	(P) 8	29.0	54	(P) 50.1	±1.8	29.6	18.1	2.5
		(C) 11			(C) 46.8				
Traeth Melynog Abermenai Point July 1976	LWN	(P) 10 (C) 10	29.0	54	(P) 54.1 (C) 52.9	±0.8 ±0.5	26.3 22.7	24.0 26.2	3.8 4.0
Traeth Melynog April 1977	HWN	(P) 7 (C) 7	58.0	111	(P) 98.8 (C) 90.6	±2.7 ±2.5	— —	— —	— —
		(P) 8 (C) 13			(P) 107.8 (C) 106.7	±0.9 ±1.0	50.9 44.6	48.3 51.9	8.5 10.2

TABLE 2. Growth bands in shells of *Cerastoderma edule* kept for known periods on a raft in the Menai Straits.

Condition	No. of shells examined for peripheral (P) & crosslamellar (C) layers	Duration of experiment (days)	Number of tidal periods	Total number of bands (mean)	Standard error		Mean no. of bands	
					strong	intermediate	strong	intermediate
Continuously immersed Natural photoperiod 10 June-25 July 1977	(P) 12 (C) 16	45	87	(P) 99.3 (C) 71.1	3.0 3.8	36.3 30.1	25.2 16.6	37.9 24.4
Continuously immersed Continuous darkness 10 June-25 July 1977	(P) 7 (C) 11	45	87	(P) 112.1 (C) 72.9	7.0 3.5	49.1 35.5	33.3 17.3	29.7 20.2

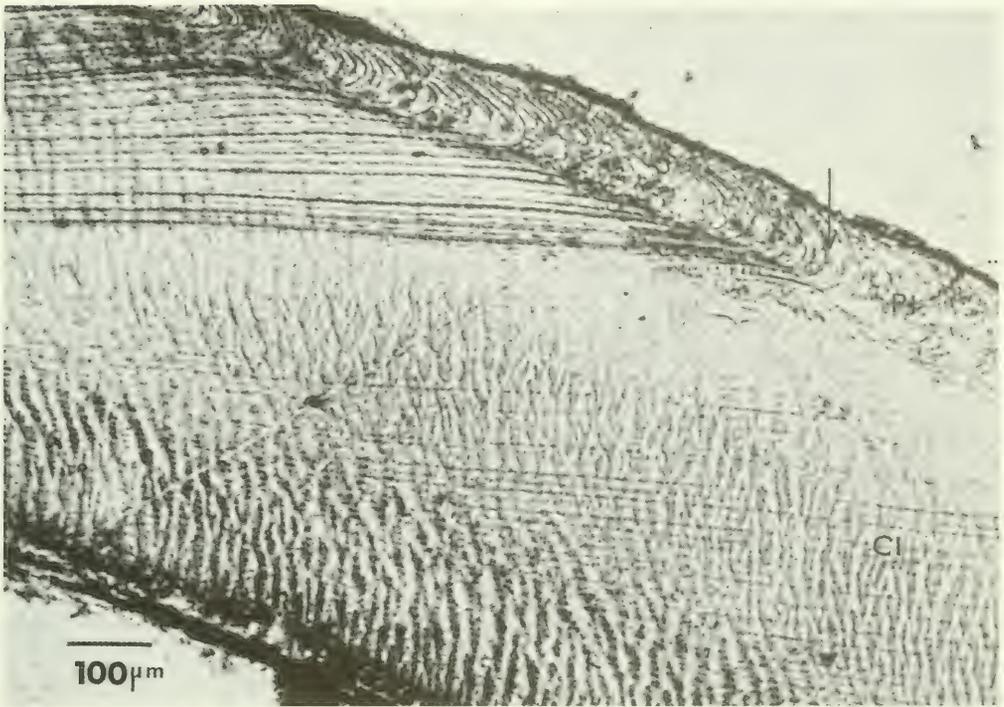


FIG. 3. Acetate peel from an experiment where *Cerastoderma edule* was transferred from the intertidal zone to a raft with continuously submerged conditions and natural photoperiod. Arrow marks transition. Very distinct and narrow growth bands laid down intertidally are seen to the left, sharply demarcated from the broader and less distinct bands laid down on the raft where continuous feeding was possible. PI—peripheral layer; CI—crosslamellar layer.

difference in the number of bands in either layer between those animals exposed to submarine daylight and those kept in the dark box.

(3). Laboratory experiments.

The appearance of the peels from the laboratory experiments in essence confirmed those from the field and the raft. Fig. 4 clearly illustrates the effect of tidal conditions on the growth bands of laboratory grown animals and parallels Fig. 3. In this particular experiment the inlet valve, which closes at simulated low tide and thus prevents the inflow of water, failed to close for 32 hours, which included 2 presumptive low waters. As a result, the animals were kept beneath a depth of flowing sea water throughout. The valves were then reactivated, producing 4-hour periods of emersion once again. As can be seen from the photograph, well defined bands were produced under both periods of tidal conditions, but only weak bands were formed when the animals were immersed. The graph to the right of the photo illustrates how growth rates can be measured off the peels and indicates that a higher rate of growth took place when the cockle was kept immersed. The peels of animals which were given simulated tides generally produced more distinct and regular bands than those continuously immersed, though the banding was less stark than that of animals from the intertidal zone itself. Moreover, as can be seen from Fig. 5, there was little evidence of alternation between thin and thick bands which so clearly characterised the shells of intertidally grown cockles. Nor did the cockles exposed to 12 hours light and 12 hours darkness in the laboratory show any appreciable alternation in banding.

Table 3 lists the counts taken from peels of cockles kept under the 6 possible combinations of illumination and tidal conditions offered in this experiment. The numbers of growth bands

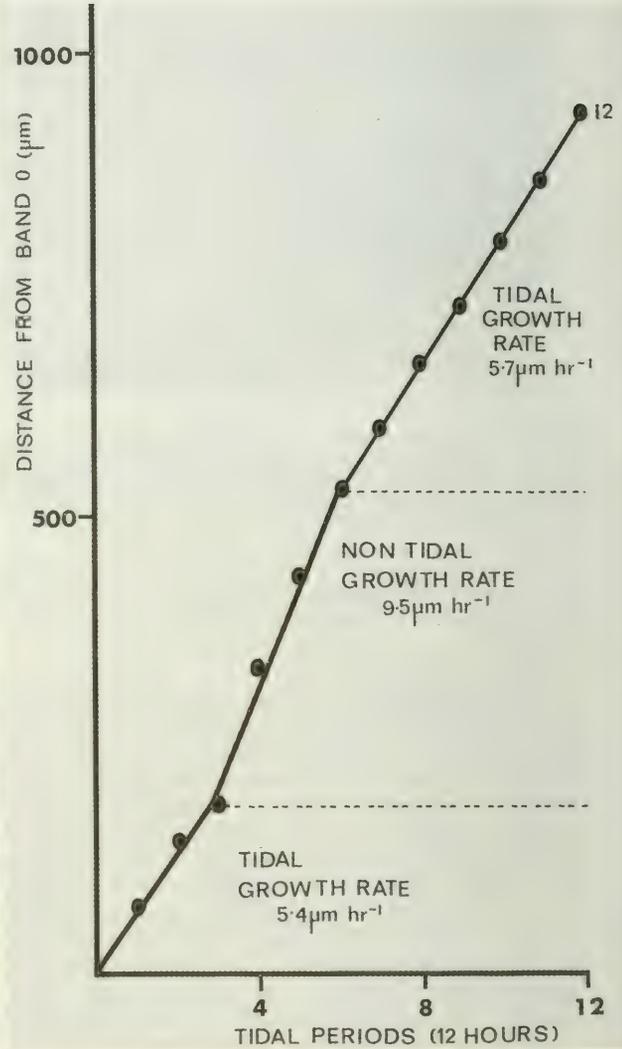
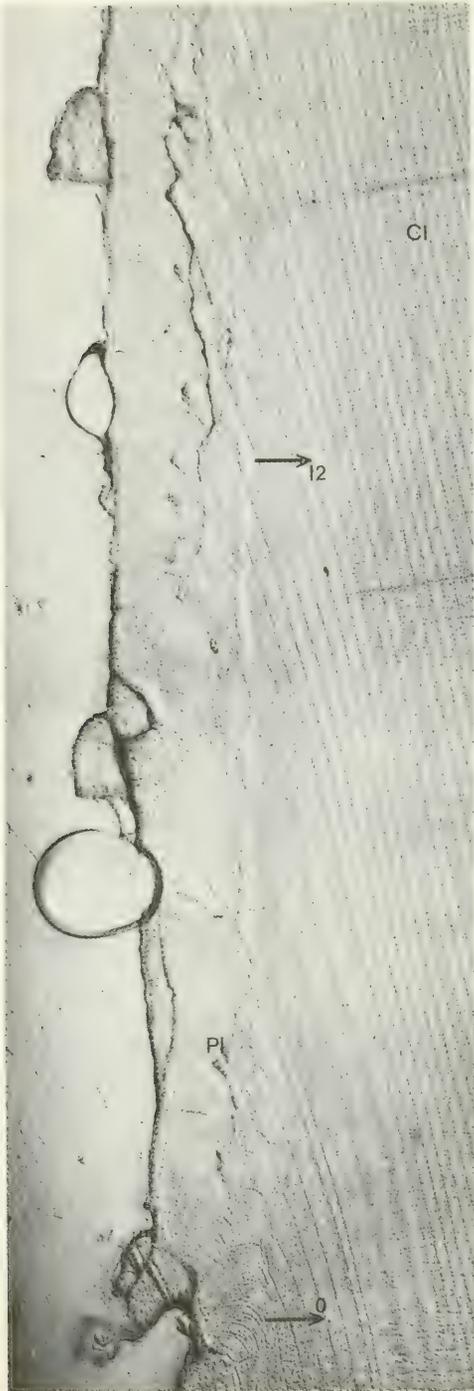


FIG. 4. Left: panel from an experiment conducted during September-October 1976, the conditions under which *Cerastoderma edule* was growing were changed from a simulated tidal regime to a continuously immersed regime for 32 hours between 2 and 4 October 1976 and then returned to tidal conditions. Note the loss of definition of the growth bands and the greatly enhanced width of the bands when continuously submerged. Right: Plot of growth against time using data from the peel and assuming each band represents one tidal period. Growth rates can be measured from the slope of the graph.

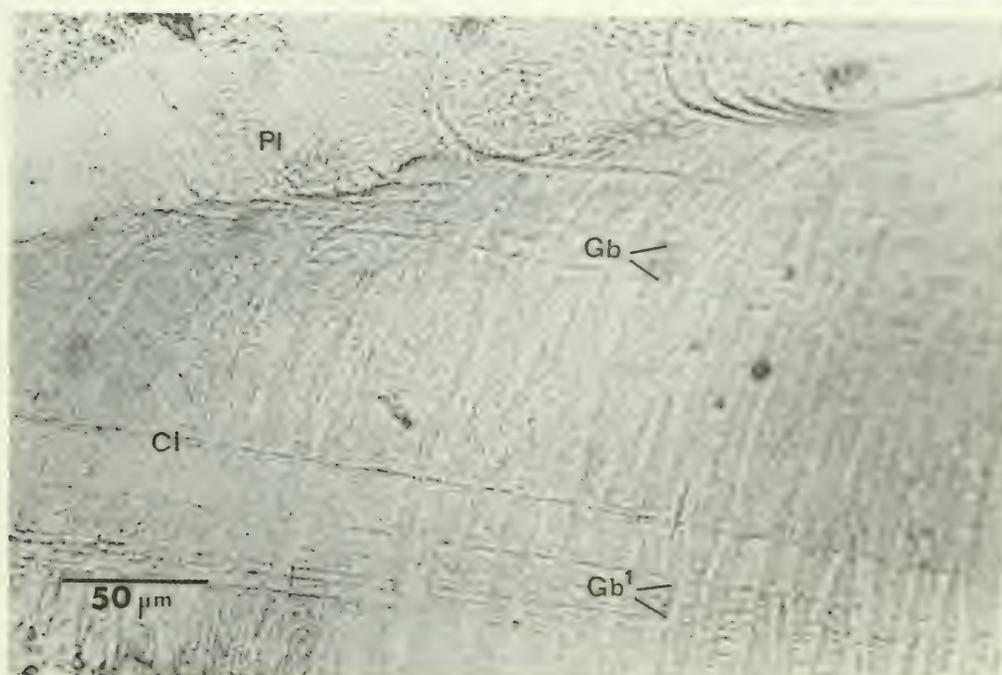
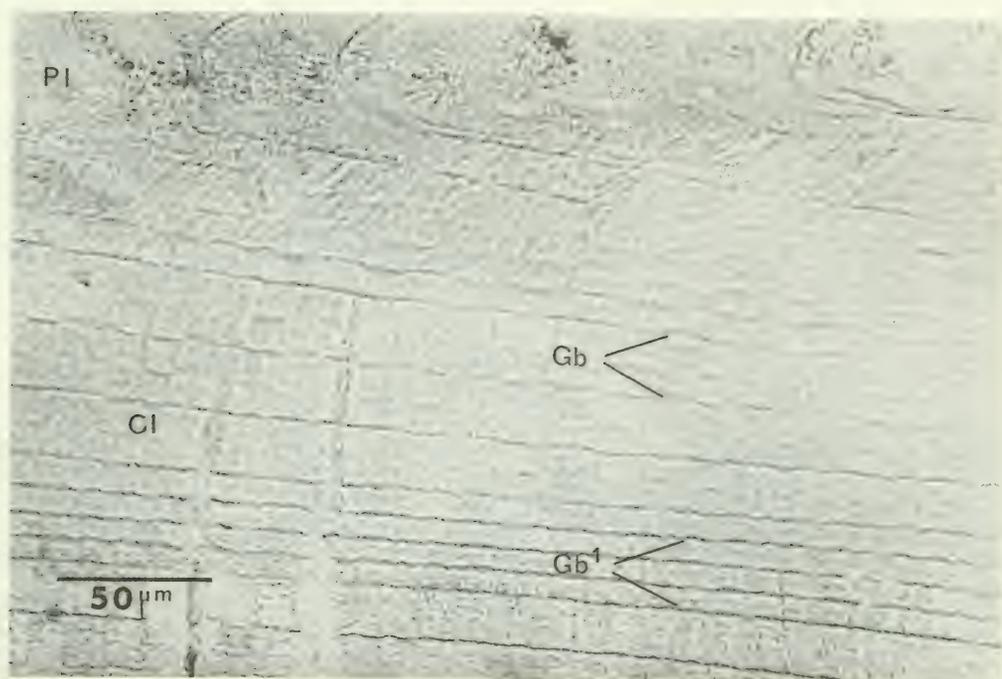


FIG. 5. Acetate peels from laboratory experiment 1 conducted in continuous darkness. The upper figure shows a peel from a cockle given tidal emersion every 4 hours in 12, the lower figure shows a peel from a continuously immersed cockle. Note the clearer definition and greater regularity of the bands in the cockle from the tidal regime. Gb—growth bands; Gb¹—narrowing of increments corresponding to an occasion when the algal food was not replenished; PI—peripheral layer; CI—crosslamellar layer.

TABLE 3. Shell growth bands in *Cerastoderma edule* kept under controlled laboratory conditions of immersion and illumination. Experiment 1: 27.IX.28.X.1976; experiment 2: 1-23.XI.1976.

Condition	No. of shells examined for peripheral (P) & crosslamellar (C) layers	Duration of experiment (days)	No. of emersions or tidal periods	Total no. of bands		Mean number of bands		
				mean	standard error	strong	intermediate	weak
Tidal emersion 12 h. light, 12 h. dark	(P) 6	30.5	61	(P) 61.2	±1.3	30.2	18.2	12.5
	(C) 10			(C) 59.0	±0.7	32.3	18.9	7.7
24 h. dark	(P) 8	30.5	61	(P) 62.9	±1.0	33.1	19.5	11.5
	(C) 10			(C) 58.3	±0.8	36.7	14.5	7.1
24 h. light	(P) 9	21.5	43	(P) 41.3	±1.9	23.4	12.9	5.0
	(C) 12			(C) 39.7	±1.5	24.6	9.5	5.6
Continuously immersed 12 h. light, 12 h. dark	(P) 11	30.5	61	(P) 45.1	±1.2	28.6	11.5	5.1
	(C) 12			(C) 41.6	±1.3	30.3	9.1	2.2
24 h. dark	(P) 12	30.5	61	(P) 74.3	±2.4	22.2	18.6	33.2
	(C) 15			(C) 48.5	±2.0	19.4	12.2	16.9
24 h. light	(P) 12	21.5	43	(P) 65.0	±5.2	18.0	21.6	25.4
	(C) 14			(C) 49.7	±2.7	23.5	12.6	13.6
24 h. light	(P) 15	21.5	43	(P) 47.9	±1.8	20.4	12.2	15.3
	(C) 16			(C) 40.4	±1.8	19.1	10.2	11.1
24 h. light	(P) 15	21.5	43	(P) 43.1	±2.6	13.5	13.5	16.1
	(C) 16			(C) 34.3	±1.5	16.6	9.7	8.0

of the tidally immersed group gave a precise fit to the number of tidal emersions, all falling within the expected confidence limits that $P < .05$. The numbers of bands seen in the peripheral layer usually exceeded those in the crosslamellar layer, but the difference was very small, generally less than $\pm 2\%$ of the numbers expected. Few of the bands were classified as weak. There was no obvious influence of illumination. In the group of cockles fed from the same source but continuously immersed, the standard errors between counts were somewhat greater than in the tidal group. However, the main difference was in the discrepancy between counts in the peripheral layer and in the crosslamellar layer. Whereas the former exceeded, the latter fell short of the number of tidal periods experienced, in both cases the differences being usually significant. Also the number of weak bands recorded was greater and the number of strong bands less than in the tidally emerged group. Otherwise no differences within the continuously immersed group could be discerned, illumination again being without apparent effect.

DISCUSSION

Table 4 summarises the results of all experiments in a form which makes them comparable. The numbers of growth bands are expressed as a percentage of the expected numbers, assuming that they each represent a single tidal period.

All cockles exposed to tidal conditions, whether natural or simulated, gave counts in both shell layers which were very close to expectation. The only exceptions were the cockles exposed at high water neap tide where growth was small. Another group of cockles exposed very late in the season (October) gave deficient numbers of bands compared with expectation, no doubt because growth rates were so small that the bands could not reliably be distinguished. It is possible that the small number of bands counted by Farrow (1971) in Thames estuary cockles, which he ascribed to daily growth, could have been underestimated because he included winter periods of virtually no deposition in the counts. Moreover, cockles which were living at the high water of neap tides would only just be covered at high tide, or might even remain uncovered in the absence of waves or swell during the smallest tides. The underestimate might therefore be real, representing a failure to deposit any increment unless immersed and able to feed for a certain period of time. As a result, 2 or more consecutive emersion bands might coincide. It must follow therefore that *Cerastoderma edule*, like the intertidal barnacles *Balanus balanoides* and *Elminius modestus*, lays down 1 growth band per tide when growing over most of its intertidal range. However, as in barnacles, bands may be missed under circumstances where growth does not take place. It is not fully justifiable therefore to equate band numbers to tidal periods in individual fossil shells where the conditions of growth cannot be assumed. The maximum number of bands are however likely to coincide with tidal frequency.

There are 2 other features of growth banding which accord with this conclusion. First, the clarity and definition of tidally formed bands are greatest in intertidal cockles, intermediate in laboratory simulated tidal animals, but dubiously present in shells of continuously immersed animals. In Table 4 the entries 3 and 4 represent this last group and are quite similar, showing an excess of bands (over 100%) in the peripheral and a deficit in the crosslamellar, due mainly to a large number of weak bands being counted in the former.

Secondly, the occurrence of an alternation of strong and weak bands in naturally growing intertidal cockles, was found by dating to have a lunar periodicity coinciding with spring tides. During these highest and lowest tides, the animals will be exposed to the air for longer periods, presumably thereby forming stronger bands. At neap tides they will be exposed only briefly causing the formation of a series of weaker bands. However, the reason for alternation of band strength must still be explained.

Table 4 allows comparison between the shells of cockles grown under natural photoperiod on the shore, under artificial photoperiod in the laboratory, and under submarine illumination on the raft to be compared with cockles grown in continuous darkness on the raft or in continuous light and darkness in the laboratory. All comparisons between different regimes of illumination but otherwise similar conditions show that illumination exercises no appreciable effect on the growth bands. Not only does this result further refute any lingering claim that the banding itself reflects a daily cycle of light and dark, but it also throws doubt on the possibility that a light-dark diurnal influence interacts with a semi-diurnal tidal periodicity to produce

TABLE 4. Summary of growth band data, expressed as mean number of bands and calculated as percentage of expectation if tidally produced. P: peripheral, C: crosslamellar layers.

Condition	Total no. & standard error	Strong	Intermediate	Weak
1. Intertidal Zone				
HWN	(P) 89.0 ± 2.4 (C) 81.6 ± 2.3		Too close for assessment Too close for assessment	
MTL	(P) 96.2 ± 2.2 (C) 95.1 ± 1.5	50.7 46.5	38.8 42.1	6.8 6.5
LWN	(P) 98.7 ± 1.2 (C) 97.0 ± 0.9	47.3 41.3	44.0 47.6	7.4 8.8
2. Laboratory tidal simulation (8 h. immersed, 4 h. emersed)				
12 h. light, 12 h. dark	(P) 100.3 ± 2.1 (C) 96.7 ± 1.1	49.7 53.0	29.8 31.0	20.5 12.6
Continuous darkness	(P) 99.5 ± 3.0 (C) 94.0 ± 2.4	54.4 58.7	30.0 23.0	15.2 12.3
Continuous light	(P) 104.9 ± 2.8 (C) 96.7 ± 3.0	66.5 70.5	26.7 21.1	11.9 5.1
3. Raft exposure				
Natural photoperiod	(P) 114.1 ± 3.4 (C) 81.7 ± 4.4	41.7 34.5	29.3 19.2	43.1 28.0
Continuous darkness	(P) 128.9 ± 8.0 (C) 83.8 ± 4.0	56.2 41.1	38.5 20.2	34.2 22.5
4. Laboratory				
Continuously immersed				
12 h. light, 12 h. dark	(P) 121.8 ± 3.9 (C) 79.5 ± 3.3	36.4 31.8	20.5 20.0	54.4 27.7
Continuous darkness	(P) 109.0 ± 6.4 (C) 87.7 ± 4.3	38.5 41.5	31.9 22.1	38.6 24.0
Continuous light	(P) 100.2 ± 6.0 (C) 79.8 ± 3.5	31.4 38.6	31.4 22.6	37.4 18.6
5. Mean and S.D. of all bands from both layers				
Intertidal	92.9 ± 6.5	46.4 ± 3.9	43.1 ± 3.7	7.3 ± 0.8
Simulated intertidal	98.6 ± 3.65	58.5 ± 8.1	26.9 ± 4.1	12.9 ± 5.0
Raft exposed	102.1 ± 23.2	43.3 ± 9.2	26.8 ± 9.0	32.0 ± 8.8
Laboratory immersed	96.3 ± 17.1	36.4 ± 4.0	27.4 ± 7.6	33.5 ± 12.8

lunar periodic alternations. If some other diurnal factor which is known to influence banding can be found, it would be a more likely candidate.

Intertidal cockles whose peels successfully included the most recently deposited band at the delicate growing point of the shell offered strong evidence that the thinner, weaker bands were formed on the early morning spring ebb, and the alternating stronger and darker bands on the afternoon ebb. It is likely that during and after the heat of a summer day the stress of high temperature and potential desiccation will cause the shells to close more firmly and for longer than in the cool of the morning. If shell closure were the proximate cause of the formation of shell bands in bivalves, as has been demonstrated in barnacles (Bourget & Crisp, 1975 a,b), one would expect the broader band to be formed on the afternoon ebb. During the spring tides, when alternations are observed in shell banding, the morning ebb occurs between 0400 and 0700 and the evening between 1600 and 1900 hours. Thus in summer it is light at both ebbs. The neap tides occur at 1000 to 1300 and 2200 to 0100 hours, and these have been shown to correspond with more uniform and weaker banding. Though the cockles will be exposed to the air and high temperature only briefly at neap tides, they will receive a much greater difference in illumination than they will at spring tides. Hence illumination itself appears not to be a

factor causing difference in band thickness. We suggest rather a combination of insolation and duration of emersion. In North Wales, the conditions producing the difference would be greatest in the period preceding the spring tide maximum when a reasonably long low water occurs either in the heat of the early afternoon, or at dawn. This view is supported by the experiments under laboratory simulated tidal regimes with light and dark conditions. A temperature differential was carefully excluded and very regular band thicknesses were observed without any alternations.

The experiments conducted on the raft and under constant immersion in the laboratory ought not, on the above theory, to have produced any growth bands. True, the banding was less distinct and regular, but some bands did appear. Furthermore, although the 2 shell layers gave different results, the average agreed very well with the number of tidal periods. This average included a very high variability between counts (Table 4, Section 5, see standard deviations) whereas the band counts of all cockles under tidal conditions showed little variability.

The tidally periodic influences which are possibly at work in all fully immersed experiments would be (1) gravitational force and (2) food availability. The former cannot be ruled out, but seems intuitively an unlikely 'Zeitgeber.' The latter would certainly be present in all laboratory experiments, because the cockles were pulse fed at each simulated high tide. The algal food would be gradually filtered and diminish in concentration until the next high tide when its concentration would be restored abruptly. On the raft, a tidal periodicity in the food supply is less likely, but possible. The abundance of algal food might vary within water mass oscillating up and down the Menai Straits. Also, water current, by renewing the supply of food adjacent to the inhalant siphon, is known to influence the intake of food in bivalves and hence their growth rate. However, the maximum tidal flux is roughly quadri-, not semi-diurnal, though ebb and flood currents are not always equal, especially near shore in narrow channels where counter eddies are frequent.

A 3rd possibility exists, namely that a persistent endogenous rhythm causes regular phases of growth and rest in the activity of the shell secreting epithelium. In the intertidal zone these phases might normally be entrained by periods of emersion, but when continuously immersed, they might display their own innate rhythm. Such a theory would explain the persistence of regular banding in shells from low water mark.

It is interesting that a similar persistence of weak growth bands was found by Bourget & Crisp (1975b) in barnacles grown on a raft or under continuous immersion in the laboratory. These authors also were forced to entertain the possibility of an endogenous rhythm in shell growth.

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WHAT IS THE FUNCTION OF THE SHELL ORNAMENTATION OF *TELLINA FABULA* GMELIN?

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ABSTRACT

Tellina fabula Gmelin is unique amongst the European Tellinidae in the possession of diagonal striae on the surface of the right valve only. What purpose this ornamentation serves is unclear. Scanning electron micrographs revealed that the striae consist of a series of flat ridges overlapping from anterior to posterior. They were not observed on the early stages of the shell and became prominent only after the first growth ring. The striae appeared to originate from the concentric rings on the shell and there was a significant correlation between the number of striae and the length of the valve. However, there was no correlation between the number of striae and the weight or the thickness of the valve. There was no significant difference in the weight of right and left valves. It is unlikely, in view of the burrowing behaviour and life position adopted by *T. fabula* that the striae assist directly in burrowing, but it is possible that they strengthen the right valve against the stresses set up by the contraction of the pedal musculature. Other possible explanations of the presence of striae such as predator deterring, animal orientation in a current, or retention of a primitive ancestral condition, are also discussed.

INTRODUCTION

The possession of oblique striae on the valves is by no means common in the Tellinidae (Table 1). Exceptions to this rule occur in tropical members of the subgenus *Scissula* and the genus *Strigilla* in which most species have striations on both valves (Moore, 1969; Stanley, 1970; Keen, 1971).

Tellina fabula is exceptional in that not only is it the sole British tellin with striae (Tebble, 1966) but also the striae occur on one valve, the right valve, only. What purpose this ornamentation could serve was unclear, so this present study was set up to investigate the morphology of the striae by means of the scanning electron microscope (SEM) and to attempt to relate morphology to possible function.

METHODS

Specimens were obtained from a site 6.0 m below Chart Datum in Kames Bay, Millport, Scotland. The valves were cleaned by removing the flesh in boiling NaOH solution (Rees, 1950) and washing in distilled water.

TABLE 1. Number of tellinid species with or without diagonal striae on one or both valves.

Number with striae	Number without striae	References
1*	9	Wood (1848-1882)
1*	3	Brøgger (1901)
3	23	Allan (1950)
1*	11	Heering (1950)
0	4	Ockelmann (1958)
1*	8	Tebble (1966)
14	73	Moore (1969)
3	15	Stanley (1970)
14	60	Keen (1971)

**T. fabula*

For the SEM 12 valves (1 left valve and 11 right valves, of varying sizes) were mounted on a metal stub and shadowed with gold.

A further 28 specimens were chosen for size and weight analysis. The left and right valves were weighed on a microbalance, and the length (L) and breadth (B) of the right valve only was noted. The number of striae on the right valve were counted at the extreme edge of the valve. An estimation of the thickness (T) of the right valve was obtained in the following way. The valve was assumed to be elliptical in shape and of density (D) of 2.80 g/ml (Taylor & Hayman, 1972; Currey, 1975). Then, since

$$\text{Weight (W)} = D \times \frac{\pi}{4} LB \times T \dots\dots\dots 1$$

$$T = \frac{4W}{2.80 \pi LB} \dots\dots\dots 2$$

The T values obtained approximated to the "mean" thickness obtained by direct measurement by Trueman (1942) for the closely related *Tellina tenuis*.

Least squares regression analysis (Bailey, 1959) was used to test for correlation between the number of striae and the length of the valve; number of striae and \log_{10} weight of the valve; and number of striae and thickness of the valve. The Sign Test (Siegel, 1956) was used to compare the weights of right and left valves.

RESULTS

Figs. 1 and 2 are the left and right valves respectively of the same specimen of *T. fabula*: the diagonal striae on the right valve can be clearly seen.

At higher magnification (Fig. 3), the striae are revealed as a series of flat ridges of constant height, overlapping from anterior to posterior. The striae did not seem to be present on the early stages of the valve. They became prominent only after the 1st growth ring, and appeared to originate from the concentric rings (Fig. 4). Occasionally they did not originate from a concentric ring (Fig. 5) or they disappeared at a growth ring (Fig. 6). The angle of approach of the striae to the edge of the shell was altered not only at the edge itself (Fig. 2), but also at the growth rings (Fig. 6), although the striae subsequently resumed their previous angle after each ring. There was also evidence of distortion of the striae around areas of damage (Fig. 7).

Size and weight analysis (Table 2) revealed that there was a significant correlation between the number of striae and the length of the valve (Fig. 8, Table 3), although there may be appreciable variation in the number of striae on valves of the same length (Table 2, animal no. 8 and animal no. 10). However, there was no significant correlation between the number of striae and the \log_{10} weight of the valve or the number of striae and the thickness of the valve (Table 3). There was no significant difference [$P = 0.38$ (Siegel, 1956)] in the weight of left and right valves (Table 2).

DISCUSSION

The ridges of the striae have their edges aligned roughly at an angle of 45° to the anterior/posterior axis, and are consequently at right angles to the line of penetration when the animal is burrowing (Trueman et al., 1966). This results in a "ratchet" profile being presented to the sand. The striae of *Tellina similis*, of similar shape and orientation to those of *T. fabula* but present on both valves, are thought to aid the animals' burrowing by the "ratchet" alternately gripping and sliding through the sediment (Stanley, 1970). In theory, then, the right valve of *T. fabula* should go down faster than the left, until the animal lies horizontal in the sand on its right valve. In fact, like most of the Tellinidae, *T. fabula* burrows at an angle with the right valve uppermost, and eventually lies in the sand in a near horizontal position on its left valve (Holme, 1961).

This preference for burrowing at an angle may result in an unequal stress on the valves, and the striae are therefore required to strengthen the right valve. It has been suggested by other workers that the ribs on mollusc shells provide strength with economy of material (Stanley,



FIGS. 1-7. *Tellina fabula*. 1. Left valve, ca. X17. 2. Right valve, showing striae, ca. X17. 3. Close-up of striae, showing shape, ca. X560. 4. Origin of striae (arrowed), ca. X68. 5. Origin of a stria between concentric rings (arrowed), ca. X140. 6. Disappearance of striae at growth rings (arrowed) showing also change of angle, ca. X68. 7. Distortion of striae around an area of damage, ca. X34.

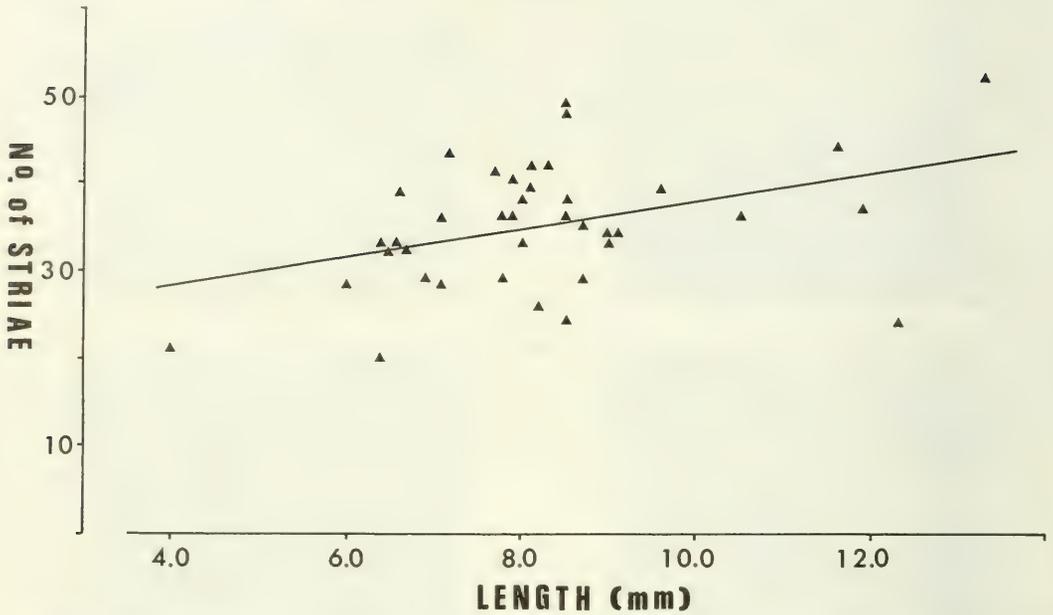


FIG. 8. *Tellina fabula*, number of striae versus length of right valve. For equation of line see Table 3.

TABLE 2. *T. fabula*. Number of striae, length (L) and breadth (B) of right valve, weight of left (LV) and right (RV) valves and thickness (T) of right valve.

Animal number	Number of striae	L (mm)	B (mm)	W (μ g)		T (μ m)
				LV	RV	
1	24	12.3	7.8	29.35	28.97	137.24
2	36	10.5	6.4	17.27	17.90	121.08
3	39	9.6	6.1	12.28	12.40	96.34
4	34	9.0	5.5	11.14	11.49	105.28
5	33	9.0	5.7	10.48	10.58	93.64
6	35	8.7	5.3	9.33	9.02	88.92
7	29	8.7	5.6	8.90	9.01	84.04
8	48	8.5	5.2	9.58	9.46	97.28
9	36	8.5	5.3	8.59	8.49	85.68
10	24	8.5	5.3	8.61	8.57	86.48
11	42	8.3	5.1	8.46	8.38	90.00
12	26	8.2	5.0	7.54	7.61	84.36
13	42	8.1	5.0	8.29	8.58	96.28
14	39	8.1	5.2	8.65	8.56	91.72
15	38	8.0	5.0	8.29	8.75	99.44
16	33	8.0	5.1	9.38	8.09	90.12
17	36	7.9	4.8	7.01	7.10	85.12
18	40	7.9	5.0	8.24	7.58	87.24
19	36	7.8	4.9	6.75	6.81	81.00
20	29	7.8	4.9	7.42	7.43	86.00
21	43	7.2	4.7	5.66	5.85	78.60
22	28	7.1	4.4	5.80	5.64	82.08
23	36	7.1	4.5	5.30	5.60	79.68
24	29	6.9	4.4	4.69	4.74	70.96
25	33	6.6	4.1	4.76	4.76	79.96
26	32	6.5	4.1	4.38	4.24	72.32
27	28	6.0	3.6	3.44	3.56	74.92
28	21	4.0	2.8	1.14	1.16	47.08

TABLE 3. *T. fabula*. Regression analysis (Bailey, 1959) of number of striae versus: (a) length of right valve; (b) \log_{10} weight of right valve; (c) thickness of right valve.

Plot	r	t	P	Equation
a	0.40	2.68	0.01	$y = 21.64 + 1.63x$
b	0.27	1.44	0.16	—
c	0.22	1.13	0.27	—

1970; Boltovskoy, 1974). However, there is no direct evidence to suggest that the striae are compensating for lack of strength in the right valve. In addition, it is unlikely that the stress on the right valve would be so much greater than on the left (Wainwright, 1969), and nothing similar has been reported in other asymmetric Tellinidae which burrow in the same manner (Holme, 1961). Lever (1958), from the evidence of differential sorting of right and left valves of *T. fabula* on the beach, postulated that the striae made the right valve more solid than the left and that the valves differed in weight. However, Table 2 shows that this is not so, and the differential sorting may be due to the respective left- and right-handedness of the valves or to the effect of the striae on the movement of the right valve in a current.

The possibility that the ornamentation affects the transport and subsequent orientation of a live animal into a favourable position for rapid re-burrowing, for example if it had been washed out of the sand by wave or current action, is remote. Firstly, the sublittoral habitat of *T. fabula* is not one which is exposed to strong current or wave action, and secondly, the orientation of *T. fabula* within the sediment is completely random, which one might not expect had the animals been lined up by a current.

The texture of the shell does not appear to affect predation by gastropods which manipulate the bivalve in their foot before boring. Ansell (1960), working in Kames Bay itself, reported no selection by *Natica alderi* either for or against *T. fabula* compared to other bivalves.

The striations do not seem to be a retention of a primitive or ancestral characteristic in which striations present on both valves aided burrowing. Firstly, there is no evidence to suggest that the condition was any more common in the past than in the present (Wood, 1848-1882; Heering, 1950; Moore, 1969) and secondly one is still faced with the problem as to why the striae should have been lost from one valve only.

Wigham (1975) correlated the shell form of *Rissoa parva* with environmental conditions, but, in assessing the extent of environmental influence on the forms of the right valve of *T. fabula*, it must be remembered that animals of the same size (and presumably age) from the same square metre of a homogenous environment, had a 2-fold difference in the number of striae. The absence of striae from the early stages of the shell does not necessarily mean that they were not present in the young *T. fabula*. The umbonal region of all the specimens was considerably worn, that is the striae may have been rubbed off and the fine detail of the larval shell lost (Fretter & Pilkington, 1971). Nevertheless, neither Rees (1950) nor Newell & Newell (1963) mention striae on the larval *T. fabula* shell. In other bivalves, such as *Donax* (A. D. Ansell, Dunstaffnage Marine Research Laboratory, personal communication) or *Cardium* (Van Benthem Jutting, 1943), the ornamentation does not develop until the adult shell is deposited. It was also noticeable that the striae altered shape at the growth rings, that is under conditions of slow or nil growth and that they were considerably distorted around previously damaged areas. It is therefore suggested that the deposition of the striae and the main shell are interlinked.

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SUMMARY

(1) The striae are a series of oblique flat ridges running toward the posterior end of the animal. Their numbers increase roughly with size, but they are not visible on the early prodissococonch stages of the valve. It is suggested that deposition of the striae is under the same control as overall shell growth and hence may be subject to environmental influence.

(2) The function of the striae is unclear, but they do not seem to aid directly in burrowing, or to facilitate reburrowing by affecting the animal's orientation if washed out of the sand, or to deter predators, or to be a retention of an ancestral character. The most reasonable hypothesis is that they may strengthen the valve against the stresses set up in burrowing.

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STUDIES ON THE GROWTH AND DENSITY OF THE CLAM
PAPHIA LATERISULCA AT KALBADEVI ESTUARY,
RATNAGIRI, ON THE WEST COAST OF INDIA

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ABSTRACT

Age and growth of the bivalve *Paphia laterisulca* have been studied by the size frequency method from August 1973 to July 1974. The clams measure 23, 38, 47 and 50 mm at the end of 1, 2, 3 and 3½ years of life respectively. Length-breadth, length-width and length-weight ratios have been studied. Monsoon checks in the form of annual rings were also used in determining age. Growth is retarded during the monsoon due to very low salinity whereas rapid growth is observed when the salinity rises again. Temperature seems to have no effect on the growth rate. Baby clams reach sexual maturity after attaining a length of 16-18 mm. The clams appear to breed from September to March with 2 peaks: November and March. Spawning intensity appears to slow down from December to February. The density of zero age clams (under 1 year old) is less in late summer than post monsoon. The density is high in between mid and low water marks. A marked decrease in zero age and marketable sized (over 37 mm shell length) clams is observed in the monsoon and is due to unfavourable environment. The density of marketable clams is high at the western side and less so at the eastern side of the estuary; it is also high in the middle part of mid and low water marks with a considerable decrease towards high water mark. Preference for intertidal substratum is muddy rather than sandy. Comparisons are made with other shellfish from the Indian coast.

INTRODUCTION

Though many species of commercially important bivalves occur along the Indian coast, little attention was paid by past workers to various aspects of growth, despite the fact that this field offers a large number of unanswered problems. Amongst these species of bivalves, clams have received some attention for the study of growth (Durve, 1970; Deshmukh, 1972; Mane, 1973). None of the observations made thus far was directed to the study of the growth and density of populations during each month of the year of the clam *Paphia laterisulca*.

P. laterisulca is an estuarine bivalve of considerable commercial importance and occurs abundantly on the west coast of India. Any biological study of this species will therefore be of interest from a scientific and also a fisheries point of view. In several markets of the coastal towns and villages of the Ratnagiri district this clam finds a ready sale, as the people there have developed a taste for them, unlike those of most other regions of India. This clam is also in great demand as fish bait for long lines and this demand is no less important than that of human food. Kalbadevi, one of the estuaries along Ratnagiri (3 km northwest of the city) has been a clam fishing centre for many years and as it is within reach of several economically important shellfish beds, it was chosen as a suitable location to carry out the work (Fig. 1). The study was undertaken with a view to understanding the biology of the species in the hope that a fuller knowledge will be of help in the development and conservation of this valuable clam fishery.

MATERIALS AND METHODS

Growth rate

Beach screenings to determine the time of settling of baby clams, the size and growth of the various year classes were carried out throughout the year from August 1973 to July 1974. The rate of growth was determined by the size frequency method. The length of the clam was used

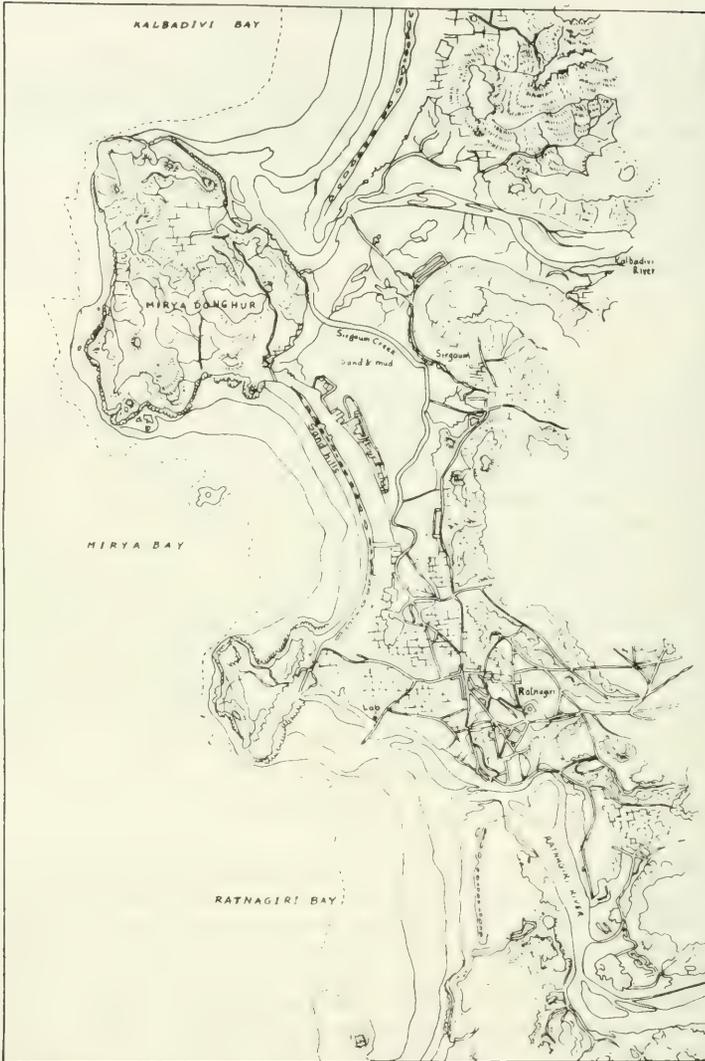


FIG. 1. Sketch map of the Ratnagiri coast showing Kalbadevi estuary. Scalloped lines indicate location of clam beds (*Paphia laterisulca*).

as a standard measure for determining the age. The data were arranged in size groups at class intervals of 3 mm. The 2 fortnightly samples were combined and converted into percent frequencies and represented separately for the monthly samples. The shifting of the mode values of different groups for each month formed the basis for interpretation of growth rate of different year classes.

Relationships

From October, 1973 to January, 1974 the clams of all size ranges from 15 to 56 mm were collected and preserved in 5% formalin in sea water. These were then grouped at 3 mm intervals. All the linear dimensions such as length, breadth and width were measured following the method adapted by Abraham (1953). Length-breadth, length-width and length-weight ratios of 1979 clams were determined. For length-weight ratios, the preserved clams were dried on blotting paper and weighed. The larger clams were individually weighed but the smaller clams from the same length groups were weighed together and the average weight of the individual was calculated.

Growth rings

The study on the growth rings was based on the method used by Weymouth et al. (1925). At Kalbadevi estuary, the clams show pronounced annual monsoon checks and regular sampling has shown that they can be considered as annuli. Those clams with rings which were difficult to read amounted to less than 10% of the samples and were discarded. The clams of population estimates of 1973-74 were retained and the distance between the monsoon rings measured to the nearest mm.

Size at sexual maturity

Periodic collections of clams of up to 24 mm shell length were made at Kalbadevi estuary and the clams were divided into 2 mm shell length groups. The gonads were removed, preserved in aqueous Bouin's fluid in sea water and histological slides prepared from the centre of the gonad were studied for stages.

Time of spawning

Adult clams were collected from the estuary in each month during the year August 1973-July 1974. The 'heel' section of the gonad was preserved in 10% formalin in sea water and the stage of the gonad development was determined from examination of smears.

Hydrographic conditions

Salinity and temperature of the sea water over the clam bed were recorded monthly at 10 day intervals during the study period. As these observations were based on mean monthly samples they could give only a general idea of the hydrographic conditions over the clam bed.

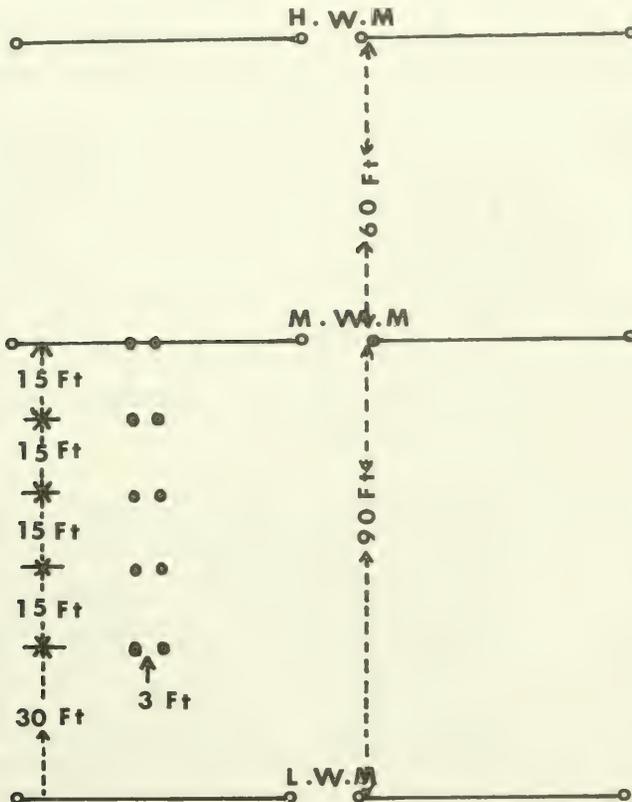


FIG. 2. Schematic diagram of transects used to assess zero age clam populations in the clam beds in Kalbadevi estuary.

Density of zero age clams

Beach screenings to determine the strength of the incoming year classes were carried out in November and December 1973 and April, May, July and August 1974 at the western part of the estuary. Samples were taken randomly from the sections of transects established to estimate the population size of the clams on the clam bed as established in the schematic diagram (Fig. 2). Each time screening was undertaken and samples were taken at regular intervals along the transects from low water tide line to the mid-intertidal zone. Almost no samples were taken above the mid-intertidal zone since no clams of zero age occur there. All samples were washed through 1 mm mesh screen, the small clams were separated and their lengths were measured to the nearest mm.

Density of adult clams (above 37 mm shell length)

Beach screenings as shown in Fig. 2 to measure the density of 2nd and 3rd year classes were carried out in July, August, October and November 1973 and January, February, April and May 1974. The collections were made with a 'Sand Pimper' (Fig. 3) which samples an area of 116 sq. cm to a depth of 20 cm. The number of sand pimper samplings (4) was kept constant irrespective to the density of the clams on the clam bed. At every time of sampling, the samples were taken from the transect established vertically in 15 ft width of the western part of the estuary and the sampling was done at a 5 ft interval from the low water mark vertically up. In October and November some additional sampling was done throughout the estuary from the middle part of the mid and low water mark at 15 ft width (Fig. 4) at intervals of 20 ft.

RESULTS

Growth rate

Spawning in *P. laterisulca* at Kalbadevi estuary starts by mid-September and lasts to the end of March, as revealed by the successive examinations of the gonadial conditions in the adult

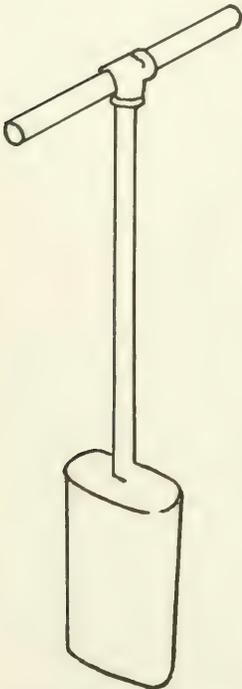


FIG. 3. Sand Pimper for clam digging.

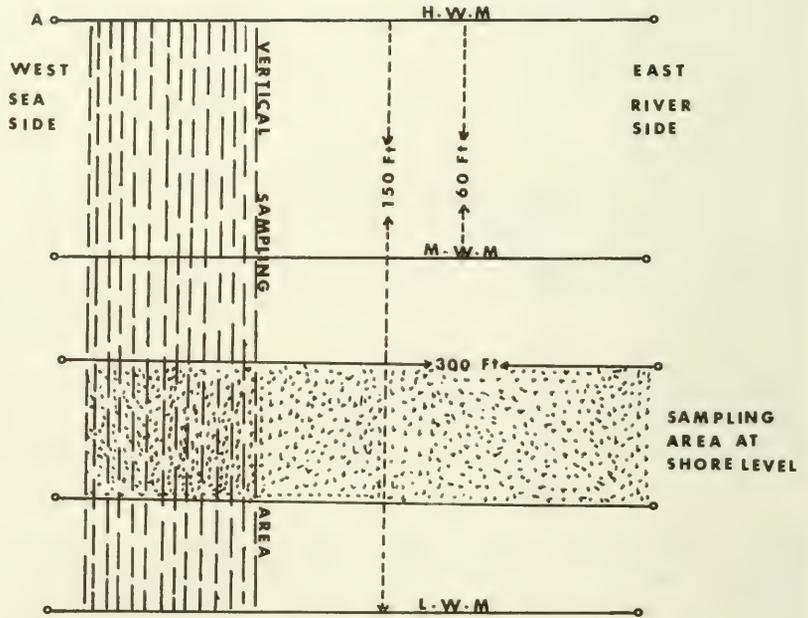


FIG. 4. Schematic diagram of transects used to assess adult clam populations in the clam beds in Kalbadevi estuary.

groups of clams. This spawning has 2 peaks—in November and in March. Juvenile clams appeared in December and May. As the spawning in this clam occurs for 5½ months, there appears to be a lot of masking effect on the adult groups of clams growing in different months and hence for the interpretation of different modes throughout the year. It was therefore necessary to follow separately the growth of clams born during the peak spawning periods, 'November born' and 'March born' clams. For the sake of convenience to express the rate of growth of the different year classes of clams, the different modes in the graph are denoted by A, B, C, D and a, b, c, d, e for November and March born clams respectively as was done by Mane (1973) for *Katelysia opima*. The letters used in the present study belong to the following year classes.

A—November born 1970	a—March born 1970
B—November born 1971	b—March born 1971
C—November born 1972	c—March born 1972
D—November born 1973	d—March born 1973
	e—March born 1974

In August 1973 seven modes appeared. The mode at 8 mm represents the year class 'd,' the mode at 20 mm represents the year class 'C,' the mode at 26 mm represents the year class 'c,' the mode at 32 mm represents the year class 'B,' the mode at 41 mm represents the year class 'b,' the mode at 44 mm represents the year class 'A' and the mode at 50 mm represents the year class 'a.' In September 1973 seven modes appeared at 11 mm, 20 mm, 26 mm, 32 mm, 41 mm, 44 mm, and 50 mm. Thus except for the growth of year class 'd' all other year classes persisted at the same modes showing no growth. In October 1973 also 7 modes appeared, wherein only the modes of year classes 'c,' 'B' and 'A' have shifted to 29 mm, 35 mm, and 50 mm, respectively. In the year classes 'd,' 'C,' 'b' and 'A' showed no growth. The year class 'd' has now completed 6 months of life showing a growth of 11 mm and the year class 'a' at 50 mm has now completed 3½ years of life. In November 1973 the year class 'd' has shifted to 14 mm, 'B' has shifted to 38 mm and 'A' has shifted to 47 mm. The rest of the year classes showed no growth. The year class 'C' has completed its 1 year age and has grown to 23 mm whereas the year classes 'B' and 'A' have completed their 2nd and 3rd year of life, respectively showing growth of 38 mm and 47 mm. In December 1973 year class 'D' has made its appearance for the first time at 5 mm mode which was born in the post monsoon of 1973. The year classes 'C,' 'c' and 'b' have now grown to 26 mm, 32 mm and 44 mm, respectively. The year class 'A' showed no growth. In January 1974 the year class 'D' has shifted to 8 mm and the mode of year class 'D' reappeared at 17 mm. The year classes 'C,' 'c' and 'B' have appeared at 29 mm, 35 mm and 41 mm modes, respectively. No growth took place in the year classes 'b' and 'A.' In February 1974 only the young clams of the year classes 'D' and 'd' have shown a growth and appeared at 11 mm and 20 mm, respectively whereas the year classes 'C,' 'c,' 'B,' 'b' and 'A' showed no growth. In March 1974 five modes appeared at 11 mm, 23 mm, 38 mm, 41 mm and 47 mm. Thus, year classes 'd,' 'c' and 'b' showed a growth of 3 mm within a month and have now completed their 1st, 2nd and 3rd year of life, respectively and appeared at the modes 23 mm, 38 mm and 47 mm, respectively. These are the clams born in the early summer spawnings of 1973, 1972 and 1971, respectively. The year classes 'D' and 'B' showed no growth. In April 1974 the clams of the year classes 'D,' 'B' and 'A' appeared at modes 14 mm, 44 mm and 50 mm, respectively. No growth occurred in the year classes 'C' and 'c.' In May 1974 newly born clams of the year class 'e' appeared at 5 mm mode which have been born in the early summer of 1974. A growth of 3 mm has now taken place in the year class 'd' appeared at the mode of 26 mm and the year classes 'D' and 'c' appeared at the modes of 17 mm and 41 mm, respectively. The modes of the year classes 'C,' 'B' and 'A' remained at 29 mm, 44 mm and 50 mm, respectively. Thus, it can be seen that the year class 'A' has now completed 3½ years of life by appearing at the mode of 50 mm. In June 1974 young clams of year classes 'e' and 'D' have now grown to 8 mm and 20 mm, respectively. The year class 'C' appeared at the mode of 32 mm. No growth occurred in the year classes 'd,' 'c,' 'B' and 'A.' In July 1974 no growth occurred in the young clams of the year classes 'e' and 'D.' The year classes 'd,' 'C,' 'c' and 'A' also showed no growth and persisted at the same modes (Fig. 5).

Thus, from the foregoing it appears that *P. laterisulca* spawns from post monsoon to early summer, the peak being in November and March. The growth of post monsoon and early

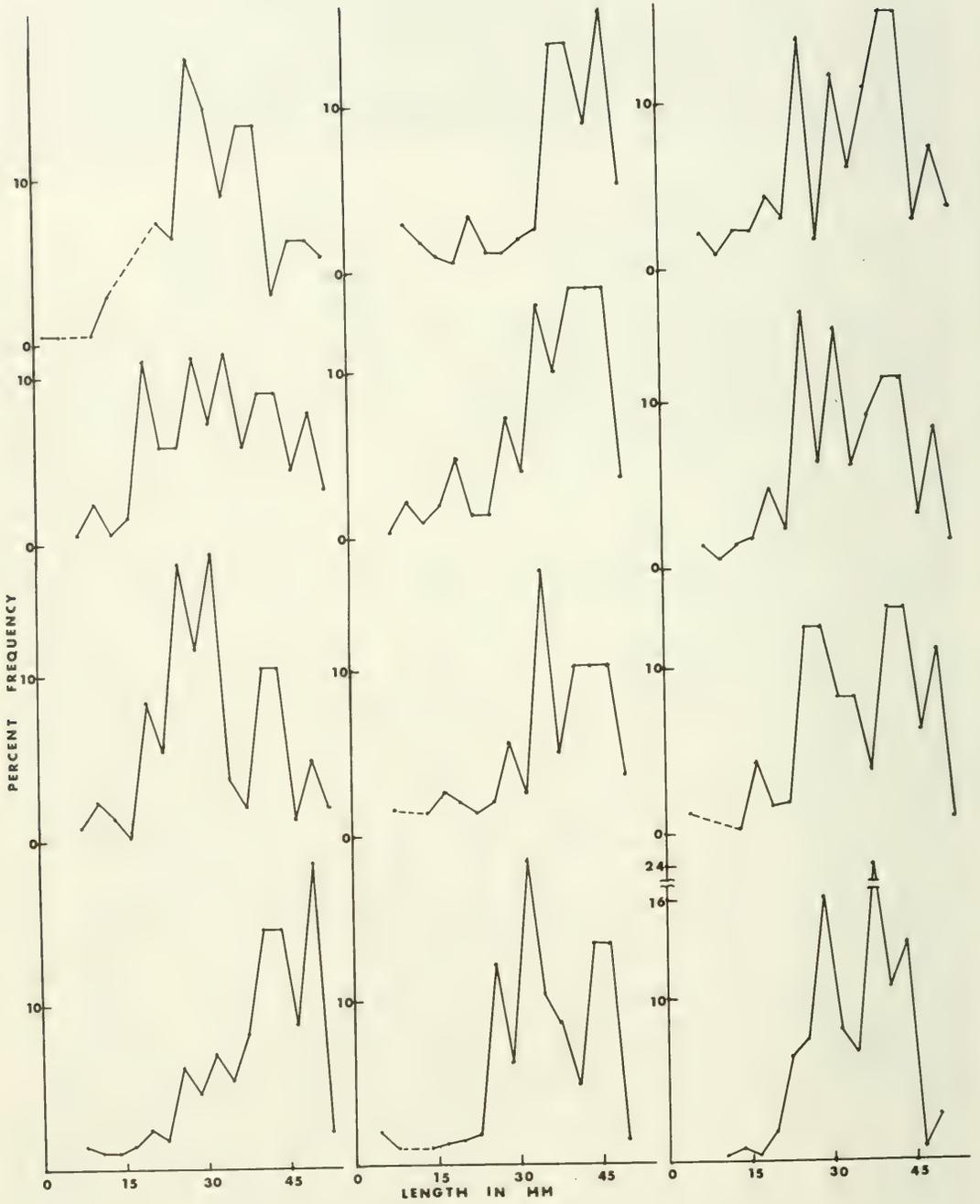


FIG. 5. Frequency distribution of clams.



FIG. 6. *Paphia laterisulca* of 12-47 mm shell length.

summer born clams in the 1st, 2nd and 3rd and also 3½ years is similar and sizes attained are 23 mm, 38 mm, 47 mm and 50 mm respectively. However, the growth in the first 6 months of post monsoon born clams (17 mm) is more than the growth of early summer born clams (11 mm) giving a difference of 6 mm. From this study it can be inferred that the clams born in early summer are faced with a period of comparatively retarded growth in the monsoon and therefore grow only to 11 mm, but growth in the later periods becomes accelerated and in the next 6 months they complete their 1st year of life representing a growth of 23 mm. However, the clams born in the post monsoon are initially favoured with a period of active growth; much rapid growth occurs before the monsoon and they grow to a size of 17 mm in the first 6 months but thereafter for the next 6 months growth takes place at a slow rate representing a growth of 23 mm during the 1st year of life. The clams of the various sizes ranging from 12 mm to 50 mm are shown in Fig. 6.

Linear relationships

In Fig. 7 the observed and calculated values of breadth and width are plotted against their respective lengths. All points for the breadth and width are more or less closely located near the fitted lines of regression. Equation for the regression line was used for the expression of length-breadth and length-width relationships in terms of calculated values i.e. $Y = a + bX$ where $X = \text{length}$, $Y = \text{breadth or width}$ and a and b are constants. Using this equation the following relationships were established—for length-breadth $Y = 1.682 + 0.6636X$ and for length-width $Y = -1.299 + 0.5237X$.

The observed and calculated weights of the clams against their respective lengths are plotted in Fig. 8. The points closely agree with the calculated weights. Using the formula $W = aL^b$ the following relationships was established— $W = 0.0003758L^{2.853}$ or $\log W = -3.224 + 2.724 \log L$.

Annuli (rings) measurements

Use of this method to determine age and growth of clams depends upon whether annuli are formed and whether they are distinguished on the surface of the shell. Most of the clams from Kalbadevi estuary have pronounced annuli. Evidence to support the view that they were indeed

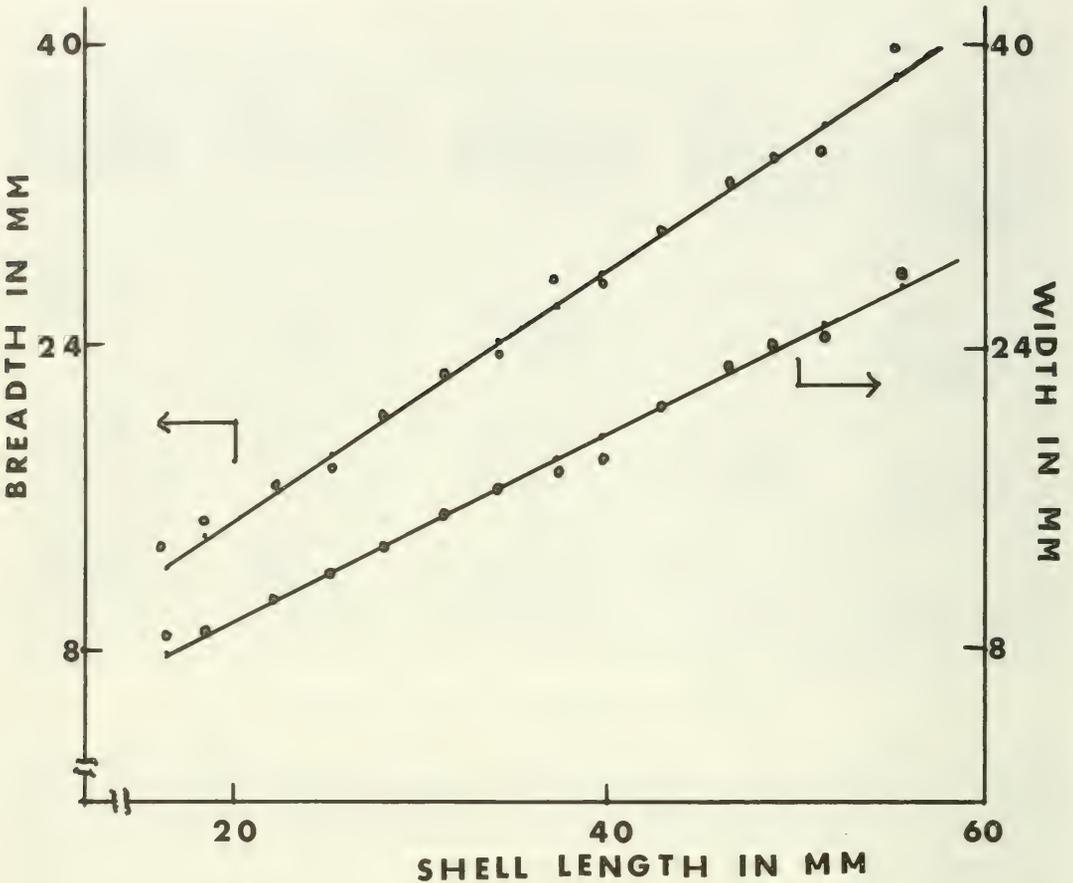


FIG. 7. Length-breadth and length-width relationships of the clam.

annuli was obtained by measuring the dominant year classes which settled during 1970-73. From the data presented in Table 1 it is clear that the occurrence of rings over the shells determine the age of the clams. The distance from first ring to umbo is more or less uniform in 2 and 3 year old clams. Similarly these distances between the 2nd ring and umbo and 3rd ring and umbo are correspondingly uniform in 2 and 3 year old clams. In the clams between 47 mm and 51 mm length 3 rings are present. From the evidence of size frequency figures the clams of this size have completed 3 years of life. The clams from 38 mm to 42 mm in length have 2 rings and are over 2 years old but have not completed 3 years age. The clams from 23 mm to 29 mm in length have only 1 ring and are over 1 year old but have not completed their 2nd year of life. The respective distances from 1st, 2nd and 3rd ring from the umbo are 16 mm, 26 mm and 32 mm, i.e. these rings were formed when the clams were 16 mm, 26 mm and 32 mm in breadth. From the fitted regression line of the length-breadth curve, it can be seen that at breadth of 16 mm, 26 mm and 32 mm, the lengths of the shells are 22 mm, 37 mm and 46 mm, respectively. These lengths nearly correspond to the year class ages of the clams. It has already been shown that the growth in the monsoon is arrested in all year classes. Thus the results show that the annuli are formed during the monsoon every year; they may be called 'monsoon checks' or 'monsoon annuli.'

Growth of the clams is faster in post monsoon born clams than early summer born clams but the formation of annuli 1 to 3 is similar for all classes in post monsoon and early summer born clams.

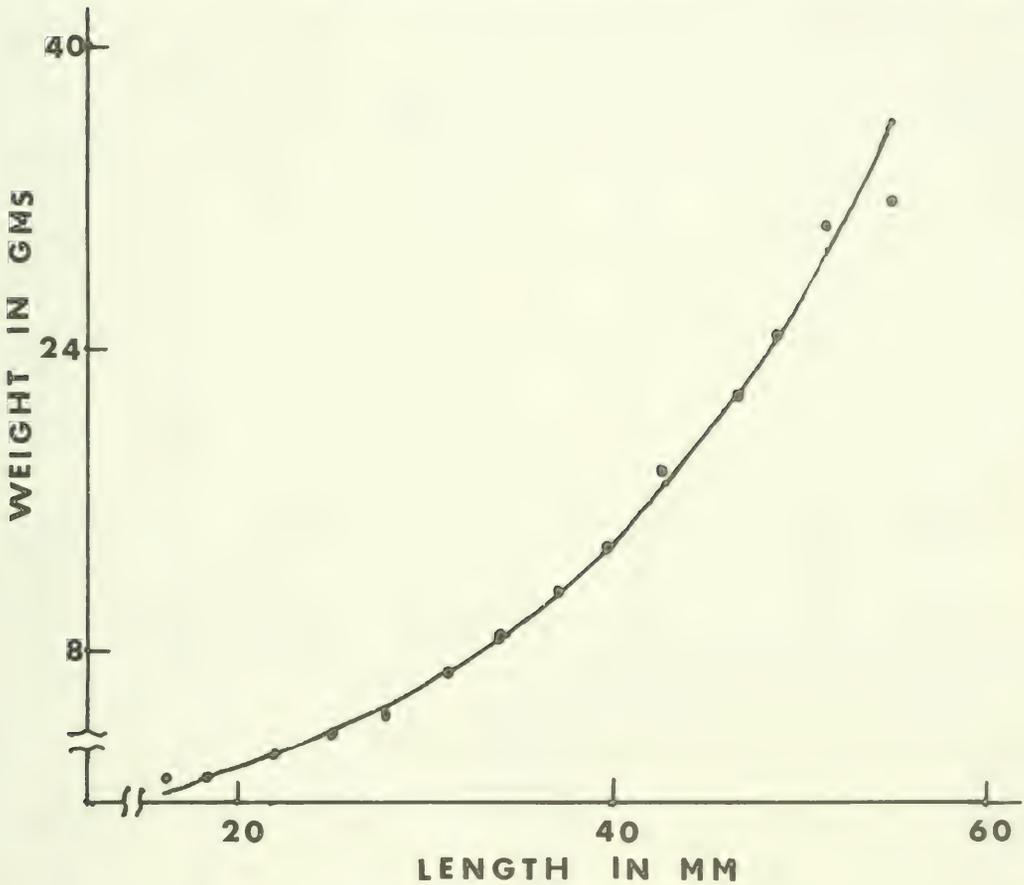


FIG. 8. Length-weight relationship of the clams.

Size at sexual maturity

This study determined the size at which the clams become sexually mature for the first time. Three categories were used to describe the stage of sexual maturity based on histological preparations. Stage I is undifferentiated, wherein there is no differentiation of sex and the gonad contains only loose vesicular connective tissue. Stage II is differentiated, wherein the gonad is in immature stage with well developed connective tissue and ramification of the follicles with ciliated ducts is seen. Some primary germ cells on the follicle wall are also observed. Stage III is developing and maturing eggs and sperm are seen.

The data are somewhat limited and unfortunately most of the collections were made in the period when the gonads of most of the adult clams are in inactive or in early part of active stage. It would have been more advantageous to have taken samples in December and to have sampled a single set as it developed to maturity. However, the data give an indication of the size at which the clams became sexually mature (Table 2). Clams up to 10 mm in shell length were in stage I and it was impossible to sex these clams. Gonads of the clams in the size group 10 mm to 14 mm were in stage II. Only 3 clams of 14 mm to 16 mm in length were in stage II whereas all others were in stage III. All the clams of 17 mm and larger were in stage III.

From the data it was concluded that the clams become sexually mature when they are about 16 mm to 18 mm in shell length.

TABLE 1. Size of *P. laterisulca* with position and number of rings.

Year classes	Length (mm)	Height (mm)	No. of rings	Distance of the ring from umbo (mm)		
				1st ring	2nd ring	3rd ring
1st	15	12	0	—	—	—
	17	13	0	—	—	—
	19	14	0	—	—	—
	20	16	0	—	—	—
	22	16	0	—	—	—
	23	17	1	15.5	—	—
	25	18	1	15.5	—	—
	26	17	1	16.0	—	—
	28	20	1	16.5	—	—
	29	21	1	16.5	—	—
2nd	38	27	2	15.5	25.0	—
	39	27	2	16.0	25.0	—
	39	27	2	16.0	25.5	—
	40	28	2	15.5	26.0	—
	40	28	2	15.5	26.5	—
	41	29	2	16.0	26.5	—
	42	30	2	16.0	26.5	—
42	30	2	16.5	26.0	—	
3rd	47	33	3	15.5	25.5	31.5
	48	34	3	16.5	25.5	31.5
	48	34	3	16.5	26.0	32.5
	49	34	3	16.0	26.0	32.0
	50	35	3	16.0	26.5	32.5
	50	35	3	16.5	26.5	32.0
	51	36	3	16.0	26.0	32.5

TABLE 2. Stages of gonadal development of *P. laterisulca* collected at Kalbadevi, 1973-74.

Size (mm)	Date of collection	No. of clams	No. of clams in		
			Stage I	Stage II	Stage III
0- 2	2-12-'73	16	16	0	0
2- 4	4-12-'73	14	14	0	0
4- 6	8-12-'73	11	11	0	0
6- 8	5-1-'74	8	8	0	0
8-10	8-1-'74	19	19	0	0
10-12	10-4-'74	31	0	31	0
12-14	21-5-'74	25	0	25	0
14-16	28-5-'74	10	0	3	4♀ 3♂
16-18	28-5-'74	17	0	0	9♀ 8♂
18-20	3-6-'74	22	0	0	10♀ 12♂
20-22	8-6-'74	17	0	0	12♀ 5♂
22-24	15-6-'74	13	0	0	7♀ 6♂

Time of spawning

The results of the gonad smear observations of the clams are summarized in Table 3. In December and May, gonads were maturing and the sexes were easily distinguished. In August, all clams were ripe and ready to spawn. In September, many clams were ripe and some spawning was observed in a few clams. This spawning reached its peak in November and the majority of the clams spawned. From December onwards spawning intensity was lowered and the regeneration in the gonad started in many clams. In January the maturation process started and by February many clams were in ripe condition. Few clams showed acceleration in spawning intensity and by March it reached its 2nd peak. In April the majority of the clams were recovering and it was difficult to sex them. In May the sexes were identifiable and in June the maturation process occurred in the majority of the clams. In July the majority of the clams showed mature eggs and sperm which are to be released in the next season which is favourable

TABLE 3. Seasonal variation in the gonadal stages of *P. laterisulca* during 1973-74.

Date of collection	Stage I: gonad filling		Stage II: gonad ripe and ready to spawn		Stage III: some spawning has taken place		Stage IV: gonad almost or completely spawned, some regeneration has taken place	
	♀	♂	♀	♂	♀	♂	♀	♂
12-8-'73	1	—	17	12	—	—	—	—
15-9-'73	—	—	15	10	4	2	—	—
13-10-'73	—	—	3	2	6	1	16	11
10-11-'73	—	—	—	—	—	—	18	15
14-12-'73	2	2	—	—	3	1	9	6
17-1-'74	8	6	—	—	—	—	3	2
12-2-'74	—	—	10	7	4	2	1	—
11-3-'74	—	—	—	—	1	—	13	9
14-4-'74	2	1	—	—	1	—	7	4
13-5-'74	11	9	—	—	—	—	2	—
15-6-'74	8	7	3	2	—	—	—	—
14-7-'74	2	1	13	11	—	—	—	—

for spawning. Thus in Kalbadevi this clam spawns from September to March with 2 peaks—November and March. The intensity of spawning was lowered in the winter season.

Hydrographic conditions

The data for salinity and temperature of sea water at Kalbadevi over the clam beds are shown in Fig. 9 with the fluctuations at its upper and lower limits. Salinity and temperature were measured at 10 day intervals during each month. Salinity considerably decreased in the monsoon season (June-September) due to the southwest monsoon. In 1974 the rainfall started at the end of June and as such there was no marked drop in salinity in this month compared to

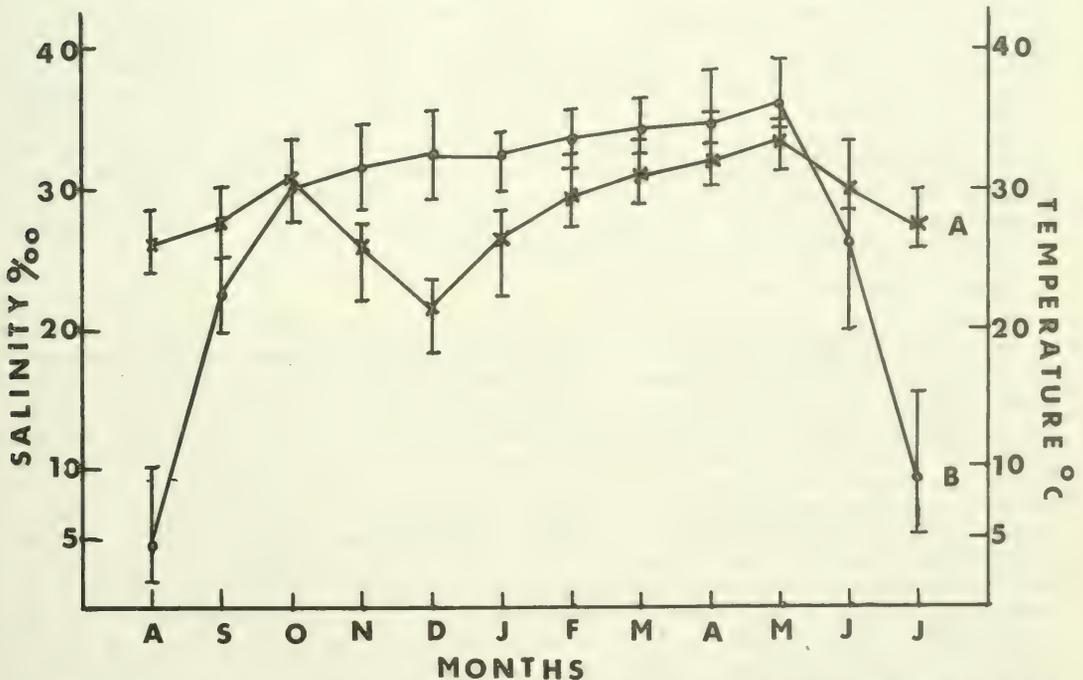


FIG. 9. Variation in salinity and temperature of the sea water over the clam beds August 1973 to August 1974.

TABLE 4. Density and mean shell length of zero age clams at Kalbadevi, 1973-74.

Distance from mid water mark (ft)	November 2-5		December 12-15		April 7-9		May 10-13		July 20-23		August 10-13	
	No. of clams /sq. ft	Mean shell length (mm)	No. of clams /sq. ft	Mean shell length (mm)	No. of clams /sq. ft	Mean shell length (mm)	No. of clams /sq. ft	Mean shell length (mm)	No. of clams /sq. ft	Mean shell length (mm)	No. of clams /sq. ft	Mean shell length (mm)
10	4	10.5	7	14.5	1	18.0	—	—	—	—	1	19.0
15	8	13.5	5	15.5	2	16.0	4	17.0	4	16.0	2	17.5
20	9	12.0	8	11.0	3	13.5	6	15.0	2	17.5	—	—
25	7	10.5	9	10.0	4	10.0	3	9.5	4	12.0	—	—
30	6	9.5	11	8.5	7	3.0	5	6.0	5	6.5	4	8.5
40	15	3.5	16	9.0	10	4.5	7	5.5	3	7.0	5	5.0
45	11	4.5	18	6.5	16	8.0	9	12.5	9	5.0	2	7.5
50	20	7.0	17	3.5	11	7.5	17	16.5	5	8.5	6	9.5
55	28	9.5	22	3.0	13	13.5	18	17.5	6	15.0	3	12.0
60	13	14.5	12	6.0	16	17.0	2	19.5	8	18.0	7	13.0
70	17	14.0	14	12.5	3	14.5	9	18.0	1	16.5	—	—
75	4	17.0	9	16.0	—	—	3	13.0	1	14.0	2	17.5
80	10	15.5	—	—	5	19.0	5	19.0	—	—	—	—
85	8	18.0	4	18.5	3	16.5	1	15.5	—	—	—	—
90	3	16.5	2	19.5	—	—	—	—	—	—	—	—

July. A maximum of 36‰ was recorded in May and a minimum of 4.1‰ in August. There was a steady rise in salinity from September to May i.e. from the end of the monsoon to the summer. The highest temperature was recorded in May (33°C) and the lowest in December (21.5°).

Density of zero age clams

Density of zero age clams has been expressed as number of clams per square foot in Table 4. The density of clams decreased in July and August, whereas the density was largest in November and December and ranged from 0 to 28. In April and May the density was from 0 to 18 and in July and August from 0 to 9. Very few small clams were found near the mid water mark and at the low water mark. The density of small clams was high in between these 2 marks. The noticeable decrease in the clam population during the monsoon probably was due to changes in turbidity and salinity of the sea water caused by the influx of river water in the estuary which produced a drastic alteration of conditions over the clam bed. Few zero age clams have been found in the screenings worked out during April and May, whereas during November and December the density was largest indicating that settlement in April and May is generally sparse and sporadic. This low abundance probably resulted in part from poor settlement and also from poor survival of the juveniles.

Density of the adult clams (above 37 mm)

Clams of 37 mm to 49 mm shell length have a very local distribution. Virtually the entire set occurred on the western zone of the estuary. Extremely high clam densities were recorded, particularly in October and November; in November it was higher than in October. Furthermore, the largest clam densities were found in the middle part of the mid and low water marks. Few clams did occur above mid water mark but mostly suffered mortality during the late summer. In the monsoon the clam density decreased at low water mark (Table 5). This decrease in July and August was probably due to survival factors principal among which are lethal salinity on the clam bed and low food abundance. This sharp decrease in the density during the monsoon coincided with the onset of monsoon storms and the mortality probably resulted from the large amount of fresh water brought by the Ratnagiri river which often causes severe alteration in the conformation of the clam beds. This agrees with the statement made by Tegelberg & Magoon (1969) who postulated that survival of juveniles is a function of size reached before the winter storms strike. A similar statement was made by Bourne & Quayle (1970) for *Siliqua patula* and by Mane (1973) for *Katylisia opima*. Vertical distribution of clams and their densities showed a striking difference in the choice of substratum and it was observed that high density occurred in the muddy parts at the area between mid and low water marks.

TABLE 5. The distribution of clams at the vertical sampling area at Kalbadevi estuary.

Distance from high water mark (ft)	April 13 1974		May 16 1974		July 12 1974		Aug 17 1974		Oct 10 1974		Nov 13 1974		Jan 8 1974		Feb 16 1974	
	No. of clams	Mean length (mm)	No. of clams	Mean length (mm)	No. of clams	Mean length (mm)	No. of clams	Mean length (mm)	No. of clams	Mean length (mm)	No. of clams	Mean length (mm)	No. of clams	Mean length (mm)	No. of clams	Mean length (mm)
5	—	—	—	—	4	37	7	41	8	37	4	40	3	37	5	41
10	—	—	—	—	2	39	5	44	6	41	7	38	2	39	3	38
15	2	39	—	—	3	42	1	38	9	37	13	37	7	42	4	39
20	1	40	—	—	—	—	3	39	4	39	4	46	10	41	12	43
25	4	37	—	—	4	43	7	45	11	44	15	44	7	47	5	46
30	3	38	2	42	14	44	8	44	13	43	19	39	11	38	13	45
35	7	45	9	46	7	37	6	38	9	39	12	47	7	42	6	40
40	5	46	3	37	3	45	3	37	3	45	11	48	9	44	14	38
45	14	44	8	39	9	46	13	37	15	47	11	48	9	44	14	38
50	11	47	13	40	13	45	12	47	7	39	22	44	18	38	20	41
55	11	47	16	44	15	39	18	46	3	38	26	39	15	46	17	42
60	18	41	17	46	17	38	13	41	14	43	20	46	13	48	19	39
65	25	44	26	38	11	44	23	39	19	42	15	44	10	45	22	47
70	29	37	32	46	17	40	17	44	16	39	14	37	29	45	30	42
75	13	36	37	44	29	39	20	45	21	46	26	40	11	39	16	41
80	38	38	30	39	21	44	15	38	18	40	12	43	22	37	17	40
85	26	44	41	39	19	43	28	44	40	42	40	39	24	40	29	38
90	10	47	27	38	29	38	26	44	24	44	19	37	50	43	32	37
95	17	34	43	44	12	39	19	48	29	43	26	48	27	42	23	49
100	28	41	42	48	27	37	14	41	28	41	33	40	29	47	20	41
105	17	38	44	47	43	44	12	39	46	42	24	37	33	37	34	42
110	48	40	48	47	39	43	43	47	55	44	39	38	27	38	53	37
115	53	46	50	46	44	47	40	39	30	38	63	40	31	39	59	39
120	39	43	37	40	30	48	37	38	52	40	49	39	40	45	46	38
125	40	38	33	38	22	39	29	39	43	38	43	47	37	44	49	43
130	28	37	27	39	17	37	17	37	31	37	38	44	30	48	28	42
135	22	42	20	42	20	38	19	44	27	36	39	42	24	39	27	43
140	20	40	16	44	13	42	11	47	16	43	17	37	23	40	18	46
145	3	49	4	47	7	44	8	48	12	42	13	38	11	37	16	37
150	8	38	10	39	4	49	3	39	8	37	11	40	7	39	9	39
					2	38	1	40	7	42	9	39	4	41	6	40

TABLE 6. Distribution of clams at the middle part of mid and low water marks in Kalbadevi estuary.

Distance from west to east in ft. (see 300 ft line in Fig. 4)	No. of clams in October 1974	No. of clams in November 1974
20	25	15
40	39	27
60	57	53
80	70	64
100	75	71
120	46	49
140	32	39
160	53	44
180	26	19
200	23	11
220	18	17
240	7	2
260	3	—
280	—	—
300	—	—

In order to assess the density of the clams at the middle part of mid and low water marks of the entire estuary, a survey was made in October and November which revealed that the greatest clam densities occurred at the western side (i.e. towards the sea side of the estuary), whereas at the eastern side (i.e. towards the river side of the estuary) the population was very sparse (Table 6). Increase in density of the clams towards the sea side confirms the marine habitat of the clam and also probably suggests an abundance of food from marine plankton.

DISCUSSION

From Indian waters the majority of the published data on growth show that very young specimens of clams grow rapidly in size and that this rate then decreases as the clams become older. The first report on the growth of commercially important species of clams by Rao (1951) on *Katelsia opima* from Madras showed that by the end of the 1st year the clams grew to 22.5 mm in length, whereas by the 2nd and 3rd year they grew to 31.5 mm and 40.5 mm. Another species of clam from the same area (*Meretrix casta*) showed a growth of 15 mm shell length within 2 months and 29.5 mm within 7 months (Abraham, 1953). Nayar (1955) showed that *Donax cuneatus* at Palk Bay on the east coast grew to a length of 14 mm within 10 months, 19 mm within 2 years and then it dies. On the other hand, *D. faba* from the east coast attained a length of 19.5 mm by the 1st year and 22.5 mm by the 2nd year of its life (Alagarwami, 1966). On the west coast of India *Solen kempfi* at Ratnagiri grew to 37.5 mm within the first 6 months and attained a length of 47.5-52.5 mm and 66 mm by the end of the 1st and 2nd years of its life, respectively (Rao et al., 1962). At Ratnagiri Mane (1973) showed that *K. opima* attained a size of 22 mm, 31 mm and 43 mm in shell length by the end of the 1st, 2nd and 3rd year of its life in Kalbadevi estuary. Talikhedkar et al. (1978) showed that *D. cuneatus* at Ratnagiri grew to 13-14 mm, 21-22 mm and 22-23 mm within the 1st, 2nd and 3rd year of life. It can be seen that the growth of *P. laterisulca* at Kalbadevi estuary at Ratnagiri is faster than in *K. opima* which occurs in the same estuary. *P. laterisulca* in the present study was found to attain a length of 23 mm in the 1st year and 38 mm and 47 mm in the 2nd and 3rd years of its life, respectively.

P. laterisulca in Kalbadevi grew to a size of 17 mm and 11 mm in the first 6 months when born in the post monsoon and early summer of the year, respectively. This difference in growth of the clams born from 2 spawning peaks is due to a widely fluctuating environment during the monsoon. The salinity over the clam bed in the monsoon lowered considerably and attained a minimum of 4‰ in August which resulted in the closing of the shell valves of the clams for most of the time and thereby retarded feeding activity of the younger clams. The clams born in the post monsoon are not influenced by this unfavourable environment at the early stages of growth, but the clams born in early summer have to face this environment at the early stages of growth which leads to retardation of the rate of growth. But the clams of both spawning peaks

attained a length of 23 mm by the end of the 1st year. Thus, it can be said that the growth in the early summer born clams is accelerated only after the monsoon. In the entire collection during the study period, few clams of 56 mm shell length were observed during November, 1973; there were only 4 in a total of 327. This probably suggests that the maximum age of the clam is 3½ years after which they die. The growth in this clam is accelerated with the rising salinity during the post monsoon season. A similar statement has been made by workers while studying the growth of marine and estuarine bivalves from the Indian coast (Nayar, 1955; Mane, 1973; Deshmukh, 1972). It has been suggested by these workers that in tropical waters changes in temperature are negligible and therefore salinity of the water plays an important role in the growth of bivalve molluscs. In the present study also there appears to be no correlation of temperature changes and rate of growth.

The linear relationship between length-breadth and length-width in *P. laterisulca* is in accordance with earlier findings (Nayar, 1955; Mane, 1973; Talikhedkar et al., 1978). The proportional increase in breadth of this clam indicates the general form to be more or less the same throughout life. The relationship between the weight and shell length followed a cube law and is close to the slope value of 3.6618. At 49 mm shell length (clams of this size are abundant throughout the year) the weight is about 20.1 g inclusive of the shell. Approximately 40% of the whole weight is usable meat; a 49 mm clam would have 8 g of marketable meat. To obtain maximum marketable meat yield from *P. laterisulca* the commercial harvest would probably be limited to summer and early monsoon when the gonads are rapidly filling or are full just prior to spawning. Since this period coincides with major efforts in the fishing of other important species, harvesting of *P. laterisulca* can be limited during the spawning periods. Thus this edible clam resource can be utilised for fishery management.

The use of annual shell rings as a means of establishing age has been employed in a variety of lamellibranchs: *Cerastoderma edule*, Orton (1926); *Tivela stultorum*, Weymouth (1923); *Venerupis pullastra*, Quayle (1952); *Anodonta anatina* and *Unio balthica*, Negus (1966); *Tellina tenuis*, McIntyre (1970); *Meretrix meretrix*, Deshmukh (1972); *Katelsia opima*, Mane (1973); *Donax cuneatus*, Talikhedkar et al. (1978). During unfavourable conditions the mantle edges of bivalve molluscs are withdrawn from the shell margin causing a cessation of growth. However, the innermost nacreous layer is continuously deposited (Seed, 1969). Thus, when growth is resumed, old and new regions of the shell are not continuous, resulting in an obvious ring. In *P. laterisulca*, similar formation of rings takes place in the monsoon season. It has been observed that during the entire life span of this clam, 3 such rings are laid down at shell lengths of 22 mm, 37 mm and 46 mm.

Recently, Quayle & Bourne (1972) stated that sexual maturity in bivalves appears to depend on size rather than age. The observations of this study are in close agreement with the findings of Nayar (1955), Mane (1973) and Talikhedkar et al. (1978). In the present study it has been observed that *P. laterisulca* at Kalbadevi estuary attained sexual maturity at 16 mm to 18 mm in shell length. This length is attained when the clams are 7-8 months old when born in the post monsoon whereas maturity is attained in summer born clams when they are 10-11 months old.

Considerable variation in spawning has been reported for different clam populations along the east and west coasts of India. Hornell (1922) believed that the normal spawning season in *Meretrix casta* was in April-May and again in September whereas Rai (1932) observed *Meretrix* to spawn from March to June at the Bombay coast. On the other hand, *M. casta* at Adyar estuary, Madras, spawned in July-August, October-November and March-April or May (Abraham, 1953). The wedge clam, *Donax faba*, from Mandapam beach spawns from November to June (Alagarwami, 1953), whereas *D. cuneatus* at Madras showed prolonged spawning from January to June. The spawning of *D. cuneatus* at Ratnagiri coast was from October to January with a peak in November and December (Talikhedkar et al., 1978). There was only one spawning period in *K. opima* from Adyar estuary (Rao, 1951) in contrast to the population of the same species from Kalbadevi at Ratnagiri (Mane, 1973), which has 2 distinct spawning periods in a year, a major one from October to November and a minor one from March to April. The present study revealed that *P. laterisulca* in Kalbadevi spawns from September to the end of March with 2 peaks—one in November and the other in March; these spawning peaks coincide with the spawning period of *K. opima* in the same estuary.

There are no reports from the Indian coast on clam density. Significant differences are

evident between the clam populations at the sea side and at the river side in the Kalbadevi estuary which show that the clams prefer the bed at the sea side as the environment most suitable to them. Similar observations for the razor clam, *Siliqua patula*, were made by Bourne & Quayle (1970) on the north and south beaches at Masset, B.C. They stated that recruitment was consistent in each year on the north beach but very low on the south beach. Clam populations on Horseshoe Beach appeared to be intermediate between the 2 beaches indicating a possible gradient of environmental factors from west to east. Tegelberg (1964) reported a similar variation on Washington State beaches; recruitment was most consistent and growth fastest on Capolis beach, the most northerly of the 3 main beaches. He postulated that the slower growth on the southern beaches may be due to greater influence of water from the Columbia river. Salinities are lower on the southern beach, particularly during the spring discharge. The sand is coarsest in the south and finer in the north. Similar oceanographic conditions might have affected clam distribution and growth in Kalbadevi estuary. During the period of maximum runoff fresh water coming into the estuary undoubtedly reduces salinity and turbidity increases considerably in the monsoon period. This water mass probably has a greater influence on the low density and growth of zero age clams and also the marketable size of the clam.

Extensive digging has taken place in Kalbadevi estuary which has resulted in fluctuation of sampling in the middle part of the vertical zone at the sea side over the clam beds. Diggers have frequently preferred digging at this area because of the abundance of the clams.

When analyzing the distribution of the clams at the vertical zone over the western part of the estuary, it can be seen that clams are abundant in the middle part of the mid and low water marks and rare above mid water mark. The reason is that clams generally prefer to settle in muddy parts of the intertidal zone rather than in the sandy area. The area above mid water mark at Kalbadevi is covered with sand and the area below it is muddy.

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HOMING IN THE GASTROPODA

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ABSTRACT

In 1950 Edelstam & Palmer suggested that the most plausible guiding senses used by gastropods to home were "smell (for terrestrial forms) and touch (for marine forms)." Since that time more information has become available, though the range of species examined has not been significantly extended. Homing gastropods may be classified into two types: those which only home in air such as *Helix pomatia*, *Onchidium floridanum* and *Limax maximus* and those which can home underwater like *Patella vulgata* and *Siphonaria* spp. The following results are from my own experiments with *Limax grossui* Lupu. Under laboratory conditions the slugs consistently home without trail following. Trails emanating from the home site are an adequate stimulus for 'homing' in the absence of the home itself, though trail following need not be involved. Failure to find a home often results in a period of trail following. The sensory sites for distant chemoreception and trail following are anatomically separate. *L. grossui* homes therefore, using an olfactory beacon with a, normally reserved, trail following capacity. This type of hypothesis can be extended to encompass the observed features of limpet homing where the prime mechanism is trail following with the olfactory beacon normally reserved. The dual nature of the mechanism is illustrated by the work of Davis. If the olfactory beacon is removed by treatment with boiling water but the trail following pheromone is unaffected then Davis' results (held to support the concept of a topographic memory) can also be interpreted in pheromonal terms. This dual pheromone mechanism may have wide relevance to the solution of gastropod homing problems. Both pheromones are in the mucus: one serves as an olfactory beacon and the second is perceived only on contact. The beauty of pheromonal homing mechanisms in the gastropods is that they depend only on sensory and nervous mechanisms known to be within the capacities of soft bodied animals. The alternatives of topographic and kinaesthetic memories both involve the precise measurement of angles and distances and the use of a detailed memory, none of which have yet been demonstrated in gastropods.

Homing is a widespread phenomenon in the Gastropoda; it has been described from the prosobranchs and the pulmonates in both aquatic and terrestrial habitats and in both shelled and shell-less forms. The features of homing have been the subject of a great deal of research over the last 100 years (Funke, 1968) but there has been little agreement over the methods used by these animals to get home.

Homing gastropods may be classified into 2 types: those that home only in air and those that can home underwater (limpets). In 1950 Edelstam & Palmer suggested that the most plausible guiding senses for homing were smell for terrestrial forms and topographic memory for marine forms. More recently Willows (1973) supported this view. It is my contention that the persistence in the literature of the involvement of memory in gastropod homing is a result of the failure of chemoreception to account for all the known features of homing and this in turn is a result of incorrect assumptions concerning the nature of the guiding chemicals.

Terrestrial homing has been demonstrated in *Deroceras* (= *Agrilolimax*) *reticulatum*, *Cepaea nemoralis* (both Newall, 1966), *Helix aspersa* (cf. Step, 1960), *Limax flavus* (cf. Taylor, 1903; South, 1965) and has been examined in depth in *Helix pomatia* (cf. Edelstam & Palmer, 1950), *Limax maximus* (cf. Gelperin, 1974) and *Onchidium floridanum* (cf. Arey & Crozier, 1921). The dominant feature of homing in all these animals is probably the distant chemoreception of home, but no critical experiments have been performed to determine whether any other factors are involved.

I have been investigating the relevance of the subsidiary factors of trail following and memory in the homing behaviour of *Limax grossui* Lupu.¹ Five animals were placed in a tank 0.58 X 1.15 X 0.15 m, the bottom of which was covered by a field of 24 glazed ceramic tiles

¹The Irish populations of '*Limax grossui*' have now been renamed *Limax pseudoflavus* Evans, 1978 (*Irish Naturalists' Journal*, 19: 173).

(porous side up). A holed brick provided the only home. Food, on a tray, was always available in the same position. *L. grossui* is strictly nocturnal and so movements were viewed under extremely dim red light with a silicon tube T.V. camera and recorded every minute on a time lapse video-tape recorder. The conclusions reached here are based on 50 nights of observations (equivalent to 3000 slug hrs of movement). Some further trail following experiments were performed using the methods of Cook (1977).

These observations indicate that strange slugs placed in an established tank can find the 'home' brick, as can strange slugs placed in a clean tank with an established 'home' brick. Finding the brick in these experiments cannot involve memory and must be based on olfaction. An excursion of a slug in an established tank rarely involves the retracing of the outward path. When the excursions of several slugs over several days are superimposed, however, definite patterns of movement emerge (Fig. 1). This pattern of movement is based on trails since the transposition of some of the tiles and the removal of the home, result in the pattern of movement changing with the position of the tiles (Fig. 2). The dependence on the trails may reflect the slugs tending to stay in an area of high trail density rather than accurate and definite trail following. Failure to find a home after its removal often results in a period of accurate trail following (Fig. 3).

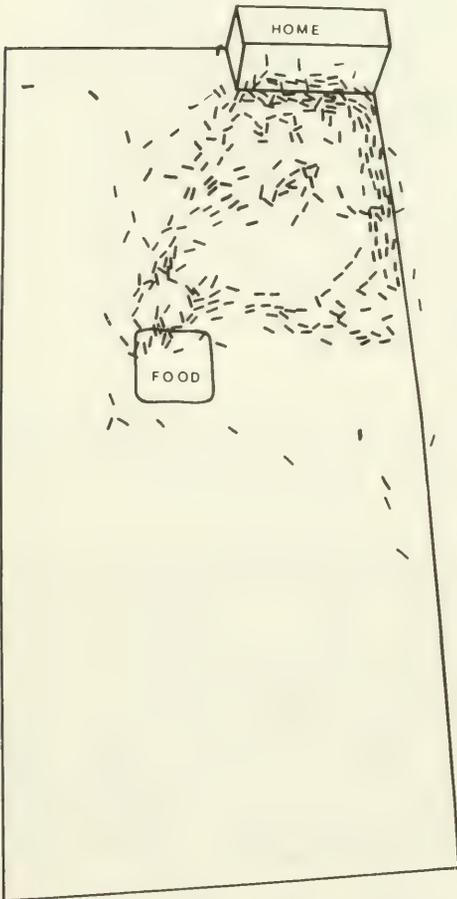


FIG. 1. The superimposed positions of 5 slugs recorded every 5 minutes for four days. Only changes in position are recorded. Scale—the home is 20 cm long.

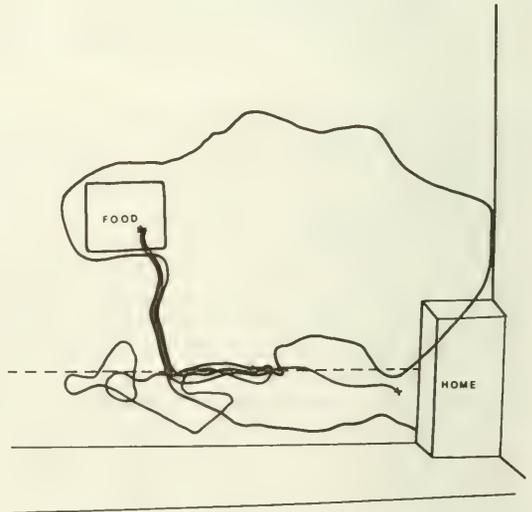


FIG. 2. The nearest row of tiles was reversed so that the former edge now runs along the dotted line and the home removed. The return track of the slug, normally along the near edge of the tank, is now influenced by the trails on the reoriented tiles along the dotted line. *home removed and tiles reversed when the slug first reached the food. Scale—the home is 20 cm long.

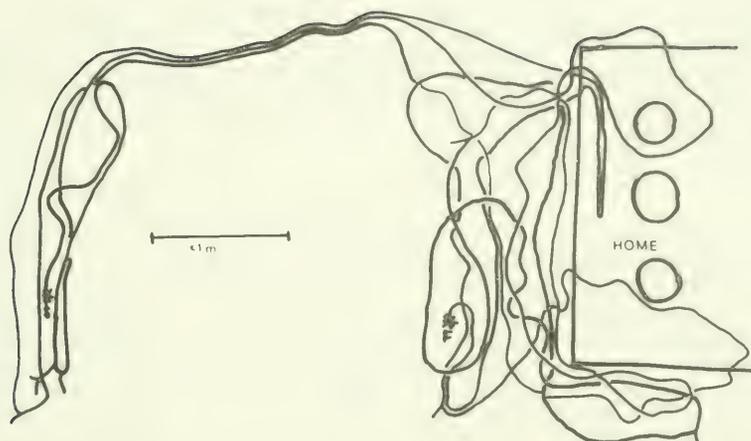


FIG. 3. The established home was replaced by a clean brick. On its return the slug searched the 'home' and the area at the base before moving back and forth along part of its return trail.

Slugs on the feeding tray of an established tank were moved to an identical but clean tank and were not able to home to the clean brick, thus demonstrating that neither topographic nor kinaesthetic memories are involved.

Removal of the optic tentacles results in a dramatic fall in homing and also a significant fall in the frequency of trail following. It has no effect on the accuracy with which a slug can stay on a trail. Removal of the anterior tentacles on the other hand, does not affect homing, nor the frequency of trail following but does significantly impair the trail tracking ability of the slug. On removal of both pairs of tentacles the animals refuse to move at all.

These results indicate that the optic tentacles are essential for homing but that they share the control of trail following with the anterior tentacles: the former having a role in the identification of the mucus and the latter being concerned with the close tracking of the trails. Distant chemoreception and trail following therefore, probably have separate sensory mechanisms.

From all these observations of *Limax grossui* it may be concluded that it homes using both an olfactory beacon based at home and the trails which emanate from home, and also a capacity to follow trails home: that is, it is a dual chemosensory mechanism, probably involving at least two pheromones, one for distant chemoreception and another for trail following. The features described here are compatible with all the experimental evidence for other terrestrial homing species.

In 1968 Funke postulated a dual trail following/distance chemoreception explanation for the homing of the limpet *Patella vulgata* but with the emphasis on the trail following. Limpets can home at all states of tide (Cook et al., 1969) and it is in this group of potentially submarine homing gastropods that most uncertainty arises concerning mechanisms. As well as *Patella*, *Siphonaria* (cf. Cook, 1969) and probably *Acmaea* (cf. Breen, 1971) home using trails. Most experiments aimed at removing trails however, do not prevent homing. Since these trails are normally not visible the only reliable way of inferring their presence is to follow the behaviour of the animals on them. A treatment which does not prevent homing therefore, could be interpreted as ineffectively removing the trails rather than requiring some further mechanism.

Davis (1970) treated rocks with boiling water and the limpets subsequently did not find home, though 50% of them got within 2 cm of home. This can either be interpreted as evidence for a topographic memory or as evidence that whilst the chemical information on the home was destroyed by boiling water, the chemical information on the trail was not. Davis' second series of experiments involved various degrees of chipping of the rock around the home. Severe chipping prevented homing whilst mild chipping failed to have this effect. The volcanic rocks of Skokholm where this work was conducted are extremely hard and it is questionable whether mild chipping would be adequate to remove all the original surface of the rock. The evidence for a topographic memory is therefore, equivocal.

TABLE 1. The effect of treating rock with 1N NaOH and displacing limpets 15 cm from home. Homing is significantly reduced ($\chi^2 = 4.74$, $p < .05$) and there is no marked tendency to move towards home. The control group differed only in the rock not being chemically treated.

	Total	Survivors	At home	Within 10 cm of home
Experiment	51	38	8	3
Control	50	38	18	2

If topographic information is used to home then no chemical treatment of the rock should be sufficient to prevent limpets returning to their home area. Cook et al. (1969) performed an experiment in which the rock was treated with 0.75N NaOH, but reported this as unsuccessful. Reanalysis of this data using χ^2 shows that this treatment did in fact significantly reduce homing, but the distance of the non-homing limpets from their homes was not recorded. I have recently repeated this experiment. Limpets were removed from the rock after having numbered both animals and their homes. The rock was washed with 1N NaOH for 15 min, 1N HCl for 5 min and then thoroughly doused with water. The limpets were replaced on the rock 15 cm from home and their positions recorded after 24 hrs (Table 1). It is clear that homing is significantly reduced ($\chi^2 = 4.74$, $p < .05$) and that limpets did not return to a "home area." These results are not compatible with the concept of a topographic memory. The other memory mechanism generally considered is a kinaesthetic one, i.e. the use of past movements to compute the future course home. Funke (1968) describes one occasion on which a moving limpet (*'Patella caerulea'*) was carefully displaced 30 cm. It then moved as if the home had also been displaced for a similar distance in the same direction. However, this was a single occurrence and limpets were normally disorientated by such a displacement. Further experiments (Funke, 1968) were inconclusive, though 3 limpets (out of 7) whose trails were artificially lengthened stopped moving before reaching the new home position. Many limpets reach home with only minutes to spare before being covered by the tide (Cook et al., 1969) and it may be that time is the important factor in such experiments rather than a memory of distance.

In conclusion it can be seen that a dual pheromone mechanism is adequate to account for all the features of homing in gastropods if these criticisms of experiments are well founded. In support of this mechanism is a consideration of the sensory and mental capacities of gastropods. Their prime senses appear to be chemical, no soft bodied animal is known to store muscular information and finally it is only recently that they have been shown to be capable of simple associative learning (Gelperin, 1974a-b), let alone the complex processes of information storage and computing required for kinaesthetic or topographic memory.

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DONNEES ECOLOGIQUES SUR DES CAECIDAE (GASTEROPODES PROSOBRANCHES) DU GOLFE DE MARSEILLE

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ABSTRACT

Seasonal samples of sand from 11 sublittoral stations (11 to 45 m) between Marseille and Cassis (Mediterranean coast of France) made it possible to obtain for the first time a very abundant material of caecid gastropods. Three species are involved: *Caecum subannulatum*, *C. auriculatum* and *C. trachea*. Analysis of sedimentary and hydrological parameters explains the distribution of these molluscs and shows that the first two species are more abundant in stations exposed to strong hydrodynamism. Comparisons are made with mesopsammic opisthobranchs and the whole mesopsammon. Vertical migrations inside the sediment as a result of increased temperature or in relation with trophic or reproductive phenomena are shown to exist in *C. subannulatum* and *C. auriculatum*. Reproductive periods are stated.

INTRODUCTION

La famille des Caecidae est très mal connue dans le monde. Le matériel en est difficile à obtenir, compte tenu des dimensions très faibles et des conditions de vie des membres de cette famille et on doit généralement se contenter d'examiner un petit nombre d'individus.

Ceci avait toujours fait obstacle à toute approche écologique de cette famille, tandis que le développement à stades multiples, unique chez les Gastéropodes, rendait sa systématique des plus difficiles. Le traitement de divers sédiments sableux du golfe de Marseille par une méthode récemment adaptée par l'un de nous (Poizat, 1975) a permis d'obtenir pour la première fois un matériel aussi abondant que représentatif de trois espèces de cette famille. La révision systématique de ces espèces et de leurs divers stades de croissance fera l'objet d'un autre travail: ici ne sont présentés que les résultats écologiques.

MATERIEL ET METHODES

Les stations étudiées, suivies depuis plusieurs années (Poizat, 1972, 1975) sont réparties entre Marseille et Cassis (Fig. 1). Dans 3 d'entre elles (Nos. 1, 2 et 4), la recherche des Caecidae a été faite pendant 6 mois à 1 an, en vue d'étudier leur cycle annuel. Les 8 autres stations (Nos. 3 et 5 à 11) avaient pour but l'étude des effets de l'hydrodynamisme marin sur la répartition des *Caecum*.

Les caractéristiques sédimentologiques et hydrologiques de ces 11 stations au moment des prélèvements, et le détail des Caecidae pris à chacune d'elles sont rassemblés dans le Tableau 1.

Les sédiments ont été prélevés à la drague Charcot (Picard, 1965) dont la profondeur de pénétration dans les sables étudiés atteint environ 12 cm, ou à l'aide de la drague "spatangue," dont le cadre métallique en forme de *Spatangus* (échinide irrégulier) ne fait qu'écrêmer les 5 ou 6 cm superficiels du sédiment.

Les Caecidae, partie intégrante du mesopsammon, ont été séparés du sédiment par la technique de Uhlig (Uhlig et al., 1973) adaptée par Poizat (1975) et permettant le traitement de 8 litres de sédiment à la fois: on laisse fondre de la glace d'eau douce au-dessus du sédiment placé dans un dispositif spécialement construit à cet effet. Le mesopsammon vagile se concentre par ses propres mouvements à la base du dispositif, dans un cristalliseur rempli d'eau de mer.

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TABLEAU 1. Paramètres écologiques aux stations étudiées dans le golfe de Marseille, et abondance numérique des stades de croissance 1, 2 et 3 des 3 Caecidae observés, des autres Prosobranches, des Opisthobranches, en fonction des dates d'observations. Les valeurs en concernent les données obtenues à la drague spatangue; les autres données sont celles obtenues à la drague Charcot.

No. Station	Prof. (m)	Date	Temp. fond (°C)	SEDIMENT			MESOPSAMMON (Nombre d'individus vivants)										Opisthobr.	Total (sauf Mollusques)
				% vase	Mode (mm)	So (Task)	<i>Caecum subannulatum</i>			<i>Caecum auriculatum</i>			<i>Caecum trachea</i>			autres Prosobranches		
							"1"	"2"	"3"	"1"	"2"	"3"	"1"	"2"	"3"			
1	20	28.5.76	14.0	0	2.80	1.30	12	18	999	285	137	981	3	0	0	772	143	20991
		21.6.76	20.0	0	2.80	1.34	0	0	72	96	690	270	0	0	0	630	126	19020
		3.8.76	16.0	0	1.77	1.31	6	6	12	192	114	384	0	0	0	426	324	15426
2	17	18.10.76	16.0	0	1.40	1.36	24	30	30	1074	138	72	0	0	0	270	477	65316
		29.11.75	15.8	0	2.25	1.20	6	18	102	14	8	56	2	0	0	566	94	21082
		23.2.76	12.0	0	1.40	1.31	3	21	93	12	0	3	0	0	6	405	252	21618
3	25	7.5.76	14.5	0	1.40	1.31	0	0	54	0	0	0	0	0	42	94	150	20480
		21.6.76	20.8	0	1.40	1.30	0	6	108	0	6	42	0	0	6	54	102	6354
		3.8.76	15.0	0	1.40	1.38	0	0	18	6	24	114	0	0	0	450	147	10626
4	11	18.10.76	16.5	0	1.40	1.29	0	0	36	30	84	90	0	0	6	564	176	42288
		3.8.76	15.0	0	2.25	1.38	480	168	120	42	24	102	0	0	0	230	110	39762
		5.12.75	14.5	0.5	0.71	1.71	1	2	8	14	1	4	0	0	0	290	30	15360
4	11	23.2.76	12.5	0.61	0.71	1.89	15	0	18	3	0	0	0	0	66	823	44998	
		7.5.76	14.0	0.79	0.50	1.73	0	6	6	0	12	36	6	12	72	221	27669	
				0	0.55	1.53	0	0	9	3	6	0	0	0	72	221	27669	
				0	0.71	1.69	0	0	12	0	0	0	0	0	3	1209	25287	
				0.77	0.50	1.73	0	6	6	0	12	36	6	12	42	102	33174	
				0.79	0.56	1.79	0	0	18	0	18	0	6	6	30	543	22884	
							0	0	18	0	18	0	6	6	24	18	10872	

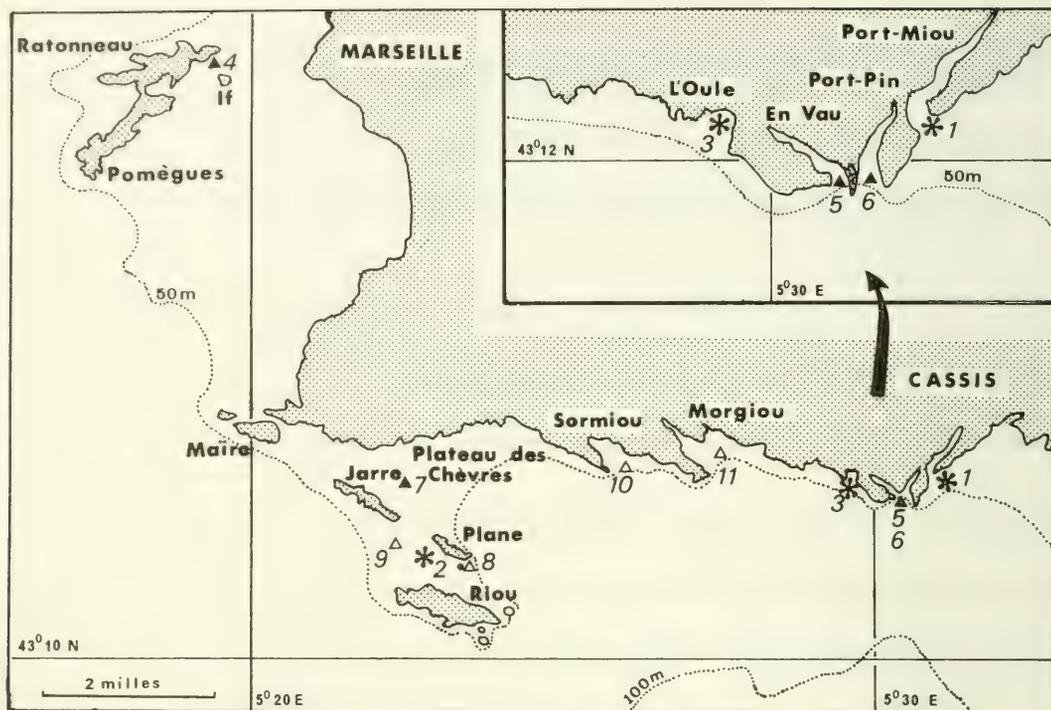


FIG. 1. Stations étudiées dans le golfe de Marseille. Δ —hydrodynamisme faible; \blacktriangle —hydrodynamisme moyen; *—hydrodynamisme élevé.

Il a été possible de classer l'ensemble des spécimens de Caecidae en 3 espèces: 2 espèces communes, *Caecum auriculatum* Folin, 1867, et *C. subannulatum* Folin, 1869, et une espèce plus rarement récoltée, *C. trachea* (Montagu, 1803), chacune représentée par divers stades de développement (Tableau 1). Disons seulement que ces stades, qui feront l'objet d'une publication descriptive particulière, sont appelés ici stade 1 (protoconque avec ou sans prolongement), stade 2 et stade 3 (adulte), correspondant respectivement au "premier âge," "deuxième âge" et "troisième âge" définis par Folin (1875).

Les Prosobranches (Caecidae compris) et Opisthobranches sont ainsi triés in vivo après légère coloration au rouge neutre. Le reste du mesopsammon (Copépodes, Annélides, Nématodes, etc.) est décompté ultérieurement après fixation à l'alcool 70% et coloration au rose Bengale. Tous les résultats sont exprimés en fonction d'un volume sédimentaire de 48 litres (par multiplication de nos comptages par 6), chiffre voisin du "volume minimum" défini par Picard (1965) pour les études de macrobenthos en Méditerranée (50 litres).

Des mesures sédimentologiques et de température de l'eau de mer apportent des informations sur les conditions hydrodynamiques et leurs variations saisonnières dans les stations. Les sédiments sont définis ici (Tableau 1) par leur pourcentage de vase (proportion de la fraction inférieure à 50 μ m), leur mode (classe dimensionnelle dominante) et l'indice de tri de Trask ($So = \sqrt{Q_3/Q_1}$; Q_3 et Q_1 étant des paramètres traduisant les dimensions atteintes par 25 et 75% des particules).

ANALYSE DES RESULTATS PAR STATION

Le sédiment enregistré plus ou moins selon les stations les fluctuations de l'état de la mer liées à la météorologie. La tendance générale est à une augmentation de la granulométrie durant la mauvaise saison, de novembre à mars (hydrodynamisme marin plus élevé); au contraire, il y a

affinement des sédiments et augmentation de leur hétérogénéité durant la belle saison, de mars à octobre. Mais cette réponse du sédiment est modulée par la situation géographique de chaque station par rapport aux deux vents dominants du golfe de Marseille: le vent d'Est et celui de NNW ou "mistral" (Poizat, 1972).

On peut classer les 11 stations étudiées en 3 ensembles selon l'intensité de l'hydrodynamisme: hydrodynamisme élevé (stations 1 à 3), moyen (stations 4 à 7) ou faible (stations 8 à 11).

Caecum trachea étant peu représenté dans nos récoltes, les commentaires suivants ne concernent, sauf exception, que les deux autres espèces.

Stations à hydrodynamisme élevé

—station 1 (débouché de la calanque de Port Miou).

Son orientation l'expose aux vents d'Est alors que l'influence du mistral y est beaucoup plus modeste. La rareté exceptionnelle du vent d'Est pendant la période d'étude a entraîné un hydrodynamisme plus faible que d'habitude, d'où affinement très important du sédiment (mode passant de 2,8 à 1,4 mm) sans augmentation notable d'hétérogénéité (indice de tri assez stable). Les circulations d'eau sur ce fond, bien que diminuées, n'ont donc pas cessé, assurant une bonne oxygénation du sable et interdisant le dépôt de particules fines (envasement nul).

L'importance numérique des Opisthobranches a augmenté bien que le mesopsammon ait accusé un léger affaiblissement au milieu de l'été (août 1976).

Cette station s'est montrée remarquablement riche en *Caecum auriculatum* à tous les stades de croissance (traduisant une abondante reproduction); *C. subannulatum*, quoique beaucoup moins abondant, y a toujours été bien représenté. Néanmoins, comme pour le mesopsammon, une diminution numérique des deux espèces s'est manifestée en période estivale.

—station 2 (dans la passe entre les îles Plane et Riou) (Fig. 2).

Station sous l'influence à la fois des vents d'Est (renforçant le courant marin général d'Est) et du mistral (qui perturbe cet écoulement en créant souvent de puissants remous). Mais la station est située dans la partie occidentale de cette passe, dont les profondeurs augmentent vers l'Ouest: aussi le courant d'Est n'a-t-il qu'un effet moyen de lessivage des sédiments, en tout cas plus faible qu'à la station 1, malgré une bathymétrie légèrement moindre. Le schéma de variation saisonnière de la granulométrie reste donc le même qu'à la station 1 (mode 2,25 mm en hiver et 1,25 mm au début de l'été).

Les Opisthobranches mésopsammiques et le reste du mesopsammon passent par un maximum numérique en mai, puis accusent (moins cependant qu'aux autres stations) un minimum numérique estival (juin 1976). Une baisse de la température de l'eau, l'augmentation du mode et de la maille sédimentaire, une meilleure oxygénation des interstices sableux entraînent un second maximum du mesopsammon, à l'exception des Opisthobranches qui décroissent vers leur minimum hivernal.

Comme la station 1, cette station est très favorable à *C. auriculatum* et *C. subannulatum* qui y vivent en abondance, s'y reproduisent et montrent un net minimum en juin (comme le mesopsammon total, Opisthobranches compris). Chez *C. subannulatum*, ce minimum est suivi d'une remarquable phase de reproduction en octobre. On note (par comparaison drague Charcot/drague spatangue) une concentration de *C. subannulatum* et *C. auriculatum* dans le film sédimentaire au moment du maximum thermique estival, suivie d'une phase de reproduction des deux espèces. Ce phénomène se manifeste aussi pour les Opisthobranches et le reste du mesopsammon, au printemps puis en été.

—station 3 (calanque de l'Oule).

Cette station semble représenter l'extrême degré d'hydrodynamisme compatible avec la vie des mollusques mésopsammiques. La maille sédimentaire est très vaste; le sédiment, très grossier, est très violemment remanié sur une grande épaisseur, ce qui crée un milieu défavorable à un riche mesopsammon. Le seul prélèvement qui y a été fait a cependant montré une abondance notable de *C. auriculatum* et *C. subannulatum*, et l'absence de *C. trachea*.

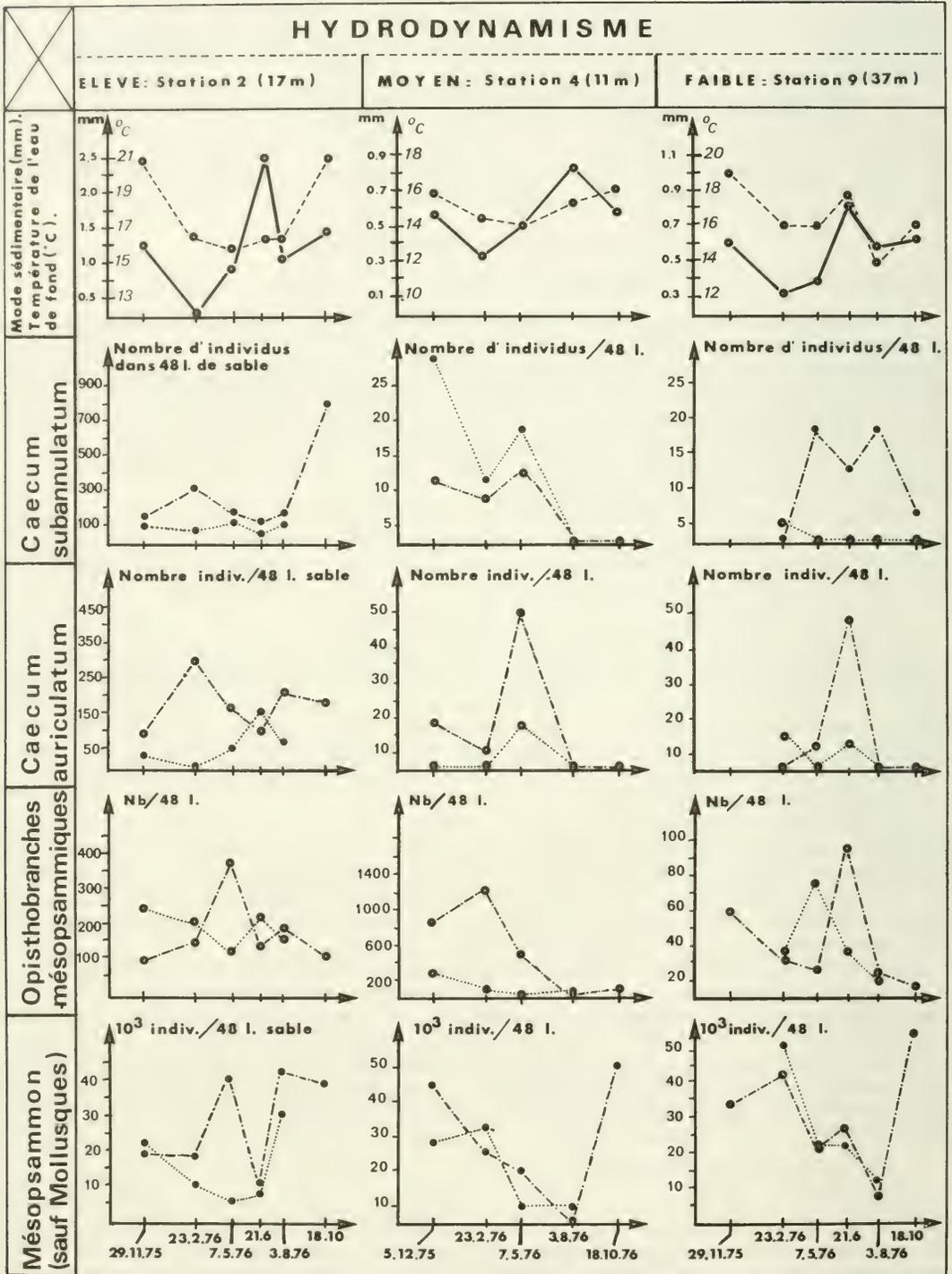


FIG. 2. Variations saisonnières des paramètres sédimentaires (-----) et thermiques (——) et de l'abondance numérique (.-.-.-: Drague Charcot;: Drague spatangue) de *Caecum auriculatum* et de *C. subannulatum*, des Opisthobranches et du reste du mésopsammon, en fonction des 3 degrés d'hydrodynamisme.

Stations à hydrodynamisme moyen

—station 4 (dans la passe entre les îles If et Ratonneau) (Fig. 2).

Station protégée du mistral par l'île Ratonneau et du vent d'Est par l'île d'If. Les eaux y sont chaudes, peu oxygénées et polluées. Par suite des températures estivales plus élevées qu'ailleurs (28°C en surface et 23°C au fond, fin juin 1976) et de la stratification thermique, l'oxygène disponible diminue; corrélativement la granulométrie du sédiment s'affaiblit (mode passant de 0,7 mm en hiver à 0,5 mm au début de l'été).

Il y a un effondrement quantitatif du mesopsammon, Opisthobranches compris, et disparition totale des 3 espèces de Caecidae (moins abondantes qu'en hydrodynamisme élevé) qui avaient montré cependant une nette phase de reproduction (présence de stades 1 et 2). Notons que, depuis la fin de l'hiver, les individus de *C. auriculatum* et *C. subannulatum* s'étaient concentrés dans le film sédimentaire superficiel avant cette disparition.

—stations 5 (calanque d'En Vau), 6 (calanque de Port Pin) et 7 (plateau des Chèvres).

Malgré une bathymétrie notable, l'hydrodynamisme des stations 5 et 6 est sous l'influence de courants de décharge (undertows) en régime de vent d'Est, auquel font face ces deux stations. De même, la station 7 est abritée par les mattes d'herbiers de Posidonies qui atténuent localement les puissants courants de fond, d'azimut Est, de règle dans cette zone. La maille sédimentaire est assez vaste, pratiquement jamais colmatée par les fractions fines.

Dans ces 3 stations, le mesopsammon (Opisthobranches compris) est très riche. Comme à la station 4, les Caecidae (sauf *C. trachea*) se reproduisent normalement (et même intensivement pour *C. auriculatum*) puis disparaissent totalement en début d'été (sauf pour *C. subannulatum* à la station 7).

Stations à hydrodynamisme faible

A cette catégorie appartiennent la station 8 (Est de l'île Plane), abritée par les herbiers de Posidonies et les récifs de la pointe Est de l'île Plane, et les stations 9 (Riou), 10 (Sormiou) et 11 (Morgiou), à hydrodynamisme faible (Fig. 2) du fait de leur profondeur plus grande (30-37 m). Les sédiments de ces stations sont "moyens" ou "peu grossiers," avec un pourcentage de vase atteignant parfois 5 à 8% (stations 10 et 11). Le mesopsammon (Opisthobranches non compris) montre des variations analogues à celles observées en hydrodynamisme moyen (cf. Fig. 2, station 4).

Les Caecidae sont présents en nombre moindre qu'en hydrodynamisme élevé, mais analogue à ce qui a été observé en hydrodynamisme moyen. Seule de ces stations la station 9 a fourni des stades jeunes, y compris pour *C. trachea*, mais ce recrutement ne semble pas y être suivi d'un maintien durable. Les conditions de milieu (exiguïté et colmatage du milieu interstitiel) de ces stations et probablement leurs conditions trophiques sont évidemment à la limite de la survie des 3 espèces de *Caecum*.

On note pour *C. subannulatum* et *C. auriculatum* une migration vers le film sédimentaire, à la fin de l'hiver (février), suivie d'une prolifération. Lors du maximum thermique estival, il y a décroissance des deux espèces; mais, contrairement à ce que l'on observe en hydrodynamisme élevé et moyen, leur maximum numérique coïncide avec le maximum thermique estival. Ce maximum numérique est suivi d'une diminution brutale de *C. subannulatum* et de la disparition de *C. auriculatum*.

CONCLUSIONS

(1) *Caecum trachea* n'est pas abondant dans les milieux sableux analysés et ne s'y reproduit guère. Cette espèce est peut être inféodée aux herbiers de Posidonies.

(2) Du point de vue écologique, *C. subannulatum* et *C. auriculatum* se distinguent difficilement et sont généralement observés dans les mêmes milieux. Leur répartition bathymétrique est analogue dans les limites des stations étudiées entre 11 et 45 mètres de profondeur.

(3) *C. subannulatum* et *C. auriculatum* prolifèrent particulièrement bien dans des milieux

sableux non envasés, liés à un hydrodynamisme élevé et où sédimentent des sables grossiers à très grossiers ("sables à amphioxus"). Dans ce type de biotope, il y a abondante reproduction et maintien des 2 espèces toute l'année, malgré un net appauvrissement au moment du maximum thermique (exemple: station 2).

Dans les milieux à hydrodynamisme moyen, caractérisés par des sables plus ou moins grossiers ("sables détritiques côtiers") la reproduction des 2 espèces est normale mais il peut y avoir localement (station 4) destruction de toute la population au moment du maximum thermique, paraissant liée à la diminution d'oxygène de l'eau interstitielle.

Dans les milieux à hydrodynamisme faible ou très faible et où sédimentent des sables moyens à peu grossiers renfermant parfois une certaine proportion de vase, les 2 espèces de *Caecum* sont moins abondantes qu'en hydrodynamisme élevé (elles sont même en état de survie pércalaire à la station 8), du fait soit de l'exiguïté de la maille sédimentaire, soit d'un début de colmatage de celle-ci.

(4) Des migrations verticales ascendantes amènent les 2 espèces de *Caecum* à se concentrer plus ou moins dans le film sédimentaire superficiel. Elles semblent de 2 types:

—Migrations "printanières," qui s'observent dans les 3 intensités d'hydrodynamisme, et sont généralement suivies d'une prolifération des *Caecum*, des Opisthobranches et du reste du mésopsammon. Ces migrations semblent être des migrations trophiques et reproductrices.

—Migrations "estivales," En hydrodynamisme élevé, la concentration des 2 espèces dans le film est suivie d'une nouvelle phase de reproduction. En hydrodynamisme moyen, les 2 espèces de *Caecum* disparaissent lors du maximum thermique; enfin, en hydrodynamisme faible, on observe soit la disparition des 2 espèces (station 8), soit la disparition de *C. auriculatum* (station 9).

Ce deuxième type de migration doit être relié, dans les 3 catégories d'hydrodynamisme, à un déficit en oxygène dissous dans la sous-strate sédimentaire.

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THE POPULATION DYNAMICS AND EXPRESSION OF SEXUALITY
 IN *BALCIS SHAPLANDI* AND *MUCRONALIA FULVESCENS*
 (MOLLUSCA: GASTROPODA: AGLOSSA) PARASITIC UPON
ARCHASTER TYPICUS (ECHINODERMATA: ASTEROIDEA)

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ABSTRACT

In Hong Kong the intertidal starfish *Archaster typicus* (Müller & Troschel) is parasitised by two aglossan gastropods, *Mucronalia fulvescens* (A. Adams) (Stiliferidae) and *Balcis shaplandi* Melvill (Eulimidae). The population dynamics of the starfish and its parasites have been studied for a period of 2 years from April 1973 to March 1975 inclusive. The starfish population at Tai Tam Bay, Hong Kong, comprises 4 age classes with new recruits arriving in the population in late summer and old individuals dying each winter when the population of starfish as a whole lies buried in the sand. *A. typicus* breeds in early summer (April-June); a form of "copulation" takes place with males overlying females, with arms alternating. Both parasites are protandrous consecutive hermaphrodites which leave the host in the late summer months. At other times the parasites are on the starfish but are spatially separated from each other—*B. shaplandi* on the aboral, *M. fulvescens* on the oral surfaces (Morton, 1976). When on the host (i.e. September-June), both parasites occur in discrete clusters which comprise 2 age classes; typically a single large female is surrounded by a variable number of smaller males. Copulation takes place first in the spring (April-May). Following egg laying (in saucer shaped capsules) the older females die and the young males undergo a process of sexual change (with transitional phases recognisable) to become female. These in turn are fertilised in a 2nd reproductive phase in autumn (September-November) by their now hatched and growing (male) progeny. Both species thus complete their cycle in approximately 15 months, but the pattern of 2 breeding seasons per annum ensures a safety margin essential for species maintenance.

The male and female reproductive systems of both species are very similar. The male system comprises a vas deferens emptying into a seminal vesicle which in turn discharges into a seminal groove surrounded by a postrate gland. Only *B. shaplandi* possesses a penis. The female system comprises an oviduct that discharges into a seminal receptacle via the intermediary of an albumen gland. The seminal receptacle opens into a pallial oviduct. The egg capsule of both species is thought to be secreted by a large modified hypobranchial gland that occurs only in the female. The gametogenic cycle of both species is described including the hermaphroditic transitional stage.

INTRODUCTION

Little is known of the life cycle of the parasitic aglossan gastropods. Thus Vaney (1913) and Fretter (1955) were only able to find females of *Eulima equestris* and *Balcis devians* respectively. Hoskin & Cheng (1969) noted that in general terms females of *Mucronalia nitidula* were larger than the males. This, however, was based on an examination of 10 specimens only. Lützen (1972) and Gooding & Lützen (1973) have described how *Stilifer linckiae* and *Robillardia cernica* are probably protandric consecutive hermaphrodites.

Megadenus cantharelloides (Humphreys & Lützen, 1972) does not exhibit sexual dimorphism because the female has to attach egg capsules to the shell of the male whereas species of *Paramegadenus* exhibit a pronounced male dwarfism. Male dwarfism is also found in *Enteroxenos oestergreni* (cf. Lützen, 1968) where the pygmy male is implanted in the wall of the pseudopallial cavity of the female. The same author also argued that this was probably also the case in the supposedly hermaphrodite *Thyonicola*, *Entoconcha* and *Entocolax* (though male dwarfism had been recognised in the latter genus much earlier: Ivanov, 1945).

Male dwarfism might be regarded as an evolutionary indicator of a highly specialised obligate endoparasite, typically occurring in few numbers. How such an adaptation arose in the Aglossa,

however, might be revealed by an understanding of the sexual cycle in less specialised ectoparasitic relatives. In Hong Kong the very common intertidal sandy shore starfish *Archaster typicus* (Müller & Troschel) is parasitized by two parasitic aglossans, *Balcis shaplandi* Melvill and *Mucronalia fulvescens* (A. Adams). The former is a member of the Eulimidae, the latter a member of the Stiliferidae. On the host the 2 parasites are spatially separated. *B. shaplandi* occurs on the aboral surface and feeds on the coelomic fluids, *M. fulvescens* occurs on the oral surface and feeds on the fluids of the water vascular system (Morton, 1976). Preliminary studies of these 2 parasites suggested that they occurred on the host in variable numbers and often in what appeared to be sex-paired clusters. A long term study of the 2 parasites was therefore initiated with the following aims: (1), to elucidate the structure of the reproductive systems; (2), to determine the sexual cycle; (3), to investigate the population dynamics of the parasites and the host; (4), to elucidate the relationships of the parasites, one with the other and with their common host.

MATERIALS AND METHODS

Commencing April 1973 for a period of 2 years, 100 specimens of *Archaster typicus* were collected every month from the shallow intertidal sand flats of Tai Tam Bay, Hong Kong Island. Each starfish was carefully examined, on site, for parasites and where these were found they were removed and placed in labelled tubes. A record was kept as to whether or not the individuals of each species were solitary or were in clusters of two or more. Upon return to the laboratory the parasites were measured along their greatest length to the nearest 0.5 mm and, following fixation in Bouin's fluid, were sectioned at 6 μ m and stained in either Mallory's triple stain, Heidenhain's haematoxylin or periodic acid-Schiff (PAS).

Each month length-frequency histograms of the population of each species of parasite were constructed and each parasite, from an examination of the sectioned material, sexed. The sexual cycle of both males and females were divided into 5 phases. These were: (1), *initial stage*; (2), *developing stage (1)*; (3), *developing stage (2)*; (4), *ripe stage*; (5), *spawned stage*. The sections were also closely examined for transitional stages.

Every 3 months for a period of one year a much larger sample of *Archaster typicus* was collected and each member of this sample was measured from the tip of one arm to the inter-radius of the opposite two arms. From these data length-frequency histograms have been constructed which have been analysed using a Walford plot (Walford, 1946; Ricker, 1958).

Archaster typicus is unusual in that sex pairs are established during the breeding season with males overlying females (Clemente & Anicete, 1949). A record was kept each month of when "copulation" was taking place in the majority of the sample i.e. when numbers exceeded 50% of the monthly sample of 100 individuals. Records were also kept of the occurrence of newly settled and adult starfish in poor condition.

RESULTS (I)—THE HOST

In December 1974 the length-frequency histograms of the sample of *Archaster typicus* from Tai Tam suggested that the population comprised 4 age classes which could have respectively settled in 1971, 1972, 1973 and 1974 if breeding and larval settlement takes place but once a year (Fig. 1). By March 1975 the same 4 age classes were recognisable with the smallest age class present in relatively greater numbers. By June 1975 the oldest age class (i.e. that which was presumed to have settled in 1971) was no longer present in the sample and the youngest age class accounted for a much greater percentage of the total. This age class had grown from an arm-disc length of 40 mm in March to 50 mm in June. Similarly both 1972 and 1973 age classes had grown. In September 1975 a new age class of length 15 mm appeared in the sample. The 1974 age class had grown by this time to 55 mm whilst the 1972 age class was possibly overshadowed by the 1973 age class.

This analysis of the population structure of *A. typicus* at Tai Tam suggests that breeding and larval settlement takes place but once a year. This fits in well with associated data on the biology of *A. typicus* at Tai Tam. From April to June each year the starfish population as a whole grouped itself into sexed pairs with males overlying females (Clemente & Anicete, 1949). The products of this union were collected as young starfish later on in the year; in this case in September 1974.

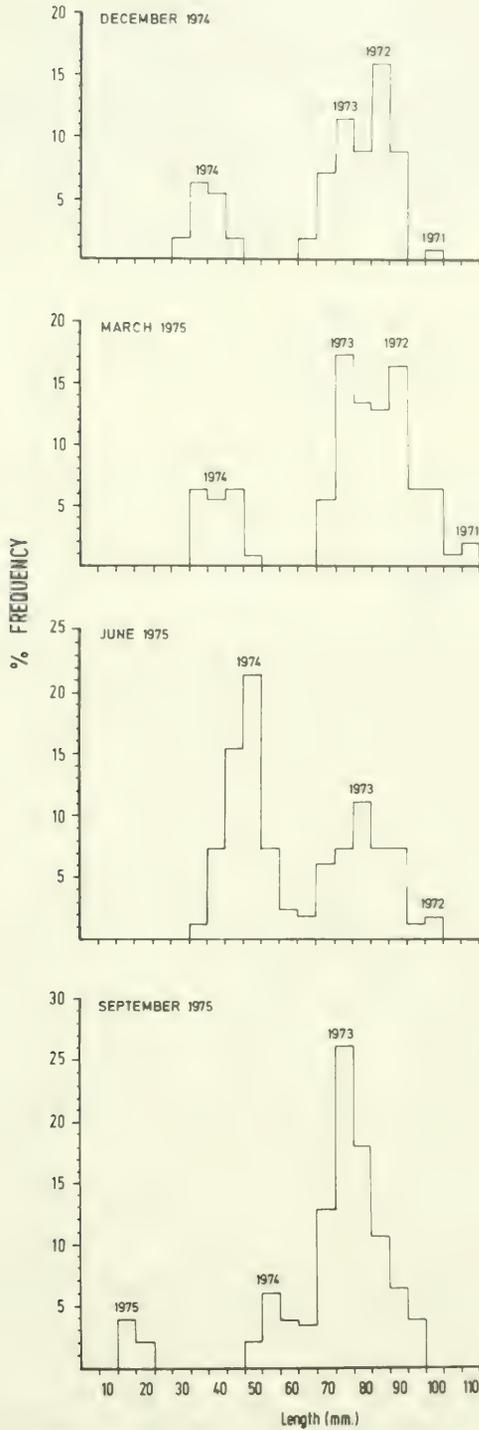


FIG. 1. Length frequency histograms showing the composition of samples of the population of *Archaster typicus* inhabiting Tai Tam bay, Hong Kong. The dates above individual peaks represent the dates at which these particular year classes settled.

During the reproductive phase the pairs are occasionally partially buried in the sand though more often they lie on the sand surface. This behaviour is not true "copulation" since no copulatory organs are possessed by either partner, but proximity may increase the chance of fertilisation especially if the release of eggs from gravid females stimulates the release of sperm by the male. During the autumn and winter months the starfish lie buried in the sand. Large, presumably old, specimens collected at this time were often in a bad condition with broken arms and damaged external surfaces. It is probable that old individuals die at this time, as is evidenced in some measure by Fig. 1 where by June the oldest (i.e. the 1971 age class) components of the population had all died.

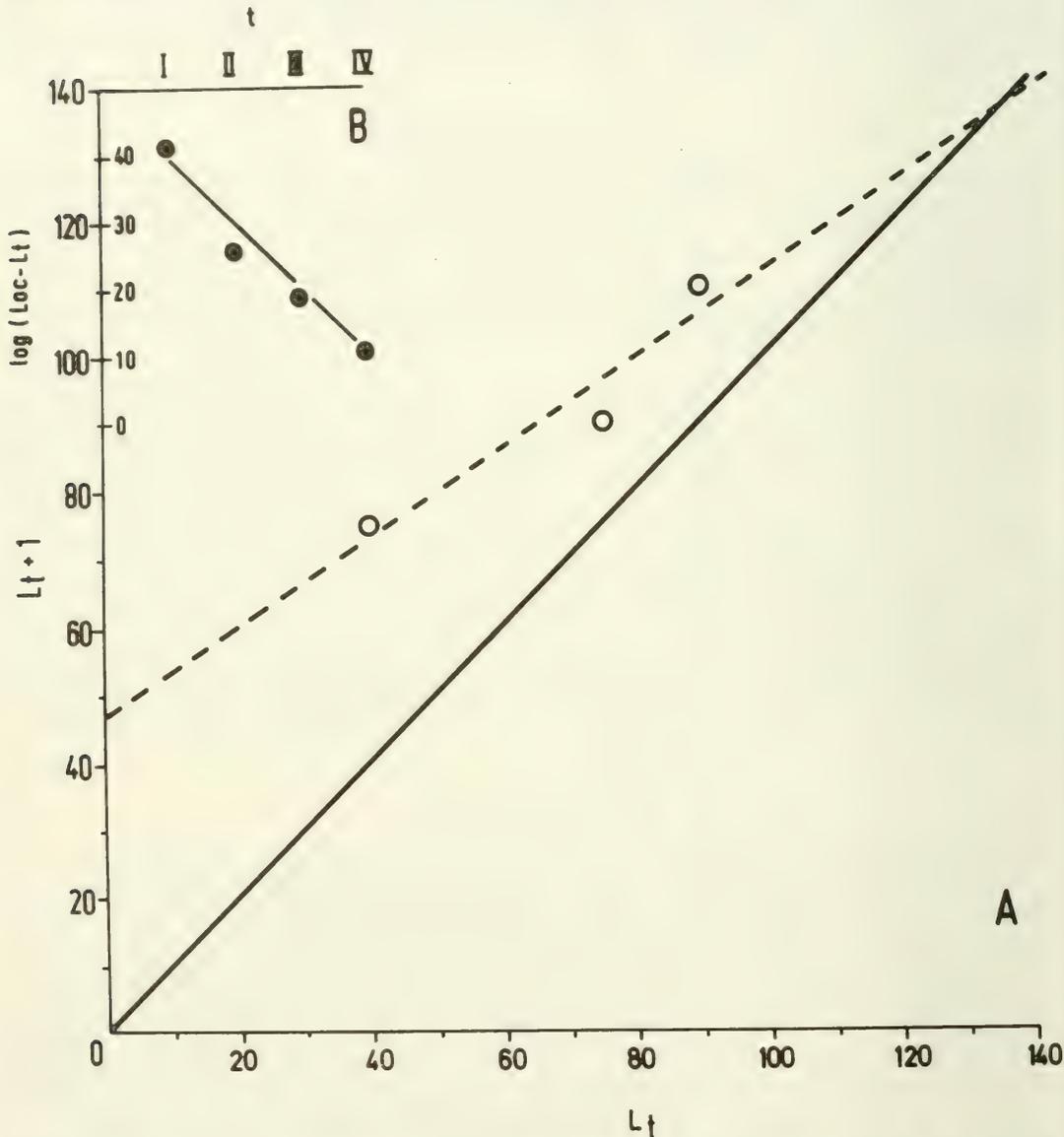


FIG. 2. (A) Walford plot for *Archaster typicus* from Tai Tam in which for each year grouping the average length (L_t) has been plotted against the average length a year later (L_{t+1}). Trial values of L_a have been used to give the best fitting line for a plot of $\log_e(L_a - L_t)$ against t . The slope K obtained from (B) has been used to calculate k . The Walford plot has then been drawn in (A) with a broken line using the calculated values of k and L_a . For $\log(\text{Loc} - L_t)$ read $\log_e(L_a - L_t)$.

The structure of the population of *A. typicus* from Tai Tam has been analysed using a Walford plot (Walford, 1946; Ricker, 1958). Fig. 2A shows that the arm-disc length of the 4 age classes of the starfish comprising the March 1975 sample approach the 45° line as expected if growth fits the Bertalanffy formula $L_t = L_a(1 - e^{-K(t-t_0)})$ (Bertalanffy, 1938) where L = length, t = age, L_a = maximum theoretical length and K = a growth constant. For this range of ages $\log_e(L_a - L_t)$ has been plotted against age (t) (Fig. 2B) to determine by trial the best value of L_a and the slope of K . The best value of L_a has been shown to be 135 mm and the regression for the slope of the Walford plot (k) has been calculated as $y = 47.1 + 0.65x$ (broken line in Fig. 2A). Such an analysis suggests that the population picture of *A. typicus* at Tai Tam as revealed in the samples is a true one and that the species can live for approximately 5 years.

RESULTS (II)—THE PARASITES

The reproductive systems

Mucronalia fulvescens

At an average length of 3.0 mm the greatest percentage of individuals of this species are male (Fig. 3A). The testis (Fig. 4A, T) occupies a dorsal position in each body whorl being enveloped by the digestive diverticula (DD). It is divided into several lobules all of which ultimately discharge into a short vas deferens which expands into a seminal vesicle (SV) 400 μ m in diameter with an epithelium composed of cells 10 μ m tall possessing nuclei 4 μ m in diameter (Fig. 6A). The lumen of the seminal vesicle contains unoriented spermatozoa, some of which were also seen with their heads embedded in the epithelial wall. A short duct connects the seminal vesicle with the mantle cavity. The duct (Fig. 4A), ultimately forming the seminal groove (SG) has a diameter of 50 μ m and comprises a ciliated cuboidal epithelium 6 μ m in height (Fig. 6B). The seminal duct is encompassed by the prostate gland (PG) which is some 350 μ m in diameter and comprises a matrix of lightly staining cells interspersed between which are granular secretory cells. These open into the seminal duct. There is no penis as in *M. nitidula* (cf. Hoskin & Cheng, 1969).

At a length of approximately 3.5 mm *M. fulvescens* changes sex. Fig. 4B is a diagrammatic representation of this transitional stage. As noted by Lützen (1972) for *Stilifer linckiae* sex reversal must take place extremely rapidly because in only one or 2 specimens out of the many hundreds sectioned, was this change at all in evidence. The testis after discharging sperm is spent. The tissues begin to break down accompanied by a degeneration of the reproductive tract except for, most importantly, the seminal duct and surrounding glandular mass. From a dorsal position in each body whorl the primordial cells of the ovary begin to develop and, growing rapidly, occupy the space left by the degenerating testis. The female ducts develop; it is not known if they represent new structures or if they develop from broken down but restructured male ducts.

At a length of between 4.0-5.0 mm most specimens of *M. fulvescens* are female (Fig. 3A).

The female reproductive tract is simple and comprises an oviduct which connects up with a seminal receptacle (Figs. 4D, SR) via the intermediary of an albumen gland. The albumen gland (Fig. 6C) comprises a darkly staining columnar epithelium composed of cells 60 μ m tall possessing short cilia (5 μ m long) and a basally located nucleus 5 μ m in diameter. The gland has overall dimensions of 450 \times 150 μ m with a narrow lumen. The seminal receptacle (Fig. 6D) has a diameter of 230 μ m and comprises a densely ciliated epithelium composed of cells 55 μ m tall each possessing a basal nucleus 5 μ m in diameter. After copulation the seminal receptacle is packed with spermatozoa (S) each oriented outwards with its head embedded in the epithelial lining. From the seminal receptacle arises a duct that opens into the mantle cavity as the pallial oviduct (Fig. 4, GD). The pallial oviduct (Fig. 6E) has the same structure as the male seminal duct (Fig. 6B) except that the prostate gland cells of the tissue surrounding the duct are not present. Into the mantle cavity opens the capsule gland which arises as 2 histologically differentiated regions of a single structure [Fig. 4D, CG(1); CG(2)] formed on the dorsal region of the mantle. Proximally the gland comprises cells [CG(1)] 100 μ m tall densely staining in Heidenhain's haematoxylin. Distally the gland comprises lightly staining cells [CG(2)] some 250 μ m tall. Both components of the gland, however, possess a similar structure (Fig. 6F) in that elongate secretory cells (SC) with a basal nucleus 5 μ m in diameter are interspersed with inversely conical "supporting" cells (SU) each possessing cilia 5-7 μ m long and an apically located nucleus 4 μ m in diameter. Both components of

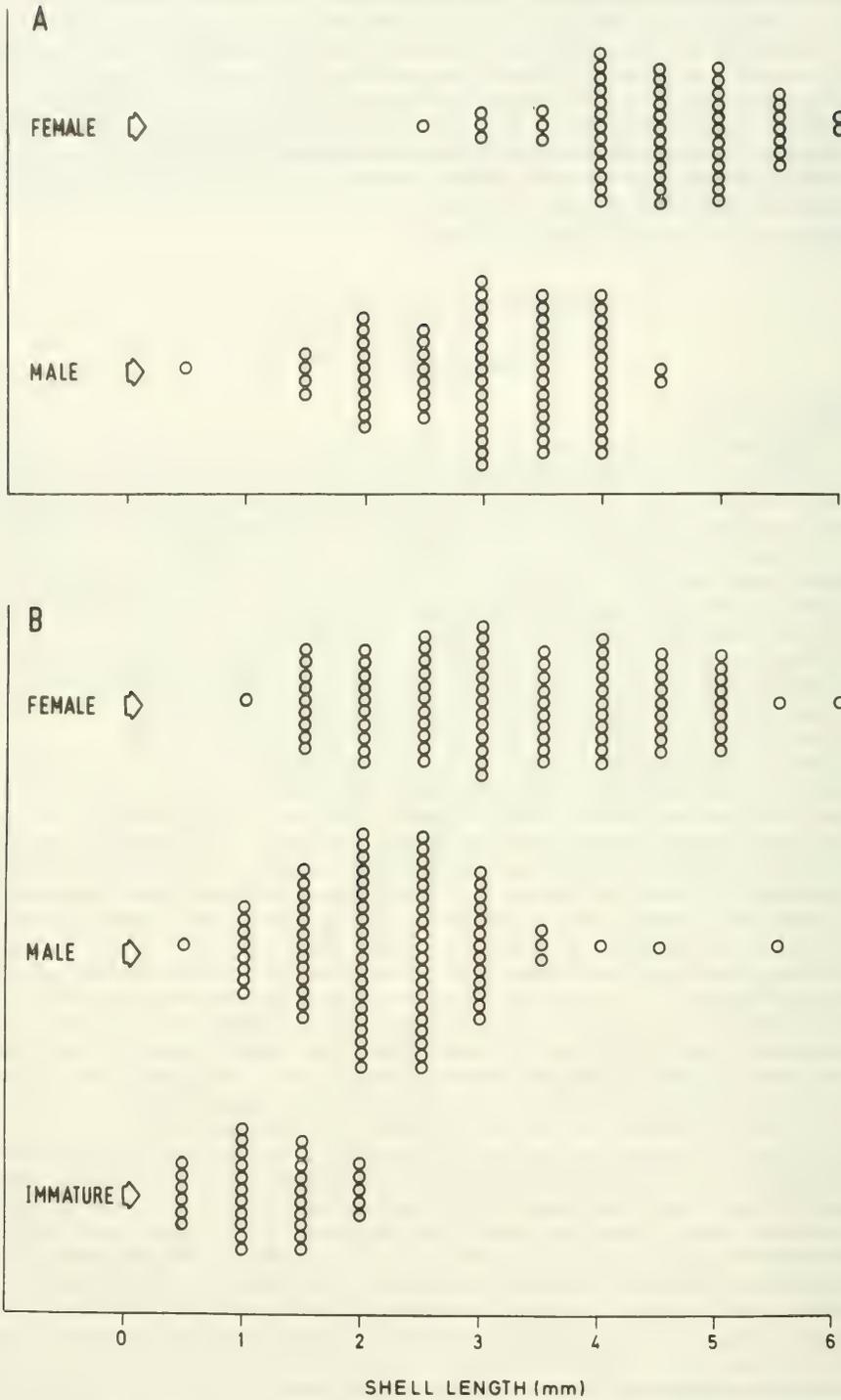


FIG. 3. Size-frequency analysis of (A) *Mucronalia fulvescens* and (B) *Balcis shaplanti* showing the relative incidence of immature, male and female individuals.

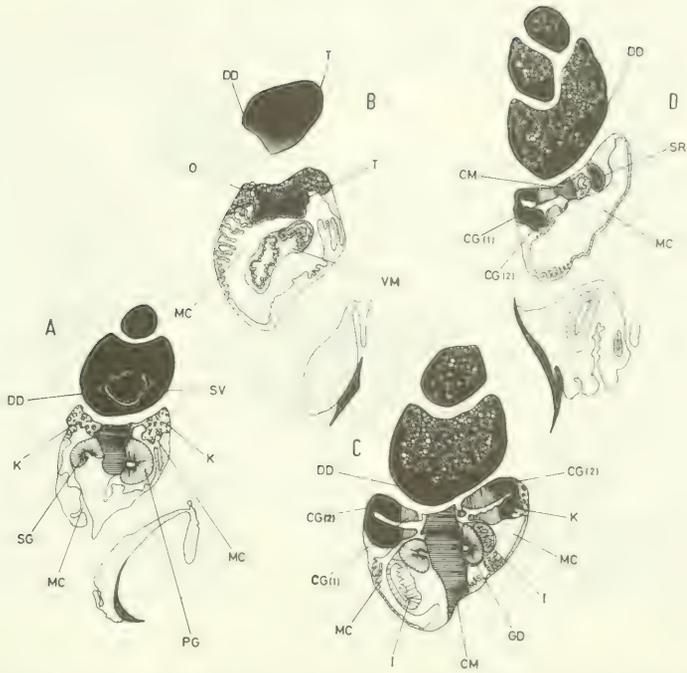


FIG. 4. *Mucronalia fulvescens*. Sections through (A), male phase (3.0 mm shell length); (B), transitional stage (3.5 mm shell length); (C), female phase (4.0 mm shell length) and (D), female phase (5.0 mm shell length), showing a full seminal receptacle (for abbreviations see text).

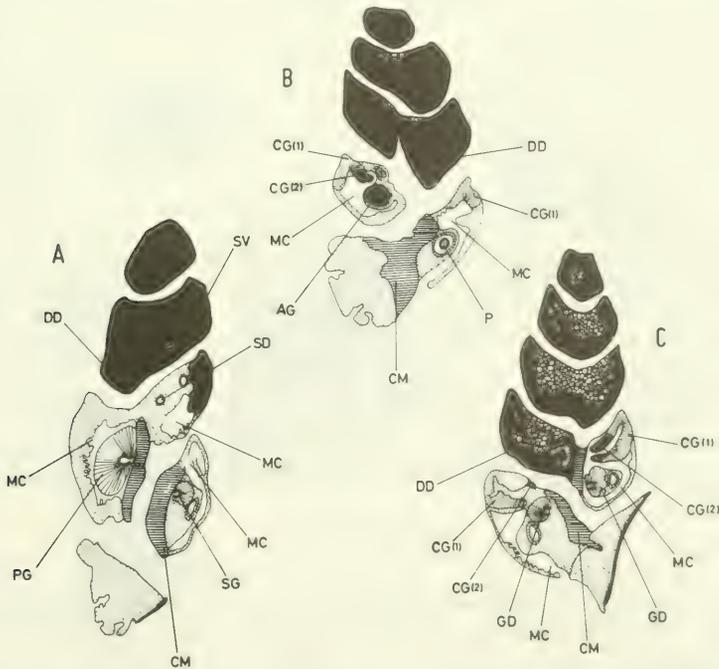


FIG. 5. *Balcis shaplandi*. Sections through (A), male phase (2.0 mm shell length); (B), transitional stage (2.5 mm shell length) and (C), female phase (3.5 mm shell length) (for abbreviations see text).

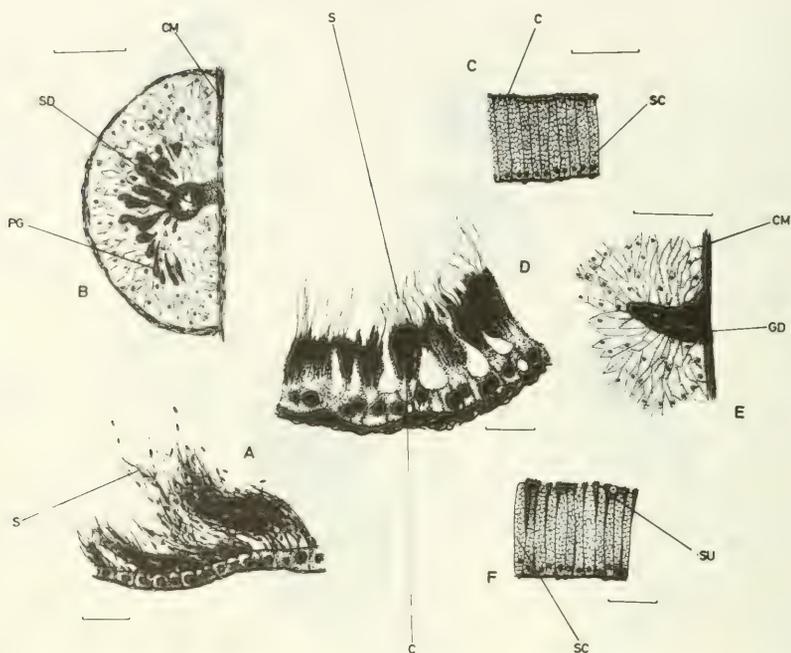


FIG. 6. *Mucronalia fulvescens*. Sections through (A), seminal vesicle; (B), seminal groove and surrounding prostate gland; (C), albumen gland; (D), seminal receptacle; (E), pallial oviduct (modified seminal groove) and (F) capsule gland (modified hypobranchial gland) (for abbreviations see text). Scales—B, E: 100 μ m; C, F: 50 μ m; A, D: 25 μ m.

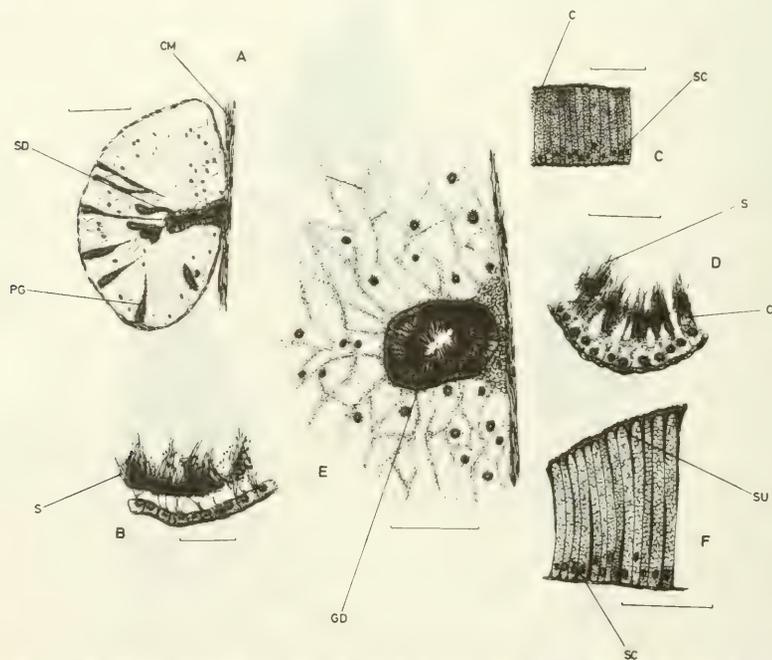


FIG. 7. *Balcis shaplandi*. Sections through (A), seminal vesicle; (B), seminal groove and surrounding prostate gland; (C), albumen gland; (D), seminal receptacle; (E), pallial oviduct (modified seminal groove) and (F) capsule gland (modified hypobranchial gland) (for abbreviations see text). Scales—A: 25 μ m; C-F: 50 μ m; B: 75 μ m. A and B should be transposed in the figure.

the gland open via a common aperture into the mantle cavity where the fertilised eggs are enveloped within a capsule. The capsule of *M. fulvescens* (and *B. shaplandi*) is in the form of an inverted saucer and is similar to that of *Littorina littorea* (cf. Fretter & Graham, 1962). Sometimes capsules were found attached to the shell of a male.

Balcis shaplandi

At an average length of between 2.0-2.5 mm the greatest percentage of individuals of *Balcis shaplandi* are male (Fig. 3B). As with *M. fulvescens*, the testis (Fig. 5A) occupies a dorsal position within each whorl other than the first, being enveloped by the digestive diverticula (DD). The testis discharges into the vas deferens which in turn empties into a large and much convoluted seminal vesicle (SV) some 160 μm in diameter and made up of a cuboidal epithelium 5 μm tall (Fig. 7A). Each cell possesses a small (3 μm) nucleus. The vesicle lumen contains a mass of unorientated spermatozoa and connects up by a long narrow duct with the seminal duct (Fig. 5A, SD) which opens into the mantle cavity (MC). As in *M. fulvescens* the seminal duct and groove is surrounded by the swollen mass of the prostate gland. The seminal duct (Fig. 7B) is some 75 μm in diameter and comprises an epithelium composed of densely ciliated cells 6 μm in height, each possessing a nucleus 3.5 μm in diameter. The encompassing prostate gland (PG) has dimensions of 250 \times 170 μm and comprises a matrix of lightly staining cells interspersed with glandular masses which open into the seminal duct. As in *B. alba* and *B. devians* (Fretter, 1955), *B. shaplandi* possesses a penis.

At a length of approximately 2.5-3.0 mm *B. shaplandi* undergoes a change of sex which as in *M. fulvescens* must take place very quickly. Fig. 5B is a diagrammatic representation of this transitional stage. Once spent, the testis and the associated duct system degenerate and from the dorsal surface of each whorl the primordial cells of the ovary begin to descend and to occupy the space left by the testis. The female reproductive tract that develops is very similar to that of *M. fulvescens*. Females occur in the population over a wide length range i.e. from 1.5-5.0 mm though they are most numerous at a length of 3 mm (Fig. 3B).

The female reproductive tract (Fig. 5C) comprises an oviduct which connects up with a seminal receptacle via the intermediary of an albumen gland (AG). The albumen gland (Fig. 7C) is some 230 μm in diameter and comprises a darkly staining columnar epithelium (SC) composed of cells 70 μm tall each possessing cilia (C) 5 μm long and a basal nucleus 4 μm in diameter. The seminal receptacle (Fig. 7D) has a diameter of 110 μm and comprises cells approximately 12 μm tall with a nucleus 6 μm in diameter. The cells are densely ciliated (C) and following copulation the receptacle lumen is densely packed with spermatozoa (S) each orientated outwards with its head embedded in the epithelium. The receptacle gives rise to a duct that opens into the mantle cavity as the pallial oviduct (Fig. 5C, GD). The pallial oviduct (Fig. 7E) is 65 μm in diameter and comprises cells 15 μm tall with long (15 μm) cilia and a nucleus 4 μm in diameter. As in *M. fulvescens*, the duct is surrounded by the same structure as in the male except that the prostate gland cells are not present. Into the mantle cavity of the female opens the capsule gland (Fig. 5C). As in *M. fulvescens* the gland is differentiated histologically into two regions [CG(1); CG(2)], but in *B. shaplandi* their position is reversed. Thus the darkly staining component of the gland [CG(1)] is located distally and comprises an epithelium 90 μm tall. The lightly staining component [CG(2)] is located proximally and comprises an epithelium 75 μm tall. The cilia of this region of the gland are somewhat shorter (7 μm) than those of the former (9 μm). Both components of the capsule gland, however, possess a similar structure (Fig. 7F) and comprise elongate secretory cells (SC) with a basal nucleus 5 μm in diameter interspersed between ciliated inversely conical "supporting" cells (SU) with an apically located nucleus 3 μm in diameter. Both components of the gland open into the mantle cavity where the fertilised eggs are encapsulated as in *M. fulvescens*.

Gametogenesis

The gametogenetic cycle in *Mucronalia fulvescens* and *Balcis shaplandi* is very similar. The following is therefore an account of spermatogenesis and oogenesis that is applicable to both species.

The acini of the testis (Figs. 8 and 9) are the sites of spermatogenesis. At first (Stage 1) each acinus (Figs. 8 and 9A) comprises a small cluster of darkly staining cells which subsequently

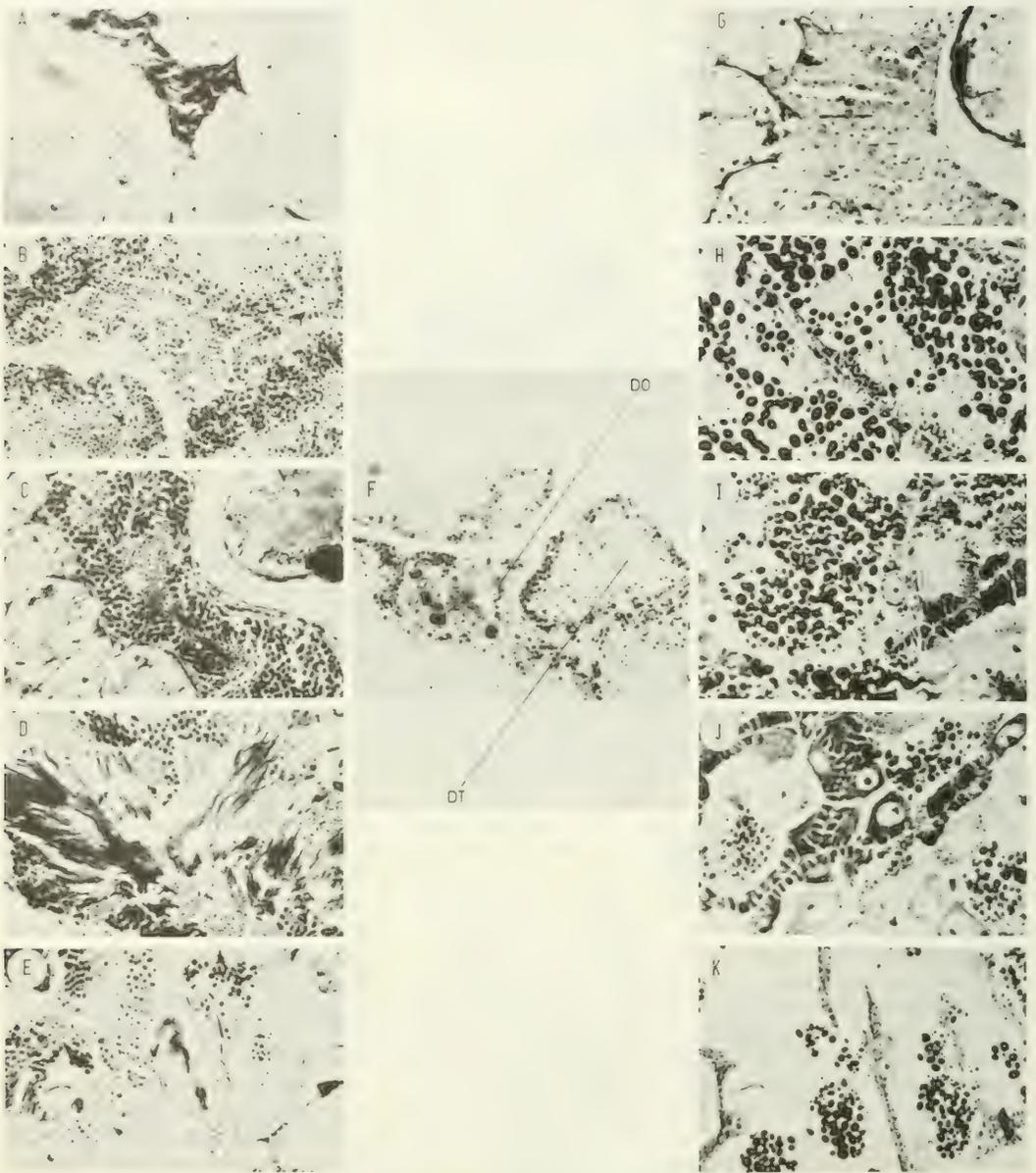


FIG. 8. *Mucronalia fulvescens*. Gametogenetic cycle (A-E = male sequence; F, transitional phase; G-K, female sequence) (for abbreviations see text).

develop into distinct tubules (Stage 2), each epithelial lining possessing a layer of spermatogonia (Figs. 8 and 9B). The tubule walls later enlarge and here can be found primary spermatocytes with nuclei smaller than those of the spermatogonia. Among the primary spermatocytes can later be found secondary spermatocytes (Figs. 8 and 9C) which give rise by meiosis to spermatids (Stage 3). Often arranged in clumps the spermatids eventually give rise to spermatozoa whose flagella densely fill the lumina of the acini. The spermatozoa remain attached to the Sertoli cells (Figs. 8 and 9D) until they are discharged into the seminal vesicle for storage until required during

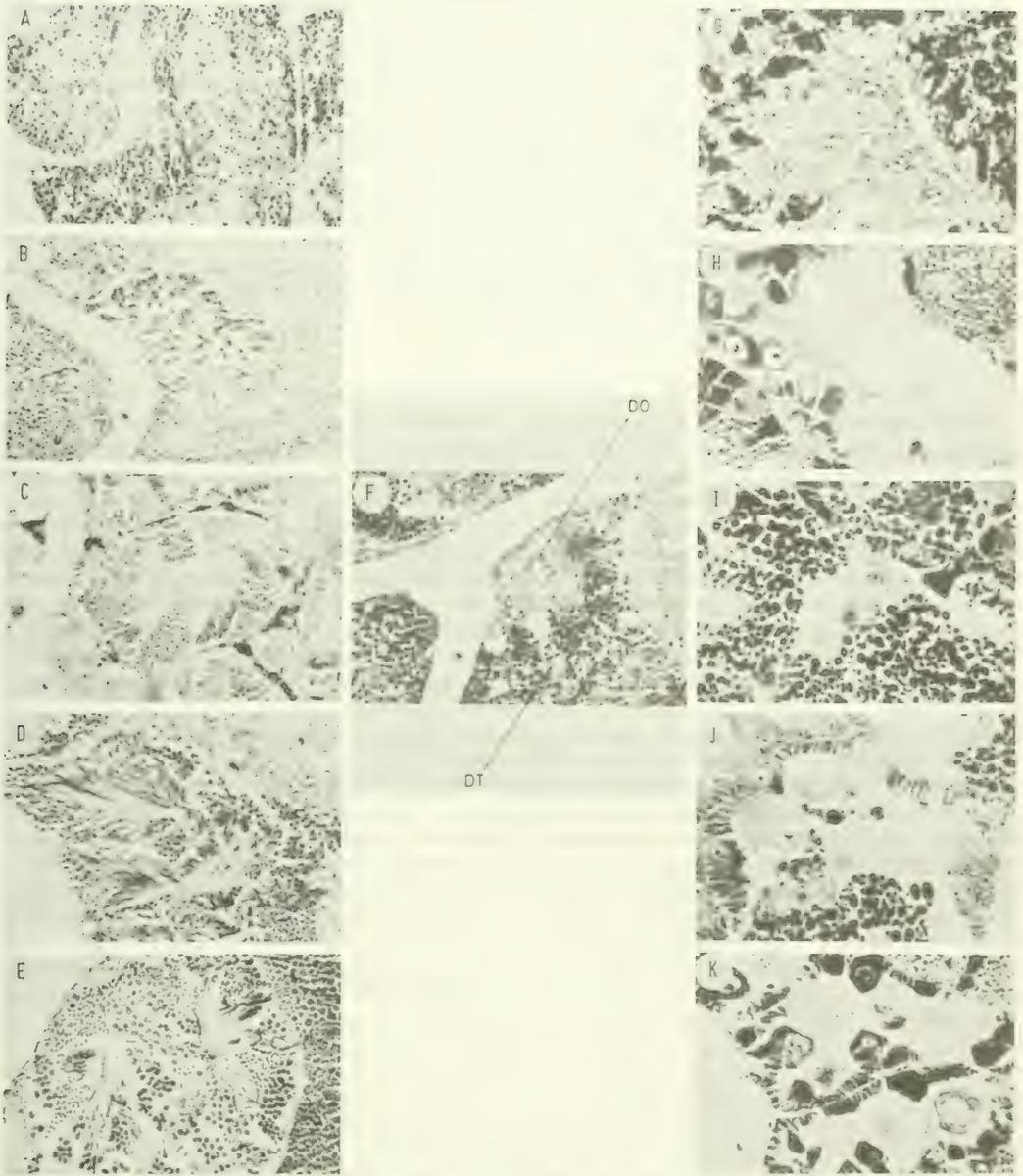


FIG. 9. *Balcis shaplandi*. Gametogenetic cycle (A–E = male sequence; F, transitional phase; G–K, female sequence) (for abbreviations see text).

copulation. At this Stage 4 the testis is mature. Once copulation has taken place the acini of the testis break down. In spawned individuals (Figs. 8 and 9E) (Stage 5) the acini lumina possess few sex cells whilst later the testis becomes a mass of degenerating cells. At this time the volume of the testis decreases as in *Stilifer* (Lützen, 1972) and its place is taken up in the visceral mass by the developing ovary (Figs. 8 and 9F). At this time both species are transitional with degenerating testis (DT) and developing ovaries (DO).

The ovary develops from the same position in the visceral mass as the testis, i.e. the dorsal

region of each body whorl. The ovary is first seen (Figs. 8 and 9G) (Stage 1) as a cluster of large, lightly staining primordial cells lying above the degenerating testis. Later (Figs. 8 and 9H) (Stage 2) a distinct alveolus forms, often with oogonia, which grows downwards into the visceral mass. The follicle walls progressively thicken and contain many darkly staining inclusions (probably glycogen) which stain positively in Heidenhain's haematoxylin and PAS. The number and size of the developing oocytes increases and at this stage are still attached basally to the follicle wall (Figs. 8 and 9I) (Stage 3). Later the oocytes are released into the follicle lumen and ovaries at this stage were considered ripe (Figs. 8 and 9J) (Stage 4). At this time the seminal receptacle was observed in some specimens to be full of spermatozoa. Few totally spawned individuals were found (Figs. 8 and 9K) (Stage 5). More common were partially spawned females with a thin follicle wall possessing few oogonia and oocytes and a lumen containing but a few oocytes. At this stage, once egg laying has been completed, death ensues and the rarity of totally spawned individuals of both species might thus be a consequence of natural mortality.

Population dynamics

Balisc shaplandi occurs on the aboral surface of *Archaster typicus* (Morton, 1976) in extremely variable numbers. From Fig. 10 it can be seen that the total number of individuals occurring on the 100 starfish (expressed as a % of the total number of individuals of this species collected over a 12 month period) changes quite considerably from month to month. Thus in April 1973 a large number of parasites were collected, but this number declined to 0 in August and remained relatively low for the months of September and October. Thereafter the number increased to a peak in January 1974. Subsequently numbers again declined (though remaining at an average frequency of 9%) with the approach of summer and very few individuals were collected in the months of May, July and September. Again numbers increased to reach a peak in February 1975. From this data it would thus seem that *B. shaplandi* is to be found on the host in greater numbers in the winter months from October/November to May and in fewer numbers in summer from June to September.

Mucronalia fulvescens occurs on the oral surface of *Archaster typicus* typically within the axis of the arms or within the ambulacral grooves (Morton, 1976).

As with *B. shaplandi*, the first sample of *M. fulvescens* collected in April 1973 registered a large number of individuals. This number declined as the year progressed to a few individuals only in November and then rose again to a small peak in February followed by a much larger peak in April and May 1974. This relatively high incidence of *M. fulvescens* was followed by a gradual decline to low numbers in October-November 1974. A final high incidence of parasites was recorded in February 1975. Thus the pattern of occurrence earlier seen in *B. shaplandi* was approximately

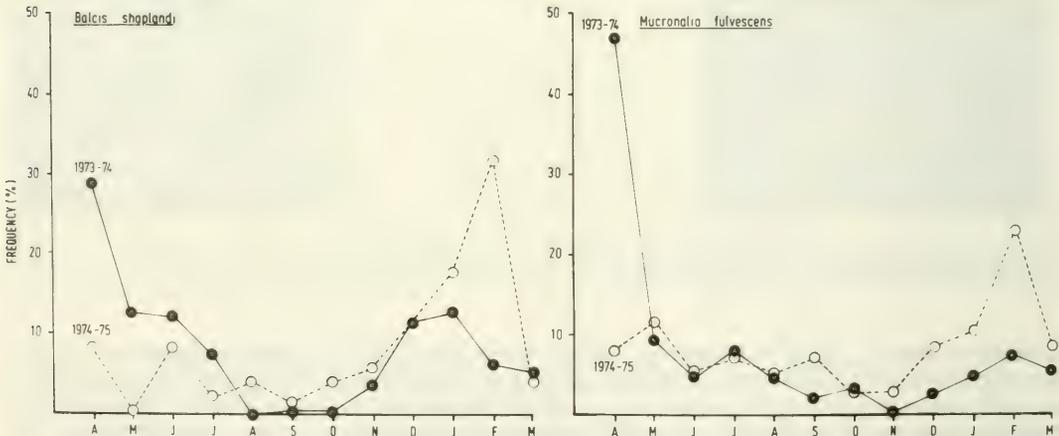


FIG. 10. The frequency of occurrence of individuals of *Balisc shaplandi* and *Mucronalia fulvescens* upon *Archaster typicus* for the years April 1973-March 1974 and April 1974-March 1975. Each monthly total is expressed as a percentage of the yearly total.

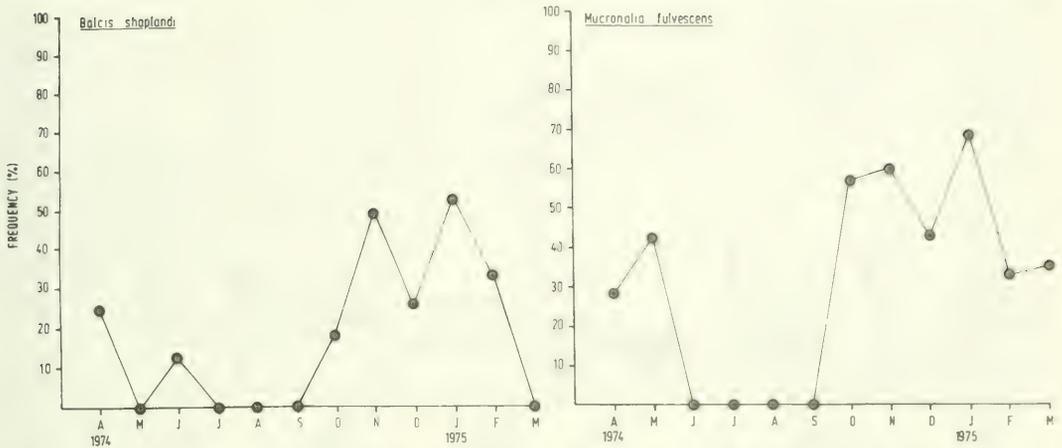


FIG. 11. The frequency of occurrence of individuals of *Balcis shaplandi* and *Mucronalia fulvescens* upon *Archaster typicus* clustered together in groups of two or more for the year April 1974-March 1975.

repeated by *M. fulvescens* with this species occurring on the host in greater numbers in the winter months from December/January to May and in fewer numbers in the late summer, i.e. from September to November.

For one year only the % number of individuals occurring in clustered groups of 2 or more individuals has been plotted as Fig. 11. In July, August and September when few specimens of *B. shaplandi* were on the host, the number occurring in clusters was few. Conversely from October to February or April, the parasites that were on the host occurred in clusters with in November and January more than 50% of the individuals so disposed. Typically at this time a single large (female) individual was associated with 1-8 smaller males.

M. fulvescens exhibited a similar pattern of behaviour. From June to September all individuals were solitary. At other times, however, corresponding to the winter months when the parasites are on the host, they occurred with greater frequency in clusters of 2, 3 or 4 with a peak in January.

As with *B. shaplandi*, a single large (female) individual was clustered with typically 1 or 2 smaller males.

The monthly samples of *B. shaplandi* (Fig. 12) have been sexed to determine first if they were either male, female or immature. In April 1973 the population comprised 2 peaks with smaller individuals being mature males and larger individuals mature females. In May 1973 a larger % of the population comprised very small immature individuals and a number of males, with only 1% of the population female. A similar pattern emerged in June but by July few individuals were immature, all clearly being either (generally) smaller males or larger females. From August to October few *B. shaplandi* were found on the host but by November the population again comprised 2 small peaks of smaller males and larger females. Small immature individuals were again recorded from December 1973 to February 1974. By April 1974, however, the sexes were once again clearly separated into smaller males and larger females. Few parasites were collected in May 1974 but immature individuals occurred on the host from June to August. In September and October 1974 the population comprised relatively well defined peaks of smaller males and larger females. Immature individuals again occurred from November 1974 until January 1975 these superimposing themselves onto an established population of small males and larger females. By February all immature individuals had sexually matured and in March the population settled down to comprise small males and larger females.

From April to June 1973 2 peaks of individuals comprised the monthly population samples of *M. fulvescens* on the starfish (Fig. 13). These were larger females and smaller males. From July to October, however, smaller individuals appeared in the population which, unlike juvenile *B. shaplandi*, were nearly always clearly recognisable as males. Few individuals were recorded from September 1973 to February 1974 but even so a clear size distinction between smaller males and larger females was often apparent. Small individuals also appeared in the population in March 1974

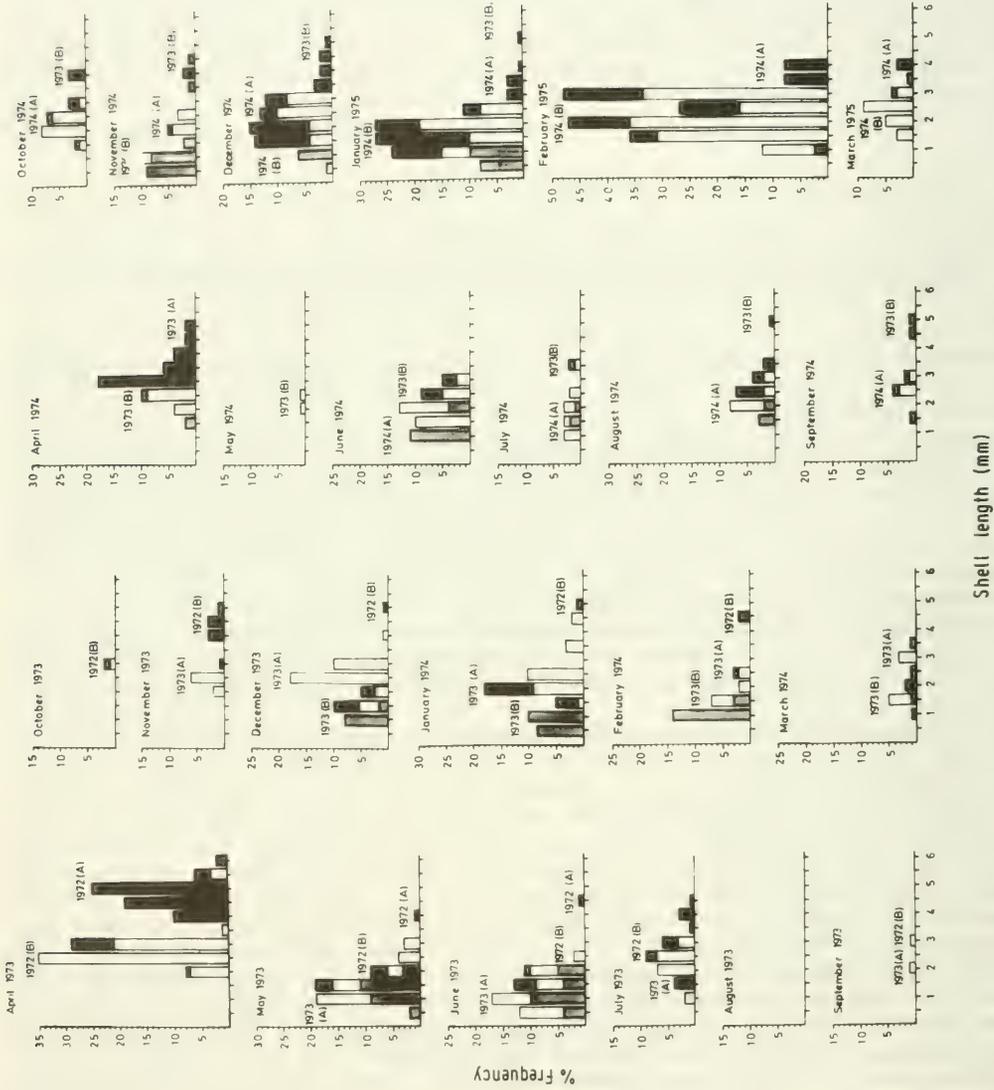


FIG. 12. Length frequency histograms showing the composition of samples of the population of *Balais shaplendi* upon *Archaster typicus*. The dates above individual peaks represent the dates at which these particular age classes hatched (grey histograms = immature individuals; open histograms = males; black histograms = females).

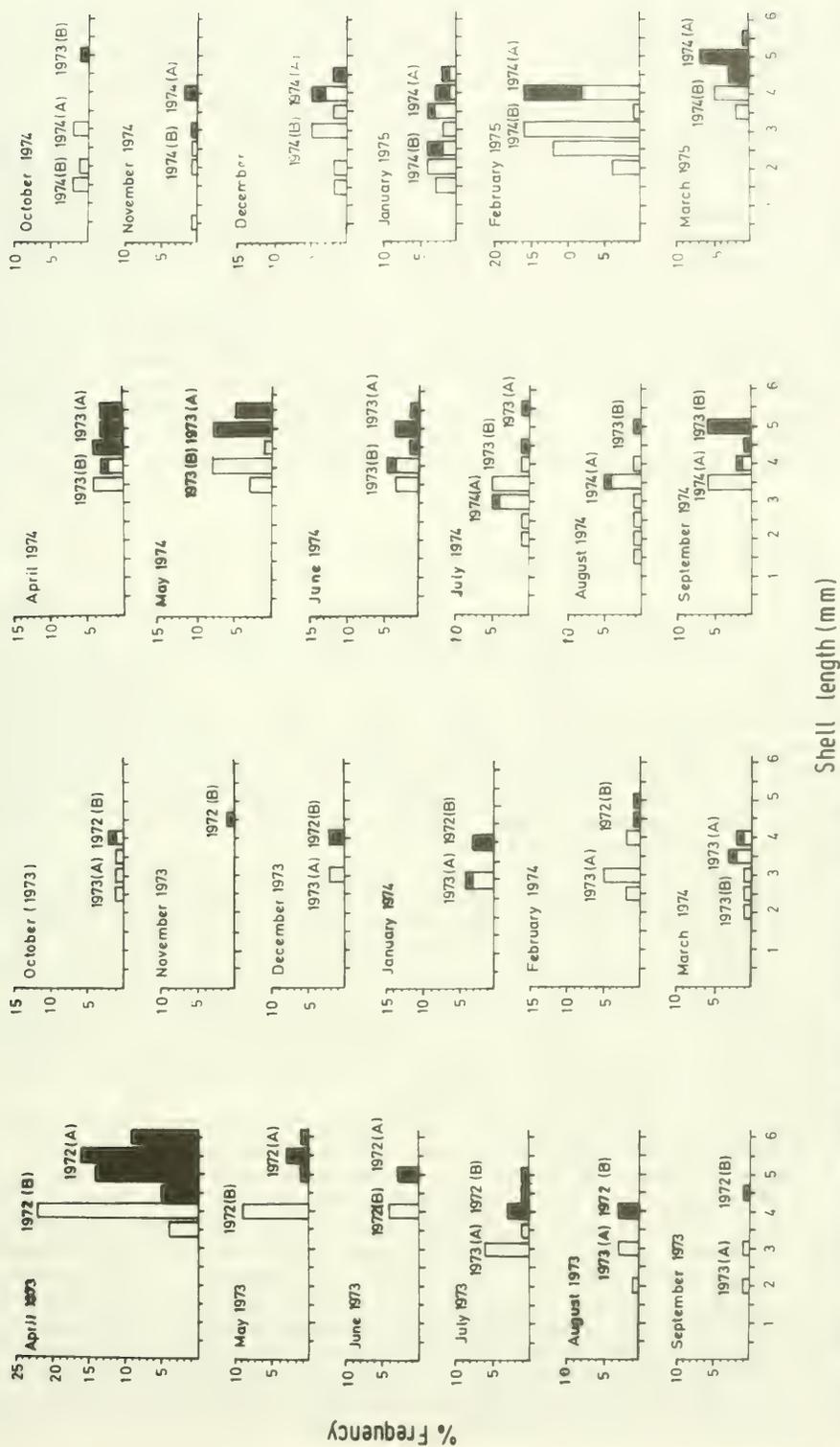


FIG. 13. Length frequency histograms showing the composition of samples of *Mucronalia fulvescens* upon *Archaster typicus*. The dates above individual peaks represent the dates at which these particular age classes hatched (open histograms = males; black histograms = females).

and these again were clearly male. In May and June 1974 the population was again approximately divisible into 2 peaks respectively comprising smaller males and larger females. Young individuals (again male) appeared in the population in July and August 1974 and finally again in October and November 1974. At all times these young individuals superimposed themselves onto an adult population which typically comprised small males and older larger females.

Each individual of both *M. fulvescens* and *B. shaplandi* comprising each monthly sample has been graded according to its state of sexual maturity earlier defined (Stages 1-5 for each sex) and shown in Figs. 8 and 9 (A-K). Figure 14 records the sexual stage (either Δ , immature; \square , male; \circ , female) that the greatest number of individuals of each age class of each species had attained in that month. Amplifying Fig. 14 it can be seen that young immature individuals arriving in the population of *B. shaplandi* in November-December and in the population of *M. fulvescens* in October-February grow rapidly and mature into males. Copulation with the larger females takes place in April in *B. shaplandi* and in May in *M. fulvescens*. Following copulation the males undergo a sexual change to become females. These females of both species grow further and mature and are fertilised in October-November in *B. shaplandi* and in September in *M. fulvescens*. Following egg laying these individuals die, having completed their life cycle in approximately 15 months. There are thus 2 phases of reproduction in both species each year with the products of one phase of reproduction maturing into males and in turn fertilising the formerly male but subsequently female parent. The resulting progeny of this fertilisation carry the process on.

DISCUSSION

The life cycle of the detritivorous starfish *Archaster typicus* is apparently straightforward. Young individuals enter the population in late summer following a phase of reproductive activity in the early summer. The species lives for approximately 4.5 years with the oldest individuals dying in the winter months of their 4th/5th year. Calculations show that it would be theoretically possible for the species to attain a disc/arm length of 135 mm and it is thus further possible that 1 or 2 individuals may survive into their 5th year. Within each age class the sexes are in the approximate ratio of 1 to 1 (Clemente & Anicete, 1949) and the species is thus dioecious. The same authors could not differentiate between the sexes on a size basis and it is thus safe to conclude that the size frequency peaks seen in these samples of *A. typicus* from Hong Kong do represent age classes and not a sexual dimorphism.

Two features of the life history are, however, unusual. First, during breeding, a form of "copulation" takes place, males overlying females with arms alternating. Thus during the early summer (April-June) in Hong Kong (and in the Philippines) sex-paired starfish closely dot the lower shore. This phase of reproductive activity coincides with the time when sea water temperatures in Hong Kong are rising (Morton & Wu, 1975) typically to a maximum of 30°C in July. Each year also the oldest age class dies, typically in the winter months when sea water temperatures are low (13°C in February). Second, following reproduction, the starfish tend to bury themselves with the approach of autumn and damaged and dying starfish were collected in the winter months. They are replaced in the population by the new recruits. These 2 factors, it is here suggested, influence the life cycle of both parasites.

Thus during the time of "copulation" in *A. typicus*, *Mucronalia fulvescens* is to be found on the host, but in declining numbers from April to June. A similar generalisation holds true for *Balcis shaplandi*. In April-May the gonads of both parasites are mature and copulation and egg laying takes place. It thus appears that following copulation both parasites leave the host and from July-September few parasites of either species were obtained. This may be partially explained by the fact that the females of both species, having once completed egg laying, probably die. Furthermore the "copulatory" activity of the starfish may physically dislodge some of the parasites. Finally, following reproduction, the oldest starfish die as the air and sea temperatures begin to fall. New recruits to the starfish population are, however, arriving at this time and it seems likely that a percentage of small male and immature, newly hatched parasites of both species, leave the host at this time, probably to find a new host. By October both parasites were recorded from the starfish in relatively small numbers but it is significant that at this time those that were on the host were in clusters and that these individuals were sexually mature. Thus in the late autumn

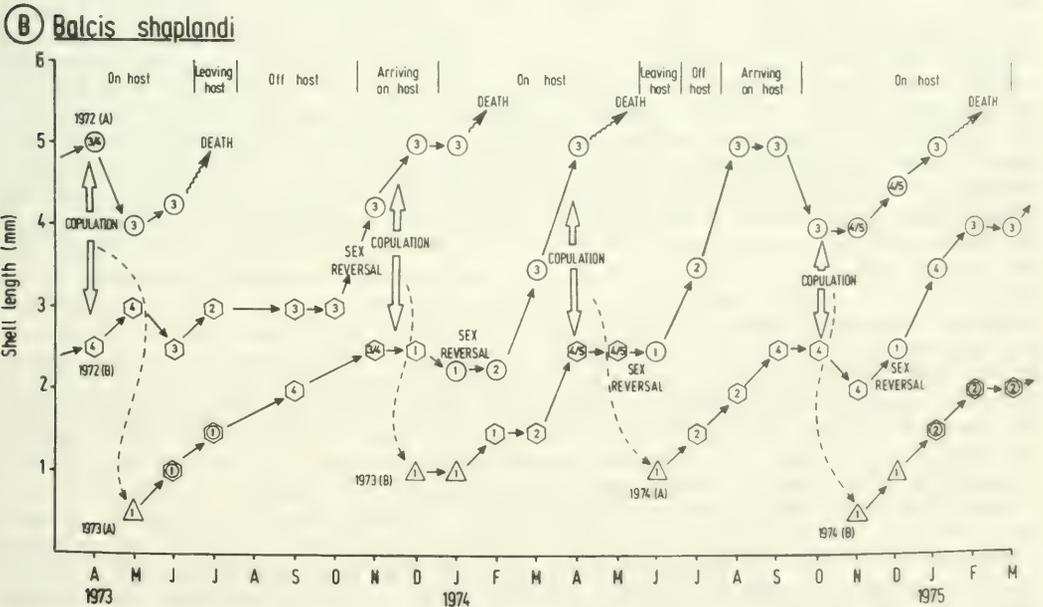
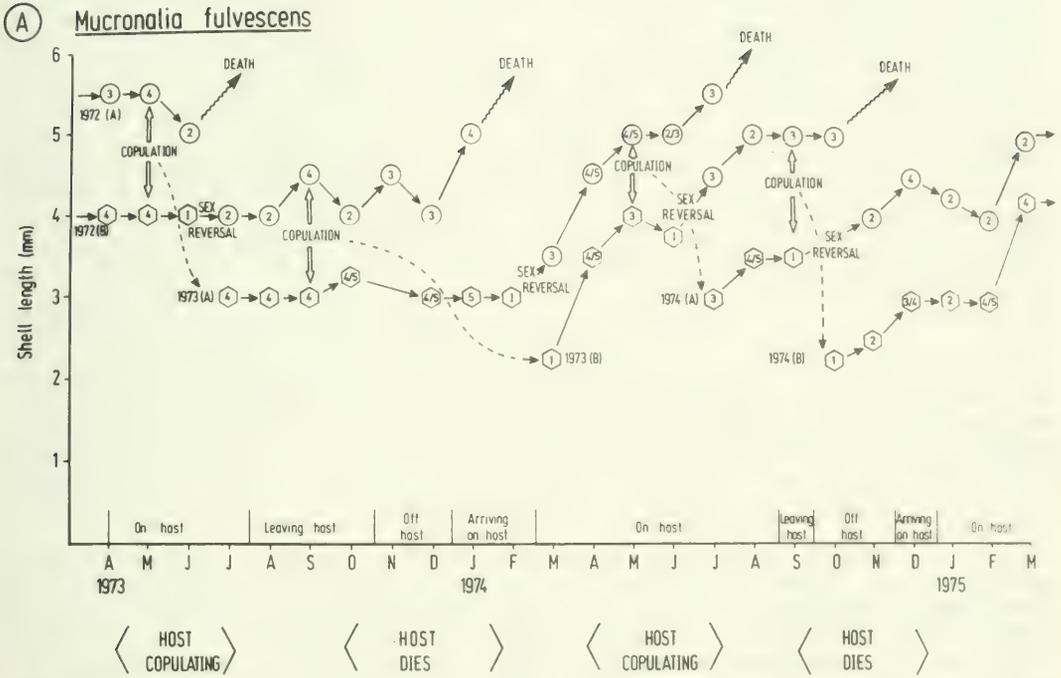


FIG. 14. Average length of each age class in the monthly samples of *Mucronalia fulvescens* and *Balcis shaplandi*. The dates represent the dates at which each age class hatched. Δ = immature individuals; \bullet = males; \circ = females. The number within each of these symbols (1-5) represents the sexual stage (either male or female) attained by a majority of the individuals comprising this age class and can be correlated with letters A-E (males) and G-K (female) of Figs. 8 and 9. The times of copulation, hatching and death are also indicated. Finally the occurrence of each parasite upon the host is correlated with the "copulatory" activity and time of death of the host.

(around October for both species) the maturing progeny of the spring (April/May) sexual union have grown sufficiently and are mature enough to mate with the sex-changed individuals who were their fathers in the spring reproductive phase but who have subsequently become female. After this second, autumn phase of reproduction and egg laying the spent females die and the young males and immature juveniles remain on the starfish typically in large numbers and in clusters for the whole winter period i.e. from October-March. At this time of year the starfish are typically buried in the sand (at least during the period of low tide when these samples were collected) and because the parasites remain on the host in relatively large numbers it is here suggested that this is the time when they are feeding, ensuring that over the cold winter months they have an adequate supply of food to see them into the next phase of reproduction that will begin again in the following spring. Thus in any one year both *M. fulvescens* and *B. shaplandi* undergo 2 phases of reproduction, one in spring, the other in autumn. This is essential because both species live for but one year during which time they are first male and then female; the females being fertilised by their own progeny. Fig. 14 sets out the sexual cycle of both parasites related (1), to the observed changes in the gametogenic cycle of each age class of each species; (2), to the recorded occurrence of the parasites upon the host, and (3), to important aspects of the biology of *A. typicus*. Though both species, in general terms, breed in spring (April/May) and in autumn (September/November) there are slight differences in the time each species undertakes these activities. This may suggest that a partial temporal separation of the 2 parasites on the host occurs, though these data clearly indicate that this mechanism of segregation is nowhere near as important as the hitherto described selective site segregation, typical of these two parasites (Morton, 1976), that subdivides the host into 2 niches.

There are only slight differences in the structure of the reproductive organs of *M. fulvescens* and *B. shaplandi*. Thus *M. fulvescens* does not possess a penis (like *M. nitidula*, see Hoskin & Cheng, 1969). *B. shaplandi* (and *B. alba*, see Fretter, 1955) does possess a penis in the male phase that is reduced to a vestige in the female. Otherwise the reproductive tract of both species is very similar with a short vas deferens leading into a capacious seminal vesicle that in turn leads into a seminal groove surrounded by the prostate gland as possibly in *Natica catena* (cf. Fretter & Graham, 1962). At a length of approximately 3.5 mm in *M. fulvescens* and of 2.5-3.0 mm in *B. shaplandi* both species undergo a change of sex (following copulation and egg laying) and become female. This process involves the degeneration and resorption of the testis and the male reproductive system, except for the seminal groove (which now becomes the pallial oviduct) and the surrounding prostate gland, which is retained except for the glandular portion of this structure which is lost. Lützen (1972) has suggested for *Stilifer linckiae* (which is similarly a protandric consecutive hermaphrodite) that the entire female system develops anew and not from a reformed broken down male system. This cannot be verified from the results of this study, but it is significant that the female system of both *M. fulvescens* and *B. shaplandi* is also simple with the oviduct enlarging to form an albumen gland and a seminal receptacle. Fertilisation is thus internal in both species though copulation in neither has been observed. In the mantle cavity of females of both species develops a large gland, divisible into two histologically distinct components that possibly secrete the chemical constituents of egg capsules, once the eggs are released into the mantle cavity. This gland is not seen in the male phase and has the general structure of the molluscan hypobranchial gland earlier described for *Diodora apertura* and *Emarginula reticulata* (cf. Fretter & Graham, 1962) and for a number of bivalves (Morton, 1977). In all of these molluscs the gland comprises large secretory cells interspersed with inversely flask-shaped, (often termed) "supporting cells," that in *Solemya* are rich in lipids and are possibly absorptive. In *Tricolia* (Fretter & Graham, 1962) a secretion from the lips of the urinogenital aperture of the female is augmented by a secretion from the hypobranchial gland and is used to entangle the egg stream within the mantle cavity. Similarly a secretion from the hypobranchial gland of *Gibbula* (Gersch, 1936) is copiously produced during the breeding season and possibly provides an embedding medium for the egg masses. In the bivalve *Nucula* the hypobranchial gland produces a secretion which furnishes nearly all the material from which brood sacs are formed (Drew, 1901). It is significant that this structure only occurs in the females of both *M. fulvescens* and *B. shaplandi* and it would appear that in both its prime function is to produce the material from which the egg capsules are formed.

Hoskin & Cheng (1969) have described the reproductive system of *M. nitidula* and found it (in both male and female) to be remarkably simple, the vas deferens developing into a sperm duct, the

oviduct into the uterus. In neither sex were associated glands described. The reproductive system of *B. devians* (Fretter, 1955) is more complex with for example in the female the oviduct debouching into a receptaculum seminis with "staining reactions suggest(ing) that the albumen is secreted by the inner closed end of the oviduct adjacent to the opening of the receptaculum seminis, and that the thick wall of the capsule is a product of the pallial region of the duct." The reproductive systems of 3 other parasitic prosobranchs i.e. *Stilifer*, *Megadenus* and *Robillardia* have been described by Lützen (1972), Humphreys & Lützen (1972) and Gooding & Lützen (1973) respectively. In these aglossans there is a consistency of structure with for example in the males a vas deferens leading into a seminal vesicle which in turn discharges into a seminal groove via the intermediary of a prostrate gland. In the females an oviduct empties into the seminal receptacle (often also with a bursa copulatrix) which in turn releases fertilised eggs into the mantle via the genital pore. An albumen gland is located between oviduct and seminal receptacle, whilst the capsule gland is found on the mantle (similarly a modified hypobranchial gland?). The reproductive organs of both *M. fulvescens* and *B. shaplandi* conform to this general plan though for neither has it been possible to histologically separate a bursa copulatrix from the seminal receptacle. Moreover the prostrate gland of both species is located around the seminal groove.

Within the prosobranch mesogastropods consecutive hermaphroditism has been recorded in the families Calyptraeidae (Orton, 1909), Ianthinidae (Ankel, 1926), Scalidae (Ankel, 1936) and the Capulidae (Graham, 1954). It has also been long suspected to occur in the ectoparasitic aglossan parasites, so that Hoskin & Cheng (1969) noted for *Mucronalia nitidula* that there were small males and large females. Lützen (1972) and Gooding & Lützen (1973) have shown how *Stilifer* and *Robillardia* are protandric consecutive hermaphrodites. This study of *M. fulvescens* and *B. shaplandi* similarly demonstrates protandric consecutive hermaphroditism in these 2 species and further describes how the population of both species is maintained. Lützen (1972) suggested for *Stilifer* that the oogonia appear by transformation of spermatogonia; this is clearly not the case in the species here under consideration. Both gonads develop from the dorsal edge of each whorl other than the first. This region of the body obviously comprises primordial cells which in a normal sequence give rise first to the testis and later to the ovary.

Franc (1968) claimed that the Eulimidae and the Stiliferidae are characterised by separate sexes. *Mucronalia fulvescens* (Stiliferidae) and *Balcis shaplandi* (Eulimidae) are protandric consecutive hermaphrodites. Such an adaptation possibly ultimately gave rise to the dwarf males in more specialised endoparasitic aglossans e.g. *Paramegadenus* (Humphreys & Lützen, 1972) and *Enteroxenos* (Lützen, 1968). *A. typicus* is not simply a source of food for *B. shaplandi* and *M. fulvescens*. On the host the reproductive activities of both parasites are co-ordinated and their life cycle is intimately bound up with the life cycle of the starfish. Because both starfish and parasites are so common, this association is an ideal one in which to investigate further the host/parasite relationship, especially since so little is known of parasitic molluscs.

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ABBREVIATIONS USED IN THE FIGURES

AG	Albumen gland	MC	Mantle cavity
C	Cilia	O	Ovary
CG(1)	Lightly staining component of the capsule gland	P	Proboscis
CG(2)	Darkly staining component of the capsule gland	PG	Prostate gland
CM	Columella muscle	S	Spermatozoa
DD	Digestive diverticula	SC	Secretory cell
DO	Developing ovary	SD	Seminal duct
DT	Degenerating testis	SG	Seminal groove
GD	Genital duct	SR	Seminal receptacle
I	Intestine	SU	Supporting cell
K	Kidney	SV	Seminal vesicle
		T	Testis
		VM	Visceral mass

EFFECT OF PESTICIDES AND NARCOTANTS ON BIVALVE MOLLUSCS¹

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ABSTRACT

The reactions of the marine bivalves *Katelysia opima* and *Donax cuneatus* (both commercially important species in India) to various pesticides and narcotants were studied under laboratory conditions. Reactions varied, but thiometon and malathion were found to have the least effect on *K. opima* and *D. cuneatus* respectively, while malathion and DDT were found to be more toxic to *K. opima* and *D. cuneatus* respectively. *D. cuneatus* was little affected by the narcotics phenobarbital sodium and hexobarbital sodium, but *K. opima* was affected by phenobarbital sodium. The commercially important freshwater bivalve *Indonaia caeruleus* was studied in detail as regards the influence of polluting substances on the neurosecretory cells, the digestive gland and the intestine. All organs were affected in different ways. The narcotics affected the neurosecretory cells and the secretory material considerably. The effect of narcotics on the hepatopancreas was not pronounced, but it was marked on the intestine. Pesticides considerably affected the hepatopancreas and intestine.

INTRODUCTION

The increasing use of pesticides in modern land and water management has posed a potential hazard not only to human beings and wildlife but also to marine organisms of economic importance as these pollutants ultimately find their way into the sea. Experiments were planned to study the effect of some pesticides and narcotants on the survival and some physiological functioning of the estuarine bivalve *Katelysia opima* and the marine bivalve *Donax cuneatus*, both commercially important shellfish in India. Phenobarbital sodium and hexobarbital sodium, commonly used narcotics, were also tested for their effect. Nervous transmission is a suitable target for pesticides since it is the basis of a coordination system without which the animal cannot live. Also, the effect of pesticides on the liver and alimentary tract have not been studied extensively in lower animals. The present study has been undertaken in order to understand the effect, if any, on the neurosecretory cells in cerebral and visceral ganglia, on the digestive gland and on the intestine passing through the visceral mass in a commercially important freshwater bivalve, *Indonaia caeruleus*.

MATERIALS AND METHODS²

Adult *K. opima* (25-30 mm) and *D. cuneatus* (20-25 mm) were collected in the Kalbadevi estuary and White Sea area respectively, at Ratnagiri on the west coast of India. In the laboratory they were placed in sea water which was changed twice a day (acclimation period: 24 h). Vital specimens were used only and no food was given. All experiments were conducted in early summer 1976. Salinity and temperature were recorded during all experiments. After acclimation the rate of particle filtration, rate of oxygen consumption and rate of ciliary beat were determined. Both species were then grouped in 8 batches. Each batch was exposed to narcotics (phenobarbital sodium: 120 *K. opima*, 150 *D. cuneatus*; hexobarbital sodium: 120

¹This is a condensed version of the original manuscript (Ed.).

²Apparatus for determining oxygen uptake cf. Galtsoff & Whipple (1930); apparatus for determining rate of particle filtration cf. Cole & Hepper (1954).

K. opima, 156 *D. cuneatus*) and pesticides (endrin, DDT, thiometon, malathion, all in absolute alcohol: 160 *K. opima*, 160 *D. cuneatus*). Apart from these, a batch in absolute alcohol and a control in sea water were also run (in all cases 160 of each species). Narcotics, pesticides and alcohol were at a concentration of 1 ppm; the water of each batch was changed 3 times a day. Rate of mortality expressed as percent mortality was recorded during all experiments. Similar sets of both species (25 individuals each) were used for determining physiological functioning.

The oxygen consumed by the clams is expressed as the amount of oxygen consumed by a clam per gram wet weight per hour per litre. The rate of particle filtration readings were converted to percent concentrations by the use of a calibration curve. All experiments in this respect were conducted on 10 individuals and average results are used for expression of data. Studies of isolated gill tissue were made to evaluate survival and activity of (a) isolated gill material suddenly transferred to narcotics and pesticides, and (b) gill material obtained from specimens under experimental conditions. In the first series of experiments 80 specimens were carefully removed from their shells; 4-5 mm wide pieces of gill tissue were taken (one from each clam) by cutting along the gill filaments. Eight batches of such gill fragments (10 in each) were exposed to 50 ml of saline solution of narcotics and pesticides. Under the microscope the terminal cilia could be readily observed in action. At 15-20 min intervals ciliary activity and survival of each bit of gill tissue were noted. For the 2nd series of experiments clams that had been exposed to various pollutants were removed periodically and gill preparations made as described above. Ciliary beat activity was rated in 3 categories: 3, normal activity; 2, somewhat reduced activity, some cilia may have stopped beating; 1, greatly reduced activity, most of the cilia have stopped beating. In these experiments the averages of each gill tissue's rate of ciliary activity and survival time are taken into consideration (cf. Vernberg et al., 1963).

Adults of the freshwater species *I. caeruleus* (55-65 mm) were obtained from the Kham River at Aurangabad and were kept in running water for a day. Healthy adults, scrubbed clean, were grouped in 8 batches of 50 each and placed in separate Plexiglas aquaria with river water. The 1st batch acted as control, whereas in the 2nd and 3rd batches phenobarbital sodium and hexobarbital sodium were added respectively, so as to reach a concentration of 1 ppm. To the batches 4-7 endrin, thiometon, malathion, and DDT were added, dissolved in alcohol (concentration 1 ppm). The 8th group received the same volume of alcohol as a control for the above. Water in all groups was changed 3 times a day. Mortality was recorded every time.

A similar set of 8 groups (50 clams each) was used for determining histological changes at LT_{50} . When this was reached the clams were removed from their shells and fixed in Bouin's fluid. Control specimens were fixed after 96 h. Cerebral and visceral ganglia were removed, hepatopancreas and intestine were dissected out, dehydrated in alcohol, cleared in xylol and stained with Gomori's chromaematoxylin phloxine (CHP) for ganglia and with Mallory's triple stain for hepatopancreas and intestine.

RESULTS

(1) Tolerance of narcotics and pesticides (33.5‰, 31°C).

K. opima was kept under observation for 80 h, *D. cuneatus* for 216 h. The solution wherein survival was 50% (and more) after 80 and 216 h respectively was regarded as tolerating range. The rate of mortality of *K. opima* increased with increasing exposure (Fig. 1):

100% mortality in malathion	after 80 h
50% mortality in malathion	after 29 h
50% mortality in endrin	after 36 h
50% mortality in DDT	after 27 h

In endrin and DDT 92.5 and 85% mortality occurred after 80 h exposure. Mortality was considerably less in thiometon; it progressively increased up to 42 h and 40% mortality occurred, but only an additional 5% mortality was registered after 80 h. In alcohol there was a gradual increase in mortality up to 68.63% after 41 h. In narcotics 90 and 72.5% mortality occurred in phenobarbital and hexobarbital sodium respectively after 80 h; 50% mortality in these 2 batches was registered after 26 and 44 h respectively. Mortality in phenobarbital

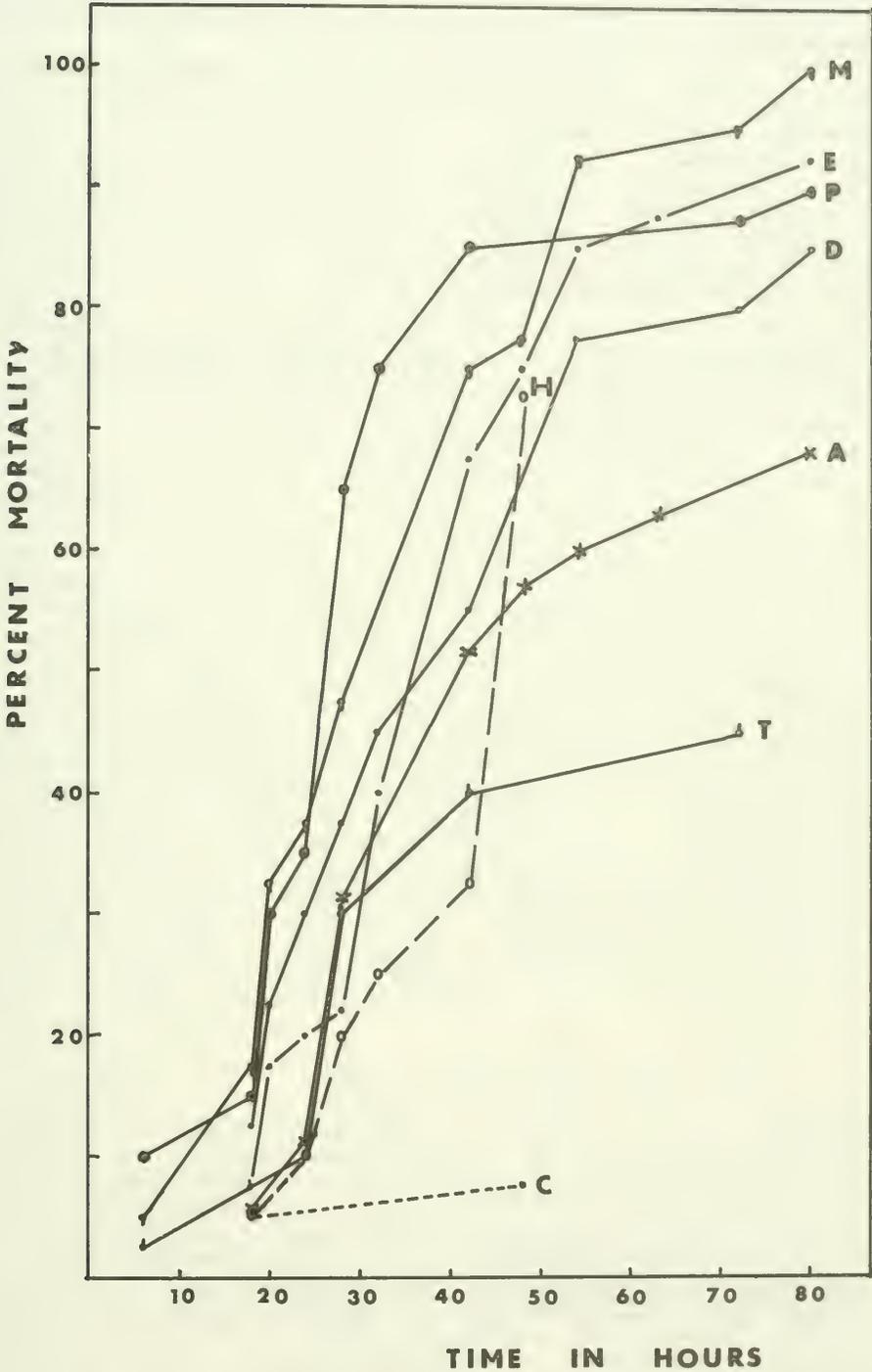


FIG. 1. Percentage of mortality of *Katelaysia opima* in 1 ppm concentrations of narcotics (A—alcohol; H—hexobarbital sodium; P—phenobarbital sodium) and pesticides (D—DDT; E—endrin; M—malathion; T—thiometon); C—control.

increased rapidly between 24 and 42 h, whereas in hexobarbital mortality steadily increased up to 42 h; after 48 h mortality increased suddenly. In the control there was 5% mortality after 18 h, which later increased to 7.5% after 48 h.

In all pesticides (except malathion) mortality of *D. cuneatus* increased with increase in time of exposure (Fig. 2):

100% mortality in DDT	after 216 h
50% mortality in DDT	after 92 h
97.09% mortality in endrin	after 216 h
97.09% mortality in thiometon	after 216 h
8.75% mortality in malathion	after 216 h
50% mortality in endrin	after 136 h
50% mortality in thiometon	after 136 h

There was a sudden increase in mortality after 132 h in both endrin and thiometon. Mortality in malathion was comparatively less and increased only after 167 h. In alcohol there was 87.78% mortality after 216 h and 50% after 149 h. There was a sudden rise in mortality after

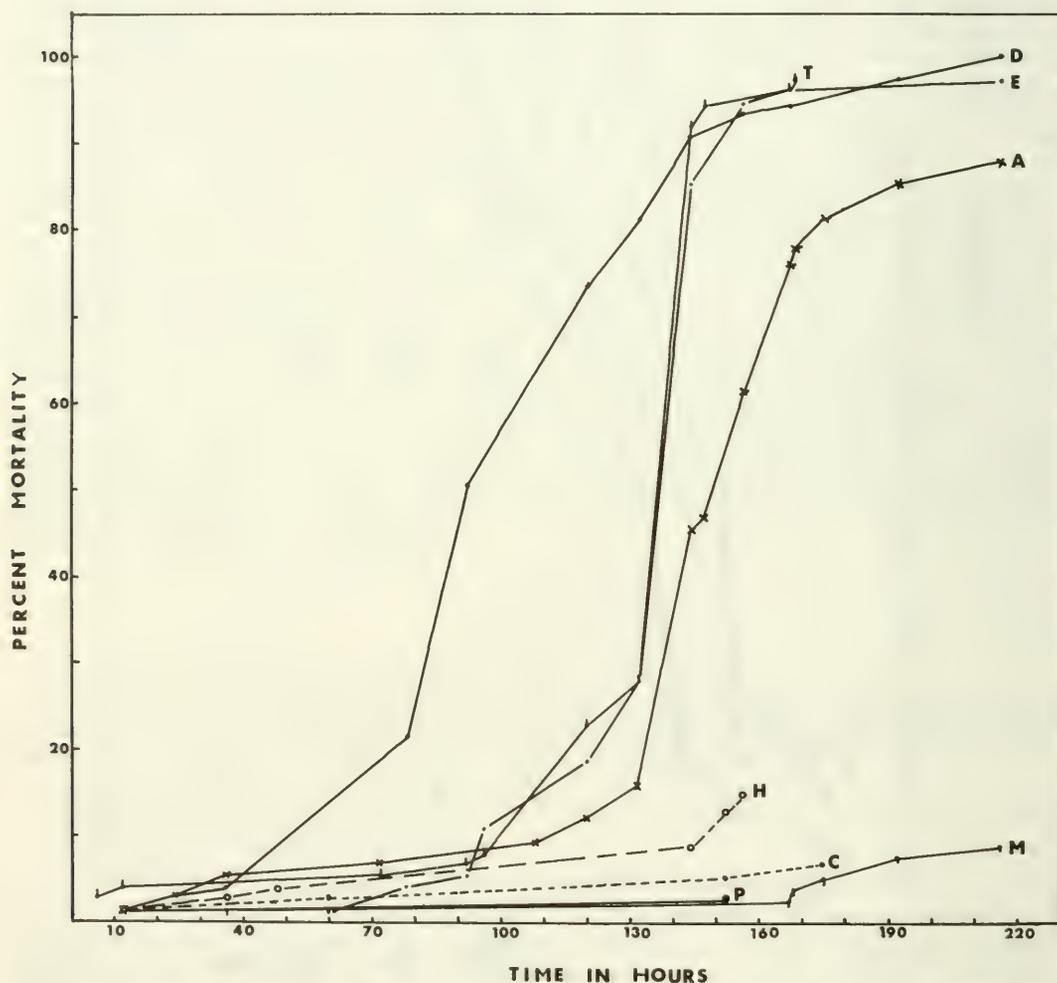


FIG. 2. Percentage of mortality of *Donax cuneatus* in 1 ppm concentrations of narcotics (A—alcohol; H—hexobarbital sodium; P—phenobarbital sodium) and pesticides (D—DDT; E—endrin; M—malathion; T—thiometon); C—control.

132 h in this batch. Mortality in the narcotants was low. Only 2.66 and 14.08% were registered after 216 h in phenobarbital and hexobarbital respectively. Mortality in hexobarbital increased from 144 h onwards. In the control there was 6.65% mortality after 216 h. Surprisingly, mortality in the control was therefore higher than in phenobarbital.

Behaviour with regard to siphon extension and accumulation of faecal matter was different with respect to the kind of water pollution. *K. opima* was completely relaxed in narcotants after ca. 42 h; complete relaxation results in a slight swelling of the body and a high rate of mortality. *D. cuneatus* was completely relaxed after 92 h without any side effects. In alcohol both species showed an increase in faecal discharge and siphon extension.

K. opima is obviously able to tolerate thiometon and *D. cuneatus* malathion. Both were least tolerant to endrin and DDT. In malathion, endrin and DDT, the siphons of *K. opima* were only extended to 3/4 of the length of those of the controls, whereas *D. cuneatus* did so in endrin, thiometon and DDT. The accumulation of faeces and pseudofaeces was more than in the alcohol batch.

(2) Oxygen consumption of specimens subjected to narcotics and pesticides (33‰, 29°C).

The normal rate of oxygen consumption of *K. opima* averaged 0.076 ml/g/h/l (measured for 72 h). In both narcotants the clams used more oxygen in the first 24 h, but later the rate decreased. After 72 h the rate decreased more in phenobarbital than in hexobarbital. In the alcohol batch the clams used more oxygen after 24 h, but after 72 h the rate decreased below that of the control specimens. In endrin and DDT the specimens showed a more or less similar trend in oxygen consumption; it did not markedly alter for 28 h, but after 32 h it decreased. This decrease was more than in the alcohol batch; decrease in DDT was more than in endrin. In malathion the rate up to 24 h was more or less the same as that of the controls, but then sharply decreased after 72 h. This decrease was more than in both endrin and DDT. Thiometon was the only pesticide that did not much affect oxygen consumption; for the first 32 h it remained more or less at the level of the controls, but after that showed a decrease.

The normal rate of oxygen consumption of *D. cuneatus* averaged 0.069 ml/g/h/l (measured for 192 h). In both narcotants the clams used more oxygen over 24 h, but then the rate slowly decreased; after 192 h it reached more or less the level of the controls. In both narcotants it did not fall below the normal rate. In alcohol there was an increase over 24 h; after that it remained steady up to 96 h and then gradually decreased. After 192 h oxygen consumption fell below the normal rate. In all pesticides (except malathion) oxygen consumption was considerably altered. After 24 h more oxygen was used, but the rate then decreased more than in the alcohol batch after 192 h. In malathion the rate increased up to the first 72 h, but later decreased and more or less reached the level of the alcohol batch after 192 h. In thiometon and endrin oxygen consumption fell below the rate of the controls (96 h) and later fell even lower. In DDT it fell below the rate of the controls at 72 h and further decreased below that of specimens in thiometon and endrin after 192 h.

The above shows that there are marked differences in the 2 species as regards their reactions to these narcotants and pesticides.

(3) Rate of particle filtration of specimens subjected to narcotics and pesticides (31.5‰, 29.5°C) (Tables 1, 2).

K. opima controls filtered 58% of neutral red solution/h after 72 h (limits 57-59%). In hexobarbital the rate of filtration was least affected: 55% after 72 h. In phenobarbital it was altered after 48 h and decreased below the rate in phenobarbital. Filtration in the alcohol batch remained more or less constant up to 24 h and then decreased to 48% after 72 h. Pesticides had a marked effect, except for thiometon, where the rate was similar to that of the alcohol batch. In DDT filtration decreased from 20 h onwards, whereas in endrin and malathion from 24 h onwards; in these pesticides filtration decreased considerably after 72 h. This was more marked in malathion than in endrin and DDT.

D. cuneatus controls filtered 47%/h after 192 h (limits 47-49%). In both narcotants the rate of filtration was more or less similar and little affected. In alcohol there was no change for 120 h, but from 144 h onwards filtration decreased; after 192 h the rate was 39%. In the pesticides (except malathion) there was a noticeable change. The rate in malathion was more or less the same as that of the alcohol batch. For the first 96 h in endrin, thiometon and DDT the

TABLE 1. Rate of particle filtration in *Katelaysia opima* when subjected to various narcotants and pesticides, expressed as percentage of neutral red removed within one hour.

Hrs	Control	Alcohol	Pheno-barbital	Hexo-barbital	Malathion	Thiometon	Endrin	DDT
6	58 ± 3	59 ± 4	59 ± 4	58 ± 4	58 ± 2	58 ± 3	58 ± 4	58 ± 2
18	58 ± 3	60 ± 4	59 ± 3	58 ± 3	58 ± 4	58 ± 3	59 ± 4	57 ± 3
20	59 ± 3	60 ± 4	57 ± 3	59 ± 3	56 ± 4	60 ± 3	56 ± 4	53 ± 3
24	58 ± 3	58 ± 4	54 ± 3	52 ± 3	53 ± 4	59 ± 3	53 ± 4	55 ± 4
28	57 ± 3	55 ± 4	56 ± 4	54 ± 3	51 ± 3	57 ± 3	51 ± 4	52 ± 4
32	59 ± 3	53 ± 4	55 ± 4	53 ± 3	49 ± 3	54 ± 3	52 ± 4	50 ± 4
42	58 ± 3	50 ± 3	55 ± 3	54 ± 4	43 ± 4	51 ± 3	49 ± 4	47 ± 4
48	58 ± 2	48 ± 4	55 ± 3	54 ± 3	40 ± 4	49 ± 3	46 ± 3	44 ± 3
54	57 ± 3	48 ± 1	49 ± 2	55 ± 3	34 ± 3	46 ± 4	42 ± 4	42 ± 4
63	58 ± 2	49 ± 3	46 ± 4	55 ± 3	30 ± 1	47 ± 4	38 ± 4	41 ± 2
72	58 ± 3	48 ± 4	45 ± 3	55 ± 1	28 ± 4	46 ± 3	37 ± 4	39 ± 3

TABLE 2. Rate of particle filtration in *Donax cuneatus* when subjected to various narcotants and pesticides, expressed as percentage of neutral red removed within one hour.

Hrs	Control	Alcohol	Pheno-barbital	Hexo-barbital	Malathion	Thiometon	Endrin	DDT
24	48 ± 2	49 ± 3	47 ± 2	48 ± 3	47 ± 2	47 ± 2	46 ± 3	46 ± 1
48	47 ± 1	48 ± 2	48 ± 2	48 ± 3	47 ± 1	47 ± 2	46 ± 2	46 ± 2
72	47 ± 2	47 ± 3	48 ± 3	47 ± 3	46 ± 3	47 ± 3	45 ± 1	45 ± 2
96	49 ± 2	47 ± 2	48 ± 3	48 ± 3	46 ± 2	46 ± 2	45 ± 4	45 ± 1
120	49 ± 2	48 ± 2	48 ± 2	48 ± 4	44 ± 3	42 ± 2	41 ± 3	40 ± 2
144	48 ± 3	44 ± 2	47 ± 2	48 ± 3	44 ± 4	39 ± 3	38 ± 1	36 ± 1
168	47 ± 1	41 ± 3	47 ± 2	45 ± 3	34 ± 2	37 ± 1	34 ± 3	30 ± 1
192	47 ± 1	39 ± 2	44 ± 3	47 ± 4	41 ± 1	34 ± 3	30 ± 1	28 ± 4

rate was not affected, but after that the DDT batch filtered at a much lower rate than in endrin and thiometon. In these 2 the rate decreased below that of the alcohol batch after 192 h.

Again there are marked differences in reaction of the 2 species.

(4) Resistance of isolated gill tissue subjected to narcotics and pesticides (32.5‰, 30.3°C) (Tables 3, 4).

Controls of *K. opima* isolated gill tissue were considered to represent category 3; survival time up to 308 min. In phenobarbital ciliary activity and survival were reduced more than in hexobarbital. In the alcohol batch activity and survival were reduced more than in the controls. Ciliary activity and survival of the pesticide batches show that in endrin, malathion and DDT gill tissue was considerably affected as compared to the alcohol batch. In thiometon ciliary activity and survival was more or less like that of the alcohol batch. In endrin, malathion and DDT ciliary activity was rated in categories 1.9, 1.7 and 2.1 respectively; survival time 140, 123 and 155 min respectively. Comparing activity and survival of isolated gill tissue obtained after 6, 24, 48 and 72 h of exposure to narcotics and pesticides shows that activity and survival decreased gradually; in phenobarbital, endrin, DDT and malathion the gill tissue was considerably affected. In the control batch ciliary activity remained the same as that of the control batch on sudden transfer, but survival time decreased after 72 h because of the increase in time of the experiments. In phenobarbital activity and survival were considerably more reduced than in hexobarbital and also than the same batch on sudden transfer. Ciliary activity in phenobarbital was rated 1.8; survival time 125 min after 72 h. In the alcohol batch ciliary activity was rated very close to that of the same batch on sudden transfer; survival was reduced to 212 min after 72 h. In the thiometon batch ciliary activity was the same as that after the sudden transfer of the same batch, but survival was similar to that of the alcohol batch after 72 h. In endrin, malathion and DDT ciliary activity and survival time were reduced more than in those suddenly transferred. After 72 h there was a more severe effect of DDT than of endrin

TABLE 3. Resistance of isolated gill tissue of *Katylisia opima* subjected to various narcotants and pesticides. Upper figures: rate of ciliary beating (average of 10 observations). Lower figures: survival time in minutes.

Hrs	Control	Alcohol	Pheno-barbital	Hexo-barbital	Malathion	Thiometon	Endrin	DDT
0	3.0 308 ± 18	2.7 270 ± 24	1.5 190 ± 11	2.4 220 ± 14	1.7 123 ± 15	2.6 195 ± 13	1.9 140 ± 29	2.1 155 ± 22
6	3.0 299 ± 21	2.8 265 ± 32	2.8 240 ± 27	2.9 253 ± 16	2.8 265 ± 21	2.9 280 ± 28	2.8 270 ± 24	2.9 280 ± 25
24	3.0 297 ± 27	2.9 239 ± 23	2.7 235 ± 11	2.9 250 ± 10	2.3 240 ± 27	2.9 255 ± 24	2.6 210 ± 17	2.5 250 ± 30
48	2.9 292 ± 18	2.7 218 ± 19	2.2 205 ± 13	2.8 215 ± 18	2.0 160 ± 37	2.7 230 ± 31	2.3 185 ± 23	2.3 217 ± 15
72	3.0 218 ± 29	2.8 212 ± 17	1.8 125 ± 30	2.6 198 ± 26	1.2 85 ± 24	2.6 212 ± 22	1.5 110 ± 27	1.9 160 ± 24

TABLE 4. Resistance of isolated gill tissues of *Donax cuneatus* subjected to various narcotants and pesticides. Upper figures: rate of ciliary beating (average of 10 observations). Lower figures: survival time in minutes.

Hrs	Control	Alcohol	Pheno-barbital	Hexo-barbital	Malathion	Thiometon	Endrin	DDT
0	3.0 260 ± 18	2.6 210 ± 21	2.8 245 ± 19	2.8 230 ± 17	2.5 250 ± 18	1.7 190 ± 19	1.7 185 ± 22	1.7 170 ± 23
48	3.0 258 ± 20	3.0 235 ± 16	3.0 253 ± 14	3.0 250 ± 19	3.0 240 ± 26	2.8 238 ± 17	2.9 230 ± 11	2.8 245 ± 21
96	3.0 252 ± 15	2.8 220 ± 12	2.8 244 ± 19	2.7 238 ± 21	2.7 230 ± 29	2.6 185 ± 18	2.8 193 ± 22	2.2 170 ± 15
144	3.0 248 ± 23	2.7 203 ± 22	2.8 237 ± 12	2.7 217 ± 27	2.4 215 ± 32	1.9 163 ± 24	2.0 142 ± 23	1.6 115 ± 18
192	3.0 248 ± 26	2.7 195 ± 16	2.8 220 ± 23	2.6 205 ± 24	2.3 210 ± 33	1.6 115 ± 29	1.4 100 ± 26	1.2 93 ± 12

and malathion. Ciliary activity and survival in these pesticides were considerably more reduced than in thiometon, which obviously has the least effect on gill tissue.

Controls of *D. cuneatus* isolated gill tissue were considered to represent category 3; survival time 260 min. In both narcotics ciliary activity was rated 2.8 with survival times of 245 and 230 min respectively. Survival time in hexobarbital was more reduced than in phenobarbital. In the alcohol batch ciliary activity was reduced to 2.6; survival time 210 min. In endrin, thiometon and DDT ciliary activity was considerably more reduced than in alcohol, i.e. to 1.7 with a considerably lower survival time. In DDT survival time was lower than in both endrin and thiometon. In malathion ciliary activity was more or less the same as that in alcohol, but survival time was longer. Obviously, malathion least affected gill tissue among these clams. Comparing ciliary activity and survival of isolated gill tissue after 48, 96, 144 and 192 h of exposure to narcotics and pesticides shows that activity and survival decreased considerably with increased exposure time; in the controls activity was not affected, but survival time decreased somewhat. Activity and survival in the narcotants were lower than in the controls and the effect was more marked after 192 h. Endrin, thiometon and DDT affected activity and survival more than in the same batches on sudden transfer; the effect of DDT was even more marked after 192 h. On the other hand, in malathion ciliary activity and survival time were least affected as compared to the other pesticides, but were reduced when compared to the same batch on sudden transfer. Activity in malathion was reduced more than in the alcohol batch after 192 h, but survival time was longer.

Once more there are considerable differences in reaction of the 2 species under discussion, e.g. in *K. opima* malathion severely affected isolated gill tissue and in *D. cuneatus* DDT did so.

TABLE 5. LT_{50} values of *Indonaia caeruleus* when subjected to various narcotants and pesticides.

Batch	LT_{50} (hours)	Dead/total
Control	—	
Phenobarbital	85	34/70
Hexobarbital	96	36/70
Alcohol	85	32/60
Endrin	85	33/70
DDT	72	37/70
Thiometon	96	29/60
Malathion	85	31/60

(5) LT_{50} values of the freshwater mussel *Indonaia caeruleus* when subjected to narcotics and pesticides (29.5-31.7°C) (Table 5).

In hexobarbital LT_{50} has higher values than in phenobarbital; in the pesticides survival was highest in thiometon. Survival in alcohol equalled that in phenobarbital, endrin and malathion. DDT showed the lowest values of all. Mortality in the control batch amounted to 20% after 96 h.

(6) Changes in neurosecretory cells from cerebral and visceral ganglia of *I. caeruleus* subjected to narcotics and pesticides.

In *I. caeruleus* 3 types of neurosecretory cells are located on the dorsal and lateral sides of cerebral and visceral ganglia. Type I are pyriform (20-25 μm) with a long axon and are directed towards the central core of the ganglia. The nucleus (diameter 8-12 μm) is round or oval, centrally or eccentrically placed. These cells are less numerous in the ganglia than the other types. Type II are oval in shape (diameter ca. 30-35 μm); the large nucleus (15-25 μm) is oval and peripheral. Vacuoles in the cytoplasm vary in number from 2-5. Type II cells are smaller than type II cells (ca. 10 μm diameter) and are generally oval, round or sometimes tapering at the ends, but the size remains constant. The nucleus (5-6 μm) is round or oval and placed centrally or eccentrically. Vacuoles are rarely seen. In all these cells neurosecretory material is seen accumulated in the cytoplasm in the form of droplets (Figs. 3a, 4a).

(6a) Neurosecretory cells from cerebral ganglia (Fig. 3).

In both narcotics neurosecretory material was released from the cytoplasm and there was no change in the morphological characters of cell types I and II. Material from type III cells did not show any change. Neurosecretory material from hexobarbital specimens was released more abundantly from both types when compared to phenobarbital specimens. There were no morphological changes in the alcohol batch, but in type I neurosecretory material was traced in the axonal part and not in the cytoplasm, whereas secretory material in type II disappeared from the cytoplasm; there was no change in type III. In thiometon secretory material disappeared from all types of cells, though traces were recorded in a few type II cells. There was some shrinkage in type I (16-20 μm), whereas type II measured 24-29 μm . Nuclear diameter decreased to 6-9 μm (type I) and 11-23 μm (type II). Type III cells were not affected. In malathion and endrin the effect was more or less similar to that of thiometon, but the degree of shrinkage was more in endrin than in malathion. In both pesticides neurosecretory material had not completely disappeared from cell types I and II and there appeared to be a considerable shrinkage in the size of all 3 cell types:

type I 14-17 μm , nucleus 4-8 μm
 type II 20-26 μm , nucleus 9-19 μm
 type III ca. 7 μm , nucleus 3-4 μm

In DDT shrinkage was more than in any other group of pesticides:

type I 11-16 μm , nucleus 3-5 μm
 type II 16-22 μm , nucleus 7-15 μm
 type III 4 μm , nucleus 2-4 μm

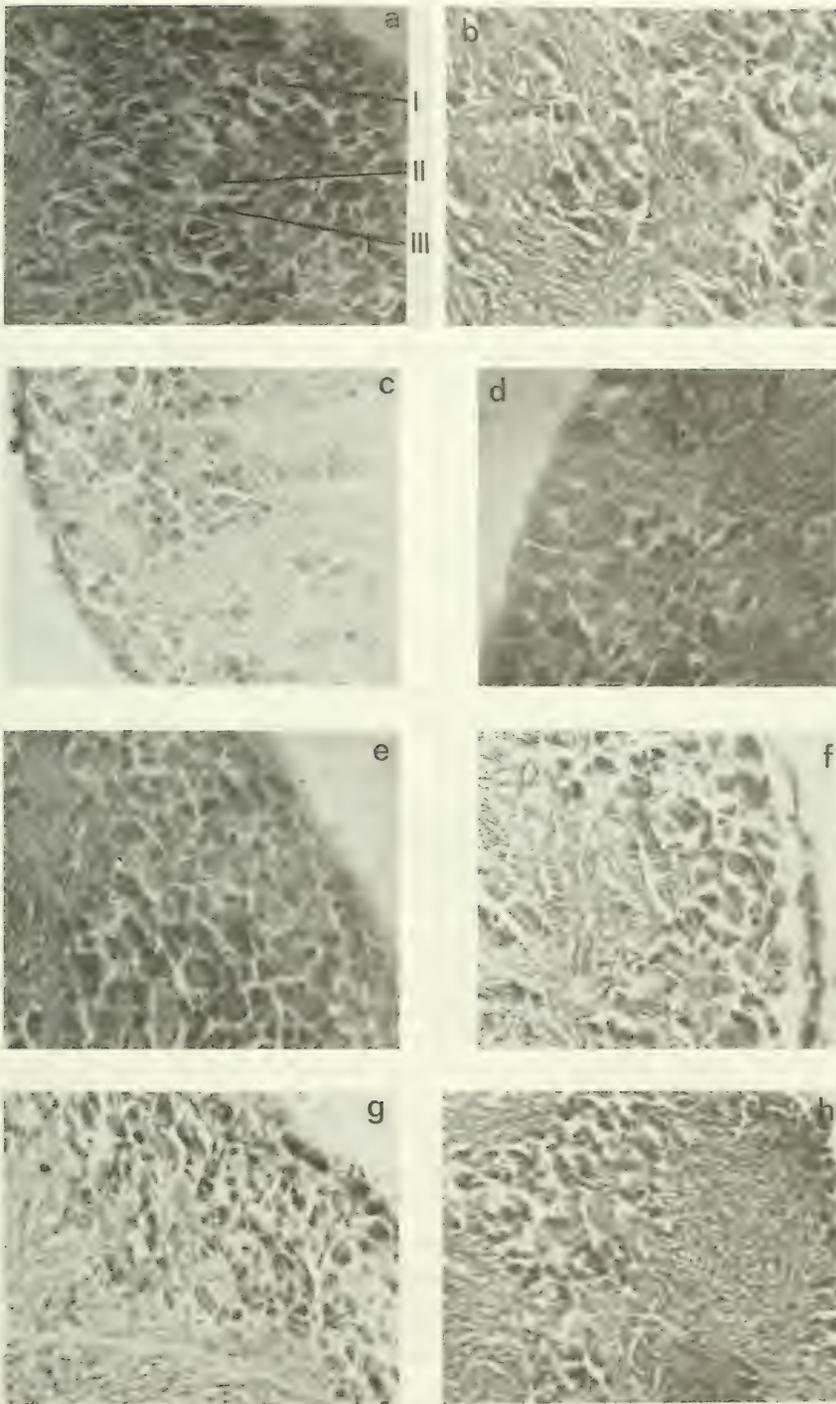


FIG. 3. Effect of narcotics and pesticides on the neurosecretory cells of cerebral ganglia of *Indonaia caeruleus* (a—control; b—phenobarbital sodium; c—hexobarbital sodium; d—alcohol; e—thiometon; f—malathion; g—endrin; h—DDT). Cell types I, II and III indicated in Fig. 3a. All figures ca. 600X.

In DDT neurosecretory material was only traced along the periphery of the nucleus in types I and II.

Obviously DDT has a considerable effect on the neurosecretory cells in the cerebral ganglia.

(6b) Neurosecretory cells from visceral ganglia (Fig. 4).

In hexobarbital neurosecretory material from many of the type I and II cells had disappeared, whereas there was no effect on cells of type III. On the other hand, there was accumulation of material in types I and II in phenobarbital, which was also shown by some type III cells. In the alcohol batch neurosecretory material from all cell types was released and there was no effect on cell and nuclear dimensions. Thiometon and malathion did not influence nuclear dimension, but there was a considerable change in cell shape. The neurosecretory material from all types had disappeared in thiometon, whereas in malathion it remained in the cytoplasm. Cell dimensions in thiometon were as follows: type I, 18-23 μm ; type II, 27-33 μm ; type III, 8 μm . Cells in the malathion batch became irregular in shape and proved difficult to measure. In endrin the neurosecretory material from all types had disappeared and cells of type I lost their shape: 17-23 μm , nucleus 7-10 μm . In DDT the material of all types disappeared and the cells lost their shape:

type I	16-18 μm , nucleus	4-7 μm
type II	23-27 μm , nucleus	11-20 μm
type III	8-11 μm , nucleus	4 μm

DDT once again has a greater effect on the neurosecretory cells of the visceral ganglia than other pesticides.

(7) Changes in the hepatopancreas of *I. caeruleus* subjected to narcotics and pesticides (Fig. 5).

The hepatopancreas consists of ducts and digestive tubules which are grouped in the form of small lobules indistinctly separated and connected by interlobular connective tissue consisting of Leydig cells and collagenous fibres.

In the present study attention has been paid to the digestive tubules of the hepatopancreas to see whether narcotics and pesticides had any effect. The cells in the digestive diverticula accept particles of filtered food and are responsible for absorption and intracellular digestion. The lumen is very small as compared to the size of the tubules. Smooth muscle fibres around each lobule effect changes in the volume of the tubule. Each tubule consists of large, lightly staining vacuolated secretory and absorptive cells and darkly staining generative cells (Fig. 5a). In both narcotants the interlobular connective tissue lost its original shape and the muscle fibres were relaxed to such an extent that the volume of the lobules increased and thereby affected the digestive cells. The pesticides (except malathion) considerably affected the digestive cells and the darkly staining cells, but the effect on the connective tissue was not severe. Of all pesticides malathion had the least effect, an effect similar to that of alcohol. These chemicals influenced the vacuolated and secretory cells which lost their shape to become irregular. There was shrinkage in the small darkly staining cells. The lumen of the lobules was shortened and sometimes became invisible. Thiometon, endrin and DDT severely affected the tubules; the lumen completely disappeared and the muscle fibres lost their connection with the lobules. The vacuolated cells completely lost their shape and vacuoles appeared in large numbers. The darkly staining cells also lost their shape while showing a considerable shrinkage.

(8) Changes in the intestine of *I. caeruleus* subjected to narcotics and pesticides (Fig. 6).

Secretory cells of the goblet type are interspersed amongst the ciliated cells in the intestine. Some of these are actively secreting mucous cells, while others are apparently regenerating. Wandering phagocytic cells occur in variable numbers in the lumen amongst the epithelial cells and in the surrounding connective tissue (Fig. 6a). In the narcotants mucous cells and muscle fibers were affected; shrinkage of the mucous cells was considerable, while the muscle fibers became relaxed and the ciliated cells appear irregular in shape. In alcohol the mucous cells lost their shape and exhibited shrinkage, whereas the phagocytic cells appeared to have increased in size. The connective tissue became irregular. In all pesticides mucous and ciliated cells were

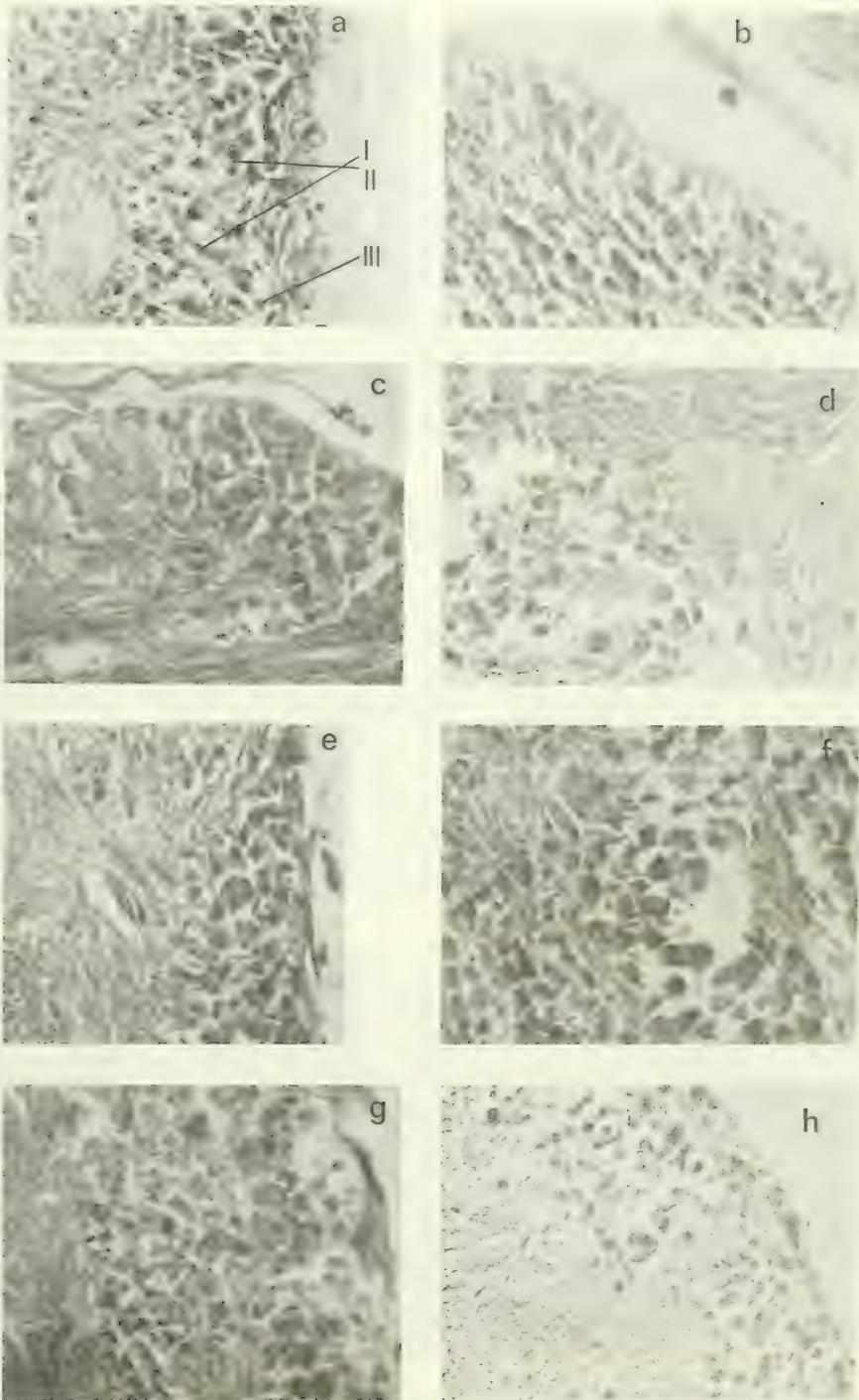


FIG. 4. Effect of narcotics and pesticides on the neurosecretory cells of visceral ganglia of *Indonaia caeruleus* (a—control; b—hexobarbital sodium; c—phenobarbital sodium; d—alcohol; e—thiometon; f—malathion; g—endrin; h—DDT). Cell types I, II and III indicated in Fig. 4a. All figures ca. 600X.

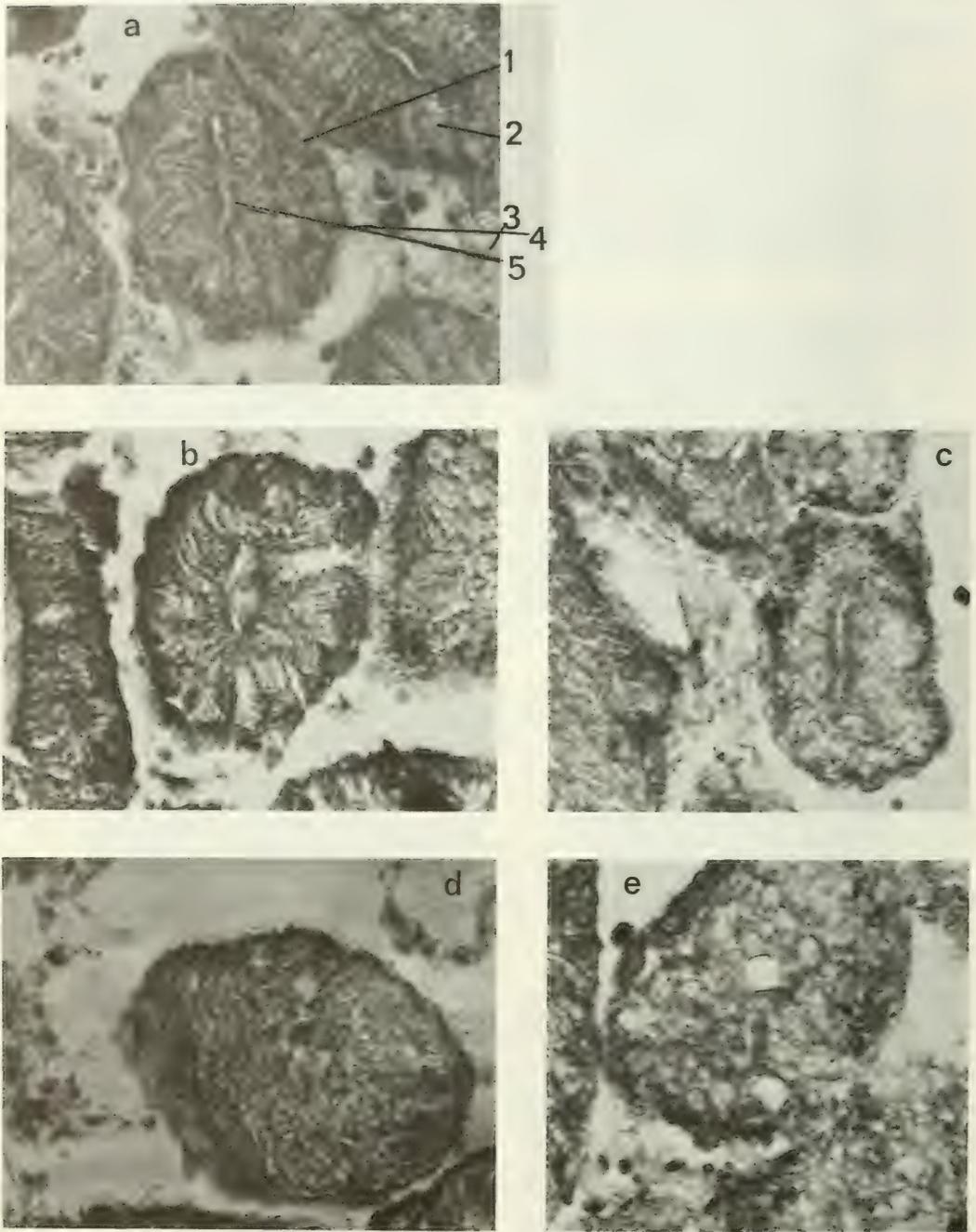


FIG. 5. Effect of narcotics and pesticides on the hepatopancreas of *Indonaiia caeruleus* (a—control; b—phenobarbital and hexobarbital sodium; c—alcohol and malathion; d—thiometon; e—endrin and DDT). Shown in Fig. 5a: 1—muscle fibres; 2—lightly staining vacuolated cells; 3—interlobular connective tissue; 4—darkly staining cells; 5—lumen of lobule. All figures highly enlarged.

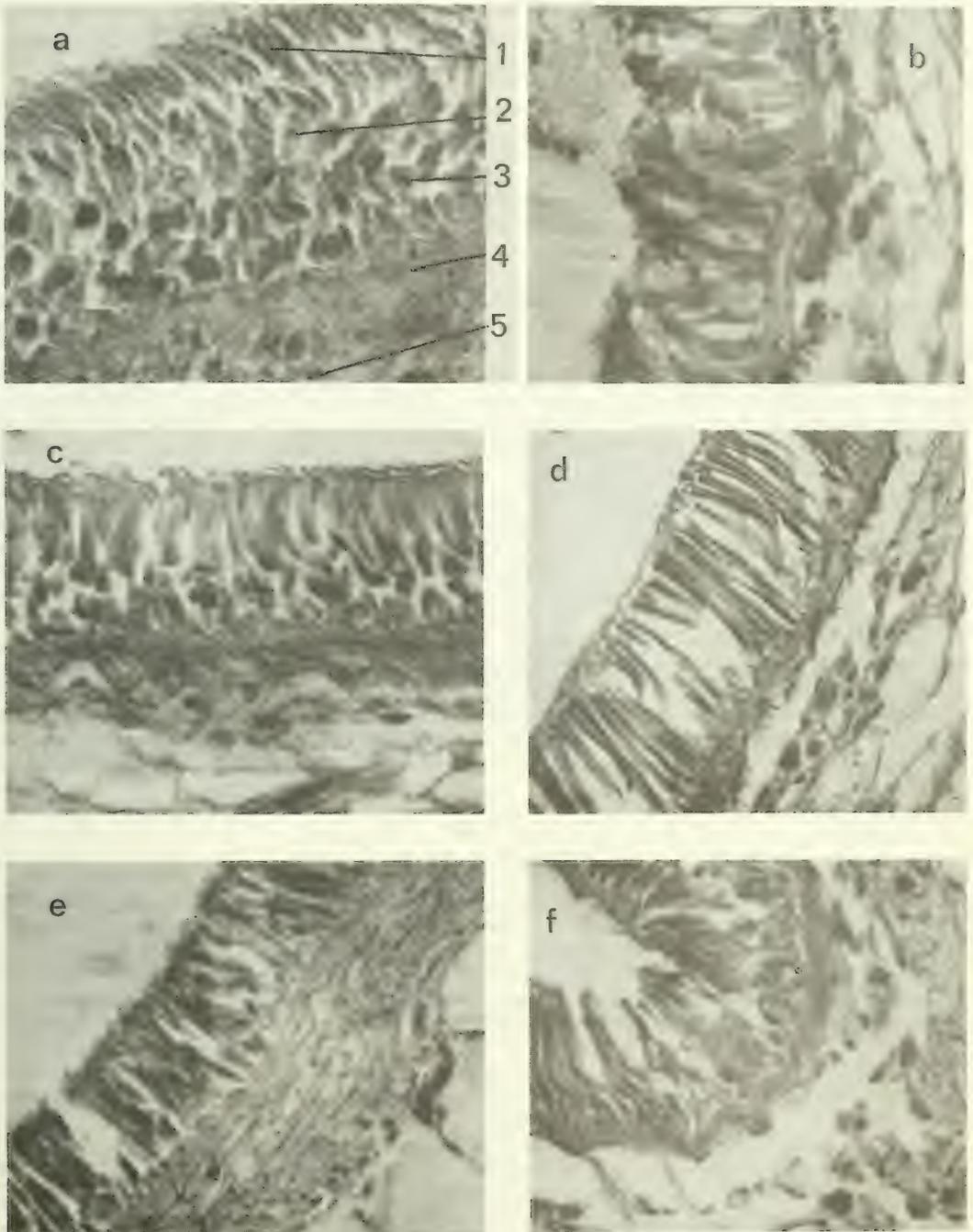


FIG. 6. Effect of narcotics and pesticides on the intestine of *Indonaiia caeruleus* (a—control; b—phenobarbital and hexobarbital sodium; c—alcohol; d—endrin and malathion; e—thiometon; f—DDT). Shown in Fig. 6a: 1—ciliated cells; 2—secretory cells (mucous cells); 3—phagocytic cells; 4—muscle fibres; 5—connective tissue.

affected; these cells lost their connection with the muscle fibers and the mucous cells were reduced in size. Again, the connective tissue became irregular. Phagocytic cells in connective tissue appeared in larger numbers in DDT and thiometon.

DISCUSSION

In general young organisms are more suitable for toxic studies than adults because they have a higher metabolic rate per unit biomass. This is even more important when the pollutants under study are of low toxicity and are applied in low concentrations. In the present study experiments to observe mortality, filtration and oxygen uptake along with survival and ciliary activity of isolated gill tissue were conducted in the summer season when the temperature of the seawater is at an optimum value for the bivalves in question. All experiments were conducted over a number of days with respect to mortality rates. Hence, the results have given a good indication of the effect of various pesticides and narcotics on the physiological activities of the clams.

Literature data indicate that shell growth may be greatly influenced by chlorinated hydrocarbon pesticides, even at low concentrations (0.007 to 0.05 ppm). In the present study all pesticides and narcotics were applied at a concentration of 1 ppm. It has been observed that with increase in exposure time different effective reaction occurred. Thiometon and malathion were found to have the least effect on *K. opima* and *D. cuneatus* respectively. Malathion and DDT were found to be more toxic to *K. opima* and *D. cuneatus* respectively. Both narcotics little affected *D. cuneatus*, but phenobarbital sodium did affect *K. opima*. It would be interesting to study and evaluate further the effect of pesticides by giving pretreatment with either of these narcotants as inducers to these clams. Furthermore, individual evaluation of each pollutant is of importance, because closely related pesticides often have widely divergent effects on the same species of animal.

Preliminary studies of the effect of pollutants on neurosecretory activity and digestive tract of *I. caeruleus* have shown the different reactions of the organism. Bivalves are filter feeders and pollutants are therefore able to penetrate the viscera of the animals. It is shown that the narcotics affected the neurosecretory material in the ganglia, whereas the pesticides affected both the neurosecretory cells and material considerably. The effect of narcotics on the hepatopancreas was not pronounced, but it was marked on the intestine. Pesticides considerably affected both hepatopancreas and intestine.

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ETUDE EN CULTURE IN VITRO DU CONTROLE ENDOCRINE DE LA GLANDE A ALBUMEN CHEZ L'ESCARGOT *HELIX ASPERSA*

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ABSTRACT

The direct effects of the hermaphrodite gland, the brain and the eye tentacle on the albumen gland of the snail *Helix aspersa* were studied using organ cultures. Fragments of albumen gland were explanted onto a solid defined culture medium at one of 2 developmental stages, organogenesis (stage of acini formation, or presecretory stage) or the beginning of secretory activity. Individually cultured acini survive for about one week at the presecretory stage, but necrotic cells are noted thereafter. When, however, the gland has already begun its secretory activity, its initial aspect is retained in culture. When cultured together with a fragment of the hermaphrodite gland, there is no increased survival in vitro of the albumen gland cells which lack secretion granules. If secretion has already begun, there is a slight stimulation of further secretion. When the albumen gland at the end of organogenesis is cultured together with cerebral ganglia from active snails, the gland is maintained in a highly satisfactory state for one week and numerous mitotic figures are seen. If secretory activity has begun before the explantation, the secretory cells are quite significantly stimulated by the cerebral ganglia. This stimulation is shown by the formation of endoplasmic reticulum cisternae and by the emission of numerous golgi vesicles in the neighbourhood of the secretion globules which fill the acini. The presence of the eye tentacle leads to a reduction in the size of the acini and volume of mesenchymatous interstitial tissue at the presecretory stage and inhibits secretory processes in glands where they have already begun. These results demonstrate a stimulatory activity of cerebral ganglia and an inhibitory action of the eye tentacle on acinar organogenesis and on the secretory activity of the gland. The gonad has a weak direct action only after the onset of secretion.

INTRODUCTION

Les observations et recherches expérimentales réalisées chez les Mollusques Gastéropodes Pulmonés ont conduit à considérer que le développement et le fonctionnement de l'appareil reproducteur sont conditionnés par des mécanismes neuro-humoraux et hormonaux. L'influence de la gonade sur le tractus génital est celle qui a été la première envisagée. En effet des escargots *Helix aspersa* présentant une castration parasitaire (Garnault, 1889; Chalaux, 1935) ont des conduits génitaux et une glande à albumen atrophiés. De même chez quelques exemplaires d'*Helix pomatia* sans glande hermaphrodite (Boulangé, 1914; Geigy, 1925) le reste de l'appareil génital demeure infantile.

Le conditionnement vraisemblablement hormonal des caractères sexuels secondaires du tractus génital par la gonade a été mis en évidence par des castrations chez *Helix pomatia* (Filhol, 1938) et chez *Limax maximus* (Abeloos, 1943). Les corrélations entre la gonade et les voies génitales et leurs glandes annexes ont été précisées par des expériences de castration et de transplantation chez des Limacidae et des Arionidae (Laviolette, 1954). En particulier la glande à albumen de jeunes *Arion rufus* implantée avec le reste du tractus chez un hôte adulte de l'espèce *Mesarion subfuscus* augmente 100 fois son volume en 30 jours. Cette glande réagit donc aux stimuli contenus dans l'hémolymphe de l'hôte. Dans ce contrôle de la croissance et de l'activité fonctionnelle de la glande à albumen la gonade a un rôle dominant car l'implantation d'une gonade adulte chez un animal impubère déclenche une différenciation précoce et rapide des annexes glandulaires du tractus génital. Ainsi la gonade semble agir par une substance hormonale qu'elle libère dans l'hémolymphe lorsqu'elle est en pleine gamétogenèse car la greffe d'une gonade juvénile est inefficace. D'autre part les organes effecteurs présentent une période

d'insensibilité au facteur gonadique au début de leur formation car la greffe d'une gonade adulte chez un hôte infantile est sans effet sur le tractus.

L'état du tractus génital n'est probablement pas contrôlé exclusivement par la glande hermaphrodite. Lusic (1961) suppose une certaine indépendance du tractus vis à vis de la gonade chez *Arion*. Des expériences combinées d'implantation de gonade et de ganglions cérébraux, de castration et d'ablation de la glande à albumen, indiquent à Gottfried et al. (1967) que le contrôle de la croissance de la glande à albumen d'*Ariolimax californicus* se fait par l'intermédiaire des ganglions cérébraux et que la gonade en développement joue un rôle principal en modulant la libération et/ou la synthèse d'un inhibiteur de la glande à albumen dans le tissu cérébral.

A la suite des travaux de Pelluet & Lane (1961) et de Pelluet (1964) qui montrent que le cerveau et les tentacules optiques influencent la production d'oeufs des limaces, Meenakshi & Scheer (1969) constatent que l'ablation des tentacules optiques chez *Ariolimax columbianus* est suivie d'une augmentation du poids de la glande à albumine et de la synthèse de galactogène. D'après ces auteurs le facteur tentaculaire agirait par l'intermédiaire de la gonade car l'extrait de tentacules inhibe la synthèse de galactogène in vivo mais il est inactif in vitro.

Des expériences comparables à celles de Laviolette (1954) réalisées par Runham et al. (1973) chez *Agriolimax reticulatus* confirment les résultats du premier auteur et les étendent car elles montrent l'existence de deux hormones sécrétées successivement. L'une responsable de la maturation de la prostate est émise pendant la spermiogenèse, l'autre contrôle les glandes de l'oviducte aux stades d'ovogenèse. L'origine des hormones est inconnue. Par la méthode des cultures d'organe Bailey (1973) n'observe aucun signe de maturation dans les cellules du tractus génital d'*A. reticulatus* cultivé en association avec la gonade ou avec le complexe cerveau/tentacule. Cependant l'association de la glande hermaphrodite et du complexe cerveau/tentacule avec le conduit du même animal agit sur la différenciation de la prostate. Ces résultats conduisent Runham et al. (1973) à supposer que les facteurs produits par la gonade déclenchent la production des hormones prostatiques et oviductaires par le cerveau.

Récemment Goudsmit (1975) obtient une activation de la synthèse de galactogène dans des explants de glande à albumen d'*Helix pomatia* en hibernation cultivés en présence du collier périoesophagien d'escargot en reproduction. Mais des expériences comparables de culture in vitro de glande à albumen de *Biomphalaria glabrata* et *Lymnaea stagnalis* en association avec le système nerveux central et ou avec l'ovotestis n'ont pas permis à De Jong-Brink et al. (1976) de démontrer de synthèse de galactogène. Aussi ces auteurs supposent qu'il y aurait nécessité d'un stimulus de nature nerveuse supprimé dans les explants dénervés.

Il apparaît ainsi que 3 sortes d'organes (gonade, cerveau, tentacules) interviennent directement ou indirectement dans le système endocrine des Gastéropodes Pulmonés qui contrôle le développement et la fonction des organes accessoires de l'appareil génital.

Pour notre part nous avons étudié les effets directs de ces organes sur la glande à albumen de jeunes escargots par la méthode des cultures in vitro.

FIG. 1. Culture d'un fragment isolé de glande à albumen au stade de formation des acini (escargot de 2,1 cm de diamètre). Au bout de 8 jours on observe des vacuoles et quelques figures de nécrose (flèches) dans les cellules de la glande.

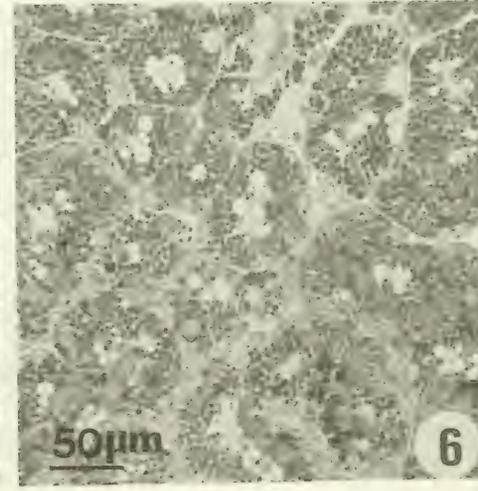
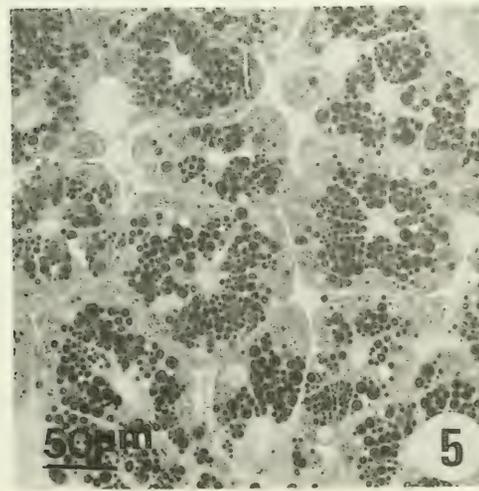
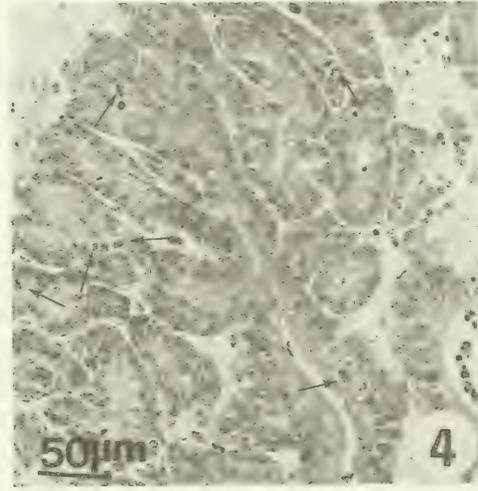
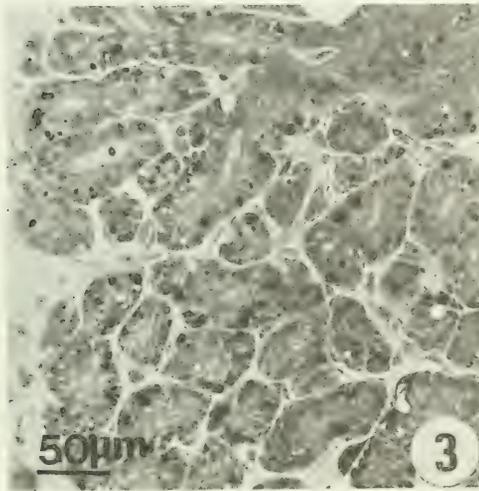
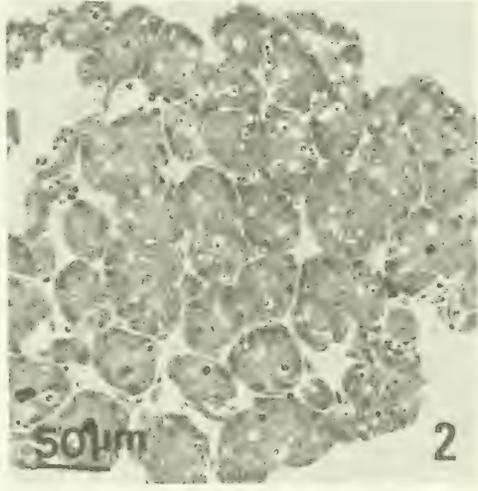
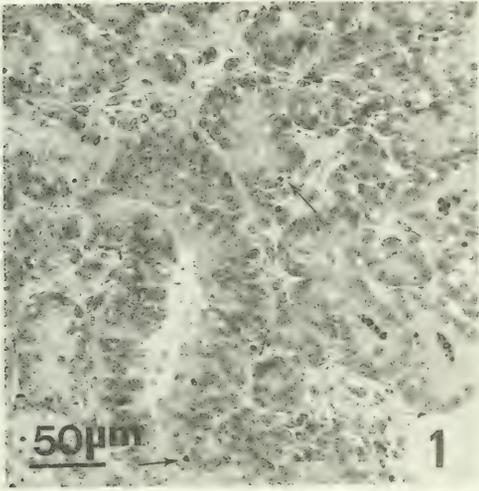
FIG. 2. Explant de glande à albumen au stade tubulaire composé (escargot de 2,1 cm de diamètre) cultivé 8 jours en association avec le tentacule oculaire d'un escargot en activité. Le tissu mésenchymateux interacineux est en régression et des cellules glandulaires meurent.

FIG. 3. Glande à albumen au stade tubulaire composé (escargot de 2,1 cm de diamètre) associée à l'ovotestis d'un escargot en activité et cultivée 8 jours. L'évolution de la glande n'est pas meilleure qu'en culture isolée.

FIG. 4. Culture de glande à albumen au stade tubulaire composé (escargot de 2,1 cm de diamètre) en association avec les ganglions cérébraux d'un escargot en activité. La glande survit très bien et on note l'apparition de nombreuses mitoses (flèches).

FIG. 5. Glande à albumen au stade sécrétoire (escargot de 2,3 cm de diamètre) cultivée pendant 8 jours. La survie est très bonne.

FIG. 6. Fragment de la glande à albumen qui a fourni l'explant de la Fig. 5. Après culture pendant 8 jours en association avec des ganglions cérébraux homologues la quantité de globules de sécrétion a nettement augmenté.



MATERIEL ET METHODE

Pour les associations autologues, des escargots juvéniles (*Helix aspersa*) ont été choisis aux stades fin d'organogenèse ou présécrétion (animaux de 1,8 à 2,1 cm de diamètre) et début de sécrétion (de 2,1 à 2,4 cm) de la glande à albumen définis par Courtot (1977). Des escargots adultes ont fourni les tentacules, le cerveau et l'ovotestis pour les associations homologues hétérochroniques.

Les cultures d'organes ont été faites selon la technique de Wolff & Haffen (1952) sur milieu gélosé adaptée par Gomot (1973) à la culture d'organes d'escargots.

Les organes témoins et cultivés sont fixés 1 h 30 dans le mélange (glutaraldéhyde à 200 mOsm: 1 vol; tampon cacodylate à 200 mOsm: 1 vol; NaCl à 300 mOsm: 1 vol) puis rincés dans la solution (tampon cacodylate à 200 mOsm: 1 vol; NaCl à 300 mOsm: 1 vol). Le lavage est suivi d'une post fixation à l'acide osmique dans le tampon cacodylate à 250 mOsm. Après déshydratation selon la technique de Luft (1961) les pièces sont incluses dans l'épon 812 ou dans l'ERL 4206 selon Spurr (1969).

Les coupes semi-fines de 1 μ m d'épaisseur sont colorées au bleu de toluidine selon la méthode de Trump et al. (1961). Les coupes ultrafines sont contrastées à l'acétate d'uranyle à 3% dans l'alcool éthylique à 50% puis au citrate de plomb selon Reynolds (1963).

RESULTATS

(1) Culture de fragments de glande à albumen isolés

Pendant les stades d'organogenèse la glande à albumen survit bien pendant les premiers jours de culture mais au bout de 8 jours on observe quelques figures de nécrose (Fig. 1).

Lorsque la sécrétion a commencé, les cellules de la glande à albumen conservent leur structure initiale (Fig. 5) pendant les 8 jours de culture.

(2) Culture de la glande à albumen en association avec le tentacule oculaire

Dans les associations de glandes à albumen en organogenèse avec le tentacule oculaire du même animal ou avec le tentacule d'un escargot adulte en activité on note une réduction importante du tissu mésenchymateux qui entoure les acini et de nombreuses cellules meurent (Fig. 2).

Dans les explants provenant de glandes en phase sécrétoire cultivés en présence d'un tentacule autologue on note une diminution du volume des acini et l'augmentation des espaces interacineux. En association avec le tentacule d'un adulte en activité on observe au contraire une augmentation du volume des acini et un changement d'aspect des noyaux qui suggère un phénomène de différenciation cellulaire. Dans les 2 cas le processus sécrétoire est arrêté.

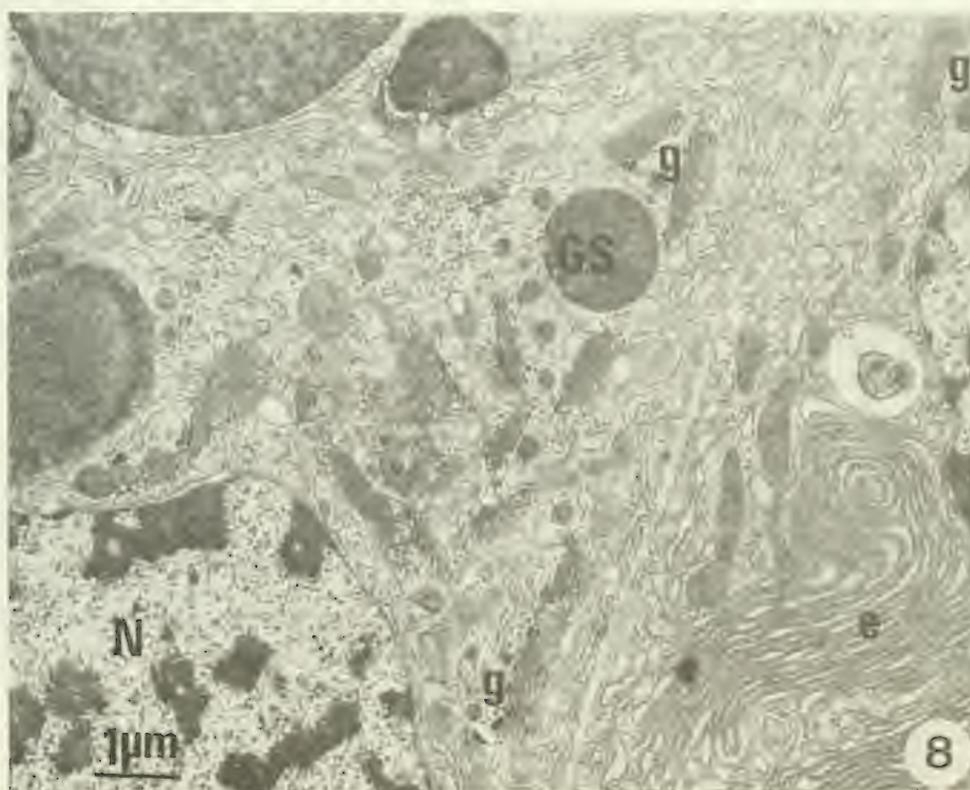
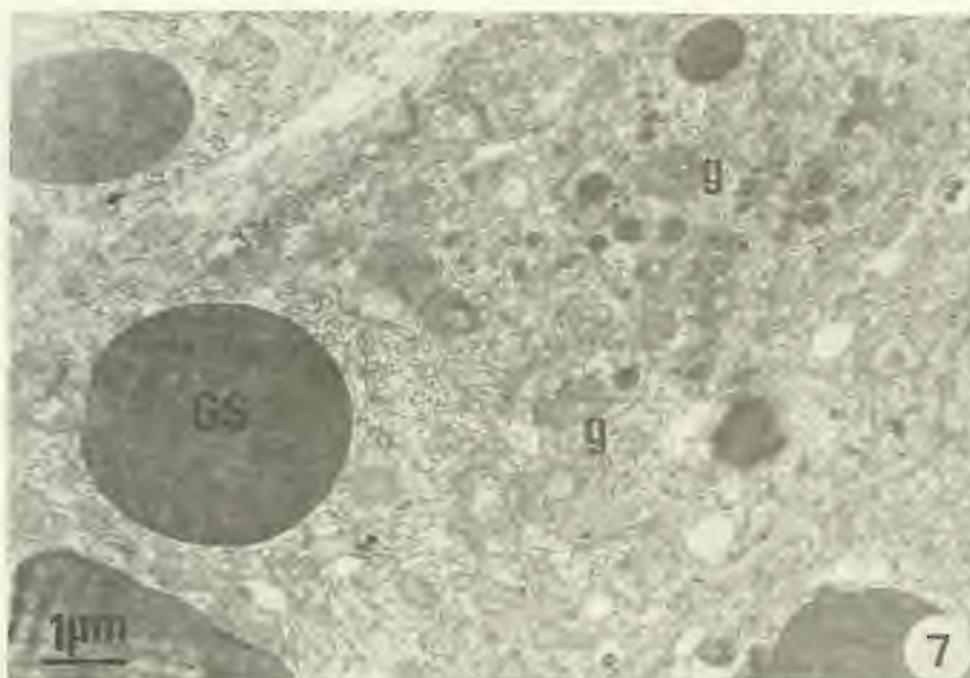
(3) Association de fragments de glande à albumen et de glande hermaphrodite

La culture d'associations autologues de glande à albumen en phase d'organogenèse avec la gonade donne des résultats identiques à ceux obtenus dans les explants de glande cultivée isolément (Fig. 3).

Par contre lorsque les cellules contiennent des grains de sécrétion la survie est très bonne et l'action de la gonade (juvénile ou d'adulte en activité) augmente légèrement l'activité sécrétrice.

FIG. 7. Aspect du cytoplasme d'une cellule sécrétrice de glande à albumen au moment de l'explantation (escargot de 2,3 cm de diamètre qui a fourni les explants des cultures représentées Fig. 5 et Fig. 6). On observe la formation de vésicules aux extrémités des saccules concentriques de l'appareil de Golgi (g) et des grains de sécrétion (GS).

FIG. 8. Micrographie électronique d'une cellule sécrétrice d'un explant de la glande à albumen représentée à la Fig. 7 cultivé 8 jours en association avec des ganglions cérébroïdes (Fig. 6). La conformation des dictyosomes s'est modifiée; les saccules golgiens (g) nombreux au voisinage du noyau (N) sont rectilignes et émettent de nombreuses vésicules à proximité des globules de sécrétion (GS). L'ergastoplasme (e) se présente sous forme de citernes circonvoluant dans le hyaloplasme.



(4) Culture de la glande à albumen en association avec les ganglions cérébraux

Les associations autologues et homologues ont les mêmes effets.

Dans les glandes en organogenèse, la survie est très bonne et de nombreuses mitoses apparaissent (Fig. 4).

Au stade sécrétoire l'activité de la glande est nettement stimulée, les cellules des acini se remplissent de globules de sécrétion et les noyaux sont comprimés contre la paroi des acini (Fig. 6). Le rapport de la surface des grains de sécrétion (dans 5 acini pris au hasard sur une coupe) à la surface totale des acini est de 61% tandis qu'il est de 45% dans les acini du fragment témoin fixé à l'explantation. Le rapport entre la surface des sections de noyaux et la surface des acini reste le même (8%). A l'examen en microscopie électronique l'augmentation de l'activité de synthèse des cellules se traduit au niveau de l'appareil de Golgi qui constitue des dictyosomes avec des saccules plus nombreux (Fig. 7 et 8). L'ergastoplasme se développe en formant des plages où de longues citernes circonvoluent dans le hyaloplasme (Fig. 8).

Dans le cas d'associations triples (glande à albumen, ganglions cérébraux, gonade) le résultat est le même que celui observé avec les ganglions cérébraux.

DISCUSSION-CONCLUSION

La culture de la glande à albumen isolée nous apporte des renseignements sur l'organogenèse et sur le fonctionnement de cette glande.

La différenciation de la glande à albumen en organogenèse ne se poursuit pas en culture isolée.

Parmi les organes associés, seuls les ganglions cérébraux ont une action directe et induisent une multiplication des cellules. A ce stade la glande hermaphrodite juvénile ou adulte est sans effet tandis que les tentacules oculaires sont inhibiteurs.

Au stade sécrétoire les cellules glandulaires ont une autonomie de survie que l'on peut attribuer à la présence de réserves dans les grains de sécrétion ou à une imprégnation plus forte en facteurs endocrines. La gonade exerce alors une stimulation effective mais modérée tandis que les ganglions cérébraux ont une action directe très nette et augmentent le nombre des globules de sécrétion dans les acini.

Le rôle du cerveau comme organe endocrine est ainsi démontré et les résultats obtenus concordent avec ceux de Goudsmit (1975) en les précisant car cet auteur associait l'ensemble du collier périoesophagien tandis que nous avons seulement explanté les ganglions cérébraux. Cependant ceux-ci sont mis en culture avec leur gaine conjonctive et les corps dorsaux. Il est donc possible que l'action mise en évidence soit due à la sécrétion hormonale des corps dorsaux qui sont attachés aux parois des ganglions cérébraux de tous les Gastéropodes (Joosse, 1972) et dont le rôle dans la différenciation des organes sexuels accessoires femelles a été démontré par Geraerts & Joosse (1975) et Geraerts & Algera (1976) chez *Lymnaea stagnalis* et par Wijdenes & Runham (1976) chez *Agriolimax reticulatus*.

Il est maintenant nécessaire d'isoler les corps dorsaux et d'étudier leur action sur la glande à albumen.

Comme d'autres auteurs cités en introduction nous avons montré que la glande à albumen est sous contrôle hormonal. De plus il apparaît que l'action directe des organes endocrines varie avec le stade de développement de l'organe considéré. Si le rôle du système nerveux apparaît nettement aux stades de développement et de fonctionnement celui de la gonade est plus énigmatique. On peut se demander si elle agit comme intermédiaire entre les tentacules et le système nerveux d'une part et la glande à albumen d'autre part ou comme un agent stimulateur des deux organes régulateurs précédemment évoqués. De nouvelles expériences doivent être entreprises pour élucider les interactions entre les organes dont l'action directe sans connexion nerveuse vient d'être démontrée.

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EVOLUTION DES RELATIONS ENTRE OVOCYTE ET CELLULES
FOLLICULEUSES AU COURS DE L'OVOGENESE DE LA PALUDINE
VIVIPARUS VIVIPARUS (GASTEROPODE PROSOBRANCHE)

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ABSTRACT

The ovary of *Viviparus viviparus* was studied ultrastructurally. Follicle cells are linked by septate junctions and surround the oogonia and young oocytes. The relations between oocytes and follicle cells are progressively modified during oogenesis:

—The premeiotic and previtellogenic phases are characterized by a narrow space between follicle cells and oocyte.

—At the beginning of vitellogenesis, the follicle envelope gradually extends next to the basal lamina, where lacunar spaces come into existence. Laterally the intercellular spaces become enlarged whereas a follicle cap persists at the apical zone of the oocyte.

—Next the follicular cells are pushed aside by the increase in size of the oocyte, which acquires a certain amount of autonomy, obtaining nutrient elements direct from the hemolymph. At this stage septate junctions arrange themselves in such a way as to assure the anchorage of the oocyte to the neighbouring follicle cells.

Peu de travaux ont été jusqu'à présent consacrés à l'étude de l'ovogenèse chez les différentes espèces de Paludines. Chez *Viviparus viviparus* l'évolution de la lignée germinale femelle et l'ovogenèse sont décrites brièvement dès 1907 par Popoff puis en 1923 par Ankel. En 1957 Yasuzumi & Tanaka recherchent à l'aide du microscope électronique l'origine du vitellus dans les ovocytes de *Cipangopaludina malleata* tandis qu'en 1972 et 1973 Bottke publie une description ultrastructurale de la morphologie de l'ovaire de *Viviparus contectus*, s'intéressant plus spécialement aux stades prévitellogénétique et vitellogénétique ainsi qu'aux cellules folliculeuses de l'ovaire adulte. Dans le cadre d'un travail expérimental visant à la compréhension des mécanismes qui régulent le fonctionnement des gonades de Paludines, j'ai été amenée à m'intéresser à l'ovogenèse de l'espèce *Viviparus viviparus* et j'ai pu constater des modifications progressives des relations entre ovocyte et cellules folliculeuses au cours du déroulement de la gamétogenèse.

MATERIEL ET METHODES

Pour les études cytologiques, l'ovaire de Paludines femelles adultes est prélevé par dissection et fixé à la glutaraldéhyde à 1% pendant 2 heures. Après lavage, il subit une postfixation d'1 heure dans l'acide osmique à 2%. Le tampon utilisé est le cacodylate de sodium et la pression osmotique des différents liquides est ajustée à 125 mOsm. Les inclusions sont réalisées à l'Epon ou dans l'ERL 4206 (Spurr, 1969). Les coupes semi-fines sont colorées au bleu de toluidine selon la méthode de Trump et al. (1961). Les coupes ultrafines contrastées à l'acétate d'uranyle puis au citrate de plomb selon Reynolds (1963) sont observées sur microscope électronique "Hitachi HU 12."

Pour la mise en évidence des espaces extracellulaires, des fragments d'ovaire de femelles adultes sont incubés pendant des durées variant de 5 min à 1 heure dans du liquide de Holtfreter enrichi de 5% de peroxydase (Sigma Chemical Company). Après fixation à la glutaraldéhyde à 1%, la visualisation de la peroxydase par la 3-3' diamino-benzidine est réalisée selon la technique de Graham & Karnovsky (1966). Après postfixation par l'acide osmique à 2% puis inclusion dans l'ERL 4206, le résultat de la réaction est observé sur coupes non contrastées.

RESULTATS

Chez *Viviparus viviparus*, l'ovaire est un tubule tapissé par un épithélium au sein duquel sont reconnaissables des cellules germinales et des cellules folliculeuses. Les cellules folliculeuses sont des cellules hautes, orientées perpendiculairement à la lame basale. Leur extrémité apicale est ornée de microvillosités tandis que leur région basale émet de longs prolongements pseudo-podiaux qui s'enchevêtrent et épousent fidèlement les contours de la lame basale. Latéralement, dans leur zone apicale, les cellules folliculeuses sont toujours réunies les unes aux autres par des jonctions septées dans le prolongement desquelles s'observent souvent des jonctions intermédiaires ainsi que des desmosomes apicaux. Au contraire les ovogonies et les ovocytes jeunes évoluent isolément, n'établissant pas de contacts jonctionnels avec les cellules qui les entourent.

L'ovogenèse peut être subdivisée en un certain nombre d'étapes en fonction de la taille des ovocytes, de la structure des organites ovocytaires et des interrelations ovocytes-cellules folliculeuses. En effet durant la préméiose puis la prévitellogenèse, plusieurs cellules folliculeuses forment un revêtement continu autour de chaque ovocyte dont elles ne sont séparées que par un espace étroit et qu'elles isolent totalement. Quelquefois cependant la base de l'ovocyte est directement en contact avec la lame basale, au niveau de surfaces réduites.

La vitellogenèse, marquée par une perte de basophilie du cytoplasme et par un fort accroissement de l'ovocyte, peut elle-même être décomposée en plusieurs stades:

La première phase vitellogénétique ou phase folliculaire, qui intéresse des ovocytes de 25 à 35 μm de diamètre environ, se déroule à l'intérieur de la barrière constituée par les cellules folliculeuses mais les espaces intercellulaires s'élargissent peu à peu sur les côtés et à la base de l'ovocyte. Durant ce stade, les gouttelettes lipidiques sont de plus en plus nombreuses, les plages polysaccharidiques de plus en plus étendues tandis qu'apparaissent les premières inclusions vitellines.

La seconde phase vitellogénétique, caractérisée par une synthèse particulièrement active des globules vitellins, est marquée par une profonde modification des relations entre les cellules folliculeuses et l'ovocyte et par un changement de la physiologie de l'ovocyte qui se libère progressivement de son enveloppe folliculeuse. Durant cette étape de paroxysme vitellogénétique la dilatation des espaces intercellulaires s'accroît, si bien qu'un ovocyte en fin de vitellogenèse n'a plus latéralement de rapports avec les cellules folliculeuses qu'au niveau de quelques courts processus. A la base se crée un système d'espaces lacuneux qui permet un contact direct entre l'ovocyte et l'hémolymphe et au niveau duquel de très nombreuses vésicules de pinocytose sont parfois visibles. A l'apex, les cellules folliculeuses sont rejetées sur les côtés par la croissance de l'ovocyte qui atteint un diamètre de 60 à 65 μm et dont le pôle fait saillie dans la lumière ovarienne où s'accumulent les résidus de nombreux ovocytes en dégénérescence. Latéralement, dans sa partie supérieure, l'ovocyte établit alors avec les cellules voisines des jonctions septées qui semblent avoir pour principal rôle d'assurer l'ancrage de l'ovocyte que rien ne retiendrait plus désormais contre la paroi ovarienne.

DISCUSSION-CONCLUSION

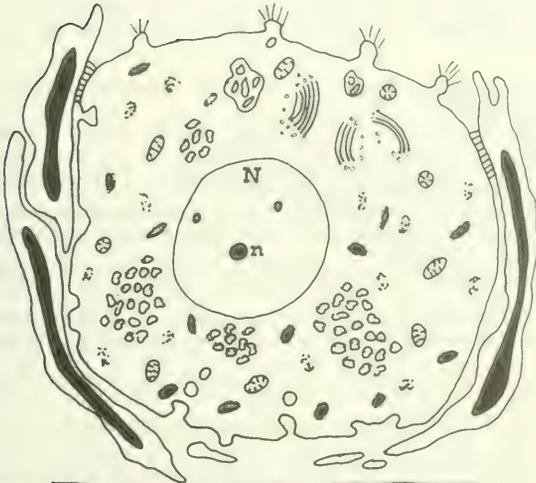
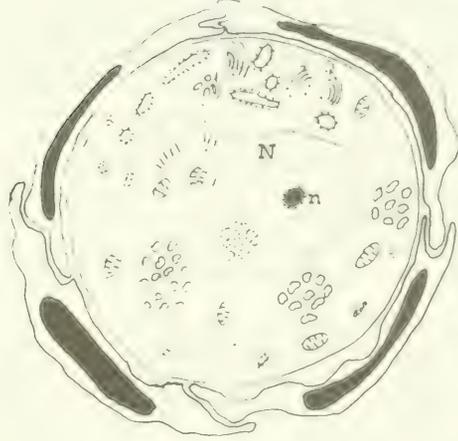
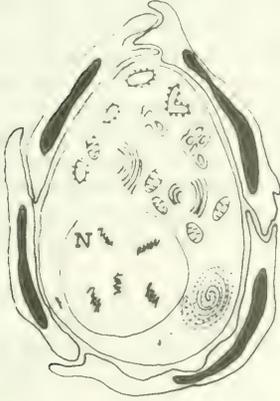
De telles modifications topographiques, schématisées sur la Fig. 1, reflètent vraisemblablement des transformations profondes de la physiologie ovocyttaire.

Durant les premiers stades de l'ovogenèse, l'ovocyte apparaît isolé à l'intérieur de son enveloppe folliculeuse. Tous ses échanges sont réglés par les cellules folliculeuses qui jouent le rôle d'intermédiaires entre la cellule germinale et les tissus voisins. Comme chez l'espèce *Viviparus contectus* (Bottke, 1972 et 1973), chez *Viviparus viviparus* divers arguments tels que l'abondance de l'appareil de Golgi dans les cellules folliculeuses, leur richesse en glycogène, vacuoles et corps multivésiculaires... suggèrent que ces cellules jouent un rôle dans le catabolisme du matériel dégénéré dans la lumière ovarienne et dans le stockage de réserves nutritives utilisables par l'ovocyte. La rareté des vésicules de pinocytose dans les zones de contact entre ovocyte et cellules folliculeuses laisse supposer que les échanges intéressent surtout des éléments de faible poids moléculaire, transférés à l'ovocyte sous une forme soluble.

Ensuite, au cours du paroxysme vitellogénétique, l'enveloppe folliculeuse éclate; l'ovocyte semble alors acquérir une certaine autonomie, puisant directement dans l'hémolymphe les

prévitellogénèse

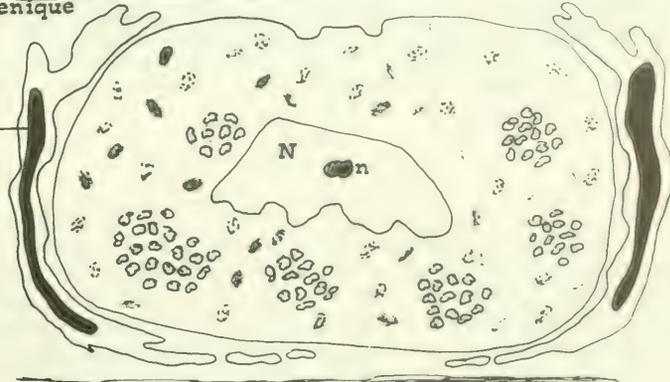
phase folliculaire



- N noyau
- n nucléole
- vesicule ergastoplasmique
- mitochondrie
- dictyosomes
- glycogène
- corps multivésiculaire
- globule lipidique
- vitellus protéique
- membrane basale

paroxysme vitellogénique

cellule folliculeuse



maturación de l'ovocyte

FIG. 1. Schema des modifications topographiques de l'ovocyte de *Viviparus viviparus* et de son enveloppe folliculeuse. Membrane basale est lame basale.

substances dont il a besoin et absorbant peut-être aussi du matériel dégénéré dans la lumière ovarienne, au niveau des villosités qui ornent sa surface.

L'apparition, après éclatement du follicule, de vésicules de pinocytose et de très nombreuses inclusions vitellines suggérait la possibilité d'une arrivée massive de produits exogènes véhiculés par l'hémolymphe et captés par l'ovocyte. La participation de facteurs exogènes, synthétisés dans des régions extra-ovariennes, à la constitution des plaquettes vitellines est en effet connue chez les Vertébrés, Insectes, Crustacés... et récemment Hill & Bowen (1976) qui ont observé des tubules de pinocytose dans les ovocytes de la limace *Agriolimax reticulatus* ont émis l'hypothèse de la présence de composants auto- et hétérosynthétiques dans le vitellus de ce Gastéropode. Pour tenter d'évaluer l'importance du phénomène d'endocytose dans l'ovocyte de *Viviparus viviparus*, j'ai fait appel à la technique de Graham & Karnovsky (1966), dans laquelle la peroxydase sert de traceur pour la mise en évidence des espaces extracellulaires et des phénomènes d'endocytose. Les premiers résultats font apparaître une double origine du vitellus, constitué:

—d'une part de matériaux synthétisés par l'ovocyte lui-même avec la participation de la plupart des organites cellulaires (appareil de Golgi et mitochondries en particulier),

—d'autre part de matériaux synthétisés en dehors de l'ovocyte et captés par pinocytose uniquement à la fin de la vitellogenèse.

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EXPERIMENTAL EVIDENCE FOR THE HORMONAL CONTROL
OF OVIPOSITION IN THE FRESHWATER PULMONATE
INDOPLANORBIS EXUSTUS

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ABSTRACT

Injection of blood or crude CNS extract from juvenile snails into intact, mature *Indoplanorbis exustus* elicits no egg-laying, whereas administration of blood or crude CNS extract from mature adults into the mature snails induces oviposition within 2 hours. Boiled extracts of CNS and crude homogenate of foot muscles from mature snails are unable to stimulate egg-laying in mature *I. exustus*. Thus it is hypothesized that a blood-borne agent which is possibly proteinaceous in nature, hormonal in character, and originating in the CNS of mature *I. exustus*, may be involved in controlling its oviposition.

INTRODUCTION

Egg-laying in the gastropod *Pleurobranchaea californica* (cf. Davis et al., 1974), which occupies a more dominant position in its behavioural hierarchy, is controlled by CNS hormone(s). In the opisthobranch *Aplysia californica* (cf. Arch, 1976) egg-laying is induced by its abdominal ganglion bag cell neurohormone. Recently, Ram (1977) has documented that in *Busycon* too, laying of egg capsules can be caused by extracts of the nervous system. Oviposition in the pond snail *Lymnaea stagnalis* is suppressed by the ablation of dorsal body and resumption of oviposition can be stimulated by dorsal body implants (Geraerts & Joesse, 1975). However, stimulation of oviposition by dorsal body hormone is due to reinitiation of vitellogenesis. It has been implicitly established that Caudo-Dorsal Cells (CDC) from cerebral ganglia of *L. stagnalis* produce ovulation and oviposition-stimulating hormone and that circadian rhythmicity in oviposition in this snail appears to be because of diurnal rhythmic release of ovulation-provoking CDC neurohormone (see Roubos, 1976). Interestingly, *Indoplanorbis exustus* of tropical freshwater also exhibits a diurnal rhythm of oviposition (unpublished data) which hinted at the possibility of occurrence of some regulatory mechanism, probably hormonal by analogy with the pulmonate brethren *L. stagnalis*. Preliminary evidence is forwarded in the present paper substantiating the above possibility by analysing the effect of CNS homogenate and blood from juvenile and mature snails on oviposition in adult *I. exustus*.

MATERIAL AND METHODS

Locally collected, healthy snails of *I. exustus* were adapted to laboratory conditions ($23 \pm 2.5^{\circ}\text{C}$, 12D:12L) for 7 days before experimentation. Adults (average length, i.e. distance between top of the shell and lowest body whorl, 17-20 mm) and juveniles (average length 5-7 mm) were selected for the treatment. The blood from the snails was collected by breaking the shell and withdrawing the blood from the heart by tuberculin syringe. Blood from 20 to 30 snails was pooled. CNS (cerebral, pleural, pedal, parietal and visceral ganglia) was quickly dissected out in chilled saline (0.7%) and homogenised with glass mortar and pestle. Similarly, foot muscles were separated, weighed and homogenised. The supernatant after centrifugation (3,000 r.p.m. for 10 min) was used for administration. To test the stability of the CNS factor responsible for oviposition, the CNS extract was boiled for 15 minutes and after boiling it was centrifuged. All injections were given directly into the foot using a 28-gauge needle. Each snail received 0.1 ml of blood or tissue extract.

TABLE 1. Effect of blood and CNS extract from juvenile snails on egg-laying in mature *L. exustus* 2 hrs after injection.

Injection	No. of snails oviposited/ No. of snails injected	No. of clutches laid
1. Blood (0.1 ml/snail)	0/21	0
2. CNS homogenate (0.1 ml containing 2 CNS/snail)	1/19	1
3. Control (0.1 ml saline/snail)	0/19	0

TABLE 2. Effect of blood, CNS and foot muscle homogenate from adult snails on mature *L. exustus* 2 hrs after injection.

Injection	No. oviposited/ No. injected	No. of clutches laid
1. Blood (0.1 ml/snail)	15/15	19
2. CNS homogenate (0.1 ml containing 2 CNS/snail)	19/19	28
3. Boiled CNS homogenate (0.1 ml containing 2 CNS/snail)	0/21	0
4. Foot muscle homogenate (0.1 ml containing 55 µg muscle/snail)	0/12	0
5. Control (0.1 ml of saline/snail)	2/19	2

Preliminary investigations indicated that the egg-clutches are laid 110 to 120 min after CNS extract administration. As such the number of egg-clutches deposited was counted 2 hours after the injections. The present experiments were carried out in August 1976 between 9:00 a.m. and 12 p.m.

RESULTS

Injections of blood and CNS homogenate from juvenile snails stimulated no egg-laying (only one snail laid a single egg-clutch in CNS homogenate-injected snail) 2 hours after injections in mature *Indoplanorbis* (Table 1), whereas blood and CNS extract from mature snails initiated egg deposition in all the injected adults after 2 hours. There was no egg-laying in boiled CNS extract and foot muscle homogenate treated adults (Table 2).

DISCUSSION

The furnished data give evidence that the CNS of mature *L. exustus* contains a substance which can be liberated by homogenisation and which, when administered into other mature but intact *L. exustus*, can stimulate oviposition. Furthermore it is proposed that this oviposition-initiating substance is a blood-borne agent, i.e. a hormone which is not heat-stable and the release of which normally initiates egg-laying in this freshwater pulmonate.

L. exustus, however, is an acyclic breeder; breeding attains a peak during the monsoon, i.e. June-September (Chintawar, 1974). Micromorphological studies of the neurosecretory system have revealed the occurrence of 2 types of neurosecretory cells (A and B cells) throughout the CNS (Chintawar, 1974). Of these the B neurosecretory cells from the cerebral ganglia show activity correlated with reproduction. Moreover, in *L. exustus* which are infected with larval

trematodes, oviposition is dramatically curtailed (unpublished data) and at the same time B cells from cerebral ganglia display aberrations (Hanumante et al., 1977).

The failure of blood or CNS homogenate (if a single exception is ignored) of immature but not from mature snails to stimulate egg-laying in adults points out that the elements concerned with the production of egg-laying substance are either inactive or absent in juvenile snails. That these elements control the egg-laying not through neural impulses but through a vascular hormonal channel is indicated by induction of oviposition following blood and CNS injections from adult snails and not by foot muscle extract. The inability of boiled CNS extract to provoke egg-laying indicates that the egg-laying hormone is not heat-stable and as such may be proteinaceous in nature (polypeptide?). Incidentally, *Aplysia* egg-laying hormone is of a polypeptide nature (Arch, 1976).

The diurnal rhythmic ovipository behaviour of *I. exustus* (unpublished data) suggests that the cells producing egg-laying hormone must also be undergoing rhythmic changes in its secretory kinetics as has been demonstrated for CDC from cerebral ganglia of *L. stagnalis* (see Roubos, 1976). However, further extensive experimentation is needed to confirm this hypothesis and also to know the exact residence of egg-laying hormone. If *L. stagnalis* is taken as a model of pulmonate neuroendocrinology and on the basis of our unpublished data, the cerebral ganglia of *I. exustus* also, appear to be most promising candidates.

It is intriguing to note that a latent period of about 2 hours is required before the onset of oviposition in *Indoplanorbis* after CNS homogenate or blood injection. It is pertinent to record that in the female echinoderm, *Echinaster modestus* (cf. Turner, 1976) too, a mean latent period of 163 minutes has to elapse before ova can be released after intracoelomic injection of 1-methyladenine. In *Aplysia* also egg-laying is stimulated approximately 50 to 75 minutes after administration of a crude bag cell extract into the haemocoel (Arch, 1976). Arch is of the opinion that, even though the gonad is immediately triggered by the hormone to extrude eggs, approximately 60 minutes are required for the string to be assembled and traverse the distance down the genital tract to the exterior in *Aplysia*. Perhaps even more time is consumed by *Indoplanorbis* to complete these preovipository rituals. However, more evidence is needed to draw the firm conclusion as to how the injected homogenate might have acted either directly on the gonads or via CNS by provoking the secretory activity of intact egg-laying neurohormonal cells. Further experiments have been planned to clarify this issue.

Thus it looks quite apparent that like the lymnaeid pulmonate, *L. stagnalis* (see Roubos, 1976) the planorbid, *I. exustus*, may also join the marine gastropods *Pleurobranchaea* (Davis et al., 1974), *Aplysia* (Arch, 1976) and *Busycon* (Ram, 1977) wherein the presence of an ovipository hormone has been established.

ACKNOWLEDGEMENTS

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THE FINE STRUCTURE OF THE OOCYTE AND FOLLICLE CELLS OF
LYMNAEA STAGNALIS, WITH SPECIAL REFERENCE TO THE
NUTRITION OF THE OOCYTE

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ABSTRACT

A description is given of the configuration of the surface membrane in the 3 zones of the polarised system of oocyte and follicle cells. Thus the cell contacts and relationships between these cells are considered and speculation on the significance of surface morphology in the passage of substances into the enlarging oocyte, is included.

Studies on the fine structure of the follicle cells and the oocytes in the gonad of *Lymnaea stagnalis*, show a well defined sequence of developmental phases of the follicle cells adjacent to the oocyte, and a high degree of differentiation and polarisation of these cells. The zones thus established in the follicle cells coincide with distinctive areas of the oocyte, particularly with the configuration of the margin of the oocyte (Rigby, in press).

Thus three broad zones are recognised in the well grown oocyte of 90-105 μm diameter, and also in their follicle cells. Raven (1963, 1967) showed that only 6 inner follicle cells surround each oocyte. It is suggested that the striking morphological differences between these areas may be associated with the provision of different pathways for transfer of differing substances into the growing oocyte.

As the oocyte separates, first from the lamellate zone of the follicle cells in the apical area of the oocyte (the future animal pole) so microvilli, 0.3-0.5 μm in height, are established from the surface of the oocyte (Fig. 1a). These microvilli are shorter and more sparse in distribution than those reported in *Spisula* (Rebhun, 1962), *Barnea* (Pasteels & De Harven, 1962), *Triturus* (Hope, Humphries & Bourne, 1963) etc., but as in all these species, the microvilli are embedded in the vitelline envelope, pinocytotic vesicles form near their base and occasionally macrovilli from the follicle cells traverse the intercellular gap passing between the microvilli to fuse with the oocyte. Occasionally pores of the endoplasmic reticulum are also found between the microvilli (Fig. 1b) and in some material it is indeed difficult to decide whether vesicles in the superficial cortical layer are pinocytotic vesicles or transversely cut channels of endoplasmic reticulum. The wide range in the degree of distension of channels of the endoplasmic reticulum in the apical cytoplasm of the oocyte recorded in this work, implies fluid uptake for the expansion of the oocyte through the pores of this system.

Brummett & Dumont (1976) following investigations on the role of microvilli and endocytotic pits in the developing oocytes of *Xenopus*, stated that "microvilli with their negatively charged sialic acid moieties may be the sites of cation exchange (and perhaps small molecule uptake?)" and that evidence of the uptake of the main precursor of yolk through the mechanism of endocytosis has been obtained. These functions seem also possible in *Lymnaea*.

The lateral band succeeds the microvillar surface and extends to the base of the oocyte and it equates with the zone between the nucleus and the basal processes of the follicle cells. The band is characterised by tight junctions, gap junctions and desmosomes alternating with deeper intercellular spaces that contain translucent material or electron-dense floccular material between the oocyte and follicle cells (Fig. 2a). Abundant distended vesicles of the endoplasmic reticulum and Golgi stacks occur in this part of the follicle cells and suggest high proteinaceous and carbohydrate metabolism. Some very large vesicles that seem to extend from the endoplasmic reticulum may be discharging their proteinaceous contents to the intercellular

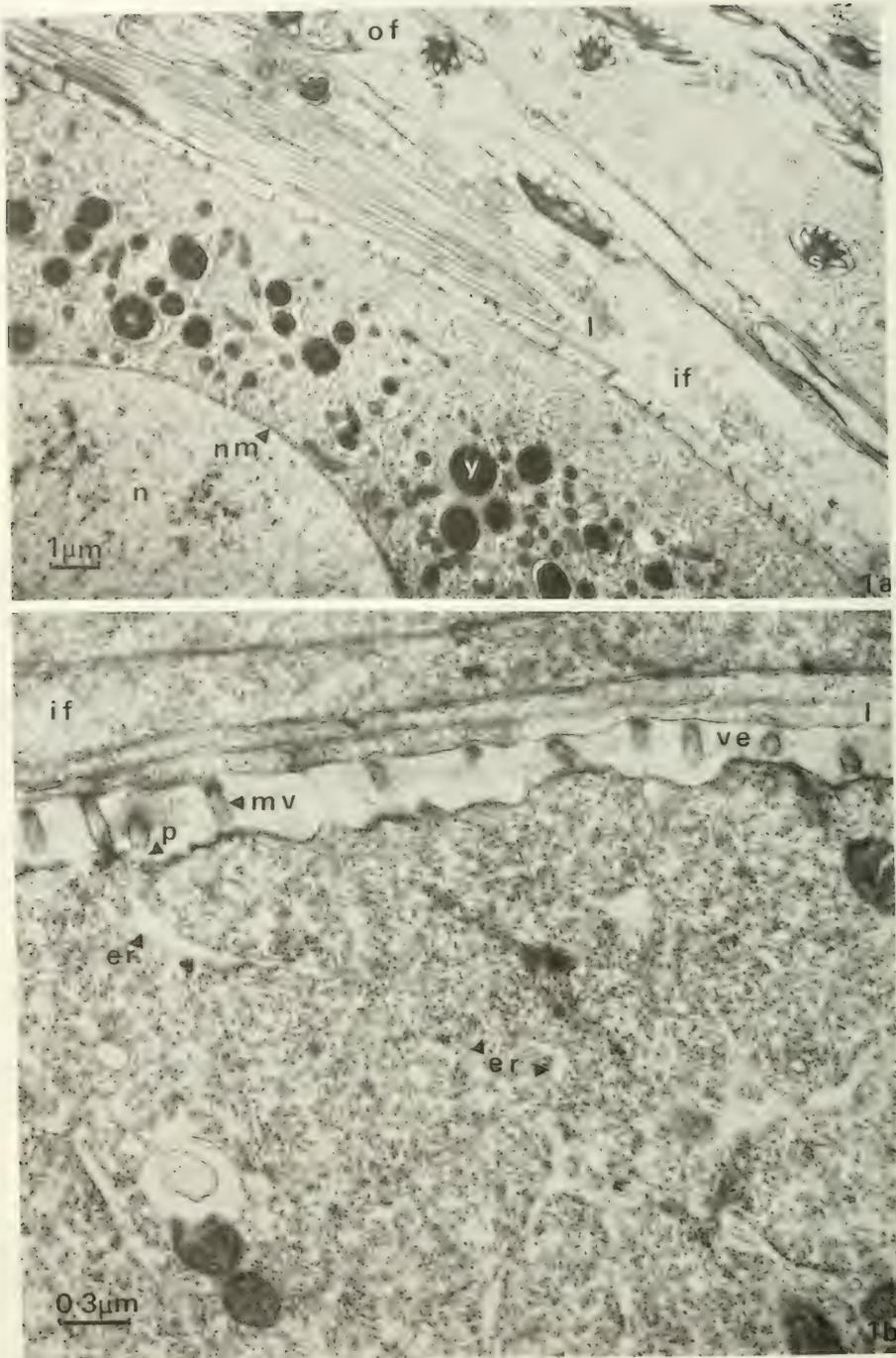


FIG. 1. (a) Apical portion of an oocyte with inner follicle cells showing their interlocking lamellae, outer follicle cells, and differentiating sperm with Sertoli cells beyond. (b) Pore of the endoplasmic reticulum, in an oocyte. Abbreviations: a—amoebocyte cell; d—desmosome; er—endoplasmic reticulum; gj—gap junction; if—inner follicle cell; im—intercellular matrix; is—intercellular space; l—lamella; mv—microvillus; n—nucleus; nm—nuclear membrane; of—outer follicle cell; o—oocyte; p—pore; s—spermatozoon; tj—tight junction; v—vesicle; ve—vitelline envelope; y—yolk granule.

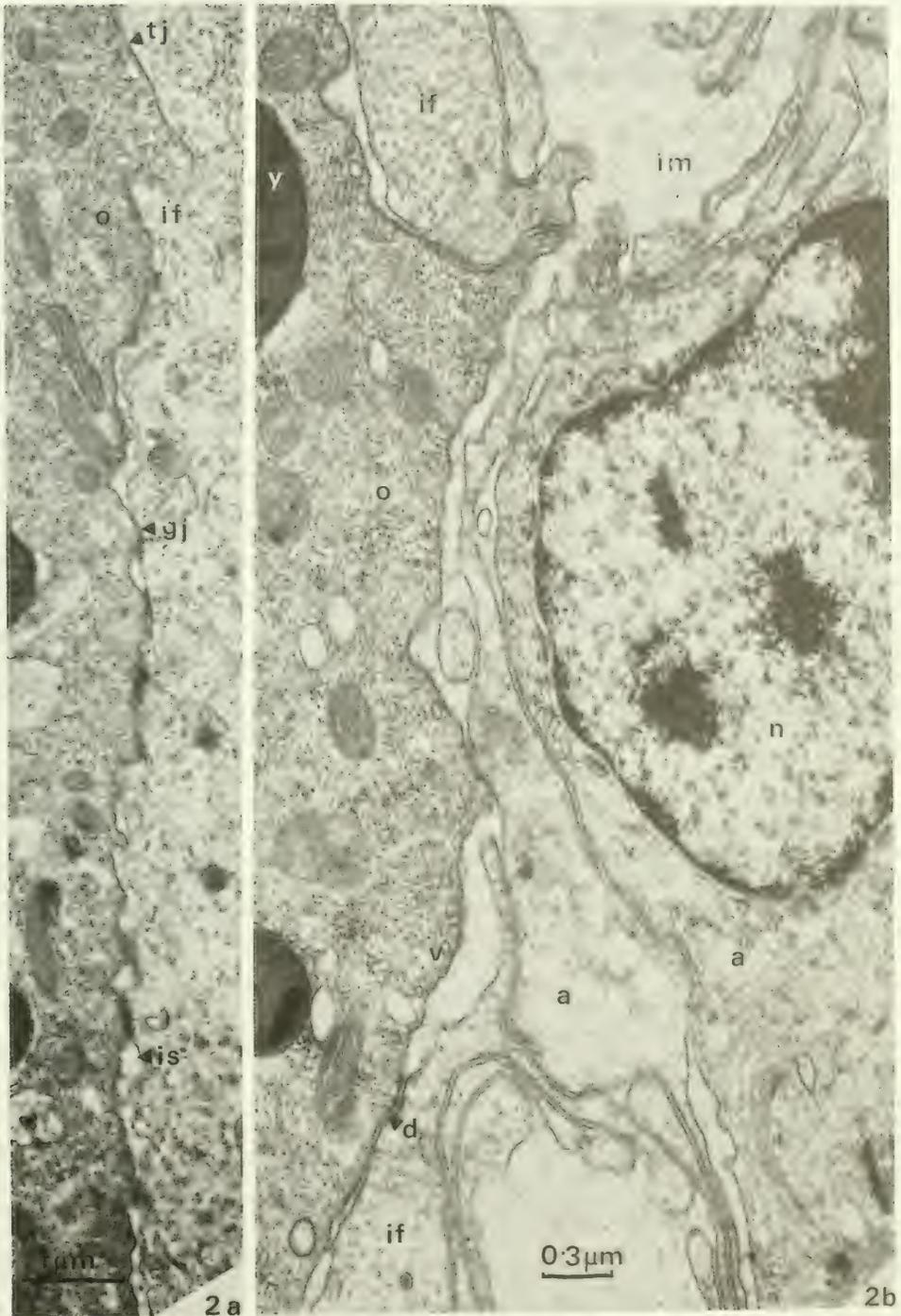


FIG. 2. (a) Portion of the lateral border of the oocyte and follicle cell. (b) Basal processes of the follicle cells and an amoebocyte cell, alongside the base of the oocyte. For explanation of lettering see Fig. 1.

spaces. Small vesicles may occur along both the follicular border and the oocyte border of the intercellular spaces. In some instances cytoplasmic projections from the follicle cells penetrate the oocyte and appear to be nipped off. Are some larger molecules of glycoprotein and nuclear protein conveyed directly by such portions of cytoplasm into the oocyte, whilst nucleotides, sugar phosphates and choline phosphates pass through gap junctions (Pitts, 1976) into the oocyte?

The growing oocyte rests on the basement membrane of the gonad which merges with the intercellular matrix between the digestive gland and the gonad. It is in this matrix that 'rhizoids,' slender projections from the base of the oocyte (the future vegetative pole), make tight junctions, gap junctions and desmosomes with elaborately branching processes which ramify through the tissue from the base of the follicle cells; the amoebocyte cells also found in the matrix, make cell contacts with the base of the follicle cells and occasionally with the oocyte (Fig. 2b). In the deep cups or capsules formed by some processes of the follicle cells, secretions aggregate and seem to be taken up by structurally different processes that penetrate into the capsule. Small vesicles are frequent along the base of the oocyte.

It is close to this basal border that the most substantial accumulation of lipid globules is found in the oocyte, these inclusions becoming membrane-bound and their contents more electron-dense. This certainly appears to be a nutritionally active area, not merely a passive anchoring region and these features link with the observations of Jooose & Reitz (1969) that yolk-containing oocytes are confined "to those parts of the wall of the acini which are apposed to the lobes of the digestive gland."

The question arises whether there is direct transfer of digested food substances across the intercellular matrix from the digestive gland to the rhizoids and pinocytotic vesicles at the base of the oocyte, whether amoebocytes facilitate the transport of substances to the oocyte and follicle cells, or whether some products of the follicle cells are discharged into capsules and taken up by processes of the oocyte. Probably all these pathways are used by some substances entering the oocyte but there are many problems to contend with to undertake feeding experiments with labelled material to resolve these points. These observations on the fine structure nevertheless indicate, how the special cytoplasmic differentiation of the mosaic egg of *Lymnaea stagnalis* (cf. Raven, 1967) may arise.

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THE STRATEGY OF COPULATION OF *STAGNICOLA ELODES* (SAY)
(BASOMMATOPHORA: LYMNÆIDAE)^{1,2}

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ABSTRACT

The copulation of *Stagnicola elodes*, a North American lymnaeid, is effected by the male from a position at the right margin of the female shell. The mode of copulation is immediately successively reciprocal, both members of a pair acting as male and female during a single meeting. During the first male to female activity, the female becomes stimulated, before the male has finished, to copulate in the reciprocal direction. The copulatory stimulus can be carried through a 3rd, 4th, etc., snail by placing the stimulated female on the shell of a snail other than the male. Females forcibly restrained from reciprocating, isolated and then reunited with the male can lose the copulatory stimulus. The female can remain stimulated to copulate up to at least 1 hour of isolation. Male activity takes approximately 2 hours, and in a reciprocating pair both snails are occupied for 4 hours. The ejaculated sperm is in the posterior part of the vagina when observed immediately after copulation. Males ejaculate most or all of their stored sperm in one copulation. Prostatic secretions are greatly depleted following copulation. Refilling of the hermaphroditic duct begins between the first and 2nd day following copulation. Two days following copulation, the duct is approximately $\frac{1}{4}$ to $\frac{1}{2}$ full. A copulatory plug, with a probable effective existence of 2 to 3 hours, is formed in the female at the area of juncture of the spermathecal duct and vagina. The plug is composed of secretions from the male reproductive tract of the male. Two roles are suggested here for the secretions of the male reproductive system, i.e., the formation of a copulatory plug and implication in the stimulus of male activity in the female.

INTRODUCTION

Members of the higher limnic Basommatophora are hermaphroditic, and many such snails are capable of self-fertilization as well as cross-fertilization. A hermaphroditic animal capable of self-fertilization must be prepared for either eventuality, mating when possible and self-fertilizing when mating is not possible. Therefore, mating is a major strategy in the reproductive biology of these animals. A major difference between the two types of reproduction is the origin of the sperm. In self-fertilization, all reproductive materials, sperm included, are provided by one animal. In cross-fertilization, the provision of the sperm, and possibly other materials, is undertaken by an animal other than the one which lays the eggs.

However, the mechanism of mating has further implications than a simple transfer of sperm. It has been shown that freshwater pulmonates will use foreign sperm in preference to autosperm (e.g., Boycott et al., 1930; Cain, 1956; Wu, 1972). Some planorbids (DeWitt & Sloan, 1959; Lo, 1967) and *Ancylus fluviatilis* (Bondesen, 1950) will not reproduce normally without cross-fertilization. It also appears that paired snails begin laying eggs earlier in life than isolated snails (Boycott et al., 1930; Noland & Carriker, 1946; DeWitt, 1954; Horstmann, 1955; DeWitt & Sloan, 1958, 1959). The stimulus for an earlier initiation of oviposition by snails which have mated is ascribed to a sperm factor resorbed from the spermatheca following copulation (Horstmann, 1955).

Although freshwater pulmonates can be induced to mate, the physiological basis for mating

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is not explained. Snails reunited after isolation will often copulate (Noland & Carriker, 1946; Duncan, 1959); *Lymnaea stagnalis appressa* copulates readily when the food supply has recently been depleted (Noland & Carriker, 1946) and *Helisoma trivolvis pseudotrivolvis* and *Physa gyrina* "brought from lower (below 10°C) to higher temperatures" will copulate and lay eggs within 36 to 48 hours (Roney, 1943). Duncan (1959) has also shown that *Physa fontinalis*, when brought into the laboratory from the field, goes through a period of intense mating activity, presumably due to the change of environment.

The types of pairing during the copulation of freshwater pulmonates are varied. Unilateral, chain and reciprocal mating have been described as primary modes of mating among members of the Planorbidae (e.g., Hazay, 1881; Precht, 1936; De Larambergue, 1939; Boettger, 1944; Malek, 1952; Paraense & Deslandes, 1956; Pace, 1971). The primary means of mating in members of the Lymnaeidae, Physidae and Ancylidae are unilateral or in chains (Karsch, 1846; De Lacaze-Duthiers, 1899; Boettger, 1944; Noland & Carriker, 1946; Barraud, 1957; Duncan, 1959; Marcus & Marcus, 1962; and others). Occasional reports of reciprocal mating among the Lymnaeidae exist (Karsch, 1846; Künkel, 1908; Noland & Carriker, 1946; Barraud, 1957), but reciprocal mating is not reported to be a primary means of mating in these three families. Thus, among the members of the higher freshwater pulmonates, differences exist in mating behavior, even within the same family.

Phenomena of mating in the North American lymnaeid *Stagnicola elodes* (Say) will be described in this report in order to more clearly delineate the mating mechanism of this snail.

MATERIALS AND METHODS

Stagnicola elodes adults were collected from a roadside pond on Liberty Road, 2.2 miles west of Zeeb Road, Ann Arbor, Washtenaw County, Michigan. Snails used in this study were laboratory raised descendants from the field collected snails. Snails were maintained in 5-gallon aquaria containing aerated tap water and an aquarium filter, and fed with lettuce throughout the period of study.

Sexually mature snails were isolated for 48 hours in plastic drinking glasses containing 50-100 ml of previously aerated tap water. Lettuce was added to each covered dish. After 48 hours, the snails were placed in pairs in the same water in which they had been isolated and were allowed to copulate. Occasional observations were made on non-isolated snails taken directly from the stock tanks.

Reproductive tracts used for histological sectioning were fixed in Heidenhain's Susa. The reproductive tracts were severed posterior to the spermatheca and the anterior portion embedded in Paraplast (melting point 56°C), following dehydration in a graded series of ethyl alcohol and clearing in dioxane or xylene. Sections were prepared at 6 μ m and stained with methyl blue-picric acid-hematoxylin (Lillie's Allochome without periodic acid-Schiff, Lillie, 1965), hematoxylin-eosin, bromphenol blue or Alcian blue 8GX (pH 2.5)-eosin.

Voucher specimens from the laboratory populations are in the Museum of Zoology, University of Michigan (UMMZ): shells UMMZ 246000, alcohol specimens UMMZ 246001.

RESULTS

Spontaneous male copulatory activity

When the snails were placed in pairs after the isolation period, 8 experiments showed that 28 of a total of 98 pairs exhibited spontaneous male activity. If spontaneous male activity was not initiated within approximately 2 hours of placing the snails in pairs, it seldom occurred during the period of observation (usually 8-12 hours).

Prior to copulation, the 2 snails move about the dish. The male-acting animal (hereafter referred to as the male) crawls upon the shell of the female-acting animal (the female). The male continues to crawl around on the shell of the female until it reaches a position on the right margin of the shell. During this time, the male gonopore region of the male becomes slightly dilated and appears as a white dot, in contrast to the barely visible male gonopore observable in a non-copulating animal. The white area is often visible before the male has crawled upon the shell of the female. The white area enlarges and the penial complex becomes partially everted. The male everts the penis and probes under the shell of the female in search of the female gonopore. During the period of male probing, the female apparently does not change its behaviour and moves about the dish and may continue to feed. The female suddenly

withdraws the right side of the body partially into the shell. This withdrawal is only temporary, and the female returns from the withdrawn position and copulation proceeds. It is thought that this withdrawal by the female represents the time of insertion of the male penis. Both snails remain motionless for some time after the female partial withdrawal and return. The male is tightly attached to the shell, and its tentacles are laid against its body.

Reciprocation and stimulation of male copulatory activity in females

Reciprocation begins with the female becoming active approximately 90 minutes from the beginning of copulatory activity, while the penis is still inserted into the female gonopore. The female at this time starts to act as a male. The penial movement and characteristics are similar to those observed when the male originated copulation. The female moves its body in such a way that the anterior region is attached to the shell of the male at the right anterior margin of the shell, and begins probing with its penis. During this time the male penis is still inserted into the gonopore of the female and the male is still tightly attached to the right margin of the female's shell. The female continues to move upon the shell of the male until the female switches places with the male.

The actual point of removal of the male penis was not observed, but it was noted that the male penis occasionally was still inserted after the female had assumed the mating position. The male penis was not observed to remain in the female vagina during reciprocation. To do so would create problems due to the positions of the respective gonopores. Copulation then proceeds in the reciprocal direction. Following the reciprocation, the pair separates. The male does not recopulate.

The male copulatory activity of the female can be directed towards a 3rd snail, rather than towards the male. The female was prevented from copulating with the male by placing the stimulated female, with the male still attached, in contact with the shell of a 3rd snail. The female quickly attaches to this shell and begins to copulate with the 3rd snail rather than with the male. After the original male ends its activity, it moves off the original female and shows no further interest in the remaining two snails.

The original female, now acting as male, continues to copulate with the 3rd snail. The 3rd snail becomes stimulated to act as a male in the same manner as the first female became stimulated. By placing the 3rd snail in contact with the shell of a 4th snail, the 3rd snail begins copulatory activity with the 4th. The original female finishes its male copulatory activity and departs. The male activity in females can probably be carried on through a large number of snails. In one experiment, the activity was shown through 5 snails, all females assuming male activity in turn before the experiment was terminated.

Male copulatory activity in the female occurred in nearly every case observed (40 of 42). Two females did not respond and were found upon dissection to contain no sperm in the hermaphroditic duct. Time of day apparently was not important, for observations of male activity in females were recorded throughout the day and evening.

The average male activity for 45 copulations was 123 minutes. This includes all male activity, i.e., spontaneous, reciprocations and male activity induced through 3 or more snails.

Status of the female reproductive tract subsequent to copulation

Dissection and/or sectioning of the reproductive tract immediately following copulation showed that the vaginal region, spermathecal duct and spermatheca are very dilated. The walls are extremely thin, and the above organs are fluid filled. The fluid contains small patches of material other than sperm, but the composition of the material was not determined. The posterior portion of the vagina and the anterior portion of the oöthecal gland contain the ejaculated sperm. Peristalsis of the dilated vagina and spermathecal duct was observed.

A white mass of material fills the vaginal lumen and sticks to the wall of the vagina at the junction of the vagina and spermathecal duct. Snails fixed and sectioned immediately following copulation showed the material to consist of areas of granules, areas of homogeneous-appearing material and areas which have a coagulated appearance (Fig. 1). Sperm were not observed to be

mixed with the mass. The granules stain bright yellow to yellow-green with methyl blue-picric acid. The granular material and most of the other material is protein as indicated with bromphenol blue. Mucopolysaccharides, indicated with Alcian blue 8GX (pH 2.5), are present in small amounts.

Snails fixed and sectioned 2 hours after acting as females showed that the granular appearance is somewhat reduced. By 3 hours after acting as females the mass of material appears to have decreased in size and is not tightly bound to the vaginal wall (Fig. 2). Four to 5 hours after acting as females, the material was usually displaced. The material was identified in the spermathecal duct and spermatheca (Fig. 3). Some animals observed from 4 to 5 hours after acting as females still had the mass in place but it was easily removed.

In one instance the male was interrupted before completing copulation. The female contained sperm but no mass, indicating that this material is passed to the female after ejaculation of the sperm.

Duration of the copulatory stimulus in activated females

Females were forcibly restrained from reciprocating until the male had finished and departed. The females were then isolated for 15 or 30 minutes and reunited with the male. If copulatory activity by the female after reunion with the male was observed, the female was re-isolated and again reunited. This procedure was repeated until no copulatory activity by the female was observed (Table 1).

TABLE 1. Duration of male copulatory activity in nine stimulated females isolated after male had finished copulation.

Snail	Time of isolation, minutes	Time to attempt copulation after reunion, minutes	Comments
1-3	15		No copulatory activity
4	15	10	Attempted copulation. Re-isolated
	15		No copulatory activity
5	15	20	Attempted copulation. Re-isolated
	15		No copulatory activity
6	30		Attached to partner, then moved off. No copulation
7	30	25	Copulated with partner
8	15	15	Attempted copulation. Re-isolated
	15	10	Attempted copulation. Re-isolated
	30		No copulatory activity
9	15	50	Attempted copulation. Re-isolated
	15	10	Attempted copulation. Re-isolated
	15	10	Attempted copulation. Re-isolated
	15	15	Attempted copulation. Re-isolated
	15		No copulatory activity

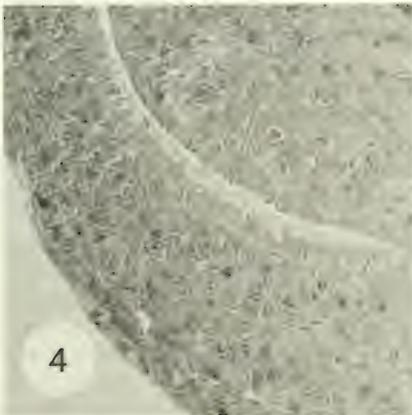


FIG. 1. Copulatory plug shortly after copulation. (1) Vaginal wall; (2) note the close adhesion to the wall; (3) granular areas; (4) gap probably due to fixation. Methyl blue-picric acid, hematoxylin.

FIG. 2. Copulatory plug in vagina, 3 hours following copulation. Bromphenol blue.

FIG. 3. Copulatory plug in spermathecal duct, 5 hours following copulation. Spermathecal duct is still somewhat dilated. Bromphenol blue.

FIG. 4. Prostate after 48 hours of isolation. Methyl blue-picric acid, hematoxylin.

FIG. 5. Prostate immediately following copulation. Methyl blue-picric acid, hematoxylin.

Status of the prostate and hermaphroditic duct
in males following copulation

Following copulation, the prostate of males is flaccid looking, in contrast to a turgid-appearing prostate prior to copulation. The secretory products of the prostate are depleted in most areas (Figs. 4, 5), although some prostates retained a substantial amount of secretory material following copulation. It appears that, often, copulation following 2 days of isolation nearly empties the prostate. The variation in amount of secretion remaining after copulation is presumably dependent on that present prior to copulation.

Prostates sectioned 1 day following copulation, in animals that had acted only as males, showed that the prostate contained more secretory products than those immediately following copulation. Sections of prostate made 2 days following copulation, in animals which had acted only as males, showed secretory products approximating the amount seen prior to copulation.

Hermaphroditic ducts from 26 snails were checked by dissection within 2 hours after completion of male activity. Of these 26 ducts, 19 were empty and 7 were depleted but were not empty. Thus, the duct may retain sperm in relatively large amounts but, more often than not, most or all of the sperm is passed during a single copulatory act. The amount of sperm remaining after copulation may depend upon how much was present prior to copulation.

Five animals which had acted only as males were isolated for 24 hours and 5 were isolated for 48 hours following copulation, and the contents of the hermaphroditic duct were checked by dissection. Animals isolated for 24 hours had little or no sperm in the hermaphroditic duct. Animals isolated for 48 hours had more sperm, and the duct was about $\frac{1}{4}$ to $\frac{1}{2}$ full.

DISCUSSION

Since members of the Lymnaeidae, Physidae, Planorbidae and Ancyliidae are generally simultaneously hermaphroditic, copulation can occur in various manners. It is deemed desirable to define terms for the various types of pairing which can occur among pulmonate snails.

Copulation: sexual intercourse between 2 snails. Copulation will thus be defined to include all male activity which occurs during the sexual intercourse of two snails.

Unilateral copulation: one member acts as male and the other member acts as female. A special case of unilateral copulation occurs in chain formation. Chain copulation is actually a series of unilateral copulations, since between any 2 members of the chain, copulation is unilateral.

Reciprocal copulation: both members act as male and female with each other.

(a) Simultaneously reciprocal copulation: both members act as male and female at the same time.

(b) Successively reciprocal copulation: both members act as male and female, in turn.

(1) Immediately successive reciprocal copulation: the pair does not separate before reciprocation occurs.

(2) Delayed successive reciprocal copulation: the pair separates before reciprocation occurs.

These categories may serve to describe any type of copulation which could conceivably occur between members of a pair.

The copulation of *Stagnicola elodes*, in snails which have been isolated for 48 hours and therefore have not copulated for that period of time at least, is nearly always an immediately successive reciprocation. The initial male to female behavior is very similar to the unilateral copulatory behavior described by Barraud (1957) for *Lymnaea stagnalis*.

Reciprocation occurs by the stimulation of the female to act as male. In the 2 instances in which the female failed to respond, it was observed that the hermaphroditic duct of the female contained no sperm. The same stimulation can be used to form chain, i.e., multiple unilateral, copulations.

It has been occasionally noted that immediately successive reciprocal copulation occurs in lymnaeids (Karsch, 1846; Künkel, 1908; Boettger, 1944; Noland & Carriker, 1946; Barraud, 1957). Except for Künkel (1908), the reports state that lymnaeids copulate unilaterally, with only occasional reciprocation.

It is probable that stimulation of male copulatory activity in the female occurs in other lymnaeids as well, as evidenced by the occasional reports mentioned above. Also, Barraud's (1957) description of multiple copulation in *Lymnaea stagnalis* indicates that stimulation of copulatory activity in the female had occurred, since the same type of chain formation was easily performed in this study by preventing reciprocation by the female and placing the female in contact with the shell of a 3rd snail.

The stimulus for male activity, both spontaneous and by stimulation, may be hormonal. This is indicated by the fact that (1) although male activity is originally spontaneous, male activity in the female is not, and (2) a male is not restimulated to male activity after a female has reciprocated. The male had not acted as female prior to its acting as male and could conceivably be receptive to a mechanical stimulation, if the stimulation to male activity in the female was solely dependent upon mechanical stimulation from the male penis. A hormonal transmitter would probably be depleted in the male after male activity. Whether such a hormone would be released in the female due to mechanical stimulation or by a secretion passed from the male, or both, is not known. The stimulus to copulate could be hormonally mediated via the brain, much as oviposition is stimulated in *Lymnaea stagnalis* (see Geraerts & Bohlken, 1976).

A component of the male secretions may stimulate male copulatory activity in female snails. It may do so directly or it may stimulate the release of some substance by the female which would then stimulate male activity. This would indicate that male copulatory activity can be stimulated in different ways. Spontaneous male activity is stimulated by unknown factors and induced male activity by factors which may include secretions from the male.

Since the female of spontaneously copulating pairs had also been isolated for 48 hours, it is possible that the male copulatory activity by the female was spontaneous rather than stimulated. However, isolated animals would act as females and assume male activity, even though they did not show spontaneous male activity, and 97% (40 of 42) of all females assumed male activity. These 2 facts speak in favor of a stimulation of male activity in the female.

Although it was not determined how long an interval after copulation is necessary before a snail can be stimulated to copulate again, the facts that a male is not stimulated to recopulate after reciprocation, that the hermaphroditic duct is usually severely depleted of sperm and that the prostate secretions are greatly depleted after copulation indicate that copulation cannot be restimulated to occur except after some time. In spontaneously male acting snails isolated after copulation, the hermaphroditic duct had filled enough after 2 days for copulation to occur again. However, 1 day after copulation the prostate contains secretory products, and it is possible that the prostate had recovered sufficiently for copulation to occur again. Also, not all snails passed all sperm in one copulation and these could conceivably copulate again without refilling of the hermaphroditic duct.

Whether or not the stimulation of copulatory activity in the female is mediated by secretions of the male reproductive system, the white mass in the vagina of the female is formed from male secretions. Proteins are a major constituent of the mass. The secretions of the male reproductive tract in *Stagnicola elodes* are overwhelmingly proteins (Rudolph, 1976), and the granules of the mass stain similarly to those of the prostate with methyl blue-picric acid. There is little doubt that the major part of the material comes from the prostate, although the rest of the male reproductive tract may also contribute to the mass. The proteinaceous mass in the vagina of the female is interpreted here as a copulatory plug. A coagulum formed from prostate secretions and situated at the junction of the vagina and the spermathecal duct in *Lymnaea stagnalis* was recognized by Horstmann (1955), and he suggested that it prevented the outflow of sperm. The materials which made up the coagulum in *Lymnaea stagnalis* were passed to the female after the ejaculation of the sperm. De Larambergue (1939) described, in *Bulinus contortus* (= *truncatus*), a "coagulum" which contained sperm and was thought to contain secretion from the prostate.

The plug in *Stagnicola elodes* contains no sperm and is neither the remains of a spermatophore nor used to hold the penis in place during copulation. One female was seen to contain sperm but no plug, and the penis of *Stagnicola elodes* possesses a penial knot which, in cooperation with the vaginal sphincter muscle, serves as a holdfast mechanism during copulation (Walter, 1969).

Although the formation of a copulatory plug is known from rats (Mann, 1964), insects (Parker, 1970) and snakes (Devine, 1975), it has not been interpreted as such in basommatophorans. The copulatory plug has a counterpart in other gastropods in the form of spermatophores. These are common in slugs and other Stylommatophora. Spermatophores are also present in prosobranchs (Hyman, 1967), opisthobranchs (Tardy, 1966), the lower basommatophoran families Chiliniidae (Harry, 1964) and Siphonariidae (Abe, 1940; Hubendick, 1944; Sumikawa & Onizuka, 1973), but not in the Lymnaeidae, Physidae, Planorbidae or Ancyliidae. According to Parker (1970), a spermatophore can function in the same manner as a copulatory plug.

Two possible functions of a copulatory plug (Parker, 1970) include prevention of sperm leakage following copulation and the prevention of a second successive copulation by another male and subsequent competition between the sperm of two males to fertilize the eggs of the female. Neither of these possibilities can be ruled out in *Stagnicola elodes*. During and immediately following copulation, the vagina, spermatheca and spermathecal duct are greatly dilated, transparent, fluid filled and turgid. It appears that a pressure builds up in the female tract and plug formation could aid in keeping this system intact. However, the gonopore region of *Stagnicola elodes* is heavily muscularized (Walter, 1969) and appears able to prevent sperm leakage.

The solidity of the plug and its adherence to the wall of the vagina immediately following copulation indicate that the plug could prevent a 2nd male from copulating following the first, at least for 2 or possibly 3 hours. This may be a critical period since, in *Lymnaea stagnalis*, sperm destined to fertilize the eggs move through the oviduct to the hermaphroditic duct in the first 2 to 3 hours following copulation (Horstmann, 1955). During the period of reciprocation in *Stagnicola elodes*, approximately 2 hours, the sperm from the first mating is probably moving up the female reproductive tract and the original female is very quiet. The original female is vulnerable to a 2nd mating when quiet, probably more so than when actively moving. The copulatory plug is in position during this time, however, and a copulation by another male would be prevented. The plug has a relatively short effective existence, however, since 3 hours following copulation the structure of the plug has changed and by 4 to 5 hours following copulation the plug is often displaced.

The combination of the 2 mechanisms, the stimulation of male copulatory activity in the female and the formation of a copulatory plug would insure insemination of both partners during the same mating by reciprocation and yet prevent competition from other males mating with either of the two reciprocating snails. This would be most effective when there are numerous spontaneous copulations occurring at the same time and in a restricted area. The arrival of conditions favorable to a burst of mating, such as during spring or precipitation after a dry spell, could constitute such a period.

The combination of these 2 mechanisms also suggests a way in which a copulatory stimulus could be passed through part of a population even though relatively few animals start to copulate spontaneously. If the snails were in close proximity to each other, it is possible that a female would copulate with a 3rd snail rather than reciprocate with the male. The presence of the plug in the female could prevent the 3rd snail from reciprocating and force it to move on to a 4th snail, etc. The stimulus could be maintained through a number of snails until either all had copulated, the stimulus was lost or until a snail was encountered which had acted as male but not female, and the cycle would stop.

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THE GONAD AND ITS DEVELOPMENT IN *DEROCERAS RETICULATUM*
(PULMONATA: LIMACIDAE)

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ABSTRACT

At hatching the gonad of *Deroceras reticulatum* consists of 1-3 acini and during the undifferentiated stage of the gonad this number increases to 70 to 180. In later stages the acini increase in size and often become lobed. The first formed acini consist of a lining epithelium of gonadal stem cells that are continuous with the epithelium in the ductule and a small group of cells filling the lumen. As the cells divide the acinus swells and cells similar to those in the lumen appear under the epithelium. The cells in the lumen differentiate into sperm, those under the epithelium into oocytes and follicle cells and the epithelial cells differentiate into sertoli cells. Around the entry of the ductule into the acinus the gonadal stem cells persist as the germinal epithelium. Further follicle cells and oocytes appear to differentiate from the edge of the germinal epithelium. Following castration, in young animals, complete and rapid regeneration from the hermaphrodite duct occurs and this parallels closely the early development of the gonad.

INTRODUCTION

Numerous studies have been undertaken on many aspects of the pulmonate gonad, however, as emphasised by recent reviews (Gomot, 1971; Luchtel, 1972), we are still far from understanding gametogenesis and the other functions of this organ. Gomot (1971) has clearly distinguished 3 fundamental processes that are involved in gametogenesis: the segregation of the germinal cells, the entry of germinal cells into gametogenesis, and the orientation of gametogenesis to either spermatogenesis or oogenesis. As yet none of these processes has been clarified. The authors wishing to study the endocrine relationships of the gonad of *Deroceras reticulatum* required a baseline study of its morphology and normal mode of functioning. The present paper is a preliminary report of our findings.

MATERIALS AND METHODS

Deroceras reticulatum, collected in the Bangor area, were fixed for light microscope studies in Susa fixative, embedded in Fibrowax, sectioned at 7 μ m and the sections stained in Azan. For studies in the electron microscope tissues were fixed in a mixture of osmium tetroxide and glutaraldehyde (Hirsch & Fedorko, 1968) and embedded in either Epon 812 or Araldite. Semi-thin sections were stained in toluidine blue and thin sections in lead citrate and uranyl acetate.

Scale models of acini were prepared from vinyl linoleum. Serial sections were photographed, enlarged and tracings made on the linoleum. These were then cut and glued together with Thixofix contact adhesive. After painting, the location and dimensions of the germinal epithelium and oocytes were marked on their surfaces.

RESULTS

Origin and initial development of the gonad

During the first few weeks after hatching the reproductive system is difficult to study as it is extremely thin. At hatching it consists of a simple blind ending duct opening to the exterior at

the genital pore. The cells lining the tract are cuboidal to columnar in shape and contain a basal nucleus with dense aggregations of chromatin in a granular matrix (Fig. 1). Within their cytoplasm numerous free ribosomes, clear vesicles and multivesicular bodies are present. From the luminal surface project microvilli and most cells possess 1 or 2 cilia, indicative of primary ciliogenesis. After about 7 days the majority of cells have developed accumulations of granules resembling glycogen. The closed end of the duct differentiates into the gonad. This is first seen as a slight swelling at the tip, and in some animals this is the state of the gonad at hatching. The swelling forms a terminal acinus. In other animals one or two tubular outgrowths close to the terminal acinus are also present at this time and acini develop at their tips.

Histologically it can be seen that the early acini are lined by an epithelium continuous with that in the remainder of the duct (Fig. 2). It is proposed to term the cells of the lining epithelium in the gonad region gonadal stem cells (G.S.C.) as they appear to differentiate into all of the cell types found in the mature gonad. Within the developing acinus proliferation of



FIG. 1. Transverse section of hermaphrodite ductule from animal at hatching (osmium/glutaraldehyde fixation, lead citrate-uranyl acetate staining). L—lumen with sections of cilia, G—gonadal stem cells.

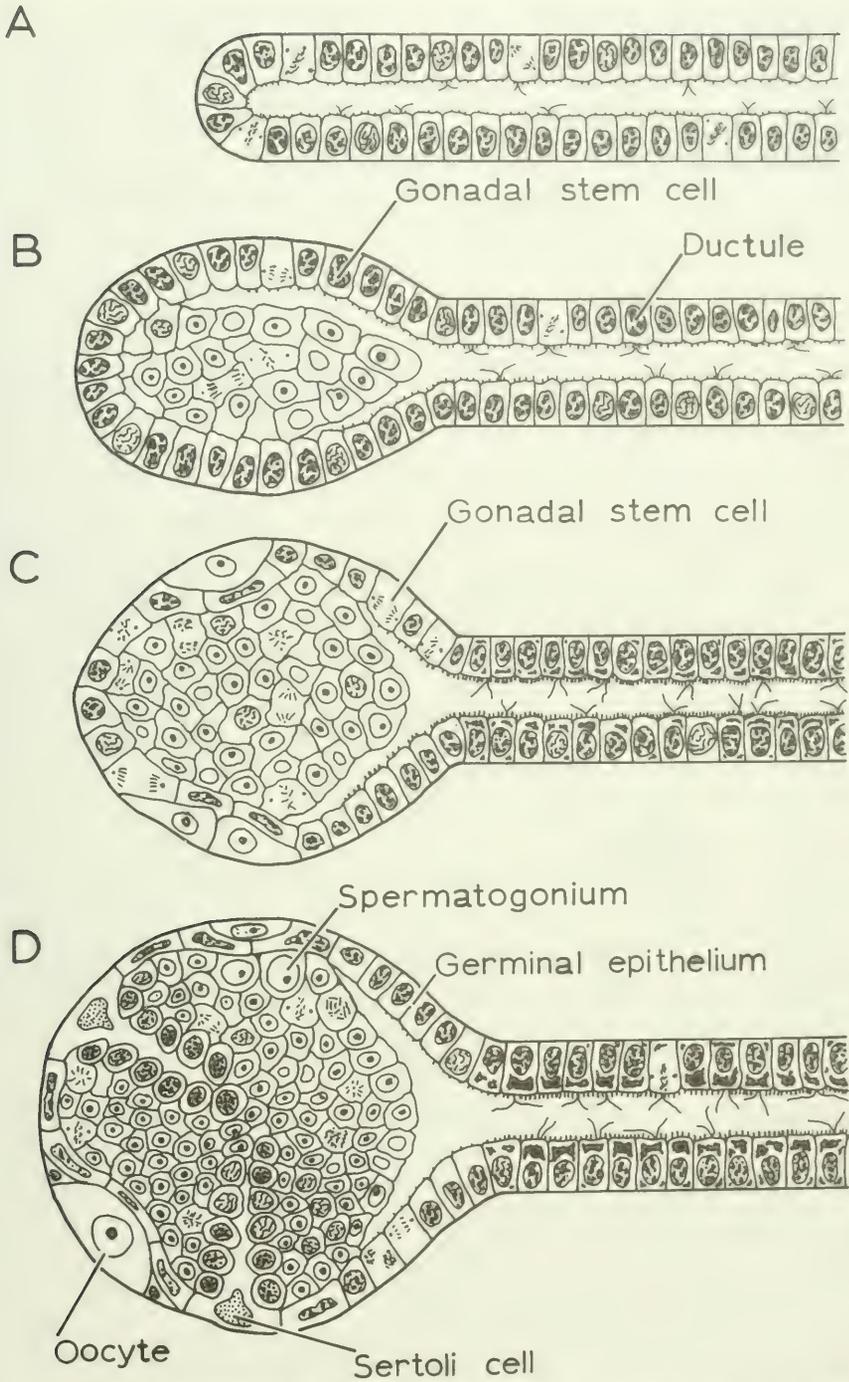


FIG. 2. Diagrams illustrating the early development of the acinus. A. Closed end of ductule. This stage has only been seen in the regenerating gonad. B. Tip of ductule swollen with proliferating cells. This is the stage of acinus development found at hatching. C. Further proliferation of cells filling the lumen and the appearance of cells beneath the epithelium. D. Spermatocyte stage acinus. The lumen is filled with developing sperm and under the continuous layer of sertoli cells are developing oocytes and follicle cells.

cells from the G.S.C. layer leads to an accumulation of cells in the lumen (Fig. 2 and 4). These cells are irregular in outline with low numbers of ribosomes and a clear nucleus (Fig. 3). Similar cells appear beneath the G.S.C. layer and are accompanied by a few very small cells. The G.S.C. persist in the ductules and as an epithelial layer around the neck of the acinus termed the germinal epithelium, but the remaining G.S.C's lining the majority of the acinus differentiate into sertoli cells. The cells filling the lumen of the gland differentiate into spermatogonia while those between the sertoli cell layer and basement membrane evolve into oocytes and follicle cells.

Further development of the gonad

In a previous study it was shown that the relative proportion and arrangement of gametes varies throughout the life of the animal (Runham & Laryea, 1968). The development of the

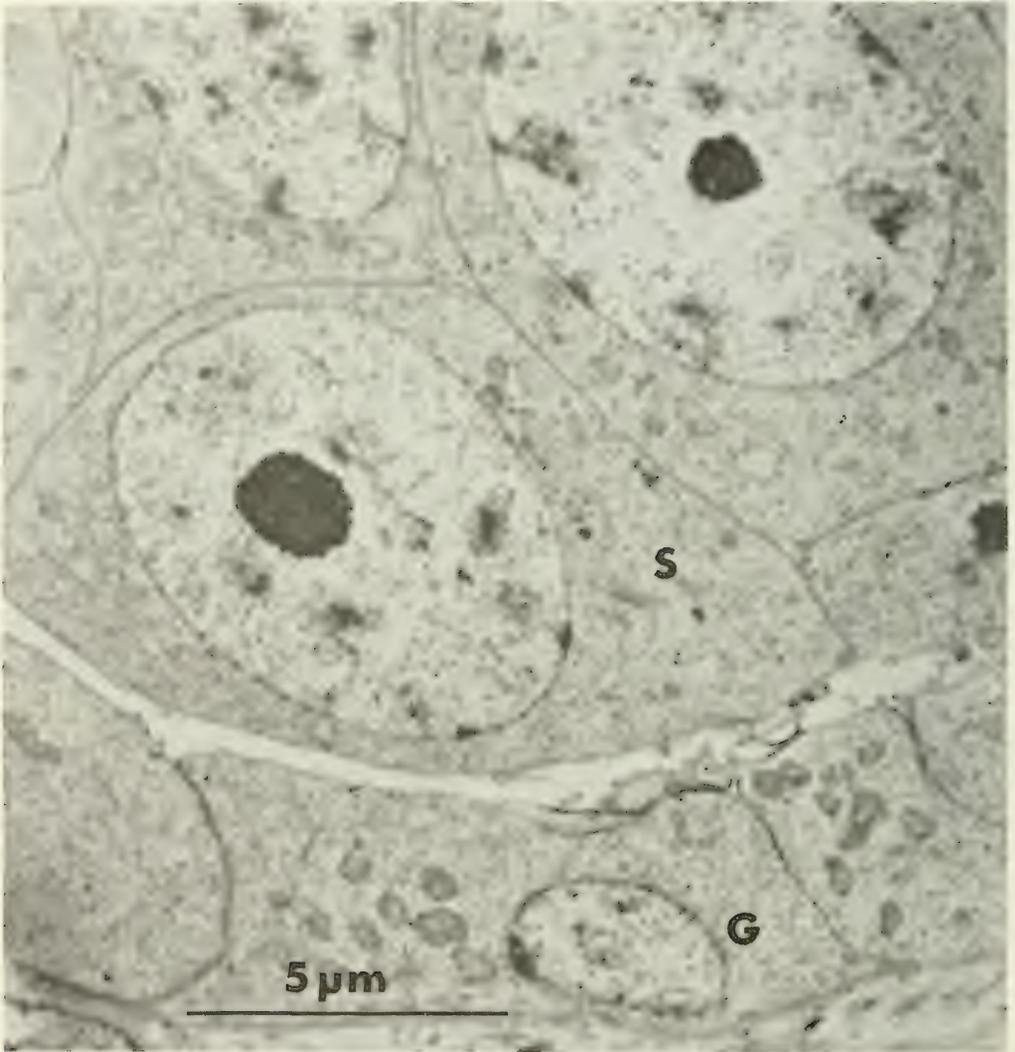


FIG. 3. Section of wall of acinus from newly hatched animal (osmium/glutaraldehyde fixation, lead citrate-uranyl acetate staining). G—gonadal stem cells. S—cells in lumen of acinus which will differentiate into sperm.

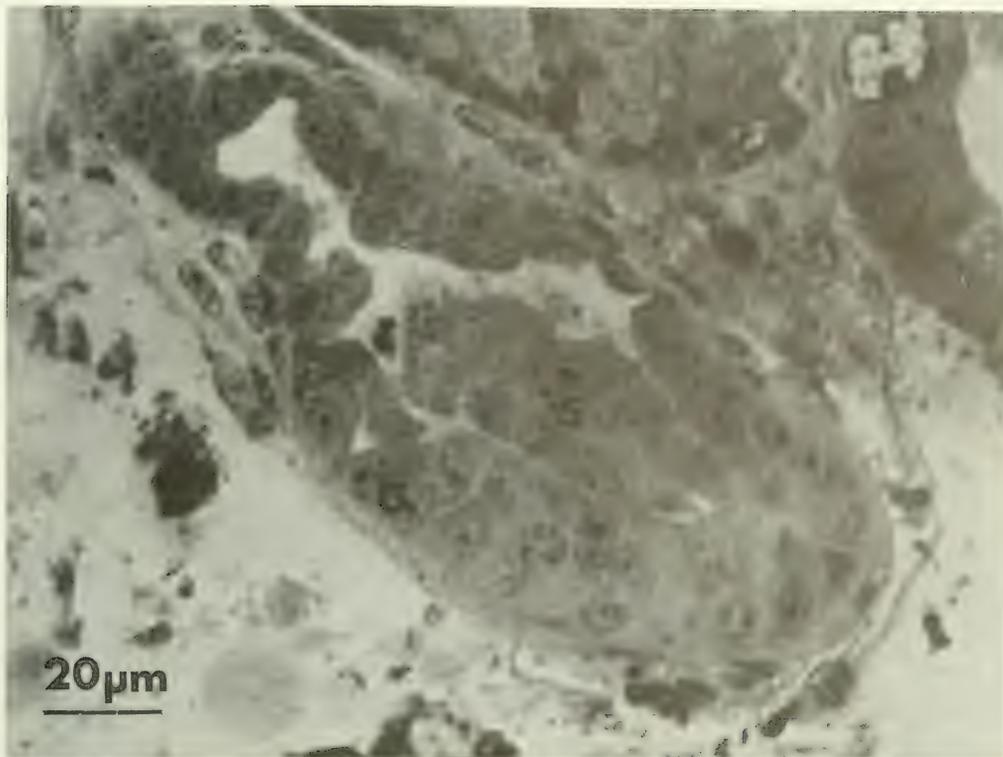


FIG. 4. Longitudinal section of acinus from animal at hatching (osmium/glutaraldehyde fixation, $1\ \mu\text{m}$ section stained toluidine blue). G—gonadal stem cells, S—cells in lumen of acinus which will differentiate into sperm.

gonad was therefore subdivided into 8 stages: (a) undifferentiated, (b) spermatocyte, (c) spermatid, (d) early spermatozoon, (e) late spermatozoon, (f) early oocyte, (g) late oocyte, and (h) post reproductive. During (a) it now appears that processes leading to proliferation of acini predominate leading to formation of between 70 and 180 acini of the typical gonad structure. Enlargement of the acini continues during (b) to (e) stages largely because of the predominance of spermatogenesis. By the (f) stage the acinus starts to empty of sperm and many mature oocytes are present.

From examination of random sections of the gonad it is difficult to understand how cells are arranged within the acinus, so scale models of acini were constructed (Fig. 5). From these it was evident that the smallest oocytes were located close to the edge of the germinal epithelium (the germinal ring) and that oocyte size increased with distance from the epithelium. The largest oocytes were therefore always located at the base of the acinus opposite the ductule opening. When degenerating oocytes were present they were situated between the large oocytes.

Gamete maturation

Oogenesis appears to continue throughout the life of the animal, but mature oocytes first appear towards the end of the late spermatozoon stage and are present in large numbers throughout the oocyte stages. A study of oogenesis and vitellogenesis has been completed and will be published elsewhere, but in outline they are very similar to the processes described for *Biomphalaria glabrata* (De Jong-Brink et al., 1976). Spermatogenesis is also very similar to that reported in other pulmonates (e.g., De Jong-Brink et al., 1977).

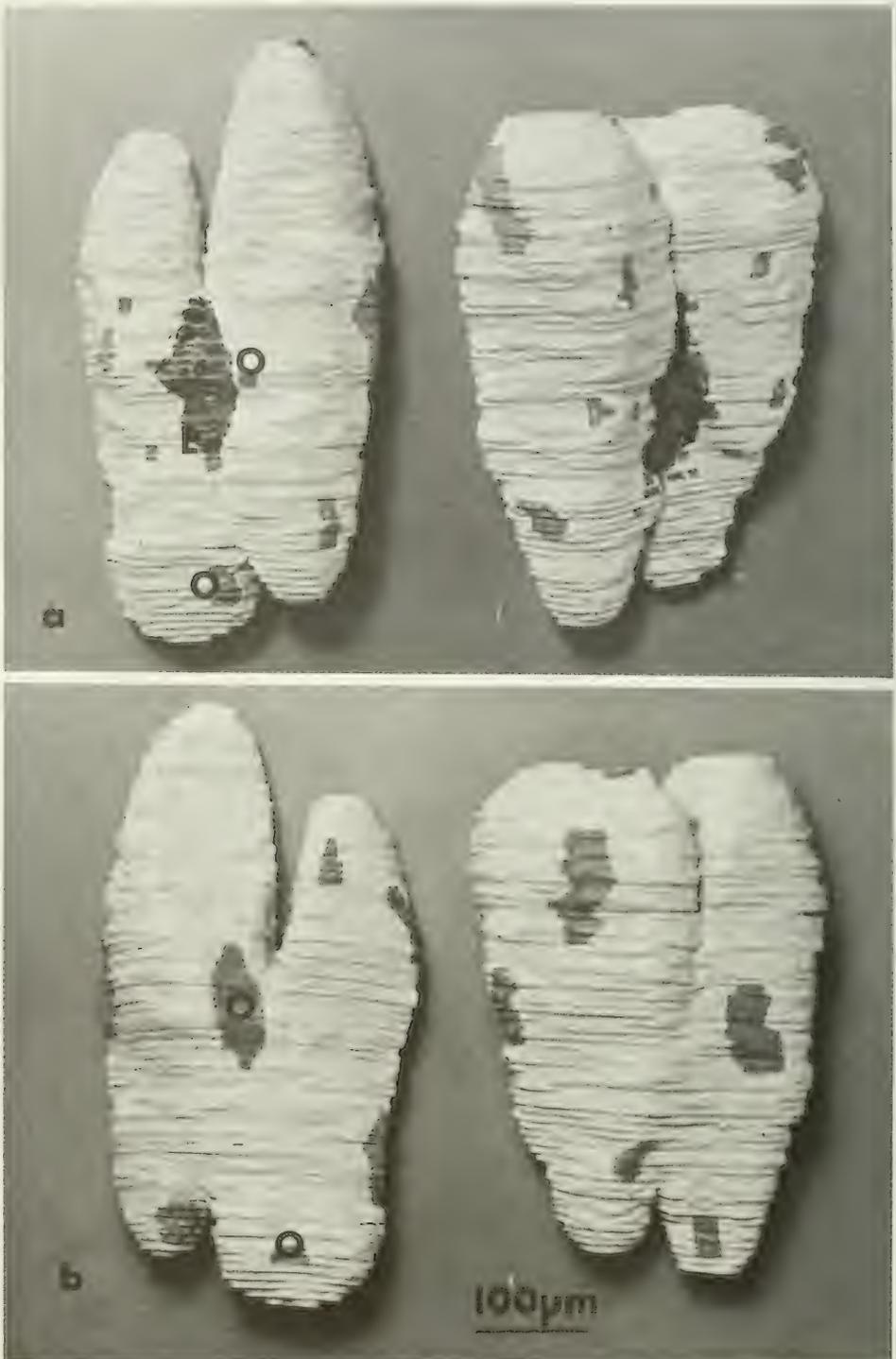


FIG. 5. Reconstruction of two acini from an early spermatozoan stage gonad. a. Top of the acini, b. Bottom of the acini. E—germinal epithelium around entry of the hermaphrodite duct. O—oocytes.

Regeneration of the gonad

While studying the effects of castration on the development of the reproductive tract (Runham, 1976 and unpublished) it was found that very rapid regeneration of the gonad occurred during the undifferentiated and spermatocyte stages, but was slow during the spermatid stage and largely absent from all older animals. In some spermatozoon stage animals 1 or 2 minute acini had formed after 5 months.

A short time after castration muscular contraction in the wall resulted in sealing of the hermaphrodite duct. Proliferation of connective tissue elements led to the formation of a mass of tissue around the wound. Subsequently, the duct cells dedifferentiated. Outgrowths of the duct into the surrounding mass of connective tissue were at first tubular and then acini developed at their tips. The sequence of differentiation within these regenerated acini closely mirrored the development of normal acini during the undifferentiated stage (Fig. 6). The reason for the lack of regeneration in the older animals is as yet unknown, but it may be significant that during the early spermatozoon stage the lining epithelium normally differentiates; the cells becoming either ciliated or non-ciliated cells.

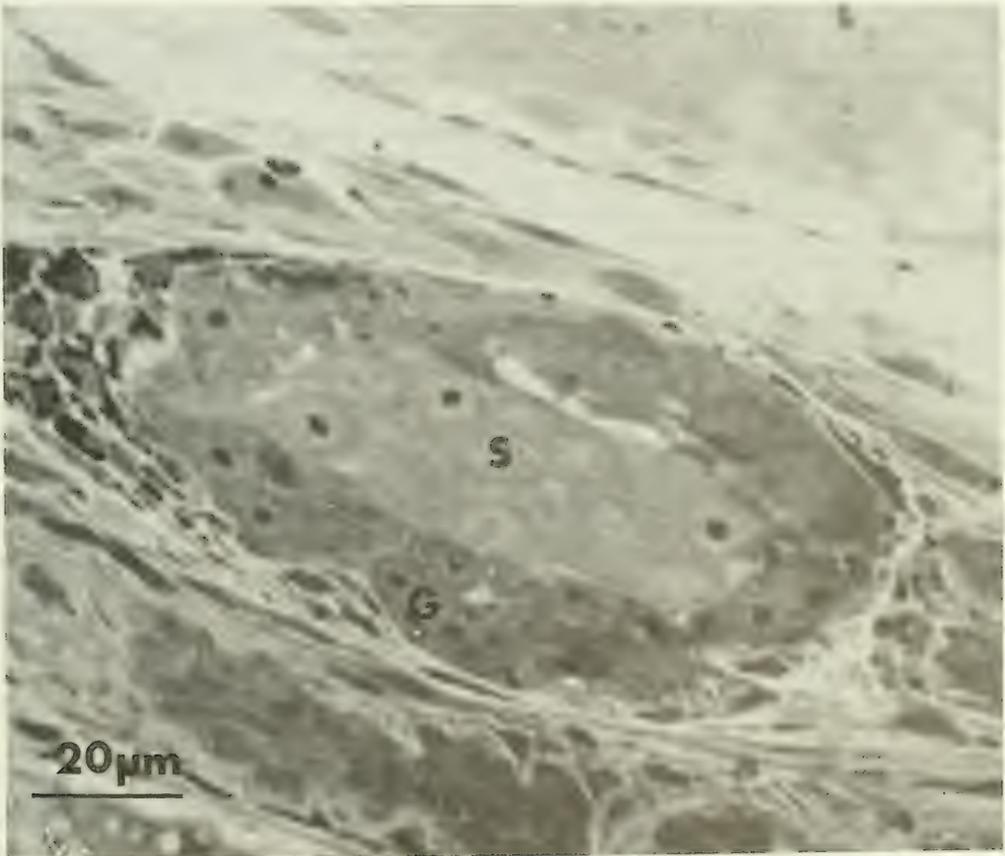


FIG. 6. Longitudinal section of regenerated acinus (osmium/glutaraldehyde fixation, 1 μ m section stained toluidine blue). G—gonadal stem cells, S—cells in lumen which will differentiate into sperm.

DISCUSSION

The embryological origins of the gonad and reproductive tract in pulmonates has proved very difficult to study (Martoja, 1964). Much misinterpretation may be due to poor fixation resulting in difficulties in differentiating it from the accompanying blood vessels and nerves.

Our observations indicate that the reproductive system has a single origin and as the simple tube is continuous with the skin of the animal we presume it is ectodermal in origin. The gonad originates from the closed end of this tube and apparently only the cells of the lining epithelium participate in the formation of the acinar contents. We are therefore essentially in agreement with Laviolette (1954) and Luchtel (1972a).

Luchtel (1972a and b) denied the existence of a germinal epithelium in *Arion circumscriptus*, *A. ater rufus* and *Deroceras reticulatum*. This study provides strong evidence for its existence in *D. reticulatum*: firstly, all the cells of the gonad including the germ cells derive from the G.S.C. which forms the germinal epithelium in the fully formed acinus; secondly, mapping of oocytes indicates a clear size gradient, the smallest being next to the epithelium and the largest furthest away from it; lastly, despite the absence of a pool of indeterminate germ cells regeneration of the gonad can occur from the severed hermaphrodite duct.

As the cells lining the hermaphrodite duct are gonadal stem cells, it is not surprising that development of the regenerated gonad is so similar to the gonad's initial development. The stages of regeneration reported here are similar to the three stages described by Laviolette (1954); namely, (1), wound nodule formation; (2), digitation; (3), appearance of crypts (acini). Laviolette also reported that the germ cells arose from dedifferentiated epithelial cells. Our results are in accord with this view. The reduced ability to regenerate in older animals may be related to the state of differentiation of the hermaphrodite duct as reported above, or to the levels of reproductive hormones in the circulation.

In their description of the gonad of *Lymnaea stagnalis* Jooisse & Reitz (1969) state that there is active migration of gametes around the wall of the acinus from the germinal ring where they are first formed to the base of the acinus. While such a process may be necessary in *Lymnaea* with its very extended period of reproduction, particularly in the laboratory, it is not necessarily present in *D. reticulatum*. The enlargement of the acini through the spermatocyte to the spermatozoon stages is so great that this could account for the observed distribution in size of the oocytes, the largest oocytes arising when the acinus was small and remaining at the base of the acinus without any active movement. At the moment it is not possible to distinguish between these 2 possibilities.

All recent work appears to indicate that the acinus is subdivided into 2 compartments; the developing sperm being located towards the centre, and the oocytes around the outside against the basement membrane. The 2 groups are separated by a layer of sertoli and follicle cells. How the 2 gametes become distributed in this way is not yet clear, but it is possible that proliferation of the G.S.C. leads to delamination, the most basal layer forming the oocytes.

The remarkable similarity between oogenesis in *B. glabrata* and *D. reticulatum* makes it likely that the processes involved are similar in other pulmonates. Hill & Bowen (1976) and Hill (1977) have reported their studies on the oocytes of *D. reticulatum* and the role of the accessory cells. They describe a process of extensive yolk sequestration by vacuolation and a sertoli cell oocyte relationship involving thin cytoplasmic bridges and large intercellular spaces. We have never observed such features in well-fixed material.

We are grateful to Miss Carole Roberts for making the reconstructions.

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INFLUENCE DU JEÛNE ET DE LA RENUTRITION SUR L'OVIPosition ET LES GAMÉTOGÈSES CHEZ LE PLANORBE *BIOMPHALARIA GLABRATA* (GASTÉROPODE PULMONÉ BASOMMATOPHORE)

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ABSTRACT

In the planorbid *Biomphalaria glabrata* (Pulmonata, Basommatophora) starvation causes a progressive decrease in the number of egg masses and eggs produced by the animals; however, the average number of eggs per egg mass does not change. Ten to 12 days starvation are necessary to stop oviposition completely. In the ovotestis starvation causes the terminal stages of spermatogenesis and oogenesis to disappear. As soon as the animals are being fed again they start depositing progressively more egg masses and eggs. First oviposition after resumed feeding depends on the length of the period of starvation, i.e. occurs earlier after shorter periods of starvation. Male and female gametogenesis will again run their normal course and the process is completed simultaneously.

INTRODUCTION

Chez le Planorbe *Biomphalaria glabrata*, si la privation de nourriture dure suffisamment longtemps, l'oviposition est bloquée. S'ils sont ultérieurement renourris, les animaux peuvent à nouveau pondre et donc se reproduire.

Le présent travail a pour but de:

- suivre la diminution de la fécondité lorsque les animaux jeûnent;
- suivre la restauration du pouvoir reproducteur après renutrition;
- préciser les conséquences du jeûne puis de la renutrition sur les gamétogènes mâle et femelle.

MATÉRIEL ET TECHNIQUES

J'ai utilisé des *Biomphalaria glabrata* de souche brésilienne, sexuellement mûrs. Les expériences ont été organisées de la façon suivante:

—Première expérience

Première partie

Soixante Planorbes féconds sont répartis en 6 groupes numérotés 1 à 6, de 10 individus chacun; chaque animal est élevé isolément dans 600 ml d'eau. Avant le début de l'expérience tous les individus sont nourris.

Au temps 0 de l'expérience, les animaux du groupe 1 sont soumis au jeûne, les autres continuant à recevoir de la nourriture. Au bout de 5 jours, les Planorbes du groupe 2 sont à leur tour privés de nourriture, ceux du groupe 1 le demeurant. Chaque 5 jours un nouveau groupe est soumis au jeûne. Cette première partie de l'expérience dure 25 jours. On dispose alors de 6 groupes numérotés 1, 2, 3, 4, 5 et 6 qui ont jeûné respectivement 25, 20, 15, 10, 5 et 0 jours.

Durant cette première partie de l'expérience, la fécondité est enregistrée pour chaque animal tous les 5 jours. Pour caractériser la fécondité, 3 valeurs sont utilisées: (a) nombre moyen de pontes par animal; (b) nombre moyen d'oeufs par animal; (c) nombre moyen d'oeufs par ponte.

Deuxième partie

Au bout de 25 jours, les animaux des 6 groupes reçoivent tous de la nourriture. La fécondité est enregistrée, non plus chaque 5 jours mais journalièrement.

Une 2ème expérience a été réalisée. Elle est comparable à celle qui vient d'être décrite mais chaque groupe est constitué par 10 Planorbes élevés dans un même bac et non plus isolés les uns des autres.

Les résultats obtenus sont soumis à une analyse statistique. La méthode principalement utilisée est l'analyse de variance. A l'intérieur d'un ensemble de moyennes, la comparaison des moyennes 2 à 2 est réalisée selon les cas par la décomposition orthogonale partielle ou, le plus souvent, par le test de Tukey (1949).

RÉSULTATS

Dans la première expérience, on connaît la fécondité de chaque animal. Pour chaque période, la fécondité d'un groupe peut donc être exprimée par une moyenne et un écart-type, ce qui permet ultérieurement d'effectuer des tests statistiques. Ce sont donc essentiellement les résultats de cette expérience qui seront exposés.

Première partie

(1) On constate que 25 jours de jeûne n'entraînent aucune mortalité.

(2) Dans chaque groupe, après l'instauration du jeûne, on assiste à une diminution progressive du nombre de pontes par animal et d'oeufs par animal. Si le jeûne a une durée suffisante (de 10 à 20 jours selon les groupes), la réduction de la fécondité peut aller jusqu'à un blocage total de l'oviposition, la fécondité étant alors nulle.

(3) La diminution du nombre de pontes est significative dès le 5ème jour de jeûne. Quelle que soit la période considérée, entre 0 et 5 jours mais aussi entre 5 et 10 jours de jeûne, on a des différences statistiquement significatives (Tableau 1). Au delà du 10ème jour, les moyennes ne diffèrent plus. Ceci ne signifie pas que le jeûne est devenu sans effet sur la fécondité mais au

TABLEAU 1. Comparaison, tous les 5 jours, des nombres moyens de pontes par animal. Le signe < indique une différence statistiquement significative, tandis que le signe = en indique l'absence. La virgule placée entre deux moyennes indique que la comparaison n'est pas autorisée. Pour chaque moyenne, le chiffre placé en indice correspond au numéro du groupe. Les moyennes situées dans une même bande diagonale correspondent à un jeûne de même durée. m: nombre moyen de pontes par animal pour une durée de 5 jours; Gr.: groupe.

	Gr. 1	Gr. 2	Gr. 3	Gr. 4	Gr. 5	Gr. 6	
0 à 5	m ₁	< m ₂	, m ₃	, m ₄	, m ₅	, m ₆	
5 à 10	m ₁	< m ₂	< m ₃	, m ₄	, m ₅	, m ₆	
10 à 15	m ₁	= m ₂	< m ₃	< m ₄	, m ₅	, m ₆	
15 à 20	m ₁	= m ₂	= m ₃	< m ₄	< m ₅	, m ₆	
20 à 25	m ₁	= m ₂	= m ₃	< m ₄	< m ₅	< m ₆	
durée du jeûne		25	20	15	10	5	0

TABLEAU 2. Comparaison, tous les 5 jours, des nombres moyens d'oeufs par animal. Mêmes explications que pour le Tableau 1, mais m: nombre moyen d'oeufs par animal pour une période de 5 jours.

	Gr.1	Gr.2	Gr.3	Gr.4	Gr.5	Gr.6
0 à 5	m_1	$= m_2$	$= m_3$	$= m_4$	$= m_5$	$= m_6$
5 à 10	m_1	$< m_2$	$< m_3$	$, m_4$	$, m_5$	$, m_6$
10 à 15	m_1	$< m_2$	$= m_3$	$< m_4$	$, m_5$	$, m_6$
15 à 20	m_1	$= m_2$	$= m_3$	$< m_4$	$< m_5$	$, m_6$
20 à 25	m_1	$= m_2$	$= m_3$	$= m_4$	$< m_5$	$< m_6$
durée du jeûne		25	20	15	10	5

contraire que le nombre de pontes est très réduit voire nul et qu'il en est ainsi tant que les animaux sont privés de nourriture. D'ailleurs, au 25ème jour, si l'on compare les nombres totaux de pontes déposées depuis le début de l'expérience, on constate qu'entre 0 et 25 jours, 5 jours de jeûne entraînent des différences significatives. Cinq jours de jeûne provoquent une différence significative du nombre d'oeufs par animal sauf pour la période 0 à 5 jours. Comme pour les pontes, l'action du jeûne continue à se faire sentir après 10 jours. La fécondité qui est fortement réduite ou même nulle, le demeure tant que le jeûne ne cesse pas (Tableau 2).

(4) Au terme de la première partie, les nombres totaux de pontes et d'oeufs de chaque groupe montrent une corrélation linéaire hautement significative avec la durée du jeûne: pour les pontes, on a $r = 0,97$ et pour les oeufs, on a $r = 0,96$ alors que dans les 2 cas on a $r_{0,01} = 0,92$. Les animaux produisent d'autant moins de pontes et d'oeufs que le jeûne subi a été long (cf. Fig. 1).

On obtient un résultat comparable avec les animaux groupés de la 2ème expérience. Les nombres totaux de pontes et d'oeufs sont supérieurs à ceux de l'expérience 1 car dans ce dernier cas, on a un léger effet inhibiteur de la fécondité dû à l'isolement (Vianey-Liaud, 1976).

(5) Le jeûne entraîne une diminution du nombre de pontes et d'oeufs. Une analyse statistique révèle qu'en revanche le nombre moyen d'oeufs par ponte n'est pas affecté par la privation de nourriture. Au fur et à mesure que le jeûne se prolonge, le nombre d'oeufs par ponte correspond à des nombres de pontes et d'oeufs de plus en plus réduits, mais il n'est pas lui-même modifié.

Deuxième partie

(1) Après renutrition, on enregistre une mortalité non négligeable puisqu'elle atteint 32% des animaux en 25 jours.

(2) La renutrition est suivie d'un retour à la situation d'origine. Les animaux qui avaient présenté une réduction du nombre de pontes et d'oeufs, voient ces nombres augmenter progressivement. Ils déposent de plus en plus de pontes et produisent de plus en plus d'oeufs. La fécondité du groupe 6 qui n'a jamais jeûné demeure sensiblement constante.

(3) Si l'on procède aux même analyses statistiques que durant le jeûne, on constate que les différences alors mises en évidence se maintiennent au plus 20 jours; 25 jours après la renutrition, les effets du jeûne sur le dépôt des pontes et la production des oeufs ont disparu. Ces nombres ne redeviennent cependant pas semblables à ce qu'ils étaient au début de l'expérience. Ceci est la conséquence de l'isolement des animaux (Vianey-Liaud, 1976).

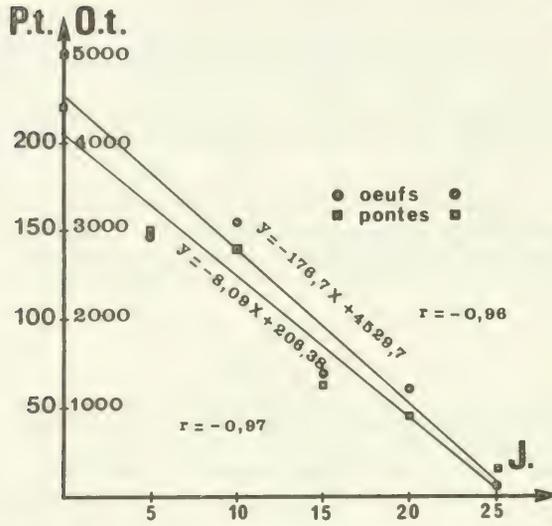


FIG. 1. Fécondité totale des Planorbes de chaque groupe, à la fin de la première partie de l'expérience. Pour les oeufs et les pontes, est donnée l'équation de la droite de régression. J.: durée du jeûne, exprimée en jours; O.t.: nombre total d'oeufs produits; P.t.: nombre total de pontes déposées; r.: coefficient de corrélation linéaire.

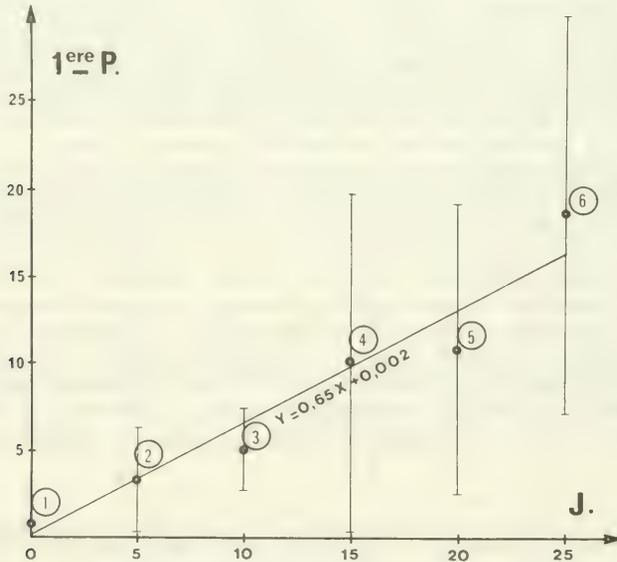


FIG. 2. Délai séparant la renutrition de la première oviposition, en fonction de la durée du jeûne préalable. Les chiffres inscrits dans un cercle correspondent au numéro du groupe. Les limites de l'intervalle de confiance sont calculées pour la probabilité $P = 0,05$. J.: durée du jeûne, exprimée en jours; 1ère P.: délai séparant la renutrition du dépôt de la première ponte, exprimé en jours.

D'ailleurs dans la 2ème expérience, la fécondité redevient très proche de ce qu'elle était avant le jeûne.

(4) Le comptage journalier des pontes et des oeufs permet de savoir, pour chaque animal, combien de jours après la renutrition apparaît la première ponte. Ces résultats sont présentés dans la Fig. 2.

Les données numériques servent à effectuer une analyse de variance portant sur la régression. Il ressort de cette étude que la durée du jeûne préalable a un effet significatif sur le délai qui sépare la renutrition de la première ponte et qu'entre 0 et 25 jours ces 2 durées sont proportionnelles. Cinq jours de jeûne préalable entraînent un retard compris statistiquement entre 2,1 et 4,3 jours. Plus le jeûne a été long, plus les animaux tardent à pondre après renutrition.

J'ai effectué une étude histologique de l'ovotestis des Planorbes durant le jeûne et après renutrition.

La gonade d'un animal qui a jeûné au moins 25 jours diffère sensiblement de celle d'un témoin. Elle renferme les stades initiaux des gamétogenèses mâle et femelle. Sont présents: les spermatogonies et les spermatocytes 1 en prophase de méiose ainsi que les ovocytes de 50 μm de diamètre (taille maximum des ovocytes: 100 μm) qui n'ont pas achevé leur vitellogenèse. Les stades plus évolués (spermatocytes 1 en métaphase et anaphase, spermatocytes 2, spermatides, ovocytes de plus de 50 μm) manquent. Ceci crée l'aspect "vide" de l'ovotestis d'un Planorbe sous alimenté. Il arrive que soient présents des spermatozoïdes, d'ailleurs jamais très abondants; ils ont été formés avant le jeûne et n'ont pas encore été évacués.

L'ovotestis d'un Planorbe qui jeûne est donc une gonade active dans laquelle spermatogenèse et ovogenèse n'aboutissent pas à la production de cellules sexuelles mûres.

Après la renutrition, les stades manquants des gamétogenèses mâle et femelle apparaissent à nouveau tandis que des cellules sexuelles jeunes continuent d'être élaborées. Il n'y a pas de décalage dans la restauration de la spermatogenèse et de l'ovogenèse. En une dizaine de jours, les 2 gamétogenèses sont intégralement représentées dans la gonade.

DISCUSSION

Chez *Biomphalaria glabrata*, Christie et al. (1974) par une étude biochimique et Jong-Brink (1973) par une étude histochimique et ultrastructurale, montrent que la privation de nourriture retentit profondément sur le fonctionnement des tractus génitaux.

Le jeûne provoque une diminution du nombre de pontes et d'oeufs produits. Si sa durée est suffisante, l'oviposition est complètement bloquée.

Les calculs statistiques que j'ai effectué témoignent bien de l'influence du jeûne, à la fois sur le nombre d'oeufs produits et sur la quantité de pontes déposées. Le jeûne agit à 2 niveaux:

- sur l'ovotestis (qui produit les oeufs),
- sur les tractus (qui produisent dans la ponte tout ce qui n'est pas le zygote).

Ces 2 points d'impact de la privation de nourriture sont relativement indépendants l'un de l'autre. En effet:

—l'action du jeûne sur les tractus génitaux (nombre de pontes) précède celle sur l'ovotestis (nombre d'oeufs). D'ailleurs, l'étude histologique de l'ovotestis au bout de 5 jours ne révèle pas de changement notable alors que le nombre de pontes a déjà chuté significativement.

—si le jeûne n'agissait que sur l'ovotestis, on pourrait obtenir des pontes stériles, sans oeuf (phénomène possible chez *Biomphalaria*), ce qui n'est jamais le cas dans les présentes expériences.

—10 jours après renutrition, alors que la totalité des gamétogenèses est rétablie dans l'ovotestis, si le jeûne a été long (au moins 25 jours), les animaux ne déposent aucune ponte. La première oviposition survient au moins une semaine après la restauration des gamétogenèses.

Ceci montre donc que le jeûne agit d'abord sur les tractus génitaux et plus tardivement sur l'ovotestis. A l'inverse, après renutrition, c'est sur l'ovotestis que les effets cessent en premier; ils persistent plus longtemps sur les tractus génitaux.

L'ovotestis et les tractus génitaux fonctionnent donc de façon relativement indépendante. Le jeûne agit cependant sur les 2 organes, ce qui réduit ou même bloque totalement la reproduction.

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THE CONTROL OF SEXUAL DIFFERENTIATION BY THE CEPHALIC
COMPLEX IN THE SLUG *ARION SUBFUSCUS* DRAP.
(GASTROPODA PULMONATA)

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ABSTRACT

The problem of determination of sexual differentiation in the slug *Arion subfuscus* has been approached through the organ culture method. Cultures concern young gonads obtained from animals 8-10 days old, i.e. at the earlier post-embryonic stage. A cytological study of these infantile gonads has been made at light and electron microscope levels. Three different cell types are distinguished: (a) at the periphery of the gonad, a cortical layer whose nuclei (3-4 μm diameter) are rich in chromatin, uniformly distributed in coarse masses. These are *stock cells*. (b) Some of these cortical nuclei show an increase in diameter (4-5 μm) and correlatively their chromatin scatters. They represent the *protogonia*. (c) Centrally positioned are cells (6-8 μm diameter) with a pale-staining nucleus in which chromatin is scarce and peripherally located. They have the characteristics of *spermatogonia*. Our results indicate: (1) When cultured in isolation, in an anhormonal medium, there is a tendency for an infantile gonad to progress towards the female line. (2) Cultures of gonadial material associated with autologous or heterologous cephalic complexes demonstrate the part of this complex in sex realisation of *A. subfuscus*. (3) The optic tentacles exert an inhibiting influence on female differentiation during the infantile phase (their presence in the culture medium prevents appearance of female cells) but are ineffective during the female phase. (4) The brain seems to stimulate the female line but its role must be approached carefully on account of the isolated gonad feminization tendency. These results are in line with those previously obtained by means of in vivo experiments concerning the role of the optic tentacles.

INTRODUCTION

In *Arion subfuscus*, optic tentacle extirpation, performed at hatching or on juvenile castrated specimens, results in a clear feminization of the gonad (Wattez, 1973). These data led us to approach the problem of the determination of sexual differentiation through the organ cultures method about which 2 reports concerning adult genital glands of slugs were known (Badino, 1967; Bailey, 1973). Our paper considers sexual differentiation; gonads were taken from young animals at the infantile stage, i.e. at the earlier post-embryonic stage. During our experiments we cultured isolated infantile genital glands and associations of gonads with the whole or parts of the cephalic complex (optic tentacles-brain) taken either from the genital gland donor (autologous associations) or from slugs in the female phase of their genital cycle (heterologous associations).

MATERIALS AND METHODS

Slugs

Laboratory-reared (according to Laviolette, 1954) specimens of *A. subfuscus* were used throughout this study. They were supplied daily with lettuce.

Organ culture method

The technique is based on that developed by Wolff & Haffen (1952), the agar synthetic medium including 199 solution (Institut Pasteur). Cultures were maintained in darkness for 15 to 21 days at 20°C.

Histological studies

For light microscopy, explants were fixed in Bouin-Hollande fluid and embedded in paraffin wax. Serial sections (6 µm) were stained with Prenant triple coloration or haemalun and eosin.

For ultrastructural studies, infantile gonads were fixed for 3 h at 4°C in 3% phosphate buffered glutaraldehyde (Taab). They were then washed overnight in 0.3 M buffer before being post-fixed in 1% Osmium tetroxide (Lyon-Alemand Louyot) at laboratory temperature for 1 h. The gonads were subsequently dehydrated in a series of acetone baths and finally embedded in araldite. Ultrathin sections were cut with glass knives on a Porter-Blum MT 1 Ultramicrotome and stained with Uranyl acetate and Lead citrate prior to examination in a Siemens electron microscope. Semi-thin sections stained in Toluidine Blue were helpful to establish a correlation between the cellular types found in light microscopy and at the ultrastructural level.

RESULTS

To determine the sexualization potentialities, it is better to study the gonad before the 2 sexual categories arise. So, we cultured infantile gonads either isolated or in the presence of whole or dissociated cephalic complexes, which were either autologous or heterologous (taken from slugs in female phase).

The genital gland is explanted from animals 8-10 days old at a stage defined as infantile. At this earlier post-embryonic stage, the gonad is represented by small groups of cells on the external wall of the genital artery and comprises (Fig. 1):

(a) at its periphery, cells whose nuclei (3-4 µm diameter) are rich in chromatin, distributed in coarse masses: the *stock cells*;

(b) a 2nd cellular type is constituted by cells whose nucleus is 5 µm in diameter. This growth is correlated with a scattering of chromatin. They also contain in their cytoplasm "granular bodies" of undetermined nature. They represent the *protogonia* (Fig. 2);

(c) centrally located are some cells with a diameter of 6-8 µm. They have a pale-staining nucleus in which chromatin is scarce and peripherally located, the center being occupied by a single and prominent nucleolus. They have the characteristics of *spermatogonia*.

Considering the impossibility to cut up the gonad with regard to its size (100 µm), the genital gland from a slug of the same age from the same batch of eggs is considered as control.

(1) Culture of isolated infantile gonads

A histological study of such an explant cultured for 3 weeks (Fig. 3) shows the appearance of numerous oocytes, which are completely absent when the organ is put in culture. Nevertheless, feminization is not complete, some spermatogonia still being found.

(2) Associations of the infantile gonad with the whole or parts of the cephalic complex

(a) Autologous associations

After 21 days of culture, most of the complete associations stay at a *status quo*. However, it is not uncommon to note the presence of 1 or 2 oocytes. So, it appears that only 2 organs are concerned with sexual differentiation in these slugs: the optic tentacles and the brain. Accordingly, cultures of gonads associated with the tentacles or with the brain were carried out.

In the case of gonad-tentacles associations fixed after 15 (Fig. 4) or 21 days of culture, it is shown histologically that the presence of the tentacles alone prevents oocytes appearing.

Close association of the brain with gonadal material causes appearance of oocytes in the acini of the genital gland, which are scarce. This is already noticeable after 15 days of culture.

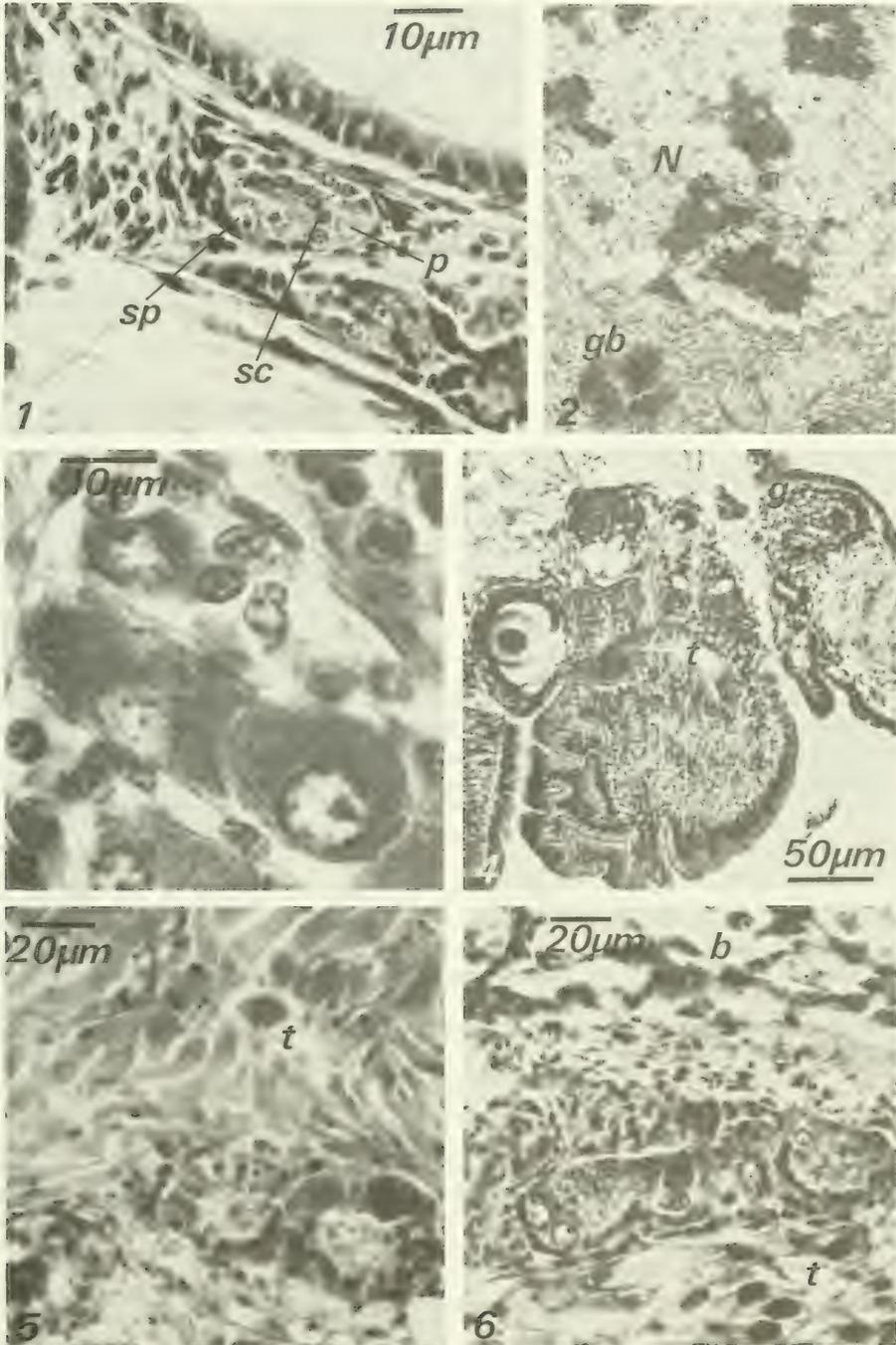


FIG. 1. Section of the gonad from a slug 8-10 days old. Three cell types are present. FIG. 2. Electron micrograph of a protogonium (ca. 24,000X). FIG. 3. Isolated infantile gonad after 21 days of culture. Note the appearance of oocytes. FIG. 4. Autologous association gonad-optic tentacles at the infantile stage, cultured for 15 days. The infantile tentacle has an inhibiting effect on the differentiation of the female line. FIG. 5. Aspect of an infantile gonad cultured for 21 days in association with optic tentacles taken from slugs in the female phase. FIG. 6. Triple association infantile gonad-infantile optic tentacles—"female" brain after 21 days of culture. Abbreviations: b, brain; g, gonad; gb, granular bodies; N, nucleus; p, protogonium; sc, stock cell; sp, spermatogonium; t, optic tentacle.

(b) Heterologous associations

The cephalic complex, taken from animals in the female phase of their genital cycle, was tested *in vitro* by heterologous associations of infantile gonad-"female" cephalic complex.

At the end of the culture period (21 days), infantile gonads associated with cephalic complexes or only with the tentacles (Fig. 5) present clear oocyte development.

Infantile gonads cultured in association with brains taken from slugs in the female phase evolve towards the female line. After 21 days of culture, we can notice the presence of oocytes.

These 3 types of heterologous associations demonstrate the loss, during the female stage, of the inhibiting power of the tentacle on female differentiation. Confirmation can be obtained by doing the triple association infantile gonad-"female" tentacles-infantile brain test; after 21 days of culture the female line in the gonad is strongly developed.

CONCLUDING REMARKS

The present experiments provide some data related to sexual differentiation in slugs:

(1) Differentiation of the female line

The female autodifferentiation demonstrated in Crustacea by Charniaux-Cotton & Ginsburger-Vogel (1962) is likely to be the rule in Gastropoda as well (see Guyard, 1971, and Streiff, 1967), but as it applies only to undifferentiated germ cells, this explains that if infantile gonads of slugs are cultured in isolation, they produce numerous oocytes as well as some spermatogonia.

(2) Role of the optic tentacles

This series of cultures shows evidence of the inhibiting effect of the infantile tentacles on the differentiation of the female line, a result parallel with the one obtained by Guyard (1971) by means of *in vitro* cultures of *Helix aspersa*. Moreover, it is demonstrated that these organs are ineffective during the female phase, which confirms results previously obtained *in vivo* after tentacles removal eventually followed up with injections of tentacular extracts (see Wattez, 1973 and 1975).

(3) Influence of the brain

The role of the brain is somehow difficult to elucidate on account of the isolated gonad feminization tendency; it is indeed not possible to dissociate autonomous action of this organ from an effect consecutive to absence of the optic tentacles. Nevertheless, it has to be pointed out that in case of association of an infantile gonad with a brain (either infantile or "female") we may expect to find after 3 weeks of culture in some explants, despite the presence of the infantile tentacles (see Fig. 6), 1 or 2 oocytes per acinus in addition to the presence of spermatogonia.

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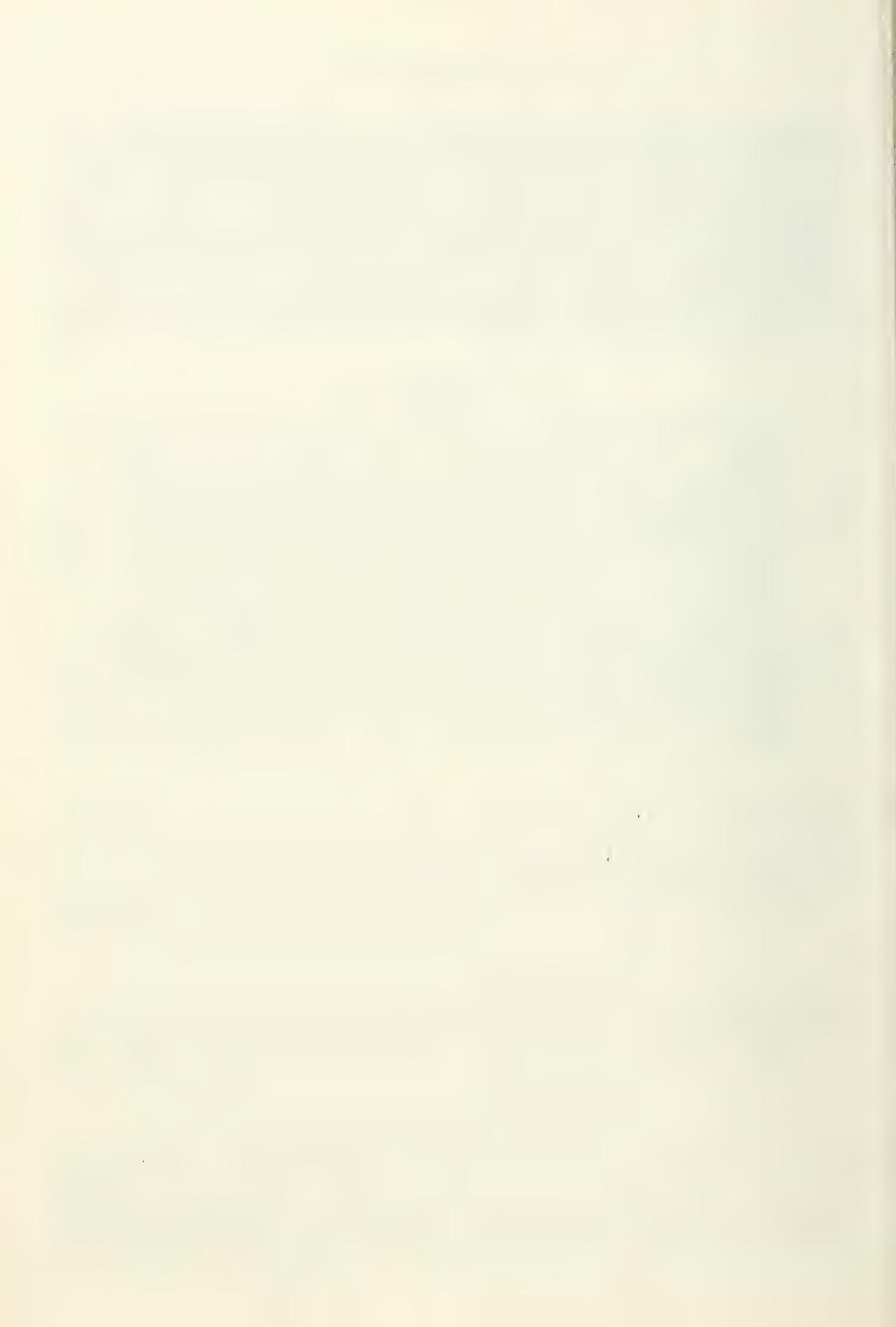
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RÉSUMÉ

Le problème du déterminisme de la différenciation sexuelle chez la limace *Arion subfuscus* a été abordé par la méthode des cultures organotypiques. Des gonades infantiles, prélevées sur des animaux âgés de 8 à 10 jours, c'est-à-dire au stade post-embryonnaire le plus précoce, ont été effectuées sur milieu gélosé à base de solution 199. Une étude cytologique de ces jeunes glandes génitales a été réalisée en microscopie photonique et au niveau ultrastructural, 3 types cellulaires y ont été retrouvés: (a) à la périphérie de la gonade, une couche corticale dont les noyaux (d'un diamètre de 3 à 4 μm) sont riches en chromatine, uniformément répartie en grains grossiers. Ce sont les *cellules souches*. (b) Certains noyaux voient leur diamètre augmenter (4-5 μm) et corrélativement leur chromatine se disperser. Ils représentent les *protogonies*. (c) En position centrale se trouvent des cellules d'un diamètre de 6 à 8 μm à noyau clair et à chromatine rare. Elles ont l'allure de *spermatogonies*. L'ensemble des cultures effectuées permet d'établir les points suivants: (1) Une gonade infantile cultivée isolément en milieu an hormonal évolue dans le sens femelle. (2) Les cultures de glandes génitales associées avec des complexes céphaliques autologues ou hétérologues complets ou dissociés démontrent la participation de ce complexe dans la réalisation du sexe chez les limaces. (3) Les tentacules oculaires exercent une influence inhibitrice sur la différenciation de la lignée femelle mais perdent ce pouvoir chez les animaux en phase femelle. (4) Le cerveau semble stimuler la lignée femelle mais son rôle est plus difficilement interprétable eu égard à la tendance que présente une gonade isolée d'évoluer dans le sens femelle.



ELEKTRONENMIKROSKOPISCHE UNTERSUCHUNGEN ZUR REGENERATION BEI NUDIBRANCHIERN¹

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ABSTRACT

The regeneration of the cerata was studied in *Aeolidiella soemmeringi* (Leuckart, 1828) by light and electron microscopy. After amputation of the tips of the cerata the edges of the epidermis and of the hepatopancreas are drawn together by muscular contraction. The restitution of the cnidosac and of the hepatopancreas begins within groups of reserve cells, which are to be found in the hepatopancreas at each time. The restitution of the epidermis starts from undifferentiated replacement cells in the epidermis. Undifferentiated cells, which probably are fibroblasts and amoebocytes form a loose, spongy blastema. The amoebocytes become more and more loaded with phagocyte material and often show a pycnotic nucleus with very densely packed chromatin, while the fibroblasts predominantly form the fibrocytes of the mesenchym of the growing ceras. Thus it seems, that to a certain extent each tissue, i.e. ectoderm, entoderm and mesoderm, retains its individuality and is repaired from its own elements.

Die Nudibranchier zeichnen sich unter den Gastropoden durch eine vorzügliche Regenerationsfähigkeit aus. Diese wurde bereits Ende des vorigen Jahrhunderts beobachtet (vgl. z. B. Hecht, 1896; Glaser, 1910) und von Cuenot (1907) elegant zum Nachweis des exogenen Ursprungs von Cleptocniden genutzt; er entfernte die Cerata bei Aeolidiern und konnte nach 2 Wochen neue Kolbenanlagen beobachten. Neuerdings prüfte Bürgin-Wyss (1961) die Regeneration in Zusammenhang mit der Musterbildung, Wolter (1967) und Baleyrier (1969) die Rhinophorenregeneration und Kress (1968) lichtmikroskopisch die Regeneration nach Kolben-Autotomie bei verschiedenen *Doto*-Arten, also einer Gattung aus der Unterordnung der Dendronotoidea, die keinen Cnidosack besitzt.

Unsere Untersuchungen wurden an *Aeolidiella soemmeringi* (Leuckart, 1828) vorgenommen (24 adulte Versuchstiere aus dem Watt von Roscoff; Material und Methodik vgl. Schmekel & Weischer, 1973), einem Vertreter aus der Unterordnung der Aeolidioidea. Die Cerata der Aeolidioidea stellen finger- oder kolbenförmige, bei 14 untersuchten Neapler Arten blindgeschlossene Ausstülpungen des Ektoderms dar, in die hinein ein Ast des Hepatopankreas zieht. Das distale Ende des Mitteldarmdrüsenastes ist zu einem Cniden der Futterhydroiden speichernden Organ, dem Cnidosack differenziert. Zwischen Mitteldarmdrüse und Körperepithel finden sich Nerven, Muskeln und ein sehr lockeres Bindegewebe. Bei unseren Versuchstieren schnitten wir mit einer Schere bei etwa einem Fünftel aller Kolbenreihen das obere Drittel der Cerata ab. Obwohl wir im Unterschied zu Kress (1968) also nicht die Autotomie ausnutzten, sondern stets viel größere Wunden setzten, bestätigen unsere Ergebnisse lichtmikroskopisch voll einige klare Befunde von Kress: auch bei *Aeolidiella* wird Mitteldarmdrüsenepithel dominierend aus Mitteldarmdrüsenepithel gebildet und Epidermis von Epidermis (Reygrobelle, 1970, berichtet demgegenüber von Dedifferenzierungen bei Limacidae und Arionidae).

Der Regenerationsvorgang sieht lichtmikroskopisch bei *Aeolidiella* folgendermassen aus: Unmittelbar nach dem Abschneiden des oberen Kolbendrittels kontrahieren sich die Cerata-Muskeln und schliessen die Wunde bis auf eine zentrale Öffnung, die meist rasch von Mucus überzogen wird, der vermutlich das Austreten weiterer Zellen verhindert. Der Prozess der Kontraktion geht in den ersten 24 bis 48 h kontinuierlich weiter. Dabei werden Epidermiszellen der Wundränder in Richtung Wundzentrum geschoben und oft abgeflacht. Im allgemeinen ist am 2ten Regenerationstag die Wunde durch ein Epithel aus alten Epidermiszellen abgeschlossen.

¹Mit Unterstützung durch die Deutsche Forschungsgemeinschaft.

Dieser Vorgang lässt sich leicht beobachten, weil die reifen Epidermiszellen auf Grund ihrer charakteristischen Vakuolen klar von undifferenzierten oder jungen Epidermiszellen zu sondern sind. Vom zweiten Tag an beobachtet man basal in der Epidermis Mitosen in undifferenzierten Zellen, die dort beständig vorliegen (Schmekel & Wechsler, 1967). Die Mitoserate bleibt während der ganzen ersten Woche klein, wobei zu bedenken ist, dass auch die unverletzten Kolben von *Aeolidiella* stark dehnbar sind, sodass ein dominierend mechanisches Verschliessen der Wunde keine Schwierigkeiten bereitet. Bereits am 2ten Tag beginnt in einigen der jungen Zellen die Ausbildung der charakteristischen Vakuolen. Die weitere Differenzierung der Epidermiszellen stimmt mit der für *Trinchesia granosa* beschriebenen überein (Schmekel & Wechsler, 1967).

Auch der Prozess des Schliessens des Mitteldarmdrüseneschlauches erfolgt zunächst mechanisch. Das Hepatopankreasepithel besitzt beständig einzelne oder mehrere, zu Nestern konzentrierte, undifferenzierte Zellen am Ende seiner Buchten. Hier beobachtet man vom zweiten Regenerationstag an Mitosen. Eine oder mehrere dieser Zellgruppen in Wundnähe bilden kleine Zonen embryonal erscheinenden Epithels, von denen in der Folge eine terminale sich gegen die Epidermis vorwölbt und später blindsackartig gegen diese vorwächst. Aus den übrigen embryonalen Zonen geht meist normales Mitteldarmdrüsenepithel hervor. Es können aber auch zwei oder drei solcher Anlagen sich stärker gegen das Körperepithel vorwölben und Cerataanlagen bilden, was dann zu den bekannten Missbildungen mehrendiger Cerata führt. Die Hauptanlage, die zunächst aus einem einschichtigen Epithel kleiner, stark basophiler Zellen besteht, wächst fingerförmig aus. Füttert man kontinuierlich, so lassen sich am 4. Tag nach der Amputation in den Zellen am Ende des Fortsatzes die ersten phagozytierten Cniden beobachten. Die Zellen an der Basis des Fortsatzes dagegen behalten ihren undifferenzierten Charakter, womit die definitive Form des Cnidosackes mit distaler Cnidenspeicherregion und proximaler Sprossungszone entstanden ist (Kälker & Schmekel, 1976). Die weitere Differenzierung der Mitteldarmdrüsenzellen erfolgt ähnlich wie bei *Trinchesia granosa* (Schmekel & Wechsler, 1968).

Wesentlich schwieriger zu klären als die Geschehnisse in Epidermis und Mitteldarmdrüsenepithel, sind die Vorgänge im Zwischengewebe mit seinen zahlreichen bis heute noch unbekanntem Anteilen. Auf die Regeneration von Nerven und Muskulatur kann im Rahmen dieses Referates nicht näher eingegangen werden. Von den bei den Aeolidiern bekannten Elementen des interstitiellen Bindegewebes scheinen die Spezialzellen (Schmekel, 1972) überhaupt nicht, die Blaszellen (= pore cells, Sminia, 1972) nur ausnahmsweise an der Wundschliessung beteiligt. Beide sind nur selten, und dann wohl eher zufällig, im Wundbereich anzutreffen. Dort dominieren zwei andere Zelltypen, die Amöbocyten und die Fibroblasten. Die Zellbezeichnungen werden in Übereinstimmung mit Sminia (1972) verwendet. Die Bezeichnung Amöbocyt umfasst sowohl die Wanderform dieser Zellen in der Hämolymphe, wie auch die mehr festsitzende Form im Bindegewebe, die ich 1972 Mesenchymzelle nannte. Ob Amöbocyten und Fibroblasten eventuell auf eine gemeinsame Ursprungsform zurückgehen oder sich ineinander umzuwandeln vermögen, kann nicht entschieden werden. Vom 2ten Regenerationstag an sind beide Zelltypen feinstrukturell deutlich trennbar.

Die Fibroblasten (Fig. 1) (vermutlich Lymphocyt I von Kress, 1968 und cellules interstitielles von Nicaise, 1972; vgl. auch Sminia, 1972, und Wondrack, 1969) sind kleine Zellen, die, einzeln oft abgerundet oder spindelförmig erscheinend, sich im Wundbereich ansammeln und relativ dicht, blastemartig zusammenlegen, manchmal vermischt mit einigen Amöbocyten. Sie zeichnen sich als junge Zellen durch eine stark zugunsten des Kerns verschobene Kernplasmarelation aus. Der im Umriss meist ovale Kern zeigt ein relativ kompaktes Karyoplasma und deutliche Nucleoli. Der schmale, dichte Cytoplasmasaum enthält einen kleinen Golgiapparat, wenige kleine, glatte ER-Vesikel, kleine Mitochondrien, kleine Zonen rauhen endoplasmatischen Retikulums und relativ viele freie Ribosomen, die häufig rosettenförmig angeordnet sind. Grössere Vakuolen mit telosomalen Inhaltskörpern kommen nur vereinzelt vor. Die Zellen bilden in wechselndem Ausmass einige wenige, meist grössere Fortsätze. Das Plasmalemm verläuft gerade bis leicht gewellt und besitzt oft einen schmalen Mantel aus feinstfibrillärem Material. Soweit erkennbar, gehen aus diesen weitgehend undifferenzierten Zellen die ebenfalls am zweiten Regenerationstag deutlichen Fibrocyten (Fig. 2) hervor. Die Fibrocyten weisen mehr, längere und schmalere Zellausläufer auf als die Fibroblasten, besitzen mehr und grössere Vesikel mit telosomalem Inhalt, einen grösseren Golgiapparat, meist kernnah

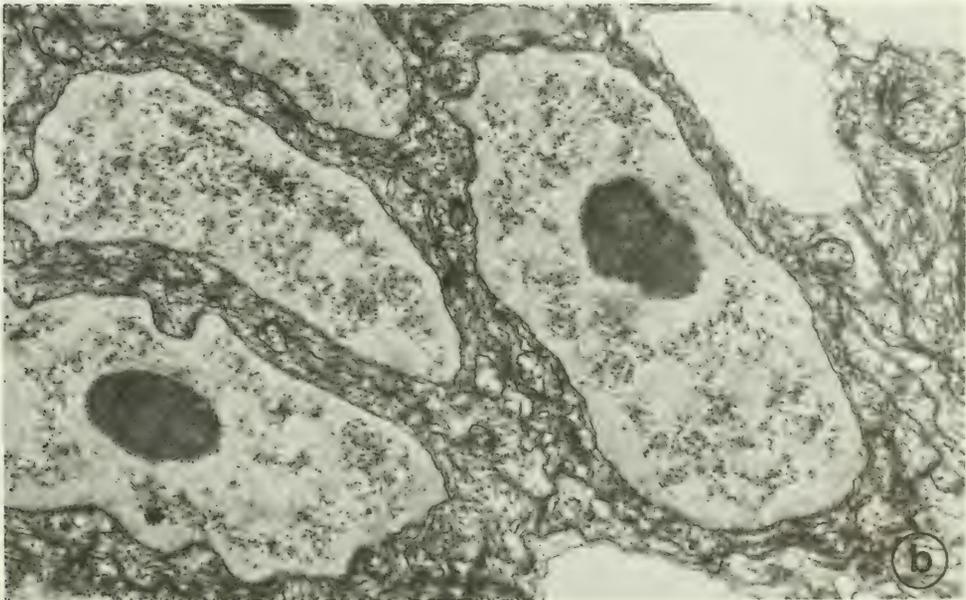
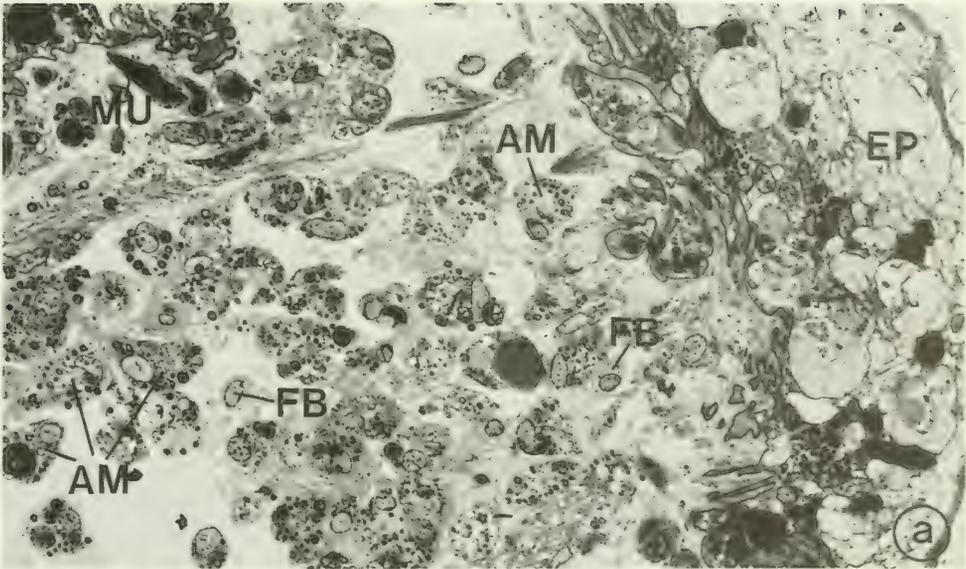


FIG. 1a. Blastemartige Zellansammlung unter der Epidermis (EP), 2. Regenerationstag. AM—Amöbocyt, FB—Fibrocyt, MU—Muskulatur (Glutaraldehyde, OsO_4 , Vergr. 700X). 1b. Vier undifferenzierte Blastemzellen ($\text{K}_2\text{Cr}_2\text{O}_7$, OsO_4 , Vergr. 12 000X).

eine markante Region rauhen endoplasmatischen Retikulums und manchmal auch erhebliche Glykogenansammlungen. Im Cytoplasma und in Vesikeln sind in unterschiedlichen Ausmass Filamente anzutreffen. Charakteristisch sind die der Zelloberfläche aufsitzenden Büschel von ca. 100 bis 300 Å dicken, im Querschnitt ein dunkles Zentrum aufweisenden Kollagen Fasern. Ein Mantel von sehr viel feineren, unregelmässig vernetzten Fibrillen lässt sich ausserdem häufig beobachten.

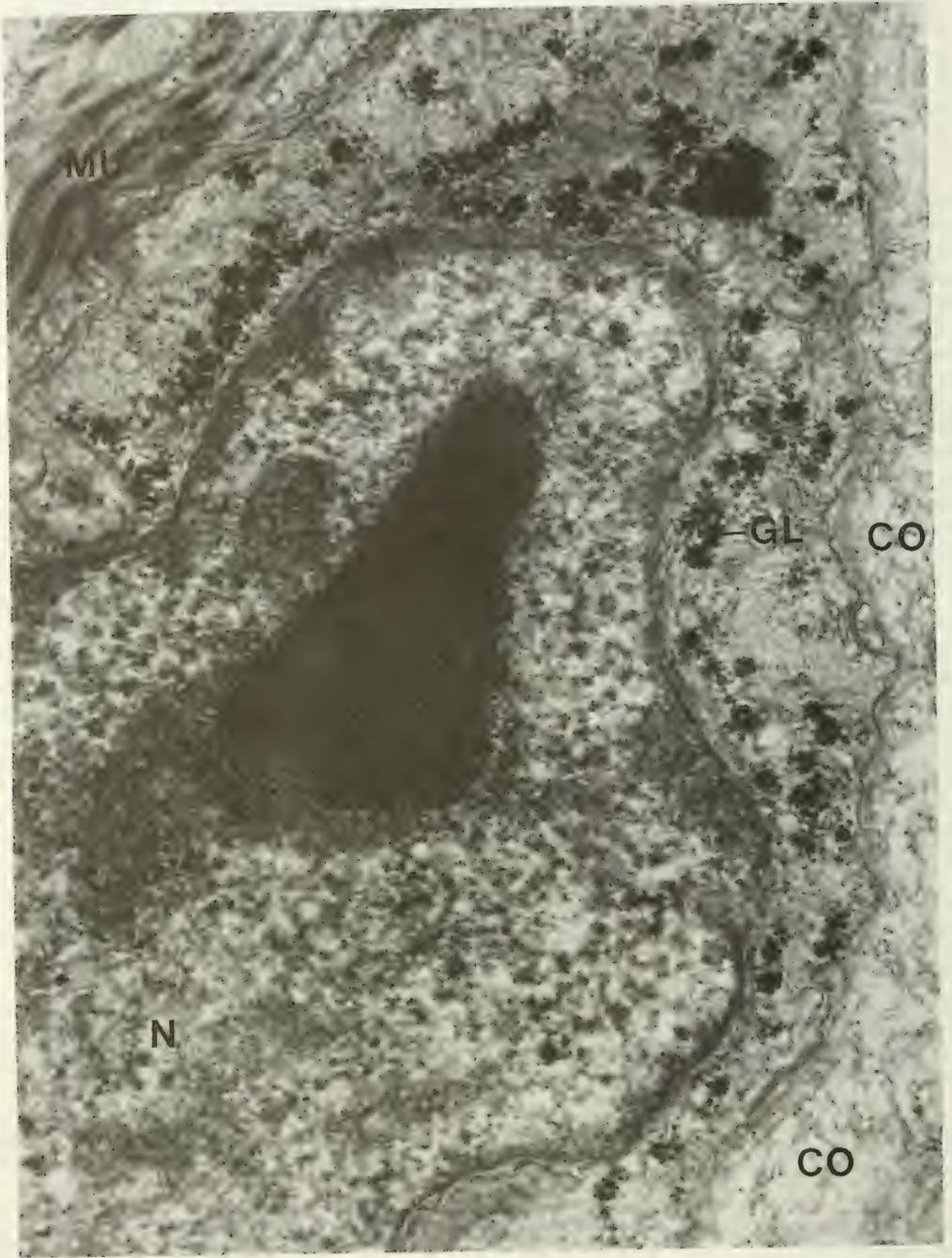


FIG. 2. Junger Fibrocyt, 6. Regenerationstag. CO—Collagen, GL—Glycogen, MU—benachbarte Muskelzelle, N—Kern (Glutaraldehyd, OsO_4 , Vergr. 37 000X).

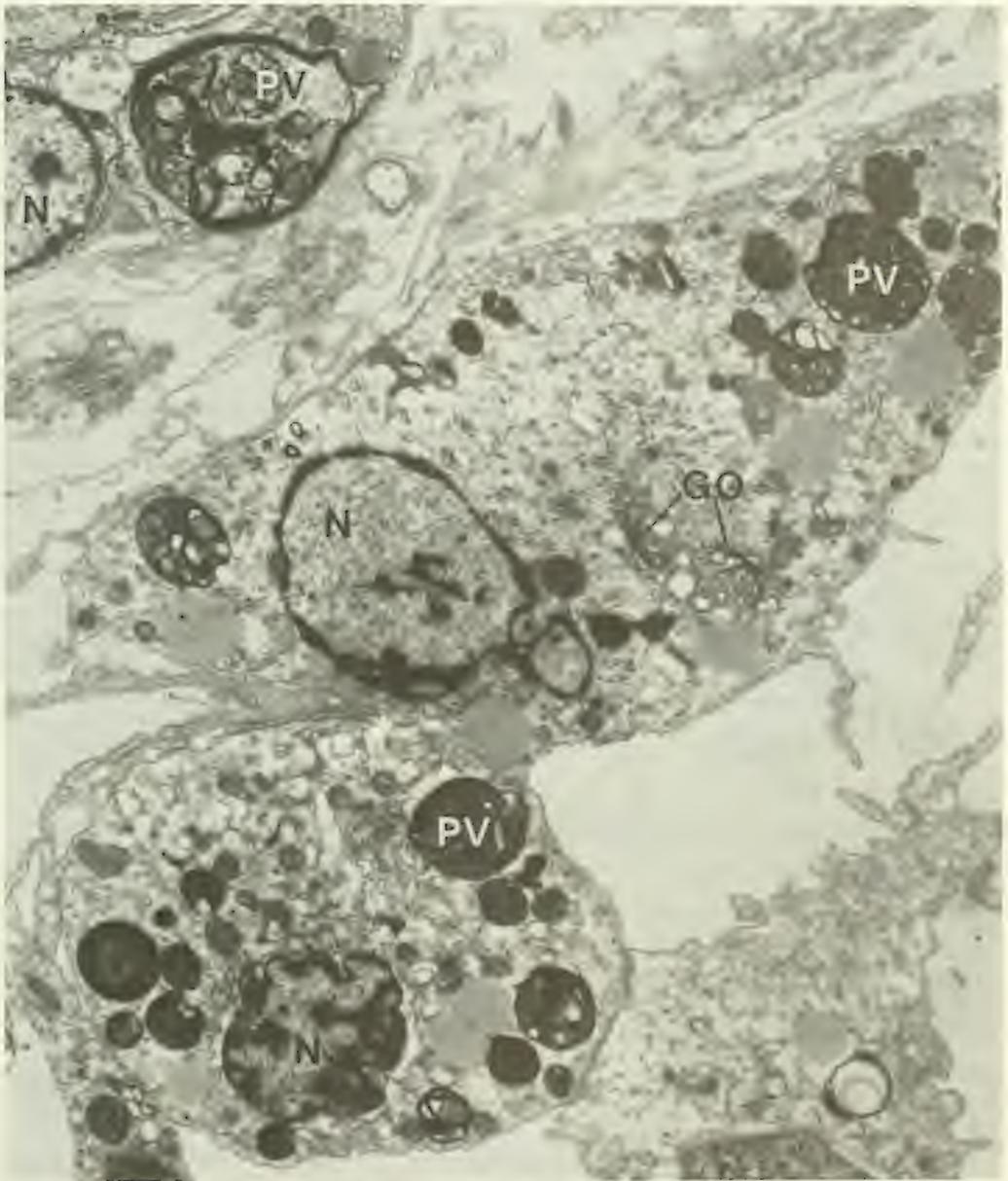


FIG. 3. Amöbocyt, 6. Regenerationstag, GO—Golgiapparat, PV—Phagozytose Vakuolen (Glutaraldehyd, OsO_4 , Vergr. 8 000X).

Die Amöbocyten (Fig. 3) (Stang-Voss, 1970) zeigen im allgemeinen eine zugunsten des Cytoplasmas verschobene Kernplasmarelation. Sie können mehrfach so gross wie die Fibrocyten werden. Die Amöbocyten liegen teils einzeln (z.T. zwischen den Fibroblasten), teils in Gruppen und haben lange, pseudopodienartige Fortsätze, mit denen sie sich häufig anderen Organen oder Amöbocyten dicht anlagern. Besondere Spezialisierungen des Plasmalemmis fehlen. Das an freien Ribosomen arme Cytoplasma weist oft Tonofilamente auf. Es enthält neben kleinen

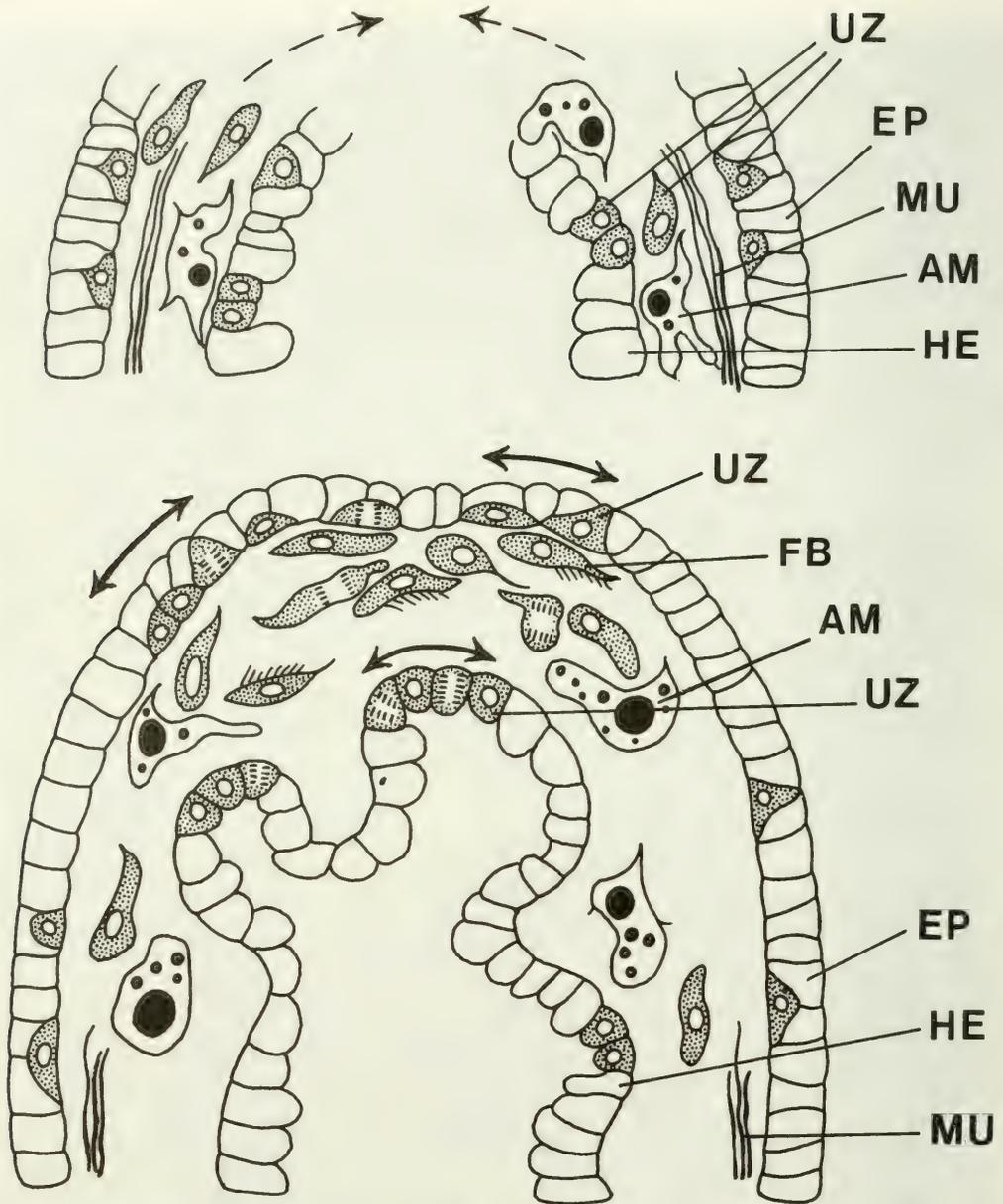


FIG. 4. Regenerationsschema. Oben ist der Zustand unmittelbar nach der Amputation wiedergegeben, unten am 3. Regenerationstag. Die unterbrochenen Pfeile symbolisieren die Kontraktionsrichtung, die durchgehenden Pfeile das Wachstum der embryonalen Zonen. AM—Amöbocyt, EP—Epidermis, FB—Fibroblast und Fibrocyt, HE—Hepatopancreas, MU—Muskulatur, UZ—undifferenzierte Zellen.

Mitochondrien viele glatte ER—Vesikel und kleine bis sehr grosse Phagocytose-Vakuolen mit unterschiedlichen Inhaltskörpern. Am zweiten Regenerationstag lassen die Vakuolen häufig noch ihre Herkunft erkennen, d.h. man beobachtet in ihnen Reste von Zellkernen, Iridocyten, Mitochondrien oder Vakuolen der Epidermiszellen. Nach 6 Tagen überwiegen kompakte, stark osmiophile, kugelige telosomale und postlysosomale Körper mit zahlreichen myelinartigen, dichtgelagerten Lamellenstrukturen. Zu dieser Zeit lassen die Kerne eine wechselnde Kondensa-

tion stark osmiophilen Materials an ihrer inneren Membran erkennen, werden kleiner und in einigen Zellen pyknotisch.

Amöbocyten und Fibrocyten sind also vom 2. bis 6. Regenerationstag nebeneinander anzutreffen. Dabei nimmt die Zahl wenig differenzierter Fibroblasten mit zunehmender Regenerationsdauer ab, diejenige vollaktiver, ausdifferenzierter Fibrocyten und diejenige pyknotischer Amöbocyten aber zu. Das weitere Schicksal dieser Zellen mit pyknotischem Kern ist weitgehend ungewiss. Ein Teil von ihnen wandert, wie Markierungen von Millott (1936) zeigten, über den Enddarm aus. Aus den Fibrocyten geht, soweit erkennbar, das Bindegewebe des auswachsenden Kolbens hervor. Sie scheinen aus dem benachbarten Bindegewebe, wo sie vereinzelt jederzeit anzutreffen sind, zur Wunde zu wandern.

Zusammenfassend (Fig. 4) lässt sich feststellen: der Wundverschluss erfolgt durch Muskelkontraktion. Eine Dedifferenzierung bereits voll ausdifferenzierter Elemente liess sich an den hier beschriebenen Organen und Zellen nicht beobachten. Die Restitution des Cnidosack und des Mitteldarmdrüsengewebes erfolgt von Nestern undifferenzierter Zellen aus, die stets in den Buchten des Hepatopankreas anzutreffen sind. Die Restitution der Epidermis geht von undifferenzierten Epidermiszellen aus. Fibroblasten und wenig differenzierte Fibrocyten bilden kurzfristig einen blastemartigen Wundverschluss, an dem auch Amöbocyten beteiligt sind. In der Folge ist das Geschick dieser beiden Elemente aber grundverschieden. Die Abbauprodukte phagozytierenden Amöbocyten mit zunehmend pyknotischen Kernen stehen am Ende ihres Lebensweges. Die Fibroblasten differenzieren sich zum Bindegewebe des auswachsenden Ceras. Bei der Regeneration des Aeolidierkolbens zeigt sich demnach, wie z.B. unter den Spiraliern auch bei vielen Anneliden (Herlant-Meewis, 1964), eine gewisse "Unantastbarkeit der Keimblätter." Im Regelfall werden die ektodermalen Anteile aus undifferenziert gebliebenen, ektodermalen Reservezellen, die mesodermalen aus undifferenziert gebliebenen Mesenchymzellen und die entodermalen aus den beständig vorliegenden, undifferenzierten Entodermzellen gebildet. Ob und wie weit darüberhinaus undifferenzierte Zellen eines Keimblattes in ein anderes wandern, bleibt offen.

Ich danke für die guten Arbeitsmöglichkeiten am Institut Biologique de Roscoff.

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PRELIMINARY STUDIES ON THE NATURAL DIET AND CARBOHYDRASES
IN THE DIGESTIVE GLAND OF THE TROPICAL AQUATIC
PULMONATE SNAIL *LYMNAEA LUTEOLA* LAMARCK

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ABSTRACT

Some observations were made on the natural diet and the carbohydrases in the digestive gland of the tropical aquatic pulmonate snail *Lymnaea luteola* Lamarck. *L. luteola* was found to live associated with aquatic vegetation, especially *Chara* sp. The animals feed by rasping with the radula on epiphytic organisms present on the stems and leaves of plants. Dissections of the digestive tract of fresh animals have shown diatoms, small fragments of filamentous algae and fine gritty sand. The digestive gland of *L. luteola* contains enzymes like amylase, cellulase, α -glucosidase, β -glucosidase and pectinase. The amylase activity was found to be very prominent in the digestive gland of the snail and this suggests that the animal mainly depends on the starch reserves of the consumed algae. A prominent cellulase activity was also noticed in the digestive gland which suggests that the cellulose components of the ingested plant material may also be digested.

INTRODUCTION

Studies on the digestive system of pulmonates have been extensive and papers published on this subject have been well reviewed by many workers (Owen, 1966a, 1966b; Hyman, 1967; Purchon, 1968; Runham, 1975). However, investigations on the digestive enzymes in freshwater snails are very scanty (Carriker, 1946). In the present investigation an attempt has been made to study the natural diet and carbohydrate splitting enzymes in the digestive gland of the local common aquatic snail *Lymnaea luteola* Lamarck.

MATERIAL AND METHODS

Observations were made on snails collected from ponds located in the vicinity of Guntur town ($16^{\circ}18'N$ $80^{\circ}29'E$), India. The food and feeding habits of the animal were observed both in the field and in the laboratory. The feeding mechanisms were observed by watching the animal while it is feeding on plant (*Chara* sp.) material in a dish of water under a binocular microscope. Fresh snails were dissected to examine the alimentary canal and determine the food of the animals.

Enzyme extracts of the digestive gland were prepared in distilled water. A 1% digestive gland extract was used to carry out these experiments. To prepare the extract, pieces of digestive gland were dissected, mixed with ice cold distilled water, homogenized with a tissue homogenizer at 2000 r.p.m. for 15 min and centrifuged at 1000 r.p.m. for 5 min. The supernatant fluid was used in the experiments.

To establish the presence of sacroclastic enzymes, substrates like starch, cellulose, sucrose, cellobiose, melitose, lactose, pectin, trehalose, and inulin were used. A 1% substrate solution is mixed with an equal quantity of the enzyme extract, and the mixtures, after adding one drop of toluene to prevent bacterial action, were incubated at $35^{\circ}C$ for 24 h. Controls with boiled extract were also maintained simultaneously. The end products of carbohydrate digestion were tested qualitatively by the methods described by Oser (1965).

RESULTS

Field studies have shown that *L. luteola* is mainly associated with aquatic vegetation. The animals, however, were most abundant on *Chara* sp. ranging in numbers from 85 to 475/kg wet weight of the plant. They are also found on other plants, *Utricularia* sp., *Ceratophyllum* sp., *Polygonum* sp., *Typha* sp., *Eichornia crispus*, and *Ipomoea aquatica*, but in very small numbers

(≤ 50 /kg wet weight of the plant). The animals were found usually attached to the stems and leaves of the submerged plants.

Observations on the feeding mechanisms of the animal have shown that the animals feed by rasping with the radula on the epiphytic organisms present on the stems and leaves of the plants. Dissection of the digestive tract of fresh animals have shown diatoms, small fragments of filamentous algae and fine gritty sand.

Table 1 shows the results of the experiments carried out to study the carbohydrases in the digestive gland of *L. luteola*. The results show that enzymes like amylase, cellulase, a-glucosidase, B-glucosidase and pectinase are present in the digestive gland of the snail. It is also clear that the amylase activity is very prominent in the digestive gland of the animal.

TABLE 1. Carbohydrases in the digestive gland of the aquatic pulmonate snail *Lymnaea luteola* Lamarck.

Enzyme	Substrate	Linkage	Reaction
Amylase	Starch	1,4, and 1,6,a-glucose	+++
Cellulase	Cellulose	1,4, B-glucose	++
a-glucosidase (sucrase, invertase)	Saccharose (sucrose)	a-glucoside (fructoside)	+
B-glucosidase	Cellobiose	B-glucoside	+
a-galactosidase	Melitose (raffinose)	a-galactoside	-
B-galactosidase	Lactose	B-galactosidase	-
Pectinase	Pectin		+
Trehalase	Trehalose	a-glucoside	-
Inulase	Inulin	1,2-fructofuranose	-

+++ very prominent action: ++ prominent action: + weak action: - no action.

DISCUSSION

A comparison of the food and feeding habits of *L. luteola* with those of *L. peregra* indicates that the former is mainly associated with *Chara* sp. while the latter with the Canadian pond weed, *Elodea canadensis* (see Calow, 1970). Both species feed on epiphytic organisms, especially diatoms and filamentous algae along with some gritty sand, by rasping with the radula (Calow, 1970). Carriker (1946) suggested that the sand in the gizzard of the snails helps in pulverizing the food.

L. luteola is a herbivore like the other snails *L. peregra* (see Calow, 1970) and *L. stagnalis* (see Carriker, 1946). The digestive gland is the main source of enzymes in *L. luteola* as in *L. stagnalis* (see Carriker, 1946). Carriker found that the digestive cells in *L. stagnalis* function in secretion, assimilation, intracellular digestion and excretion.

The presence of a strong amylase in the digestive gland of *L. luteola* suggests that the snail mainly depends on the starch reserves of the consumed algae. A prominent cellulase activity in the digestive gland also suggests that the cellulose components of the ingested plant material may also be digested.

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THE ORGANISATION OF BEAK MOVEMENTS IN *OCTOPUS*

P. R. Boyle,¹ K. Mangold² and D. Froesch³

ABSTRACT

Two types of 'biting' activity in preparations of the isolated buccal mass of *Octopus vulgaris* have been observed;

(a) 'spontaneous biting,' which may continue for some hours together with movements of the radular apparatus, and

(b) 'evoked biting,' a single 'bite' cycle elicited by electrical stimulation of the bundle of nerves containing the interbuccal connective.

Mechanical records of the movement of the upper beak relative to the lower one have allowed division of the 'biting' cycle into closing, closed and opening phases. An 'evoked bite,' however, always began this sequence with a rapid opening phase. The duration of the 'evoked bite' cycle was significantly shorter than 'spontaneous bites.' Myogram records from mandibular muscles, correlated with mechanical records of beak movements have helped to ascribe functions to these muscles. It is concluded that the 'biting' cycle of beak movements can be organised and controlled from the inferior buccal ganglion.

INTRODUCTION

With the exception of the bivalves, the buccal mass of the Mollusca is composed of a complicated musculature which works the radula and accessory structures. Typically, the basic feeding movement involves the protrusion of the radula through the mouth and its retraction over supporting structures which erect the radula teeth to give a rasping action. In the gastropods particularly, feeding movements have been studied physiologically with the general aim of understanding the organisation and control of these complicated though stereotyped actions.

The cephalopod buccal mass offers an unusually interesting subject. In the octopus, radula, buccal palps, salivary papilla and submandibular gland, together with supporting muscles are enclosed within a capsule formed by a pair of large mandibles or beaks and their associated musculature. This capsule occupies a space within the bases of the arms just in front of the brain, and is only lightly anchored to surrounding tissue. Closely associated with the buccal capsule are two nervous ganglia, the subradular and the inferior buccal, which are linked to the brain by relatively long nervous connectives.

The anatomy and functioning of the enclosed radular and salivary apparatus, beaks and mandibular musculature is nowhere fully described. Probably the main reason for this is the very tough, chitinous nature of the beaks. The general layout of the parts of the buccal mass and its innervation is shown in Fig. 1, redrawn from published accounts (Young, 1965a; Altman & Nixon, 1970). The nervous supply to these structures has been thoroughly described by Young (1965a).

In the present paper, a preliminary account is given of the beak movements of the isolated buccal mass of *Octopus vulgaris*.

METHODS

Octopus vulgaris specimens were trawled from the Catalan Sea and maintained alive in the seawater circulation system at Laboratoire Arago.

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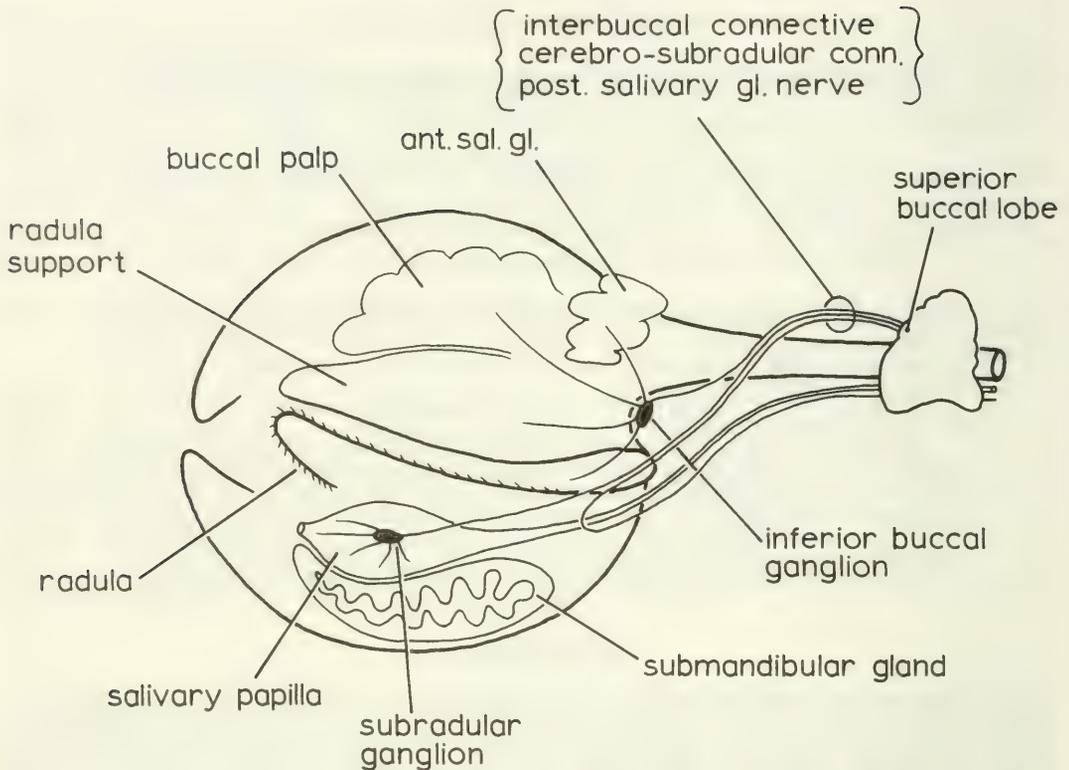


FIG. 1. Composite diagram of the buccal mass of *Octopus vulgaris* and its main nerves, redrawn from Young, 1965a.

After various trials, the following experimental procedure was adopted. Individual animals were anaesthetised in ethanol (3%) in seawater, until breathing and arm movements ceased. A mid-line incision was made to expose the brain and buccal mass. The supra-oesophageal brain was bisected and the interbuccal connective of each side located. This connective runs between the superior buccal lobe of the brain and the inferior buccal ganglion and it is bound up together with the cerebro-subradular connective and the posterior salivary gland nerve of each side. This bundle of nerves was ligatured close to the brain and cut free from it.

The entire buccal mass was freed from the arms and transferred to fresh seawater. The anterior salivary glands were removed and the lips cut away. The lower beak was then gripped firmly in a small clamp and the whole preparation mounted in a bath of flowing seawater (Fig. 2). When it was necessary to interrupt the flow of seawater for recording, the same water was diverted through a jacket to the bath, thus minimising the temperature fluctuations (15-17°C). Opening and closing movements of the upper beak relative to the lower one were registered using a strain gauge attached by a thread and a small hook to the upper beak. One of 2 alternative transducer beams were used requiring 1 g or 10 g loading for maximum deflection.

Electrical stimuli consisting of trains of squarewave pulses could be applied to the ligatured nerve bundle through a pair of silver hook electrodes. Fine, flexible copper wires, insulated almost to the tip could be inserted into the mandibular muscles and used to record electrical activity in the muscle at that point. Recording from the nerve bundle using etched silver hood electrodes was also attempted. Events were displayed on an oscilloscope and records made on magnetic tape or Polaroid film.

Whole buccal masses from adult animals with complete beaks were fixed in seawater-Bouin solution. The best sections were obtained from celloidin-embedded material cut transversely at 90 µm thick and stained with Masson's Trichrome.

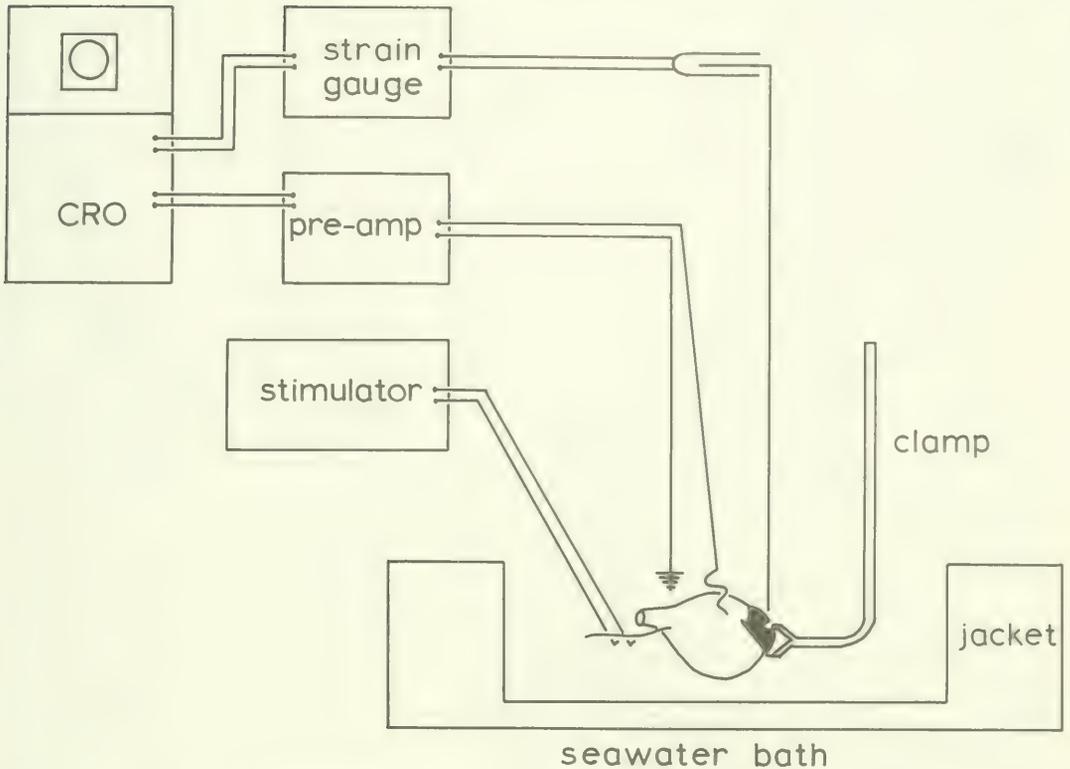


FIG. 2. Arrangement of the isolated buccal mass preparation for recording and stimulation.

RESULTS

The isolated buccal mass of *Octopus vulgaris* would execute 'biting' movements of the beaks for periods of up to 2 hours when simply immersed in cool aerated seawater. Although most preparations showed some 'biting' activity, the inter-bite interval was quite variable (10 sec to 2 min) and usually tended to increase as the preparation aged (Fig. 3). In comparison the isolated buccal mass of *Eledone cirrosa* rarely made any 'biting' movements.

Each 'bite' consisted of a cycle of activities. Starting from a resting position about three-quarters open, the upper beak closed progressively until contact was made with the lower beak. The beaks remained closed, the upper mandible fitting inside the lower, while the upper one was drawn backwards relative to the lower one. An opening movement completed the 'bite' cycle and the upper beak returned to the resting position. Typical records of these 'spontaneous bite' cycles are shown in Fig. 4A, D and F.

By dividing the duration of the 'bite' cycle into 3 phases consisting of a closing time, 'biting' time and opening time, measurements of the relative duration of these phases were made from the mechanical records (Table 1). There was considerable variation in the duration of these phases, particularly in the length of the closed period. In some preparations the beaks were held closed with considerable force while the upper beak was drawn backwards; others began to reopen soon after closing.

A total of 18 successful preparations were made in which 'biting' activity of the buccal mass was evoked by electrical stimulation of the interbuccal connective of one side (in a bundle together with the cerebro-subradular connective and ascending arm of the posterior salivary gland nerve). Trains of stimuli at 50-60 Hz and lasting for 1 or 2 seconds were effective in evoking a characteristic 'biting' cycle. This 'evoked bite' invariably began with a defined

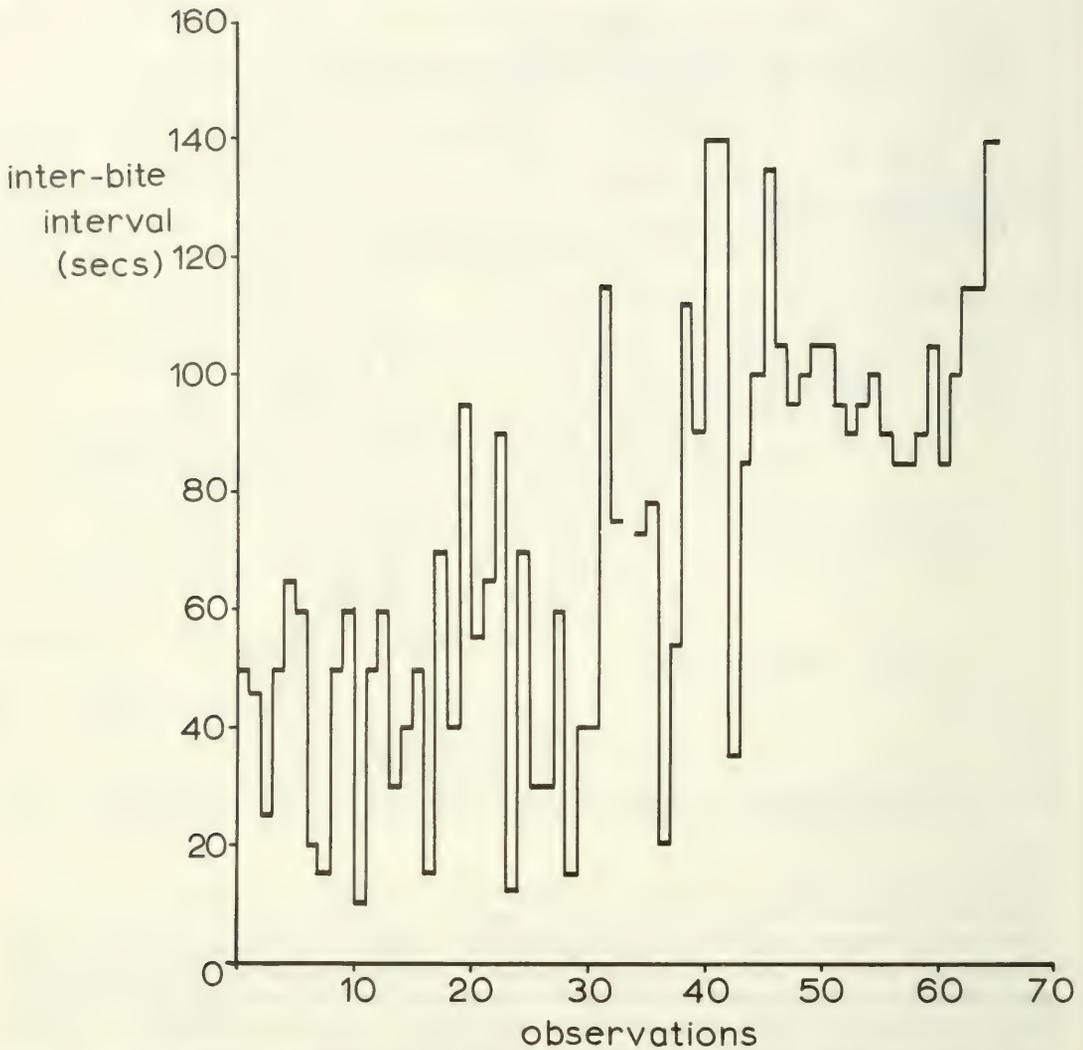


FIG. 3. Variation of successive inter-bite intervals of one 'spontaneously biting' preparation.

opening movement (Fig. 4B, C and E), but the following movements were significantly faster than 'spontaneous bites' except for the final opening phase. The duration of components of the bite cycle was measured from photographs taken at slow oscilloscope speeds. Consequently the accuracy of the time estimation was usually no better than ± 0.1 sec, but in view of the rather arbitrary division of the cycles into distinct phases this accuracy was considered adequate.

A minimum stimulus train duration and voltage were required to evoke a 'bite' cycle although the cycle would run to completion even while continuously stimulated (Fig. 4C). Careful separation and cutting of the cerebro-subradular connective where it by-passed the inferior buccal ganglion made no apparent difference to the activity. As well as the 'bite' cycle of activity of the mandibles, electrical stimulation of the connective trunks could also evoke a general contraction of the buccal mass. This response consisted of a clamping of the upper and lower beaks together and continued for the duration of the stimulus train. These evoked contractions, characteristic of aging preparations, could always be distinguished from the 'biting' cycle because there was no opening of the mandibles.

TABLE 1. The mean duration \pm SD of components of the 'spontaneous' and 'evoked bite' cycles (N = 25 and N = 32 respectively).

time (secs)	'spontaneous bite'	'evoked bite'	P
total	18.5 \pm 9.2	11.0 \pm 5.7	0.001
closing phase	6.1 \pm 2.8	2.6 \pm 1.8	0.001
biting phase	6.6 \pm 6.5	3.1 \pm 2.4	0.01
opening phase	5.6 \pm 2.3	4.8 \pm 3.9	N.S.
latency		\sim 0.4	
initial opening		\sim 1.2	

During a 'biting cycle' electrical activity was recorded from the superior and lateral mandibular muscles (Fig. 5) of the ipsi- or contralateral side to the stimulated nerve bundle. Activity persisted throughout the closing and closed phases (Fig. 4E and F) or was present only during closing (Fig. 4D and F). As far as our identification of electrode position would allow, it seemed that activity in the superior mandibular muscle persisted while the beaks were held closed (Fig. 4E), but was only present in the lateral mandibular muscle during closing (Fig. 4D). Simultaneous records from the two muscles confirmed this division of function (Fig. 4F).

DISCUSSION

The normal actions of the beaks and radula, their relative roles in feeding are not fully understood (Altman & Nixon, 1970). Thus it is not possible to assess the extent to which movements of the isolated buccal mass described here resemble its normal activity in the intact animal. However, since the isolated buccal mass will perform a credible sequence of 'biting' movements, then these must be controlled by the inferior buccal ganglion. In gastropods where radula movements have been studied, alternating activity in separate groups of motor cells in the buccal ganglion control the radula protractor and retractor muscles respectively (Kater & Rowell, 1973). These central motor rhythms are clearly important in the control of gastropod radula movements (Rose, 1971, 1972; Davis, Siegler & Mpitsos, 1973), but the presence of sensory receptors, described anatomically and physiologically (Laverack, 1970; Kater & Rowell, 1973) suggests that these actions are not entirely centrally programmed (Heyer & Kater, 1973).

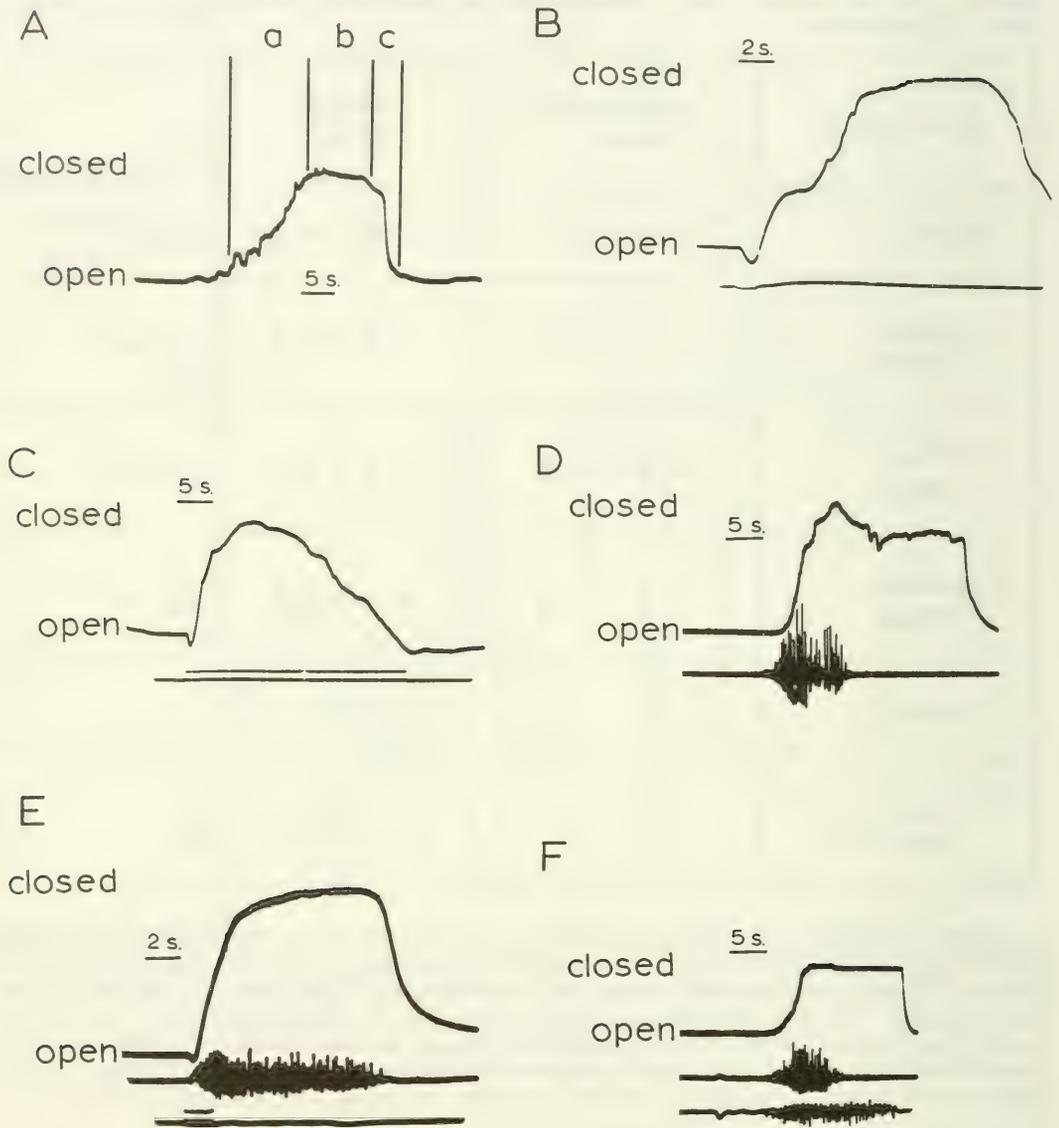


FIG. 4. Mechanical records of 'bite' cycles and associated muscle activity. (A) 'Spontaneous bite' showing division into 3 phases (see text); (B and C) 'Evoked bites,' stimulus train shown on lower trace; (D) 'Spontaneous bite' with activity recorded from lateral mandibular muscle; (E) 'Evoked bite,' activity recorded from superior mandibular muscle; (F) 'Spontaneous bite' with activity recorded from lateral (upper trace) and superior (lower trace) mandibular muscles. In (D) and (F) muscle spikes re-touched.

The evoked 'bite' cycle with its initial opening phase, faster and less variable movements, probably more closely resembles normal activity. This finding that the interior buccal ganglion is responsible for the direct control and sequence of mandibular movements fits in with the idea that feeding in the octopus is controlled by a succession of nervous centres (Young 1965a, 1965b).

Our preliminary results of muscular activity suggest that the superior mandibular muscle, active while the beaks were held closed could be responsible for the relative backwards as well

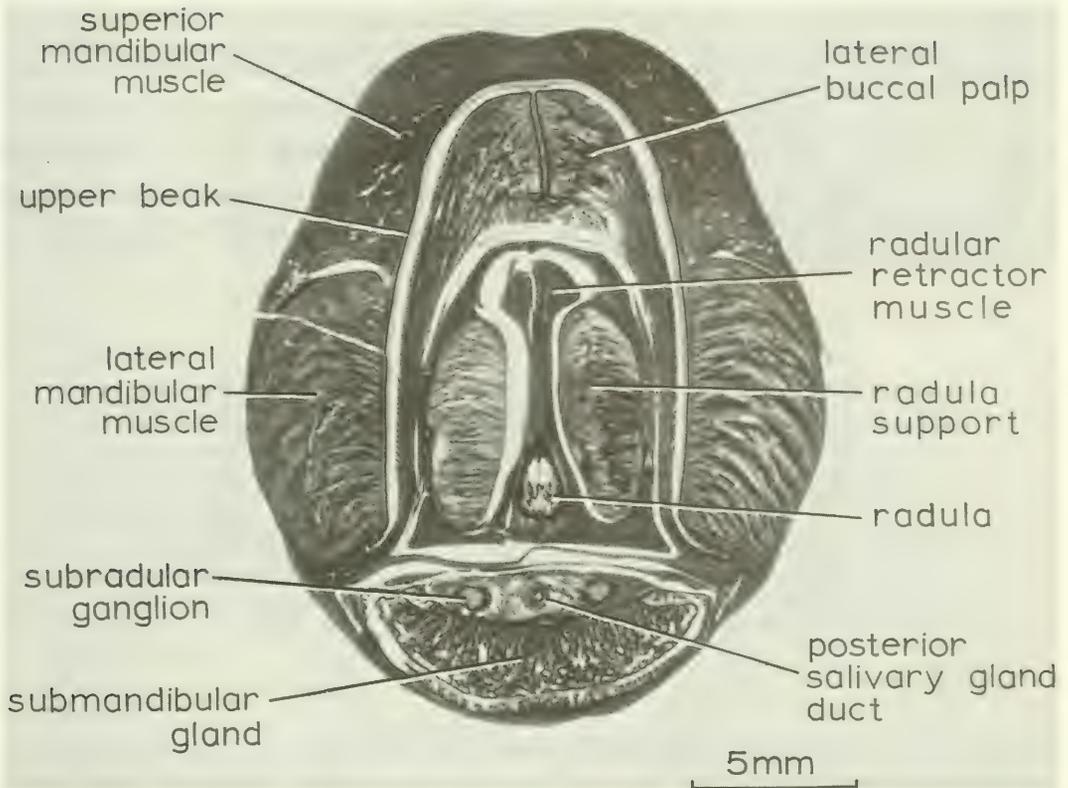


FIG. 5. Transverse section of the buccal mass.

as closing movements of the upper beak. The lateral mandibular muscle showed activity only during closing. It is perhaps significant that no myogram activity could be recorded from the external muscles of the buccal mass associated with an opening movement. Unlike earlier phases of movement, the final opening phase did not differ significantly in spontaneous and evoked 'bites,' and it could be suggested that this final phase is mainly passive. Occasional simultaneous myogram records from bilaterally symmetrical positions showed that activity in the two sides was identical.

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HOLE-BORING IN SHELLS BY *OCTOPUS VULGARIS* CUVIER IN THE MEDITERRANEAN

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ABSTRACT

The holes that *Octopus vulgaris* bores in *Mytilus edulis* and *Murex trunculus* have different characteristics. In *Mytilus* a single hole is made close to the umbo, with an average diameter of 1.1 mm and a penetration hole of 0.2 mm or less. Several holes are usually bored in *Murex*, with an average surface diameter of 0.6 mm increasing in size with the weight of the octopus, and the penetration hole is often almost as large as the surface one. The differences in the holes bored in these two molluscs is probably related to their hardness.

The radulae of individual octopuses are compared with the holes bored. At most only the rachidian tooth, or part of it, could enter the inner, penetration hole. It has been suggested that the salivary papilla may play a part in drilling. This is a mobile, muscular, plastic organ carrying at its tip the eversible end of the posterior salivary gland duct. There are small teeth on the surface of the papilla and slightly larger ones on the end of the duct.

INTRODUCTION

Aristotle observed that octopuses fed on lobsters and molluscs and left the shells in middens (see D'Arcy Thompson, 1910). In Japan, in 1916, Fujita found that *O. vulgaris* could bore holes in the shell of the pearl oyster. Pilson & Taylor (1961) reported on the boring activities of *O. bimaculoides* and *O. bimaculatus* in small abalones, and also demonstrated the paralytic effect of venom on the prey. In 1969 there were two reports on the activity of *O. vulgaris* in America in boring holes in shells (Arnold & Arnold, 1969; Wodinsky, 1969). Striations were found on the walls of the holes, suggesting use of the radula, and this was supported by sounds of rasping received through a hydrophone.

The present study was undertaken to find out whether *O. vulgaris* from the Bay of Naples would drill holes in shells and how they do it.

METHODS

Animals. *Octopus vulgaris* Cuvier, 40-630 g, caught in the Bay of Naples, were kept isolated in tanks with circulating sea water, 24-27°C. The animals were kept for the entire period on either a diet of crabs (*Carcinus maenas*, 30-40 mm across the carapace), mussels (*Mytilus edulis*, 11-46 mm long), or gastropods (*Murex trunculus*, 35-80 mm in height). On one day all the *Mytilus* and *Murex* shells were examined before being given to the octopuses; not one hole was present that in any way resembled those subsequently found after the occupant had been eaten by the octopuses.

Feeding. (1) Normal octopuses. One food animal was dropped into each tank and the reaction of and mode of capture by the octopus noted. Prior to giving another animal any debris was removed from the tank and examined, and the mollusc shells retained. If the animal was still alive it was removed and again dropped into the tank. Often this provoked the octopus to attack and eat it. This procedure was repeated for 3 or 4 days after which fresh food was introduced.

(2) Operated octopuses. Before surgical procedures the octopuses were each fed one animal

TABLE 1. The molluscs taken and the number and size of the holes bored in each by the octopuses on these diets. The percentage of food items eaten can be compared with the crabs eaten. C, clean, all tissue removed; D, dirty, much tissue adhering; PE, fairly clean, some tissue remaining.

Diet	Octopus no.	Body weight		No. items No. days	% eaten	No. holes No. shells	Holes per shell	Greatest diameter of hole on shell surface		Shell cleaning						
		initial g.	loss or gain g.					range mm	average mm	No hole bored			Hole bored			
										D	PE	C	D	PE	C	
<i>Mytilus</i>	124	45	0	15/16	93	13/15	0.8	1.0-1.6	1.2	—	2	—	2	2	5	6
	144	70	-20	17/21	81	13/17	0.7	1.0-1.3	1.1	—	2	—	2	9	2	2
	142	80	-20	18/21	85	17/18	0.9	0.8-1.5	1.0	—	—	1	—	8	3	5
	146	85	-19	14/17	82	8/14	0.6	0.7-1.5	1.1	—	1	1	4	—	2	6
	447	220	-35	8/25	32	5/8	0.6	1.0-1.5	1.2	—	1	2	—	2	2	—
	448	280	-72	10/25	40	9/11	0.8	1.0-1.5	1.2	—	1	—	1	7	—	2
	317	280	-80	20/25	80	17/19	0.9	0.8-1.5	1.1	—	1	2	—	7	7	3
Average	—	—	-35	—	70	—	0.7	—	1.1 Total	6	9	37	21	24	—	
<i>Murex</i>	130	40	-11	3/17	17	9/3	3.0	0.3-1.0	0.5	—	—	—	—	—	—	—
	123	85	+30	11/17	64	26/11	2.3	0.3-1.0	0.5	100	—	—	—	—	—	—
	122	85	+25	14/22	63	22/14	1.5	0.3-1.0	0.5	85	—	—	—	—	—	—
	120	85	+5	10/22	45	23/10	2.3	0.3-1.0	0.6	60	—	—	—	—	—	—
	425	190	+15	11/26	42	48/11	4.3	0.5-1.0	0.7	66	—	—	—	—	—	—
	426	195	+5	11/26	42	42/11	3.8	0.3-1.0	0.6	66	—	—	—	—	—	—
	442	200	+70	4/19	21	12/4	3.0	0.5-1.3	0.8	91	—	—	—	—	—	—
	318	226	+19	10/26	38	29/10	2.9	0.4-1.5	0.7	86	—	—	—	—	—	—
	424	270	-45	6/26	23	17/6	2.8	0.5-1.0	0.8	88	—	—	—	—	—	—
	Average	—	+12	—	—	39	—	2.8	—	0.6	—	—	—	—	—	—
<i>Carcinus</i>	467	45	+50	19/19	100	—	—	—	—	—	—	—	—	—	—	—
	468	85	+33	19/19	100	—	—	—	—	—	—	—	—	—	—	—
	151	110	+32	16/16	100	—	—	—	—	—	—	—	—	—	—	—
	453	170	+125	22/22	100	—	—	—	—	—	—	—	—	—	—	—
	452	180	+130	22/22	100	—	—	—	—	—	—	—	—	—	—	—
	450	190	+130	22/22	100	—	—	—	—	—	—	—	—	—	—	—
451	210	+40	16/22	72	—	—	—	—	—	—	—	—	—	—	—	
Average	—	+77	—	—	96	—	—	—	—	—	—	—	—	—	—	—

% holes on aperture face of shell
(see Fig. 3)

of the type selected for them to eat. After removing the anterior part of the salivary papilla the feeding procedure was the same as above.

Surgical procedures. Fourteen *O. vulgaris* were divided into 3 groups and each group was fed on the diet selected for them several days prior to operation. The beaks of the anaesthetized animals (3% urethane in sea water) were opened and the tip of the salivary papilla held with forceps while the muscular part of the papilla was cut as far back as possible. After recovery the animals were given the same diet as before the operation.

Post-mortem. The excised buccal mass was fixed (10% neutral formalin) and from the normal animals the rostrum of the beak was removed to expose the radula. In the operated

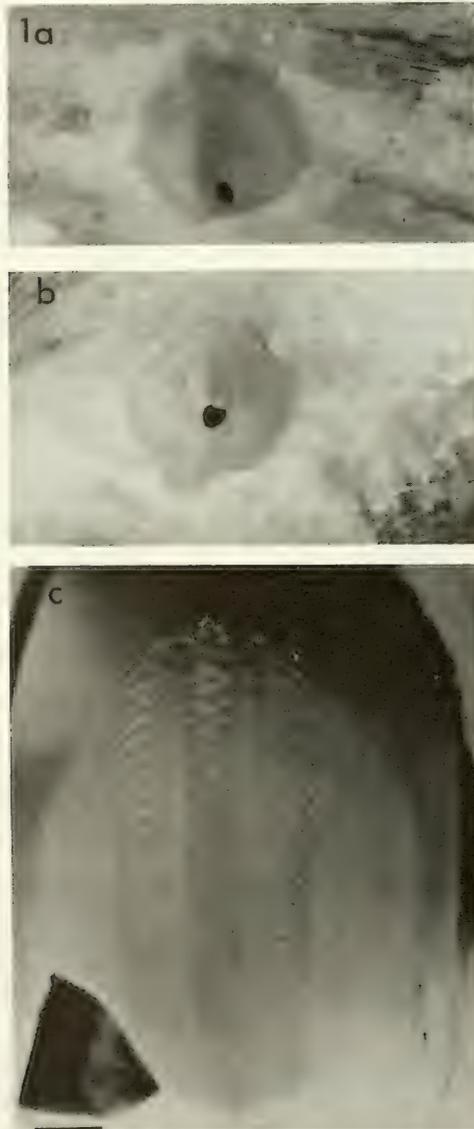


FIG. 1. Holes bored in *Mytilus* (a & b) by the radula (c), all taken at the same magnification. A slight lip can be seen on the surface of the shell. The greater size of the surface opening of the hole drilled can be compared with the narrower part where the sides slope down to where it penetrates the shell. Rasp marks are visible on some parts of the walls of the hole. The bar represents 500 μ m.

group the lower beak was removed and a median antero-posterior cut made with a surgical blade so that the condition of the salivary papilla could be seen.

RESULTS

1. *Normal group.* All but 1 octopus fed with crabs ate them all, the range being 72-100%, and gained weight (Table 1). None of the octopuses fed on molluscs ate all those given, the range for *Mytilus edulis* being 32-93% and for *Murex trunculus* 17-64%. Furthermore all the octopuses fed with *Mytilus* lost weight except one, while 7 of the 9 given *Murex* gained weight (Table 1).

(a) *Mytilus edulis.* Table 1 shows the number of occasions on which mussels were opened and their occupants eaten. Five of the octopuses took, bored a hole in and ate a *Mytilus* within 24 hours of being given the prey; the others took 2 or 3 days to do this (Table 2). One octopus took only 8 of 25 mussels given, while another ate 15 of 16 (Table 1). Of the shells opened the majority had a bore hole (60-90%) but every octopus in the group was also able to open a mussel without boring a hole. A crude analysis of the state of the shell after feeding was made using a three-point scale (Table 1). The shells could be left completely clean and free of adhering tissue whether or not the octopus had bored a hole. Out of 102 shells opened 81 had 1 bore hole and 1 had 2 bore holes (Table 2). The hole was usually close to the umbo or occasionally a little further along the edge of the shell.

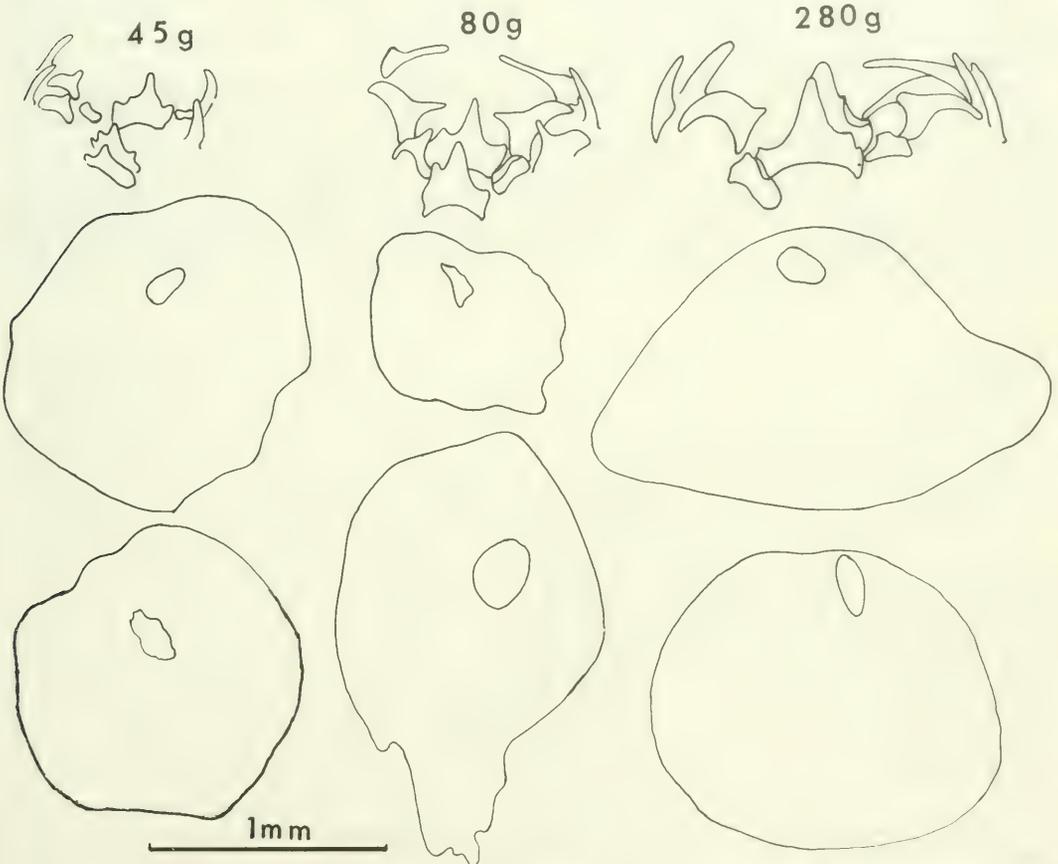


FIG. 2. The radulae from octopuses of different weights (given above); below each are, in outline, the perimeter of the surface and penetration holes drilled in *Mytilus* shells.

Two holes drilled by an octopus of 220 g were photographed at the same magnification (Figs. 1a & b) and, using a camera lucida, the periphery of the outer and inner holes was drawn to show any differences present in holes drilled by 3 octopuses of 45 g, 80 g and 280 g. The diameters of the outer holes were similar in size although drilled by octopuses of different body weights, the range in the diameter being 0.7-1.6 mm (Table 1). Figs. 1 and 2 also show the radulae from the octopuses responsible for the holes illustrated, made at the same magnification in each case. Thus all of the teeth (in the transverse rows) from an octopus of 45 g could enter the upper region of the holes it had made, but this is not so for the radulae of the larger animals (Figs. 1 and 2). Furthermore below the upper region the walls of the holes slope quite steeply down to the always small penetration hole (Fig. 1a,b), of 0.09-0.22 mm into which at most only the mesocone of the central rachidian tooth could enter.

The surface aperture, usually ovoid, varied in shape even when made by the same octopus (Figs. 1a,b, 2). On the surface of many of the shells was a lip, presumably the position of the central tooth during drilling. Striation marks could be seen on the walls of some of the holes, just discernible in Figs. 1a & b, perhaps reflecting the action of the teeth, but not necessarily of the radula (see Section 3). The walls of the holes, usually wide near the upper surface, became narrower and tapered down to the site of penetration (Figs. 1a,b); these variations in the form of the hole probably reflect the composition of the shell (see Discussion).

(b) *Murex trunculus*. One octopus ate 3 of 17 *Murex* given and another 11 out of 17 (Table 1). The shells and opercula when removed from the tanks were always clean and entirely free of adhering tissue.

Of the *Murex* eaten 22% of the shells had 1 hole and 78% more than 1 bored, the range being from 1-8 in each though 1 octopus bored 17 holes (see Section 2). That it was not essential to make so many holes is shown well by octopus 122 (Table 2) which bored only 1 hole in each of 8 of the 14 it ate.

Not 1 octopus consumed its first *Murex* in the laboratory in less than 2 days, and of the others 3 took 3 days, 1 took 7 days and another 12 days (Table 2). Subsequently the octopuses took 1-7 days to eat another *Murex*. The holes were bored near the aperture on the body whorl and on the smaller whorls above (Fig. 3). To ascertain whether this was the preferred area for boring, each shell was held with the aperture facing and the number of holes then visible counted and shown as a percentage of the total number of holes (Table 1). The range was from 60-100%, so clearly there is selection, the aperture and apex of the shell

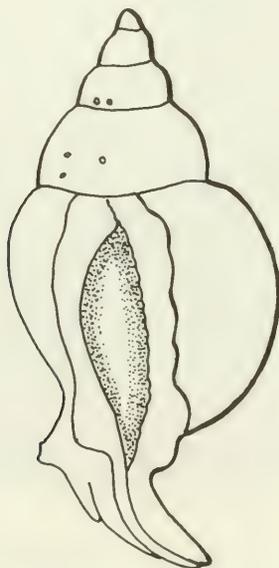


FIG. 3. Drawing of the aperture face of *Murex* on which the majority of the holes were bored.

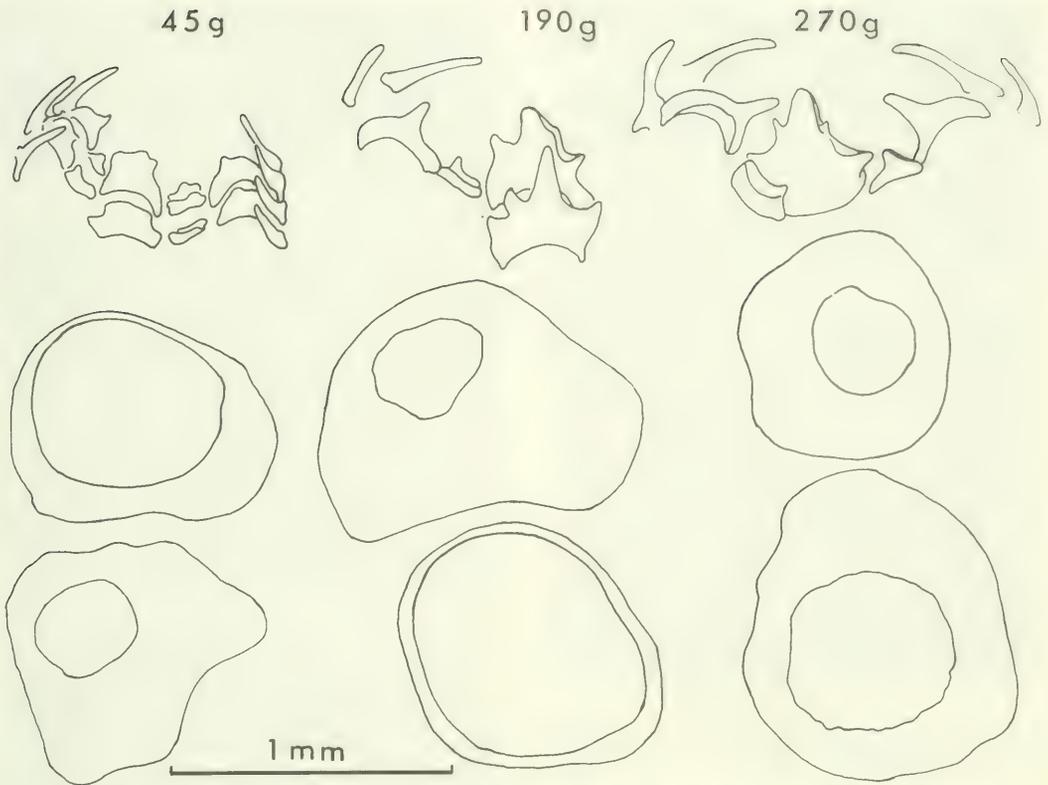


FIG. 4. Radulae from octopuses of different weights, given above; below are outlines of the surface and inner holes drilled in *Murex*. The holes are variable in shape and size even when made by the same octopus.

presumably forming landmarks for orientation, but there is little indication of an optimum site within this area.

The long axis of the bore hole on the upper surface of the shell was 0.3-1.5 mm, but the average for each octopus increased with its body weight (Table 1). The outlines of the surface and penetration holes have been drawn in outline together with the radulae responsible (Fig. 4) and photographed (Fig. 5). The penetration hole is often almost as large as the surface hole, this may reflect shell thickness. However, Fig. 6 shows several holes bored by 1 octopus quite close together on the same body whorl of a *Murex* and there are still differences.

(c) *Carcinus maenas*. The octopuses all took and ate all the crabs given leaving the exoskeleton separated and completely free of tissue (Altman & Nixon, 1970) except 1 octopus which left a crab alive for 6 days but then took it and subsequently ate it and others normally (Table 1). The cleaned exoskeletons were examined but no obvious holes found, and none that in any way resembled those found in the mollusc shells. To try to ascertain if holes were made 2 crabs were given to 2 octopuses and removed after 30 sec. The crabs had been paralysed (see Ghiretti, 1960) but examination with a binocular microscope did not reveal any holes, although this does not preclude the possibility of small puncture holes, perhaps in the joints, made either by the mesocone of the rachidian tooth or by the very small teeth present on the tip of the posterior salivary gland duct (see Section 3).

2. *Operated group—no salivary papilla*. Pre-operatively these octopuses were given the food selected for them and all were successful in killing and eating it appropriately. One octopus made 17 holes in 1 *Murex*, although 3 days elapsed between giving it and finding the shell empty, so each hole took, on average, 4 hours to drill.

TABLE 3. Food before and after removal of the anterior part of the salivary papilla and the state in which this was found at post-mortem. A, salivary papilla absent; B, anterior part of salivary papilla removed; C, shell clean, all tissue removed; Op, day of operation; PE, partly eaten food; UN, food untouched.

Diet	Octopus no.	Body weight		Pre-operation food state	Num-ber days post-op.	Post-operation			Last 3 days of experiment						Post-mortem salivary papilla						
		initial g.	loss or gain g.			Shells given	Shells opened	Holes bored	State of shells		crab		crab			crab					
									D	PE	pois-oned	eat-en	pois-oned	eat-en		pois-ened	eat-en				
<i>Mytilus</i>	510	190	-5	2/2	19	7	4	0	-	2	2	No	PE	No	PE	No	PE	A			
	508	350	-20	1/1	14	8	7	0	-	4	3	Yes	C	Yes	C	Yes	C	B			
	148	250	-5	1/1	14	6	4	0	1	2	1	Yes	C	Yes	C	Yes	C	B			
	150	310	-30	1/1	14	7	1	0	-	1	-	No	PE	Yes	PE	No	PE	A			
	426	490	-210	1/1	14	9	5	0	1	-	4	Yes	C	Yes	C	Yes	C	B			
<i>Murex</i>	505	180	-40	12/2	17	5	1	0	1	-	-	Yes	C	Yes	C	Yes	C	B			
	502	220	-30	21/2	17	5	1	0	1	-	-	Yes	C	Yes	C	Yes	C	B			
	425	235	-35	3/1	30	7	1	0	1	-	-	Yes	PE	Yes	PE	Yes	PE	B			
	513	240	-40	1/1	17	5	UN	0	-	-	-	Yes	PE	Yes	PE	Yes	PE	B			
	511	310	-65	3/1	23	7	UN	0	-	-	-	Yes	C	Yes	C	Yes	C	B			
<i>Carcinus</i>	514	220	0		17					August											
	515	300	-20		17	Op.	PE	C	C	23	24	25	26	27	28	29	30	31	September		
	516	335	+5		17	Op.	UN	UN	UN	1	2	3	4	5	6						
	512	380	+30		17	Op.	PE	PE	C	C	C	C	C	C	C	C	C	C	C	C	C

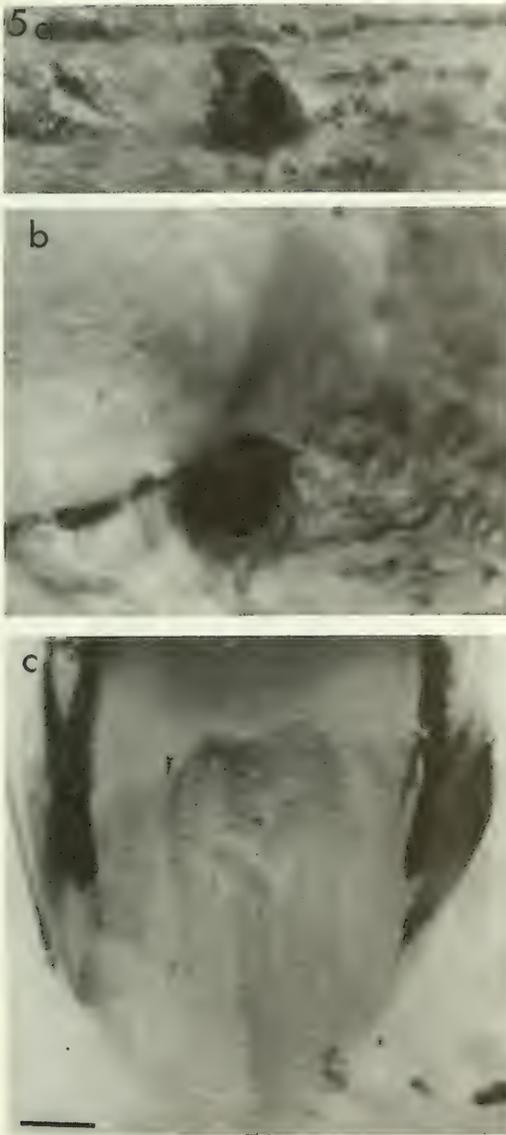


FIG. 5. Holes bored in *Murex* (a & b) and (c) the radula responsible (broken during preparation for photography). The surface region of the hole is small but the penetration hole made is large compared with those made in *Mytilus* (see Figs. 1a & b taken at same magnification). The bar represents 500 μm .



FIG. 6. Seven of the 17 holes made by 1 octopus in a single *Murex* so that the differences in shape and form can be easily compared. The bar represents 2000 μm .

(a) *Mytilus edulis* can be opened by the octopus without boring a hole in it. The 5 animals on the diet each opened 1-7 mussels in periods of 17-22 days post-operatively (Table 3). The condition of these shells can be compared with those taken from the normal octopuses (Table 1). The operated animals opened 21 shells in a total of 75 days compared with 21 in 137 days by normal octopuses, thus the operated animals opened more shells in this way than did normal animals who were able to bore holes. Not 1 shell opened by an operated animal had a hole in it although they could still eat and often left the shell entirely free of tissue (Table 3).

For the last 3 days each octopus was given a crab. All but 1 were able to paralyse a crab and eat all, or most, of its tissue (Table 3).

(b) *Murex trunculus*. Not one octopus bored a hole post-operatively; only 3 *Murex* were

opened and a little flesh eaten, the opercula had been pulled off with muscle still adhering.

On the last 2 days the octopuses were given a piece of dead fish and then a live crab. All took and ate the fish and all could paralyse a crab and leave the exoskeleton clean, except 1 which only partly ate 1 crab (Table 3).

(c) *Carcinus maenas*. Three octopuses, fed 2 days post-operatively, partly ate the crab tissue but did not separate the exoskeleton at the joints (Altman & Nixon, 1970). On the following

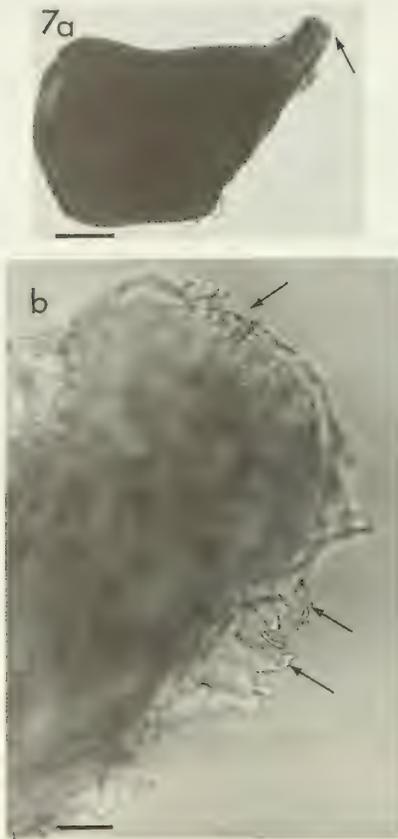


FIG. 7. (a) The everted tip of the duct of the posterior salivary gland, taken from the side, with small teeth covering it but barely visible at this magnification which is the same as that of the radulae and the holes they bored in *Mytilus* and *Murex* shown in Figs. 1 and 6. The bar represents 500 μ m. (b) The tip at higher magnification to show the small teeth on it. The bar represents 50 μ m.

days the crabs were separated at the joints and the exoskeleton left clean (Table 3). It was 7 days before the remaining octopus ate a crab leaving it only partly cleaned but on the remaining days it left them separated and clean.

3. *The radula and salivary papilla.* The size of the central rachidian teeth has a close relationship with the body weight ($r = 0.92$, Nixon, 1973). The average size of the hole at the surface of the *Murex* shell increased with the body weight of the octopus (Table 1). This was not so with *Mytilus* where the average size of the surface of the hole was similar irrespective of the size of the octopus that had bored it (Table 1). The penetration hole in *Murex* varied in size but could be almost as large as the surface hole whereas in *Mytilus* it was always small (Figs. 1, 2, 4, 5 and 6). The rachidian teeth could have entered the penetration holes bored in

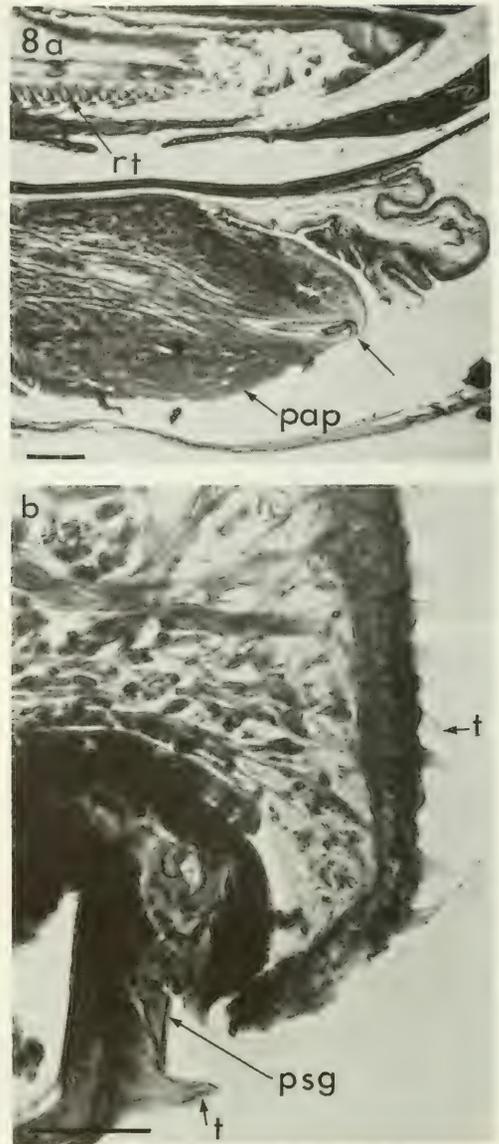


FIG. 8. (a) Sagittal section through the buccal mass of *Eledone cirrosa* showing part of the radula and its teeth (rt), the salivary papilla (pap.) and the inverted tip of the posterior salivary gland duct (psg). This region is shown at higher magnification below. (b) The teeth (t) on the surface of the papilla are small while those on the inverted tip of the duct (psg) are larger but still small when compared with the radular teeth. The bar represents 500 μ m in (a) and 50 μ m in (b).

Murex shells, but only the mesocone could have entered those in *Mytilus*. These observations and the results from the operated octopuses led to a histological examination of the papilla.

The anterior part of the papilla excised from 2 octopuses was kept and subsequently examined microscopically. One, from an octopus of 240 g is shown in Fig. 7, taken at the same magnification for comparison with the radulae and holes in Figs. 1 and 5. In the buccal masses from the operated octopuses, cut sagittally, the whole of the papilla or at least the anterior half was found to have been removed in every case (Table 3). The tip of the posterior salivary gland duct, projecting forwards from the papilla (Fig. 7), has some small teeth on its (now) external surface (these were first noted by J. S. Altman, personal communication, when operating in this region). The tip of the duct is everted, as it presumably is when secretion from the posterior salivary gland is ejected, and it is closer in size to the small penetration hole made in *Mytilus* than are the radular teeth. These teeth on the duct are almost transparent like those of the radula, and are small with a simple rod-like form (Fig. 7) although in section (Fig. 8b) they appear more complex; only the scanning electron microscope will reveal their shape and form. The cuticular covering of the salivary papilla is also endowed with teeth. These are restricted to the anterior part of the papilla and are smaller than those on the eversible tip of the duct (Fig. 8). The salivary papilla is very muscular and at its tip there is a rich plexus of nerve fibres below the epidermis (Young, 1965). In isolated preparations the papilla is very mobile (Dilly & Nixon, in prep.) and its muscular activity made it difficult to excise it under anaesthesia in the current experiments, due probably to the sensory and motor nerves present (Young, 1965).

The other cuticular teeth found in the buccal cavity are on the medio-anterior faces of the lateral buccal palps (Nixon, 1968). These teeth are backward pointing and almost certainly concerned with the transport of food to the oesophagus.

DISCUSSION

The differences found between the holes made in *Mytilus* and *Murex* are probably due to the composition of the shells. *Mytilus edulis* has an outer conchiolin layer, a middle prismatic layer of calcite (75%) and an inner layer of aragonite (25%) (Vinogradov, 1953; Degens & Spencer, 1966; Travis, 1968), whereas *Murex brevifrons* is 100% aragonitic (Degens & Spencer, 1966) and *M. trunculus* may be too. Aragonite is 3.5-4 on Moh's scale of hardness and calcite 3.0 (diamond is 10; Weast, 1976). This greater hardness could account for the smaller hole in *Murex* and the very small penetration hole in *Mytilus*. *Octopus bimaculatus* drilled holes of similar shape to those of *Mytilus edulis* in *Haliotis fulgens* (Pilson & Taylor, 1961); another species, *H. cracherodi*, is 70% calcite and 30% aragonite (Degens & Spencer, 1966). The hardness of the radular teeth of the hole-boring gastropod have been tested; the marginal teeth were found to be harder than the rachidian teeth and their range of hardness overlapped those of the prey shells (Carriker, 1969). The teeth of the radula, salivary papilla and duct of *Octopus vulgaris* need to be tested for comparison with the shells.

Not one octopus made a hole in a shell after the removal of the salivary papilla. This suggests it has some role, but whether it is the teeth on the papilla, and/or those on the tip of the duct, and/or the glandular secretion ejected through the duct that is involved in drilling activities, we do not yet know. The muscular papilla and duct tip must be plastic and capable of small, delicate movements which must be important in directing secretion from the glands. Furthermore the papilla could fit much more readily into the holes bored than does the radula, particularly in the lower and less accessible regions where its mobility would be of great value. The holes drilled by *Urosalpinx* are about 1.5 mm but are large when compared with its radula which has plenty of space to move in whilst rasping away the shell (see fig. 17, Carriker & Van Zandt, 1972).

The posterior salivary glands were still functional in the operated octopuses as they could paralyse crabs, and more surprisingly the duct was still patent in spite of cutting away much of the papilla. This indicates that the tip of the duct is not used to puncture the exoskeleton of the crab. This supports Lo Bianco's (1908) suggestion that the secretion from the posterior salivary glands is ejected close to the branchial hole of the crab to cause paralysis (see Ghiretti, 1960). Secretion in the form of a viscous mucus was found in bore holes (Wodinsky, 1969), though whether from the posterior salivary glands or from the other buccal glands is not known.

The mucus was found to be of pH 8.0 (Arnold & Arnold, 1969). Secretion presumably occurs during the silent periods recorded from the hydrophone (Arnold & Arnold, 1969; Wodinsky, 1969), and would provide the necessary lubrication for drilling. It is not possible to say whether the secretion acts in any way upon the shell, and accounts for the smoothness of the walls of some holes (Wodinsky, 1969). In *Urosalpinx* the accessory boring organ secretes carbonic anhydrase which was found essential for dissolving mollusc shells; the secretory activity alternated with the rasping action of the radula (Carriker & Van Zandt, 1972). *Urosalpinx* cannot bore holes after excision of its accessory boring organ, or after ablation of its radula. The effect of the removal of the radula was not tested here. In a previous experiment octopuses fed almost normally without it when given crabs or fish to eat (Altman & Nixon, 1970).

Fujita (1916) suggested that the salivary secretion weakened the muscles of the prey and Pilson & Taylor (1961) found an abalone, although still alive, unable to adhere firmly to the substrate after a hole had been bored in its shell by *Octopus* sp. Extracts from the posterior salivary glands, injected in the foot of an abalone, had similar effects. Another function of the secretion in *Octopus vulgaris* is in loosening the tissues from the joints on the shell of the prey, perhaps the action of hyaluronidase (Romanini, 1952, 1954) on the cement substance. The shells and opercula of *Murex* were left quite free of tissue after a hole had been bored, although *Mytilus* shells could be left clean whether or not a hole had been bored—however, a bivalve once opened is entirely exposed to its predator. There is no complete external digestion since recognizable fragments of gills, hepatic caecae and small cubes of muscle are found in the crop (Altman & Nixon, 1970).

Octopus vulgaris can capture a crab at a distance of 45 cm in 4 sec (Maldonado, 1963), paralyse it in 30-45 sec (MacGinitie, 1938) and eat it within 60 minutes depositing the debris outside its home (Hornell, 1893; Altman & Nixon, 1970). Pilson & Taylor (1961) watched an octopus (*O. bimaculatus* or *O. bimaculoides*) drill an abalone and detach it from the wall of the aquarium; this took 3 hours although the abalone was not left for the octopus to feed on. *O. vulgaris* took 20-30 min to enter *Oliva reticularis*, 40-60 min for *Strombus gigas*, 60-80 min for *S. raninus* and 90-120 min for *Livona pica* (Wodinsky, 1969). Thus in general it takes longer for octopus to drill and eat a mollusc than a crab. *O. vulgaris* has been shown to have a preference for crabs and crustaceans, followed by lamellibranchs and then gastropods (Taki, 1941). It is evident that *O. vulgaris* is an opportunist feeder. Indeed another octopus, *Paroctopus apollyon*, was found feeding on mussels when living on beds of them and on *Pecten* when living close to these (MacGinitie, 1938).

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NOTE ADDED IN PROOF

Since this article was submitted another experiment has been carried out in Naples in 1978. In this the functional part of the radula was removed and the octopuses were still able to drill holes in mussel shells; presumably mainly by the action of the teeth of the salivary papilla. This is in marked contrast with the effect of ablation of the salivary papilla reported above. The holes drilled, after ablation of the radula, closely resembled those bored by the control animals (Nixon, in preparation) and by the normal octopuses examined here.

The scanning electron microscope has shown the very small teeth on the anterior face of the salivary papilla to be closely packed, numerous and conical in form. It seems probable that the papilla acts like a countersink with a rasping surface (Nixon, 1979).

A further study with the scanning electron microscope has demonstrated that the tooth-covered salivary papilla does fit the holes drilled. Furthermore the tip of the posterior salivary gland duct could enter the small penetration holes made in *Mytilus edulis*. After drilling the tablets, of the inner layer of the shell, of aragonite have a rough and irregular surface indicating that some chemical action occurs (Howell, Maconnachie & Nixon, in preparation).

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THE EFFECT OF ACTIVITY ON THE OXYGEN UPTAKE OF *NASSARIUS RETICULATUS* (GASTROPODA, PROSOBRANCHIA)

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ABSTRACT

Oxygen uptake of individual *Nassarius reticulatus* was continuously measured using a polarographic oxygen electrode. Rates of uptake during activity did not differ significantly from resting rates. The maximum probable increase in oxygen uptake attributable to locomotion was about 20%. A similar figure was obtained for proboscis eversion. These increases are small compared with those which occur in response to food odours and feeding.

INTRODUCTION

Nassarius reticulatus, like many other neogastropods, is a scavenger. It feeds intermittently, locating its food by a series of responses to chemical stimuli and water currents, as described for *N. obsoletus* by Dimon (1905) and Copeland (1918). The olfactory reactions of *Nassarius* to stimuli normally indicative of food are suppressed by recent feeding (Crisp, 1978). Food odours, and feeding itself, also affect oxygen uptake to a marked degree (Crisp, Davenport & Shumway, 1978). In response to odours the increase of 3 to 4 times the starved uptake is transient. It lasts 2-4 days after feeding. The locomotor activity of starved whelks exposed to food odours is also initially increased and the proboscis is often everted. The present investigation is addressed to the question of how far the increases in activity can account for the observed increases in oxygen uptake. Newell & Pye (1971a and b) have reported differences of 300-500% in the oxygen uptake of *Littorina littorea* measured by Gilson respirometry. High rates of oxygen uptake were displayed by active winkles and low rates by inactive ones.

MATERIALS AND METHODS

Nassarius reticulatus were supplied by the Marine Biological Association of the United Kingdom from "White Patch," Plymouth. They were kept in running seawater at 16°C without food for a fortnight or more before use.

The oxygen uptake of individual *Nassarius* was followed using the apparatus described by Crisp, Davenport & Shumway (1978). The closed chamber was of 55 ml capacity and allowed the animals to move freely. At the start of each experiment 1.4 ml of a chemical stimulus (1.0 M glycine or a standardised crab extract) was liberated into the chamber. The behaviour of the whelk was closely observed and periods of movement, inactivity, proboscis eversion and withdrawal were marked on the oxygen uptake record.

For comparison between the rates of oxygen uptake during different states of activity the first few minutes after release of the chemical stimulus were ignored. The slope of the trace during each behavioural state yielded an estimate of oxygen uptake during that state.

RESULTS

Control experiments showed that the electrode consumed a negligible amount of oxygen compared with the animal and that during the period of the experiment, the crab extract did not cause the consumption of detectable amounts of oxygen.

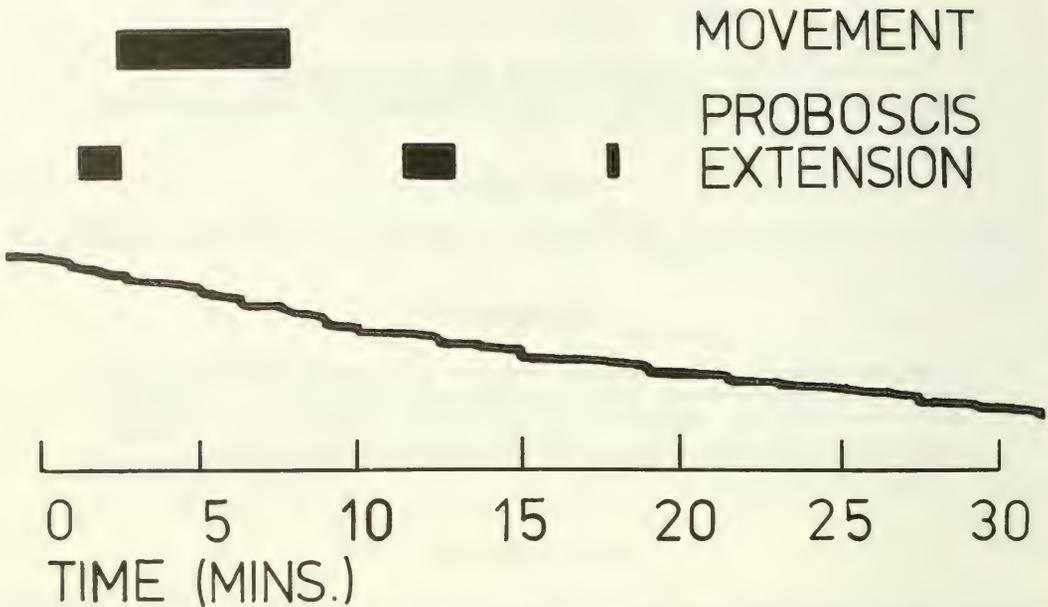


FIG. 1. Change of oxygen uptake with time by *Nassarius reticulatus*, individual B. The upper black band indicates a period of movement. The lower black bands indicate periods of proboscis extension.

Fig. 1 shows the trace from the animal referred to as 'B' in Tables 1 and 2. Changes in the slope of the trace, corresponding to changes in an animal's activity were rarely if ever evident. Since the uptake of oxygen fell as the oxygen tension was lowered (cf. Crisp, Davenport & Shumway, 1978), the oxygen tension-time curves were convex to the time axis. That is, measured rates of oxygen uptake normally fell during the experiment. Therefore from each curve the oxygen consumption rates for periods of rest and activity were separately averaged during a given continuous period. The results were selected in the form of matched pairs where observations of periods of activity and rest could be compared consecutively or intermittently in the same animal over the same continuous period of time. Table 1 gives rates for moving and inactive whelks. Table 2 gives rates for whelks during proboscis eversion and withdrawal.

The matched pairs *t* test showed no significant difference (at the 0.05 level) between rates of oxygen uptake during activity and inactivity. Using the sample standard errors obtained, the *t* test was used to estimate confidence limits for the increase caused by activity at $p = 0.95$. These were small. The average uptake of inactive animals was $475 \mu\text{l oxygen g dry wt}^{-1}\text{h}^{-1}$ and the maximum likely increase due to activity was $97 \mu\text{l oxygen g dry wt}^{-1}\text{h}^{-1}$, an increase of about 20%. Whelks with the proboscis withdrawn had an average uptake of $305 \mu\text{l oxygen g dry wt}^{-1}\text{h}^{-1}$, and the maximum likely increase due to proboscis eversion was $55 \mu\text{l oxygen g dry wt}^{-1}\text{h}^{-1}$, or an increase of 18%.

TABLE 1. Comparison of oxygen uptake of *Nassarius reticulatus* during movement and rest ($t = 2.23$ N.S.).

Animal	State of proboscis	Time after start of experiment during which measurements made (mins)	Average rate of oxygen uptake ($\mu\text{l O}_2 \text{ g dry wt}^{-1}\text{h}^{-1}$)		Difference $\Delta = (r_m - r_s)$ $\mu\text{l O}_2 \text{ g dry wt}^{-1}\text{h}^{-1}$
			During movement (R_m)	During rest (R_s)	
A	withdrawn	3.5-12.5	974	974	0
B	withdrawn	2.5-11.0	365	285	+80
C	withdrawn	4.0-30.0	273	256	+17
C	everted	7.0-15.0	298	278	+20
D	everted	30.0-45.0	679	581	+98

TABLE 2. Comparison of oxygen uptake of *Nassarius reticulatus* during proboscis eversion and withdrawal ($t = 1.686$ N.S.).

Animal	Moving (M) or station-ery (S)	Time after start of experiment during which measurements made (mins)	Average rate of oxygen uptake ($\mu\text{l O}_2 \text{ g dry wt}^{-1} \text{ h}^{-1}$)		Difference $\Delta = (R_E - R_W)$ $\mu\text{l O}_2 \text{ g dry wt}^{-1} \text{ h}^{-1}$
			During proboscis eversion (R_E)	During proboscis withdrawal (R_W)	
B	S	7.5-18.0	205	238	-33
C	M	6.0- 8.0	298	298	0
C	S	8.0-30.0	278	234	44
D	S	36.0-55.0	577	540	37
E	S	5.0-13.0	387	408	-21
F	S	5.0-17.0	223	193	30
G	S	30.0-50.0	169	173	-4
H	M	30.0-45.0	325	240	85
J	S	30.0-36.0	263	131	132
K	S	30.0-60.0	163	198	-36
L	S	30.0-60.0	591	577	14
M	S	30.0-53.0	469	430	39

DISCUSSION

The results show that the increases in oxygen uptake of *Nassarius* which result immediately from increased motor activity were small, if, indeed, they existed. The changes in uptake which result from certain chemical stimuli or from feeding (Crisp, Davenport & Shumway, 1978) were of quite a different order and could not therefore have been caused by enhanced locomotor or proboscis activity. These latter are also much smaller than the increases reported by Newell & Pye (1971a and b) for *Littorina littorea*.

Increases in metabolism with activity are naturally related to power output. When this is high, as for example in a flying insect, metabolism is enormously increased. A flying bee consumes 50-200 times the oxygen used at rest (Krogh, 1941). When power output is less, even though movements may still be vigorous, the increase in oxygen uptake is correspondingly less. Polychaetes irrigating their tubes, for example, consume oxygen at 2-15 times their resting rates (Mangum, 1976). The movements of *Nassarius* in the respirometer chamber were leisurely. Only a small effect on oxygen uptake is therefore to be expected.

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HABITUATION CHARACTERISTICS OF THE TENTACLE CONTRACTION REFLEX OF THE POND SNAIL *LYMNAEA STAGNALIS* (L.)

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ABSTRACT

Habituation experiments were carried out on the tentacle contraction reflex of *Lymnaea stagnalis*. The relations between habituation and dishabituation of the contraction amplitude and the number of action potentials of an identified tentacular motoneurone (element I) were studied. Two locations of the habituation were found: one at the input level of the motoneurone and one at its terminals. Dishabituation occurred only in the periphery.

INTRODUCTION

Interactions between the central and peripheral nervous systems in molluscs can be studied by habituation experiments. Habituation (decrement of the response) can occur in both systems (Jacklet & Lukowiak, 1975). A habituated response mediated via the peripheral nervous system can be dishabituated (restored) by input via central neurones, as is shown by Lukowiak & Jacklet (1972) for the siphon withdrawal response of *Aplysia californica*. Reflexes mediated via the central nervous system can be dishabituated by giving a novel stimulus. Pinsker et al. (1970) for example found in an *Aplysia* preparation that tactile stimuli applied to the siphon cause habituation of the resulting gill contractions. Dishabituation of the responses could be established by interpolating a head stimulus in the series of siphon stimulations.

In the present study an attempt was made to investigate the contribution of central and peripheral neural circuits to habituation and dishabituation in the tentacle contraction reflex of *Lymnaea stagnalis*. This contraction is part of the defensive withdrawal of the snail (Lever et al., 1977) and can be evoked via both central and peripheral pathways. In the reflex an identified motoneurone (element I) is involved (Lever et al., 1977; Lever, 1977b), receiving its sensory synaptic input (from the tentacle and from other skin areas) within the tentacular nerve (Lever, 1977a). The habituation and dishabituation of the tentacle contraction caused by tactile stimuli applied to the mantle and the tentacle were correlated with responses of element I.

MATERIALS AND METHODS

Snails were used with a shell height of 30 ± 2 mm. They were bred in the laboratory under standard conditions (Van der Steen et al., 1969).

The preparations consisted of the left two-thirds of the mantle, the left mantle nerve, the central nervous system, the left tentacular nerve and the left tentacle. These were kept in a recording chamber filled with physiological saline (NaCl 30 mM, KCl 1.5 mM, MgCl₂ 2 mM, CaCl₂ 4 mM, Na₂HPO₄ 0.25 mM, NaHCO₃ 18 mM).

Recordings of the electrical activity in the tentacular nerve were made with an 'en passant' electrode, and those from the tension in the tentacle by means of a force transducer.

Pressure stimuli were applied to the tentacle or the mantle with a glass probe (tip diameter 0.25 mm.) driven by a moving coil current meter. Dishabituating stimuli were applied with a small brush.

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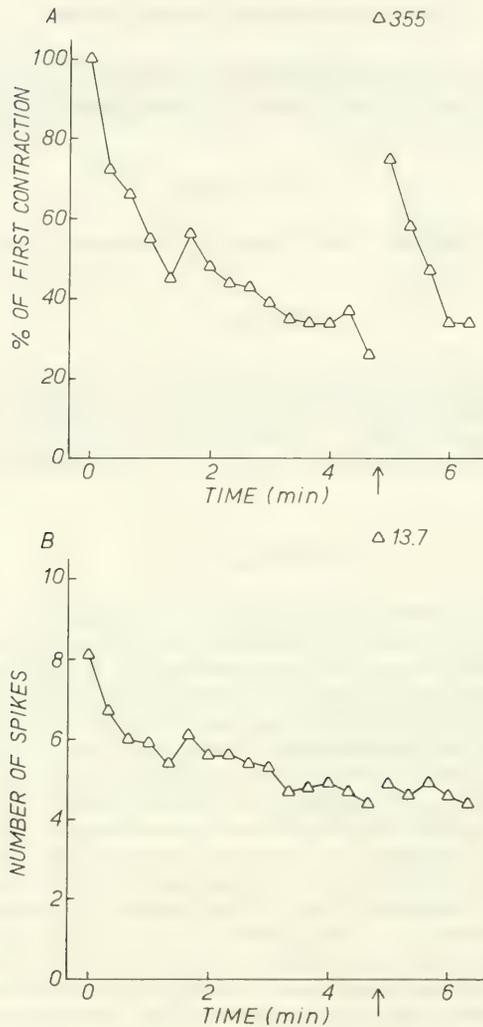


FIG. 1. Averaged (9 experiments) amplitudes of the tentacle contractions caused by repetitive pressure stimulation of the mantle (3 stimuli/min) expressed as the percentage of the first contraction (A), and the means of the frequencies of the simultaneously evoked spikes of element I expressed as the number of spikes recorded during the first half second after the stimulus (B). After the 15th stimulus a stroke stimulus was applied to the tentacle (arrow).

RESULTS

The tentacle contraction reflex can be evoked by tactile stimulation of the tentacle and of other skin areas in intact animals and in preparations. If the tentacle is stimulated, the contraction is mediated via both central motoneurons and the peripheral nervous system. The response of the central motoneurons can be measured in the tentacular nerve. It mainly consists of action potentials of element I. If the mantle is stimulated the reflex is mediated via the central nervous system, usually by the activation of element I only.

1. Habituation of the tentacle contraction reflex by repeated stimulation of the mantle.

Repetitive pressure stimuli (strength ± 32 mg, duration 10 msec, intervals 20 sec) were applied to the mantle. During successive stimulations the amplitudes of the tentacle contrac-

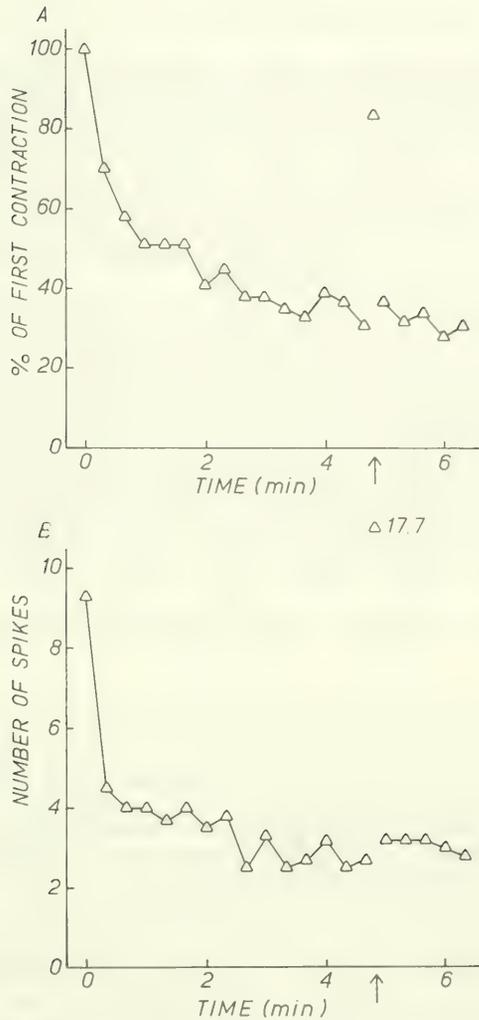


FIG. 2. Averaged (6 experiments) amplitudes of the tentacle contractions caused by repetitive pressure stimulation of the tentacle (3 stimuli/min) expressed as the percentage of the first contraction (A), and the means of the frequencies of the simultaneously evoked spikes of element I expressed as the number of spikes recorded during the first half second after the stimulus (B). After the 15th stimulus a stroke stimulus was applied to the mantle (arrow).

tions (Fig. 1A) and the number of action potentials of element I (Fig. 1B) decreases. When, during an interval, one stroke stimulus is applied to the tentacle a larger contraction and an increased number of action potentials of element I are evoked. Thereafter only dishabituation of the contraction and not of the number of spikes of element I, is seen (Fig. 1). The dishabituated contraction amplitude habituates again.

2. Habituation of the tentacle contraction reflex by repeated stimulation of the tentacle.

Also during repeated pressure stimulation (strength ± 12 mg, duration 10 msec, intervals 20 sec) of the tentacle, the amplitude of the tentacle contraction reflex (Fig. 2A) as well as the number of spikes of element I (Fig. 2B) decreases. An inserted stroke stimulation of the mantle causes one larger contraction of the tentacle and an increased number of action potentials of element I. Neither dishabituation of the contraction amplitude of the tentacle, nor of the number of element I spikes (Fig. 2) was found.

DISCUSSION

The experiments show that repetitive tactile stimulation of the mantle and of the tentacle causes a habituation of the contraction amplitude of the tentacle and of the number of element I spikes. Dishabituation of the contraction amplitude occurs only when a tactile stimulus upon the tentacle is interpolated in a series of mantle stimulations, but not in the reverse experiment. In both experiments no dishabituation of the number of element I spikes is found. This indicates that, in the first experiment, the increase of the contraction amplitude effected by the dishabituating stimulus is caused by heterosynaptic facilitation of peripheral neurones ending upon the terminals of element I; no post-tetanic potentiation was caused by the large number of spikes in response upon the interpolated stimuli.

The present investigations show that this system has, at least, 2 sites of habituation: one at the input level of element I, and another at its terminals. Dishabituation occurs only at the terminals. This is unlike the result of Kandel and his co-workers (Castellucci et al., 1977; Kandel, 1971; Hawkins et al., 1977). They found heterosynaptic facilitation at the central synaptic input level of an identified motoneurone in *Aplysia*. A dishabituating influence of peripheral neurones upon central neurones is also found by Lukowiak & Jacklet (1972, 1975) in *Aplysia californica*. In addition a dishabituating influence of central neurones upon habituated peripheral responses was observed. In the present study no dishabituating effect of responses mediated by the central nervous system (in response to mantle stimulation) upon habituated responses in the periphery (caused by repetitive stimulation of the tentacle) was found. Further experiments are in progress; the results will be published elsewhere (Lever & Bloemen, 1978).

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MICROELECTRODE INVESTIGATIONS OF LEARNING PHENOMENA IN SNAIL (*HELIX POMATIA*) NEURONES

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ABSTRACT

We have examined changes of postsynaptic potentials and of pattern activity of the identified silent and oscillatory snail neurones in *Helix pomatia* during conditioning. Local changes of EPSP or IPSP have been recorded during association following the first stimulus in the silent cells, whereas spike discharges could be observed in response to the 2nd stimulus. In the oscillatory neurones changes of pattern activity have been recorded following the 2nd stimulus, while the first stimulus proved to be ineffective. The formation of temporary connections of snail neurones seemed to be a specific phenomenon, because it was necessary to pair stimuli of different inputs for the development of these modifications. These plastic changes seemed to depend on the interstimulus as well as on the intertrial intervals. Our experimental data underline the probable role of the stimulus parameters and of the electrical properties of neurones during the formation of learned neuronal responses.

INTRODUCTION

In the last years numerous papers were published dealing with the problems of learning phenomena in the invertebrate nervous system. It has been demonstrated that snail neurones can well be used as models of the formation of temporary connections (Kandel & Tauc, 1965; Kandel & Spencer, 1968; Sokolov, 1969; Baumgarten & Hukuhara, 1969; Epstein & Tauc, 1970).

In our earlier experiments (Puszta & Ádám, 1974; Puszta et al., 1976b) the possibility of the formation of temporary connections was demonstrated by delayed pairing of 2 stimuli according to the classical conditioning technique in the giant neurones of the snail *Helix pomatia* L.

In the present work we examined the changes of postsynaptic potentials and of the activity pattern of the identified silent and oscillatory snail neurones during conditioning.

METHODS

The experiments were performed on the identified giant neurones of the suboesophageal ganglions of the snail *Helix pomatia* L. The basic methods of preparation, recording and identification of neurones are described in preceding papers (Puszta & Ádám, 1974; Puszta et al., 1976a).

The stimulation of the peripheral nerves was performed using bipolar silver electrodes, fixed in a special chamber. Fig. 1 shows the oesophageal ganglia placed in this chamber, the nerves being sited in separate channels containing the bipolar electrodes. The black points indicate the silver electrodes.

RESULTS

The results are recorded in Table 1; 66 neurones were examined of which 32 were silent and 34 oscillatory cells.

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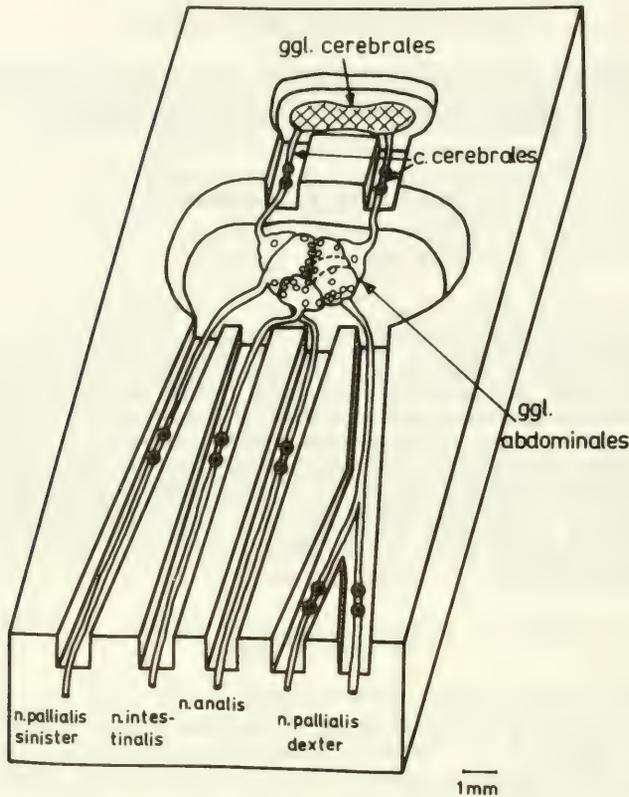


FIG. 1. Schematic drawing of the oesophageal ganglia placed in a special plastic chamber. The nerves are sited in separate channels containing the bipolar electrodes. ○—identified neurones, ●—silver electrodes.

In the silent neurones typical changes of postsynaptic potentials can be observed as a consequence of conditioning. For example the cell V_3 (Fig. 2, row 1) reacted to the stimulation of the nervus analis by producing EPSPs, whereas the stimulation of the left pallial nerve produced action potentials (Fig. 2, rows 2-3). During association of the 2 stimuli the frequency and magnitude of EPSPs increased, especially in the intertrial intervals (Fig. 2, rows 4-5). After 30 associations the stimulation of the nervus analis by itself developed action potentials (Fig. 2, row 6). Subsequent stimulation of the anal nerve (rows 7-8) has been applied after 15 and 30 min.

These changes of EPSPs seemed to depend strongly on the frequency of the paired stimulations. Fig. 3 shows the changes of the relative amplitude of the EPSPs, i.e. denotes in % the ratio of EPSP after association as compared to the EPSP evoked by stimulation of the anal nerve before conditioning.

TABLE 1. Results of conditioning of *Helix* identified neurones.

Neuron	No. of cells	Type of cell activity	No. of pairings	Duration of CR/min
V_3	13	silent	30-80	10-30
V_7	8	silent	40-60	5-15
V_9	7	oscillatory	30-60	10-40
V_{16}	11	oscillatory	5-25	15-40
LPa_3	11	silent	30-60	15-45
RPa_2	7	oscillatory	20-80	15-45
RPa_3	9	oscillatory	10-60	10-30

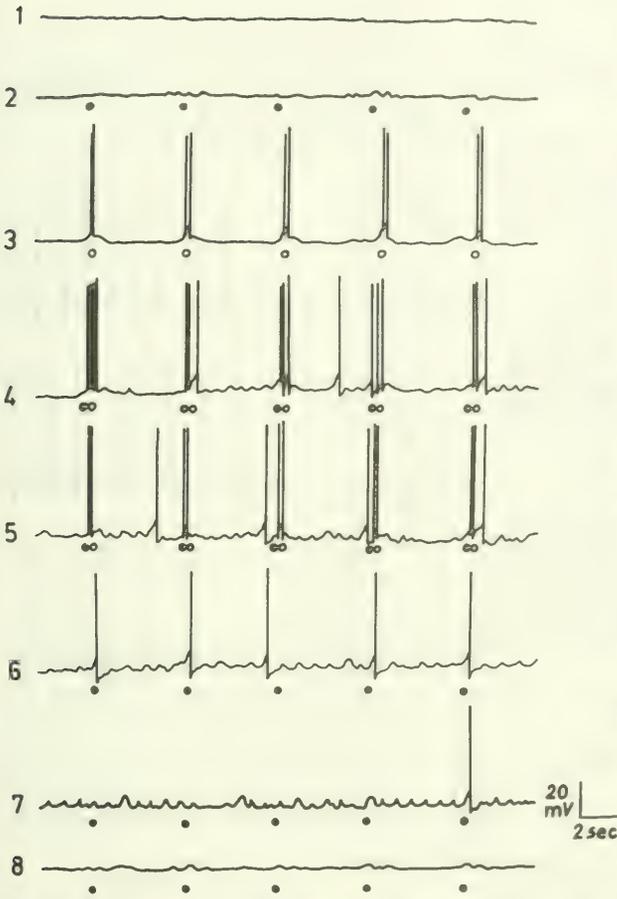


FIG. 2. Elaboration of conditioned response (CR) in the neurone of the visceral ganglion V_3 . 1, spontaneous state; 2, effect of the anal nerve stimulation (\bullet); 3, effect of the left pallial nerve stimulation (\circ); 4, 5, pairing of the two stimuli; 6, 7, 8, CR immediately after association, then after 15 and 30 min.

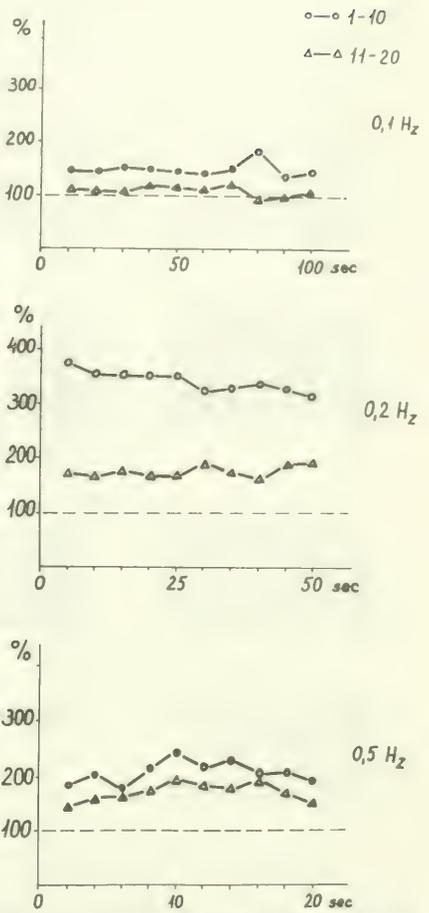


FIG. 3. Influence of different frequency of pairings on the changes of relative amplitude of EPSPs. Symbols: \circ - \circ test stimuli immediately after associations, \triangle - \triangle then after 20 min.

The first 10 tests stimuli (\circ - \circ) were realized immediately after the associations, whereas the 2nd 10 test stimulations (\triangle - \triangle) were performed subsequently after 10-25 min.

A growth of the EPSPs of 250-300%, its absolute value being of 8-15 mV amplitude, often has been enough to develop an action potential.

According to our results the changes of the firing pattern and frequency of giant neurones during and following conditioning could be classed in 4 groups; 1—firing frequency increased; 2—firing frequency decreased; 3—pattern activity changed; 4—frequency and magnitude of synaptic potentials changed (see Figs. 4, 5 and 6).

DISCUSSION

In our earlier works, as in other papers, the changes of the EPSPs and of the firing activity during and following the pairings, namely in the intertrial intervals, have not been examined. Therefore, in this series we studied the optimal conditions of the elaboration of the temporary connections on the neuronal level, using microelectrode technique.

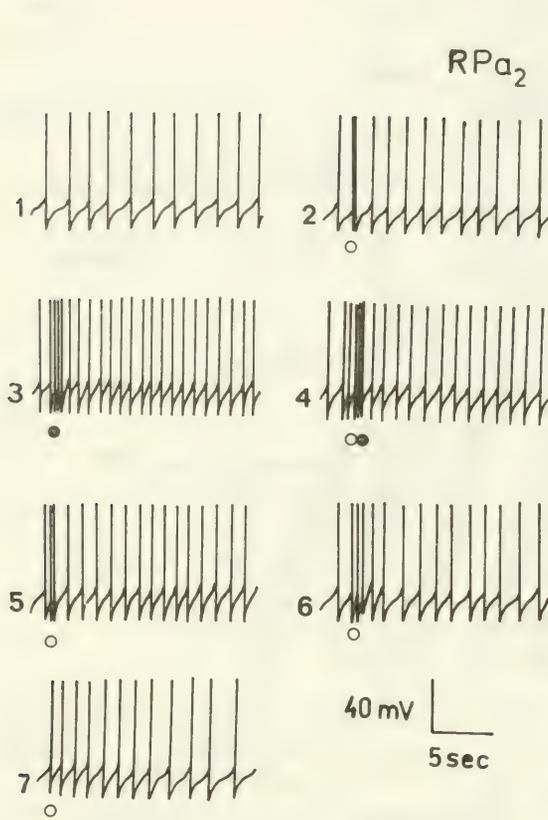


FIG. 4. Development of a learned response in the neurone of the right parietal ganglion RPa_2 . 1, spontaneous spikes; 2, effect of the left pallial nerve stimulation; (○); 3, effect of the right pallial nerve stimulation (●); 4, pairing of two stimuli; 5, 6, 7, CR 5, 15 and 30 min after the pairings.

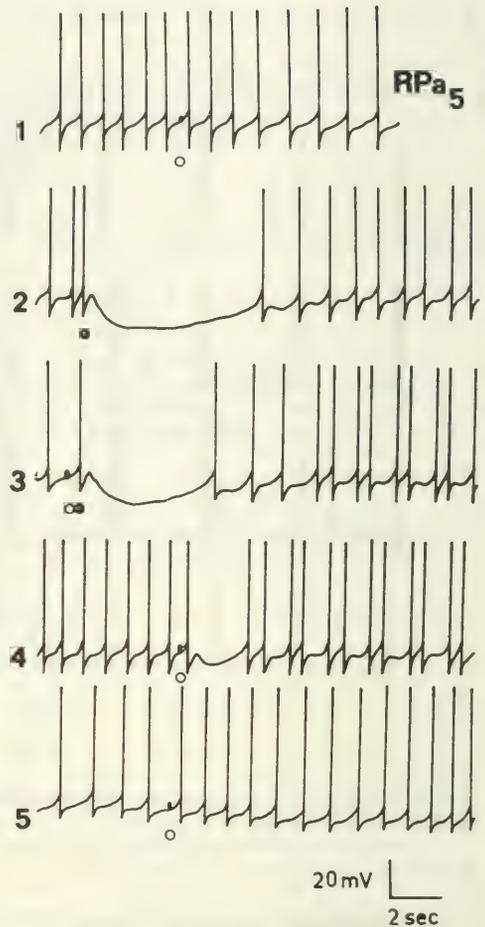


FIG. 5. Learned response in the cell RPa_5 . 1, spontaneous activity and stimulation of the anal nerve; 2, effect of the intestinal nerve stimulation; 3, pairing of two stimuli; 4, 5 CR immediately after the pairing, then after 20 min. Legend in other details identical to that of Fig. 4.

It has been demonstrated that the optimal delay between the 2 stimuli in our conditions is 500 msec (the interstimulus interval was altered from 300 to 800 msec). The increase of the amplitude of EPSPs in our experiments seemed to depend on the frequency of the paired stimulation, the optimal local changes appearing during association with a frequency of 0,2 Hz (see Fig. 3).

The formation of the above mentioned temporary connections seemed to be specific, since the paired stimulation of different inputs was necessary for the development of this learned phenomenon. Our data underline the probability of the role of local electrical changes in the membrane during the formation of learned neuronal responses.

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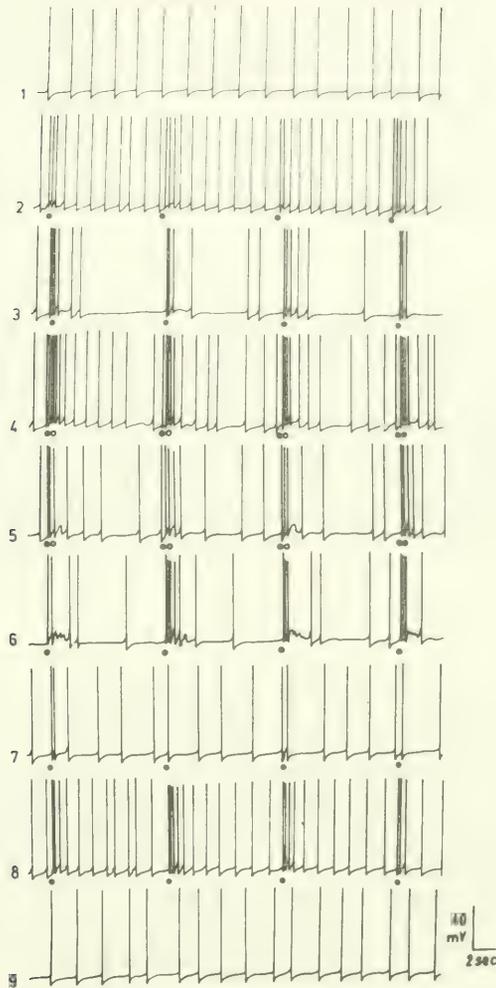


FIG. 6. Elaboration of a conditioned response in the neuron V_9 . 1, spontaneous activity; 2, effect of the right pallial nerve stimulation; 3, effect of the intestinal nerve stimulation; 4, 5, pairing of two stimuli; 6, 7, 8, CR after pairings 5, 20 and 40 min; 9, spontaneous activity of neuron V_9 45 min after pairings. Legend in other details identical to that of Fig. 2.

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PERIPHERAL AND CENTRAL PHOTORECEPTION IN *APLYSIA FASCIATA*

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ABSTRACT

Extraocular photosensitivity in *Aplysia fasciata* was studied in the skin and in the central nervous system (CNS). Local illumination causes contractions of the muscles of the body wall, which are obviously mediated by the peripheral nervous system (PNS). Afferent sensory activity is supposedly mainly dependent on stretch reception. Light-induced peripheral reflexes habituate after repetitive stimulation in preparations in which the CNS is present. In preparations without CNS light-induced contractions are remarkably stronger and do not habituate after repetitive stimulation. Central responses to peripheral stimulation could be evoked by both "light on" and "light off" stimulation, indicating that 2 types of photosensitive elements are present in the periphery. Observations on isolated CNS-preparations revealed that in the central ganglia photoreceptive elements are also present. Here, too, elements responding to the onset as well as elements responding to the offset of light have been detected.

INTRODUCTION

Extraocular photoreception is a widespread phenomenon within the phylum Mollusca. It has been demonstrated in many bivalves and gastropods, for instance, and it has become evident that several different ways of photoreception may account for extraocular photosensitivity. The receptors may be located in the CNS (Kennedy, 1960; Arvanitaki & Chalazonitis, 1961; Hisano et al., 1972) or may be peripheral sensory cells (Dijkgraaf, 1935; Föh, 1932; Crisp, 1972; Stoll et al., 1976). In both cases the existence of distinct receptors for "light-on" and for "light-off" have been reported (Arvanitaki & Chalazonitis, 1961; Block & Smith, 1973; Stoll, 1976).

In *Aplysia fasciata* (= *A. limacina*, cf. Eales, 1960), sudden illumination of the body causes reflexes of the body wall musculature (Dijkgraaf, 1935), which are particularly prominent in the large parapodia. The present observations deal with the location and physiological properties of extraocular photoreceptors in this species.

MATERIALS AND METHODS

Specimens of *Aplysia fasciata* were collected in the Gulf of Naples and studied in the Zoological Station. Preparations were made by removing the visceral mass and pinning out the head-foot and parapodia in a dish with sea water. Part of the preparation was illuminated by means of a microscope lamp with white light (infrared-filtered) of about 2500 lux. Muscular contractions were registered with a force-transducer which was attached to the illuminated body-part; whole-nerve responses were recorded with a suction-electrode which was connected with either the central or the peripheral stump of the nerve to be studied (Fig. 1).

PERIPHERAL RESPONSES

Illumination of the parapodial area innervated by the posterior parapodial nerve (Hughes, 1971; Stoll et al., 1978) causes muscular contractions and sensory nerve responses; simultaneous recording revealed that muscle contractions precede the sensory responses (Fig. 2).

Hence it appears that stretch-sensitive elements rather than photoreceptive elements contribute to the nerve responses. This observation is compatible with the observation of Lukowiak

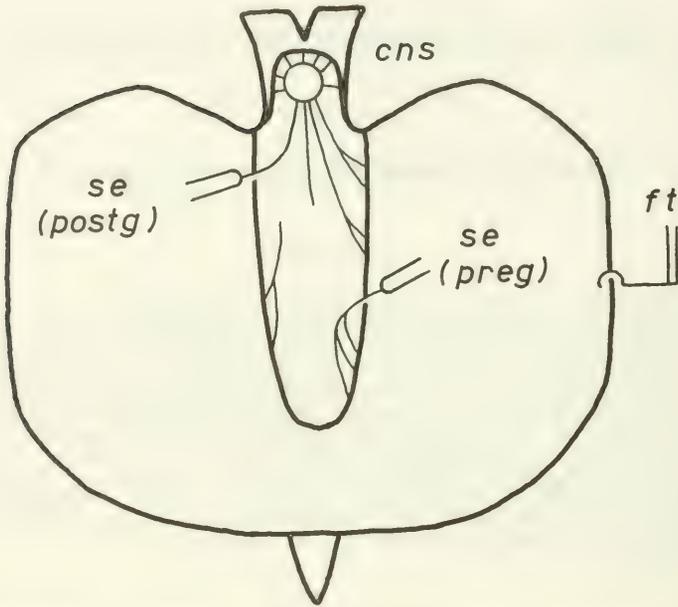


FIG. 1. Schematic drawing of a preparation, showing different ways of recording of nerve activity. CNS: central nervous system; se (postg.): suction electrode for registration of postganglionic responses, connected with the central stump of a nerve; se (preg.): suction electrode for registration of preganglionic responses, connected with the peripheral stump of a nerve; ft: force-transducer for registration of parapodial contractions.

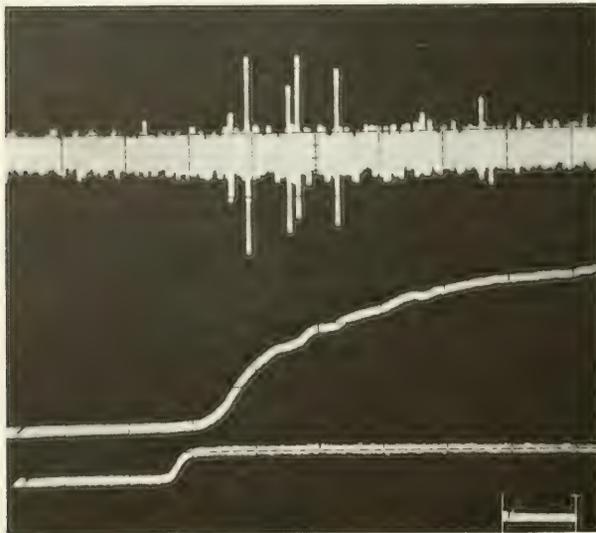


FIG. 2. Peripheral effects of illumination of the right parapodium. Lower trace: light-on stimulus. Middle trace: parapodial contraction. Upper trace: sensory activity recorded from the right posterior parapodial nerve. Time cal.: 1 sec.

& Jacklet (1975) on *Aplysia californica*, that light-induced contractions of the siphon occur in absence of any photosensory activity in the siphonal nerve. However, the possibility cannot be excluded that photoreceptive elements with a very small amplitude, usually not exceeding the noise level of the extracellularly recorded nerve activity, are present in the nerve responses. Indeed, such elements have been established both in a direct and an indirect way in *Lymnaea stagnalis* (Stoll et al., 1976).

Nevertheless, the photoreceptive cells are part of a peripheral nerve net involved in parapodial contractions, for light-induced reflexes persist in absence of the CNS (Dijkgraaf, 1935). Since it was observed that direct illumination of muscles inside the parapodia was not effective in evoking contractions, it is concluded that photoreceptive processes take place in the epidermis, like it was shown in a prosobranch gastropod, *Nassarius reticulatus* (cf. Crisp, 1972).

In spite of the fact that light-induced reflexes are a peripheral event, the CNS exerts an inhibiting influence on the contractions (Dijkgraaf, 1935). In the present experiments it was observed that in preparations without CNS light-induced contractions are stronger and more tonic than in preparations in which the connections with the CNS are intact. Furthermore it was observed that in the presence of the CNS the contractions habituate upon repetitive stimulation, whereas in the absence of the CNS they fail to do so (Fig. 3).

CENTRAL RESPONSES

Since from the above results the question arises whether the CNS receives photosensory input from the periphery, postganglionic responses to parapodial stimulation were studied in the central stumps of some pedal and pleural nerves; particular attention was paid to the relative latencies of the parapodial contractions and the postganglionic nerve responses, respectively. Unfortunately, postganglionic responses never preceded the parapodial contractions and these nerve responses, therefore, may have been induced either via photoreceptors or via secondarily

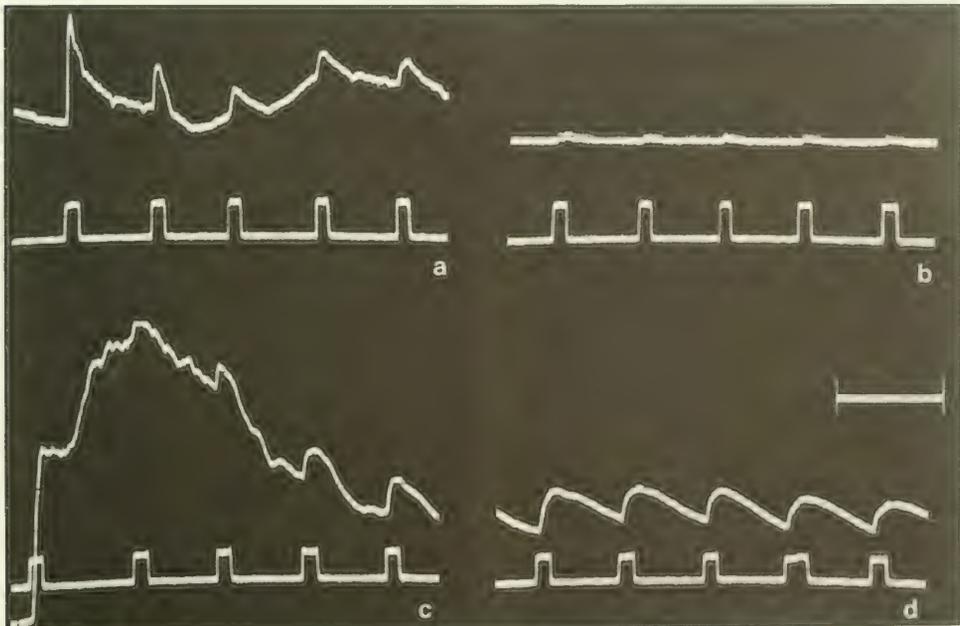


FIG. 3. Effect of repetitive light-stimulation of a parapodium in a preparation with (a,b) and without (c,d) CNS. Lower traces: light stimulus. Upper traces: parapodial contraction. (a) CNS intact; light-stimulus nr. 1-5. (b) CNS intact; light-stimulus nr. 11-15. (c) CNS removed; light-stimulus nr. 1-5. (d) CNS removed; light-stimulus nr. 11-15. Time-cal.: 10 sec.

activated stretch receptors. Surprisingly, during the experiments on postganglionic responses the observation was made that not only "light on" stimulation, but also "light off" stimulation of the preparation is followed by postganglionic responses in the pedal and pleural nerves (Fig. 4a,b). These off-responses are certainly not evoked via stretch-receptors, because light-off stimulation does not induce contractions in the parapodia. Since, moreover, the optic nerves were cut in these experiments, these results show that indeed extraocular photoreceptors are responsible for these off-responses. This suggests that two types of functionally different photoreceptive elements co-exist in the skin, like in *Lymnaea stagnalis* (Stoll, 1976). Postganglionic light-on and light-off responses are different, which also indicates that two receptor systems exist in the periphery.

Finally, after complete isolation of the CNS postganglionic responses to light-on could be easily obtained (Fig. 5a), demonstrating that on-receptors are also present in the CNS. Postganglionic off-responses were seldom obtained in isolated CNS-preparations. However, in a

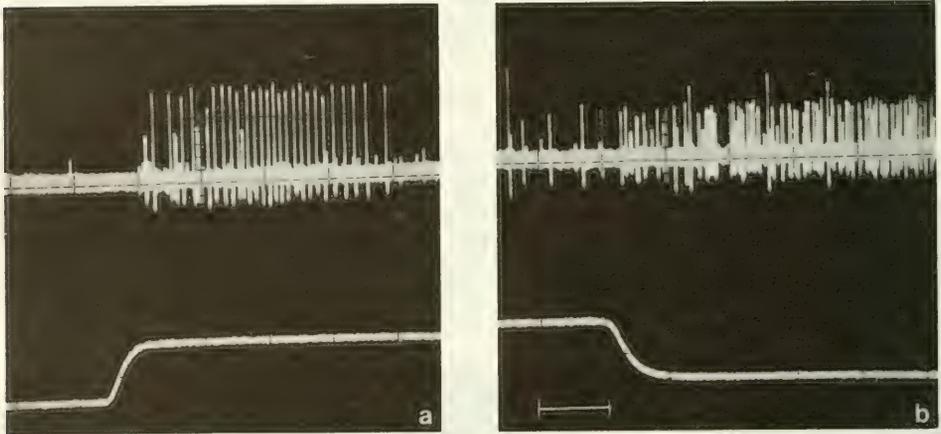


FIG. 4. Postganglionic responses to photic stimulation of a preparation with CNS, recorded from right pleural nerve. (a) On-response. (b) Off-response. Lower trace: Light-stimulus. Upper trace: Nerve response. Time cal.: 1 sec.

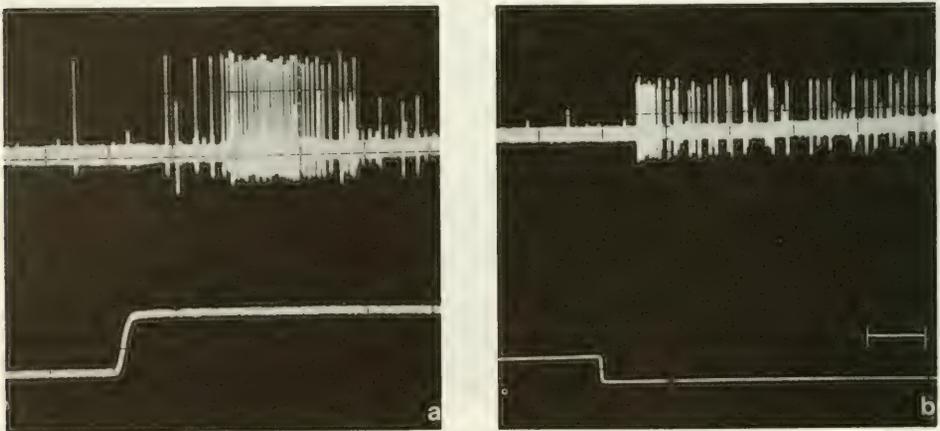


FIG. 5. Postganglionic responses to photic stimulation of an isolated CNS, recorded from right pleural nerve. (a) On-response. (b) Off-response. Lower trace: Light-stimulus. Upper trace: Nerve-response. Time cal.: 2 sec.

few cases the CNS clearly responded to a light-off stimulus (Fig. 5b). It should be assumed, therefore, that off-receptors are present in the CNS as well (cf. Block & Smith, 1973).

Summarising, the present results show that in *Aplysia fasciata* extraocular photoreception takes place in the skin and in the CNS. In both cases distinct receptive systems for "light-on" and "light-off" detection are likely to exist.

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5-HT INDUCED ACCUMULATION OF 3',5'-AMP AND THE
PHOSPHORYLATION OF PARAMYOSIN IN THE ABRM
OF *MYTILUS EDULIS*

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ABSTRACT

In *Mytilus edulis* 5-Ht induced relaxation of a muscle in catch is preceded by an increase in 3',5'-AMP content. In vitro two proteins of the contractile apparatus are phosphorylated by 3',5'-AMP dependent protein kinases. The 295000 dalton protein cannot be identified, the other one is paramyosin. Phosphorylated paramyosin inhibits actomyosin ATPase of smooth mollusc muscles at low and high Ca^{++} concentrations.

INTRODUCTION

Molluscan smooth muscles may display prolonged stretch resistance. During this tonic contraction or "catch" the active state is absent and energy consumption is only about 10% above resting level. The molecular basis of catch is still unknown. However, the molecular structure and the arrangement of the filaments in the muscle cells favour the linkage hypothesis. This theory supposes that the actin myosin cross bridges break very slowly during catch (for details see Twarog, 1976).

In vitro catch can be induced by acetylcholin. Tension development is Ca^{++} dependent. However, the catch itself seems to be rather independent of the concentration of free Ca^{++} in the myoplasm (Atsumi & Sugi, 1976; Marchand-Dumont & Baguet, 1975). The relaxation of a muscle in catch can be best initiated by serotonin (5-Ht). The relaxation process is preceded by a fourfold increase in the concentration of the cyclic nucleotide 3',5'-adenosine monophosphate (3',5'-AMP). The 5-Ht-induced accumulation of 3',5'-AMP depends on the presence and concentration of 5-Ht and is enhanced by low concentrations of free Ca^{++} (Achazi et al., 1974; Higgins, 1974). Additional experiments with dibutryl-3',5'-AMP, theophyllin, and skinned muscle fibers (Marchand-Dumont & Baguet, 1975) suggest that this cyclic nucleotide is the second messenger in the 5-Ht-mediated relaxation process. The reaction chain initiated by 5-Ht may proceed in the following way: 5-Ht induces the accumulation of 3',5'-AMP, this nucleotide activates a protein kinase, which in turn phosphorylates a protein. This protein affects the actin myosin interaction and relaxation starts.

Evidence of the protein kinase system will be presented elsewhere. This paper is concerned with (a) the nature of the protein to be phosphorylated and (b) the way by which this phosphorylated protein affects the actin myosin interaction.

MATERIALS AND METHODS

Mytilus edulis was collected at the Northsea coast. After dissection the anterior byssus retractor muscle (ABRM) and/or the posterior adductor muscle in the cold, paramyosin was isolated by (1) selective extraction of thick filaments (Szent-Györgyi et al., 1971), (2) from ethanol-ether dried powder (Bullard et al., 1973), or (3) by reducing the ionic strength of a crude actomyosin preparation from 600 mM to 350 mM. The phosphorylation of paramyosin with associated or purified protein kinase was performed according to Pratje & Heilmeyer, 1972, at a

salt concentration of 600 mM KCl to avoid precipitation of paramyosin. Analytical gel electrophoresis followed the procedure of Weber & Osborn, 1969, and electron microscopy the method outlined by Bullard et al., 1973.

RESULTS

(A) Phosphorylation of paramyosin

In *Mytilus* preparations of myofibrils can be phosphorylated by associated and purified protein kinases. For the initial identification of the phosphorylated protein phosphorylated crude actomyosin was subjected to analytical gel electrophoresis. After staining and determination of the molecular weight of the individual bands, the gels were sliced and the radioactivity of the fraction assayed in an scintillation counter. Only proteins of 2 different sizes are labelled: a still unidentified protein of a molecular weight of 295000 ± 5000 daltons ($n=7$) and a smaller one (106000 ± 2500 daltons, $n=7$). The latter seemed to be paramyosin.

The identity of this phosphorylated protein with paramyosin can be proved by isolating paramyosin according to the methods outlined above. As shown in Fig. 1 and Fig. 2 only protein with a molecular weight of paramyosin is phosphorylated. The activation of the phosphorylation by cyclic AMP is not very high. This is probably caused by the unspecific activation of the kinase by the high salt concentration in the assay. Paramyosin prepared by method (2) is far better phosphorylated than paramyosin isolated by other methods. The maximum incorporation amounts to 2515 pmoles/mg protein, respectively 2770 pmoles/mg protein in the presence of 3',5'-AMP. This indicates that every 4th, respectively every 3rd, monomer is labelled. The paramyosin monomers of *Mytilus* smooth muscles do not seem to have identical molecular weights. Analytical gel electrophoresis reveals three subunits of 104000, 106000 and 108000 daltons. The latter ones are present in equimolar ratios.

Final evidence for the identity of paramyosin and the phosphorylated protein was achieved by the formation of paracrystals. The electron micrographs of paracrystals of the phosphorylated protein exhibits a pattern with the periodicity of 14.6 nm. This agrees quite well with the periodicity of paramyosin reported elsewhere (Bullard et al., 1973; Sobieszek, 1973). The pattern of paracrystals of nonphosphorylated protein is slightly irregular (Fig. 3).

(B) Inhibition of actomyosin ATPase by phosphorylated paramyosin

Cross bridge turnover can be assayed as actomyosin ATPase activity at high (10^{-3} M) and low (10^{-6} M) Ca^{++} concentrations. The inhibitory activity of phosphorylated paramyosin was tested by coprecipitation of paramyosin and actomyosin prepared by method (3). The addition of non-phosphorylated paramyosin affected the ATPase activity of the actomyosin preparation insignificantly. The activity changed at 10^{-3} M Ca^{++} from 912 ± 61 nmoles/mg protein.min to 792 ± 171 nmoles/mg protein.min, and at 10^{-6} M Ca^{++} from 89 ± 2 nmoles/mg protein.min to 114 ± 31 nmoles/mg protein.min. However, the addition of phosphorylated paramyosin decreased the activity of the actomyosin preparation to 442 ± 39 nmoles/mg protein.min at 10^{-3} M Ca^{++} and 26 ± 0 nmoles/mg protein.min at 10^{-6} M Ca^{++} . This and comparable experiments prove that phosphorylated paramyosin inhibits actomyosin ATPase at low and high Ca^{++} concentrations.



FIG. 1. Separation of 50 µg phosphorylated paramyosin isolated by selective extraction of thick filaments in gels of 3.5% acrylamide concentration. (a) protein phosphorylated in the presence of 3', 5'-AMP; (b) protein phosphorylated in the absence of 3', 5'-AMP.

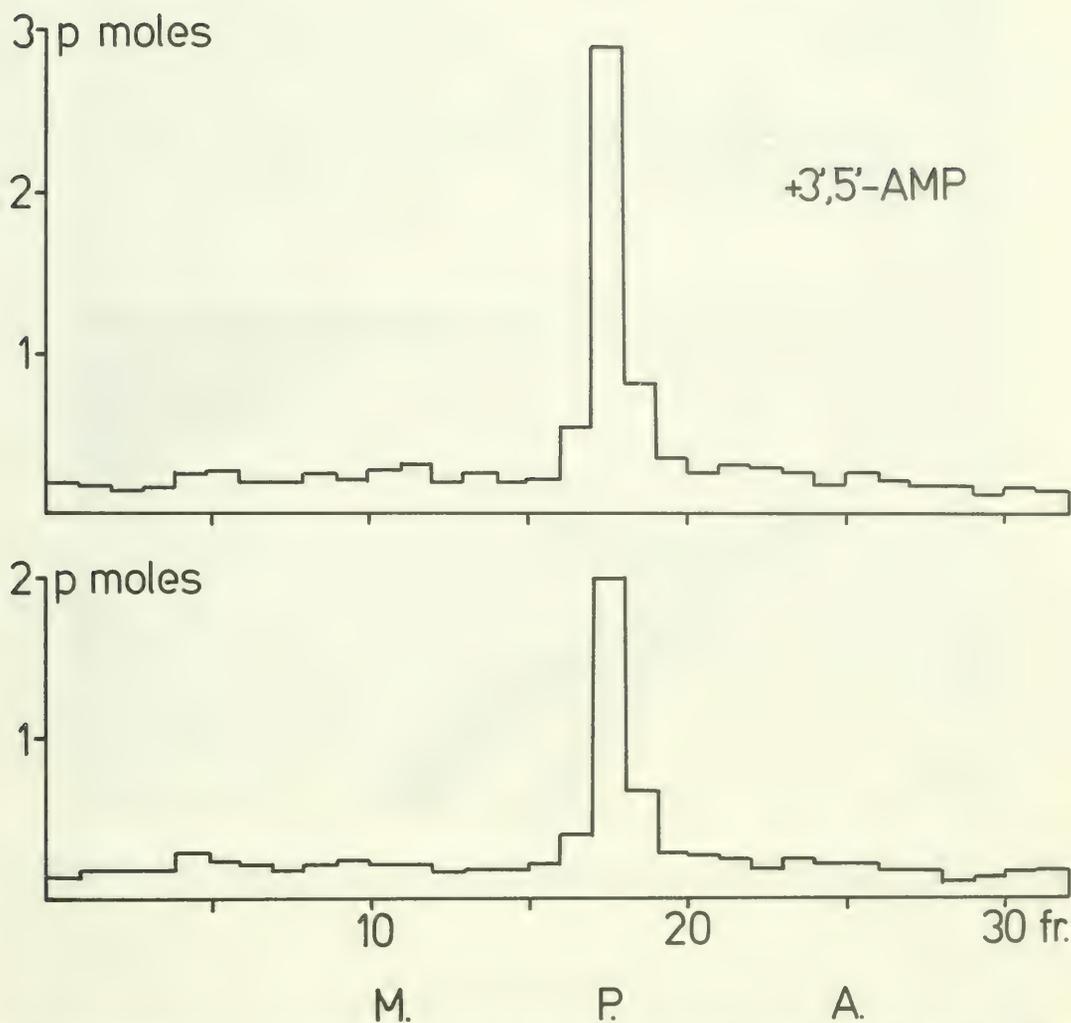


FIG. 2. Radioactivity of the fractions of gel (a) and (b) of Fig. 1 in pmoles P^{32} /slice. The position of the protein bands are marked by the abbreviations M (myosin), P (paramyosin), and A (actin) (fr. = fraction).

DISCUSSION

In vitro paramyosin reduces the activity of the actin activated ATPase, as the ratio of paramyosin to actomyosin is increased (Szent-Györgyi et al., 1971). However, *in vivo* the regulation of the actin myosin interaction has to proceed in a different way. A more conceivable model of the relaxation of muscles in catch can be derived from the results presented above. 5-Ht may activate protein kinases, by induction of the second messenger 3',5'-AMP. These enzymes phosphorylate paramyosin, which in turn inhibits the actomyosin ATPase and thus produces the relaxation. Further evidence for the validity of the scheme proposed can only be derived from investigation of the ATPase cycle.

I thank Prof. Dr. G. Beinbrech for help with electron microscopy and A. Weidner for excellent technical assistance.

(a)



(b)

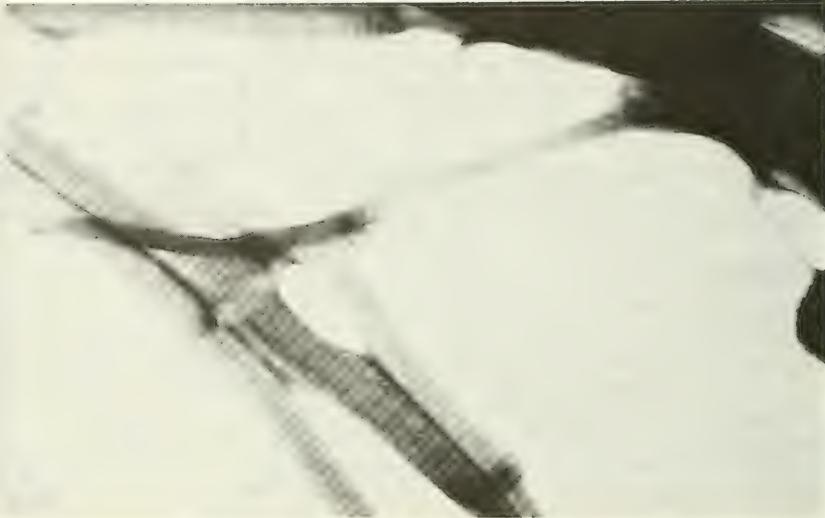


FIG. 3. Electron micrographs of negatively stained paracrystals of phosphorylated (a) and nonphosphorylated (b) 10600 dalton protein isolated from ethanol-ether dried powder of smooth mollusc muscles ($\times 91,000$).

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NERVOUS AND LOCAL MEDIATOR CONTROL OF MUCOCILIARY TRANSPORT IN A BIVALVE GILL

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ABSTRACT

Stimulation of nerves supplying the gill of the mussel *Mytilus edulis* increased mucus secretion and particle transport on the frontal epithelium. Touching the surface and adding heavy suspensions of particles had a similar effect. Electron microscopy revealed a nerve supply to this region and fluorescence microscopy indicated that its fibers contained serotonin and dopamine.

INTRODUCTION

Schlieper & Kowalski (1958) suggested that the frontal cilia of *Mytilus edulis* are stimulated to beat faster by the presence of food particles in the water, and it is common experience that dissecting a mussel to remove the gill causes the secretion of large amounts of mucus. Paparo (1972), Paparo & Finch (1972) and Stefano & Aiello (1975) reported the presence of monoaminergic nerves in the frontal region of the filament. It seemed reasonable, therefore, to test the gill for its response to tactile stimulation, to examine the proposed innervation by electron microscopy, and to see if electrical stimulation of the nervous system could elicit any response from the frontal epithelium.

MATERIAL AND METHODS

Mytilus edulis was collected from Long Island Sound and maintained in Instant Ocean artificial sea water at 17-20°C for several weeks. For experiments, one demibranch was removed with the visceral ganglion and a piece of the cerebrovisceral connective still attached and pinned to a rubber mat glued to the bottom of a dish. Forty ml of artificial sea water was added. Prodigal graphite particles (5-15 µm diam) were added in suspension. Transport velocities were measured with an eyepiece micrometer and stopwatch under a dissecting microscope at 10X or 30X and are expressed as ranges. Electrical stimulation was done with a Grass stimulator. Biphasic pulses of 2 msec duration at 5/sec for 2 min were applied through platinum wire electrodes. Fluorescence microscopy employed the Falck-Hillarp method as described earlier (Stefano & Aiello, 1975). Electron microscopy followed standard procedures (Paparo, 1972).

OBSERVATIONS AND RESULTS

Tactile stimulation. When the gill was clearing fine particles from dilute suspension, particles were transported by the frontal epithelium at rates which varied randomly within a particular range for that preparation. When heavy suspensions were added, the first few readings always gave rates that were 20 to 50% higher than the rest, and this could be repeated several times. At the end of some experiments, some particles remained stationary on the frontal surface but would begin moving again if heavy suspensions were added. Touching the surface with a fine probe re-activated transport on that filament but not adjacent ones unless they were all covered by a common layer of mucus. Touching the frontal surface of several filaments with a blunt probe evoked a copious secretion of mucus which was cleared off the gill in about 1 min. The

same results were obtained repeatedly, even after removal of the visceral ganglion, but could have involved an axon reflex or the release of a local mediator.

Nerve stimulation. The cerebrovisceral connective was stimulated at 2 V while observing a stream of mucus traverse a small gap between 2 adjacent filaments, passing from one food groove to the other. After an initial slight gill movement, which always widened the gap, the width of the stream increased from 0.05 to about 0.08 mm, and at the end of stimulation slowly returned to the original width. Ten V and 20 V stimulation gave larger initial movement and wider streams, the last increase being to 0.15 mm in width. The stream appeared to move faster but this could not be measured. Frontal particle transport during the 20 V stimulation decreased from 8.5-14.8 to 5.4-10.3 mm/min and returned to 7.4-14.8 mm/min 10 min later. The widening of the stream must have been due to increased secretion of mucus. The increased mucus secretion with increased voltage is attributed to the activation of more fibers supplying filaments that contributed mucus to the stream. Similar results were obtained with 2 other preparations; 5 other attempts failed to elicit a significant change.

The branchial nerve was stimulated while observing a stream 0.15 mm wide traverse a 2 mm wide gap at a velocity of 10.2-12.7 mm/min. Stimulation at 10 V produced some gill movement and widening of the stream. At 30 V the stream widened to 0.4 mm and the velocity increased to 13.5-14.8 mm/min. Additional mucus, secreted in response to the stimulation, was carried by the terminal cilia in a separate stream trailing off into the medium. Branchial nerve stimulations frequently accelerated particle transport on the frontal surface. Stimulation at 20 V clearly caused an increase from 15.9-22.2 to 22.1-36.0 mm/min on about 10 filaments in one region without affecting other filaments in the field of view. Subsequent tests at 15 min intervals raised the rate from 13.9-20.0 to 21.5-35.0 mm/min and from 12.9-20.0 to 15.7-21.2 mm/min. Four other preparations gave the following increases: 13.2 to 20.0 (averages); 11.2-12.8 to 13.5-16.7; 24.7-37.0 to 29.6-49.3; and 19.3-24.7 to 33.6-34.2 mm/min. In two other preparations in which transport was slow and erratic, and particles were seen to become stationary on the surface, stimulation at 20 V caused the resumption of transport, but in one of these transport stopped again within a few minutes after each of 4 periods of stimulation at 15 min intervals. Because the cilia appeared to be beating all the time when examined under 80X, the effect may have been caused by the release of mucus rather than the acceleration of cilia.

Fluorescence and electron microscopy. Histochemical fluorescence microscopy revealed small yellow, green and yellowish green specks between the ciliated epithelium and the supporting rod. They appear as fine lines in sections made along the axis of the filament and are interpreted as sections of 5-HT (yellow) and DA (green) containing nerves. Blood cells contain 5-HT and fluoresce golden yellow. They appear at random in most of the blood channel, consistently at its frontal end, and frequently on the epithelial side of the supporting rod and have been mistaken for nerve cells (Paparo, 1972). Localization of nerve fibers under particular cells is unreliable in these preparations but the fibers are in the same location as those seen in electron micrographs.

Transmission electron microscopy consistently revealed the presence of a nerve (3-5 μm diam) under the frontal epithelium on each side near the laterofrontal cells (Fig. 1). Each nerve contained 30 or more axons (mostly 0.2-0.3 μm diam and relatively empty), and several larger fibers (1.5 μm diam) containing mitochondria, many small clear vesicles (0.08-0.1 μm diam) and several dense core vesicles (0.3 μm diam) (Fig. 2). Most of the nerve was surrounded by what appeared to be glial cells but several axons made close contact with adjacent epithelial cells. No synapses were identifiable. A separate nerve, as described by Aiello & Guideri (1965) and by Paparo (1972), was always seen under the lateral epithelium.

DISCUSSION

The large numbers of nerve fibers supplied to the frontal epithelium of the gill raises the possibility that mucus secretion and transport could be integrated with other activities of the gill and of the whole animal in response to changes in the environment. Senius (1975) suggested the neuronal control of thermal resistance adaptation by frontal ciliated cells, and Jørgensen (1975) pointed out the advantage the organism would have if its laterofrontal cirri were under



FIG. 1. Electron micrograph of frontal region of *Mytilus edulis* gill cut in cross section showing a nerve (N) lying at the base of several frontal cells, the supporting rod (SR) and a blood cell (BC) in the blood channel.



FIG. 2. Enlarged view of part of the nerve shown in Fig. 1, showing many small, relatively empty fibers (F) and several large fibers, some of which contain clear and dense core vesicles (arrows).

nervous control. The experiments described above suggest that mucus secretion and particle transport, and probably frontal ciliary beating, can be influenced by nervous and tactile stimulation.

The active substances involved probably include 5-HT and DA as neurotransmitters, 5-HT released from blood cells, and acetylcholine released from still unidentified sites. The cilioexcitatory action of 5-HT and DA on frontal cilia has been reported by Malanga (1974, 1975) and by Jørgensen (1975, 1976) and the effect of acetylcholine on frontal cilia and mucus secretion by Bülbring and others (1953). Unpublished data by Aiello and Jean-Baptiste support a stimulatory effect of 5-HT and DA on mucus secretion. The gill appears to have many autonomously functioning units and the apparatus for their control and integration by nerves and local mediators, but how it all works is still a mystery.

ACKNOWLEDGEMENTS

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AXONAL PATHWAYS AND SYNAPTIC INPUTS OF THREE IDENTIFIED NEURONS IN THE BUCCAL GANGLION OF *HELIX POMATIA*

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ABSTRACT

The axonal pathways and the synaptic inputs of the identified neurons B1 through B3 in the buccal ganglia of *Helix pomatia* were studied. The axons of neurons B1, B2 and B3 were found to run invariably within the ipsilateral posterior oesophageal nerve, ipsi- and contralateral salivary gland nerves, and ipsilateral cerebrobuccal connective, respectively. Synaptic responses could be elicited by stimulation of most of the nerves of the buccal ganglia. These consisted of an early depolarization which was most frequently followed by a longlasting de- or hyperpolarization. The shape of the synaptic response proved to be related to the different neurons.

Cottrell & Macon (1974) as well as Schulze et al. (1975) identified three neurons in the lateral part of the buccal ganglion of *Helix pomatia* on the basis of topographical and electrophysiological criteria. Schulze et al. (1975) labeled these neurons B1, B2 and B3 in the frontopedal-caudodorsal direction. In the present investigations the axonal pathways and the synaptic inputs of these neurons were investigated.

The experiments were carried out on hibernating and non-hibernating snails. The buccal ganglia with their nerves were isolated from the animals and fixed to a wax bottom of an experimental chamber. The nerves of the buccal ganglia were stimulated by single electrical impulses with the aid of suction electrodes. Bioelectrical activity was recorded from the cell bodies of the neurons B1, B2 and B3 through an intracellular microelectrode. A second microelectrode was inserted into the same cell to change the resting membrane potential by current injection.

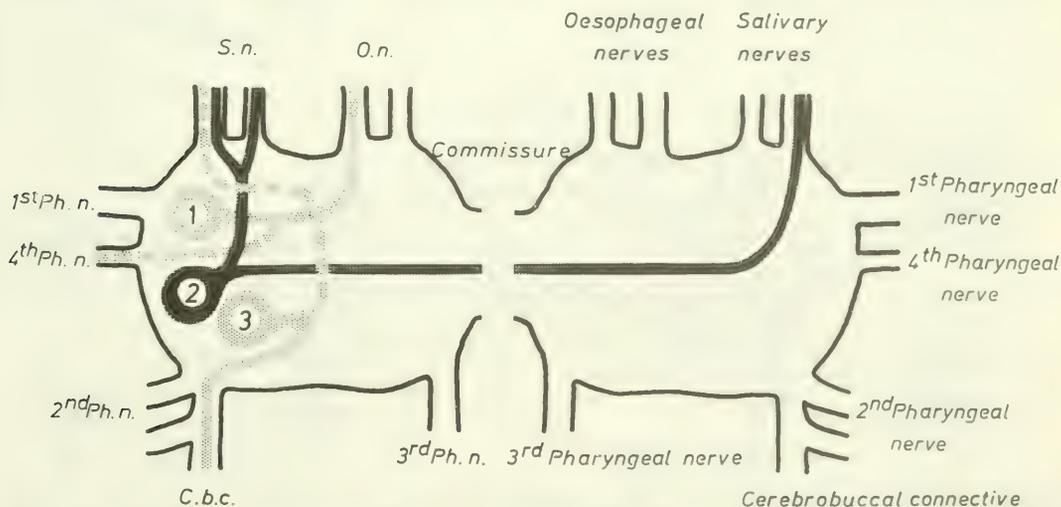


FIG. 1. Axonal pathways of neurons B1, B2 and B3 in the buccal ganglion of *Helix pomatia*. The bodies and axons of the neurons are marked by hatched areas within a schematic contour of the buccal ganglia. The appearance of the interrupted axons is variable.

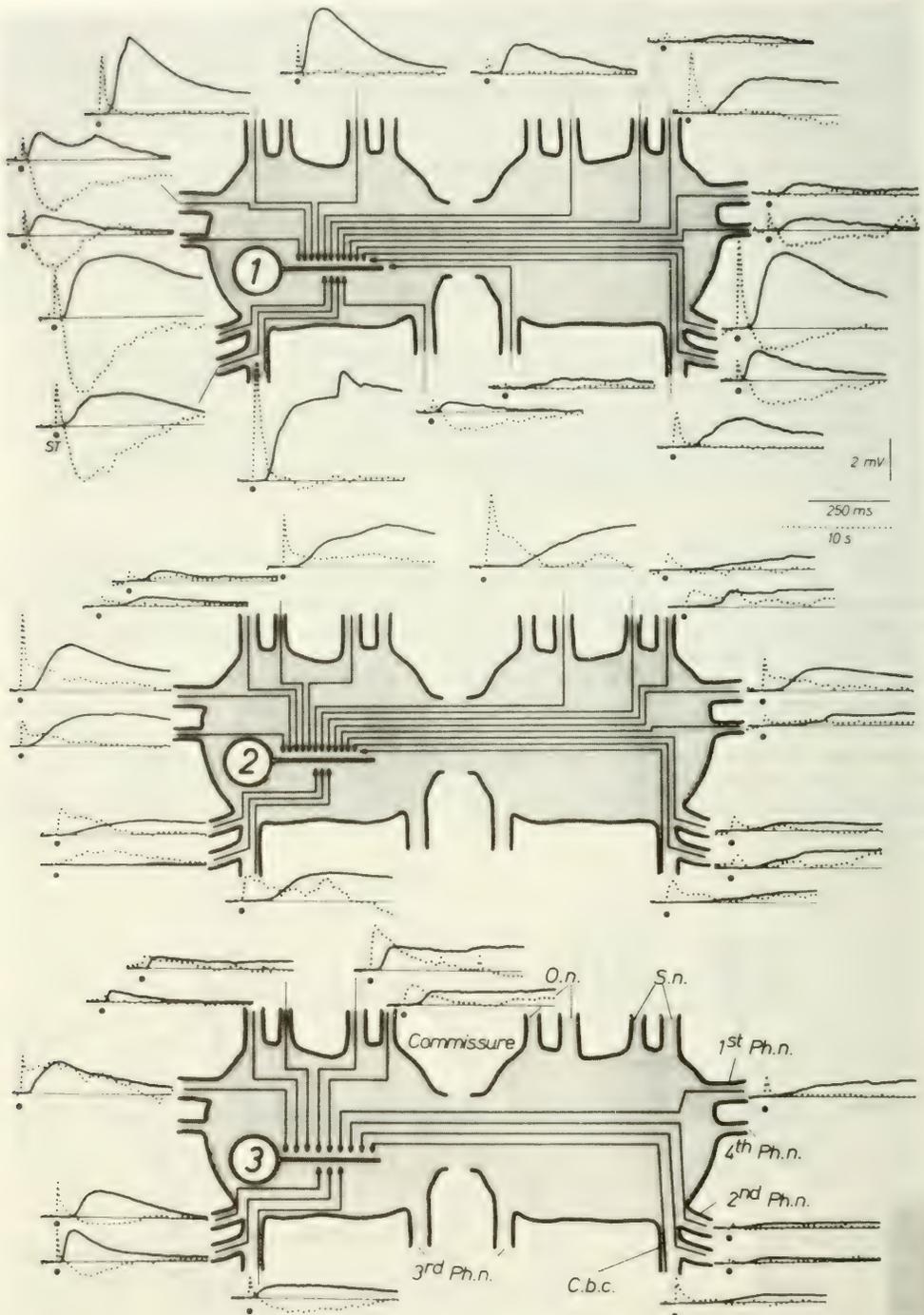


FIG. 2. Synaptic inputs of neurons B1, B2 and B3 in the buccal ganglion of *Helix pomatia*. The neurons are presented unilaterally in the schematic contours of the buccal ganglia. The arrows pointing to the axons of the neurons come from those nerves the stimulation of which evoked synaptic responses. The synaptic reactions are presented in the periphery of the ganglia in connexion with the corresponding nerves. Two recordings with different time scales are superimposed (continuous and dotted curves). ST = nerve stimulation; C.b.c. = cerebrobuccal connective; O.n. = oesophageal nerves; Ph.n. = pharyngeal nerve; S.n. = salivary gland nerves.

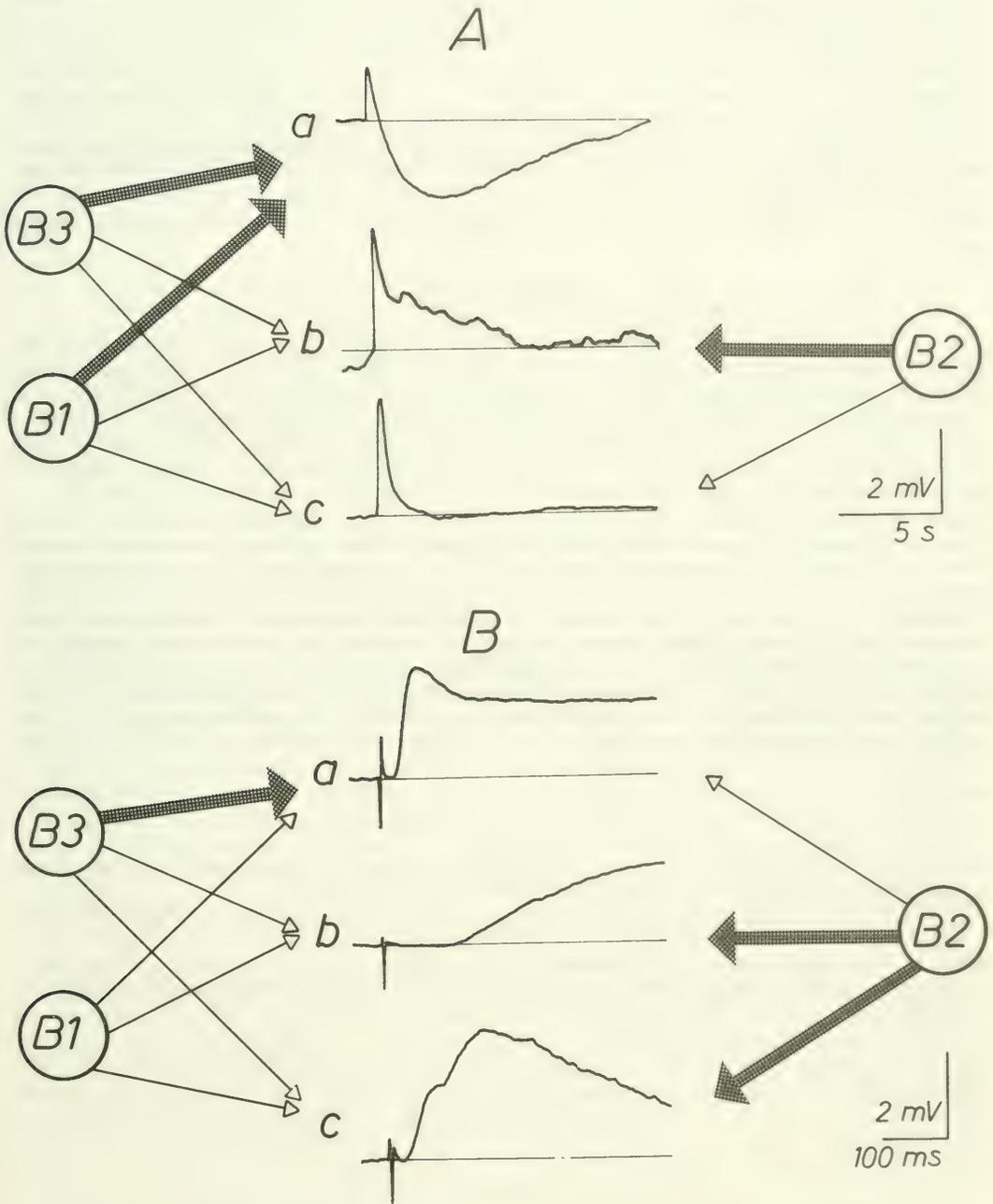


FIG. 3. Different types of the synaptic response of neurons B1-B3 to nerve stimulation. Classified by means of the time course and shape of the whole response (A, a-c) and by means of the slope of rise of the early synaptic event (B, a-c). The probability of appearance of these potential types in the identified neurons is indicated by the width of the arrows.

1. Axonal pathways

The axonal pathways of the three neurons were identified with the aid of antidromic invasion of action potentials into the cell soma after nerve stimulation. The antidromic action potentials were distinguished from orthodromic responses using the criteria for antidromicity defined by Kerkut et al. (1975). The electrophysiological findings obtained by the described method were substantiated by intracellular staining using Procion yellow dye. The histological and the electrophysiological results proved to be in agreement. The findings are summarized in Fig. 1. The axons of neuron B1 are regularly located within the ipsilateral posterior oesophageal nerve and sometimes within the 4th pharyngeal nerve. The axons of neuron B2 run within the ipsi- and contralateral salivary gland nerves, and the axons of neuron B3 are found within the ipsilateral cerebrobuccal connective and sometimes also within the first ipsilateral salivary gland nerve.

2. Synaptic inputs

In order to examine the synaptic inputs to the 3 neurons concerned, the nerves of the buccal ganglia were stimulated successively, and the corresponding orthodromic responses led from the cell bodies were analyzed. A survey of the orthodromic responses is given in Fig. 2. It is conspicuous that neurons B1 and B2 get nearly symmetric synaptic inputs from the nerves of both buccal ganglia. In contrast to that, the synaptic inputs to neuron B3 are asymmetric. These predominate from the side on which the neuron is located. Fig. 2 shows, furthermore, that the total number of inputs to neurons B1 and B2 markedly exceed that to neuron B3.

The evaluation of time course and shape of the synaptic responses shows that the first bioelectric reaction consists in a depolarization. This early synaptic event is most often followed either by a long lasting depolarization or by a slow hyperpolarization. In a few cases a late component is missing. These 3 basic types of the synaptic response are summarized in Fig. 3A. As indicated by the width of the arrows, the probability of appearance of these potential types is related to the three different neurons. Besides this classification based on the polarity and amplitude of the *late* potential shift, 3 types of synaptic reactions can also be differentiated concerning the slope of rise of the *early* response. Among the early depolarizations steep, flat, and compound shifts of the membrane potential are distinguishable. As shown by Fig. 3B, the probability of appearance of these potential types is also characteristic for each of the 3 cells.

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THE FINE STRUCTURAL ORGANIZATION OF SENSORY NERVE ENDINGS IN THE LIP OF *HELIX POMATIA* L.

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ABSTRACT

The fine structural and cytochemical characteristics of sensory nerve cells have been studied in the lip of *Helix pomatia*. A ruthenium red positive cuticular layer was found on the surface of the sensory epithelium. Among the undifferentiated epithelial cells two types of sensory dendrites were observed, namely ciliated and non-ciliated ones. A large amount of smooth surfaced endoplasmic reticulum, microtubules and ribosomes were present in the neuroplasm of the sensory dendrites. However, rough surfaced endoplasmic reticulum, mitochondria, and electrondense bundles of long filaments were characteristic in the simple epithelial cells. The cell bodies of the sensory dendrites lie subepithelially among the muscle cells and they generally contain empty or dense core vesicles.

It is well-known that the body wall of molluscs is abundantly supplied with nerves—especially with receptors (Bullock & Horridge, 1965). Light microscopical studies clarified the histological organisation of the sensory cells (Veratti, 1900; Smidt, 1902; Retzius, 1892).

Recently published electron microscopical investigations (Crisp, 1971; Rogers, 1971; Zylstra, 1972a, 1972b; Storch, 1972; Wondrak, 1975; Emery, 1975) provided new information, particularly on the fine structural feature of sensory dendrites in different species of mollusc, but as regards the skin of the snail *Helix pomatia*, there are mostly light microscopical studies available both for epithelial and epidermal sensory cells (Schulz, 1938). In spite of unsatisfactory morphological data, detailed physiological experiments were carried out to study the mechanism of reception in the lip of *Helix pomatia* (Kieckebush, 1953; Salánki & Bay, 1975).

Results of these physiological experiments undoubtedly suggest that the receptors of the lip in the snail can distinguish among different chemical substances. The question arises: what is the morphological basis of these different chemical sensitivities? To answer this question it seemed useful to employ electron microscopy techniques, in order to identify the fine structural organisation of receptor cells and their processes.

MATERIAL AND METHODS

Adult specimens of *Helix pomatia* L. were used for the investigation. Tissues were fixed 4 hrs in either 2% OsO₄ s-collidine buffered (pH 7.3) solution at 4°C or in a freshly prepared mixture of glutaraldehyde (2.5%) and OsO₄ (2%) in s-collidine buffered solution (pH 7.3) at 4°C. Ruthenium red staining was carried out according to Luft (1971). After dehydration, tissues were embedded in Durcupan ACM. Ultrathin sections were stained with Reynolds lead citrate, and examined with a TESLA BS 500 electron microscope.

RESULTS

Intra-epithelial receptor cells were not found in the epidermis of the lip, however, a great number of sensory dendrites were observed (Fig. 1), especially on the ventral surface of the lip. Sensory dendrites could be clearly distinguished from the simple epithelial cells on the basis of their ultrastructural features. The simple epithelial cells contain a relatively large number of cell organelles (mitochondria, pigment granules, rough surfaced endoplasmic reticulum) in the

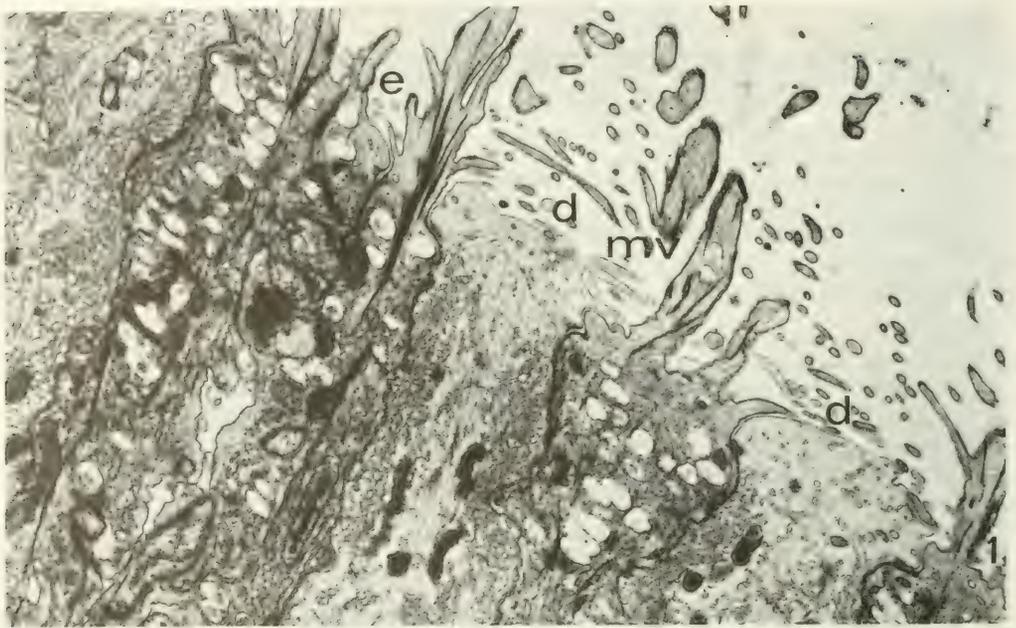


FIG. 1. Sensory dendrites (d) among simple epithelial cells (e). Only microvilli (mv) can be seen on the top of dendrites (X12,000).



FIG. 2. Ciliated sensory dendrite (cd) in the lip of *Helix pomatia*. Note smooth surfaced endoplasmic reticulum (ser) (X12,000).



FIG. 3. Ruthenium red staining of the sensory epithelium. The cuticular layer (c) is strongly stained, as well as the sensory dendrites (d) (X12,000).

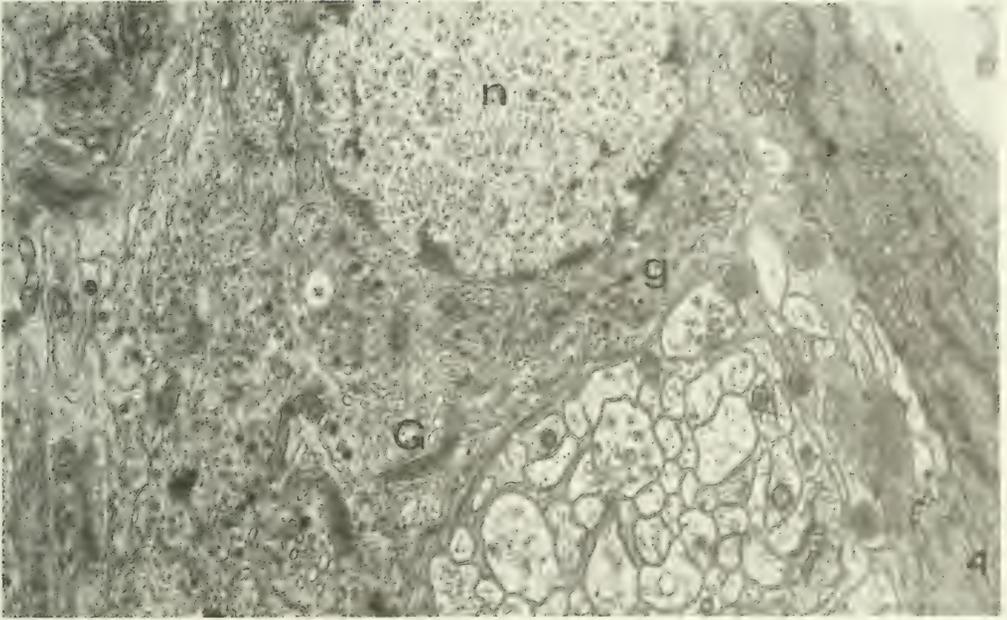


FIG. 4. Nucleus (n) and cell body of a sensory neuron in the lip. Golgi apparatus (G) and a great number of dense core vesicles (g) can be seen in the cell ($\times 12,000$).

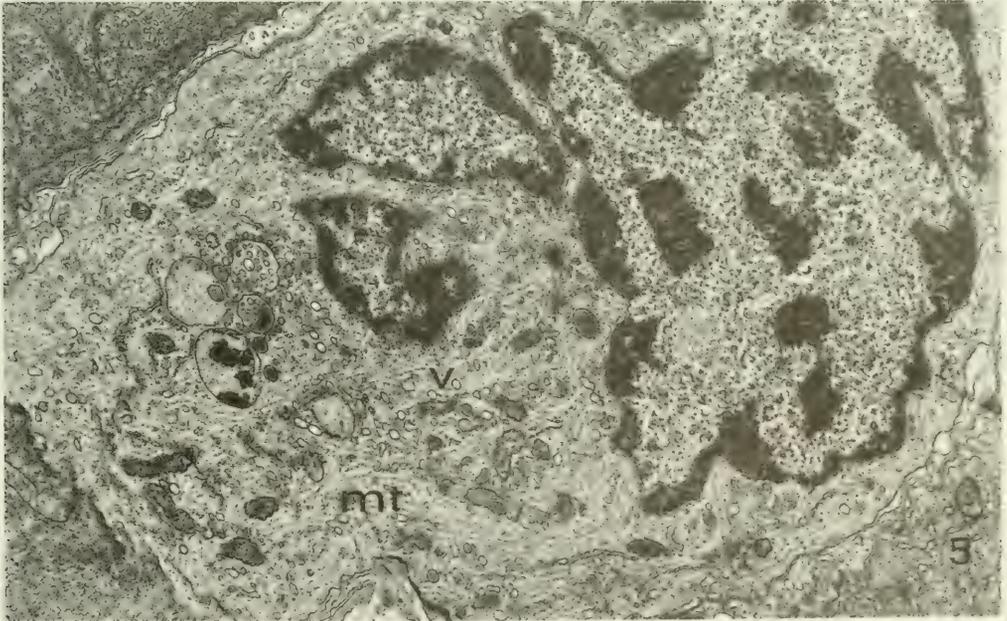


FIG. 5. The nongranulated sensory neuron is rich in empty vesicles (v) and microtubules (mt) ($\times 12,000$).

electron dense cytoplasm (Figs. 1, 2). Cytoplasmic processes of the epithelial cells are always branched and often contain bundles of long filaments. The neuroplasm of the sensory dendrites is electron transparent (Figs. 1, 2). Mitochondria, smooth surfaced endoplasmic reticulum, microtubules and free ribosomes are the characteristic cell organelles of sensory dendrites (Figs. 1, 2).

Both on the free surface of the simple epithelial cell and sensory endings there is a rather thick, fine granulated cuticular layer. After ruthenium red staining this cuticular layer became very electron dense (Fig. 3). The ruthenium red positivity of the sensory endings was also much higher than that of the cytoplasm of the simple epithelial cells (Fig. 3). At the top of the sensory dendrites microvilli, cilia or both may occur (Figs. 1, 2). The cell body of the sensory cells lies subepithelially among the muscle cells in small groups (forming small ganglia) or solely surrounded by electron dense supporting cells (Figs. 4, 5). Most of the sensory cells contain a great number of dense core vesicles (Fig. 4), while the other type of the sensory cell (Fig. 5) contains a large amount of empty vesicles in the cytoplasm.

DISCUSSION

To describe the ultrastructural organisation of sensory cells of the lip the first basic requirement was the satisfactory preservation of the tissue. Among the different fixatives, glutaraldehyde + OsO₄ mixture assured the best preservation, providing moreover the differentiation of nervous elements from the simple epithelial cells. As regards the cell organelles in the neuroplasm of sensory endings the abundant occurrence of the smooth surfaced endoplasmic reticulum, and the relatively small amount of ribosomes are conspicuous. Unfortunately, we know very little about the functional role of the smooth surfaced endoplasmic reticulum in the process of reception.

The strong ruthenium red positivity of the cuticular layer of the lip was not surprising. The unexpected strong staining of the sensory dendrites with ruthenium red, however, reflected the high glycoprotein content of these cells. Further cytochemical experiments may elucidate this phenomenon.

One of the most discussed questions is the types of the sensory endings. On the basis of their ultrastructural characteristics, different authors (Crisp, 1971; Zylstra, 1972b; Storch, 1972; Emery, 1975) have described different types of dendrites. Besides the presence or absence of cilia, the rootlet is the most important cell organelle which serves as base for the identification and classification of the sensory dendrites. In the case of the snail *Helix pomatia*, ciliated and non-ciliated dendrites may be observed without difficulty. However, serial sections were not made to prove, whether a so-called non-ciliated dendrite did bear cilia or not. As we could hardly detect the strongly developed rootlets in the sensory dendrites of the snail, which were observed by Zylstra (1972b), we could identify only 2 types of sensory dendrites: the ciliated and non-ciliated ones. Until now also 2 types of sensory cell body were found in the subepithelial layer of the lip. In the future we will try to find the so-called "middle" part of the sensory dendrites too, because in this case we would be able to reconstruct the "whole sensory cell." Knowledge of the morphological character of the sensory cells perhaps might enable us also to determine what type of reception is accomplished.

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ELECTROPHYSIOLOGY OF "YELLOW CELLS," NEUROSECRETORY NEURONES IN *LYMNAEA*

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ABSTRACT

The thirty Yellow Cells of *Lymnaea* show single, double and other extra spike modes of firing. Yellow Cell bursts consist of various combinations of single, doublet and triplet spikes whose number per burst varies spontaneously. Single spike firing modes of activity can be converted into doublets or bursts (and *vice versa*) by applying steady currents of the appropriate polarity. Spike activity is basically endogenous although it is modulated by low frequency synaptic input originating from within the brain. Interburst interval is affected by the number of spikes occurring in the preceding burst. This varies spontaneously or can be induced by applying appropriately timed current pulses or occurs following synaptic input. Excitatory synaptic input often induces bursts which far exceed the duration of the input and which are followed by long periods of inhibition.

INTRODUCTION

As part of our investigation of the electrophysiology of neurosecretory neurones in the pond snail, *Lymnaea stagnalis*, we have carried out intracellular recordings from Yellow Cells in the visceral ganglion of this snail. Yellow Cells burst endogenously, but the bursts are unusual in that they contain single spikes, doublets and other additional spike groupings (Benjamin & Swindale, 1975). As will be shown in the present report, Yellow Cells also receive a periodic excitatory synaptic input (input 3 of Winlow & Benjamin, 1976) which can evoke bursts if it occurs anywhere in the interburst interval of the endogenous rhythm. At frequencies of input approaching that of endogenous bursting every Yellow Cell burst is initiated by a synaptic potential so that bursting activity is entirely timed by the occurrence of excitatory post-synaptic potentials (EPSPs).

MATERIALS AND METHODS

Intracellular recordings were carried out on Yellow Cells in the visceral ganglia of *Lymnaea* using techniques which have been described elsewhere (Benjamin & Swindale, 1975; Benjamin, Swindale & Slade, 1976; Benjamin, 1978).

RESULTS AND DISCUSSION

Yellow Cells are usually active on penetration and the type of activity recorded depends on the tonic level of membrane potential. There are 2 states of single spiking, one at high levels of membrane potential (low frequency single spiking) and the other at low levels (high frequency single spiking), while at intermediate levels bursting activity occurs.

An important factor in burst formation appears to be the after-depolarising potential which follows single spikes at low frequency (Benjamin, 1978). If membrane potential was decreased, this after-potential gave rise to a second spike arising from the falling phase of the first thus forming a doublet. Bursts are formed by additions of further spikes by the same mechanism. Bursts are terminated by a rapid hyperpolarisation which after a few seconds is followed by a gradual depolarisation leading to the next burst.

Much of the burst activity seen in *Lymnaea* Yellow Cells seems to be due to an endogenous mechanism because in many preparations the frequency of synaptic potentials is too low to account for bursting. That Yellow Cells are capable of endogenous bursting is further supported by the following observations. (A) The phase of burst activity is delayed by the application of hyperpolarising square pulses of current in the interburst interval. (B) The duration of the interburst interval is related to the number of spikes in the previous burst. Thus if the number of spikes in a burst is increased by applying a depolarising pulse during a burst then this increases the duration of the following interburst interval. Conversely, if the number of spikes in a burst is decreased by applying hyperpolarising pulses during a burst then the duration of the following interburst interval is reduced. (C) The mean frequency of spike activity is a function of the level of applied depolarisation.

The endogenous activity of Yellow Cells is, however, modulated by periodic EPSP input which varies considerably in its frequency in different preparations. It may occur every 30-40 seconds or as high as every 10-20 seconds. This low amplitude (a few mV) compound EPSP can initiate a burst at any point in the endogenous interburst interval. If the EPSP frequency is much lower than the endogenous burst frequency then the input evokes a burst whose duration usually exceeds that of the EPSP, and this synaptically evoked burst delays the onset of the next endogenous burst. So the occurrence of low frequency input to Yellow Cells continuously resets the endogenous rhythm. If the EPSP frequency approaches that of the endogenous burst rhythm then the input entirely determines the timing of the Yellow Cell bursts. Each EPSP evokes a burst and no bursts are endogenously generated. The maximum frequency at which synaptic inputs have been seen to drive Yellow Cell bursting is about 0.2 Hz but theoretically it should be possible to evoke higher frequency bursting than this.

This appears to be the first time that the effects of periodic EPSP inputs interacting with an endogenous bursting mechanism have been described in molluscs although it is known that inhibitory synaptic inputs can rapidly synchronise the bursting activity of a population of bursting cells in *Aplysia* (Pinsker, 1977).

ACKNOWLEDGMENTS

I thank the S.R.C. for financial support and the European Training Programme in Brain and Behaviour Research for providing a grant towards attending this conference.

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THE RE-FORMATION OF CONNECTIONS IN THE NERVOUS SYSTEM OF *LYMNAEA STAGNALIS* AFTER NERVE INJURY

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ABSTRACT

Changes in the tentacle reflex pathway of the pond snail *Lymnaea stagnalis* induced by peripheral nerve injury were studied with behavioural and electrophysiological techniques. After nerve injury regeneration of sensory axons is obtained in 6-12 days, suggesting an axonal outgrowth at a rate of 1 mm per day. Recovery of the tentacle reflex takes much more time indicating that synaptic efficacy is affected considerably by the period of sensory deprivation following nerve injury.

INTRODUCTION

Changes in the nervous system occurring after nerve injury have been studied mainly in vertebrates (Sunderland, 1968; Guth, 1974) and in arthropods (Bittner & Johnson, 1974; Palka & Edwards, 1974). It appeared that not only the severed neurones, but also postsynaptic neurones can be subject to dramatic changes after nerve injury.

Only a few papers deal with effects of nerve severance on molluscan neurones (Eakin & Brandenburger, 1970; Eakin & Ferlatte, 1973; Flores Scarso & Pellegrino de Iraldi, 1973; Flores Scarso et al., 1977). These cells react with degenerative and regenerative changes. Dramatic post-synaptic effects as found in vertebrates and arthropods have not been described, although changes of membrane properties of postsynaptic neurones after injury of central pathways have been reported (Wald, 1976).

The present electrophysiological and behavioural study deals with direct and indirect effects of nerve injury on the tentacle reflex pathway of the pond snail *Lymnaea stagnalis*. The tentacle reflex is very suitable for this purpose because of its simplicity and the small number of neurones involved (Lever, 1977).

METHODS

Laboratory bred snails (shell length 20-30 mm) were used for the experiments. Operations were performed as described by Joosse & Lever (1959). After the operation the animals were kept in glass jars placed in running tap water (18°C).

Recordings of nervous activity were made with suction electrodes and conventional electrophysiological apparatus. Muscle contractions were recorded with a force transducer made in the laboratory. Touch stimuli were delivered with a glass rod with a round tip (diameter 0.5 mm).

RESULTS

When *L. stagnalis* is gently touched on the skin, a very conspicuous reflex which can be observed is a downward movement of the ipsilateral tentacle. This reflex is evoked by sensory input of only one nerve if the posterior foot part, which is exclusively innervated by the posterior branch of the inferior pedal nerve (Janse, 1974), is touched.

The tentacle is innervated by the tentacular nerve and by the superior cervical nerve (Janse, 1974). In the present study it could be shown that the motor neurones involved in the tentacle

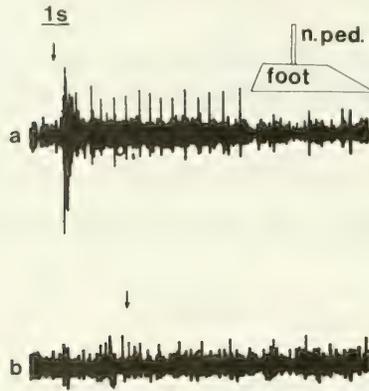


FIG. 1. Sensory responses in an intact (a) and an injured (b) inferior pedal nerve upon touching the foot. The inset shows a scheme of the preparation, n.ped.—inferior pedal nerve.

TABLE 1. Recovery of the tentacle reflex after injury of the inferior pedal nerve. During the recovery process animals were randomly sacrificed for other experiments.

	Days after operation																
	2	5	8	11	14	15	17	20	23	26	29	32	38	45	50	53	57
% of snails showing tentacle reflex	0	0	0	0	0	0	0	0	0	0	0	10	10	41	48	89	96
Number of snails	43	42	41	40	39	38	37	36	35	32	30	30	30	27	27	27	25

reflex have their axons in the tentacular nerve; animals in which the tentacular nerve was surgically cut did not show the reflex, while animals in which the cervical nerve was cut normally showed the tentacle reflex. The following part of this study deals with changes in the pathway of the tentacle reflex described above after injury of the inferior pedal nerve.

In experimental animals the left inferior pedal nerve was injured just centrally of the branching point by pinching with a pair of tweezers (the nerve sheath was not cut). This effectively severs the axons as can be concluded by comparing the activity of an intact and an injured nerve after foot stimulation (Fig. 1). Moreover, the tentacle reflex could not be evoked by touching the posterior part of the foot.

Recovery of the tentacle reflex was studied in a group of 43 successfully operated animals. Table 1 shows that the first signs of recovery occur at about 30 days after the operation. The majority of the snails were recovered 10-15 days later.

The recovery of the reflex was also studied with electrophysiological techniques. The preparations consisted of the left foot, the left tentacle and the mantle connected with the central nervous system (CNS) by the left inferior pedal nerve, the left tentacular nerve, and the left pallial nerve, respectively (see inset of Fig. 2). En passant post-ganglionic activity was recorded in the tentacular nerve simultaneously with the recording of tentacle contractions. Fig. 2-1 shows recordings of a preparation made of an unoperated animal when touched on the mantle (a) and on the foot (b). In both instances post-ganglionic activity is accompanied by tentacle contractions.

Preparations of operated animals which did not yet show (behaviourly) a tentacle reflex often showed upon stimulation of the foot comparatively incomplete post-ganglionic responses in the tentacular nerve which could be accompanied by weak muscle contractions (Fig. 2-IIb). Such preparations did show normal post-ganglionic responses and tentacle contractions upon stimulation of the mantle (Fig. 2-IIa). Preparations of animals with recovered tentacle reflexes showed normal post-ganglionic responses and tentacle contractions upon touching the mantle (Fig. 2-IIIa) and the foot (Fig. 2-IIIb).

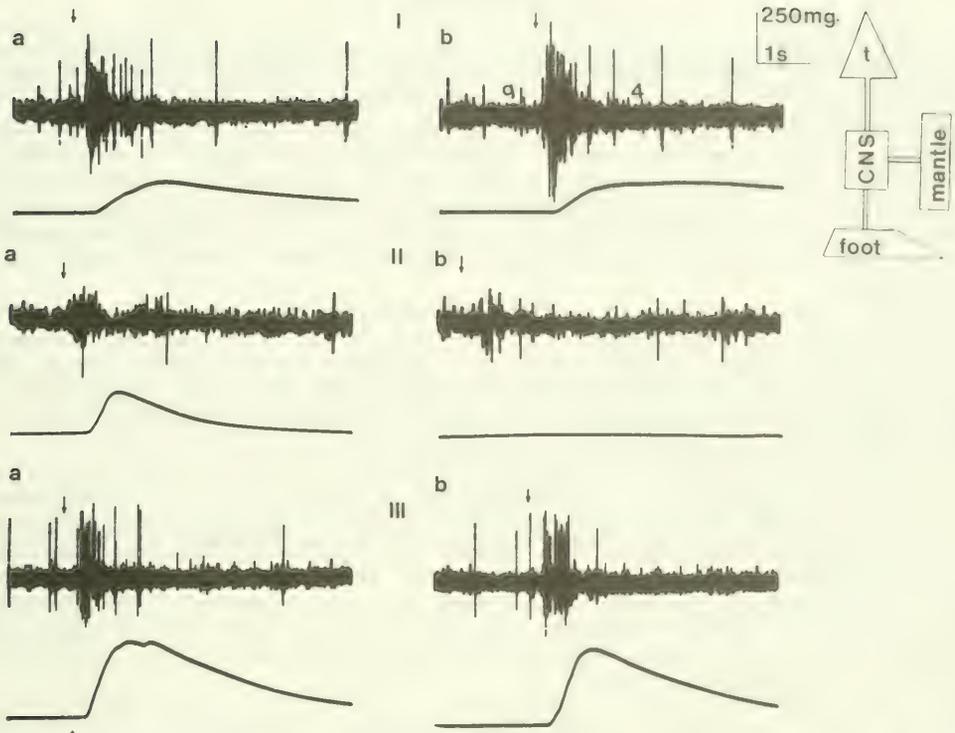


FIG. 2. Post-ganglionic responses in the tentacular nerve (upper traces) and contractions of the tentacle (lower traces) upon touching (arrows) the mantle (a) and the foot (b). I: preparation of an unoperated animal. II: preparation of an animal which did not yet show recovery of the tentacle reflex (36 days after the operation). III: preparation of an animal which showed a totally recovered tentacle reflex (43 days after the operation). The inset gives a scheme of the preparation. n.t.—tentacular nerve; n.p.—pallial nerve; n.ped.—inferior pedal nerve; t—tentacle.

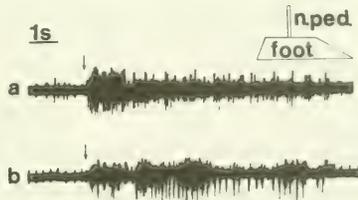


FIG. 3. Responses in the inferior pedal nerve upon touching the foot in a normal animal (a) and of an animal 6 days after nerve injury (b). The inset shows a scheme of the preparation. n.ped.—inferior pedal nerve.

To investigate the recovery process of the sensory connections, preparations were made of operated animals (from another series than those of Table 1) consisting of the foot and the left inferior pedal nerve. Recordings were made just centrally from the injured spot of the nerve. The first sensory responses upon tactile stimulation of the foot were observed 6 days after the operation, indicating that regeneration had occurred (see Fig. 3). From 12 days after the operation all preparations showed normal sensory responses in the operated inferior pedal nerve indicating that from that date full recovery is obtained.

The experiments described above show that the recovery of the complete tentacle reflex takes much more time than that of the sensory connections in the injured nerve.

DISCUSSION

The most striking observation is that in *L. stagnalis* recovery of the tentacle reflex after nerve injury is not simply obtained by the re-establishment of sensory connections; after the sensory input has been regenerated it takes a considerable time for the tentacle reflex to recover.

During the recovery process normal post-ganglionic responses and tentacle contractions (in preparations as well as in intact animals) can be obtained by stimulation of skin areas other than that of the posterior part of the foot. This demonstrates that the long-lasting absence of the tentacle reflex is due to a deficiency of information transport in the pathway between the regenerated sensory neurones and the tentacle motor neurones. In view of the time needed for reflex recovery, it is likely that the deficiency is caused by ineffectiveness of central synaptic transmissions.

Changes in effectiveness of synaptic transmission have been reported to occur in vertebrates as well as in invertebrates after blocking of afferent input. In cat cortical neurones this has been observed after temporary closure of one eye (Wiesel & Hubel, 1963) and in cricket interneurones after removal of particular cerci (Palka & Edwards, 1974). Probably the long lasting absence of the tentacle reflex in *L. stagnalis* is also due to sensory deprivation causing ineffectiveness of synaptic transmission in the reflex pathway.

With respect to the process of regeneration of sensory connections some remarks can be made. In *L. stagnalis* outgrowth of axons of severed central neurones has been observed (Roubos, 1973). Taken with the fact that the sensory axons of the peripheral nerves have their cell body in the CNS (Janse, 1974, 1976; Janse & Van Swigchem, 1975), this suggests that in *L. stagnalis* regeneration of sensory connections takes place by axonal outgrowth. This means that a distance of about 10 mm (from the injured nerve spot to the foot skin) was bridged in 6-12 days, and that regeneration takes place at a rate of 1 mm per day. A similar rate of axonal outgrowth was found for sensory axons in vertebrates (Jacobson & Guth, 1965) and in crustaceans (Bittner & Johnson, 1974).

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FUNCTIONAL CHARACTERISTICS OF AN IDENTIFIED PAIR OF NEURONES IN THE CNS OF THE POND SNAIL (*LYMNAEA STAGNALIS* L.)

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ABSTRACT

The properties of 2 giant electrically coupled neurones (A10 and P1) identified in the visceral and right parietal ganglia of *Lymnaea stagnalis* were examined. The active and passive electrical parameters of the neurones, as well as the junction between them were measured. The main peripheral and interneuronal connections of the neurones were demonstrated using both electrophysiological and morphological methods. It is shown that the coupled cells are not neurones of the same function, but they are asymmetrical ones. This finding is supported by the following results: (1) the axonal pathways of neurones A10 and P1 are different; (2) there are significant differences in their afferent and efferent connections; (3) though the electrical junction between them is bidirectional, the junctional electrical characteristics prefer P1-A10 transmission. According to the electron microscopic results both neurones are possible neurosecretory cells. The differences demonstrated between the 2 giant neurones may have significance concerning their role in a special neuronal network.

In our studies aimed at mapping the *Lymnaea* CNS several dorsal giant neurones of the visceral and right parietal ganglia have been identified (Kiss & Salánki, 1977). During this work we found 2 cells which had some peculiar properties; visually they can be well identified on the basis of their localization, diameter and whitish colour. Their activity shows a 1:1 coupling. In our map we marked these cells as A10 and P1. The same cells have also been examined by Winlow & Benjamin (1976). On the basis of our personal experience A10 and P1 seem to be identical with their VD1 and RPD2 cells, respectively.

MATERIAL AND METHODS

Experiments were carried out on adult specimens of *Lymnaea stagnalis*, which were kept in running Balaton-water until used. Electrophysiological measurements were performed on 100 snails and histological investigations on 12 snails in every season of the year. Intracellular recording and current application was made with glass microelectrodes connected with a FET input amplifier in the same way, as has been described by Kiss & Salánki (1977). The activity of nerves was recorded using suction electrodes. Intracellular and retrograde CoCl_2 staining were carried out in a conventional way (Pitman et al., 1972; Kiss & Salánki, 1977).

The electron microscopic investigations were carried out both on intact ganglion and isolated cells. The material was fixed in a solution containing 2.5% glutaraldehyde and subsequently in 2% OsO_4 .

RESULTS

Fig. 1 shows the position and axonal pathways of the 2 neurones, as summarizing the data obtained with intracellular and retrograde CoCl_2 staining, as well as the main electrophysiological findings. Neurone A10 has a branching axon. The proximal region of the axon is thickened to a high degree and several dendritic spines originate from it. The longer axonal branch runs into the right parietal ganglion, while the shorter one is terminated in the visceral

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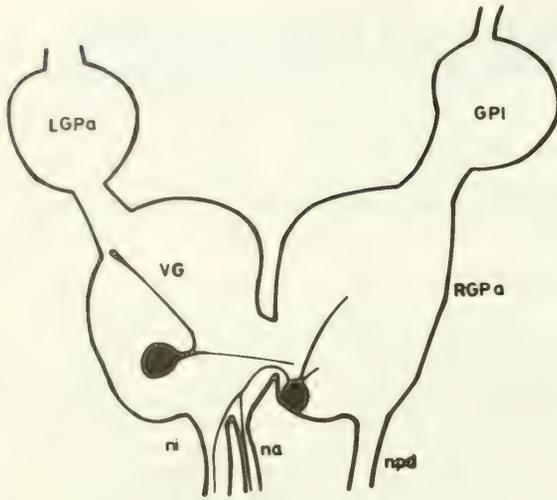


FIG. 1. Scheme of the position and axonal pathways of neurone A10 in the visceral ganglion (VG) and of neurone P1 in the right parietal ganglion (RGP a); GPI = pleural ganglion, LGPa = left parietal ganglion, na = anal nerve, ni = intestinal nerve, npd = pallial nerve.

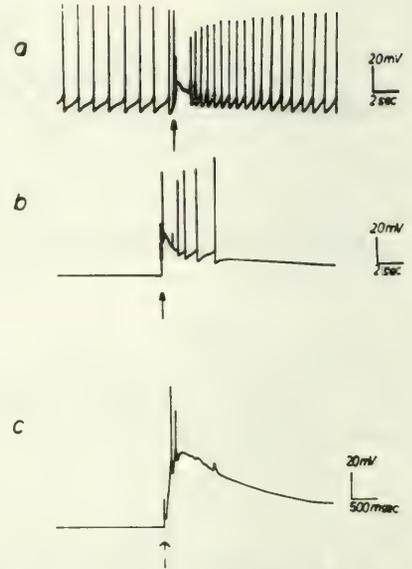


FIG. 2a. Effect of stimulation of the nervus pallialis dexter on the spontaneous activity of neurone A10 at the level of the resting potential; b. the same effect at the level of -70 mV hyperpolarization; c. The same effect at the level of -120 mV hyperpolarization.

ganglion showing a swelling. Neurone P1 has 3 processes, one of which is directed to the visceral ganglion, where it ramifies again; furthermore these branches run into the intestinal and anal nerves, respectively.

The spontaneous activity of these cells shows 3 main types: frequency of continuous regular distribution, irregular distribution and burst activity, which show a clear seasonal alternation. From early Spring onward the exogenous synaptic influence was declining, while the endogenous potential generation (continuous regular pacemaker and burst) was increasing.

Neither of the cells have direct efferent output in any nerve originated from the visceral and right parietal ganglia. When stimulating the same nerves neurone A10 does not respond with antidromic spike, but with complex EPSP-s in every case (Fig. 2). On the other hand neurone P1 generates a slow, depolarizing wave following the stimulation of intestinal and anal nerves, which evokes a spike, when exceeding the firing threshold (Fig. 3). If one records the spontaneous activity of this cell parallel with the activity of intestinal and anal nerves, it can be often observed that an increase in the firing rate of the cell is preceded by unit or burst discharge (Fig. 3d-e). These findings suggest that P1 probably is a 2nd or higher order sensory neurone. Neurones A10 and P1 are coupled electrotonically; This is supported by the following. The 2 cells have synchronous spontaneous activity (Fig. 4a). Either of the cells can trigger the activity of the other one (Fig. 4b). The delay between the synchronous spikes is smaller than what would be expected in the case of chemical transmission (Fig. 5). There is a bidirectional transmission for both the depolarizing and hyperpolarizing transient potentials (Fig. 6).

According to Bennett's equivalent electrical network (Bennett, 1966) of an electronic synapse, if one applies an i_1 current to the first cell and records v_2 electrotonic potential from the coupled one, the electrical characteristics of the supposed junction can be estimated on the basis of the i_1-v_2 relation and the time constant of potential transients recorded from both sides. The values of coupling coefficients are presented in Table 1. It is interesting to note that "k" depends on the direction of transmission, as well as on the stimulating current. The highest value is obtained, if the transmission of a depolarizing pulse occurs from P1 to A10 neurone. In Table 2 the calculated passive electrical characteristics of the 2 cell membranes and

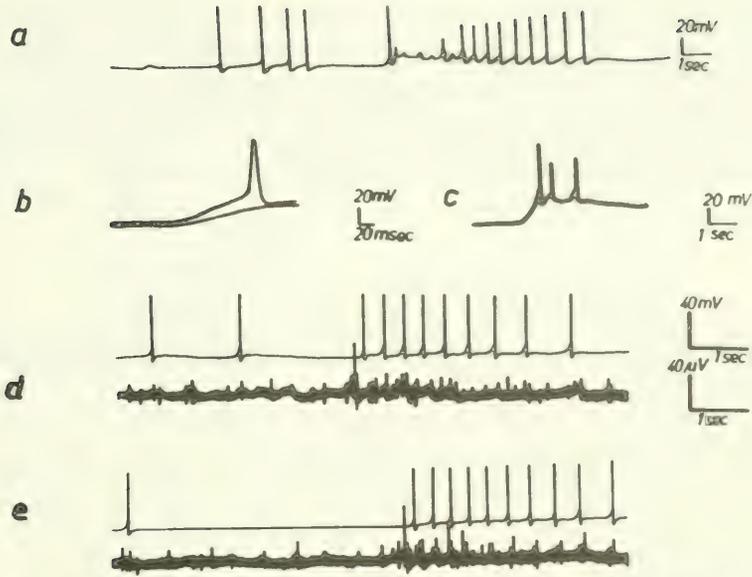


FIG. 3a. Response of neurone P1 to the stimulation of the anal nerve; b. Depolarization evoked by the stimulation of the anal nerve (lower trace) resulted sometimes in the appearance of a spike (upper trace); c. Response of the neurone to the stimulation of the intestinal nerve; d, e. Relationship between the activity of neurone P1 and the intestinal and anal nerves respectively.

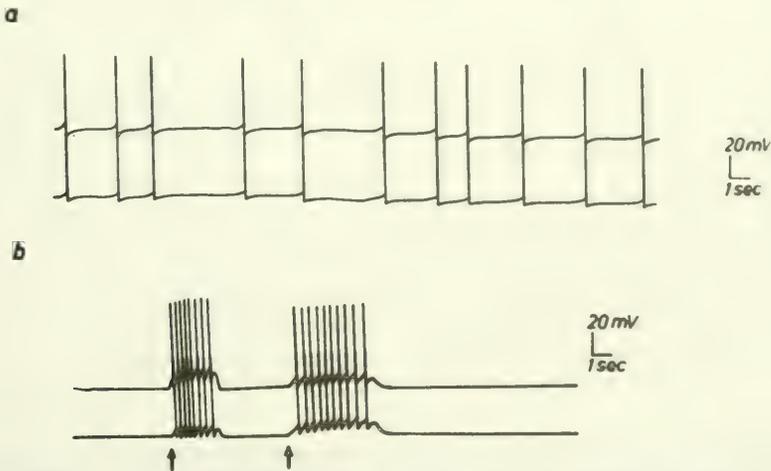


FIG. 4a. Synchronous spontaneous activity of neurones P1 and A10; b. Artificial depolarization of neurone A10 (first arrow) and neurone P1 (2nd arrow).

TABLE 1. Coupling coefficients.

Negative impulse		Positive impulse	
$\frac{V_1}{V_2}$	$\frac{V_2}{V_1}$	$\frac{V_1}{V_2}$	$\frac{V_2}{V_1}$
$0,13 \pm 0,08^*$	$0,19 \pm 0,12$	$0,35 \pm 0,14$	$0,21 \pm 0,02$

$V_1 = V_\infty$ value of potential transient recorded from neurone P1;

$V_2 =$ the same recorded from neurone A10;

*SD.

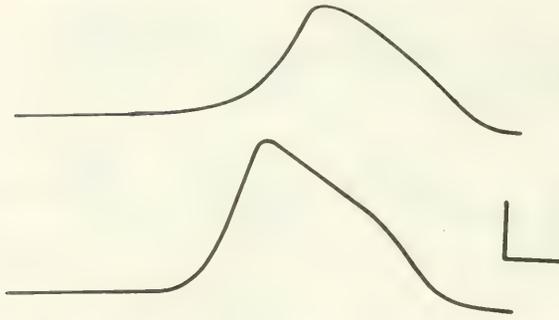


FIG. 5. High speed recording for measuring the delay between the synchronous spikes. Calibration: 40 mV, 5 msec.

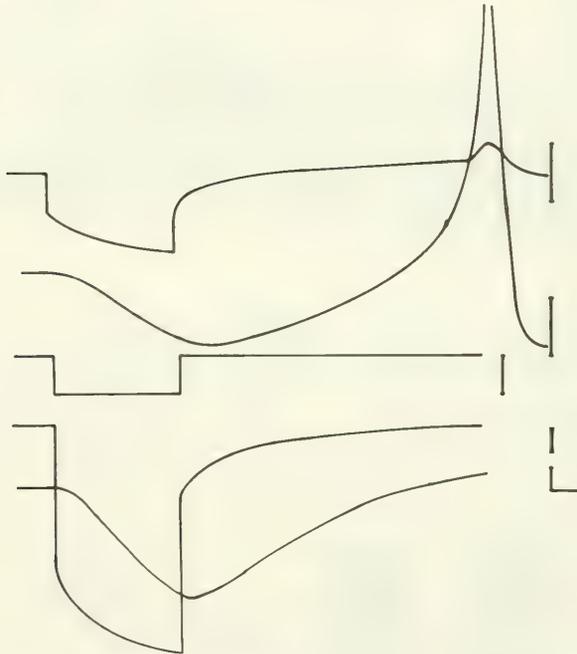


FIG. 6. Transmission of the transient potential evoked by hyperpolarizing square pulses from P1 to A10 (upper) and from A10 to P1 (lower traces). Following the firing of neurone A10 an electrotonic PSP can be recorded from neurone P1. Calibrations: upper traces: P1—100 mV; A10—10 mV; lower traces: A10—20 mV P1—5 mV; square pulse: 10^{-8} A, 50 msec.

TABLE 2.

R_1 ($\times 10^7$ Ohm)	R_2 ($\times 10^7$ Ohm)	C_1 ($\times 10^{-9}$ F)	C_2 ($\times 10^{-9}$ F)	C_j ($\times 10^{-9}$ F)
$3,68 \pm 0,94^*$	$3,25 \pm 0,70$	$2,08 \pm 0,76$	$2,07 \pm 1,51$	$5,92 \pm 3,62$

R_1 and R_2 = input resistance of neurone A10 and P1, respectively;
 C_1 and C_2 = membrane capacity of neurone A10 and P1, respectively;
 C_j = junctional capacity;
 *SD.

the junction are shown. At first it can be seen that the junctional resistance is much higher than that of the soma membrane, thus it is the factor which mainly determines the transmission. Secondly, as the value of junctional capacity is quite high, the junction should behave like a stimulus-dependent impedance, rather than a simple ohmic resistance. The current-dependence of junctional resistance underlines the current-dependence of the coupling coefficient (Table 3, Fig. 7). One of the functional consequences of the junctional properties, which prefer the P1 to A10 transmission, is that usually neurone P1 behaves as pacemaker and

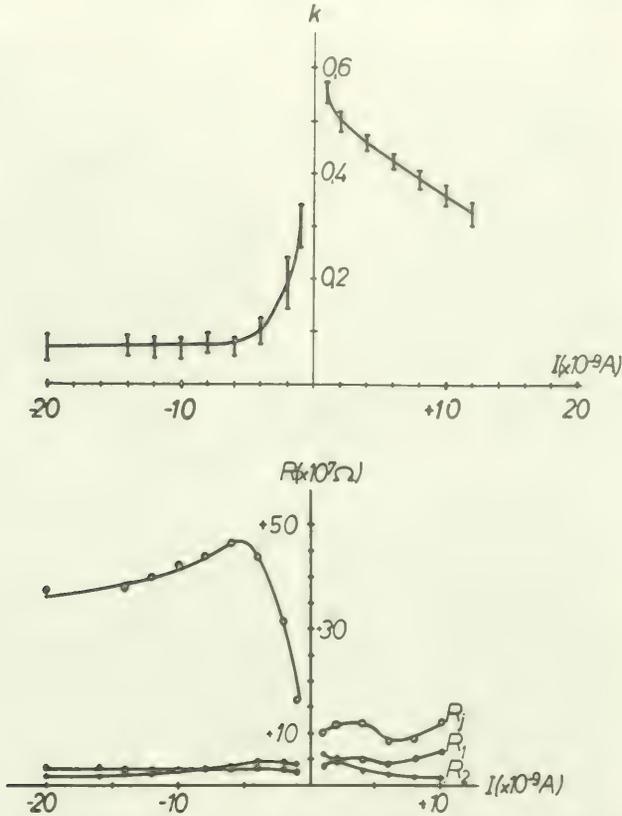


FIG. 7. Changes in the coupling coefficient (k) and junctional resistance (R_j) depending on the stimulating current in the case of P1 to A10 transmission. R_1 and R_2 are the resistances of the soma of A10 and P1 neurones, respectively.

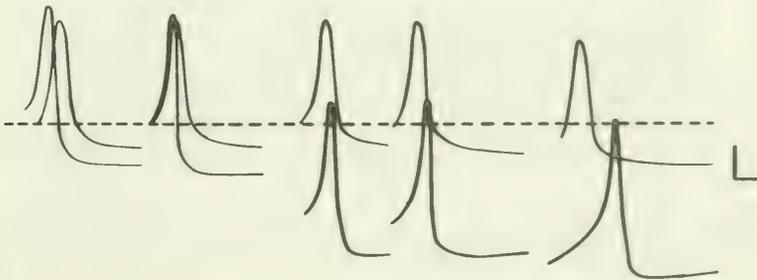


FIG. 8. Changes in the delay of synchronous spikes of the two examined neurones as a result of the hyperpolarization of neurone P1. Upper trace: A10; lower trace: P1. Broken line shows the level of the resting membrane potential. Calibration: 20 mV, 20 msec.

TABLE 3. Junctional resistance for transmission of different direction R_j ($\times 10^7$ Ohm).

Negative impulse		Positive impulse	
P1 \rightarrow A10	A10 \rightarrow P1	P1 \rightarrow A10	A10 \rightarrow P1
37,57 \pm 9,05*	27,29 \pm 7,42	10,68 \pm 1,64	23,41 \pm 6,11

*SD.

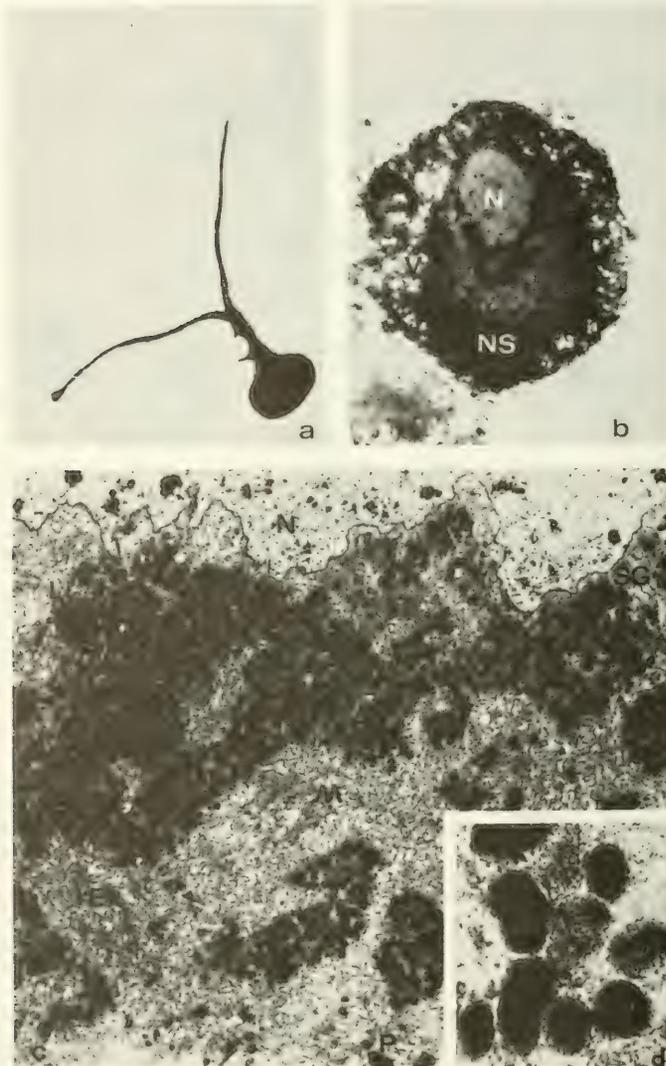


FIG. 9a. Neuron A10 marked with intracellular CoCl_2 staining; b. A light-microscopic section of neuron A10. Note the piles of dark-stained neurosecretory material (NS) in the cytoplasm. Toluidine blue staining, $\times 300$. c. Electron micrograph taken from cell A10. In the surface of the nucleus (N) there are a number of invaginations. In the immediate vicinity a large amount of secretory granules (SG) can be seen. The cytoplasm is rich in rough-surfaced endoplasmic reticulum (rER). Besides a couple of mitochondria (M), lipid droplets (Li) and pigment granules (P) occur in the cell; $\times 7200$. d. High power electron micrograph: the granules are variable in shape, their fine-granulated inner content is delimited by a unit membrane; $\times 43,000$.

initiates the activity of A10. However, a hyperpolarizing shift in the membrane potential can reverse this relation (Fig. 8); even an uncoupling may occur.

Further we examined the ultrastructure of both neurones, which was found to be basically similar (Fig. 9, 10). One of the most characteristic fine structural properties was the pronounced invaginated nature of the nucleus membrane. In the cytoplasm a highly developed rough endoplasmic reticulum and Golgi complex could be observed. The soma of both neurones is characterised by a high quantity of elementary secretory granules, surrounded by unit membrane. Some of them contained electron-dense material, the other ones had a lower electron-density. Their average diameter proved to be in a range of 1300-2000 Å. They were

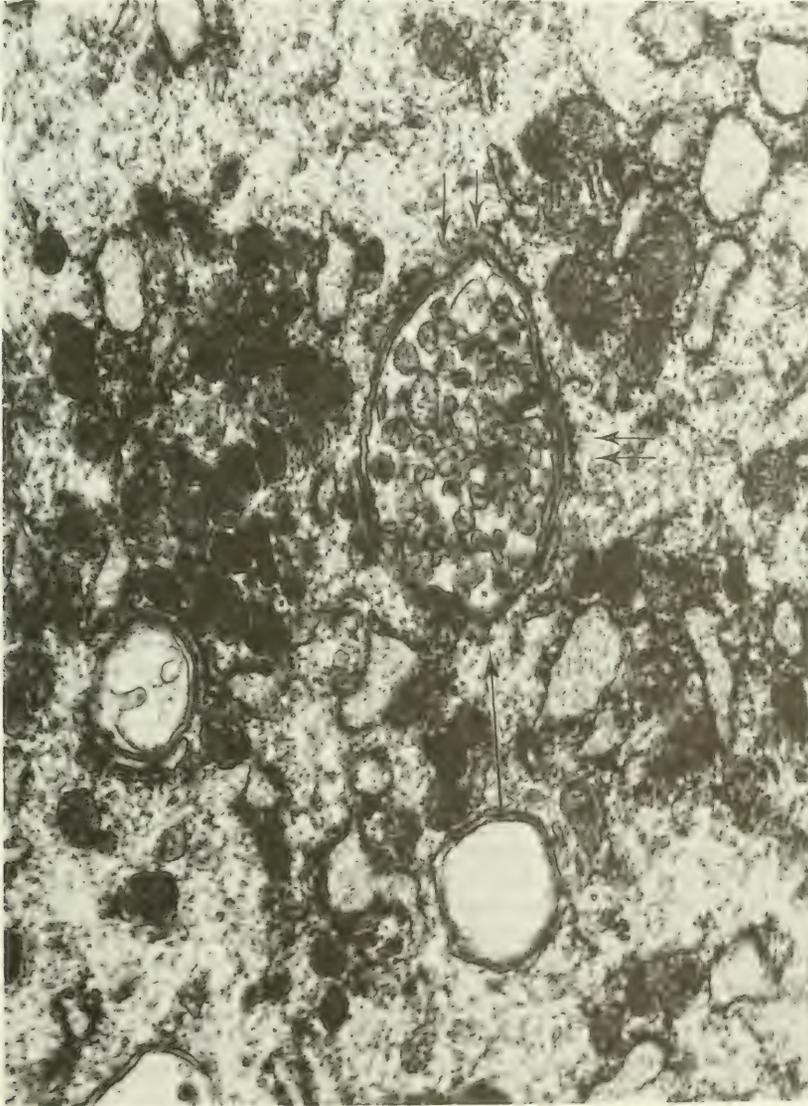


FIG. 10. Invagination of a synapse-like axon on the surface of the soma of GRP₁ (arrows). Populations of neurosecretory-like granules with dense or granulated material can be seen in the perikaryon (double arrows point to the RER elements in the vicinity of the synapse-like axon) $\times 36,000$.

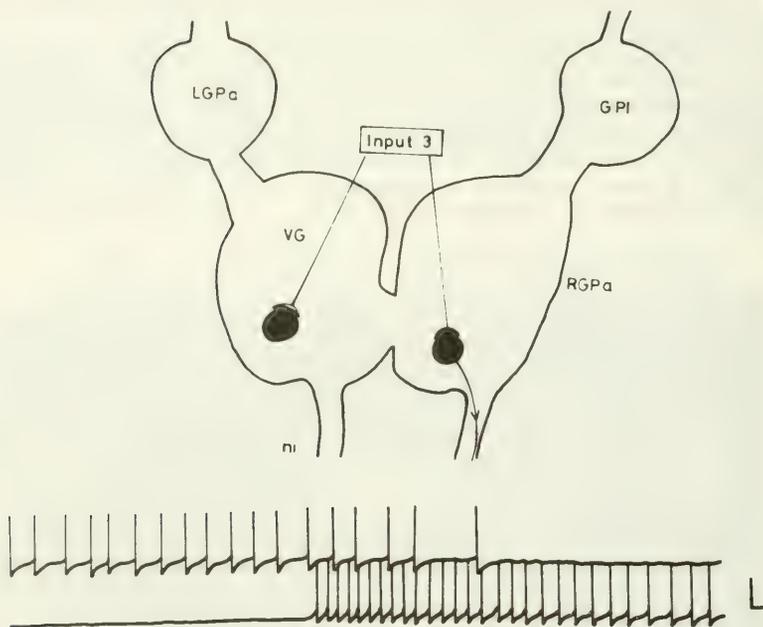


FIG. 11. Scheme of the supposed correlation between neurone A10 in the visceral ganglion and an unidentified neurone in the right parietal ganglion. We use "input 3" for marking a common input according to the scheme of Winlow & Benjamin (1976); right = excitatory, left = inhibitory synaptic input. Bottom: alternating activity of the right parietal neurone (upper trace) and neurone A10 (lower trace). Calibration: 40 mV, 1 sec. Abbreviations as in Fig. 1.

often found to be clumped together. Synapse-like structures (Roubos, 1975) often invaginated the soma (Fig. 10); however, typical true synaptic connections were not observed.

In order to understand the special function of these paired neurones we recorded their activity parallel with other neurones. The most interesting correlation we found was an alternating firing of A10 and an unidentified neurone (Fig. 11), which is an "A" group cell using the nomenclature of Winlow & Benjamin (1976). It seems to be quite an important finding, if we consider that the A10 neurone is inhibited by dopamine applied to the cell. The results suggest that these 2 neurones receive a common synaptic input from a double action interneurone, which might be identical with RGDC described by Winlow & Benjamin, if we would be able to demonstrate an exciting action of dopamine on this unidentified cell. At the same time we did show that the iontophoretically applied dopamine had no effect on neurone P1, and this may be considered as a further possibility for uncoupling of P1 and A10 cells.

So, the main conclusion of the present findings that P1 and A10 appear to be functionally asymmetrical, but ultrastructurally symmetrical giant neurones. Considering the electron microscopic results they have a clear neurosecretory character (cf. Kiss & Benedeczky, 1977). Boer et al. (1977) described a giant dark-green cell in the very same position of the right parietal ganglion of *Bulinus truncatus* (GDGC). Our P1 cell can be considered as a giant dark-green cell being homologous with GDGC in *Bulinus truncatus*. It is a difficult and open question why other authors did not show this cell using Alcian blue-Alcian yellow methods (Wendelaar-Bonga, 1970; Swindale & Benjamin, 1976; Boer et al., 1977). The function of neurone P1 is rather peculiar, as it has a 2nd or higher order sensory and at the same time secretory function.

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THE CENTRAL NERVOUS CONTROL OF THE ADDUCTOR BEHAVIOUR OF LAMELLIBRANCH MOLLUSCS

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ABSTRACT

In *Egeria radiata* (L.) and *Mutela dubia* (Gmelin) decerebration does not result in tonus of the posterior adductor muscle and each species continues to exhibit its characteristic rapid and slow rhythms. Excitatory and inhibitory nerve pathways originating in the cerebral ganglia terminate on the visceral ganglia. The cerebral ganglia alone do not exhibit any rhythm; the anterior adductor muscle remains relaxed after excision of the visceral ganglia.

The mid-dorsal and the anterior lobes of the visceral ganglia in *Scrobicularia plana* (Da Costa) control all adductor activity. Groups of potentials in the posterior adductor nerves originate from the different lobes and are separate physiological mechanisms.

INTRODUCTION

The adductor muscles of lamellibranch molluscs exhibit periodic alternation of activity and quiescence (Marceau, 1909; Barnes, 1955; Morton, 1970). Activity consists of rhythmic rapid contractions and slow relaxations; at quiescence the muscles are either continuously contracted for long periods of time in some species or are relaxed in others. The rhythm of the active period is the rapid rhythm and the alternation of active with quiescent periods, the slow rhythm (Barnes, 1955).

In *Anodonta cygnea* (cf. Barnes, 1955) each paired cerebral or visceral ganglion possesses an intrinsic rhythm which controls the rapid rhythm of the nearest adductor muscle and brings about the prolonged contraction of the tonic portion of the local adductor muscle during quiescence. Only the cerebral ganglia can inhibit the tonus of the two adductor muscles to allow the next period of activity to begin. Tonus of the posterior adductor muscle results from decerebration. In *Scrobicularia plana* (cf. Odiete, 1976a) the visceral ganglia alone control the tidal, rapid and slow rhythms. Excision of the visceral ganglia results in the relaxation of the anterior adductor muscle and the cerebral ganglia alone, unlike in *Anodonta*, do not exhibit any rhythm.

Anodonta cygnea and *Scrobicularia plana* are the only 2 species of bivalves in which ganglionic control of adductor rhythms has been investigated and the results do not agree. Other species need to be investigated before generalisations can be made on the nature of the central nervous control of adductor behaviour in the Lamellibranchiata. The results of experiments in two freshwater bivalves, *Egeria radiata* (L.) (Tellinacea) and *Mutela dubia* (Gmelin) (Unionacea), form part of this paper.

MATERIALS AND METHODS

The methods of recording adductor behaviour of an animal fixed by one shell valve has been described (Barnes, 1955; Odiete, 1976a). Animals fixed in glass tanks were covered with well-aerated pond water in which the animals had been living. Adductor behaviour was recorded for about three days after which an adductor was cut across and the local paired ganglia removed with a pair of fine forceps through the gape at the corresponding extremity of the animal and recording resumed. Electrophysiological and kymograph records were made to ascertain which lobes of the visceral ganglia controlled adductor behaviour. The details of the method of recording from the posterior adductor nerves have been described (Odiete, 1976b).

Posterior activity in a decerebrate *Scrobicularia* was recorded for some time and the 2 mid-dorsal lobes removed with the aid of a pointed glass probe and recording resumed. Next, the visceral ganglia were cut across, leaving only the posterior dorsal lobes and recording continued.

Ganglia were fixed and stained in formalin containing 0.3% thionin (Gurr, 1962) and paraffin sections examined.

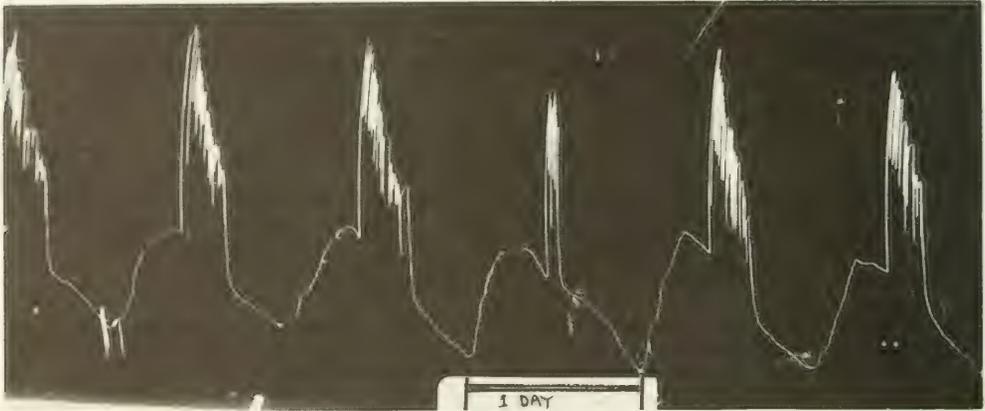


FIG. 1. The adductor rhythm of *Egeria radiata*. Part of 1 week of activity. Adduction downward in the traces. Scale = 24 h.

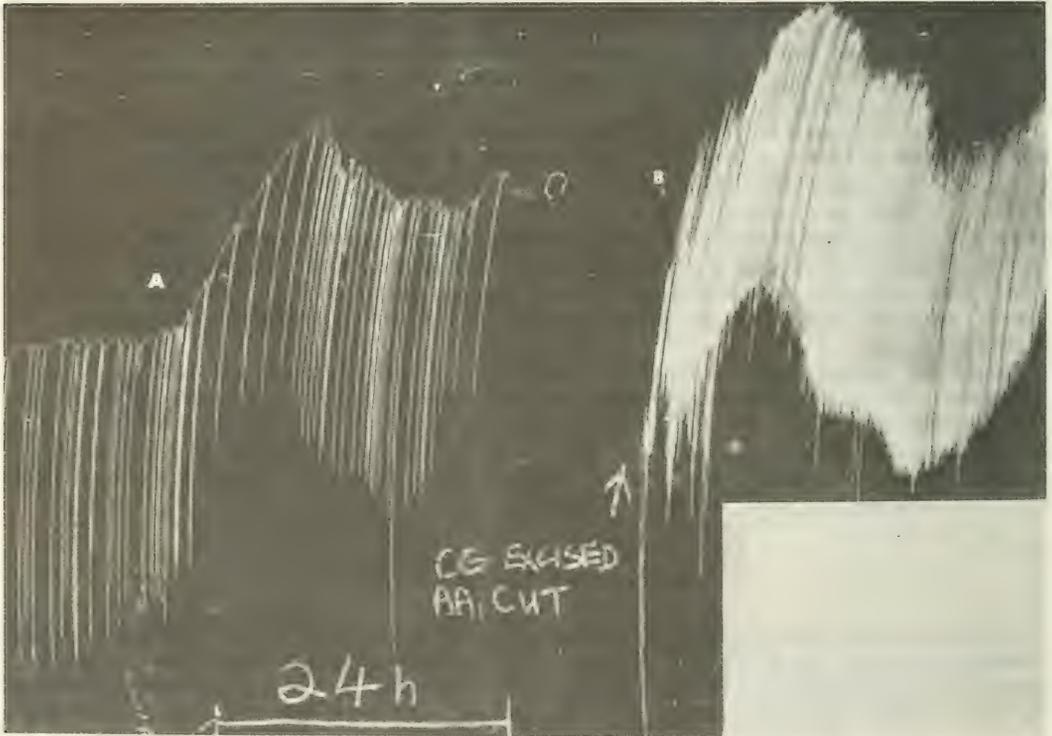


FIG. 2. The effect of decerebration on the adductor rhythm of *Mutela*. A, normal and B, rhythm after decerebration. At arrow the cerebral ganglia (GC) were excised and the anterior adductor muscle (AA) cut across. Adduction downward.

RESULTS

Egeria exhibits an endogenous rhythm fairly similar to those of *Anodonta* and *Unio* (Morton, 1970) except for part of the quiescent periods. The shell valves close completely for 1-3 h after which they steadily open for 6-8 h before the rapid rhythm begins. A small and slow

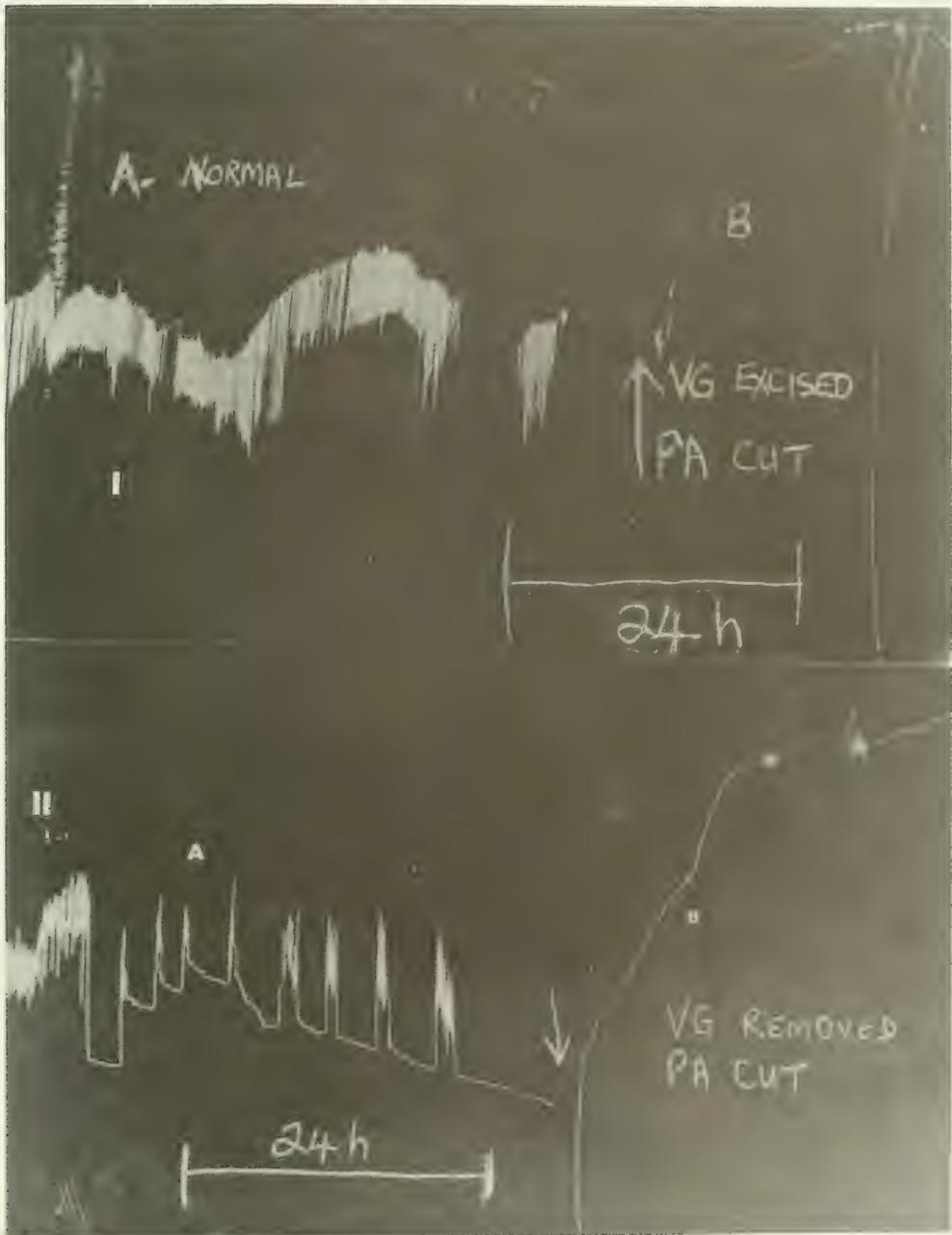


FIG. 3. The effect of the excision of visceral ganglia on the adductor rhythm. At arrow the visceral ganglia (VG) were excised after the posterior adductor muscle (PA) was cut across. I, Rhythm in the mutelid *Mutela dubia*. II, Rhythm in *Egeria radiata*.

adduction always precedes activity (Fig. 1). *Mutela* exhibits a rhythm similar to that of a permanently immersed *Scrobicularia* (Odiete, 1976a). Between periods of activity (consisting of 4-12 phasic contractions per hour) there are 1-2 h of quiescence during which the shell valves are gaping.

When the cerebral ganglia of the 2 freshwater bivalves are excised, the animals continue to exhibit the rapid and slow rhythms characteristic of each species (Fig. 2). The strength and frequency of phasic adductions are increased. Excision of the visceral ganglia results in the gradual relaxation of the anterior adductor muscle until the shell valves gape fully and remain so except for a few spontaneous phasic contractions (Fig. 3).

Decerebration does not produce tonus of the posterior adductor muscle in any of these two bivalves. Faradic stimulation of the cerebrovisceral connectives (CVC) produces contraction of the posterior adductor muscle, the contraction being maintained throughout stimulation. On the

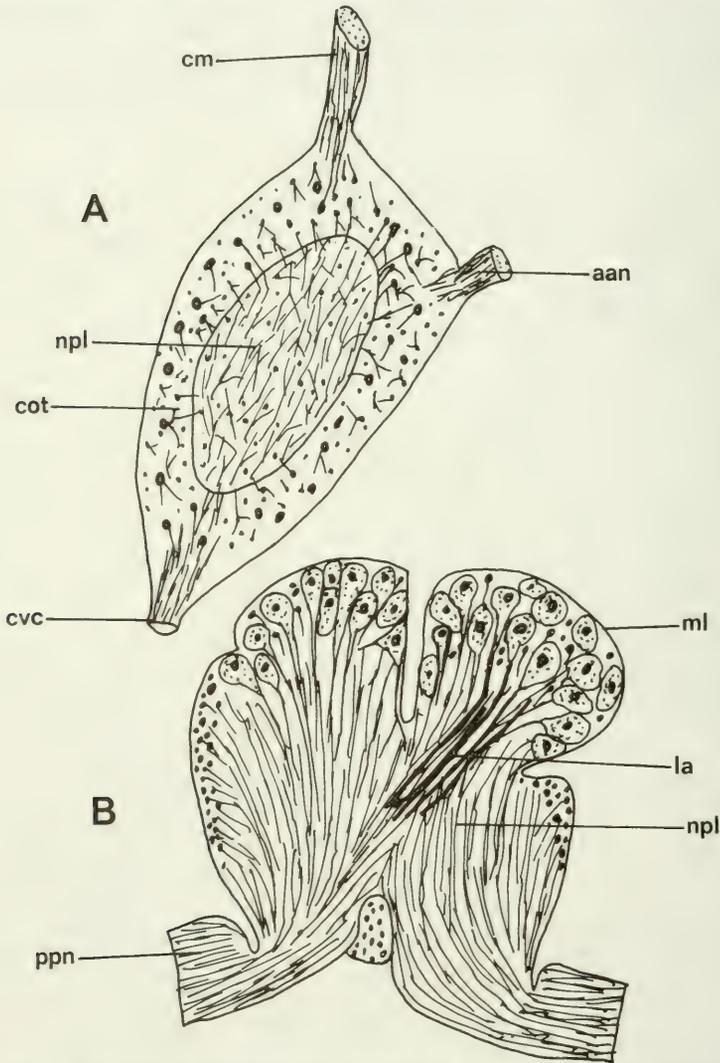


FIG. 4. (A) Parasagittal section through the cerebral ganglion of *Mutela*. (B) Transverse section of the fused visceral ganglia, drawn to the same scale as (A); aan, anterior adductor nerve; cm, cerebral commissure; cvc, cerebrovisceral connective; cot, cortex; fnpl, fused neuropil; la, large axon; ml, mid-dorsal lobe; npl, neuropil; ppn, posterior pallial nerve.

cessation of stimulation the muscle gradually relaxes much more fully. The anterior adductor muscle similarly contracts during faradic stimulation of the CVC.

These results indicate that excitatory nerve pathways connect the 2 paired ganglia. The increase in size and number of phasic adductions following decerebration and the fact that the posterior adductor muscle remains relaxed for long periods following faradic stimulation of the CVC, suggest that the cerebral ganglia have the means to inhibit the visceral ganglia or the adductor muscle fibres.

All these results agree with the findings in *Scrobicularia* (Odiete, 1976a). Decerebration in this species does not produce a tonus of the posterior adductor muscle and the visceral ganglia control all adductor rhythms unlike in *Anodonta*.

Microscopic examination of the ganglia in *Egeria* and *Mutela* has revealed that, as in *Scrobicularia*, the cerebral ganglia contain cortices with evenly distributed neurones surrounding the neuropils while in the visceral ganglia the neurones are concentrated in lobes located mainly on the dorsal part (Fig. 4).

Investigations have been conducted to ascertain which lobes of the visceral ganglia are responsible for controlling adductor activity. *Scrobicularia* is more suitable for these investigations because its shell valves are easy to cut and the nerves are not entangled in masses of connective tissue as in larger species. The removal of the paired mid-dorsal lobes (XI cut, Fig. 5) results in the decrease in the number of phasic adductions from 15 to 1 per hour (Fig. 7). This reduction corresponds to the disappearance of the large group A potentials (Odiete, 1976b) from the posterior adductor nerve (Fig. 6b). When the mid-dorsal lobes have been removed in fresh preparations and the visceral ganglia are then cut across, leaving only the posterior lobes (XII cut, Fig. 5), no adductions occur. The shell valves remain gaping (Fig. 7). This, again, is correlated with the absence of nerve action potentials in the adductor nerves as shown in Fig. 6c. The results suggest that both the mid-dorsal and the anterior lobes of the

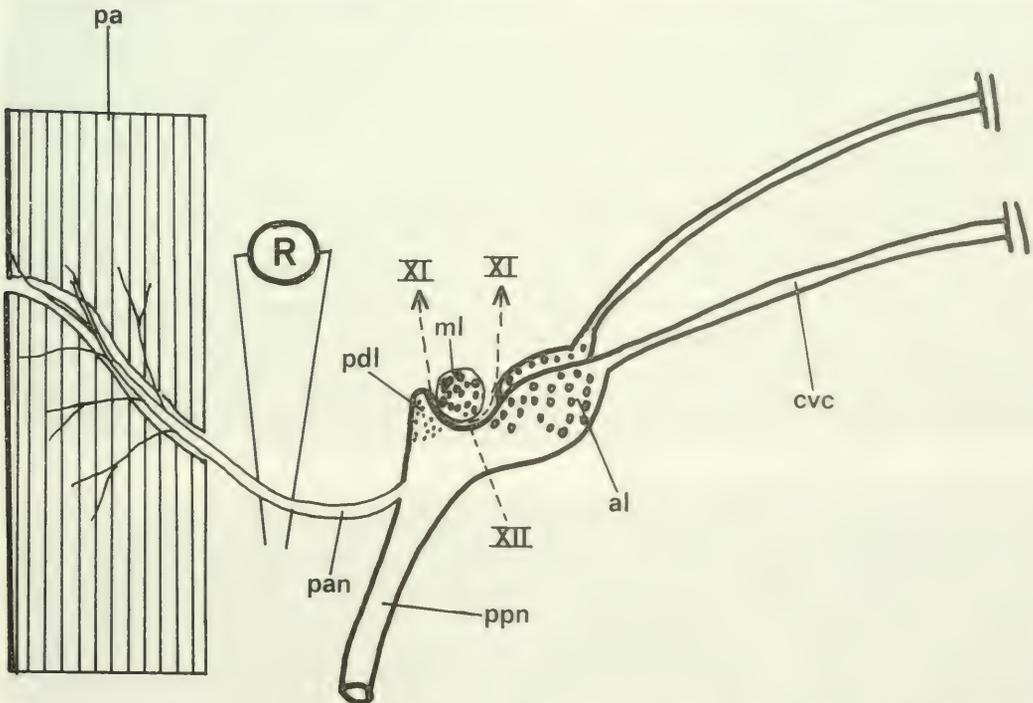


FIG. 5. Position of recording, R, during excision of lobes of the visceral ganglia in a decerebrate *Scrobicularia*. XI cut, separates mid-dorsal lobes; XII cut, separates anterior lobes. Lettering as in Fig. 4; al, anterior lobes; ml, mid-dorsal lobes; pa, posterior adductor muscle; pan, posterior adductor nerve; pdl, posterior dorsal lobes.

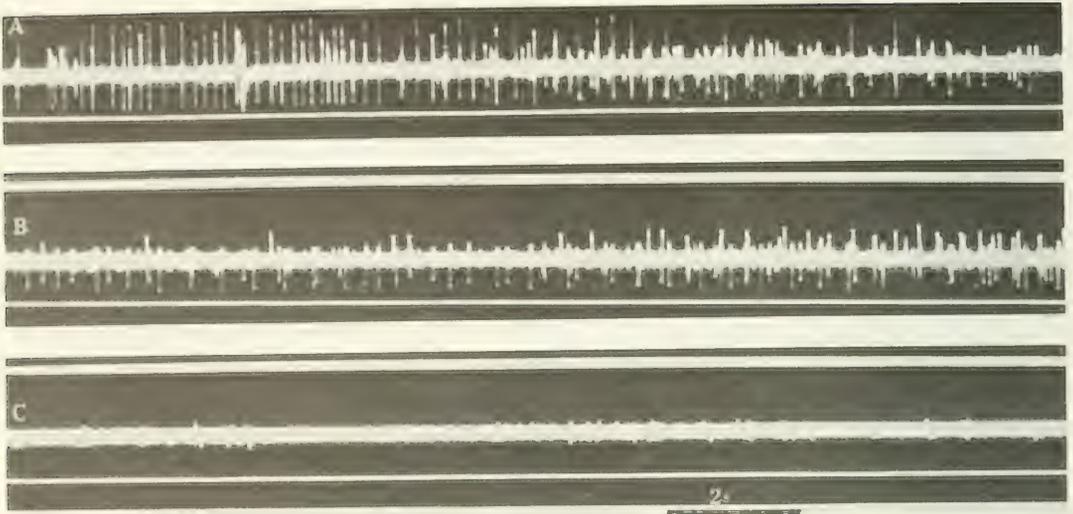


FIG. 6. A, Spontaneous activity in the posterior adductor nerve of a decerebrate *Scrobicularia*. Three sizes of potentials present. B, Activity after removal of mid-dorsal lobes. C, Activity after cutting visceral ganglia across as at XII.

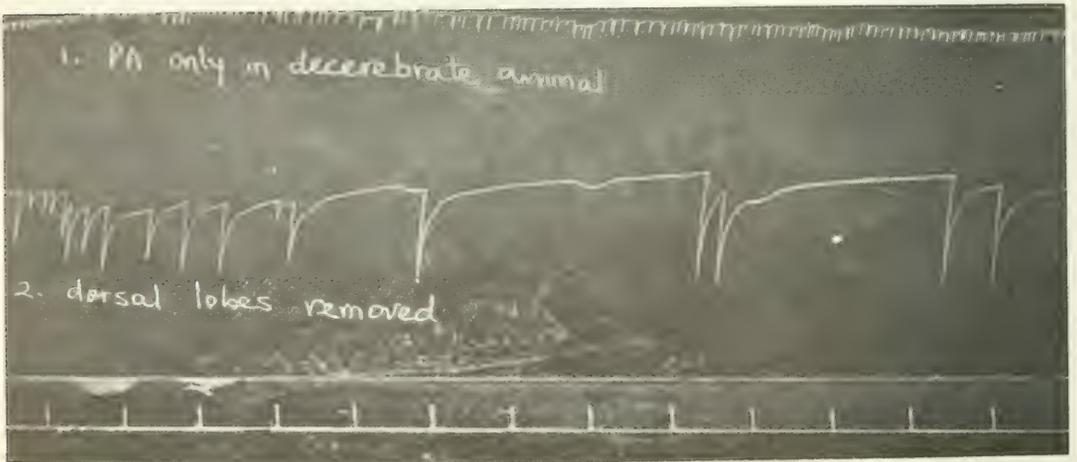


FIG. 7. Kymograph records of (above) the effect of the removal of the mid-dorsal lobes (2) upon posterior activity (1) in a decerebrate animal, and (below) record of activity resulting from XII cut; (1) posterior activity and (3) record after cut.

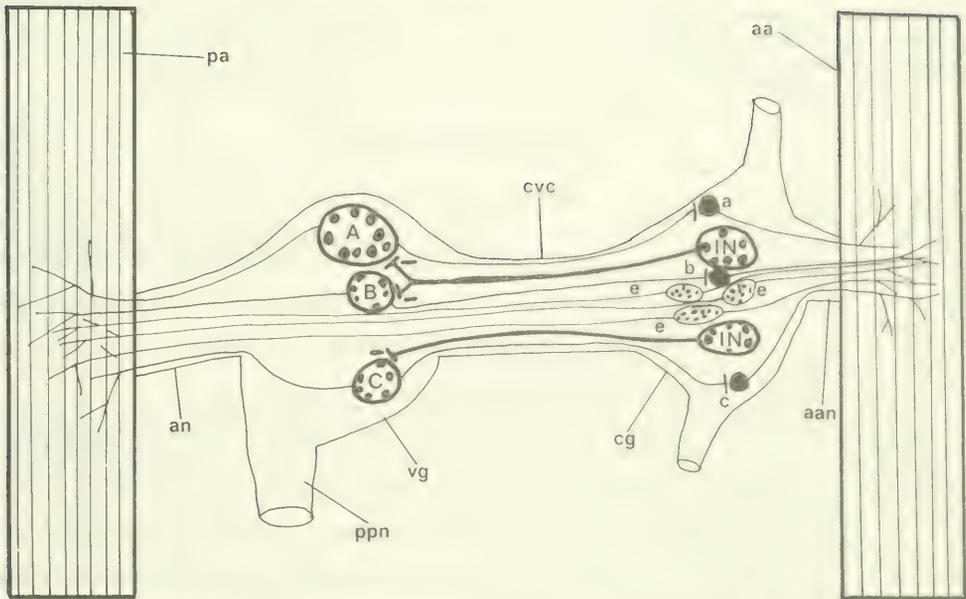


FIG. 8. Schematic representation of some of the nerve fibre pathways associated with adductor rhythmicity in the Lamellibranchiata. A, B, and C are centres in the visceral ganglia which produce groups A, B and C nerve action potentials respectively and which control adductor rhythmicity. They form excitatory synapses (a, b and c) in the cerebral ganglia. IN, are inhibitory nerve centres with which visceral ganglia activity are inhibited when necessary. Excitatory nerve pathways (e) also connect the cerebral with the visceral ganglia or the adductor muscle fibres; aa, anterior adductor muscle; aan, anterior adductor nerve; an, posterior adductor nerve; cg, cerebral ganglion; cvc, cerebrovisceral connective; pa, posterior adductor muscle; ppn, posterior pallial nerve; vg, visceral ganglion.

visceral ganglia in *Scrobicularia* and possibly in the two freshwater bivalves control adductor behaviour. Group A potentials originate from the mid-dorsal lobes and the small group C and the intermediate size group B potentials (Odiete, 1976a) come from the anterior lobes.

DISCUSSION

In *Egeria radiata*, *Scrobicularia plana* and *Mutela dubia* the visceral ganglia control adductor rhythmicity. Unlike in *Anodonta cygnea* decerebration does not produce tonus of the posterior adductor muscle in any of the 3 species and the cerebral ganglia alone do not exhibit any rhythms. Excitatory nerve pathways connect the 2 paired ganglia or the opposite adductor muscle fibres (Fig. 8). The cerebral ganglia can inhibit the visceral ganglia. Since the former control digging in the bivalves that have been investigated (Drew, 1908; Nadort, 1943) inhibition of the visceral ganglia is necessary and the posterior end of the animal should be closed during the process of burrowing. It is probable that the inhibitory process is initiated by the pedal ganglia.

The visceral ganglia in *Gari* (Graham, 1934), *Egeria*, *Scrobicularia* and *Mutela* possess lobes concentrated with neurones. *Pecten* (Dakin, 1910) possesses large and highly complex visceral ganglia. It is thus probably a characteristic feature of the central nervous system of the lamellibranch molluscs for the visceral ganglia to be more complex in anatomical organization than the cerebral ganglia. This increase in complexity is partly due to the fusion of several paired ganglia (paired parietals and viscerals) and is correlated with the fact that the posterior ends of bivalve molluscs are influenced first by changes in the environment. As adductor activity must clearly be correlated with siphonal and other activities in the posterior part of the animal it is not surprising that visceral ganglia should assume control of all these activities.

Excision experiments suggest that the mid-dorsal and the anterior lobes of the visceral ganglia control adductor activity. Groups of potentials recorded in the posterior adductor nerves originate from different lobes.

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EFFECTS OF IONIC ENVIRONMENTAL CHANGES ON THE LIGHT-EVOKED DEPOLARIZATION OF AN IDENTIFIED *HELIX POMATIA* NEURON

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ABSTRACT

In a previous work we have described the effects of photostimulation on 4 identified neurons in a *Helix pomatia* ganglion. It was found that 3 of the cells reacted to illumination with a depolarization while in one cell the onset of light induced hyperpolarization. In the present experiments the effect of illumination on one of the photosensitive nerve cells in the left parietal ganglion was investigated with the aim to obtain data about processes involved in the light-evoked depolarization (LED). The experiments were carried out on isolated circumesophageal ganglia of *Helix pomatia*. The ganglia were dissected from active animals kept previously for several weeks in the laboratory under conditions of natural light. The experiments were carried out on neuron C (e.g. D 1) in the left parietal ganglion. KCl filled glass capillary microelectrodes connected to a microelectrode amplifier equipped with a bridge circuit were used for recordings together with a pen recorder. The light source used for photostimulation was a halogenic lamp (12 V, 100 W) focused on the cell by a system of lenses. The effects of illumination were first established in the regular snail solution. After that the medium was changed and the same procedure was repeated. Testing the effect of sodium free solution on LED it was found that its effect depended on the nature of the substituent. Lithium could partly replace the sodium in LED, whereas the replacement of sodium by TRIS (hydroxymethyl-aminomethan) abolished reversibly the cell's reaction to light. Illumination induced the depolarization in K^+ free solution but the return to the steady state membrane potential was in some cases modified. The amount of Ca^{++} ions in the medium modified LED. Ouabain suppressed the LED, as well as cooling of the ganglion. The possibility that illumination enhances the activity of electrogenic Na^+ pump is discussed.

In our previous work the effects of photostimulation on *Aplysia depilans* and *Helix pomatia* neurons were investigated (Pašić et al., 1972; Pašić et al., 1975). In the experiments on garden snail nerve cells we confirmed the findings of Arvanitaki and her collaborators (Arvanitaki et al., 1968) that photosensitive neurons are present in ganglia of this species. However as those experiments were carried out on unidentified neurons, we recently described the effects of photostimulation on four identified nerve cells in *Helix pomatia* subesophageal ganglia (Pašić et al., 1977 in press).

In the present work the reaction of one of the identified photosensitive neurons to illumination was further analysed. The effects of intermittent photostimulation are here described as well as the reaction of neurons to light in different ionic media.

METHODS

The experiments were carried out on the isolated subesophageal ganglionic complex of *Helix pomatia*. The snails were collected locally and prior to experiments kept for several weeks under laboratory conditions.

The circumesophageal ganglia were dissected from snails by means of the usual dissecting technique and mounted individually on Sylgard in a small recording chamber with circulating snail solution (Kerkut et al., 1975). After establishing the position of the chosen nerve cell under a binocular microscope, it was penetrated with a KCl filled glass capillary microelectrode with resistances ranging from 5-15 MOhms. A microelectrode amplifier (Grass P16), an

oscilloscope (Tektronix 502A) and a pen recorder (Beckman Dynograph RS) were used for recordings. In cases when the microelectrode was used simultaneously for membrane polarization and recording, it was connected to a bridge circuit.

The light source was a halogenic lamp (12V, 100W) focused on the cell by a system of lenses, the size of the spot being less than 0.5 mm. The intensity of light was kept constant during most experiments.

The thermal effects of light were avoided by continuous circulation of snail solution above the preparation. In most experiments a small thermocouple was placed just beside the preparation and the temperature was monitored continuously.

As stated above the control perfusion was carried out with regular snail solution. Replacement of Na was made with the appropriate amount of iso-osmotic TRIS (hydroxymethylaminomethan). KCl was substituted by NaCl and CaCl_2 was compensated by MgCl_2 .

RESULTS

As described in our previous work (Pašić et al., 1977) illumination induces a depolarization and an increment of action potential frequency in 3 of the 4 identified photosensitive neurons tested, while in one of the cells at the onset of light a hyperpolarization occurs. Testing the overall reactivity of the cells to light it was observed that the identified neuron in the left parietal ganglion (neuron C in Fig. 1) is highly photosensitive. This neuron reacted to

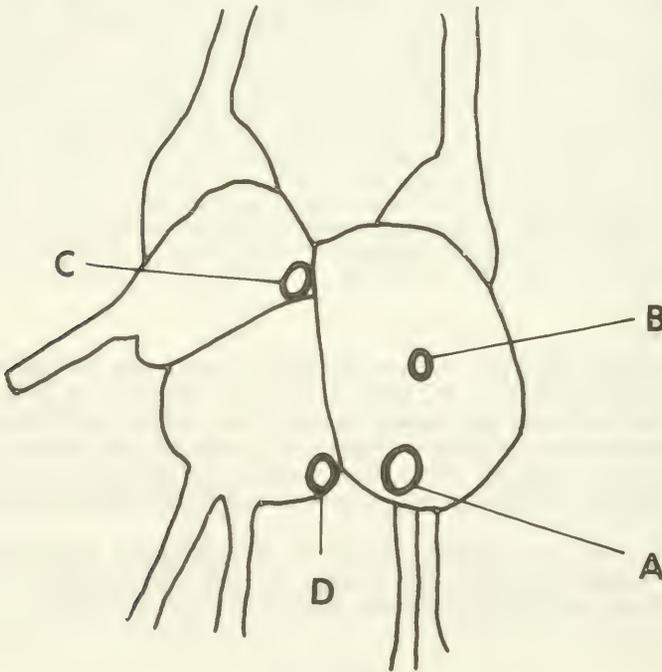


FIG. 1. Schematic presentation of subesophageal ganglia of *Helix pomatia*. Note the position of neuron C in the left parietal ganglion.



FIG. 2. Effect of illumination on neuron C; light on and off at arrows.

illumination in all cases when the microelectrode was successfully impaled into it. In 40 experiments a reaction to illumination of neuron C was recorded.

In Fig. 2 the effect of photostimulation on the spontaneous spike activity of neuron C is presented. In this experiment several light/dark cycles, each lasting 1 minute, were applied and, as seen, the cell reacted to each of them. The onset of light induced a depolarization with an increment of action potential frequency which persisted during the whole 1 minute period of illumination. When the light was switched off the membrane hyperpolarized and the action potential frequency decreased. A shift to hyperpolarization during illumination as well as a decrement of action potential frequency after a maximum was reached can be noticed in this and other records. However as stated in our previous work (Pašić et al. 1977) where the analysis of cells spike activities was carried out by means of an earlier described method (Ristanović & Pašić, 1975), the action potential frequency remained significantly higher at every moment during illumination than during the subsequent dark periods. As stated there, the photosensitive neurons apparently behave as a slowly adapting receptor.

Investigating the effects of intermittent photostimulation on unidentified *Helix pomatia* neurons (Pašić et al. 1975) we found that such stimulation induces changes in the photosensitive cells reactivity to light. In Fig. 3 the results of an experiment where intermittent photostimulation was applied to the identified neuron A (e.g. Br neuron, Fig. 3) are presented. From the regression line in the upper part of this figure it can be seen that during

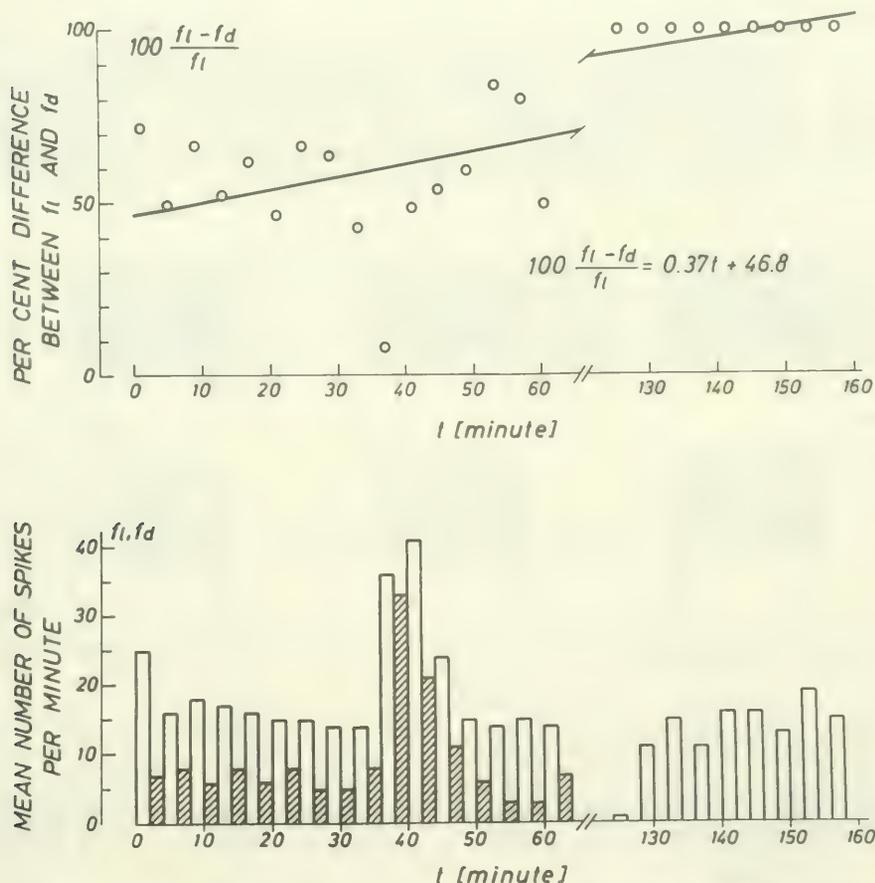


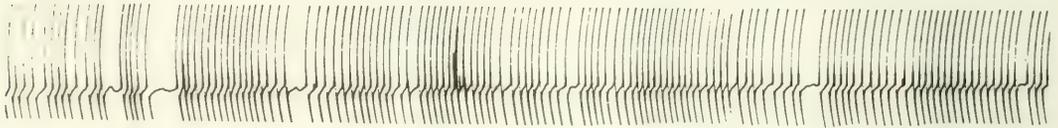
FIG. 3. Effects of illumination on the Br neuron (neuron A) of a dormant snail. Abscissa: minutes from the beginning of the illumination. Columns in lower graph: mean numbers of spikes per minute during illumination (white) and during darkness (shaded). Each value in the upper graph is calculated from the differences between adjacent light and dark periods.

intermittent photostimulation the percentage difference between the action potential frequencies during illumination and subsequent dark periods progressively increased in the course of the experiment. The lower part of the graph in the same figure, where the mean numbers of spikes per minute calculated for each of the induced light (f_1) and dark (f_d) period are shown, however, reveals that this increment is mainly due to the decrement of action potential frequency during the dark periods while the frequency remained nearly constant during periods of illumination. At the still later stage of experiment no action potentials were generated after the light was switched off.

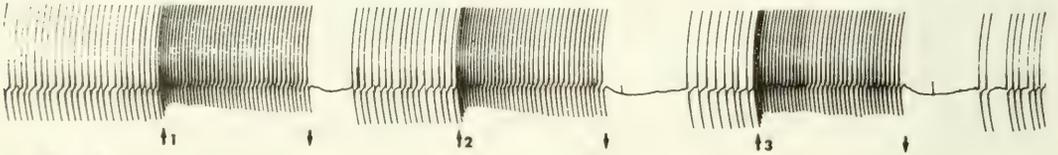
In the record in Fig. 4 which was obtained from neuron C it can be observed that repeatedly induced light actually enhanced the hyperpolarization which developed after illumination. Its amplitude and especially its duration increased in the course of the experiment extending at the end to the whole dark period. In 10 other cells where the photosensitivity was high (the difference between f_1 and f_d being at least 50% at the beginning of the experiment) similar effects of intermittent photostimulation were obtained. In Fig. 5 another record is presented where an enhancement of hyperpolarization is seen even after 3 light flashes lasting only 10 seconds. It should be stated that a fairly high variation of amplitude (1-2.5 mV) and duration of the postillumination hyperpolarization occurred from cell to cell but it was recorded whenever the cells reaction to light was, as stated above, well expressed.

The membrane mechanism involved in the light evoked depolarization (LED) and in postillumination hyperpolarization was next considered. According to several earlier works on

INITIAL D



D/L 1,2,3



D/L 26, 27, 28

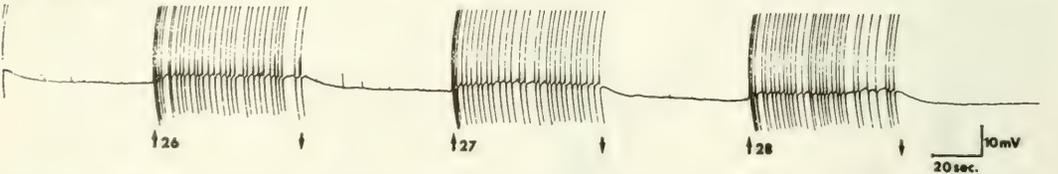


FIG. 4. Effects of intermittent photostimulation on neuron C. Note the enhancement and prolongation of hyperpolarization in the 3rd record.

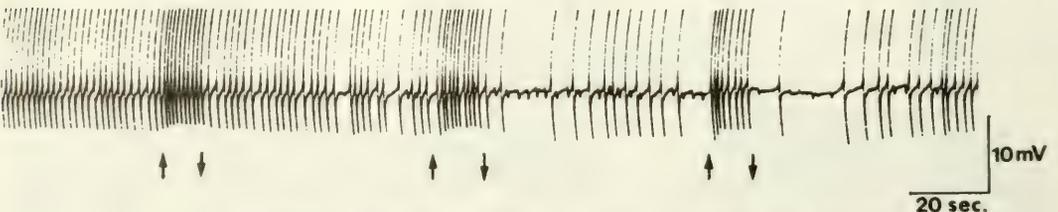


FIG. 5. Effects of 10 second illumination followed by 1 minute dark periods.

invertebrate photoreceptors (e.g. Smith et al., 1968) the light evoked depolarization in *Limulus* photoreceptors is mainly due to a conductance increment to sodium ions. On the other hand postillumination hyperpolarization analyzed in barnacle photoreceptors (Koike et al., 1971) was ascribed to activation of the electrogenic Na^+ pump which occurs as a result of sodium influx during illumination.

The hyperpolarization of membrane following the light-evoked depolarization in neuron C and especially its enhancement during intermittent photostimulation suggested that illumination could also here induce activation of the electrogenic Na^+ pump which increases as the cell

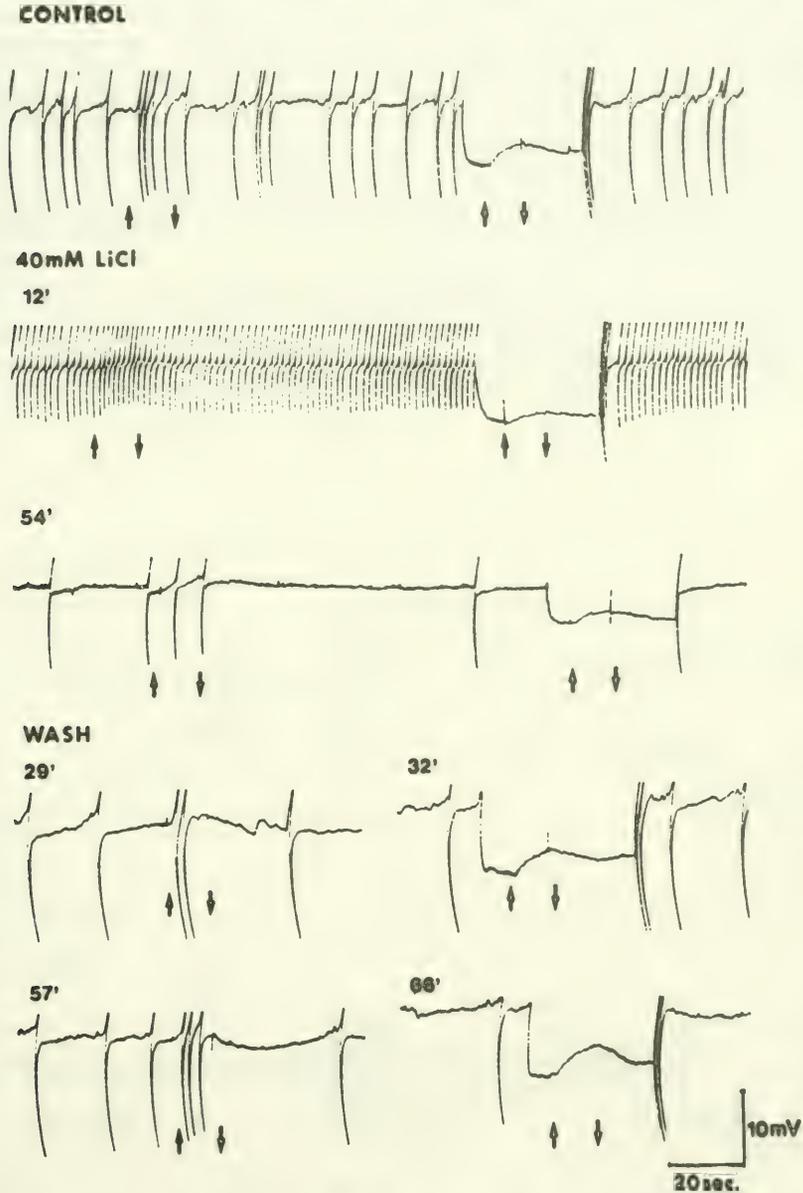


FIG. 6. Effect of partial substitution of Na^+ with Li^+ ions. The cell was hyperpolarized in order to enhance the light-evoked depolarization (see right hand side of records). The peaks of action potentials were cut in this and other pen records.

becomes loaded with sodium after several illuminations. To examine this possibility, first the effects of sodium substitution on LED and postillumination hyperpolarization were tested. In Fig. 6 an experiment where half of Na^+ ions was exchanged for Li^+ ions is shown. As seen, the LED diminished but it could still be elicited during the whole 60 minutes incubation in this solution. However no shift to hyperpolarization was recorded neither during illumination nor after it. A complete restitution of LED and postillumination hyperpolarization occurred only after washing. In 2 experiments where one half of Na^+ was replaced by Li^+ and the other by TRIS as well as in 3 other tests where TRIS was the only substitute for sodium (Fig. 7a,b) it was shown that lithium can partly replace Na^+ ions in LED.

Removal of K^+ ions from the incubation medium, addition of cardiac glycoside ouabain as well as cooling are also procedures which abolish the electrogenic Na^+ (e.g. Thomas, 1972; Carpenter, 1973; Ayrapetyan, 1973). In 7 experiments the ganglia were perfused with K^+ free solution. One of these experiments is presented in Fig. 8. As in this case it was consistently noted that LED was present in K^+ free solution while the shift to hyperpolarization during illumination and the postillumination hyperpolarization, which were here absent, could be still recorded in potassium free solution but their size was mostly diminished.

In Fig. 9 the effect of treatment with ouabain on neuron C and LED is presented. As seen, ouabain depolarized the cell and abolished the postillumination hyperpolarization before abolishing completely the LED.

As it was shown that Ca^{++} ions are involved in the transduction processes in photoreceptors as well as in photosensitive neurons (Brown & Brown, 1973; Brown et al., 1977) the effect of the removal of these ions on LED was tested. In Fig. 10 it can be seen, as was the case in 2 other experiments, that although the removal of Ca^{++} ions from the perfusion medium depolarized the cell, LED and a membrane hyperpolarization after illumination were still present.

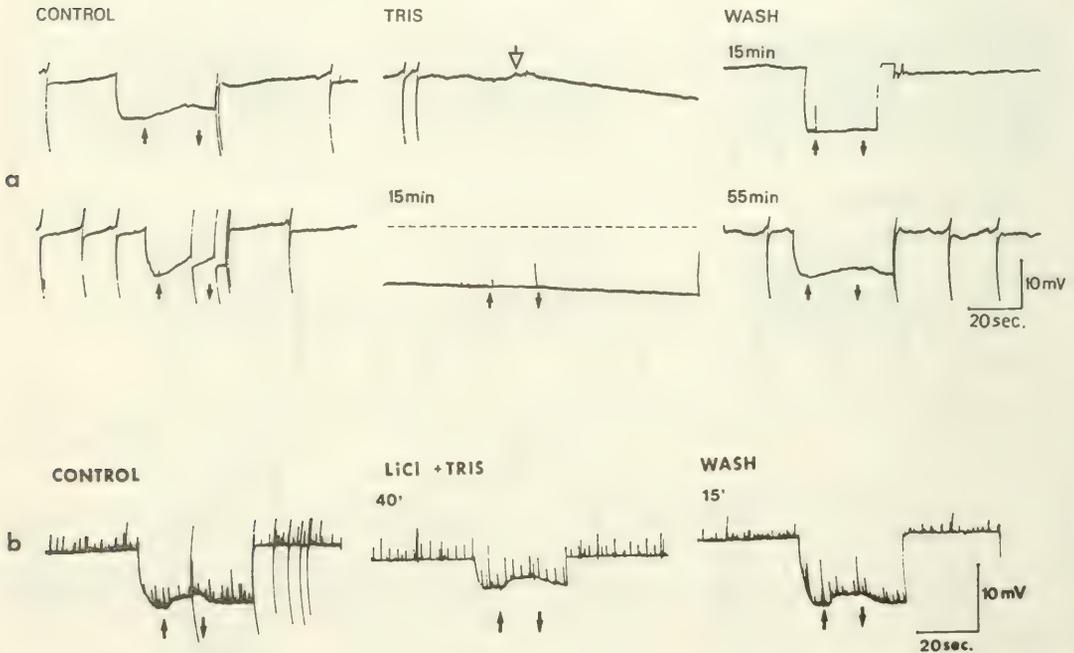


FIG. 7. Effect of substitution of sodium ions in the perfusion solution with TRIS on light-evoked depolarization (a) and of a solution containing 40 mM of lithium in addition to TRIS (b).

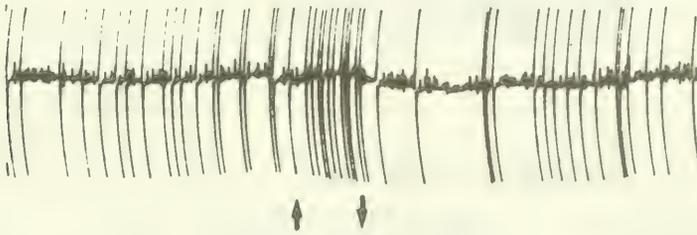
DISCUSSION

In the present experiments it was demonstrated that illumination induced in the photo-sensitive neuron in the left parietal ganglion of *Helix pomatia* a depolarization and an increment of action potential frequency which was followed by hyperpolarization of cell membrane. Although a variation of amplitude as well as in the time course of postillumination hyperpolarization was observed, its increment during intermittent photostimulation was constantly obtained.

Considering the present results together with our earlier data on unidentified photosensitive neurons, which reacted to light with depolarization, the possibility that sodium entry during illumination activates the electrogenic pump was tested.

As stated above, partial or total replacement of Na^+ by Li^+ did not abolish the LED in neuron C. Investigating the effects of sodium replacement on sensitivity of *Limulus* photo-receptors to light Smith's group (Smith et al., 1968) found that Li^+ as well as TRIS can partly replace Na^+ in light evoked depolarization. In the present experiments the substitution of Na^+

CONTROL

NoK⁺

15'



WASH

30'

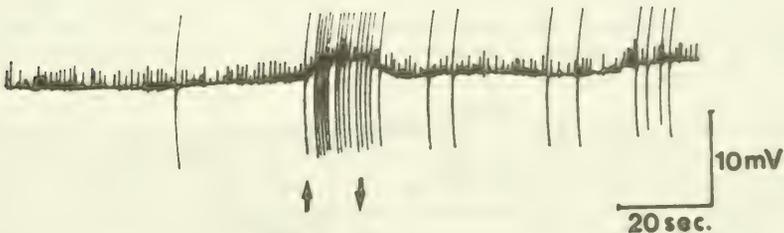
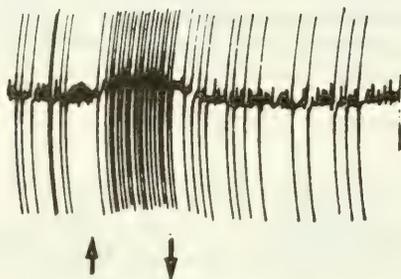


FIG. 8. Light-evoked depolarization and the postillumination hyperpolarization in K^+ free solution.

with TRIS proved to be ineffective in eliciting LED. The observation made that substitution of Na^+ with Li^+ abolished the postillumination hyperpolarization is consistent with the fact that lithium can substitute for Na^+ along the passive channels, but it is actively transported out of the cell at a much slower rate (Keynes & Swan, 1959). Also according to Partridge & Thomas (1976) partial substitution of Na^+ with Li^+ in snail solution decreased the intracellular sodium content of the nerve cells. That procedure would thus decrease the pump activity.

It was shown in many experiments that removal of K^+ ions from the external medium inhibits the activity of the electrogenic sodium pump (Thomas, 1972). Our results concerning the

CONTROL



OUABAIN $2 \times 10^{-4} \text{M}$

15'



WASH

10'

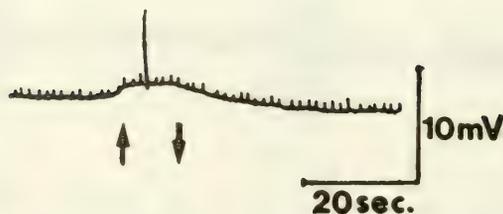
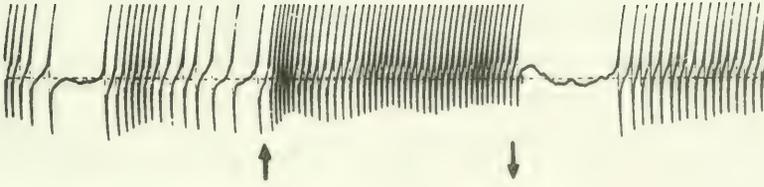
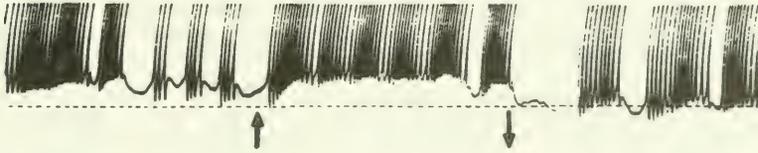
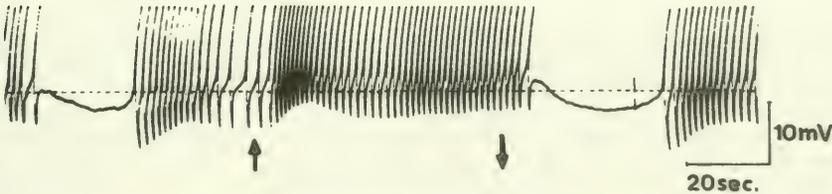


FIG. 9. Effect of incubation in ouabain on light evoked depolarization.

CONTROL

No Ca^{++} 17'

WASH 15'

FIG. 10. Light-evoked depolarization in Ca^{++} free medium.

effects of K^+ free solution on LED and hyperpolarization after illumination are, however, not completely unambiguous. Although it could be observed that the shift to hyperpolarization during illumination and after it was diminished in K^+ free solution and although in 2 experiments we could not observe its enhancement with repeatedly induced light, the fact that postillumination hyperpolarization still occurs in K^+ free medium points to the possibility that an increment of potassium conductance after illumination can also occur and account for membrane hyperpolarization. It was indeed proved in *Aplysia* giant neurons that a post-stimulus hyperpolarization can be due to a slow potassium conductance increment (Brodwick & Junge, 1972). In some preliminary experiments we tested the membrane conductivity during and after illumination but no changes were observed. However, more experiments are needed to ascertain this finding.

The experiments where ouabain was applied to the cell support the view that the electrogenic sodium pump may be activated during illumination. The preliminary observations made concerning the effects of cooling of the ganglia also point to such conclusions. It was observed that a prolonged incubation at a low temperature (e.g. 6°C) tended to enhance the hyperpolarization after illumination and also that it was decreased when the ganglia were cooled to 10°C . However, more experiments are needed to really confirm this observation.

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THE INFLUX OF TRYPTAMINE INTO SNAIL (*HELIX POMATIA*) GANGLIA:
COMPARISON WITH 5-HYDROXYTRYPTAMINE

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ABSTRACT

Isolated ganglia possess the ability to concentrate tryptamine from an external medium by a process which is temperature sensitive and independent of sodium and other cations. Kinetic analysis of the accumulation process showed the influx of tryptamine to be a single mechanism with K_m and V_{max} values of $1.4 \times 10^{-4}M$ and 5×10^{-8} mole/g/min. The influx of tryptamine is an unspecific process and is insensitive to a number of metabolic inhibitors and various analogues. The process of tryptamine influx is thus similar in principle to the low affinity uptake mechanism for 5-HT, thus demonstrating indirectly the specificity for the high affinity uptake mechanism for 5-HT (see Osborne et al., 1975). The present data, which include some experiments on the release of 5-HT and tryptamine, are discussed from the point of view of a functional role for 5-HT and tryptamine in the snail CNS.

INTRODUCTION

Recently the presence of tryptamine has been established in bovine and rat brain (Martin et al., 1971; Saavedra & Axelrod, 1972; Snodgrass & Horn, 1973; Osborne & Neuhoff, 1973; Philipps et al., 1974; Sloan et al., 1975), where the concentrations are generally very low. The level of tryptamine in nervous tissue of invertebrates is also generally very low with the exception of the echinoderms (Juorio & Robertson, 1977). Among the molluscs, the concentration of tryptamine in *Helix* nervous tissue is between 0.2-1.5 nmol/g (Wu & Juorio, 1975; Osborne & Neuhoff, 1973) and recent experiments have shown the amine to be formed in vitro from tryptophan (Cardot, 1974). The hydroxylated metabolite of tryptamine, 5-hydroxytryptamine (or serotonin), also occurs in the snail CNS but in a concentration at least 50-fold greater. Moreover, impressive evidence is available suggesting that 5-hydroxytryptamine (5-HT) functions as a neurotransmitter in the molluscs (Gerschenfeld, 1973; Cottrell & Macon, 1974; Osborne & Neuhoff, 1974; Osborne, 1974). In contrast, nothing is known about the functional significance of tryptamine in the snail CNS (see Robertson & Juorio, 1976).

The present work is concerned with the influx of tryptamine into snail ganglia and in particular the possible existence of a specific high affinity uptake mechanism. Specific high affinity uptake systems have been demonstrated for several transmitter substances in nervous tissue where they are thought to represent a mechanism for the inactivation of the released substance (see e.g. Iversen, 1970, 1971). Since a high affinity uptake system for 5-HT in *Helix* CNS has already been demonstrated and partly characterised (Osborne et al., 1975; Osborne & Neuhoff, 1977; Stahl et al., 1977), a comparison between the influx of tryptamine and 5-HT is discussed.

MATERIALS AND METHODS

Helix pomatia were obtained from Robert Stein, Donau, Germany, and kept at room temperature in a moist atmosphere for 24 hours before use. Suboesophageal ganglia from a number of animals were rapidly dissected and placed in a beaker containing cold snail saline. The snail saline (Meng, 1960) consisted of NaCl (3.45 g/l), KCl (0.43 g/l), CaCl₂ (1.17 g/l),

NaHCO_3 (1.0 g/l) and MgCl_2 (1.55 g/l). Each ganglion was subsequently blotted dry on filter paper, weighed (4-6 mg) and placed in a vial containing 2 ml ice-cold snail saline. After a preincubation for 15 minutes at 25°C in a shaking water bath, various amounts of ^3H -tryptamine (Radiochemical, Amersham 1 Ci/mmol) were added to the incubation medium and the incubation continued for varying periods of time. At the end of the incubation the ganglia were recovered with forceps and rinsed twice in 20 ml ice-cold saline. It was previously established that no significant release of radioactivity from the tissue occurred during the washing process. Individual ganglia were then placed in vials containing 0.5 ml tissue solubilizer (Soluene-350, Packard). Radioactivity was measured in a Packard Liquid Scintillation Spectrometer. A small amount (100 μl) of radioactive incubation mixture dissolved in 10 ml Dimilume was also counted. The counting efficiency was monitored by internal standards of ^3H -toluene and the appropriate correction applied. The counting efficiency of tritium was about 25%. Tissue/medium (T/M) ratios were calculated as cpm in 1 g tissue per cpm in 1 ml medium. The amount of amine accumulated by the tissues was calculated from values obtained from the T/M ratios at 0°C and 25°C (Shaskan & Snyder, 1970). Unlabelled tryptamine was added to the labelled tryptamine to produce the solutions containing higher concentrations of amine required for the kinetic studies. Kinetic constants were determined by computer, where an iterative method was used to fit the data directly into rate equations for a two or a single carrier model.

Ganglia were also incubated in ^{14}C -tryptamine for 25 min. at 25°C , the tissue homogenised in 0.01 M HCl/acetone (1:1 by vol.) and the supernatants, after centrifugation at 1500 g, applied to Silica precoated plates (Merck). Small amounts of carrier tryptamine were added to chromatograms which were developed an ascending way using either n-butanol/pyridine/acetic acid/water (15:2:3:5 by vol.), n-butanol/acetic acid/water (12:3:5 by vol.) or methyl acetate/isopropanol/ NH_3 25% (45:35:20 by vol.). Tryptamine was identified by spraying the chromatograms with ninhydrin solution. Autoradiograms from the chromatograms were subsequently prepared, the ^{14}C -tryptamine and ^{14}C -metabolite spots eluted from the silica with methanol/water (1:1 by vol.), and the eluate dried and counted in a scintillation spectrometer. From the results the percentage of ^{14}C -tryptamine metabolised was calculated.

In order to study the efflux of radioactivity from nervous tissue, single ganglia were incubated in 2 ml snail solution containing 10^{-7}M ^3H -tryptamine for 25 min. at 25°C . The ganglia were recovered, rinsed rapidly and then transferred to vials each containing 1 ml snail saline for a definite length of time. The 1 ml saline samples were then placed in Quicksint²⁹⁴ solution (Koch Light) and the radioactivity counted.

Adenosine triphosphate (ATP) was assayed in tissues using luciferase and a liquid scintillation spectrometer as described by Stanley & Williams (1969).

RESULTS

Time course of ^3H -tryptamine accumulation

The time course of ^3H -tryptamine accumulation in snail ganglia incubated with 10^{-7}M radioactive amine at 25°C is illustrated in Fig. 1. There is an initial main phase of accumulation (0-40 min.) followed by a slower phase (40-50 min.). The maximum accumulation of radioactivity in the tissue occurred after 40 min. when a T/M ratio of about 19 was achieved. The extracellular space of the tissue was determined by incubating ganglia for various periods with ^3H -inulin. After 30 min. a T/M ratio of 0.5 ± 0.05 (mean \pm S.E.M., $n = 4$) was obtained. It would therefore appear that the amount of ^3H -tryptamine accumulated into the extracellular space is small compared with the total accumulation at 25°C . The results presented in this paper have therefore not been corrected for the radioactivity accumulated into the extracellular space. The accumulation of ^3H -tryptamine at 35°C is greater than at 25°C (see Fig. 1) and again 2 distinct phases can be resolved. However, at 0°C the accumulation is markedly reduced in comparison with higher temperatures and is linear from 5-50 minutes.

In order to achieve maximal accumulation of ^3H -tryptamine, normal air is sufficient. Bubbling normal air (approx. 98% nitrogen) through the incubation medium has little influence, whereas bubbling a mixture of O_2/CO_2 (95:5 by vol.) reduced the influx of ^3H -tryptamine

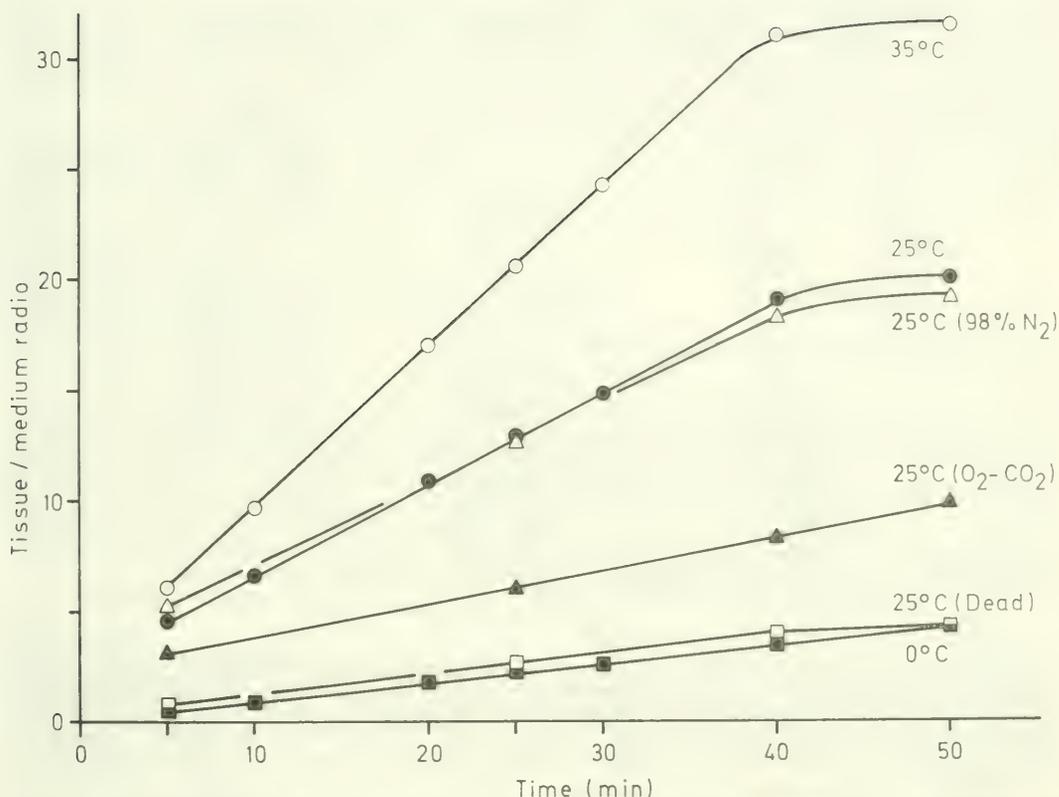


FIG. 1. Time course for the accumulation of ^3H -tryptamine by subesophageal ganglia of *Helix pomatia* at varying temperatures and conditions. Incubation media contained 10^{-7}M ^3H -tryptamine. Values are means of at least 6 determinations. For radio read ratio.

drastically (see Fig. 1). It can also be seen that the accumulation of ^3H -tryptamine by dead tissue (ganglia boiled for one minute) parallels the accumulation process observed at 0°C , suggesting that the influx of amine at 0°C is a purely diffusible component.

Kinetics of ^3H -tryptamine accumulation

Ganglia were incubated either at 0°C or 25°C for 25 min. with ^3H -tryptamine at 14 different concentrations of between 0.07 and $5000\ \mu\text{M}$. The accumulation of ^3H -tryptamine at 0°C was considered to be a linear diffusible component and this was therefore subtracted from the values obtained at 25°C . As can be seen from Table 1, although the T/M ratios for tryptamine are high, they do not saturate except at extreme concentrations of amine in the incubation medium. This contrasts strongly with the accumulation of 5-HT where the T/M ratios are also high for low amounts of amine in the incubation medium but decrease with increasing amine concentrations, showing that a saturating process occurs. In order to determine the kinetic parameters for ^3H -tryptamine accumulation, an iterative method was then used to fit the corrected data at 25°C (see Table 2) directly with rate equations, using a digital computer. Models consisting of single or 2 carrier systems were examined. Assessed in terms of goodness of fit of data to the iterated model parameters, a single carrier model was preferable (see Table 2) and the kinetic estimates were $K_m\ 1.4 \times 10^{-4}\text{M}$ and $V_{\max}\ 5 \times 10^{-8}\ \text{mole/g/min}$. The predicted values derived with these parameters are listed in Table 2.

TABLE 1. T/M ratios for ^3H -tryptamine and ^3H -5-HT accumulation into the suboesophageal ganglia over a wide range of amine concentration at 25°C .

Amine concentration (μM)	Tryptamine	5-HT*
0.01	—	30.30 ± 1.62
0.02	—	26.58 ± 1.64
0.05	—	21.90 ± 1.66
0.07	15.04 ± 2.69	—
0.10	—	17.30 ± 1.89
0.17	12.38 ± 0.34	—
0.20	—	12.38 ± 1.58
0.50	11.99 ± 1.26	12.55 ± 1.09
1.0	11.99 ± 0.54	9.66 ± 0.47
2.0	—	8.97 ± 0.71
5.0	11.50 ± 1.15	8.22 ± 0.78
10.0	10.71 ± 1.07	7.23 ± 1.28
50.0	10.56 ± 0.77	5.35 ± 0.76
100.0	8.84 ± 0.36	3.57 ± 0.12
200.0	6.71 ± 1.16	—
300.0	6.59 ± 0.12	—
500.0	4.26 ± 0.31	—
1000.0	3.09 ± 0.21	—
2000.0	2.07 ± 0.05	—
5000.0	1.66 ± 0.17	—

*Data taken from Osborne et al. (1975).

TABLE 2. Tryptamine accumulation by snail ganglia *in vitro*. Ganglia were preincubated at 37°C for 15 min and then incubated at either 25°C or 0°C for a further 25 min with radioactive tryptamine at the concentrations shown. The influx of tryptamine was then calculated (observed values) by subtracting the values at 0°C from that at 25°C . Each experimentally determined value is the mean \pm S.E.M. for 6-13 estimations. The predicted values are those for a single carrier system with the following kinetic parameters: $K_m 1.4 \times 10^{-4}\text{M}$ and $V_{max} 5 \times 10^{-8}$ mole/g/min.

Exogenous ^3H -tryptamine (μM)	^3H -tryptamine accumulation into ganglia (nmol/g wet weight/min)	
	Observed	Predicted
0.07	0.034 ± 0.001	0.02
0.5	0.25 ± 0.02	0.17
1.0	0.46 ± 0.01	0.35
5.0	1.94 ± 0.18	1.73
10.0	3.40 ± 0.12	3.35
20.0	5.80 ± 0.30	6.28
50.0	11.79 ± 1.79	13.22
100.0	18.28 ± 0.66	20.91
200.0	31.52 ± 1.74	29.47
300.0	44.32 ± 1.41	34.13
500.0	51.32 ± 3.50	39.08
1000.0	57.31 ± 3.86	43.84
2000.0	57.99 ± 9.02	46.69
5000.0	66.91 ± 2.33	48.58

The effect of different ions on tryptamine accumulation

The effect of cations and anions on the accumulation of ^3H -tryptamine is shown in Table 3. It can be seen that none of the anions influences the accumulation process while of the cations Cl^- slightly stimulates the accumulation process. The insensitivity of ^3H -tryptamine accumulation to sodium is particularly interesting since it contrasts with the uptake process of 5-HT (see Osborne et al., 1975), where sodium is of prime importance.

TABLE 3. Effect of anions and cations on ³H-tryptamine accumulation at 25°C (in percentage of control). Sucrose was substituted for the cations omitted from the incubation medium. The composition of the incubation medium is described in the methods. The concentration of ³H-tryptamine in the incubation medium was 7 × 10⁻⁸ M.

Incubation medium	³ H-tryptamine accumulation
Normal incubation medium	100%
Na ⁺ free	101%
K ⁺ free	98%
Mg ⁺⁺ free	94%
Ca ⁺⁺ free	95, 6%
NaCl substituted by NaBr	109, 6%
NaCl substituted by NaI	114, 7%
NaCl substituted by NaHCO ₃	90, 1%
NaCl	120%

} Incubation medium lacked CaCl₂

TABLE 4. Effect on various inhibitors on the cellular ATP concentration and tryptamine influx into snail ganglia. The incubation medium was as described in the methods. Cellular ATP concentrations were analysed after giving the ganglia an incubation for 25 min at 25°C in incubation media alone or with one of the inhibitors. To study the influx of ³H-tryptamine (7 × 10⁻⁸ M), radioactive amine was then added and the accumulation measured over a further period of 25 min. Control ATP (100%) concentration in ganglia was approx. 1 nmol ATP/mg wet weight. Control ³H-tryptamine influx (100%) was 0.034 nmol/g tissue/min.

External medium	Cellular ATP concentration (% of control)	Tryptamine influx (% of control)
Normal medium	100%	100%
+ 1 mM sodium fluoroacetate	100%	100%
+ 50 mM sodium arsenate	60%	100%
+ 10 mM sodium fluoride	100%	100%
+ 1 mM iodoacetamide	100%	100%
+ 10 mM sodium iodide	100%	100%
+ 2 mM sodium cyanide	50%	100%
+ 10 mM 2-deoxy-D-glucose	30%	100%
+ 10 mM 2, 4-dinitrophenol	15%	100%
+ 2 mM sodium azide	100%	100%
+ 0.1 mM probenecid	—	100%
+ 0.1 mM p-hydroxymercuribenzoate	—	100%
+ 0.1 mM pargyline	—	100%
+ 0.1 mM c-AMP	—	100%

The effect of pharmacological agents and ATP on tryptamine accumulation

The fact that the accumulation of tryptamine into snail ganglia does not require sodium, would suggest that the sodium pump mechanism is not directly involved as an energy source for the accumulation process. An alternative energy source may be ATP and this possibility was therefore investigated. As can be seen in Table 4, of 9 different agents tested, only 2,4-dinitrophenol, 2-deoxy-D-glucose, NaCN, and Na₂HAsO₄ had the effect of decreasing the endogenous ATP level in the snail CNS. However tissues with reduced endogenous ATP levels (achieved by incubating ganglia with the appropriate pharmacological agents) still had the normal capacity for accumulating radioactive tryptamine, as shown in Table 4. Indeed none of the agents tested, which included probenecid, p-hydroxymercuribenzoate, pargyline and c-AMP, had the slightest effect (see Table 4).

Influence of analogues on tryptamine accumulation

The effect of a number of analogues on tryptamine accumulation is shown in Table 5. Of the substances tested, all had a mild inhibition effect on the ³H-tryptamine accumulation,

showing that none of the substances competes with ^3H -tryptamine influx. Since the analogue structures are very similar to those of tryptamine, the results suggest that either the influx of ^3H -tryptamine is absolutely specific for this molecule, which is unlikely, or that the accumulation process is unspecific. The latter idea is supported by the fact that 10^{-4}M tryptamine only inhibits the accumulation of 10^{-7}M ^3H -tryptamine by 50%.

Table 5 also shows the effect of the analogues upon 5-HT accumulation. Here it can be seen that the uptake of 5-HT is drastically inhibited by tryptamine and to some extent 5-hydroxyindole too, demonstrating that the influx process for 5-HT is determined by the specificity of the molecule's structure. This would appear not to be the case for tryptamine.

Metabolism of accumulated tryptamine

Chromatographic analysis of ganglia incubated for 25 min. in the presence of 10^{-4}M ^{14}C -tryptamine showed that approx. 70% of the tryptamine remained unmetabolised or incorporated into the tissue. Three chromatographic systems were used and in each instance the accumulated ^{14}C -tryptamine was shown by autoradiographical means to be metabolised into at least 2 major substances (see Fig. 2).

Efflux of radioactivity from ganglia following loading with ^3H -tryptamine

Fig. 3 illustrates the efflux of radioactivity from ganglia which had previously been incubated with 10^{-7}M of either ^3H -tryptamine or ^3H -5-HT for 25 min and then transferred to

TABLE 5. Influence of different analogues (10^{-4}M) on the influx of $7 \times 10^{-8}\text{M}$ ^3H -tryptamine and 10^{-7}M ^3H -5-HT. Results expressed in percentage of control, and are the means of at least 6 independent determinations. The ganglia were always given a preincubation of 15 min with the analogue before adding the radioactive amine, the influx of which was then measured over a further 25 min.

	Tryptamine	5-HT
Tryptophan	85.45	—
5-Hydroxyindole acetic acid	76.69	76.5
Melatonin	84.62	78.1
N-Acetylserotonin	78.92	72.1
5-Hydroxyindole	104.79	58.2
Tryptamine	50.16	35.0
5-HT	90.0	—

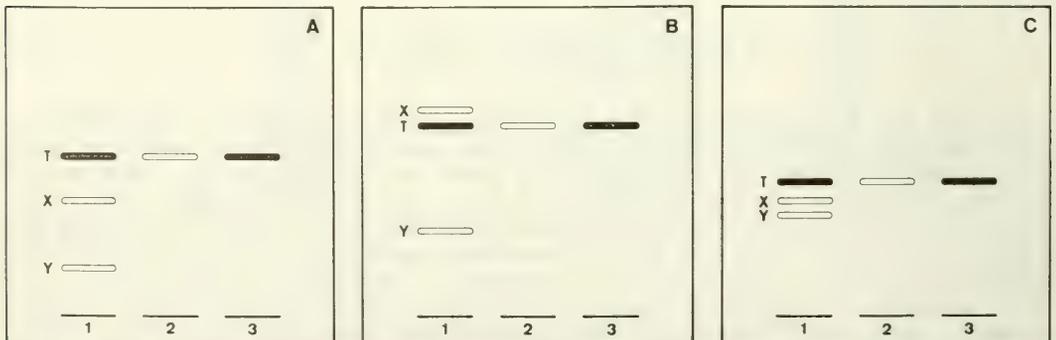


FIG. 2. Diagram showing the chromatographic separation of ^{14}C -tryptamine metabolites using 3 different solvent systems. Tissues were incubated with ^{14}C -tryptamine and thereafter the extracts applied to chromatograms as described in the text. The radioactive tryptamine (T) is metabolised by tissues into at least 2 other substances (x and y). Three chromatographic systems were used: A = n-butanol/pyridine/acetic acid/water (15:2:3:5 by vol.), B = n-butanol/acetic acid/water (12:3:5 by vol.), and C = methyl acetate/isopropanol/ 25% NH_3 (45:35:20 by vol.). In each chromatogram 1 = tissue sample incubated with ^{14}C -tryptamine, 2 = dead (boiled) tissue sample incubated with ^{14}C -tryptamine and 3 = pure radioactive ^{14}C -tryptamine.

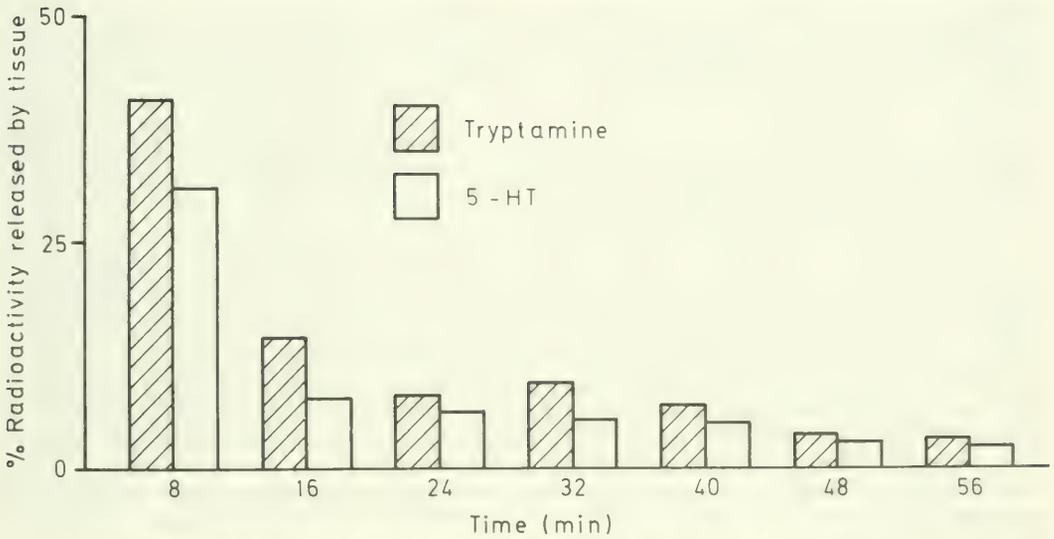


FIG. 3. Efflux of radioactivity from ganglia previously incubated with $10^{-7}M$ 3H -tryptamine or 3H -5-HT for 25 min. at $25^{\circ}C$ (see text for further details). It can be seen that the profile for release of both substances is similar, although the amount of tryptamine released is proportionally very much more.

fresh medium. There was an initial fairly rapid loss of radioactivity in each instance, in the first 8 min 40% for tryptamine and 30% for 5-HT. This was followed by a slower efflux during the next 48 min; a further 48% of radioactivity was released for tryptamine and only 30% for 5-HT.

DISCUSSION

The present results demonstrate that snail ganglia possess the ability to concentrate tryptamine from an external medium by a process which is saturable at very high concentrations of amine in the medium (see Table 1) and temperature sensitive, thus able to produce a net uptake of tryptamine. Kinetic analysis of the influx of tryptamine shows that the amine is taken up by a single transport system with a K_m value of $1.4 \times 10^{-4}M$. The influx of tryptamine into snail ganglia is thus fundamentally different from that of its hydroxylated analogue 5-HT, where the accumulation shows rapid saturation kinetics (see Table 1), is sodium dependent, slightly inhibited by ouabain and also temperature sensitive (Osborne et al., 1975). Kinetics of 5-HT uptake show the influx to be divided into a high affinity mechanism with a K_{mH} value of $8.5 \times 10^{-8}M$ (sodium sensitive component) and a low affinity mechanism with a K_{mL} value of $1.8 \times 10^{-4}M$ (sodium insensitive component) (Osborne et al., 1975). The influx of tryptamine is thus, in principle, similar in character to the low affinity uptake mechanism for 5-HT, i.e. sodium insensitive with relatively high K_m value. The difference between the K_m value for tryptamine (computer determined) and the low affinity system for 5-HT may be of no significance since the kinetic constants for 5-HT uptake were determined through a Lineweaver-Burk plot where the curves were fitted by eye (see Osborne et al., 1975). Further evidence that the tryptamine influx mechanism is similar in character to a low affinity uptake system is shown by the fact that the tryptamine influx is not influenced by a number of pharmacological agents, analogues of tryptamine and ATP. Low affinity uptake mechanisms show similar characteristics to this (see e.g. Evans, 1973; Osborne, 1976), while characterised high affinity uptake mechanisms are very sensitive to pharmacological agents (e.g. for 5-HT: chlorimipramine, imipramine), analogues (e.g. for 5-HT: tryptamine) and a variety of other substances (see Osborne et al., 1975; Stahl et al., 1976; Osborne & Neuhoff, 1977).

A low affinity uptake mechanism is generally considered to be an unspecific process (see

Iversen, 1970, 1971) due to various factors, such as passive accumulation, unspecific binding or incorporation into the tissue, while a high affinity uptake mechanism is a highly specific process (see Introduction). The present data thus argue in favour of the idea that tryptamine influx into snail ganglia is not a specific process, suggesting that should tryptamine be a transmitter, it is inactivated by some other means. It also supports the idea that tryptamine does not have a transmitter role in the gastropod CNS, where the evidence for tryptamine having such a function is nonexistent (see Introduction). It may well be that the small amounts of tryptamine in the gastropod CNS are the result of metabolic accidents or minor by-products of more important biochemical pathways.

Taken as a whole the present results, together with our knowledge on the uptake of 5-HT (see Osborne et al., 1975; Stahl et al., 1976; Osborne & Neuhoff, 1977), demonstrate the specificity of the high affinity uptake mechanism for 5-HT and support indirectly the idea that 5-HT is a neurotransmitter in the gastropod CNS. Further support for this is shown by the results from the release experiments where it can be seen that the spontaneous release of tryptamine is more rapid than 5-HT, indicating that the latter substance is more firmly bound. This is what one would expect from a transmitter, as these substances are primarily stored in vesicles which, within this form, can then participate in the release mechanism (see Axelrod, 1974; Krnjević, 1974). However, the release experiments represent an initial study and too much emphasis should not be placed on them, since some of the accumulated tryptamine is metabolised. Clearly this has to be given high consideration in the interpretation of the data from these experiments.

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CYTOLOGICAL ASPECTS OF DIFFERENT NERVE CELL SOMATA IN THE BUCCAL GANGLIA OF *HELIX POMATIA* L.

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ABSTRACT

In early September most of the neurons of the buccal ganglia of *Helix pomatia* contain neurosecretory material as membrane bound granules. There is only one, in exceptional cases 2 types of granules per cell. This suggests that different types of granules do not change into one another, and that each granule type contains a different secretory product. One granule type contains PAF-positive neurosecretory material, another one catecholamines, but most of the granules cannot be associated with special substances. The identified giant neurons B1-B4 contain granules in less density than the smaller neurons. B1 and B2 resemble each other in their granule type, whereas both B3 and B4 differ from B1 and B2.

The main subjects of this paper are the secretory granules of the somata of nerve cells from a few buccal ganglia of *Helix pomatia*, that were fixed in the first days of September. As shape and size of the secretory granules are the most striking characteristics of the neurons of these specimens, the granules shall be described and compared with one another. It should be pointed out, that in buccal ganglia fixed in different seasons, e.g. at hibernation, there are completely different cytological aspects to be found in the neurons (Steffens, 1978). By cutting alternating semithin and ultrathin sections through the whole ganglion it was tried to identify special neurons and groups of neurons both light and electron microscopically. Fixation was carried out in 2.5% glutaraldehyde in cacodylate buffer (pH 7.2), postfixation in 2% OsO₄ in cacodylate buffer.

The buccal ganglia of *Helix pomatia* contain neurons of various sizes. Four somata (B1-B4, Fig. 1; nomenclature after Schulze et al., 1975) reach diameters from 120-170 μm , while all other somata are 10 to 90 μm in size. Three of the giant neurons, B1-B3, were already and are still test specimens of neurophysiologists (Schultze et al., 1975; Altrup, Speckmann & Caspers, 1979; Zidek & Speckmann, 1979).

Most surprising was the fact that nearly all somata contain membrane-bound granules of different appearance in high amounts. But each soma contains only one or rarely 2 types of granules.

In an area near the posterior gastric nerve (Fig. 1, ngp) there is a peptidergic neurosecretory cell group of about 10 small somata with diameters from 20-30 μm . This cluster of cells is stainable with paraldehyde fuchsin (PAF) in both paraffin and semithin sections. Therefore it is possible to identify the PAF-positive material directly in ultrathin sections subsequent to semithin sections (Fig. 2a,b). The cells contain large amounts of neurosecretory granules (elementary granules with diameters ranging from 1500-2200 Å (Fig. 2c). The membrane of the granules is adjoining the electron dense contents.

Peptidergic neurosecretory cells and cell groups are components of many ganglia of gastropods (see reviews by Martoja, 1972; Boer & Joosse, 1975). In the buccal ganglia of different stylommatophores, e.g. of *Arion rufus* and *Arion subfuscus* (Herlant-Meewis & Van Mol, 1959; Van Mol, 1962), *Arion ater* (cf. Smith, 1967) and *Succinea putris* (cf. Cook, 1966), they have also been found with light microscopical methods.

Spread over the buccal ganglia there are neurons similar to the PAF-positive cells. They have the same size and contain large amounts of granules, which are to be distinguished from the PAF-positive granules at high enlargements, and which show a membrane detached from the

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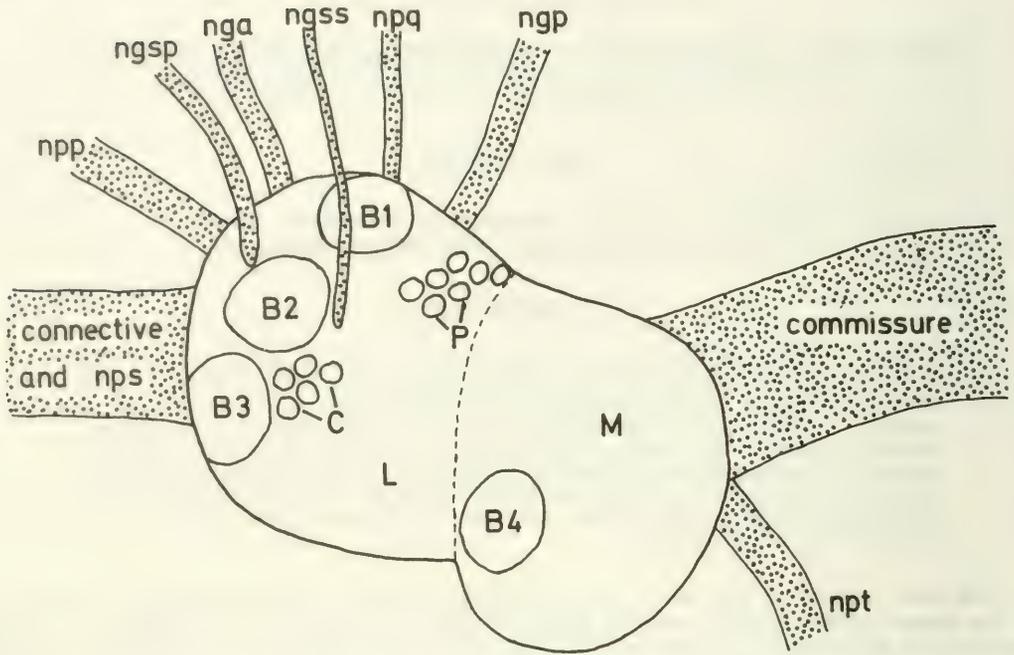


FIG. 1. Schematic drawing of the left buccal ganglion of *Helix pomatia* showing the positions of the giant neurons and 2 cell groups. B1-B4 = giant neurons; C = catecholamine containing cells; L = lateral lobe, M = medial lobe of the ganglion; P = PAF-positive cells; npp = 1st, nps = 2nd, npt = 3rd, npq = 4th pharyngeal nerve; nga = anterior, ngp = posterior gastric nerve; ngsp = first, ngss = second salivary gland nerve.

electron dense contents (Fig. 3b). Discrimination of these cells from the PAF-positive cells is simple by comparison of ultrathin and PAF stained subsequent semithin sections.

There exists a cluster of about 8 catecholamine containing neurons localized between the giant neurons B1 and B2 and the neuropile (Fig. 1). Visualization was carried out by formaldehyde induced fluorescence after freeze drying (Fig. 4). The diameters of the somata are about $30\ \mu\text{m}$. Localization, size and number of cells suggest, that electron microscopically they are identical with some neurons, which contain granules with an electron dense core (Fig. 3c). The granules have diameters from $600\text{-}1000\ \text{\AA}$. They are affiliated to the range that was given by Pentreath & Cottrell (1974), for the granules of a giant dopamine-containing neuron of *Planorbarius corneus* ($500\text{-}2500\ \text{\AA}$). Serotonin-containing neurons, as they were found in some ganglia of the circumesophageal ring of *Helix pomatia* (Dahl et al., 1966), do not exist in the buccal ganglia.

There are other small neurons with great amounts of granules, which do not stain with PAF or contain fluorescent microscopically demonstrable biogenic amines. Particularly frequent are some neurons with somata of diameters up to $50\ \mu\text{m}$ spread over the lateral lobes of the buccal ganglia. They contain membrane bound granules with poor contrast and a halo (Fig. 3d). The granules have diameters ranging from $1250\text{-}1500\ \text{\AA}$. In other small somata one can find granules with a dense core and diameters from $1000\text{-}1250\ \text{\AA}$ (Fig. 3e).

As the giant neurons are easy to find it is simple to associate granules with distinct cells. The 4 giant neurons neither are PAF-positive nor do they contain fluorescent microscopically demonstrable biogenic amines. The giant neurons do not contain granules in such a high density as the smaller neurons do. B1, which is called the "anterior cell" by Cottrell (1971), contains predominantly oval membrane bound granules with diameters ranging from $1000\text{-}2500\ \text{\AA}$. The membrane adjoins the electron dense contents (Fig. 3f). The granules of B2 ("middle cell," Cottrell, 1971) resemble those of B1 so much, that it is impossible to distinguish them morphologically (Fig. 3g). The granules of B3 ("posterior cell," Cottrell, 1971) mostly are more

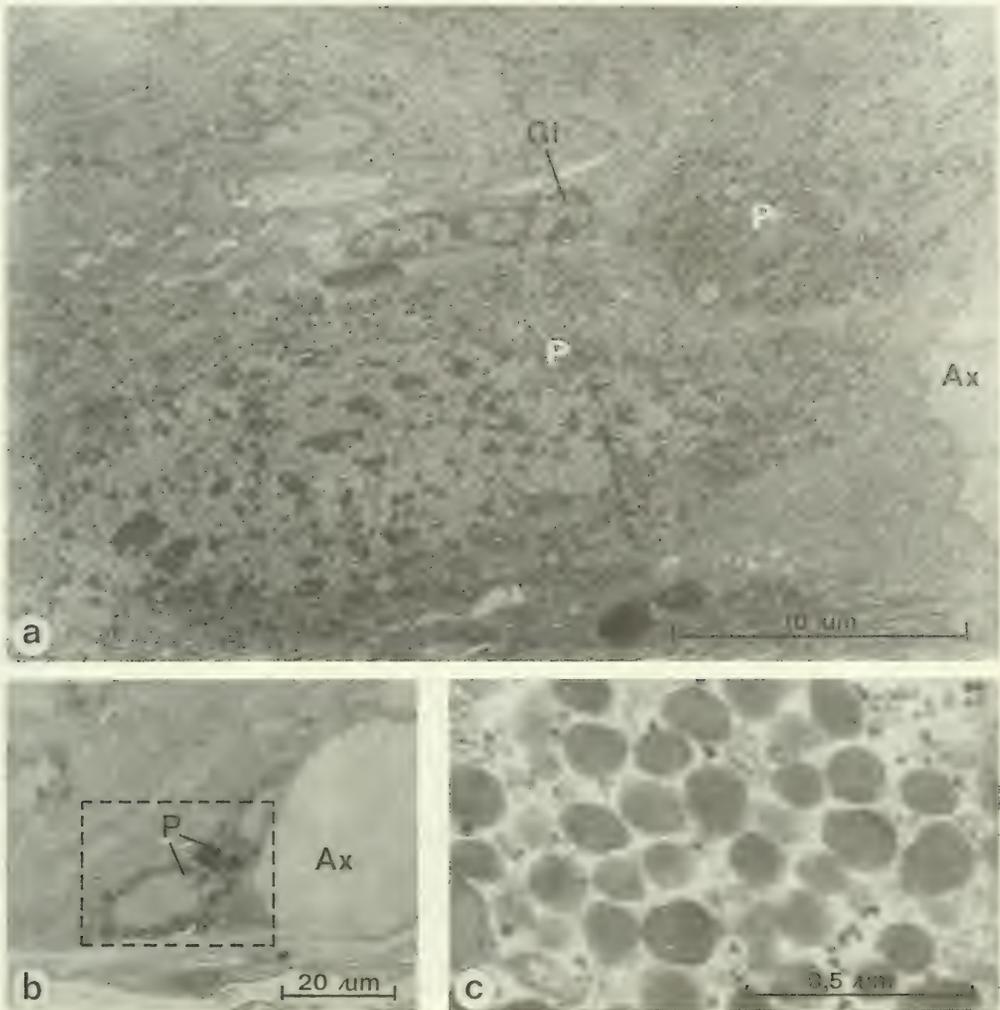


FIG. 2. PAF-positive neurons. a: low electron microscopical magnification showing a cut out of (b); b: semithin section stained with PAF for neurosecretory material; c: elementary granules of a PAF-positive neuron. Ax = axon; Gl = glial cell; P = PAF-positive cells.

or less circular. Two different types of granules are to be found in B3: one type with a weak electron dense core and the other with a strong electron dense core. Both types of granules have diameters ranging from 1500-2000 Å and they have a halo (Fig. 3h). The giant neuron B4, which has not been examined electrophysiologically until now, contains smaller granules with a tiny halo (Fig. 3i). The granules have electron dense contents and measure 1000-1300 Å. In the buccal ganglia there are some small neurons (diameter up to 35 μm), which have similar granules as B3, and there exist some medium large neurons (diameter up to 90 μm), which have similar granules as B4.

Obviously both B1 and B2 contain morphologically not distinguishable granules while B3 is different. In contrast B2 differs from B1 and B3 electrophysiologically. B1 and B3 are silent or show irregular spontaneous activity at resting membrane potential, whereas the neuron B2 tends to fire in repetitive bursts (Schulze et al., 1975).

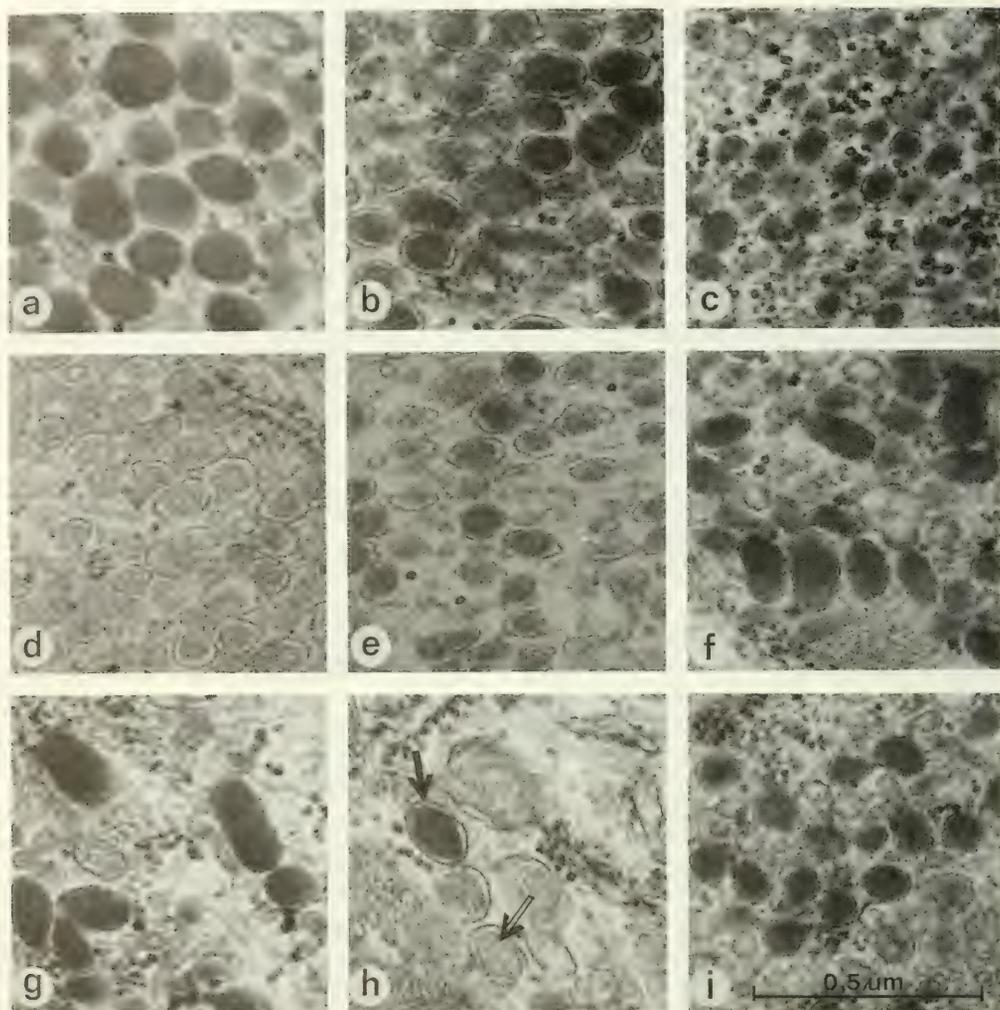


FIG. 3. Various granules of different neurons. a: PAF-positive, diam. 1500-2200 Å; b: dense contents, detached membrane, diam. 1500-2000 Å; c: possibly catecholamines, diam. 600-1000 Å; d: weak contrast core, diam. 1250-1500 Å; e: dense core, diam. 1000-1250 Å; f: granules of B1, diam. 1000-2500 Å; g: granules of B2, diam. 1000-2500 Å; h: granules of B3, weak (⇒) and high (↔) contrast cores, diam. 1500-2000 Å; i: granules of B4, diam. 1000-1300 Å.

The described variety of granule types, which occur isolated in different neurons and do not show any transitions, is faced by a variety of substances, which possibly work as transmitters or neurohormones in gastropods (Kerkut & Walker, 1975; Frontali & Gainer, 1977). So far an absolutely sure association of granules with these substances is possible only for some peptides and for some biogenic monoamines, especially if there are well located neurons, which contain these substances.

Not only the buccal ganglia seem to contain a great variety of granules, but also other ganglia of the gastropods. This was described for the circumesophageal ring of *Lymnaea stagnalis* (cf. Wendelaar Bonga, 1970) and *Bulinus truncatus* (cf. Boer et al., 1977). One can hardly compare the morphological results with one another, as the morphology of granules is dependent on fixation (Elekes, 1974).

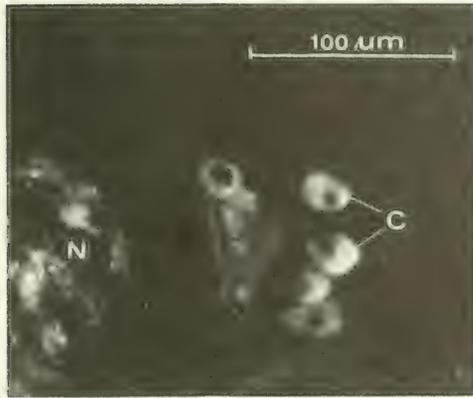


FIG. 4. Cluster of catecholamine containing cells (C), visualized by formaldehyde induced fluorescence; N = neuron.

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NEUROGENIC CONTRACTILE ACTIVITY OF THE PENIS RETRACTOR
MUSCLE OF *HELIX POMATIA* L.

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ABSTRACT

Using conventional mechanical and electromyographic recording methods 2 distinct types of neurogenically elicited activity can be observed in the penis retractor muscle (PRM) of *Helix pomatia*: (1) rhythmic, phasic contractions correlated with single or a few compound action potentials and (2) intervening, strong, prolonged contractions accompanied by sustained, high frequency electrical muscle activity. The 2 distinct types of muscle activity which seem to play a part in the normal behaviour of the PRM in the intact animal are mediated by both the central nervous system and peripheral neurons. While central neuronal structures are involved in causing the strong, prolonged contractions, the phasic activity is initiated by peripheral neuronal structures located at the proximal end of the PRM. There is evidence that the transmission of excitation at the neuromuscular level of central and peripheral origin is mediated by ACh.

INTRODUCTION

The nervous system of molluscs is known to possess in addition to the central nervous system (CNS), an extensive peripheral nerve net resembling the peripheral nervous supply of certain vertebrate viscera (Minker & Koltai, 1961). In molluscs not only normal functioning of the visceral motor system, but also the somatic motor system, involves neural control of both central ganglia and peripheral neurons. Studies of the somatic nervous supply revealed that in several molluscs, apart from direct effector innervation by centrally located neurons, central neurons may also rely on peripheral nervous sites which, in turn make direct connection to effectors (for review see Kandel, 1976). Following extirpation of central ganglia the residual peripheral nerve net remains capable of initiating and controlling a variety of effector activities, thought to be mediated by peripheral circuits of sensory neurons and motoneurons (Prior, 1972; Peretz, 1974). Due to the inaccessibility of peripheral neurons, work on these has been limited and their physiological role has remained largely obscure. The intention of the present paper is to present a molluscan visceral smooth muscle preparation supplied by a remarkably extensive peripheral nerve net, which offers the opportunity to relate effector activities with peripheral and/or central nervous input. The examined preparation is the penis retractor muscle (PRM), a thin (< 500 μ m) muscle strip of the penial apparatus of *Helix pomatia*.

RESULTS AND DISCUSSION

Central neurons sending off processes into the PRM nerve branches were located with the cobalt iontophoresis technique in the cerebral and pedal ganglia (Eberhardt, in preparation). Peripheral neurons connected to the nervous supply of the PRM could easily be distinguished by their large size (20-50 μ m) and round shape. Their cell bodies could be grouped into two types by virtue of location: (1) clusters of neurons scattered along the main branch of the extrinsic PRM nerve trunks, travelling in a thin and transparent septum (Fig. 1a) and (2) neurons that occur along intrinsic nerve branches within the muscle mass confined to the proximal end of the PRM (Fig. 1b). Because the living extrinsic neurons can be identified optically and the intrinsic neurons are characterized by their distinct location, the CNS-peripheral nervous system-PRM preparation provides an useful object for the study of contributions of central, peripheral extrinsic and peripheral intrinsic neurons on the PRM

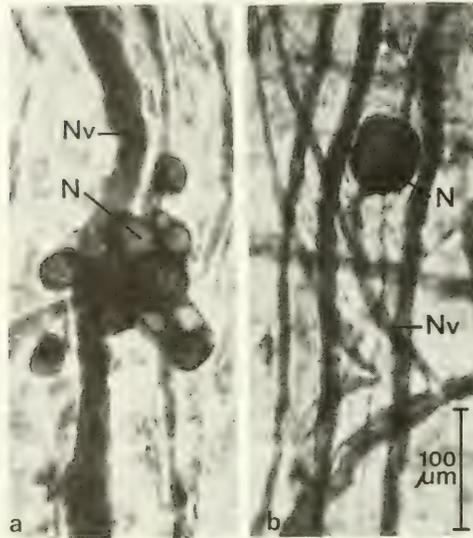


FIG. 1. (a) Cluster of peripheral extrinsic neurons (N) along a PRM nerve branch in the stretched out septum and (b) intrinsic neuron and nerve strands (Nv) at the proximal end of the PRM.

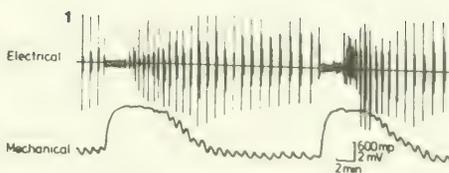
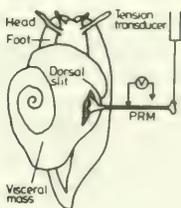
activity. The more so, because it has been proved possible to study normal functioning of the PRM in intact animals by means of long-term intracellular recordings from single muscle cells. To clarify the question to what extent the PRM is controlled by the CNS or by peripheral mechanisms 5 types of preparations at different stages of "intactness" using surgical or chemical isolation methods were examined: intact restrained animals in which spontaneous normal function of the PRM could be observed (Fig. 2A), semi-intact preparations consisting of the PRM, intrinsic and extrinsic nerve trunks and neurons but disconnected from the CNS (Fig. 2B), partially isolated PRM preparations that consisted of the PRM and the complete intrinsic nervous system including the intrinsic neurons at the proximal end but disconnected from the CNS and the extrinsic neurons (Fig. 2C), PRM preparations as in (C) but chemically denervated by high $MgCl_2$ (35 mM) and low $CaCl_2$ (0.7 mM) (Fig. 2D) and PRM preparations that consisted only of the PRM and peripheral nerve endings without any connection either to the intrinsic neurons or to extrinsic and centrally located neurons (Fig. 2E).

Using conventional mechanical and electromyographic recording methods (Wabnitz, 1975, 1976a) in the intact CNS-nerve net-PRM preparation (Fig. 2A) 2 distinct types of spontaneously occurring PRM activity were observed: rhythmic phasic contractions correlated with single or a few compound action potentials, and intervening, strong prolonged contractions accompanied by sustained, very frequent electrical muscle activity.

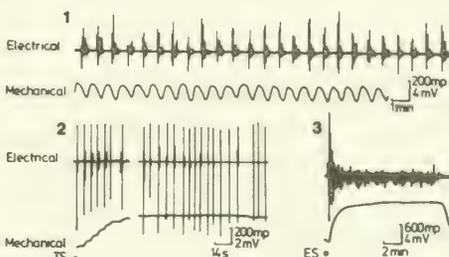
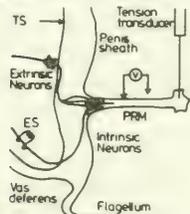
Surgical removal of the central ganglia eliminated the spontaneously occurring long duration activity. The twitch-like contractions, however, were unaffected by this surgical treatment. The functional differences between the intact and the semi-intact PRM suggest that in the absence of an obvious sensory input, central neuronal structures are involved in causing the long duration activity, while the remaining phasic rhythmicity of the PRM resides in the peripheral nervous structures. In addition to the latter activity in the semi-intact preparation, a tetanic muscle activity could be observed in response to tactile stimuli applied to the penis sheath (Fig. 2B2). The local reflex was found to be absent after removal of extrinsic neurons in preparation C (see Fig. 2) whereas the rhythmic activity still remains. Electrical stimulation of the extrinsic

FIG. 2. Diagram of the experimental arrangement for mechanical and electromyographic study of PRM preparations at different stages of isolation. Spontaneously occurring activity (1), response to tactile stimuli (TS) of the penis sheath (2) and response to electrical stimulation (ES) of extrinsic or intrinsic nerves.

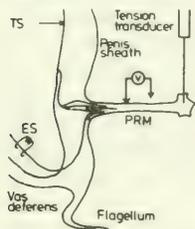
A INTACT PREPARATION



B SEMI-INTACT PREPARATION

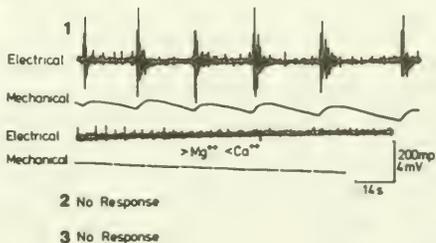
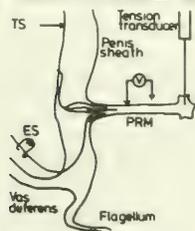


C PARTIALLY INTACT PREPARATION



- 1 Spontaneous Activity as in B 1
- 2 No Response
- 3 Responds as in B 3

D CHEMICALLY DENERVATED PRM



nerve branches in preparations (B) and (C) produced a sustained contraction, the contractile response characteristic of the intact PRM preparation.

This suggests first of all that the extrinsic neurons are involved in mediating a localized reflex and are not responsible for initiation of spontaneous rhythmic activity. Secondly, one may conclude from the muscle response to electrical stimulation that the long duration activity results from a direct link between central neurons and muscle cells or from central neurons relaying on to peripheral motoneurons, which in their turn elicit long duration activity. In the partially isolated PRM, the CNS mediated muscle activity could also be mimicked by exogenously applied adenosine triphosphate (ATP) (Wabnitz, 1976b). This observation together with the demonstration that ATP neither excites presynaptic motor nerve terminals nor postsynaptic sites at the neuromuscular junction, but exerts its action on peripheral structures, may indicate a link between CNS and the peripheral nervous system.

The remaining spontaneous activity in the partially isolated PRM preparation (Fig. 2C) may be of myogenic origin or due to repetitive activity of histologically identified intrinsic neurons. If the latter is the case the identified cells may be motoneurons directly innervating muscle fibres. The dozen or so large neurons at the base of the PRM embedded in tough connective tissue and muscle fibres have as yet resisted attempts to functionally identify them with electrophysiological methods. Therefore, to test the latter hypothesis, experiments were undertaken to disconnect the PRM from these cells by chemical denervation (D) or by surgical isolation (E). In order to block chemical synaptic transmission the PRM preparation (C) was bathed in solutions containing high Mg^{++} and low Ca^{++} . These solutions reversibly abolished synaptic transmission of any neural elements within the partially isolated PRM to muscle cells. After this treatment electrical stimulation of intrinsic nerves failed to elicit muscle activity, while, on the other hand, the nerve impulse conduction was found to be unaffected. Extirpation of the proximal muscle end (Fig. 2E) produced a complete and irreversible block of spontaneously occurring activity. The electrical stimulation of the nerve-junction preparation (Fig. 2E), however, still results in muscle contraction. Single pulse stimulation elicited single compound action potentials and twitch-like mechanical responses. Repetitively applied stimuli elicited additional spikes and a summation of the mechanical responses was observed. While the former response resembles the spontaneous activity of preparation C and D, the latter is comparable to the activity pattern of the reflective response to tactile stimuli.

Beyond that the results indicate the presence of synaptically mediated activity between a repetitive intrinsic motor innervation and PRM cells. The junctional transmission seemed to be chemically mediated.

In order to obtain further insight into the neuromuscular control exerted by central and peripheral motor elements on single PRM cells we performed an electrophysiological examination.

In the first stage it was possible to obtain long term intracellular recordings from muscle cells during the spontaneous neurogenic inherent PRM rhythmicity (Fig. 3a) as well as during the centrally initiated tonic contractions. This enables us to examine phenomena such as the mode and nature of the neuromuscular transmission. Intracellular recordings from impaled muscle cells of the partially isolated preparation (C) show spontaneous excitatory junction potentials (EJP's). When the intervals between the EJP's were short, the EJP's summed and also showed facilitation (Fig. 3b). It was found that spontaneous phasic PRM contraction resulted from the depolarization produced by summation and possible facilitation of EJP's combined with superimposed regenerative spikes. The rapid rise of the regenerative membrane potential never reaches zero potential and repolarization generally subsides in about 1 to 2 sec to subthreshold values, except when the muscle contracts tonically. In this case the repolarization passes into a plateau showing spike-like oscillations superimposed on the repolarization plateau. Applications of acetylcholine (ACh), even in the presence of high $MgCl_2$, was found to mimic the latter spontaneous activity of the intact PRM preparation. d-Tubocurarine (d-TC) in low concentrations ($< 10^{-4}M$) inhibited spike potentials and both phasic and tonic contractions, whereas the amplitudes of single EJP's and summed EJP's were only reduced as in the case of vertebrates end plate. In high concentrations of d-TC ($> 10^{-4}M$), however, the EJP's were abolished. High $MgCl$ and low $CaCl$ solutions were found to block EJP's and miniature end plate potentials.

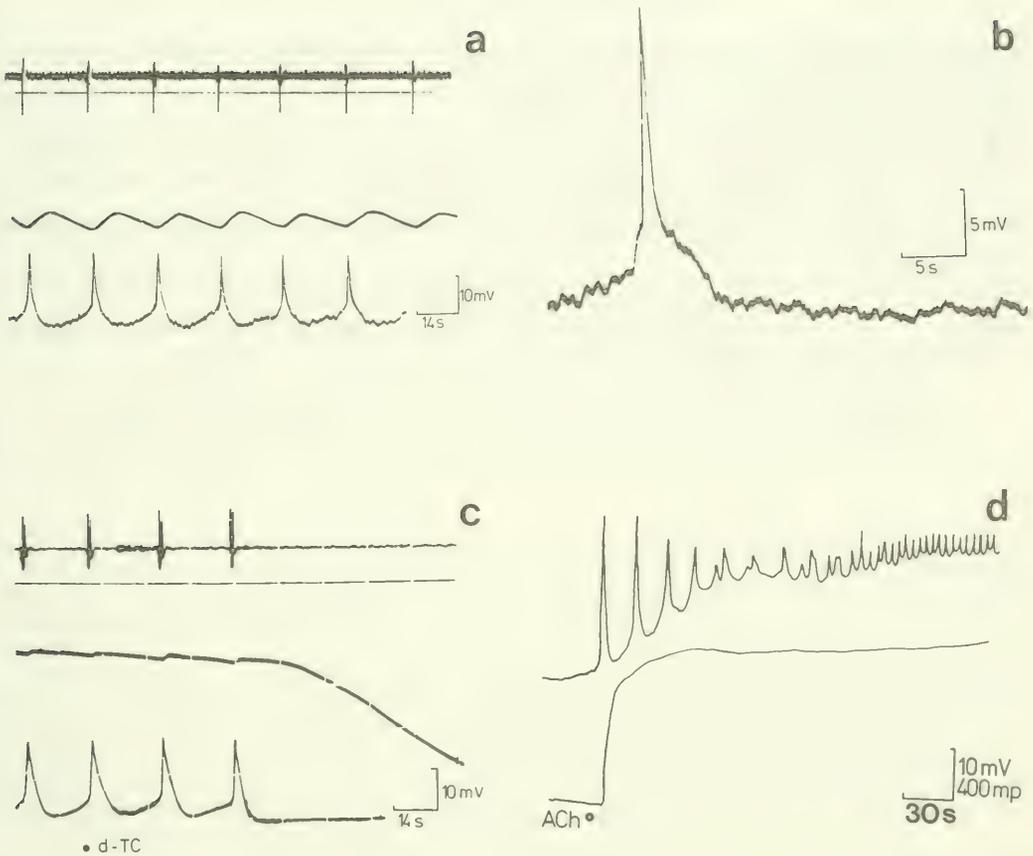


FIG. 3. (a) Simultaneous recording of PRM electromyogram (top), zero potential (2nd line), mechanical activity (3rd line) and membrane potential (bottom); (b) intracellular recording with 3M KCl electrode from PRM cell; (c) recording as in (a), dot indicates d-tubocurarine (d-TC) application; (d) intracellular recording (top) and correlated tension development after acetylcholine (ACh) application (bottom).

The data presented supplement previous neuroethological studies and neuromuscular system analysis of molluscs (Kandel, 1976; Heyer et al., 1973) and extend the following conclusions concerning the mechanisms controlling spontaneous activity in a molluscan visceral muscle:

(1) The spontaneous contractile activity in the PRM is of neurogenic origin. (2) Volleys of impulses of its nerve supply depolarize stepwise muscle membrane potential to the value of the threshold for spike generation. (3) Summation (and possible facilitation) of EJP's is an important factor to reach firing threshold, the latter is required for a regenerative non-over-shooting membrane response. (4) Spontaneous phasic PRM contraction is generally correlated to one spike potential. (5) In contrast tonic contraction is due to a rapid spike-like depolarization similar to (4) but followed by additional oscillatory depolarizations on the repolarization plateau. (6) The inherent PRM rhythmicity is due to the autoactive peripheral intrinsic nerve net. (7) The dozen or so large intrinsic neurons may be motoneurons directly innervating muscle fibers. (8) The excitatory neuromuscular transmission of both central and peripheral origin seemed to be cholinergic. (9) Central motor elements may rely on intrinsic nervous sites to modulate their endogenous activity pattern but this requires further investigations.

I thank H. H. Funk for excellent technical assistance.

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TEMPERATURE DEPENDENT MEMBRANE POTENTIAL CHANGES IN SNAIL NEURONS AND THEIR RELATION TO ACTIVE ION TRANSPORT

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ABSTRACT

The mechanisms underlying the temperature response of the resting membrane potential (RMP) were investigated in 3 identified neurons of the buccal ganglion of *Helix pomatia*. Lowering the temperature evoked a decrease of the RMP and an increase in membrane resistance, and vice versa. The temperature response of the RMP had an equilibrium potential of ca. -60 mV. It is essentially evoked by changes in the potassium conductance. Indications of an electrogenic sodium transport were not detected.

Previous investigations had shown that with normal resting membrane potential (RMP) single neurons of *Helix pomatia* depolarized if the temperature of the bath fluid was lowered. An increase of the temperature had the reverse effect (Schulze et al., 1975). The aim of the present investigations was to elucidate the basic mechanisms underlying the neuronal temperature response.

The experiments were carried out on the 3 identified neurons (B1-B3) of the buccal ganglion of *Helix pomatia* (Schulze et al., 1975). The buccal ganglia were isolated from the animals and fixed in an experimental chamber. The membrane potential of the neurons was recorded by conventional techniques. For measurement of the membrane resistance and for changing the RMP 2 separate microelectrodes were impaled to inject current and to record the bioelectric potentials independently. Intracellular injections of ions were performed by inter-barrel iontophoresis (Eccles et al., 1964).

It has already been mentioned that with lowering the temperature of the bath fluid the RMP decreased when its initial value ranged at normal (40-50 mV) levels (Fig. 1A). This temperature response can either be due to changes in the membrane conductance or to changes in an electrogenic ion transport or to both. In an initial series of experiments it was examined whether and to what extent the temperature response was evoked by conductance changes. In a first step a transient shift in temperature was performed at various levels of the initial RMP. As shown in Fig. 1A amplitude and polarity of the temperature response depended on the amount of the initial RMP. This relationship is shown in the evaluation of Fig. 1B which demonstrates that the equilibrium potential of the temperature response was in the range of -60 mV. In a second step the membrane resistance of the neurons was measured during temperature changes. Part C of Fig. 1 shows the membrane resistance displayed as a current voltage relation. It can be seen that the resistance increased with the delayed rectification simultaneously being reduced, when the temperature was changed from 22°C to 8°C . The described findings indicate that conductance changes contributed substantially to the temperature response.

Further experiments were designed to analyse the ion currents involved in the temperature reaction of the RMP. The equilibrium potential of about -60 mV already suggests that potassium and/or chloride currents may play a role. To clarify this question the potassium gradient was reversed by a 24 hours incubation in potassium free solution. After these procedures the temperature response was inverted in polarity when bath fluid with normal or elevated potassium concentrations was applied. Changing the chloride, sodium or calcium gradient across the neuronal membrane resulted in no substantial variations of the temperature response. From these findings it can be concluded that the temperature response of the RMP is essentially elicited by changes in the potassium conductance and that its equilibrium potential corresponds mainly with the potassium equilibrium potential.

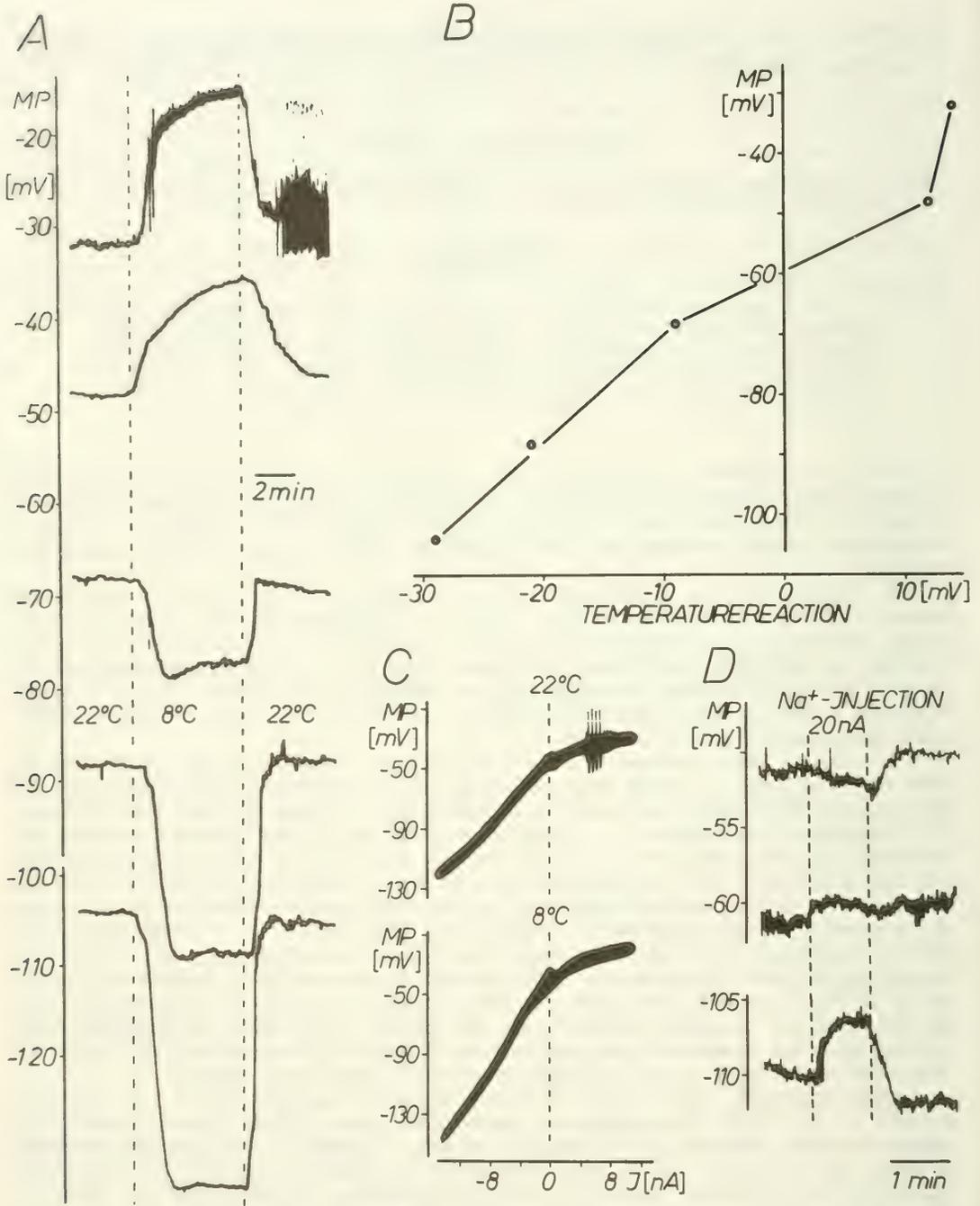


FIG. 1. Neuronal reactions to temperature changes and to intracellular sodium injection. A: Effect of temperature changes on the membrane potential (MP) at various initial MP levels. Neuron B3. B: Graphical evaluation of the experiment in A. C: Membrane resistance displayed as current-voltage relation (abscissa: current (I); ordinate: MP) at 22 and 8°C. Neuron B2. D: Effect of intracellular Na⁺ injections on the MP at various initial levels. Neuron B2.

In a final series of experiments it was tested whether and to what extent activity changes of an electrogenic sodium transport contribute to the temperature response of the RMP. A criterion for the existence of an electrogenic ion transport is most often assumed to be the rapid and distinct neuronal depolarization evoked by the application of metabolic inhibitors (cf. Kerkut & York, 1971). An admixture of ouabain or of cyanide was found to elicit a depolarization also in these neurons. However, this effect may be due to a depression of either an electrogenic or of an electroneutral pump. Using the potassium-dependent temperature response as an indicator, it could be shown that ouabain and cyanide decreased the potassium equilibrium potential by about 15-20 mV. Such a shift gives a sufficient explanation for the drug-induced depolarization. Therefore, the depolarization evoked by metabolic inhibitors need not without fail be attributed to the inhibition of an electrogenic pump. As to the electrogenic ion transport, it was examined furthermore whether the finding, that an intracellular injection of sodium ions induces a hyperpolarization, indicates the existence of such a transport mechanism in any case (cf. Thomas, 1972). Fig. 1D demonstrates that sodium injection evoked a hyperpolarization at normal RMP (-50 mV) also in these neurons. This effect was associated with a reduction of the membrane resistance and exhibited an equilibrium potential in the range of -60 mV (Fig. 1D). This equilibrium potential was shifted to lower levels after ouabain application. These experiments point to conductance changes as essential mechanism underlying the effect of intracellular sodium injection. As a whole, from the described results no direct evidence of electrogenic ion pumps could be detected in these neurons. Therefore, also no indication is apparent that the activity change of such a mechanism contributes to the temperature response.

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FORMATION DE LA COQUILLE DES MOLLUSQUES: LES PROBLÈMES POSÉS PAR LA PRÉSENCE ET LE COMPORTEMENT DE CELLULES LIBRES DANS LA COQUILLE NORMALE ET RÉGÉNÉRÉE CHEZ *AGRIOLIMAX RETICULATUS* (GASTÉROPODE PULMONÉ)

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ABSTRACT

The presence of cells on the inner surface of the normal shell of *Agriolimax reticulatus* again set the problem of their possible rôle in the shell-forming process. These cells synthesize and release on the inner surface of the shell a great number of minute organocalcic granules. The released granules show a continuous spherulitic growth by concentric precipitation from extrapallial fluid, to form an inner discontinuous shell layer which is interpreted as a reduced hypostracum. It is assumed that this spherulitic type of calcification is initiated by the activity of these cells which are always present, under normal conditions, at the shell level. The same cells are also found in great number in shell regenerates. Histological observations at the mantle level during the first stages of shell regeneration suggest that these cells arise from the mantle subepithelial connective tissue.

INTRODUCTION

Dans l'étude des mécanismes de formation de la coquille des mollusques, il ne fait aucun doute, à l'heure actuelle, que le manteau est responsable de l'élaboration des constituants fondamentaux de cette structure (Wilbur, 1972). Néanmoins, certains auteurs ont avancé l'hypothèse de l'intervention "in situ" de cellules libres, assimilées à des amœbocytes, dans l'apport des matériaux constitutifs de la coquille. La plupart des travaux portent sur l'étude de la régénération de la coquille après ablation d'un petit fragment de celle-ci et les interprétations données par les auteurs sont très divergentes. L'idée générale qui s'en dégage est que ces cellules n'ont qu'un caractère régénératif sans rapport avec les mécanismes morphogénétiques normaux. La coquille d'*Agriolimax reticulatus* s'est révélée comme un matériel particulièrement favorable (Fournié, 1976b). La Fig. 1 montre les relations anatomiques qui existent chez cet animal entre les divers compartiments concernés par le processus de formation de la coquille.

PRESENCE DE CELLULES DANS LA COQUILLE NORMALE

L'observation "in toto" de la coquille normale après décalcification à l'EDTA et diverses colorations topographiques a permis de déceler à ce niveau la présence de cellules libres. Leurs caractéristiques morphologiques et histochimiques nous ont conduit à décrire (Fournié, 1976a) deux types cellulaires en apparence distincts. Les cellules de type A, de petite taille, ont des caractéristiques de cellules jeunes. Les cellules de type B présentent, à la surface interne de la coquille, divers états qui traduisent une capacité de synthèse et d'accumulation de microgranules organocalciques. Au terme de cette évolution, l'éclatement de ces cellules conduit à la libération de ces microgranules à la surface interne de la coquille.

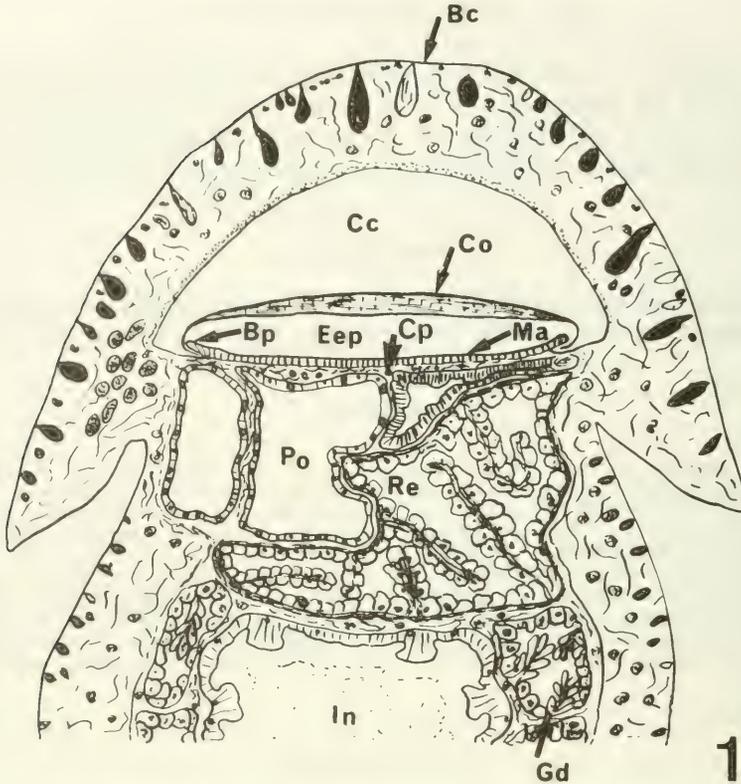
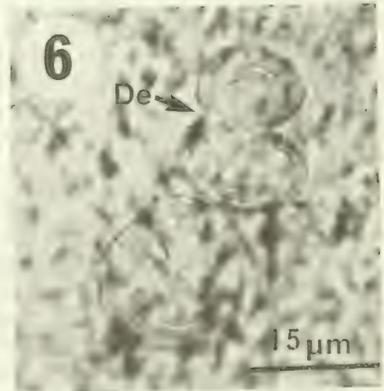
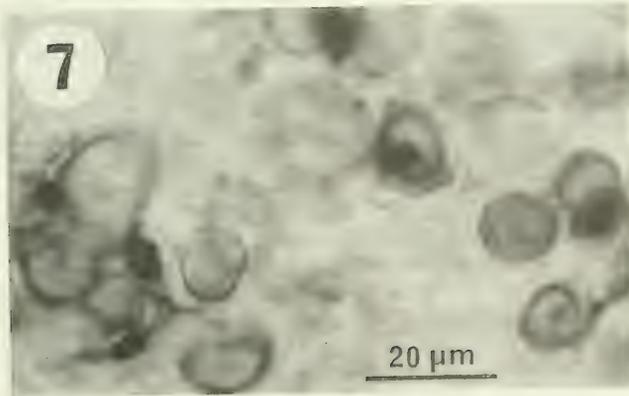
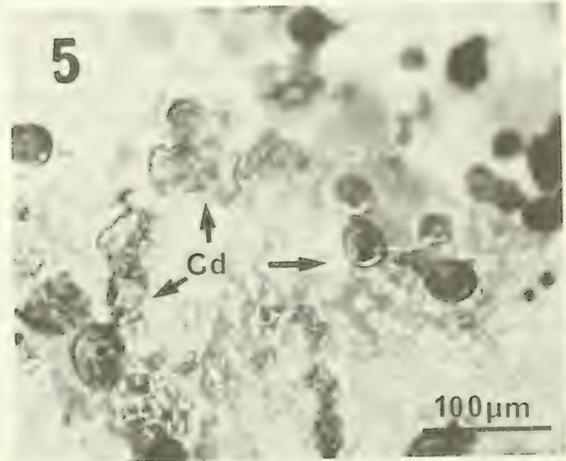
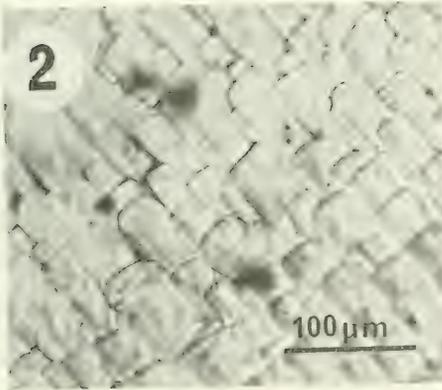


FIG. 1. Coupe transversale schématique d'*Agriolimax reticulatus* montrant les rapports de la coquille avec les tissus mous alentour. Bc: bouclier cephalique; Bp: bourrelet palléal; Cc: cavité de la coquille; Co: coquille; Cp: tissu conjonctif sous-palléal; Eep: espace extrapalléal; Gd: glande digestive; In: intestine; Ma: manteau; Po: poumon; Re: rein.

EVOLUTION DES MICROGRANULES SUR LA COQUILLE NORMALE

Par les différentes techniques histochimiques du calcium, il a été possible d'étudier (Fournié, 1979a), au niveau de la coquille normale, l'évolution des microgranules après leur libération. Il est curieux de constater (Fig. 3) que ces microgranules continuent leur croissance à l'état libre par apposition de couches concentriques successives. La persistance du processus donne lieu à des figures d'accolement de 2 ou plusieurs éléments; on obtient ainsi (Fig. 4 et 6) des figures en forme de bâtonnet, d'haltère ou de double éventail. Ceci conduit finalement à la mise en place (Fig. 5) d'une couche organocalcique discontinue, à la surface interne de la coquille, entre le liquide extrapalléal et la couche de calcite organisée en plaques cristallines. Cette couche en plaques n'est visible sur les montages "in toto" en vue interne qu'aux endroits où la couche interne sphérulitique est absente. La Fig. 8 explique la disposition relative de ces deux couches. Les techniques histochimiques du calcium, en particulier la technique de Kashiva pour le calcium facilement ionisable, indiquent que dans la couche sphérulitique le sel de calcium est sous une forme plus labile que dans la couche en plaques. Sur des préparations décalcifiées, la trame organique des sphérulites restant après décalcification (Fig. 7) montre bien leur mode de croissance et les figures obtenues par leur fusion. Cette calcification sphérulitique est donc le résultat de la précipitation de certains constituants du liquide extrapalléal autour des nuclei primordiaux représentés par les microgranules élaborés et libérés par les cellules à la surface interne de la coquille normale.



FIGS. 2-7. Vues internes de la coquille d'*Agriolimax reticulatus* observées sur des montages "in toto." Fig. 2. Aux endroits où il n'y a pas de formations sphérulitiques, la couche ostracale cristallisée en plaques est visible. Noter l'arrangement des plaques caractéristique de la calcite. Figs. 3-6. Mise en place des formations sphérulitiques à la surface interne de la coquille à partir des microgranules (Gr) libérés par les cellules. La croissance sphérulitique des granules conduit à des figures en bâtonnet (Ba), haltères (Ha), doubles éventails (De) et finit par donner une couche discontinue (Cd). 3: technique de Kashiva; 4: observation directe sans coloration; 5: technique de Stoelzner; 6: technique à l'alizarine. Fig. 7. Trame organique des sphérulites restant après décalcification à l'EDTA. Noter les couches concentriques et les figures d'accolement des sphérulites.

MORPHOGENESE REGENERATIVE DE LA COQUILLE

Chez *Agriolimax reticulatus*, l'ablation totale de la coquille est suivie par une régénération très rapide (Fournié, 1979b). Cette régénération commence par la mise en place d'une formation organique membraneuse. Cette membrane de régénération est ensuite colonisée par un très grand nombre de cellules dont l'aspect et le comportement sont identiques à ceux observés sur la coquille normale. Des microgranules sont libérés par ces cellules, ils initient là encore un processus de calcification sphérulitique. Sur les régénérats, ce processus s'accomplit simultanément à la mise en place de plaques calcitiques identiques à celles de l'ostracum de la coquille normale. Il n'est pas possible d'établir une filiation entre ces 2 types de calcification. Il semble plutôt qu'elles s'accomplissent de manière indépendante et parallèle.

Une étude histologique au niveau du manteau après une ablation totale de la coquille chez *Agriolimax reticulatus* (cf. Fournié, 1979b) montre que 20 minutes après l'ablation le manteau manifeste par l'augmentation de la hauteur de ses cellules et leur forte pyroninophilie, une hyperactivité de synthèse. Celle-ci conduit à l'élaboration et à la libération dans l'espace extrapalléal d'un matériel organique qui se condense pour donner une trame membraneuse. Cette trame est ensuite colonisée par des cellules qui sont toutes de petite taille et comparables aux cellules de type A observées sur la coquille normale. Ces cellules proviennent du tissu conjonctif sous-palléal qui est alors le siège d'une importante prolifération cellulaire. Ces cellules d'origine conjonctive traversent l'épithélium du manteau.

DISCUSSION

Des cellules libres sont présentes au niveau de la coquille normale d'*Agriolimax reticulatus* et elles sont identiques à celles observées sur les régénérats chez cette espèce; elles sont aussi comparables, par leur morphologie et leur comportement à celles décrites dans les régénérats d'autres espèces. La mise en évidence de cellules au niveau de la coquille normale d'*Agriolimax* écarte manifestement l'hypothèse jusqu'ici admise d'un rôle uniquement régénératif; elle soulève donc à nouveau le problème de leur signification et de leur rôle éventuel dans la morphogenèse de la coquille. Les microgranules libérés par les cellules à la surface interne de la coquille normale d'*Agriolimax* sont responsables de la mise en place de la couche interne de la coquille qui a une nature discontinue et dans laquelle la calcification est de type sphérulitique. Abolins-Krogis (1968) a décrit des formations analogues dans les régénérats de coquille d'*Helix pomatia*. Leur existence dans la coquille normale d'*Agriolimax* pose le problème de leur signification. La couche des plaques calcitiques a manifestement chez cette espèce (Fig. 8) la disposition et la nature cristallographique d'un ostracum. La position interne de la couche sphérulitique par rapport à la couche ostracale est celle d'un hypostracum. Dans les quelques cas où il a été décrit, l'hypostracum des Pulmonés est connu pour avoir, comme dans le cas étudié ici, une nature réduite et sphérulitique. Le problème qui se pose est celui de la signification de la coexistence de ces 2 types de calcification au niveau de la coquille. Les observations effectuées sur les régénérats de coquille d'*Agriolimax* où l'on observe simultanément les sphérulites et les plaques calcitiques semblent indiquer que la calcification en plaques et la calcification sphérulitique se déroulent de manière contemporaine et indépendante. D'un simple point de vue morphologique, il n'est pas possible d'établir une filiation entre les sphérulites et les plaques calcitiques. Chez l'animal normal, cette couche interne mise en place par une activité cellulaire pourrait représenter un moyen de mise en réserve du calcium au niveau de la coquille plus efficace et plus rapide que la cristallisation physicochimique du carbonate de calcium. Les résultats histochimiques indiquent que dans la coquille normale et régénérée les formations sphérulitiques sont plus labiles que les plaques cristallines. Il est possible que le carbonate de calcium mis rapidement en réserve par une activité cellulaire au niveau de la couche sphérulitique, puisse être redissous et réutilisé pour la précipitation cristalline au niveau de la couche ostracale.

Pendant la régénération de la coquille d'*Agriolimax reticulatus*, de jeunes cellules venant du conjonctif sous-palléal traversent l'épithélium du manteau, évoluent à l'état libre dans l'environnement muqueux du liquide extrapalléal et arrivent à la surface interne de la coquille. Les cellules qui traversent le manteau sont toutes comparables aux cellules de type A que l'on

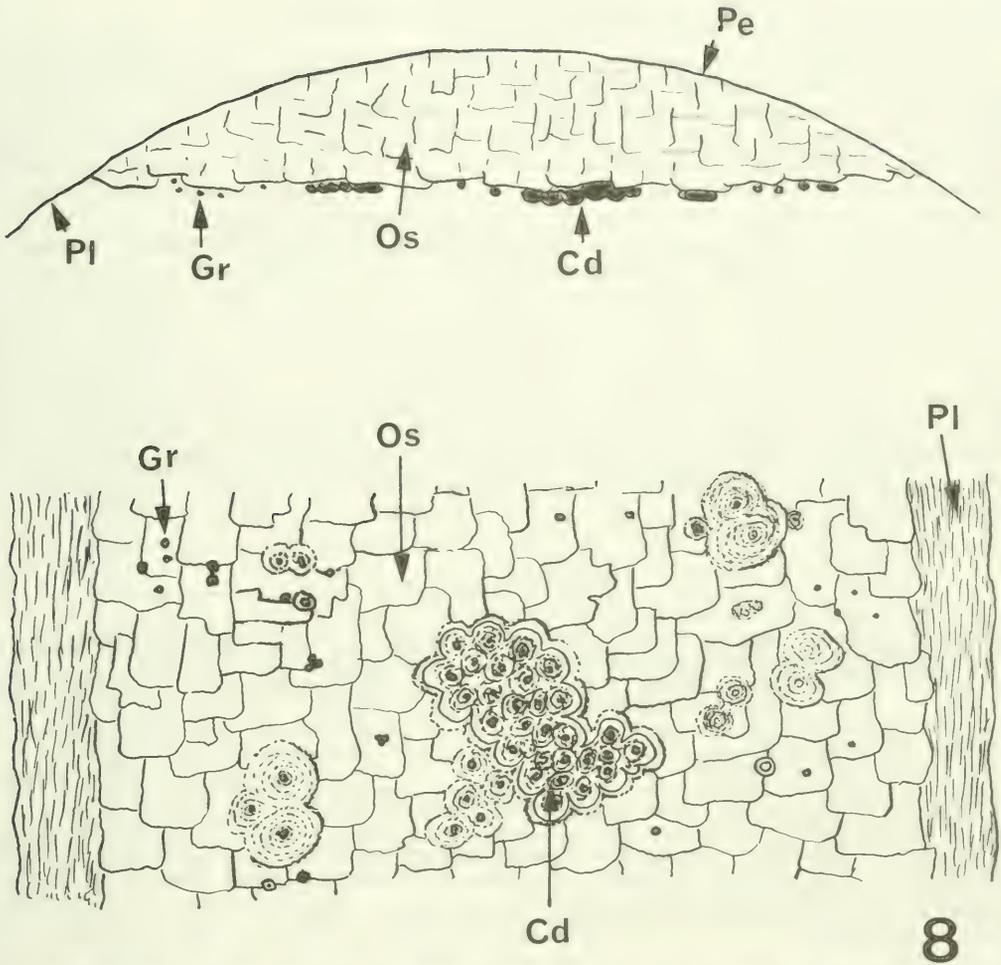


FIG. 8. Représentation schématique de la position relative des différentes couches de la coquille. En haut: coupe transversale, en bas: vue interne partielle. Cd: couche sphérolitique discontinue; Gr: granules; Os: ostracum; Pe: périostracum; Pl: bord libre du périostracum.

trouve au niveau de la coquille normale. Cette dernière observation indique que les 2 types A et B observés sur la coquille normale et interprétés jusqu'ici (Fournié, 1976a) comme 2 catégories cellulaires différentes, sont en fait 2 stades successifs d'une même lignée cellulaire. L'état A présente toutes les caractéristiques de cellule jeune. Il correspond à l'état dans lequel les cellules arrivent au niveau de la coquille. Les caractéristiques histochimiques des cellules de la forme A dénotent un métabolisme protéique important qui correspond certainement à la mise en place des structures responsables de l'initiation des microsphérules organocalciques. L'état B correspond alors à la phase de croissance des microsphérules et de précipitation "in situ" du carbonate de calcium à leur niveau; l'accroissement continu de ces microsphérules conduit fatalement à l'éclatement de la cellule et à leur libération. Dans la plupart des travaux sur la régénération de la coquille des mollusques, les auteurs interprètent les cellules observées sur les régénérats comme des amœbocytes apportant depuis les tissus mous vers la coquille tous les matériaux, et en particulier le calcium, nécessaires à l'édification de celle-ci. Les phénomènes observés pendant le régénération de la coquille d'*Agriolimax reticulatus* sont plutôt en faveur de l'intervention de cellules conjonctives qui arrivent à la surface interne de la coquille sous une forme juvénile. Il n'y a pas, dans le cas étudié ici, transport de calcium et l'accumulation de cet

élément se fait lorsque les cellules sont parvenues au niveau de la coquille et à partir des éléments qu'elles puisent dans le liquide extrapalléal.

L'origine conjonctive des cellules de la coquille et leur capacité d'accumuler des microgranules calciques sont 2 caractéristiques qui les rapprochent des cellules à calcium généralement décrites dans le tissu conjonctif des mollusques. En effet, Richardot (1976, 1979) a montré que les cellules à calcium du tissu conjonctif de *Ferrissia wautieri* dérivent de cellules conjonctives totipotentes: les cellules à sillons. Néanmoins, par la petite taille des microgranules qu'elles élaborent et par le fait qu'elles métabolisent les produits du liquide extrapalléal, les cellules de la coquille apparaissent comme un type bien particulier de cellule à calcium.

Il est intéressant de constater enfin que l'étude comparative de la coquille normale et régénérée chez *A. reticulatus* laisse voir des caractéristiques fondamentales communes à ces 2 structures. Dans les 2 cas, les mêmes cellules interviennent et elles initient une calcification de type sphérolitique toujours contemporaine à la formation des plaques calcitiques. Il semble donc que la morphogenèse normale de la coquille et sa régénération font intervenir les mêmes mécanismes. Ces derniers sont amplifiés pendant la régénération par le besoin de reconstruction rapide de la structure manquante et cette exagération permet de les observer plus facilement que chez l'animal normal où ils sont plus lents et plus discrets. Chez *A. reticulatus*, l'origine conjonctive des cellules de la coquille a été mise en évidence pendant la régénération. Il reste à examiner si, sur ce point particulier, la morphogenèse normale de la coquille fait aussi intervenir les mêmes mécanismes.

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THE FUNCTIONAL MORPHOLOGY OF THE EMBRYONIC SHELL-GLAND IN THE CONCHIFEROUS MOLLUSCS

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ABSTRACT

Judging from the literature 3 functions of the embryonic shell-gland seem to be possible: (1) It encloses the first embryonic shell and will rupture later on (Gegenbaur, 1852); (2) It is a real gland secreting a fluid which after hardening will be the embryonic shell (Fol, 1875, 1876); (3) The central part of the shell-field needs a resting stage because it is not able to secrete periostracal material. This function is restricted to the marginal cells of the shell-field outside. Thus the formation of a pellicle with a central aperture is prevented (Ziegler, 1885; Naef, 1923). As recent studies on *Lymnaea stagnalis*, *Marisa cornuarietis* and *Mytilus galloprovincialis* have shown, the latter interpretation certainly holds true for ectocochleate conchifera. These exhibit a stage in which the shell-gland seems to be closed. At that time the first pellicle is secreted on the outside, i.e. only by the not invaginated cells of the shell-field. As could be demonstrated by electron microscopy the invaginated cells show no secretion. Thus it seems that the acquisition of a shell-gland stage in the ontogeny enables the embryo to secrete a periostracum without a central opening. Tight closure of the future crystallization space is guaranteed. Endocochleate forms attain the same closure by overgrowing the shell-field with an ectodermal fold.

INTRODUCTION

As is well known the outer surface of the molluscan mantle is responsible for the elaboration of the shell. It is not surprising that a large number of papers have dealt with the embryonic origin of this secretory epithelium, the shell-field. The literature on morphology, development, and function of the embryonic shell-field and the shell-gland has recently been reviewed (Kniprath, 1979a). In this review it is shown that fundamental differences in the explanations of the morphology of the shell-gland stages made it impossible to give a general interpretation of its function. Based on new studies on the shell-gland of some molluscan species, a re-interpretation of the functional morphology is attempted in the present paper.

PREVIOUS INTERPRETATIONS

The first morphologically recognizable 'Anlage' of the shell-field is a thickening in the dorsal posttrochal region of the trochophore. Soon afterward, the central region of this thickening invaginates to form a deep depression which seemingly closes. This stage was first described in 1852 by Gegenbaur. Judging from his observations on *Limax*, *Arion*, *Clausilia*, and *Helix* he thought that all shells develop within this depression and hence are internal. Hescheler in 1900 followed this interpretation in Lang's textbook on zoology. But as the other literature and recent studies have shown, this is not generally true, perhaps apart from a few species (*Clausilia*: Gegenbaur, 1852; Schmidt, 1895; *Succinea*: Schmidt, 1895; *Achatina*: Ghose, 1962; *Archachatina*: Brisson, 1968).

A new explanation of the shell-gland (the name shell-gland was given to this invagination in 1873 independently by both Lankester and Ganin) was published in 1875 and 1876 by Fol. He thought it to be a true gland. The shell material would accumulate as a liquid within the

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shell-gland. Later, by evagination of the shell-gland, this liquid would be spread over the dorsal epithelium of the embryo and harden by contact with the surrounding medium. Blochmann (1883) was the first to reject the idea that the shell-gland was a true gland. He said that the chitinous material seen by Fol in the entrance of the shell-gland was an artifact. As seen in the electron microscope, the fine structure of this material in the shell-gland of *Lymnaea* suggests that it is albumen (Kniprath, 1977). This albumen has been "swallowed" during the invagination of the shell-gland. It is taken up by the shell-gland cells before the larval shell is secreted.

A variety of publications appearing after Fol and Blochmann caused much confusion concerning 3 aspects of shell-gland function: (1) Which cells secrete the first shell? (2) When is it secreted? (3) How does evagination proceed? The last question is not relevant to the present study and is not discussed here. The questions 1 and 2 are directly correlated. Fig. 1 shows graphically the differences between the concepts. Some authors are convinced that only the marginal cells of the shell-gland which are not invaginated secrete actively (Fig. 1, a and b) whereas others attribute this function only to the central cells (Fig. 1, c-e). It should be mentioned here that only some of the papers state exactly whether "first shell" means first calcareous shell or first pellicle. Of course, some of the differences are due to the difficulty of discerning the pellicle by light microscopy. Other differences depend more on different fixation methods and less exact cutting direction than on the real conditions. *Lymnaea stagnalis* is a good example for this. For this species the literature offers 4 of the possibilities shown in Fig. 1 (a-d).

NEW RESULTS AND THE 3RD INTERPRETATION

As it was impossible to generalize the morphology and function of the molluscan shell-gland from the literature, some ectocochleate species were studied by light and electron microscopy

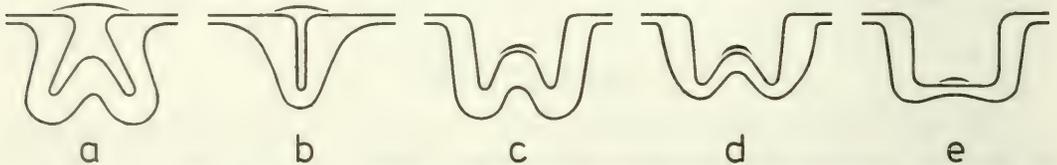


FIG. 1. Various concepts of the secretion of the shell rudiment in molluscs. a, Secretion by not invaginated cells; evagination before flattening. b, Secretion by not invaginated cells; no evagination but spreading. c, Secretion by central, yet thickened, cells during (after) evagination. d, Secretion by central, just flattened, cells during (after) evagination. e, Secretion by central, just flattened, cells; no evagination, but radial spreading (part of Fig. 1 in Kniprath, 1979a).

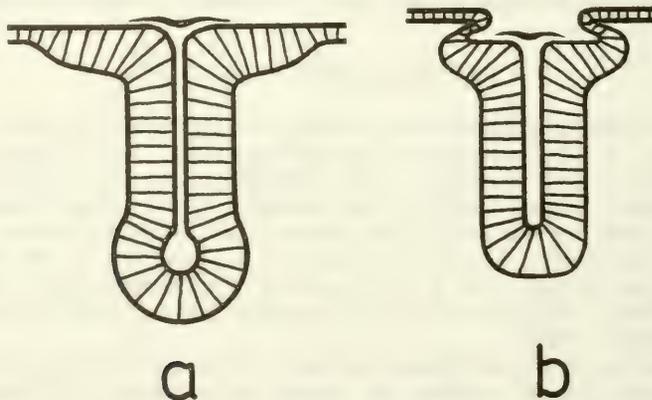


FIG. 2a. Shell-gland stage of *Lymnaea stagnalis* (from Kniprath, 1977). b, Shell-gland stage of *Marisa cornuarietis* (after Kniprath, 1979b).

and then compared. These were the freshwater pulmonate *Lymnaea stagnalis* (see Kniprath, 1977), the freshwater prosobranch *Marisa cornuarietis* (see Kniprath, 1979b), and the bivalve *Mytilus galloprovincialis* (see Kniprath, 1979c).

Lymnaea (Fig. 2a) shows a well "closed" shell-gland. The electron microscope demonstrated that the first exclusively organic shell is secreted only by the cells which have not invaginated. This shell covers the entrance of the shell-gland. None of the invaginated cells takes part in periostracum secretion but become active in calcium secretion long after evagination (Kniprath, 1977).

Marisa (Fig. 2b) has a much more complicated shell-gland stage. The "closure" lasts only a very short time. As in *Lymnaea*, only the cells which have not invaginated form the initial organic shell. The deeply invaginated central cells remain inactive until calcium secretion starts (Kniprath, 1979b). *Marisa* demonstrates one of the possible sources of the different interpretations shown in Fig. 1. Just before maximum invagination is reached the marginal part of the shell-field is overgrown by the surrounding ectoderm. Sections which are not precisely oriented may erroneously indicate internal elaboration of the shell. In other species this overgrowth may go further, perhaps up to an apparent closure. Then the shell seems to grow internally, regardless of the cutting direction. But in this case the space in which the shell grows is not identical with the shell-gland.

The 3rd species, *Mytilus galloprovincialis*, shows the shell-gland which has a really closed stage (Kniprath, 1979c). At that time the first pellicle is secreted only by the cells which are not invaginated.

The results may be summarized as follows. All 3 species have a stage in which the shell-gland appears to be closed. About that time the first organic shell is secreted only by the cells which have not invaginated. Together with Stempel (1900) I am convinced that this stage is common to all (at least embryonically) ectocochleate conchifera. The cases in which the shell of ectocochleate forms is reported to grow internally are interpreted as depending on a partly or entire overgrowth of the shell-gland by non-secretory ectoderm.

From these statements it is easy to give an interpretation of the functional morphology of the molluscan shell-gland. It is not surprising that it is not new. Indeed, in 1885, 2 years after Blochmann had rejected the idea that the shell-gland was a true gland, Ziegler published this 3rd interpretation. From his studies on *Cyclas* he concluded that the central, i.e., the invaginated part of the shell-gland, is not able to secrete periostracum. The temporary secretory inactivity of these cells and their invagination are correlated. Only if the part of the shell-field which does not secrete is invaginated, a pellicle without a central hole can be elaborated.

Without mentioning Ziegler, Naef (1923, 1924) came to the same conclusion. Describing the ontogeny of the gastropod *Lithoglyphus naticoides* he wrote: Only the marginal cells of the shell-field which meet in the shell-gland pore secrete the pellicle. The remaining cells are liberated from this function by invagination.

It seems curious that the extensive literature does not enter into this functional interpretation of Ziegler and Naef. The authors preferably discuss the question on the first appearance of the shell. This question has been clearly answered by the reported EM studies on *Lymnaea*, *Marisa*, and *Mytilus* (Kniprath, 1977, 1979b, c). Simultaneously it has been demonstrated that there is no better interpretation of the functional morphology of the conchiferan shell-gland than that of Ziegler and Naef.

Recent studies (Haas et al., 1978) on the embryonic development of the shell plates of *Lepidochitona cinerea* have shown that the chitons also have a homologous stage. The long microvilli of the marginal cells of the individual plate-field form a covering over its central part. Below this covering the first portion of the calcareous plate is secreted.

CONCLUSIONS AND SUMMARY

During embryonic development the shell-field of the conchiferan molluscs passes through a shell-gland stage. This stage in which the marginal cells of the shell-field meet in the shell-gland pore is functionally necessary. Only these cells are able to secrete the first organic shell, the pellicle. By the concentration of the pellicle-secreting cells at one point it is guaranteed that a periostracum without a central hole is secreted. The central cells of the shell-field, which are

deeply invaginated in this stage are not involved in periostracum secretion. After evagination or spreading of the shell-gland the central cells secrete calcium into the extrapallial space. This crystallization space is fully sealed up against the surrounding medium by the adherence of the pellicle margin to the marginal cells of the shell-field. Thus undisturbed crystallization of the first layer of calcium carbonate is guaranteed.

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THE LIMPET *FERRISSIA WAUTIERI*, A MODEL FOR STUDIES ON CALCIFICATION MECHANISMS IN MOLLUSCS: SOME RESULTS

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In *Ferrissia wautieri* (Ancyliidae) only submature subjects can support drought periods by developing a septum, partially closing the shell aperture (septifer form), whereas juveniles and adults retaining their ancyliid shell shape (ancyloid form) disappear. When conditions are favourable again, estivation is over and septifers start to form a new shell around the constricted shell aperture, and then reproduce (postseptifer form) (Richardot, 1974, 1976).

The septum is formed by the posterior part of the mantle edge. There is a complete similarity in crystalline structure of both shell and septum (Richardot et al., 1972).

We are now able to obtain, at will, under laboratory conditions, septum development and, later, resumption of shell growth (Richardot, 1976, 1977a, 1977b, 1978).

By initiating septum formation we stop the shell formation activity of the anterior part of the mantle edge (shell growth is interrupted), whereas an hyperactivity of the posterior part is triggered. On the other hand, interruption of estivation initiates general activity of the mantle edge. *Ferrissia* thus becomes very suitable material for the study of the mechanisms involved in external calcification.

Moreover, when submature limpets are kept under unfavourable conditions, just before septum formation, a rapid loading of the connective tissue with intracellular calcospherites occurs. *Ferrissia* thus becomes also very suitable material for the study of the mechanisms involved in intracellular calcification.

The observations suggest that the calcospherites may store calcium salts and act as a reserve supply in the calcium economy of the organism. Many ultrastructural and cytochemical observations suggest that the groove cells of the connective tissue may turn into calcium cells.

The results obtained using cytochemical techniques¹ led us to assume that the mantle epithelium intercellular spaces are involved in calcium transfer between the connective tissue and the shell or the septum (Figs. 1, 2) (Richardot, 1976). These results are in agreement with those of Neff (1972) and Vovelle (1973).

Moreover histochemical studies performed at light microscope level provide evidence that there is a calcium loading of ovocytes in reproductive limpets.

It can be inferred from the results obtained by ecological, histological, ultrastructural and histochemical studies that the calcium economy seems to consist of a complex regulatory system which maintains an equilibrium between the different calcium compartments: external and internal milieus, intracellular spherules, external calcareous sheaths (shell and septum), kidney and ovocytes. A series of different figures shows the possible different ways of calcium movements in limpets kept under favourable conditions (fast growth and reproduction) and unfavourable conditions (hibernation and estivation) (Fig. 3) (Richardot, 1976).

¹Light microscope level: technique of Kashiwa (1966). Ultrastructural level: technique of Carasso & Favard (1966): prefixed pieces incubated in 0,5% lead acetate, for 15 minutes, at 37°C. Controls were performed on prefixed pieces, by decalcification with a EDTA disodium salt buffered according to Kashiwa (1966) or with 0,4 M sodium citrate buffer, pH 4,8.



FIG. 1-2. Intercellular and extracellular spaces labelled by lead salts precipitates; technique of Carasso & Favard. Ultrathin sections stained with uranyl acetate only. 1, Dorsal epithelium of the mantle, septifer; note the loading of epithelial cells with glycogen, a typical feature of estivating (or hibernating) subjects (ca. $\times 18000$); 2, Ventral epithelium of the mantle (close to the belt), septifer with a septum in course of construction (septum length 0.08 mm) (ca. $\times 17600$).

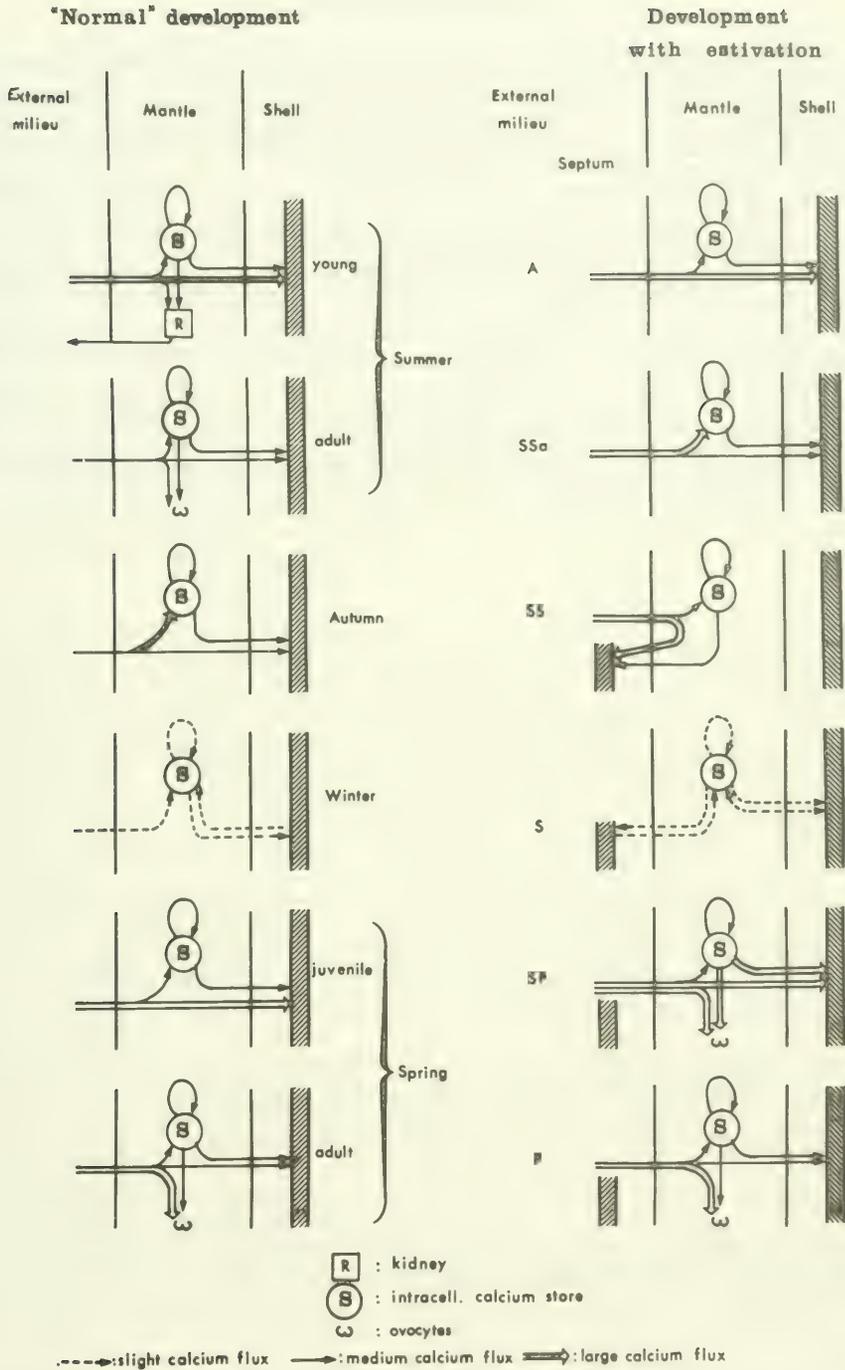


FIG. 3. Possible shifting of the calcium equilibrium in *Ferrissia wautieri* throughout development. The kidney plays a role in the calcium elimination in all cases; however, in order to simplify the drawing, the kidney is figured once only. A: ancyloid; SSa: subseptifer (the lateral borders of the shell become constricted inwards); SS: septifer with septum in course of construction; S: septifer with a complete septum (estivation); SP: estivation is over and growth is resumed; P: postseptifer (reproduction).

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APPROCHE HISTOPHYSIOLOGIQUE ET CYTOLOGIQUE DU ROLE DES
CELLULES A SPHERULES CALCIFIQUES DU REPLI OPERCULAIRE CHEZ
POMATIAS ELEGANS (MÜLLER), GASTEROPODE PROSOBRANCHE

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ABSTRACT

Previous histochemical data on cells with calcareous spherules in the conjunctive tissue underlying the epithelium of the opercular fold in *Pomatias elegans* have been completed with the help of marking techniques and data from ultrastructural cytology. The labile mineral component has been visualized by the use of tetracyclin and Ca^{45} in vivo. The cells delineated by a continuous basal lamina and a discontinuous larger peripheral cytoplasm differentiate irreversibly. Particular tubular structures join the outside of the cell to a central reservoir with spherules.

Cette étude correspond à une nouvelle approche d'un matériel abordé précédemment (Vovelle, Grasset & Meunier, 1976), le Prosobranchie calcicole *Pomatias elegans* (récolté en Provence), et de la zone pédieuse génératrice de son opercule minéralisé. Elle concerne les cellules à sphérules calciques du conjonctif sous-jacent à l'épithélium du "renflement externe" impliqué dans la sécrétion de l'opercule calcaire superficiel. Même présentes ailleurs dans la masse pédieuse, leur densité et leur situation tendraient à désigner localement ces cellules comme des jalons du calcium déposé ensuite par l'épithélium de transfert sur la zone de croissance de l'opercule. Sans vouloir en conclure prématurément, on a tenté la caractérisation fonctionnelle de ces cellules par l'emploi de marqueurs et par une étude cytologique ultrastructurale.

Avant d'en développer les résultats, on rappellera la description antérieure de ces cellules par Prenant, 1924 (qui propose une hypothèse de la formation des sphérules) et les résultats histochimiques qui les caractérisent par rapport aux 2 autres catégories cellulaires voisines dans le conjonctif (cellules à glycogène et cellules pigmentaires ou "de Leydig"), notamment par la présence d'une trame organique alcianophile des sécrétions, identifiable comme un mucopolysaccharide peu acide.

On soulignera en outre le caractère labile du calcium immobilisé au niveau des sphérules:

—en grande partie déplacé par l'incubation (en milieu aqueux) préalable à la visualisation terminale des activités phosphomonoestérasiques par la méthode de Gomori aux métaux lourds,

—très atténué à la réaction de Stoelzner sur l'animal inanité, maintenu captif plus de 6 mois après sa récolte,

—entièrement effacé non seulement par un fixateur acide aqueux comme le Bouin mais aussi par un fixateur acide anhydre comme le Carnoy.

On soulignera enfin une densité particulière de calcium ionique "circulant" révélé dans toute la zone du "renflement externe" par la méthode de Kashiwa et al. au GBHA, les cellules à sphérules calciques apparaissant alors très surcolorées sur le pourtour et dans les interstices de leurs inclusions.

EXPERIENCES DE MARQUAGE DU CALCIUM

Elles comportent l'une comme l'autre le maintien de l'animal en survie pendant une période de 2 à 10 jours dans une enceinte où le marqueur en solution aqueuse diluée imbibe le substrat (coton).

(a) L'emploi de la tétracycline, inspiré des travaux qui, après Milch et al. (1958) mettent en vedette sa substitution au dépôt calcaïque, a été suivi d'une fixation anhydre (acétone) et d'une inclusion aux résines acryliques, pour observation microscopique de la fluorescence aux Ultra-Violets après réalisation de lames minces minéralogiques conservant en place parties dures et parties molles. Il permet de reconnaître pour un temps moyen de survie une fluorescence intense et élective de la zone du "renflement" (épithélium et conjonctif) et de marquer la dernière strie de croissance de l'opercule minéral. Un lot d'animaux maintenu 4 jours au contact du marqueur indique une localisation plus précise (conformément aux résultats que l'on connaît pour l'ossification) et révèle les seules cellules à sphérules. Notons que cette réponse n'est pas limitée au renflement, mais concerne autant, sinon plus, les cellules du même type de toute la masse pédieuse. La Calceïne, employée un temps court (2 jours) fournit des résultats comparables au premier type de réponse à la tétracycline, par contre l'alizarine naturelle, proposée sous forme de bouillie de poudre de racines de garance et tolérée 10 jours, marque d'une fluorescence rosée les cellules elles mêmes. On soulignera l'intérêt théorique de cette dernière catégorie de résultats, puisqu'il s'agit de techniques réservées jusqu'à présent au marquage des "parties dures" (dépôts squelettiques).

(b) L'emploi du Ca^{45} (1 M Ci de cation radioactif sous forme de chlorure, en 4 ml de solution aqueuse) a permis un examen radioautographique ultérieur à l'échelle photonique (après fixation à l'alcool-formol et exposition des coupes 3 semaines et plus), et même à l'échelle ultrastructurale (après fixation à la glutaraldéhyde-osmium et exposition 2 à 6 mois). Dans le premier cas, la réponse positive, particulièrement intense après 2 jours de traitement, concerne les seules cellules à sphérules (de tout le conjonctif pédieux) et contraste avec les images obtenues précédemment chez *Astrea* (Vovelle, 1973), diffuses dans le conjonctif et nettes à l'apex de l'épithélium de transfert. Dans le 2^{me} cas, la labilité du calcium sphérolaire laisse peu d'impacts à observer, au niveau de quelques petites sphérules en formation (et rarement, de la jonction sous-apicale des cellules épithéliales).

EXAMEN CYTOLOGIQUE DES CELLULES A SPHERULES AU NIVEAU ULTRASTRUCTURAL

—Définition par comparaison avec les 2 autres catégories cellulaires du tissu conjonctif.

La confusion entre les grosses cellules (20-30 μm) remplies de glycogène, à noyau arrondi non altéré, et les cellules un peu plus petites (15-20 μm) à sphérules calcaïques est impossible. Les cellules de Leydig (9-13 μm), dont les grains pigmentaires sont accompagnés ou non de plages de glycogène, présentent à leur pourtour les différenciations caractéristiques (sillons et vésicules) des "pore-cells" (Plummer, 1966; Sminia, 1972) ou "cellules à sillon" (Nicaise, 1966). Elles sont souvent en contact intime avec les cellules à sphérules, mais gardent leur délimitation propre. Leurs inclusions, plus grosses et plus régulières que les sphérules, présentent un contraste aux électrons non renforcé par la méthode de Carasso-Favard qui révèle la composante minérale des sphérules calcaïques. Chez *Pomatias* il n'y a donc pas d'indice de filiation entre cellules de Leydig et cellules à sphérules calcaïques, comme l'hypothèse a été avancée pour d'autres gastéropodes (Richardot sur *Ferrissia*, 1976).

—Bilan des structures cytoplasmiques en rapport avec l'élaboration des concrétions organo-minérales et leur accumulation (réversible?).

La cellule à sphérules à maturité apparaît comme une formation creuse, délimitée par une membrane basale continue enveloppant de minces plages cytoplasmiques interrompues par endroits et prolongées vers l'intérieur par quelques trabécules cytoplasmiques ténues. A la périphérie seulement on observe, outre un ergastoplasme dense, avec des indications de reticulum granuleux, d'autres inclusions et organites: quelques figures myéliniques et flaques de triglycérides, rares traces de glycogène, pas de Golgi. Le noyau lorsqu'on l'observe, est excentré, collé contre la basale avec très peu de cytoplasme périphérique. Plus ou moins avancée, sa pycnose le présente très chromatique, rétréci et anguleux, suggérant l'irréversibilité de l'évolution cellulaire.

Les sphérules de tailles diverses, dépassant rarement 2 μm , sont réduites par la préparation à la trame organique, qui dessine un double système concentrique et rayonnant surtout à leur pourtour. La composante minérale peut être visualisée par la technique de Carasso-Favard,

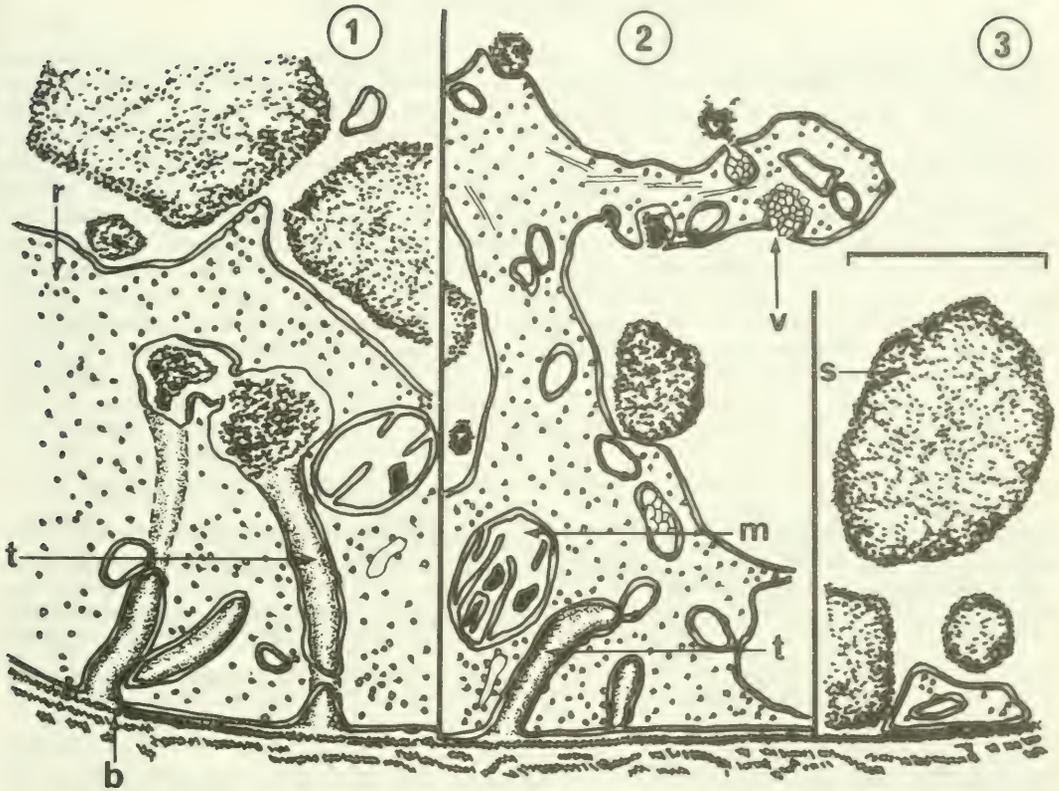


FIG. 1. Détail de l'ultrastructure des cellules à sphérules. 1 et 2, plages cytoplasmiques périphériques d'une cellule jeune, avec tubules (t) et vésicules alvéolées (v). 3, plage cytoplasmique résiduelle d'une cellule à maturité (b, lame basale; m, mitochondrie; r, ribosomes; s, sphérules). Echelle 1 μ m.

souignée parfois d'une auréole de diffusion du calcium labile. La régularité des contours des sphérules et la densité de leur trame varient d'une cellule à l'autre d'un même animal. Leur contact fréquent avec la membrane basale laisse deviner les voies immédiates de la restitution de calcium ionique circulant au conjonctif.

Dans cette cellule définitivement réduite à un réservoir central, délimité intérieurement par la membrane plasmique, les voies de l'insolubilisation de la fraction minérale des sphérules et de l'élaboration de la composante organique sont à coup sûr moins directes. L'observation exceptionnelle de cellules jeunes implique dans cette double sécrétion des structures originales, sans qu'on puisse encore la jalonner complètement. Il s'agit d'abord de formations tubuleuses d'un diamètre de 700 à 1200 Å, invaginées à partir de la membrane plasmique externe, dont elles conservent la délimitation sur presque tout leur trajet, marquée de place en place par des macules osmiophiles. Ces tubules centripètes en réseau dans les plages cytoplasmiques périphériques s'ouvrent-ils dans la lacune centrale, par l'intermédiaire de dilatations où la trame des nouvelles sphérules apparaît au contact de membranes moins bien délimitées? Certains trabécules cytoplasmiques présentent dans cette situation des vésicules aux parois marquées d'un réseau polygonal (à la façon des "vésicules alvéolées" des cellules à sillons, cf. Nicolas, 1973) sans qu'on puisse assurer leur continuité ou leur situation terminale par rapport au système de tubules.

INTERPRETATION ET CONCLUSIONS

Réservoirs, au double sens de l'accumulation et de la restitution, d'un calcium minéral très labile, les cellules à sphérules étudiées dans le "renflement" operculaire de *Pomatias* peuvent être admises comme un jalon privilégié dans l'itinéraire du cation avant sa traversée de l'épithélium de transfert localisé qui élabore l'opercule externe (cette vocation locale étant compatible avec la présence de cellules de même type dans toute la masse pédieuse). D'où vient le calcium insolubilisé? On sait la haute teneur en calcium du milieu intérieur de ce Prosobranch calcicole (cf. revue in Delhaye, 1974), on n'oublie pas non plus qu'il possède au voisinage même de la zone étudiée cette glande pédieuse particulière, "tubuleuse" que Delhaye (1974) et Bensalem (1976) interprètent de façon contradictoire mais dont les images évoquent bien celles d'autres structures impliquées dans un transfert ionique rapide. Ainsi un circuit rapide du calcium déposé dans l'opercule peut-il être envisagé à l'intérieur de la masse pédieuse de *Pomatias*, dont les cellules à sphérules étudiées représenteraient une ultime étape.

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RESUME

Des données antérieures obtenues par l'histochimie concernant les cellules à sphérules calciques du conjonctif sous-jacent à l'épithélium du repli operculaire chez *Pomatias* ont été complétées par des techniques de marquage et des données de cytologie ultra-structurale. La composante minérale labile a été visualisée par l'emploi in vivo de la tétracycline et du Ca^{45} . Les cellules délimitées par une basale continue et un cytoplasme périphérique discontinu, se différencient irréversiblement. Des structures particulières, tubuleuses, réunissent l'extérieur de la cellule au réservoir central à sphérules.

PHYSIOLOGICAL CHANGES IN GASTROPODS DURING EGG SHELL CALCIFICATION. PART II

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Calcification of snail eggs involves the addition of a jelly coat and the deposition of crystals of CaCO_3 within the jelly during the passage of the eggs through the uterus. Three aspects of calcification of the eggs of the snails *Helix aspersa* and *Anguispira alternata* are considered: (1) the ultrastructure of the cells of the uterine epithelium concerned with calcification; (2) the calcium flux across the uterus during calcification; and (3) additional processes which may be involved in egg calcification.

(1) Structure of the uterine epithelium.

Two kinds of epithelial cells line the uterus. One type has a greatly developed cell membrane infolded from the basal portion leaving open spaces. These cells resemble those associated with ion transport in other animals. The path length for calcium in passing from blood vessels across these cells to the eggs is $3\ \mu\text{m}$ or less. The 2nd cell type is very much larger and contains large vesicles which probably provide the jelly coat of the eggs.

(2) Calcium flux across the uterus of *Helix*.

Single eggs in various positions within the uterus were analyzed for calcium. The calcium content was found to be directly proportional to the distance the eggs had moved along the uterus, indicating that each portion of the uterus is equally capable of calcifying the eggs. An egg which had traversed the length of the uterus contained 0.6 mg calcium, most of which was present as crystals within the jelly coat. Since a snail lays as many as 100 eggs, the amount of calcium transported across the uterine epithelium may amount to 60 mg. The calculated calcium flux was 3×10^{-6} moles $\text{cm}^{-2} \text{hr}^{-1}$ for *Helix* and 1×10^{-5} moles $\text{cm}^{-2} \text{hr}^{-1}$ for *Anguispira*.

The rate of uptake of ^{45}Ca by isolated uteri of *Helix* containing eggs and ligated at the ends remained constant with time for $4\frac{1}{2}$ hours but was less than 1% of the rate in vivo. The lack of circulation may reduce the flux rate due to oxygen deficiency and increased path length of calcium movement. That increased path length was a probable factor was indicated by the finding that calcium deposition was increased more than 10-fold by increasing the calcium concentration of the medium from 4.5 mM to 20 mM.

(3) Uterine potentials.

A potential of 10 mv to 12 mv (inside negative) was present across the uterus with Ringer both outside and inside. The potential was highest with aeration with 98% O_2 , 2% CO_2 . N_2 and 0.3 M DNP reversibly depressed the potential by approximately one third, demonstrating that oxidative processes are necessary for maximum potentials and that a reduced potential can be maintained under anaerobic conditions.

The following factors appear to be involved in the calcification of the snail eggs:

(1) The jelly initiates crystal nucleation since crystals are not deposited in the uterus external to the jelly.

(2) A negative potential across the uterine epithelium would favor movement of calcium from the blood toward the egg surface

(3) The concentration gradient of calcium from the blood to the egg is increased by a rise in free blood calcium during egg-laying (Tompa & Wilbur, 1977). The increased gradient would be favorable to calcification.

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DURCH PARASITEN INDUZIERTE PERLBILDUNG BEI *MYTILUS EDULIS* L. (BIVALVIA)

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ABSTRACT

In some populations of *Mytilus edulis* from the intertidal zone of the island of Sylt (North Sea) pearl formation was observed. Both general types of pearls are found: spherical pearls in the connective tissue of the gonads and the midgut gland, and ovoid or pear-shaped pearls attached to the inside of the shell. Eggs of hitherto undeterminable marine animals and larval stages (metacercariae probably of a *Gymnophallus* species) are found in the center of the pearl ("nucleus"). These nuclei are surrounded by concentric lamellae produced by the pearl sac epithelium. Concentric and radial lamellae of conchium form pockets in which the calcium carbonate is deposited. The formation of the lamellae occurs periodically. The first step in producing lamellae is the deposition of granular material in between the microvilli of the inner surface of the pearl sac. Later, this material is condensed to distinct leaflets. In the connective tissue surrounding the pearl sac ovoid particles enclosing a calcium-proteid-complex were found. This complex contains the precursor material of the aragonite crystals.

EINLEITUNG

In Miesmuscheln sind häufig Schalenkonkretionen, Schalenperlen und freie Perlen (Definition bei Götting, 1974) in grösserer Anzahl zu finden. Während über die Perlbildung bei Perlmuscheln (*Margaritifera*, *Pinctada*) eine umfangreiche Literatur vorliegt (Alverdes, 1932; Erben, 1972; Haas, 1955; Mutvei, 1970; Wada, 1966), ist zu den entsprechenden Vorgängen bei anderen Muscheln wenig bekannt. Loos-Frank (1971) erwähnt Perlen bei *Mytilus* im Zusammenhang mit parasitologischen Untersuchungen, Lauckner (1971) beschreibt Perlen aus *Cardium*-Arten.

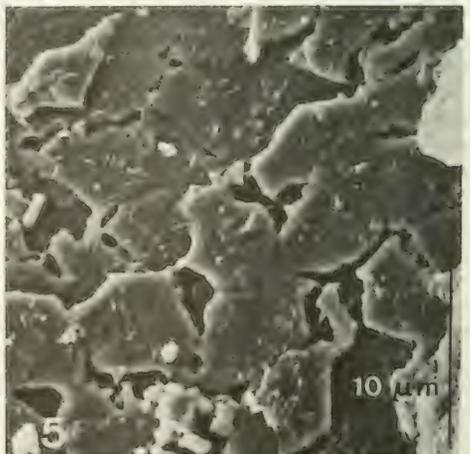
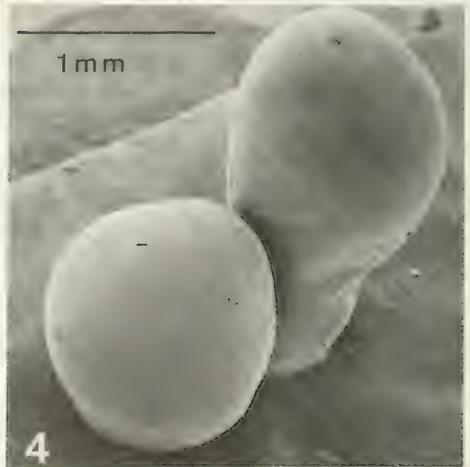
MATERIAL UND METHODE

Die für diese Arbeit verwendeten Miesmuscheln (*Mytilus edulis* L.) wurden verschiedenen Populationen der Insel Sylt entnommen. Freie Perlen wurden mit dem umgebenden Gewebe extirpiert und in den für elektronenmikroskopische Zwecke üblichen Gemischen mit OsO₄ und Glutaraldehyd fixiert, denen zum Teil ÄDTE zugesetzt war. Die Einbettung erfolgte in Vestopal W. Von freien Perlen und von Schalenperlen wurden Dünnschliffe hergestellt, die im polarisierten Licht untersucht wurden.

Die Schalenkonkretionen und Schalenperlen wurden zur SEM-Untersuchung etwa 500-600 Å dick mit Gold beschichtet (Polaron E 5000 Diode Sputtering System) und am JEOL JSM-1 abgerastert. Die Ultradünnschnitte wurden am Philips EM 300 untersucht.

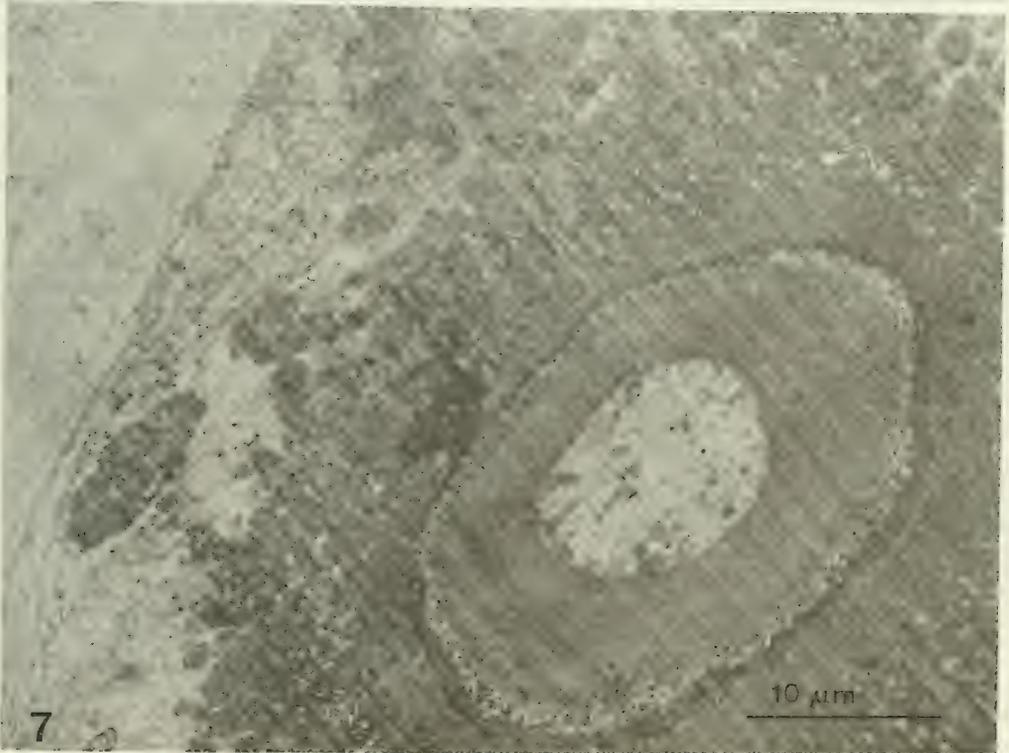
BEFUNDE

In bestimmten Populationen der Miesmuschel sind Perlen sehr häufig. So wurden in einer Schalenklappe von 70 mm Länge 60 Schalenperlen (Fig. 1) mit Durchmesser bis zu 1 mm, im gleichseitigen Mantellappen 38 freie Perlen festgestellt. Im allgemeinen ist die Anzahl geringer.



- FIG. 1. Schalenperlen auf der Innenseite der linken Schalenklappe in der Nähe des Adductors.
 FIG. 2. Kugelförmige Schalenperlen. Zwischen den beiden rechten entsteht eine Aragonit-Brücke, während die beiden linken Perlen ein späteres Stadium mit gemeinsamer Umhüllung repräsentieren (Zwillingsperle).
 FIG. 3. Zwei Schalenperlen mit einer Aragonit-Brücke in statu nascendi (vergleiche Fig. 2 rechts).
 FIG. 4. Kugel- und birnenförmige Schalenperlen.
 FIG. 5. Aragonit-Kristalle in tafelig-hexagonaler Grundform aus der Brücke zwischen 2 Schalenperlen (vergleiche Fig. 3).

- FIG. 6. Metacercarie als Nucleus einer freien Perle.
 FIG. 7. Nicht näher identifiziertes Ei als Nucleus einer freien Perle. Links oben sind die Conchin-Taschen der Aragonit-Kristalle sichtbar.



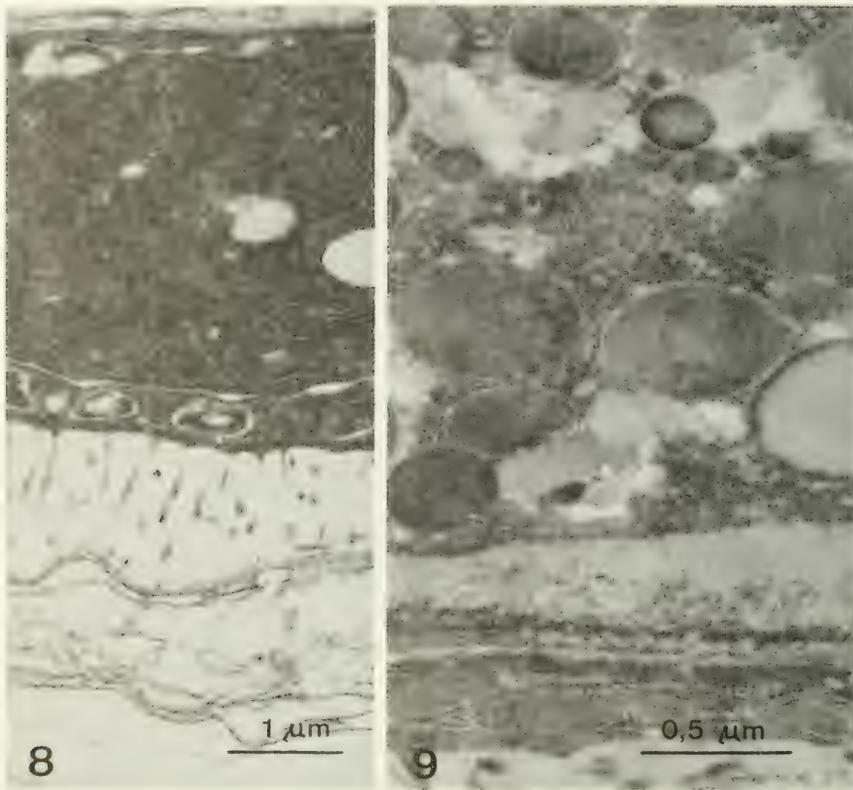


FIG. 8. Perlsack-Epithel (oben) mit Mikrovilli und den Vorstufen der Conchin-Lamellen (unten).
 FIG. 9. Ovoide Ca-Proteid-Partikeln aus dem Bindegewebe um den Perlsack. Perlsack-Epithel unten.

Stets sind jedoch Schalenperlen häufiger als freie Perlen, meist sind sie auch häufiger als Schalenkonkretionen, soweit sich ihre Herkunft beurteilen lässt.

Die Schalenperlen entstehen bevorzugt in drei Bereichen, nämlich in der Nähe des hinteren Adductors sowie in je einem dorsalen und einem ventralen, schmalen Streifen. Sie sind kugelig bis birnenförmig (Fig. 2, 4) und entstehen oft in so enger Nachbarschaft, dass sie während des weiteren Wachstums miteinander verschmelzen. Dabei wird zunächst eine Brücke aus Aragonit-Kristallen von Perle zu Perle gebildet (Fig. 3, 5). Die Brücke wird verstärkt, die Einbuchtung nach und nach aufgefüllt (Fig. 2, links), bis eine Doppelperle entstanden ist. Im lichtmikroskopischen Dünnschliff sind in solchen Zwillingasperlen die beiden ursprünglichen Nuclei deutlich sichtbar.

Der Ultradünnschnitt einer freien Perle zeigt in den meisten Fällen einen Nucleus, der aus einer granulären Masse besteht. In einigen Fällen konnten jedoch andere Verursacher der Perlbildung identifiziert werden. So waren mehrfach Metacercarien (Fig. 6) nachzuweisen, in seltenen Fällen kommen auch Eier (Fig. 7) vor. Die Nuclei werden zunächst stets in eine organische Hülle eingeschlossen. Das Perlsackepithel scheidet weiter zahlreiche konzentrische und radiäre Conchin-Lamellen ab, die sich zu Taschen für die später einzulagernden Aragonit-Kristalle zusammenschließen. Die Bildung der konzentrischen Lamellen erfolgt periodisch, so dass kugelförmige Zonen von dicht und von weiter auseinanderliegenden Lamellen entstehen.

Die Mikrovilli der Epithelzellen des Perlsacks sind lang und dünn (Fig. 8). Zwischen ihnen werden mucopolysaccharidhaltige Substanzen ausgeschieden, die zunächst netzartig erscheinen, später zu fädigen Strukturen kondensiert werden. Diese vereinigen sich zu den Conchin-Lamellen. Im Bindegewebe um den Perlsack waren ovoid Partikeln nachzuweisen (Fig. 9). Die

Mikroanalyse ergab, dass es sich um einen Ca-Proteid-Komplex handelt, der wahrscheinlich das Reservematerial für das CaCO_3 darstellt, das als Aragonit in den Conchin-Taschen der Perle kristallisiert.

DISKUSSION

Die grundsätzlichen Vorgänge der Perlbildung stimmen mit denen bei *Pinctada* (Wada, 1970) überein. Während in der Literatur auch Sandkörner als Stimulans für die Entstehung von Perlen angeführt werden, konnten solche in den bisher untersuchten *Mytilus*-Perlen nicht festgestellt werden. Vielmehr wurden als Nucleus nachgewiesen:

(1) In den meisten Fällen granuläres Material, das wahrscheinlich aus Exkreten besteht, deren Herkunft noch festzustellen ist. Möglicherweise handelt es sich um Stoffwechselprodukte der Miesmuschel, die nicht auf dem üblichen Wege aus dem Körper befördert werden. Die intensive Reaktion des Muschelgewebes auf diese Substanzen lässt jedoch eher vermuten, dass es sich um abgebaute Reste oder um Exkrete von Parasiten, speziell von Metacercarien, handelt.

(2) In mehreren Fällen konnten Metacercarien als Nucleus festgestellt werden. Es handelt sich um nicht cystenbildende Arten, also wahrscheinlich Gymnophallidae (Lauckner, 1971; Loos-Frank, 1971). Zudem legt der ultrahistologische Aspekt die Vermutung nahe, dass es sich um Metacercarien handelt, die abgebaut werden. Da die Gymnophalliden-Metacercarien sich bewegen, werden sie wahrscheinlich erst nach ihrem Tode von der Miesmuschel mit einer Conchin-Hülle umgeben, die später durch Einlagerung von CaCO_3 verstärkt wird.

(3) In seltenen Fällen waren Eier als Nucleus der Perle nachzuweisen. Die Eier sind oval und etwa $100 \times 65 \mu\text{m}$ gross. Sie stammen von bisher noch nicht identifizierten Tieren. Weiter fortgeschrittene Entwicklungsstadien waren bisher nicht zu beobachten.

Da Schalenkonkretionen und Schalenperlen häufiger sind als freie Perlen, ist es wahrscheinlich, dass die Parasiten auf dem Wege über den extrapallialen Raum in die Miesmuschel gelangen. Um den eingedrungenen Fremdkörper sammeln sich in einer ersten Abwehrreaktion amöboide Zellen, die eine fibröse Kapsel bilden (Fig. 6, 7). Um den ganzen Komplex von Fremdkörper, Kapsel und amöboiden Zellen werden dann die Conchin-Lamellen abgeschieden, zwischen die Aragonit eingelagert wird, so dass der Eindringling schliesslich isoliert ist.

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INHIBITION OF HYPERTONIC-SALINE STIMULATED NEUROSECRETORY CHANGES IN THE FRESHWATER BIVALVE *INDONAIA CAERULEUS* (PRASHAD) BY CHLORPROMAZINE AND RESERPINE

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ABSTRACT

In the present paper, the effects of reserpine (RSP) and chlorpromazine (CPZ) on the neurosecretory cells of a saline treated freshwater bivalve, *Indonaia caeruleus* have been tracked. After saline (0.1 ml of 1.5% NaCl per animal) treatment A and B cells of cerebral and visceral ganglia showed significant increase in cell area, nuclear diameter and decrease in the NSM, while administration of RSP (0.50 mg per animal) and CPZ (0.25 mg per animal), following the saline injection completely inhibited these changes. Physiological implications of these changes are discussed in the light of neurosecretory dynamics.

INTRODUCTION

Numerous neuropharmacological agents and other chemicals provoke alterations in the neurohormonal cells of both vertebrates (Gold & Ganong, 1967; Singh & Dominic, 1975) and invertebrates (Masner et al., 1970; Raina, 1974; Nagabhushanam & Hanumante, 1977). Hypertonic saline stimulates dramatic histological changes in the neurosecretory cells of vertebrates (Gabe, 1966) and numerous invertebrates e.g. the snail *Lymnaea stagnalis* (see Wendelaar Bonga, 1971), the slug *Laevicaulis alte* (see Nagabhushanam & Kulkarni, 1971), the prosobranch *Melania scabra* (see Muley, 1974) and several other species. The tranquillizers chlorpromazine and reserpine inhibit the hypertonic saline induced histological alterations in the hypothalamo-hypophysial complex of the musk shrew *Suncus murinus* (see Kulshreshtha & Dominic, 1971a, b), the spotted owl, *Athene brama* (see Singh & Dominic, 1974a, b) and in certain other vertebrates. Recently it has been demonstrated that in the freshwater pulmonate *Lymnaea auricularia* (see Hanumante et al., 1976, unpublished data) RSP successfully prevented the hypertonic saline provoked neurosecretory changes. Virtually nothing is known about the impact of these tranquillizers on the saline tested neurosecretory neurons of the freshwater bivalves. Retrospectively, the present study was designed to evaluate the effect of chlorpromazine (CPZ) and reserpine (RSP) on the histological changes in the neurosecretory cells provoked by hypertonic saline treatment in the freshwater bivalve *I. caeruleus*.

MATERIAL AND METHODS

Adult bivalves of *Indonaia caeruleus* of either sex were collected from the Godawari river (Paithan). They were maintained under laboratory conditions for about a week before the start of the experiment. The animals were grouped as follows:

Group 1. Group of 10 mussels, each mussel was injected with 0.1 ml of distilled water and served as control.

Group 2. Group of 20 animals, each mussel received 0.1 ml of 1.5% NaCl in distilled water.

Group 3. Group of 20 animals, each mussel was injected with 0.1 ml of 1.5% NaCl and 0.50 mg of reserpine (aqueous product of CIBA, India).

Group 4. Group of 20 animals, each mussel was injected with 0.1 ml of 1.5% of NaCl and 0.25 mg of chlorpromazine (Beacon Pharmaceuticals, India).

TABLE 1. Effects of reserpine and chlorpromazine on the hypertonic saline provoked neurosecretory changes in the cerebral ganglion neurosecretory cells of *I. caeruleus*.

	A cells			B cells				
	Cell area (μm^2) \pm S.D.	% change in cell area	Nuclear diameter (μm) \pm S.D.	% change in nuclear diameter	Cell area (μm^2) \pm S.D.	% change in cell area	Nuclear diameter (μm) \pm S.D.	% change in nuclear diameter
Control	629.020 \pm 37.34	—	6.406 \pm 0.137	—	437.300 \pm 49.68	—	6.112 \pm 0.034	—
Hypertonic saline (0.1 ml of 1.5% NaCl/mussel)	990.900 ^a \pm 51.18	+57.53	8.281 ^b \pm 0.027	+29.26	613.300 ^b \pm 62.10	+39.34	7.290 ^c \pm 0.026	+19.28
Hypertonic saline + RSP (0.1 ml of 1.5% NaCl + 0.50 mg of RSP/mussel)	613.300 ^d \pm 57.79	- 2.499	6.562 ^d \pm 0.031	+2.435	448.920 ^d \pm 23.16	+ 2.598	6.125 ^d \pm 0.159	+ 0.212
Hypertonic saline + CPZ (0.1 ml of 1.5% NaCl + 0.25 mg of CPZ/mussel)	674.790 ^d \pm 19.01	+ 7.252	6.510 ^d \pm 0.031	+ 1.623	414.820 ^d \pm 34.69	- 5.024	6.250 ^d \pm 0.000	+ 2.258

P values: a, $P < 0.001$; b, $P < 0.005$; c, $P < 0.05$; d, $P > 0.05$.

TABLE 2. Effects of reserpine and chlorpromazine on the hypertonic saline induced neurosecretory changes in the visceral ganglion neurosecretory cells of *I. caeruleus*.

	A cells			B cells				
	Cell area (μm^2) \pm S.D.	% change in cell area	Nuclear diameter (μm) \pm S.D.	% change in nuclear diameter	Cell area (μm^2) \pm S.D.	% change in cell area	Nuclear diameter (μm) \pm S.D.	% change in nuclear diameter
Control	617.920 \pm 61.52	—	6.093 \pm 0.027	—	295.300 \pm 47.56	—	5.458 \pm 0.029	—
Hypertonic saline (0.1 ml of 1.5% NaCl/mussel)	892.250 ^a \pm 48.77	+44.40	7.500 ^a \pm 0.000	+23.11	476.400 ^b \pm 9.00	+60.99	6.914 ^a \pm 0.029	+26.67
Hypertonic saline + RSP (0.1 ml of 1.5% NaCl + 0.50 mg RSP/mussel)	641.900 ^c \pm 41.07	+ 3.88	6.458 ^c \pm 0.023	+ 5.98	305.900 ^c \pm 41.85	+ 3.59	6.041 ^c \pm 0.028	+ 3.60
Hypertonic saline + CPZ (0.1 ml of 1.5% NaCl + 0.25 mg CPZ/mussel)	653.700 ^c \pm 41.81	+ 5.79	6.667 ^c \pm 0.028	+ 9.41	292.760 ^c \pm 45.20	- 1.19	6.250 ^c \pm 0.000	+ 7.18

P values: a, $P < 0.005$; b, $P < 0.001$; c, $P > 0.05$.

Three hours after the respective injections (which were given in the foot) cerebral, pedal and visceral ganglia were dissected out and fixed in aqueous Bouin's fluid. Blocks were prepared and cut at $7\ \mu\text{m}$ and stained with Gomori's Aldehyde Fuchsin (Ewen, 1962). Nuclear diameter and cell area were measured by the techniques described earlier (Adams et al., 1975).

RESULTS

Histological characters of the neurosecretory cells in the cerebral, pedal and visceral ganglia of *I. caeruleus* have already been described (Khatib, 1974).

In control animals (Group 1) neurosecretory cells had a considerable amount of neurosecretory material (Fig. 1). However, after hypertonic saline treatment (Group 2), A and B cells of both cerebral and visceral ganglia showed increase in cell area and nuclear diameter (Tables 1 and 2), whereas neurosecretory material was found to be decreased as compared to the control (Fig. 2).

Administration of CPZ and RSP in the mussels pretreated with the hypertonic saline (Group 3 and 4), showed an increase in the amount of neurosecretory material (Fig. 3 and 4); other neurosecretory characters were essentially similar to those of control mussels (Tables 1 and 2). Pedal ganglion neurosecretory cells did not reveal any changes following any of these treatments.

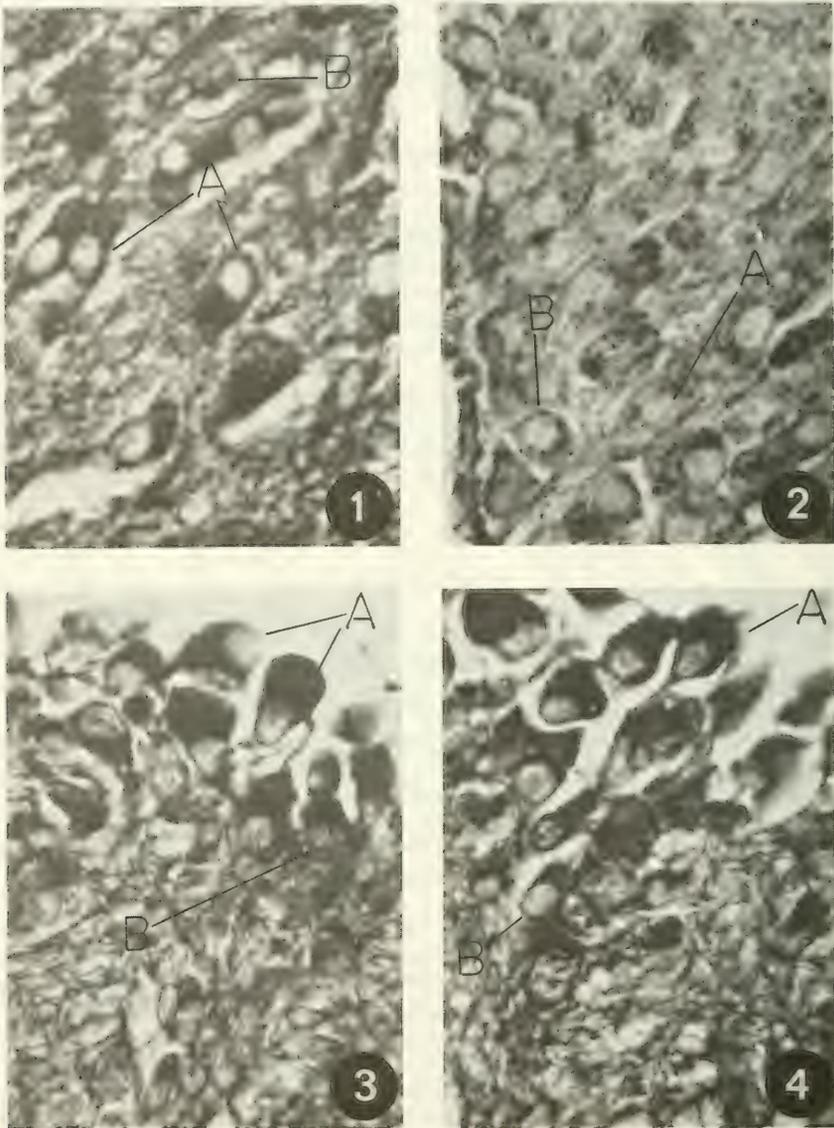
DISCUSSION

The present data show that hypertonic NaCl injection produces an increase in the synthesis of A and B cell NSM of cerebral and visceral ganglia as revealed by their enlarged nuclear diameters. Simultaneously there was an increment in the rates of axonal transport and release of NSM and these were faster than the rates of synthesis of NSM thereby causing emptying of the pericarya. These observations are in agreement with earlier data (Hanumante et al., 1976, unpublished) on *Lymnaea auricularia* wherein disappearance of NSM and enlarged nuclei had been recorded following hypertonic saline treatment.

Neurosecretory kinetics of the pedal ganglion is not altered after hypertonic saline injections, hence it is conceivable that only cerebral and visceral ganglia neurosecretory cells can pick up the messages from osmoreceptors and translate the same into the neurosecretory granules probably for shielding its homeostasis.

Since hypertonic saline administration provokes desiccation, the augmented synthesis and release of NSM from cerebral and visceral ganglia might be an effort of these bivalves to combat the stress of dehydration i.e. released NSM is having antidiuretic effects. However, this does not mean that the liberated NSM is itself an antidiuretic hormone, since it is proved that in vertebrates the histologically demonstrable NSM is not ADH (Sawyer, 1963; Scharrer & Scharrer, 1963). At the most it can be suggested that the released NSM following hypertonic saline treatment in the mussels may be a vehicle or precursor of the antidiuretic hormone-like principle. This hypothesis is supported by the fact that in mammals and birds (Gabe, 1966; Singh & Dominic, 1974b) there is a close physiological relationship between the NSM and an ADH, since both are depleted after desiccation or hypertonic saline administration and reappear when the animals are brought back to normal conditions. As such the vicissitudes in the cerebral and visceral ganglia neurosecretory cells following hypertonic saline treatment can be considered as cytological evidence of increased synthesis and release of ADH-like factor in *I. caeruleus*.

The neurosecretory profile of CPZ and RSP administered mussels, pretreated with hypertonic saline did not show any change to that of the control, thereby suggesting that both CPZ and RSP suppress the appearance of hypertonic saline induced neurosecretory changes as in higher vertebrates by RSP (Kulshreshtha & Dominic, 1971a; Singh & Dominic, 1974a), by CPZ (Kulshreshtha & Dominic, 1971b; Singh & Dominic, 1974b) and in invertebrates by RSP (Hanumante et al., unpublished). Suppression of the hypertonic saline stimulated histological changes in the HNS of the musk shrew *Suncus murinus* by CPZ (Kulshreshtha & Dominic, 1971b), RSP (Kulshreshtha & Dominic, 1971a) and ethanol (Kulshreshtha & Dominic, 1972), the owllet



FIGS. 1-4. Neurosecretory cells of the cerebral ganglion of *Indonaiia caerulea*. 1, Control (distilled water injected). Note moderate NSM in the pericarya. 2, After hypertonic saline (0.1 ml of 1.5% NaCl per animal) administration. Note the increased cell area, nuclear diameter and less NSM as compared to control. 3, After RSP (0.50 mg per animal) pretreated with hypertonic saline (0.1 ml of 1.5% NaCl per animal). 4, After CPZ (0.25 mg per animal) pretreated with hypertonic saline (0.1 ml of 1.5% NaCl per animal). All AF $\times 1000$. A = A neurosecretory cells, B = B do.

Athene brama by CPZ (Singh & Dominic, 1974b), RSP (Singh & Dominic, 1974a) and ethanol (Singh & Dominic, 1974c) and in the A cells of the freshwater snail *Lymnaea auricularia* (Hanumante et al., unpublished) by RSP has been regarded as histological reflection of the inhibition of ADH or hormonal principle secretion by these chemicals. Consequently, inhibition of hypertonic saline induced neurosecretory changes by RSP and CPZ can be regarded as the histological proof displaying decline in synthesis and release of antidiuretic hormone-like factor. Further experiments are being undertaken to test the validity of this conclusion.

Thus it is interesting to note that even though these freshwater molluscs are evolutionary separated from the vertebrates by a very wide gap in time, both groups share a common constellation of certain neurosecretory features.

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AESTIVATING SNAILS—THE PHYSIOLOGY OF WATER REGULATION IN THE MANTLE OF THE TERRESTRIAL PULMONATE *OTALA LACTEA*P. F. Newell¹ and T. C. Appleton²

ABSTRACT

Aestivating *Otala lactea* snails are able to regulate the rate of water loss from the mantle collar epithelium, losing water as slowly as an insect cuticle (Machin, 1974). Regulating animals develop an osmotic gradient in the upper part of the cells but this epithelium is not obviously structurally adapted to reduce water loss, the cells having features usually associated with absorptive cells. Cells from aestivating animals develop dense caps of lamellate vesicles which are absent in normal snails (Newell & Machin, 1976). X-ray microanalysis of freeze dried ultra-thin frozen sections of mantle collar cells has shown that there is a steep concentration gradient of K and Cl ions present in the apical 2 μm of these cells. The ionic gradient is developed apically to the lamellate vesicles and is not found in cells taken from active, non-regulating snails.

INTRODUCTION

Snails are well adapted for life on land. Often these adaptations are shown in their ability to conserve water. In many the behaviour of the animal can reduce the rate of water loss and most terrestrial molluscs are nocturnally active after rain, and during periods of drought snails become inactive and aestivate. Machin (1974) has shown that aestivating *Otala lactea* Müll. can reduce the rate at which water is lost from the mantle collar epithelium, which plugs the mouth of the shell in the withdrawn snail. Machin showed that this epithelium develops an osmotic gradient in hibernating snails equal to a depression of the freezing point of 8°C. This gradient is very steep, being around 4 moles per kg and is concentrated in the upper part of the cells; about 3 moles of this gradient being concentrated in the upper 2 μm of these cells. It has been shown that the development of the osmotic gradient in aestivating snails is related to the formation of dense caps of lamellate vesicles in the mantle collar cells (Newell & Machin, 1976).

The generation of osmotic gradients across epithelia and within cells is of fundamental importance to many living organisms, and it is thought that most fluid transport processes follow the accumulation of ions within tissues. Thus, the study of ionic concentrations within these cells has wider implications than merely to improve our understanding of the physiology of aestivating land snails.

Ionic gradients within these cells can be studied by X-ray microanalysis of freeze dried ultrathin frozen sections. Preliminary results from the X-ray microanalysis of freeze-dried ultrathin frozen sections showed that the osmotic gradient is primarily generated by potassium and chloride ions (Appleton & Newell, 1977) and this paper presents further information about this gradient.

MATERIALS AND METHODS

Hibernating *Otala lactea* snails were kept in a dry, undisturbed place in the laboratory. Other snails were kept in a glass tank with moist lettuce leaves, and these actively crawling and feeding snails served as controls to the hibernating animals. Small samples of tissue (1 mm³) were taken from the mantle collar epithelium by smashing the shell, removing small pieces of

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the mantle collar and freezing them onto small brass specimen holders with liquid nitrogen slush at -210°C . The sample preparation process was rapid and took less than 20 sec from picking up the snails from their container to freezing of the tissues.

The blocks were carefully trimmed in the cryostat so that the square block face included a vertical section through the most superficial cells of the mantle collar epithelium. The mucus covered microvilli were always positioned on the sides of the block and never on the top or bottom; thus, the knife always passed across the cells parallel to the surface of the epithelium. Ribbons of sections were cut on an ultramicrotome set at 130 nm held in a cryostat at a temperature at which ribbons of sections could just be obtained, which was -72°C for the controls and -76°C for the regulating snails. The ribbons were collected onto formvar coated nickel grids, pressed onto the grids and then allowed to freeze dry for 3 h at the cutting temperature in the cryostat in a dry nitrogen atmosphere. The sections remained in the cryostat overnight, the temperature of which slowly warmed to -15°C , and on the following day were taken to ambient temperature also in dry nitrogen. The sections were then coated with 20-30 nm of carbon to prevent the sections from rehydrating (Appleton, 1974).

The coated sections were analysed either in the AEI Cora (courtesy of Kratos Limited, AEI Scientific Instruments) or an EMMA 4 analytical electron microscope equipped with a LINK system energy dispersive detector. The grids were held in a specimen rod fitted with graphite inserts which reduced the background readings. In all cases the analytical conditions were carefully monitored and matched between both instruments and were as follows: accelerating voltage 60 Kv; beam current as measured by Faraday Cage, 4 nAmps; spot size 100 nm; overall counts per second 200-700; counting time 100 seconds.

For conventional electron microscopy small pieces of tissue, about 1 mm^3 , were fixed for 1 h in 1% glutaraldehyde and 1% acrolein dissolved in 100 mM sodium cacodylate, pH 7.4. The tissue was then rinsed in fresh buffer before being transferred to 1% osmium tetroxide made up in 100 mM sodium cacodylate for 1 h. The fixed tissue was then dehydrated in a graded acetone series and then embedded in Araldite resin polymerised at 20°C and thin sections cut on an LKB-Huxley ultramicrotome.

RESULTS

The results obtained from a comparison of the analysis of both aestivating and control snails show that in aestivating snails there is a steep ionic gradient from the top of the cell to a point between 1.5 and $2\ \mu\text{m}$ below the base of the microvilli. Initially data for many elements were collected (Appleton & Newell, 1977) but the elements primarily responsible for the osmotic gradient first observed by Machin (1974) are K and Cl. Fig. 1 presents a graph which shows the gradients measured for those 2 ions from a number of measurements from a series of frozen sections. The decrease in concentration takes place very sharply and the lowest mean levels of both K and Cl in the apex of the cell occurs between 1.5 and $1.7\ \mu\text{m}$ from the apex. The analytical data can also be presented in terms of relative mass fractions and the graphs obtained are virtually indistinguishable from that shown in Fig. 1.

Electron micrographs of conventionally fixed material show a dense apical cap of lamellate vesicles in regulating cells (Newell & Machin, 1976). The zone of the steepest ionic gradient is above the zone of the vesicles and would be in the terminal web of the mantle epithelial cells underneath the microvilli. A micrograph taken from a frozen section is shown in Fig. 2. In this figure subcellular details are clearly shown, including microvilli, nuclei, junctions and mitochondria. The sub-cellular preservation in these sections is good and the detail shown is in good agreement with that shown in conventionally fixed material shown in Fig. 3. Artefact produced during the specimen preparation procedure is minimal and so ionic gradients measured within these cells are probably characteristic of the intact cell.

DISCUSSION

The frozen sections analysed showed little ice crystal damage and the amount of ultrastructural detail visible made analysis in various sub-cellular organelles comparatively easy (Appleton & Newell, 1977). The large electron lucent areas are not holes in the sections, but are

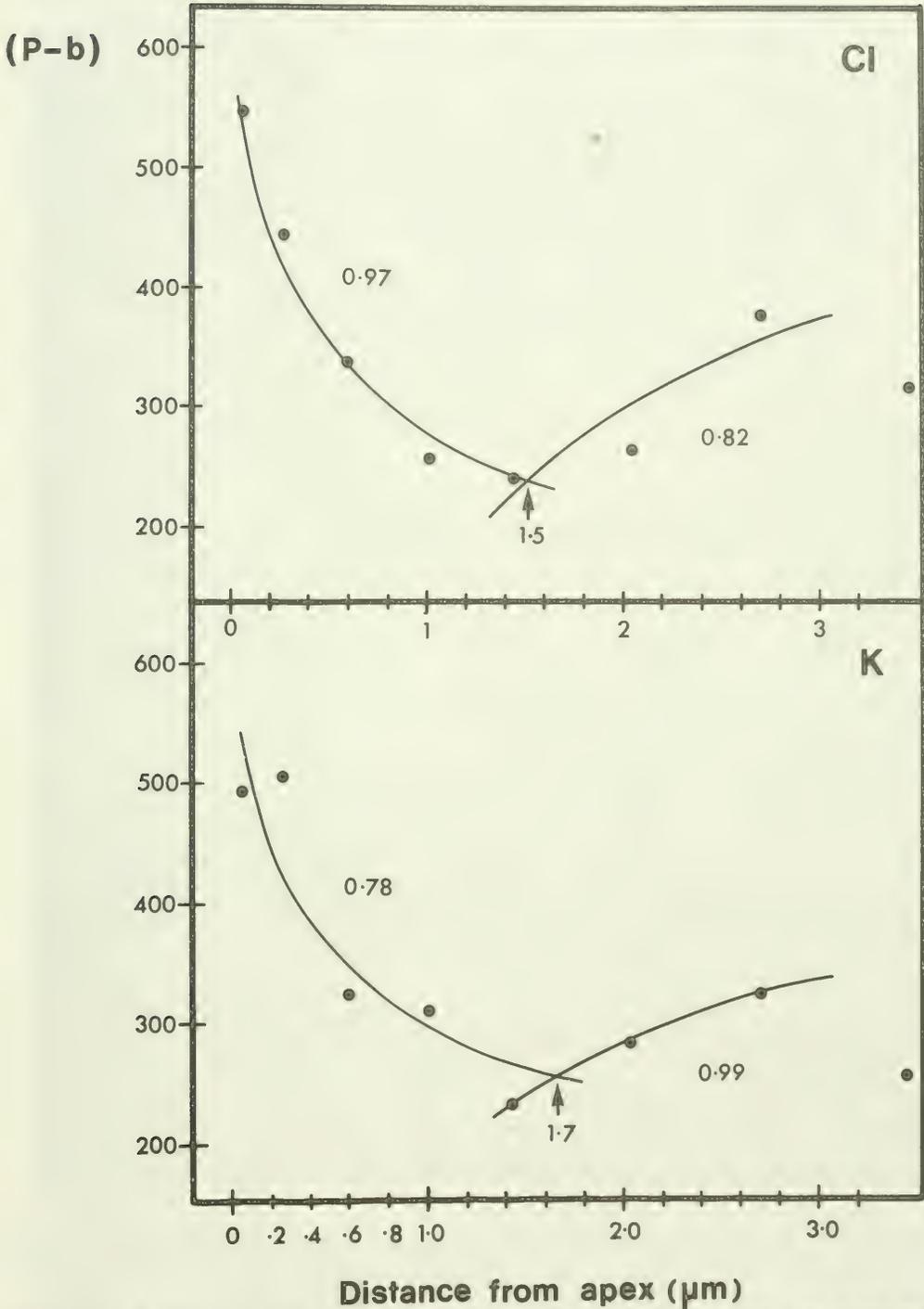
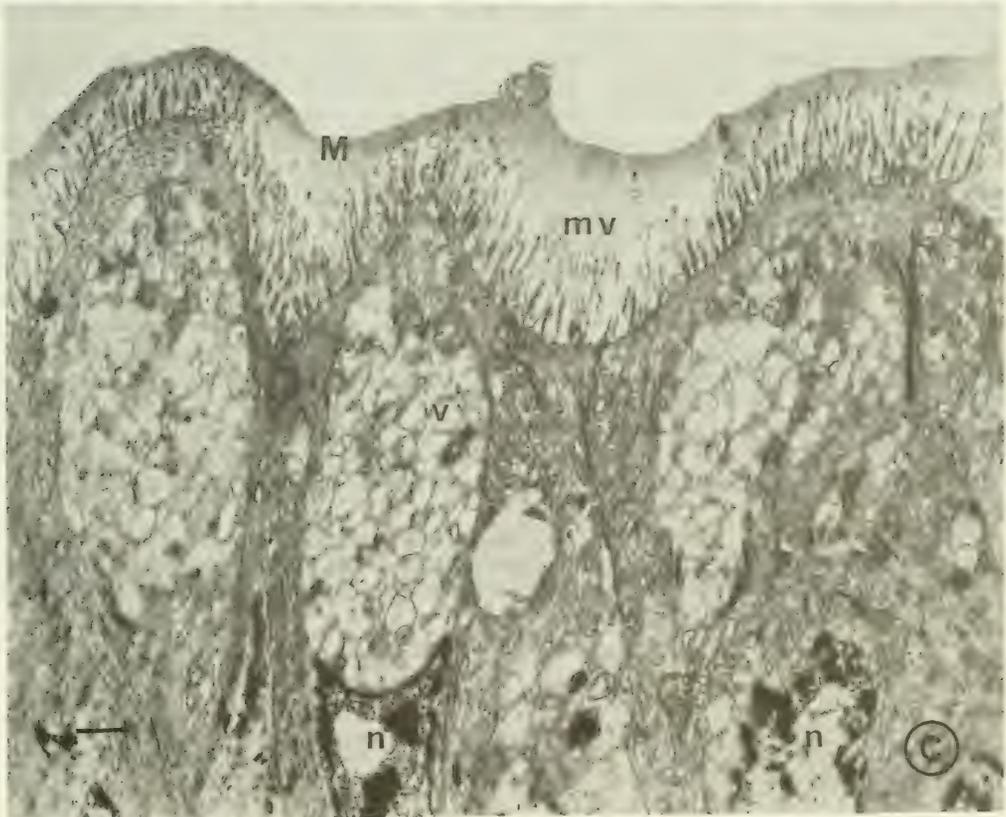
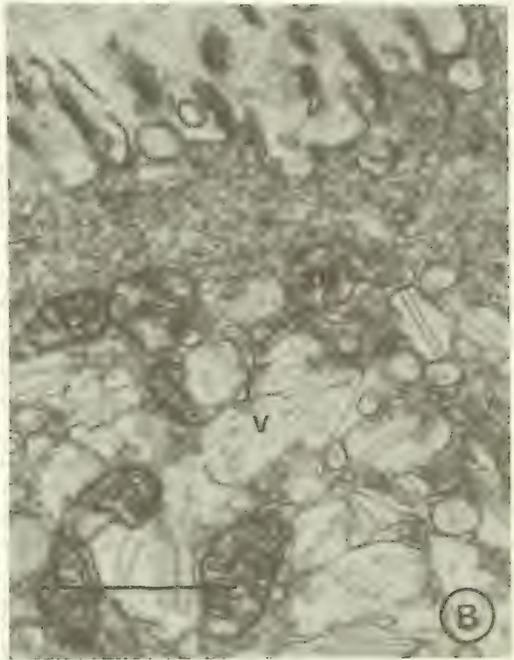
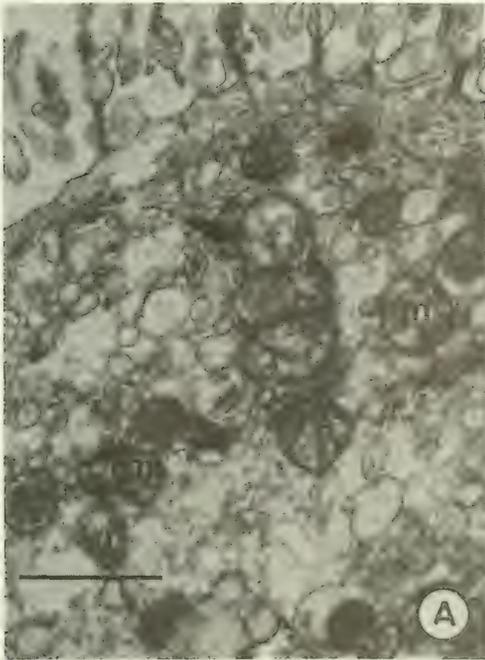


FIG. 1. Graph showing the profile of the K and Cl ionic gradients in aestivating *Otala*. The vertical axis is a measure of the concentrations of these ions per unit volume of the cell. The figures beside the fitted log curves are the coefficients of fit for the log curve (r^2), the closer the fit the nearer this figure approaches one.



FIG. 2. Electron micrograph of a freeze dried ultra-thin frozen section from an aestivating snail. The lamellate vesicles are not obvious in these sections, perhaps because in unfixed material they lack contrast with the surrounding cytoplasm. This material has not been fixed, stained or treated in any way ($\times 10,000$). j, junctional complex; m, mitochondria; mc, mucocyte; mv, microvilli; n, nuclei. Scale $1 \mu\text{m}$.

FIG. 3. Conventional transmission electron micrographs from the mantle collar epithelium of *Otala*. A, Apical area from a normal, non-aestivating snail. There is no cap of lamellate vesicles, $\times 19,000$. B, Apical area from an aestivating snail, $\times 26,000$. C, Low power micrograph taken of cells from an aestivating snail. Note the dense caps of lamellate vesicles, shown in detail in B, $\times 7,200$. m, mitochondrion; M, mucus; mv, microvilli; n, nuclei; v, lamellate vesicles. Scale $1 \mu\text{m}$.



sections of mucocytes. This interpretation is supported by our analytical results which give a characteristic elemental analysis in these areas, with a high sulphur content corresponding to mucus.

The use of ultra-thin freeze dried sections as opposed to thick frozen sections is justified by the improved resolution of the ultra-structural detail. Thus, identification and analysis of sub-cellular organelles can then be confidently made. However, it is recognised that the sectioning process may well involve a limited amount of melting of the tissue in the vicinity of the knife edge. But, although the possibility of this error is recognised, it has not been identified. The resolution available with current instrumentation is about 100 nm and we conclude that should any elemental redistribution occur during either sectioning or freeze drying, then it is below the current limits of detection. This has been tested by measuring the ionic concentrations on both sides of the membranes of the microvilli and no evidence of leakage was found. Some sections of mantle tissue were allowed to rehydrate after freeze drying and during the subsequent analysis no gradients could be detected.

The orientation of the blocks was very carefully kept so that the gradient being measured was at right angles to the plane of sectioning. This has enabled us to eliminate the possibility that the ionic gradients measured could, in any way, be related to a redistribution phenomenon occurring during the sectioning process.

The sectioning process produces ribbons of sections, and this is taken as evidence that their thickness, when cut, is fairly uniform. However, after freeze drying both the thickness and the mass of the section is not uniform, the areas of the cell with most water drying to produce a thinner specimen with less mass, than the areas of the cell with the least water which will be relatively unchanged by the freeze drying process. Thus, Hörling (personal communication) has suggested to us that it should be possible to express our results by volume ($P - b$) by not taking the continuum (W) into account to produce results expressed as the more conventional relative mass fractions ($\text{RMF} = (P - b)/W \times 100$). This seems correct and in fact almost identical curves are produced if these results are expressed by volume, as in Fig. 1 or by relative mass fractions.

If the more hydrated areas of the cell have the least mass (as measured by the continuum, W) then by plotting the mean figures for W a profile for cell mass and so perhaps the water content of the cell, can be obtained. This has been done and the curves obtained are very similar to those shown in Fig. 1, with a double log curve broken at $1.5 \mu\text{m}$ from the cell apex. Thus, the apical area of the cell size shows a dehydration gradient, the driest point being at the surface. The water content of the cellular cytoplasm increases to $1.5 \mu\text{m}$ below the surface, then decreasing at $4 \mu\text{m}$ to the same levels shown by the microvillous area.

It thus seems that hibernating *Otala* are able to regulate the rate of loss of water from the mantle collar epithelium by producing an osmotic gradient in the upper part of the epithelial cells. This gradient exists apically and to the outside of a dense band of lamellate vesicles. Somehow, water is prevented from moving freely within these cells, and it is not yet clear whether the formation of the vesicles precedes the establishment of the osmotic gradient, or vice versa. However, the process is not just a simple one of establishing a potassium and chloride gradient, as many other ions are significantly altered in hibernating snails when they are compared to active snails. These include silicon, whose rôle in the cell is not clear, and iron and zinc. Both iron and zinc are elements associated with enzymes as metallic cofactors, zinc being associated with carbonic anhydrase and iron with, amongst other things, cellular oxidative enzymes.

The ability of snails to reduce the rate at which water is lost from their mantle collar whilst aestivating has great survival value, enabling snails to colonize warm and dry areas. It is thought that such a mechanism might also be found in fresh water pulmonates, many of whom are able to survive out of water for prolonged periods.

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ENVIRONMENTAL OSMOLARITY AND NEUROSECRETORY NEURONES
IN *LYMNAEA STAGNALIS* (L.)

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ABSTRACT

Cell body volume and Alcian blue-Alcian Yellow staining properties of neurosecretory neurones in the brain of *Lymnaea stagnalis* were compared for snails kept in de-ionised water and standard tapwater. In the same experiment, the ionic content of the blood, blood volume and body weight and environmental ionic composition were measured. Five days of immersion in de-ionised water resulted in significant decreases in body weight, blood volume and blood, Na^+ and Cl^- concentrations but no change in blood Ca^{2+} , K^+ , and HCO_3^- concentrations, compared with controls. No consistently significant differences across the 5 day period were found in cell body volumes for Dark Green Cells, Yellow Cells or Light Green Cells (used as a control) when these volumes were compared for large numbers of cells from snails kept in de-ionised water and standard tapwater. However, the number of Yellow Cells which could be counted in snails kept in de-ionised water was lower than the number from standard tapwater by day 2 of the experiment and lower for Yellow-green Cells by day 5. We interpret this lower number to be the result of depletion of Alcian blue-Alcian Yellow stained neurosecretory material in these cells which made them impossible to distinguish. This was confirmed by examination of visceral Yellow Cells which could be identified on the basis of known location close to the visceral-right parietal connective.

INTRODUCTION

From the work of Wendelaar Bonga (1970b) it is known that the brain of *Lymnaea* contains a number of neurosecretory cell types that may be largely characterised according to their colour on staining light microscope sections with Alcian Blue-Alcian Yellow. These various cell types occur either scattered, in ones and twos, or in distinct clusters. There is evidence from a number of studies (Wendelaar Bonga, 1971, 1972; Roubos, 1973, 1976) that two of these cell types, the Dark Green Cells and the Yellow Cells show increased synthesis and release of neurosecretory material on exposure of animals to de-ionised water and a suppression of these activities on exposure to hypertonic saline. It has further been suggested that these changes are probably mediated, in the Dark Green Cells at least, by changes in the osmolality of the blood (Roubos, 1973, 1976). The intention of the present study was to investigate this further by making detailed measurements of changes in the concentrations of the major blood ions, together with changes in blood volume on exposing snails to de-ionised water. In the same animals 4 types of neurosecretory cells were examined in Alcian Blue-Alcian Yellow stained material under the light microscope, for indications of altered secretory activity. As well as the Dark Green Cells and the Yellow Cells, the Yellow-green Cells and the Light Green Cells also were examined. The Yellow-green Cells are found in more or less the same locations as the Yellow Cells and often in mixed clusters with them. The Light Green Cells, which were used as a control, have been shown to be involved in the neuro-endocrine regulation of growth (Geraerts, 1976). Wendelaar Bonga (1971) and Roubos (1973) both showed that significant changes in the cell body volumes of Dark Green Cells occur under the same conditions as those causing increased synthesis and release in these cells. It was thought that a similar phenomenon might also be shown by the Yellow Cells and be a useful indicator of secretory activity.

The results obtained showed a significant decrease in the visible numbers of Yellow Cells and Yellow-green Cells that could be counted after 2 days exposure of animals to de-ionised water. This was preceded by a fall in the blood concentrations of both Na^+ and Cl^- and was followed

by a drop of blood volume. No consistent, statistically significant differences were found for the visible numbers of Dark Green Cells, the cell body volumes of Dark Green Cells, Yellow Cells or Light Green Cells, or the blood concentrations of K^+ , Ca^{2+} or HCO_3^- between animals from standard tapwater or de-ionised water.

MATERIALS AND METHODS

Sixty weighed snails were immersed individually in standard tapwater (S.T.W.) and 50 in de-ionised water (D.W.) for periods of 0 to 5 days. Each animal was kept in a separate plastic beaker containing 500 ml of its particular medium, which was replaced daily. Every 24 hours, 10 animals from each group were removed and measurements made of body weight, blood volume and the concentrations of Na^+ , K^+ , Ca^{2+} , Cl^- and HCO_3^- (or more precisely total alkali). Blood samples were obtained by provoking its extrusion through the haemal pore. The maximum possible volume of blood was obtained from each animal. The brains were then removed, fixed for 24 hours in Stieve's fixative, sectioned in paraffin wax at $10\ \mu m$ and stained with Alcian Blue-Alcian Yellow (Wendelaar Bonga, 1970b).

Using the light microscope, the cell body volumes of all the Dark Green Cells, a cluster of Yellow Cells in the visceral ganglion and the 10 most ventrally situated Light Green Cells in each cerebral ganglion were measured by the method of Swindale & Benjamin (1976). Where staining was faint in the Yellow Cells, dark field rather than bright field illumination was used to increase the contrast between stained and unstained cells (Swindale & Benjamin, 1975). In each animal, the total, visible numbers of Dark Green Cells, Yellow Cells and Yellow-green Cells were measured using bright field illumination only.

RESULTS

The visible numbers of Dark Green Cells that could be counted over the 5 day period of this experiment remained very constant in both D.W. and S.T.W. groups, at a mean of about 30 per animal. For both Yellow Cells and Yellow-green Cells, the visible numbers of cells in the S.T.W. animals also remained very constant at around 25 per animal for each cell type. In D.W. animals, however, counts for both showed a clear and statistically significant drop after 2 days exposure. Clearly, stain was not being taken up by the cell bodies of these neurones in D.W. animals to the same extent as by those of the S.T.W. animals, rendering them indistinguishable from the surrounding neurones. Since the Alcian Blue-Alcian Yellow stains bind to neuro-secretory material (discussed by Swindale & Benjamin, 1976) this material was clearly being depleted from the cell bodies as a result of the de-ionised water treatment.

Measurements of the cell body volumes of the Dark Green Cells and Yellow Cells gave rather disappointing results. We were unable to detect the significant increases of these in animals exposed to de-ionised water suggested by the results of Wendelaar Bonga (1971) and Roubos (1973) for Dark Green Cells. Only on day 3 were the somas of the Yellow Cells in D.W. animals significantly larger than those of S.T.W. animals. Measurements of Light Green Cells, for which no evidence has yet been presented supporting a role in ion or water regulation, did produce results that indicated a significant difference between S.T.W. and D.W. animals, although this was not consistent. The volumes in D.W. animals were sometimes lower and sometimes higher than those in S.T.W. animals. This result indicated that rather non-specific cell body volume changes can occur under these conditions.

The blood concentrations of Na^+ and Cl^- in S.T.W. animals remained constant over the experimental period at around 55 mM and 37 mM respectively. Their concentrations in D.W. animals, however, fell from the onset of exposure, the loss reaching 25% for each ion by day 5. Further measurements made on other animals after 7 and 10 days exposure to de-ionised water showed that Na^+ and Cl^- remained at this 25% lower level.

The blood concentrations of K^+ , Ca^{2+} and HCO_3^- remained constant over the experimental period in both S.T.W. and D.W. groups and we were unable to detect any significant differences resulting from the experimental treatment. In terms of blood ion changes, then, the drop in concentration of Na^+ and Cl^- is clearly the major effect of exposing *Lymnaea* to de-ionised water.

Blood volume changes were examined in 2 ways. Firstly they were estimated from the percentage body weight change over the period of exposure. This gave rather variable results, but after day 4, D.W. animals showed a significant drop of body weight with respect to S.T.W. animals, reflecting a decrease in blood volume. This drop reached 8% of the original body weight on day 5. Secondly, direct measurements were made on the extruded blood samples. This produced very consistent results for S.T.W. animals when expressed as a percentage of the original body weight, supporting the validity of the method. D.W. animals again showed a significant drop in blood volume, falling from about 34% to 28% of the original body weight, after day 4. The results obtained using these 2 methods were therefore complimentary.

DISCUSSION

The results of this experiment give evidence for the depletion of neurosecretory material from the cell bodies of 2 cell types, the Yellow Cells and the Yellow-green Cells, on immersing animals in de-ionised water. This presumably indicates that the synthesis of neurosecretory granules is no longer keeping pace with their release. In the light of the results of Wendelaar Bonga (1972), who gave ultrastructural evidence for increased release of material from the axon terminals of Yellow Cells from de-ionised water treated snails, it is sensible to suggest that this imbalance is the result of increased release of material rather than decreased synthesis. It was somewhat surprising that we were unable to obtain any further evidence for altered secretory activity by the Dark Green Cells for either cell body volume measurements or reduced staining intensity, reflected in the counts of total visible cell bodies. It seems that cell body volume measurements, at least in the short term, are not a reliable indicator of the state of synthetic or release activity in a cell, particularly considering the rather non-specific changes shown by the Light Green Cells.

If the Yellow Cells and the Yellow-green Cells are altering their secretory activity as a result of some change in the blood osmolarity, as suggested for Dark Green Cells by Roubos (1973), then Na^+ and Cl^- levels seem to be likely candidates. Both ions show a large significant drop in concentration on exposing animals to de-ionised water while the levels of K^+ , Ca^{2+} and HCO_3^- remain very constant. They could be acting either as individual ion species, together, or as the cause of the overall change in blood osmolarity. The drop in blood volume that occurs is rather delayed with respect to the first signs of depletion of material in the Yellow Cells and Yellow-green Cells. It seems more likely therefore that this fall of blood volume is a result of the altered secretory activity of one or both of these cell types rather than the cause.

The neuronal geometries of Yellow Cells and Yellow-green Cells have been studied by Swindale & Benjamin (1976) and they have been shown to project both centrally and peripherally. It is interesting that axons containing neurosecretory granules of the diameter characteristic of Yellow Cells (Wendelaar Bonga, 1970b) have been found in the kidney epithelium (Wendelaar Bonga, 1970a, 1972) and that Yellow-green Cell type axons have been found in the heart and aorta (Plesch, 1977). Release of neurohormone in either of these sites could be important in the control of water or ion balance. The kidney, which may normally be involved in the active excretion of ions (De With, 1977) may also be important in the active reabsorption of ions when their intake from food or the environment drops. The heart is probably the site of filtration of primary urine into the pericardium (Picken, 1937). It might also, of course, merely represent a release site that ensures the rapid distribution of a hormone.

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THE PALLIAL GLAND OF *LITHOPHAGA LITHOPHAGA* (L.): A HISTOCHEMICAL AND BIOCHEMICAL APPROACH OF THE ROCK BORING PROBLEM

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ABSTRACT

The rock boring activity of *Lithophaga* has been related to secretions from the mantle. Histochemical and ultrastructural research has been carried out in order to provide a histomorphological description of the glands involved in the secretion. A biochemical analysis of the main constituents of the glands and its secretion have also been undertaken. A single type of gland is present in the mantle (pallial gland) with 2 types of cells, viz. (A) gland cells containing glycogen and glycolipoprotein (staining for the proteins is enhanced after chloroform/methanol extraction and that for the lipids after proteolytic digestion), and (B) interstitial cells containing lipid droplets stainable with Sudan Black B and Nile Blue Sulphate. Biochemical analysis confirms the presence of glycogen (0.8% W/W). The lipid fraction (13.8%) consists mainly of glycerides and cholesterol. Phospholipids, sulpholipids and glycolipids have also been separated by TLC. The amount of sulpholipids changes according to the season. Glycolipids do not contain sialic acid. Dialyzable and non-dialyzable lipid-bound peptides have been separated after chloroform/methanol extraction. These are characterized by a high percentage of lysine (1.7% of all amino-acid concentration) and a high concentration of aspartic acid and glutamic acid. The function of Ca^{++} binding activity is probably due to lipoproteic components with acidic groups ($\text{pK}_a < 3$).

The mechanism responsible for calcareous rock boring in *Lithophaga lithophaga* (L.) is still much debated. Among the papers published during the last 10 years combined mechanical and chemical mechanisms have been postulated in explaining rock boring. Acid secretions due to glycosaminoglycans (Hodgkin, 1966) or neutral mucoprotein Ca^{++} chelating (Jaccarini et al., 1968; Newell, 1964) were considered the chemical agents responsible for rock boring.

Morphological research was carried out on the pallial gland aiming at identifying the cells related to this secretion. As early as 1903 Carazzi described 3 kinds of glands (protoacid, anterior acid and posterior acid glands). Yonge (1955) attributed to the anterior gland the responsibility for producing acid mucopolysaccharides (Alcian Blue positive). The posterior gland is likely to be used to widen a hole already excavated by secretion from the anterior gland.

Owing to the lack of histomorphological data on the pallial gland and to the many hypotheses on the chemical nature of the secretion as well as its mechanism in rock boring, we thought it worth while to investigate the pallial gland of *Lithophaga* by histochemical, ultrastructural and biochemical procedures. Biochemical analysis was also performed in order to separate and evaluate glycogen (as energy storage for performing chemical work), polar lipids and peptidic components (as main constituents of the gland).

MATERIAL AND METHODS

Histochemical research

Morphological stains (Haematoxylin-Eosin, Azan-Mallory, etc.) and specific histochemical tests have been carried out on sections of glands from *Lithophaga* from Ligurian beaches: neutral and acid mucopolysaccharide reactions; reactions for proteins carried out directly or

after digestion by pepsin or extraction by chloroform/methanol; reactions for nucleoproteins. Histochemical tests for lipid with Sudan Black B and Nile Blue Sulphate were performed directly and after solvent extraction (pyridine, chloroform/methanol 1:2) or digestion with pepsine. The following enzymes were tested: succinic-dehydrogenase, lactate, isocitrate, malate, and glucose-6-P dehydrogenases; diaphorases (NAD⁺ and NADP⁺); (Na⁺, K⁺)-ATPase; acid and alkaline phosphatases; arylsulphatases; carbonic anhydrase.

Biochemical methods

Biochemical research was aimed at

- (1) Analyzing aqueous homogenate of the gland for
 - protein concentration;
 - amino-acid separation from acid hydrolysate;
 - determination of hexosamines, hexoses and sialic acid.
- (2) Analyzing chloroform/methanol extraction
 - testing polar lipid bound, sulphatides, cholesterol, hexoses;
 - TLC separation of polar lipid;
 - evaluation of amino-acids in dialyzable and non dialyzable peptidic components in chloroform/methanol extracts.
- (3) Analyzing the defatted residue for hexosamines, hexoses, amino-acids.
- (4) Extracting the glycogen.

RESULTS

(A) Histomorphological and histochemical results

In the mantle of *Lithophaga* one gland only is present. The gland appears to be histologically uniform and no ducts can be shown. Amorphous masses of secretion between alveoli are noted at several locations in the gland. Under common morphological stains in the gland there appears a single cell type only; but with Sudan Black B some triangular cells can be seen with large sudanophilic granules in their cytoplasm. This cell type is likely to be the "interstitial cells" described by the early authors. The presence of 2 types of cell was confirmed by ultrastructural research. The first cell type, which we have called "interstitial cells" is characterized by the peculiar shape, size and aspect of the secretion granules, which are all of the same kind and very electron dense. The secretion is positively produced in these cells, since only here the Golgi complex appears involved in active secretion.

In the other cell type, which we have called "storage or glandular cells" 2 quite different kinds of granules can be seen: one is electron dense and exhibits characteristics similar to those of the granules of interstitial cells, the other is much less electron dense and is found only in this type of cell.

In our opinion, the secretion originally produced by interstitial cells subsequently passes to the storage cells, where it is partially re-elaborated. The secretion granules appear to be slightly PAS positive, but negative to the usual stains for MPS. The granules react positively to reactions for protein and only slightly positive with Sudan Black B and Nile Blue Sulphate.

The most interesting results are achieved when the cryostat slices are digested with pepsine before staining with Sudan Black B or are extracted by chloroform/methanol before staining for protein; both stains appear stronger. The electron microscope pictures are supporting our view that the secretion granules pass outside through the epithelium.

Biochemical results

Analytical data concerning the gland in toto are reported in the following table:

Amino-acids ¹ (protein)	9-11	%
Lipids	13.8	%
Hexoses	4.8	%
Hexosamines	0.43	%
Sialic acid	absent	
Ca	0.073%	

¹25% are basic amino-acids (lysine 1.7%); glutamic and aspartic acids are about 1/3 of total amino-acids.

The results of the analysis obtained from crude lipid extract are reported in the following table:

Cholesterol	4.20%
Lipid bound phosphate	0.80%
Lipid bound hexosamines	0.24%
Lipid bound hexoses	0.64%
Sialic acid	absent
Sulpholipids	0.051-0.154%

No true gangliosides have been observed. Phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine are the main phospholipid compounds demonstrable by TLC, in addition to sulpholipids. The last class do not resemble true sulphatides on the basis of I.R. profile. Two-thirds of amino-acids present in hydrophobic peptide (extractable by chloroform/methanol) are glycine and alanine.

The analysis of delipidized residue (23-28% of wet weight) outlines the high protein (31.7%) and glucidic (hexoses 25%) constituents. Less than 1% of hexosamines are bound in glycopeptidic compounds. The hexoses are probably bound to glycopeptides, but a large concentration of glycogen is also extractable (0.8%).

CONCLUSIONS

The secretion from the gland cell, which takes the form of granules, was characterized on the basis of histochemical reactions and biochemical analysis as being constituted by glycolipoproteins. The protein and lipid fraction appear in some way to be bound together, at least in some granules. There are, however, secretion granules which are holoproteic, some other hololipidic. As regards the function of the secretion, it seems to have a Ca^{++} binding activity due to a lipoprotein. Lipoprotein and glycoprotein (without sialoresidues) have been demonstrated. Lipid concentration reaches 13% (wet weight). The crude lipid extract contains cholesterol (more than 1/3), phospholipids and glycolipids, but no gangliosides. Sulpholipids (not belonging to true sulphatides) are present and their concentration changes during the year. TLC separation of polar lipid confirms the absence of gangliosides and reveals the phospholipids spots corresponding to phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine. Among the peptidic components soluble in chloroform/methanol 2:1 it is worth mentioning that a non-dialyzable peptidic component contains phosphorylated amino-acid derivative. A considerable amount of glycogen has been isolated by extraction (0.8%). It is likely that the glycogen, stored in the gland as large granules, is a remote energy reservoir for chemical work, while the boring mechanism could be attributed to some Ca^{++} binding lipoproteins, which act as chelating agents.

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CYTOMORPHOLOGY AND HISTOCHEMISTRY OF THE GLANDULAR
CELLS IN THE FOOT OF *CERNUELLA (XEROMAGNA)*
CESPITUM ARIGONIS (ROSSMASSLER)

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A study was made of the various kinds of glandular cells in the foot of *Cernuella (Xeromagna) cespitum arigonis* (Rossmässler, 1854). The specimens were collected in the gardens of the San Marcos Hotel and in fields around the city of León, Spain. After identification (genitalia, see Fig. 9) they were experimentally infected with larvae I of *Neostromylus linearis* (Nematoda, Metastrongylidae), whereby we showed that the larval stages acquired their infecting power inside the snail's foot. The specimens were killed by immersion in water, fixed in Bouin and Carnoy's liquid modified by Hetherington, included in paraffin and cut horizontally, longitudinally and transversely into sections 8-10 μm thick. After treatment with several histological and histochemical techniques (viz., Böhmer's and Crazzi's Hematoxylin, Yellow Eosin, Ziehl's Fuchsin, Carmine Picro-Indigo, Alcian Blue, Leve & Spicer's formula, P.A.S., Masson & Mallory's Trichromics, Gomori for reticuline fibres, and Methyl-Pironin Green according to Unna-Pappenheim) microscopical examination demonstrated that in the foot of *Cernuella cespitum arigonis* there are various kinds of glandular cells: A, B and C. These cells differ greatly in siting, morphology, structure and chemical make-up. Further research, already underway, using the electron microscope, is expected to clarify our conclusions.

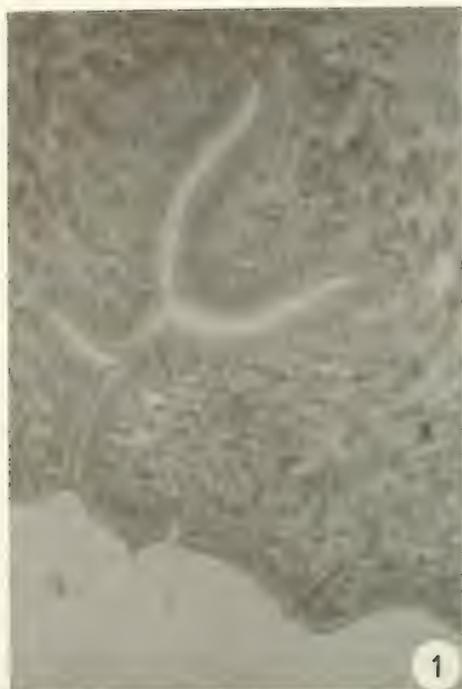
Type A is found in large accumulations at the end of the foot (Figs. 1, 4). The cells are voluminous and polygonal, with the nucleus in the centre and dense, evenly distributed chromatin. Green Pironin staining reveals a nucleolus in a central position. The nucleus is small (4-5 μm) in relation to the abundant cytoplasm in which there are tangled basophilic sections between mucous-like parts which are slightly P.A.S. positive (Figs. 1-4).¹

Type A cells are also found in the lateral areas but in groups of 2 or 3 in a dermo-epidermic position, and isolated ones are to be found deeper. Their elongated cytoplasm forms canals which allow secretion to pass through pores visible among the epidermic cells of the outer layer (Fig. 5).

Types B and C are isolated and situated in the dorsal parts of the foot. The B variety is typically shaped like a Florentine flask, the neck varying in length according to the depth of the situation. They have a spherical nucleus in a basal position, with dense granules of chromatin among which there is an easily visible nucleolus. The cytoplasm contains an acidophilous substance which turns pink, green or yellow when stained with Eosin, Picro-Indigo Carmine or Van Gieson's Picrofuchsin respectively. This secretion stains intensely with P.A.S. according to MacManus, which demonstrates the presence of neutral polysaccharids. The outside of the cell is surrounded by fibers of reticuline which can be clearly seen with Gomori's technique (Figs. 7-8).

Type C cells are oval in shape and situated in the subepithelial portion with the orifices apparent on the simple outer epithelium. The nucleus is difficult to observe, is flat and adheres to the plasmatic membrane. The contents are granular and rich in acidic mucopolysaccharids and stain intensely with Alcian Blue (Fig. 6).

¹Figs. 1-8 were submitted as coloured prints. Unfortunately colour reproduction is out of the question here (Ed.).



FIGS. 1-4. Sections of the foot of *Cerneuella cespitum arigonis* after experimental infection with *Neostromylus linearis*. 1. Larval cyst 40 days after infection. 2. Parasitic nodule with fibrous capsule 40 days after infection. 3. Parasitic nodule with characters closely resembling those of Fig. 2, 40 days after infection. 4. Tangential section of epidermis showing pores of type A cells partly in cross section. All figures highly enlarged; after coloured photos.



FIGS. 5-8. Sections of the foot of *Cermeuella cespitum arigonis* after experimental infection with *Neostromylylus linearis*. 5. Calcified larval cyst 60 days after infection. 6. Glandular cells (type C) in the dorsal part of the foot. 7. Flask-shaped glandular cells (type B) in the dorsal part of the foot. 8. Do., under higher magnification. All figures highly enlarged; after coloured photos.

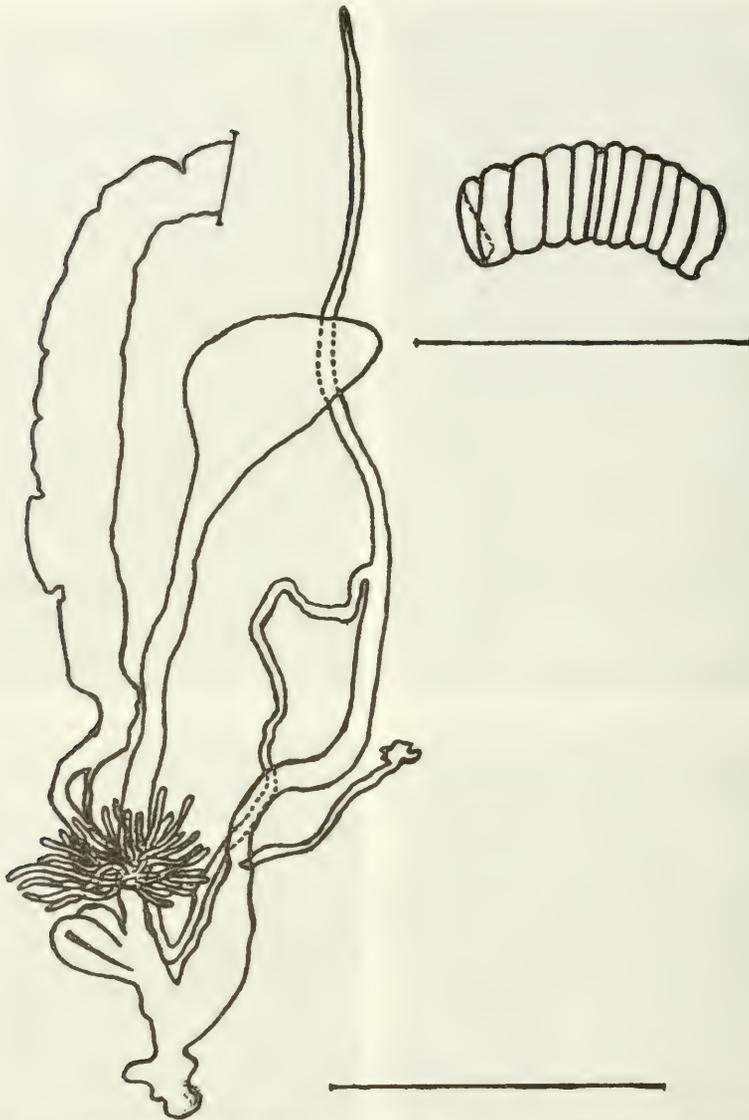


FIG. 9. Genitalia of *Cernuella cespitum arigonis* from Léon, Spain; scale 10 mm. Upper right figure depicts the mandibula; scale 1 mm.

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SOME ASPECTS OF AMINO ACID CATABOLISM IN THE FRESHWATER
PULMONATE SNAIL *LYMNAEA STAGNALIS*

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ABSTRACT

Lymnaea is known to produce both ammonia and urea as presumed excretory products. In studies estimating the ammonia produced aerobically and anaerobically by homogenates, the L-amino acids ARG, HIS, ILE, LEU, LYS, MET, PHE, TRY, and VAL gave evidence of aerobic deamination; GLY, PRO, and THR did not. Little or no ammonia was produced with any of the above substrates incubated under nitrogen. Catalase activity has also been found. The pattern of L-amino acid oxidase activity seen is in general agreement with findings of Olomucki et al. (1960) using manometry. Urease was not found. Since *Lymnaea* has an arginase, the absence of ammonia production anaerobically with arginine as a substrate is in accord with this assertion. An ADP activated glutamate dehydrogenase was found in *Lymnaea* by Sollock (1975) using a radiometric method in the direction of glutamate synthesis. The presence of this enzyme has also been observed in our studies using a spectrophotometric method. ADP activation was also noted and glutamate synthesis was demonstrably dependent upon the presence of NH_4^+ . EDTA and L-leucine also appear to stimulate the activity of this enzyme. The reaction was reversible but much less active in the direction of α -ketoglutarate and ammonia production. Aspartate aminotransferase and malate dehydrogenase also appear to be present in a coupled system that oxidizes NADH when α -ketoglutarate and aspartate are substrates. The absence of urease is in accordance with the production of urea by the snail. Also, oxidative deamination of amino acids, and the possibility of trans-deamination involving glutamate appear to have some enzymatic bases for involvement in the production of ammonia, however the pronounced tendency of glutamate dehydrogenase to operate in the direction of reductive amination, the possible operation of various modifiers, and the presence of aspartate aminotransferase indicate the possibility of alternative pathways through the accumulation and transamination of glutamate. It is also suggested by the author that the L-amino acid oxidase could function with glutamate dehydrogenase and transamination in a system indirectly aerobically oxidizing NADH with the concomitant production of NH_3 and hydrogen peroxide.

INTRODUCTION

Although *Lymnaea stagnalis* is one of the better studied snails morphologically and physiologically, biochemical and metabolic information is not readily available. One aspect of intermediary metabolism, namely that of ammonia and urea production, has been of interest to the author for some time and although major pathways remain to be determined in *L. stagnalis*, some general enzymatic patterns are now evident.

In this paper, various studies on reactions involving amino acids and derivatives will be presented and discussed in relationship to the known production of ammonia and urea by this snail (Bayne & Friedl, 1968; Friedl, 1974).

MATERIALS AND METHODS

The snails used in this study came from natural populations of *Lymnaea stagnalis jugularis* Say in Minnesota and have been maintained in the laboratory for over 15 years. Since this paper is a summary and preliminary report of several investigations, pertinent information on experiments will be presented in each section and with included figures. Preparation of homogenates, however, involved the removal of the gizzard and as much of the alimentary system of the animal as possible, with the remaining soft parts used in "whole body"

homogenates. Other preparations, with the alimentary tract removed, were subdivided into "body" (foot and viscera excluding the digestive gland) and "digestive gland" (the removed digestive glands including adherent tissues, e.g. gonadal, not easily pulled free). Such homogenates were used directly, or preserved with thymol, and when necessary, dialyzed into appropriate suspending media. Various methods of homogenization were used, including the Waring Blendor and ultrasonic disruption; preparations were kept on ice when feasible and stored refrigerated at 4°C. Protein was estimated using the Lowry Phenol Reagent Method (Lowry et al., 1951) or the Mehl Biuret Method (Mehl, 1945).

RESULTS

1. L-Amino Acid Oxidases

Studies on whole body homogenates of *L. stagnalis* have indicated that a number of amino acids are deaminated oxidatively. The studies were performed by measuring ammonia production aerobically and anaerobically in reaction mixtures containing pH 7.1 phosphate buffer over a period of three hours at 30°C. Assays with appropriate controls were incubated in air and under nitrogen.

The results of these experiments are shown in Table 1. The L-amino acids Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Tryptophane, and Valine gave evidence of aerobic deamination; Glycine, Proline, and Threonine did not. Little or no ammonia was produced by any of the above incubated anaerobically. These results indicate the presence of an L-amino acid oxidase (E.C.1.4.3.2) acting, in contrast to certain other types, preferentially on basic amino acids. This pattern of activity is in general agreement with that seen in *L. stagnalis* by Olomucki et al. (1960) using a manometric method. Preparations from other molluscs have also shown the ability to oxidize basic amino acids (Campbell & Bishop, 1970).

2. Catalase

Digestive gland homogenates of *L. stagnalis* assayed by observing decrease in H₂O₂ concentration concurrent with a decrease in absorption at 230 nm (Maehly & Chance, 1954), have given evidence of catalasic activity. It appears that this is a true catalase (E.C. 1.11.1.6) since it can be inhibited by cyanide and azide. Since the L-amino acid oxidase reaction produces hydrogen peroxide (as evidenced in *L. stagnalis* by Olomucki et al., 1960, in an ethanol coupling study), the existence of catalase as part of a total system to protect the resulting keto acid from subsequent oxidation might well be expected.

Experimental evidence for this activity is presented in Fig. 1.

TABLE 1. Deamination of L-amino acids by whole body homogenates of *Lymnaea stagnalis*. Tissues from whole animals pooled for experiments. Reaction mixture consisted of 0.5 ml Phosphate buffer (0.1 M, pH 7.1), 0.25 ml homogenate ("whole body"), and 0.25 ml substrate (containing 10-25 μ moles of L-amino acid or urea). Assays done in duplicate on one or more homogenate samples. Incubation at 30°C for 3 hrs with shaking on Dubnoff Metabolic Incubator under atmospheres of air or nitrogen. Ammonia produced estimated by micro-diffusion and Nesslerization. Homogenate protein estimated by the Lowry protein method (Lowry et al., 1951). Results calculated as μ moles ammonia produced/mg protein/hr $\times 10^{-2}$. Less than 1 microgram of nitrogen detected in assay considered as zero.

Amino acid	Ammonia produced in air	Ammonia produced under nitrogen
Arginine	19.6	0.0
Glycine	0.0	0.0
Histidine	23.9	0.0
Isoleucine	5.8	0.0
Leucine	18.7	0.0
Lysine	24.6	0.0
Methionine	10.7	0.0
Phenylalanine	14.4	0.0
Proline	0.0	0.0
Threonine	0.0	0.0
Tryptophan	12.8	0.0
Valine	3.4	0.0
Urea	0.0	0.0

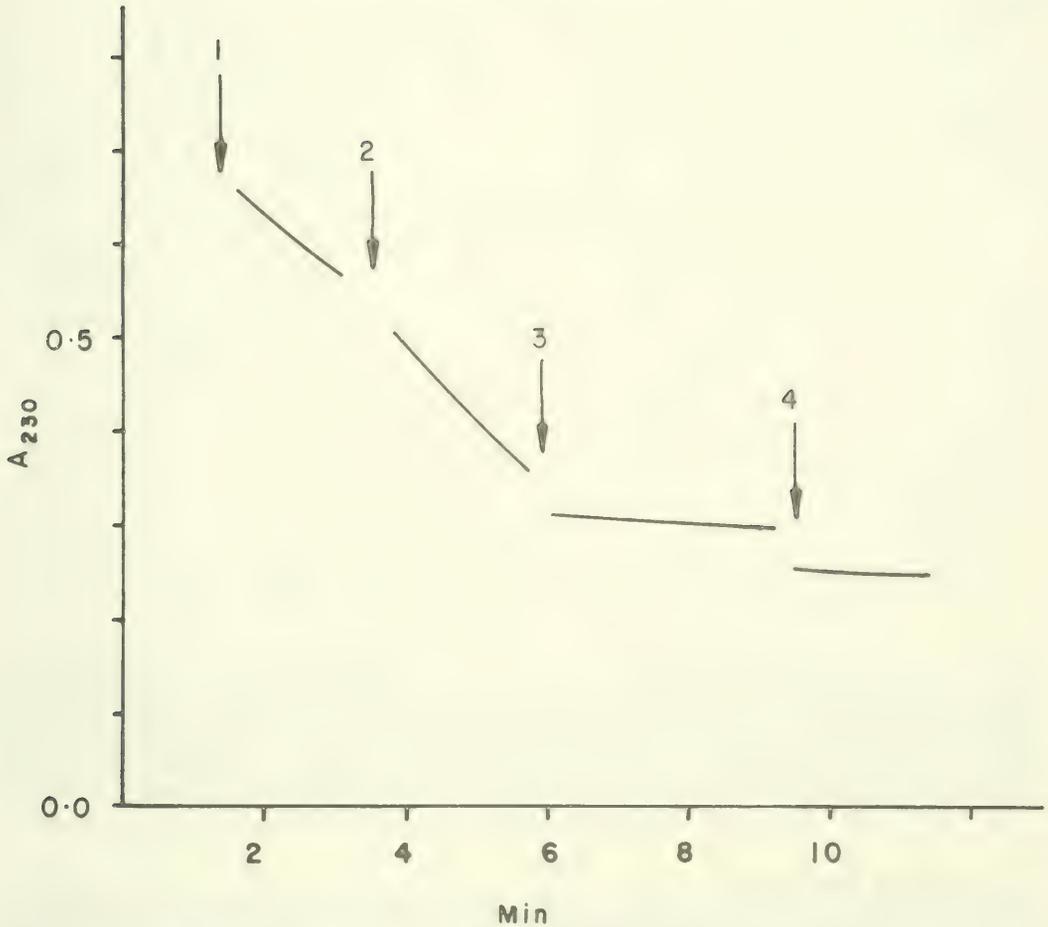


FIG. 1. Catalytic activity in *Lymnaea stagnalis* and its inhibition by cyanide. Reaction mixture consisted of 2.5 ml of 0.1 M phosphate buffer at pH 7.16 plus Hydrogen Peroxide to give stable absorption of about 0.76 at 230 nm (about 10.6 μ moles/ml in mixture). Incubation at 27°C. At points "1" and "2," 0.05 ml additions of "digestive gland" homogenate were made (about 0.23 mg protein per addition by Mehl biuret method, Mehl, 1945). At points "3" and "4," 0.05 ml additions of 0.1 M KCN were made. Decreasing absorption indicates hydrogen peroxide decomposition which was halted by cyanide. Similar results have been observed with sodium azide as an inhibitor (tracing from spectrophotometric recording).

3. Urease

Urease (E.C. 3.5.1.5) has not yet been detected in any chemical study done of *L. stagnalis* in this laboratory. It was assayed along with the L-amino acids in section 1 of this paper and essentially no ammonia was formed aerobically or anaerobically. Since *L. stagnalis* possesses an arginase, the absence of ammonia production anaerobically with L-arginine as a substrate is in accord with this. Additionally, urea appears in hemolymph (Friedl, 1961) and in ambient media in which this snail is kept (Bayne & Friedl, 1968).

4. Ornithine Cycle Enzymes

Arginase (E.C. 3.5.3.1) in *L. stagnalis* has been known for some time (Baldwin, 1935). In 1966 Friedl & Bayne examined the Ornithine Cycle system and found ornithine carbamoyl-transferase (E.C. 2.1.3.3) but no evidence for the operation of the cyclical arginine synthetic system using a chemical assay. No carbamoyl-phosphate synthases were investigated at this time.

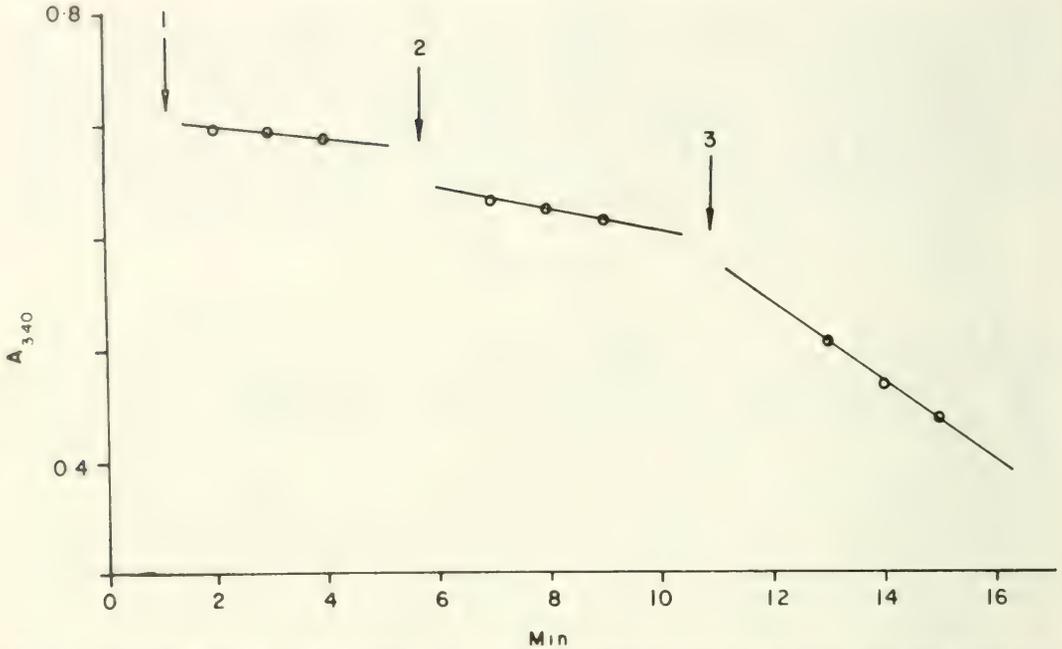


FIG. 2. Glutamate Dehydrogenase activity in *Lymnaea stagnalis*. Reductive amination of α -Ketoglutarate. Reaction mixture consisted of 2.0 ml of 0.1 M Phosphate buffer, pH 7.1, plus 0.5 ml of dialyzed "body" homogenate (about 2.24 mg protein by Mehl biuret method). At "1," 0.05 ml of NADH (about 0.25 μ mole) and 0.1 ml of 0.1 M α -Ketoglutaric Acid have been added. At "2," 0.1 ml of 1.0 M NH_4Cl was added, and at point "3," 0.1 ml of ADP (about 9.0 μ moles). Incubation at 27°C. Decrease in absorbance due to NADH oxidation upon ADP addition is notably above previous endogenous levels (data abstracted from spectrophotometric recording).

5. Glutamate Dehydrogenase

An ADP activated glutamate dehydrogenase was found in *L. stagnalis* by Sollock (1975) using a radiometric method in the direction of glutamate synthesis. This activity has also been observed in our studies using a standard spectrometric method based on the change in absorbance as NAD is oxidized or reduced. ADP activation was also noted and glutamate synthesis was demonstrably dependent upon the presence of NH_4^+ . The reaction is reversible but much less active in the direction of glutamate oxidation and deamination. Accordingly, the presence of glutamate dehydrogenase (E.C. 1.4.1.2) seems well established for *L. stagnalis*. Some of the evidence of this enzyme is shown in Figs. 2 and 3.

Lymnaea glutamate dehydrogenase appears to be inhibited by NH_4^+ in the direction of glutamate oxidation and, interestingly, is influenced by EDTA and L-Leucine. Activation by these substances has been reported by other workers on mammalian preparations (Frieden, 1965; Yielding & Tomkins, 1961; McGivan et al., 1973). It appears in *Lymnaea* that activation can be accomplished by ADP, EDTA and Leucine, with maximal activation found in the presence of all 3 components of the system. Some features of this system are illustrated in Fig. 4.

6. Aspartate Aminotransferase and Malate Dehydrogenase

Aspartate aminotransferase (E.C. 2.6.1.1) has been assayed by observing the appearance of oxalacetic acid by means of changes in absorbance at 280 nm (after Cammarata & Cohen, 1951).

Malate Dehydrogenase (E.C. 1.1.1.37) has been found using an assay based on the oxidation of NADH. No distinction between mitochondrial and cytoplasmic activity was studied. Evidence for malate dehydrogenase activity is presented in Fig. 5.

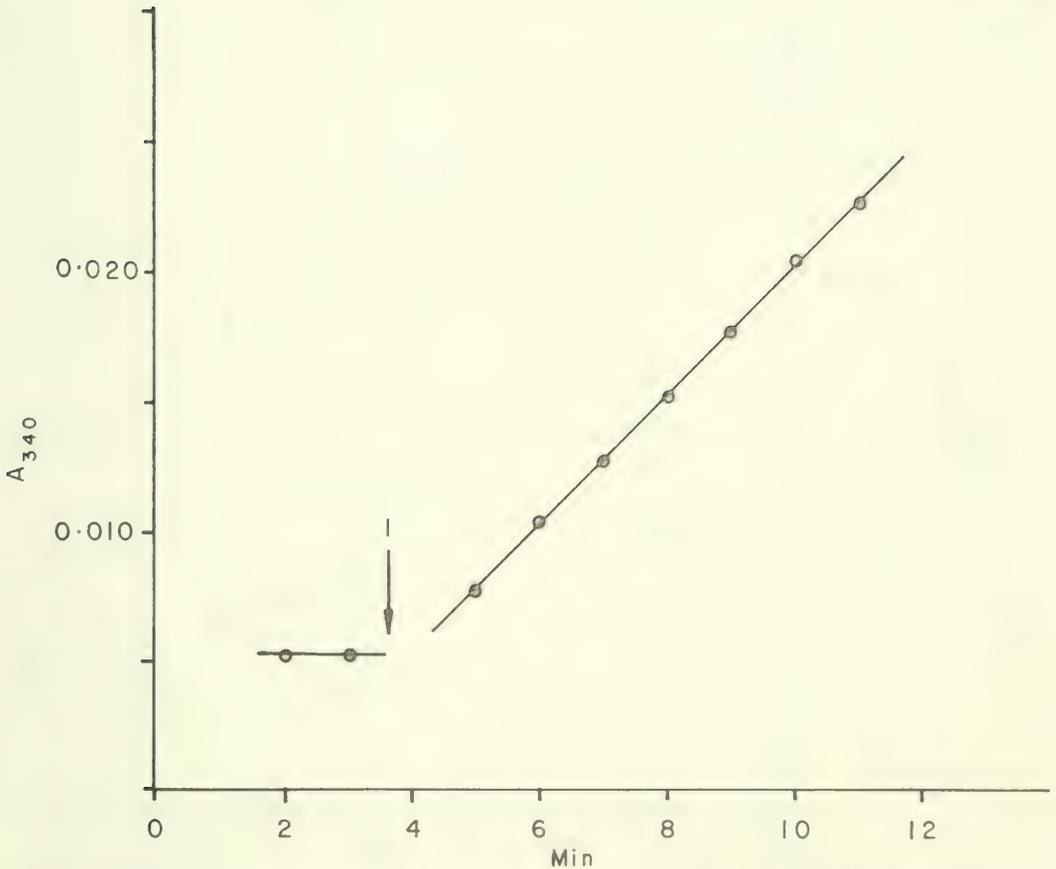


FIG. 3. Glutamate Dehydrogenase activity in *Lymnaea stagnalis*. Oxidative deamination of L-glutamate. Reaction mixture consisted of 2.0 ml of 0.1 M Phosphate buffer, pH 7.1, plus 0.5 ml of dialyzed "body" homogenate (about 2.24 mg protein by Mehl biuret method), 0.1 ml of 0.05M L-Glutamic Acid and 0.05 ml of NAD (about 0.5 μ mole). At point "1," 0.1 ml of ADP (about 9.0 μ moles) was added. Incubation at 27°C. Reduction of NAD commenced upon the addition of ADP. When compared to the rate shown for the similar experiment in Fig. 2, the ratio of reductive amination to oxidative deamination was about 11.7 (data abstracted from spectrophotometric recording).

Aspartate aminotransferase and malate dehydrogenase in *L. stagnalis* homogenates will function in a coupled multi-enzyme system that oxidizes NADH in the presence of L-aspartic acid and α -ketoglutaric acid. Superficially this resembles glutamate dehydrogenase when a pyridine nucleotide assay method is used, however, it is obviously not dependent upon NH_4^+ and requires the presence of aspartate. It accomplishes the production of glutamate and malate and the oxidation of NADH. Some characteristics of this coupled system, supporting the assay of aspartate aminotransferase as noted above, are shown in Fig. 6.

7. Other observations

Lactate dehydrogenase does not appear to be active in the preparations studied, nor has good evidence of alanine aminotransferase yet been found. One preliminary experiment indicated that added L-glutamine produced ammonia aerobically and anaerobically, however, more recent glutaminase assays have not given appreciable activity with "whole body" homogenates.

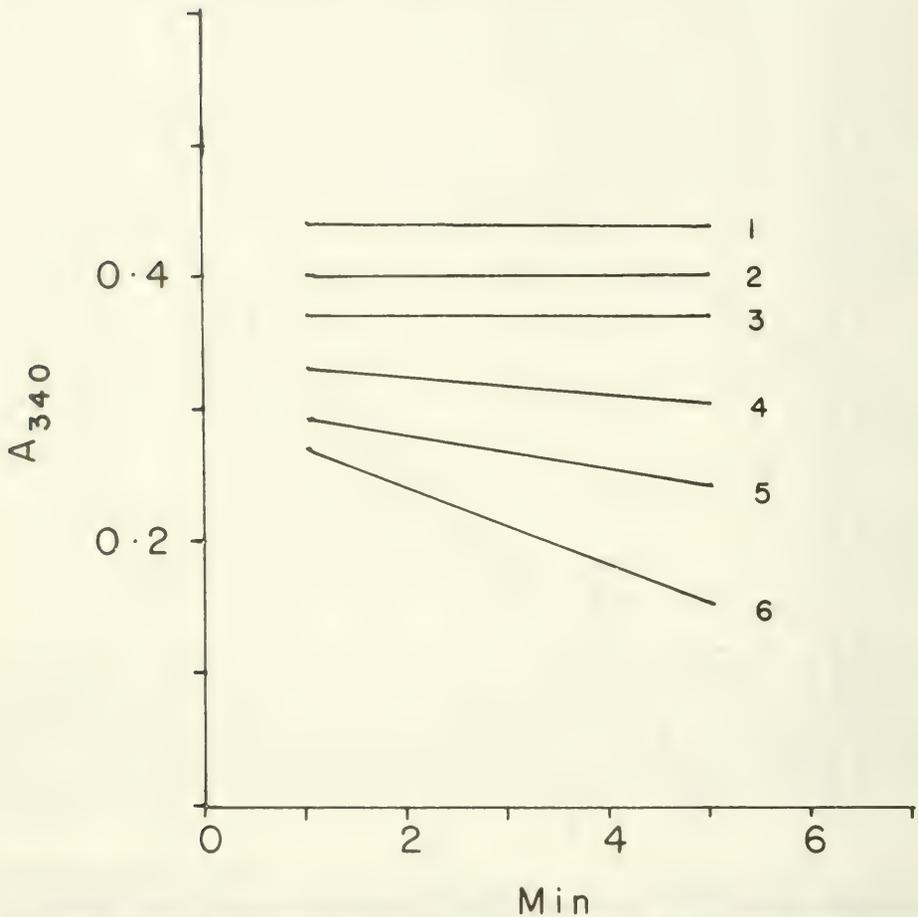


FIG. 4. Glutamate Dehydrogenase activity in *Lymnaea stagnalis*. Effects of ADP, EDTA, and L-Leucine. Reaction mixture consisted of 2.0 ml of 0.2 M Phosphate buffer, pH 7.1, plus 0.5 ml of dialyzed "body" homogenate (about 0.62 mg protein by Mehl biuret method). Incubation at 27°C. Abstracts of spectrophotometric records of slopes of reactions are shown in terms of NADH oxidized vs. time. In "1," 0.025 ml NADH was added (about 0.25 μ mole); at "2," 0.1 ml of 1.0 M NH_4Cl ; at "3," 0.05 ml of ADP (about 2.2 μ moles); at "4," 0.1 ml of 0.1 M α -Ketoglutaric Acid; at "5," 0.1 ml of 0.1 M Disodium EDTA; and at "6," 0.1 ml of 0.05 M L-Leucine. Addition of α -Ketoglutaric Acid initiates reaction, which is further augmented by EDTA and Leucine. ADP had been previously added at "3" as primary activator.

DISCUSSION

As can be seen from above, the enzymatic evidence found suggests the possibility of a number of patterns of amino acid catabolism. It is not clear how many or what segments may actually function in vivo. The absence of urease, however, is in accordance with the external production of urea by this snail.

Trans-deamination (Braunstein & Asarkh, 1945) clearly is to be considered in the production of ammonia. However, the possible involvement of leucine (and other modulators), and the pronounced equilibrium of the reaction in the direction of glutamate formation may indicate a more important alternative role of reductive amination in the total system. Such a role has been suggested by McGivan et al. (1973) for mammalian systems. The disposition of accumulated glutamate might be by transamination, perhaps to form aspartate for inclusion in a purine nucleotide cycle (Moss & McGivan, 1975, for mammalian systems). Unfortunately, as yet,

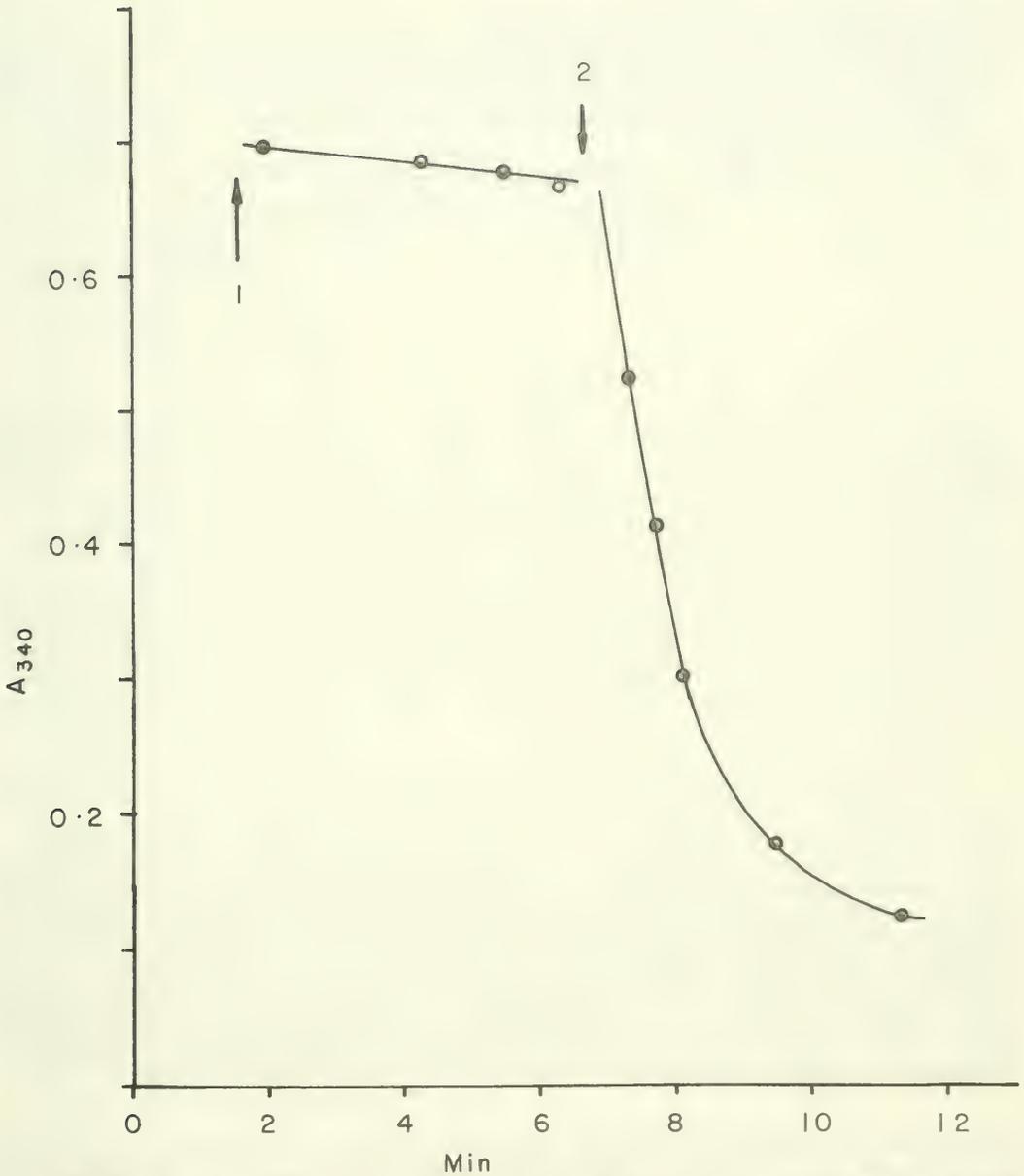


FIG. 5. Malate Dehydrogenase activity in *Lymnaea stagnalis*. Reduction of oxaloacetate. Reaction mixture consisted of 2.0 ml of 0.1 M Phosphate buffer, pH 7.1, plus 0.5 ml of "body" homogenate (about 3.0 mg protein by Mehl biuret method). Incubation at 27°C. At "1," 0.05 ml of NADH (about 0.28 μ mole) was added; at "2," 0.1 ml of 0.1 M Oxaloacetate. Abstract of spectrophotometric record shows NADH oxidation was initiated promptly upon the addition of Oxaloacetate.

preliminary studies have not indicated the presence of adenosine or adenyate deaminase activity requisite for such a purine nucleotide cycle in *L. stagnalis*. Other alternatives could be the utilization of glutamate in glutamine synthesis (Campbell, 1973), or in combination with oxidases as suggested below.

The significance of notable L-amino acid oxidase activity (possibly with catalase involve-

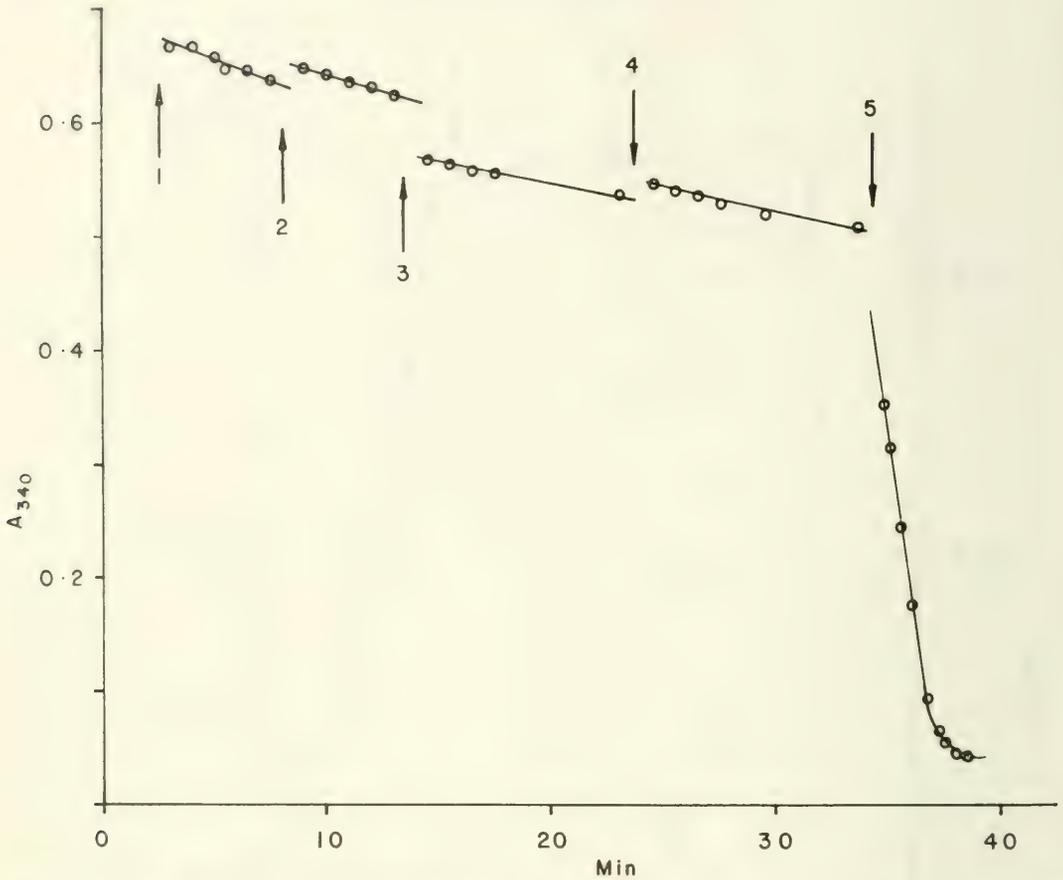
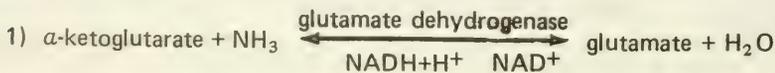


FIG. 6. Coupled Aspartate Aminotransferase and Malate Dehydrogenase activity in *Lymnaea stagnalis*. Reaction mixture consisted of 2.0 ml of 0.1 M Tris-HCl buffer, pH 7.3 plus 0.5 ml of "whole body" homogenate (about 1.95 mg protein by Mehl biuret method). At "1," 0.05 ml of NADH (about 0.26 μ mole) was added. At "2," 0.1 ml of 0.1 M Na Pyruvate; at "3," 0.1 ml of 0.1 M L-Alanine; at "4," 0.1 ml of 0.1 M α -Ketoglutaric Acid; and at "5," 0.1 ml of L-Aspartic Acid were added and the change in absorbance at 340 nm subsequently noted. Incubation at 30°C. It can be seen that some endogenous oxidation of NADH occurred, with no increase upon Pyruvate addition (suggesting little or no lactate dehydrogenase) nor with Alanine or α -Ketoglutarate (suggesting little or no N (1-carboxyethyl) alanine formation or Glutamate Dehydrogenase activity). NADH oxidation, however, starts abruptly and rapidly with Aspartic Acid addition, bearing out the premise that the coupled Aspartate Aminotransferase-Malate Dehydrogenase reaction is operable.

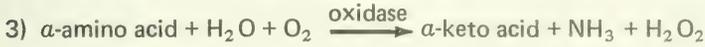
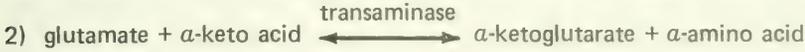
ment) in ammonia production and nitrogen catabolism in *Lymnaea* is not yet clear. The oxidase could function alone, or with transaminases and glutamate dehydrogenase in a "complementary/supplementary" mode as suggested by Sollock (1975). Alternatively or additionally, such enzymes could be part of a flavoprotein oxidation scheme utilizing oxygen.

In a similar sense, alternative cuproprotein respiratory chains leading to oxygen but not involving cytochromes or cytochrome oxidase, have been suggested (and criticized) in plants (Mahler & Cordes, 1971; Dawson & Tarpley, 1951; Malmström et al., 1975). The operation of the dehydrogenase-aminotransferase-oxidase system visualized by the author is as follows:

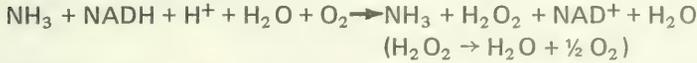
Since the reaction:



serves to oxidize NADH, oxygen could be indirectly used as a terminal hydrogen acceptor by the transamination of glutamic acid to an oxidase acceptable amino acid. The oxidation of this acid and its deamination would regenerate the transamination-acceptor keto acid again. Presumably, the presence of catalase would remove H_2O_2 so as to reduce the possibility of further degradation of the keto acid. Such a sequence of reactions could occur as follows:



Sum of reactions 1-4:



Enzymatic evidence presented in this paper supports the postulation of such a pathway. The features that would distinguish this type of system from other possible operational combinations of these enzymes would be (1) the tendency for ammonia fixation and glutamate accumulation as predicted by the equilibrium for the glutamate dehydrogenase reaction (Smith et al., 1975), although subject to modulators, (2) the disposition of accumulated glutamate in transamination systems with the regeneration of α -ketoglutarate, and (3) the ultimate involvement of a flavoprotein oxidase and H_2O_2 production (with possibly additionally coupled reactions such as peroxidative activity of catalase, Schonbaum & Chance, 1976) and NH_3 release as a secretion or excretion. Such a system could accomplish the indirect oxidation of NADH aerobically without glutamate accumulation, while anaerobically, the regeneration of NAD could take place via the coupled transaminase-malate dehydrogenase system seen to be present.

ACKNOWLEDGEMENTS

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ABBREVIATIONS USED IN TEXT

NAD ⁺	Oxidized Nicotinamide-adenine dinucleotide
NADH	Reduced Nicotinamide-adenine dinucleotide
ADP	Adenosine-5'-diphosphate
EDTA	(Ethylenedinitrilo) tetraacetic acid

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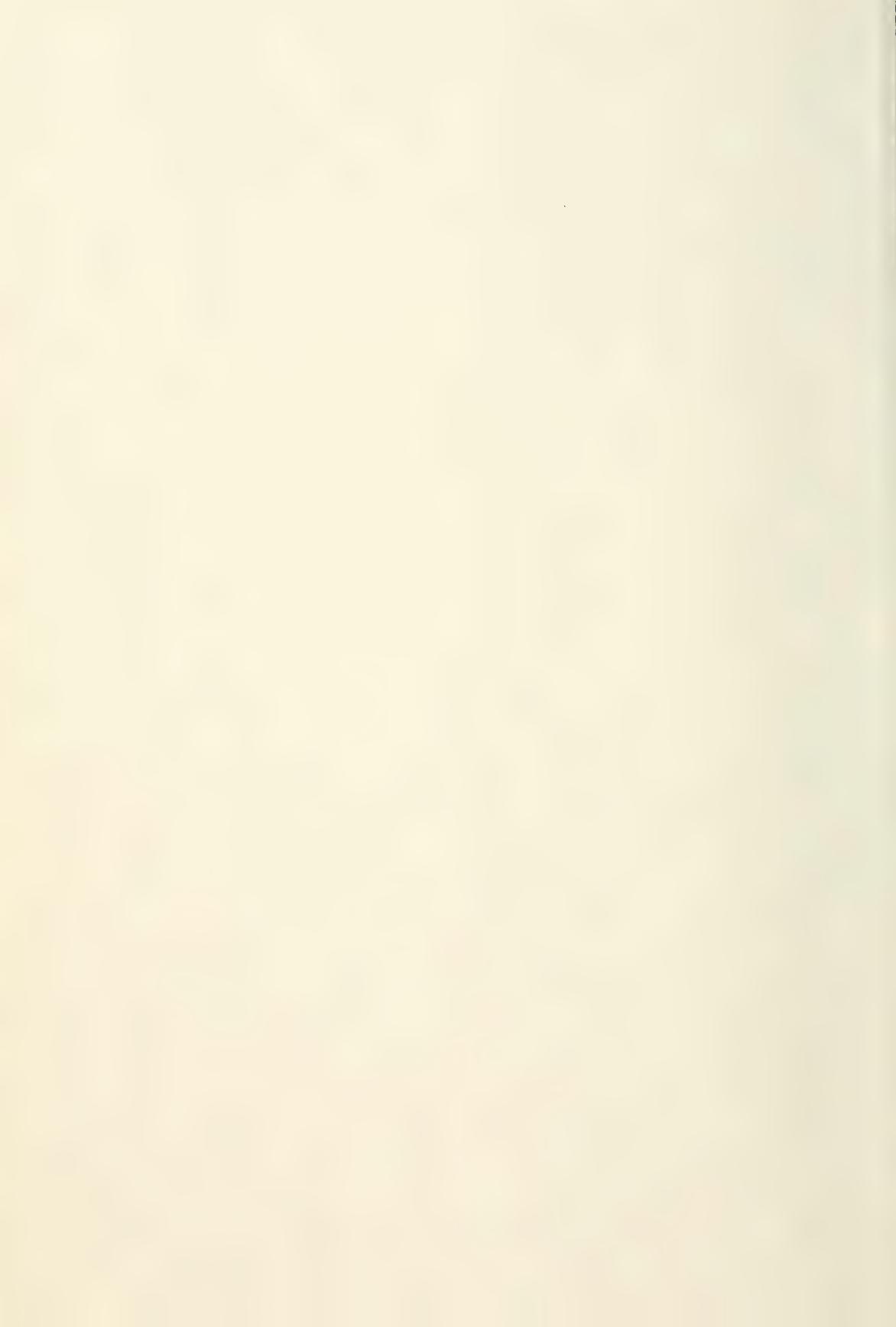
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