UNIVERSIDADE DE SÃO PAULO
FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS

BOLETIM N.º 232 **ZOOLOGIA N.º 22**

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DOMINGOS VALENTE

(Departamento de Fisiologia Geral e Animal — Universidade de São Paulo — Caixa Postal n. 2926 — São Paulo)

CONTRIBUIÇÃO PARA O ESTUDO DA NEUROSECREÇÃO NOS CRUSTÁCEOS *

(28 Figuras).

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(*) — Tese apresentada para o concurso de docência livre de Fisiologia Geral e Animal (19a. cadeira) da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo.

PREFÁCIO

Neste trabalho com que ora me apresento ao concurso de docência-livre de Fisiologia Geral e Animal da Faculdade de Filosofia, Ciências e Letras da Universidade de S. Paulo, procurei situar o assunto escolhido como tese dentro do objetivo do Departamento de Fisiologia Geral e Animal, que é o estudo da fisiologia sob os seus aspectos gerais e zoo-comparativos.

A pesquisa refere-se à neurosecreção, tema de marcante atualidade, que nos últimos anos por assim dizer, revolucionou os conceitos referentes ao contrôle nervoso e hormonal dos organismos. Relaciona-se ainda com as repercussões que estudos de neurosecreção, iniciados em vertebrados superiores, tiveram na fisiologia comparativa **senso lato**.

Estudando, assim, aspectos da neurosecreção em Crustáceos, o presente trabalho procurou contribuir, na medida do possível, para a generalização de conceitos novos e destacar eventuais particularidades encontradas no referido grupo de invertebrados que, sabidamente, no campo de tais estudos constituem bom objeto para pesquisas.

Por outro lado, escolhendo um tema de neurosecreção em crustáceo, foi minha intenção filiar-se a uma linha marcante e já antiga da pesquisa do Departamento de Fisiologia Geral e Animal, dirigido pelo Prof. Dr. Paulo Sawaya, que por motivos que não importa recordar, ficou posteriormente abandonada. Em verdade, basta folhear a lista de publicações do referido Departamento, para se verificar como os seus primeiros trabalhos, ao tratar de problemas de mudança de côr em crustáceos e peixes, de certo modo se preocuparam com a **neurosecreção**. De uns dois anos para cá, finalmente voltou ela a figurar nas nossas cogitações, como atestam os últimos trabalhos publicados.

Um trabalho de fisiologia, nos dias atuais, não se faz sem o concurso próximo ou remoto de outras pessoas ou investigadores. No caso presente, o bom andamento das pesquisas deveu-se à colaboração imprescindível de um certo número de colegas e companheiros de laboratório, que assim se tornaram cre-

dores da minha sincera gratidão: O Prof. Dr. Paulo Sawaya que orientou os meus primeiros passos na fisiologia e que com a sua experiência no campo da neurosecreção, muito cooperou na confecção dêste trabalho com sugestões e críticas justas. O colega e amigo Dr. Erasmo Garcia Mendes e o Dr. George A. Edwards, ex-professor colaborador do Departamento de Fisiologia Geral e Animal, que igualmente com sua experiência no assunto, se tornaram valiosos conselheiros nas horas difíceis. As Dras. Lic. Elisa P. Knapp e Anna Amélia Ancona Lopes e o Lic. Prof. Chaim N. Grinkraut pelo precioso auxílio prestado na parte histológica, nas microdisecções e nos bio-ensáios. Os Srs. Perclides de Oliveira e Conrado Bizarro que muito se esmeraram na captura do material. Às Sras. Gertrudes S. Alterthum e Dra. Carolina Bresslau que nos auxiliaram na tradução dos textos alemães. À Sta. Elza Farah a quem se deve a datilografia dos originais.

Finalmente, não poderia deixar de manifestar aqui o meu apreço e gratidão à companheira dos bons e dos maus momentos, a minha espôsa, D. Maria da Penha Machado Valente.

I — INTRODUÇÃO

O conceito de neurosecreção surgiu da observação de células nervosas capazes de produzir granulações e gotículas de natureza coloidal, com atividade semelhante à dos hormônios. Admite-se que estruturas nervosas sejam responsáveis pela existência de substâncias de atividade especial, como p. e., ,a acetilcolina, a simpatina, produzidas ao nível das terminações nervosas periféricas, e que, todavia, não se acham relacionadas com a neurosecreção. Esta refere-se principalmente à elaboração pelas próprias células nervosas de certos produtos de atividades definidas e determinadas que influem, p. e., no metabolismo, na mudança da côr, na atividade locomotora, etc.

E' assunto bem estudado tanto nos Invertebrados como nos Vertebrados. Nos primeiros, além dos Insetos, o grupo melhor conhecido neste particular é o dos Crustáceos, nos quais foi possível estabelecer-se íntima conexão entre as pesquisas his-

tológicas e as fisiológicas. O entendimento da neurosecreção nos Crustáceos depende de uma estreita colaboração entre morfólogos, fisiólogos e, agora, bioquímicos. De há muito que HANSTRÖM (1931) se dedica à investigação anatômica e histológica dos órgãos incretórios dêsses e de outros Invertebrados. Foi o primeiro a descrever o "órgão X" nos Crustáceos, identificando-o (1933) em **Benthesicymus, Gennadas, Sergestes, Acanthephyra Parapandulus, Virbius, Lysmata seticaudata, Spirotoncaris polaris, Pontonia syrrhena, Processa edulis, Pontophilus norvergicus** e **Parapandalus** (SAWAYA, 1939).

O órgão X é proveniente das células sensoriais da papila ocular. Em alguns Crustáceos desenvolve-se como órgão do sentido, em outros como glândula sensorial e em outros ainda como glândula endócrina (HANSTRÖM, 1938). Em trabalhos posteriores, o mesmo autor determinou as células do órgão X como sendo neurosecretoras e em pesquisas mais modernas confirmou tal asserção.

Em **Macrura Natantia** e **Brachyura,** segundo CARLISLE & PASSANO (1953), o órgão X está separado em duas porções: uma primeira porção próxima ao poro sensorial, que é denominada por êsses autores **pars distalis** do órgão X e uma segunda, situada próxima à **medula terminalis,** denominada **pars ganglionaris** do órgão X; estas duas porções estão ligadas por um nervo, **conexio X-organi.** Sòmente a **pars ganglionaris** do órgão X se conjuga com a glândula do seio.

Em **Brachyura** (MENDES, 1942; BLISS, 1951; PASSANO, 1951 e 1952) o órgão X é constituído de células neurosecretoras em número de 12 ou mais, as quais estão localizadas entre células nervosas normais, diretamente abaixo da superfície justa-ventral da **medula terminalis.** Os grandes axônios das células neurosecretoras formam uma parte do nervo sinoganglionar.

Em **Isopoda** também foram descritos elementos neurosecretores **(Lygia exotica)** por SAWAYA (1939) tendo-se aí identificado tanto a glândula do seio como o órgão X. Foi êste o primeiro trabalho realizado em nosso país sôbre êste importante

assunto, contribuindo suas conclusões para fundamentar outras pesquisas nêste campo.

Grande progresso na pesquisa da endocrinologia dos crustáceos se obteve quando PASSANO (1953b) mostrou que o órgão X descrito por HANSTRÖM (1933) como neurosecretor, produz uma secreção transportada através de um nervo para a glândula do seio. Pensou-se que essa glândula produzisse um hormônio que impediria a muda. PASSANO, porém, provou que a extirpação da glândula do seio não induzia a muda supranumerária como era esperado. Isso só foi possível com a extirpação do órgão X.

Existem ainda certas discrepâncias de natureza anatômica e citológica, entre órgão X e glândula do seio e as numerosas atividades dos hormônios armazenados na glândula do seio. Êsse problema foi em parte solucionado com os achados de grande número de grupos de células neurosecretoras localizadas no cérebro, na cadeia nervosa ventral e na parte nervosa do pedúnculo ocular. Inúmeros grupos de células neurosecretoras mandam os seus produtos para a glândula do seio (BLISS & WELSH 1952 e BLISS, DURAND & WELSH (1954). Que ocorre de fato transporte de secreção nas vias neurosecretoras revela-se pelas observações de CARLISLE (1953) realizadas numa preparação viva de camarão, onde observou movimento lento de gôtas de secreção no axoplasma dos neuritos, numa velocidade de 2 a 4 micra por minuto. PASSANO (1952) observou sistemas esféricos ao microscópio de fase no **Decapoda Sesarma,** os quais consistem de pequenos grânulos com 0,3 micra, muito refrativa à luz. Êsses grânulos envolvem uma gôta central òticamente vazia. Estas gôtas podem se reunir em gôtas maiores, o que foi observado no órgão terminal da glândula do seio e no neurônio secretório.

Coube a ENAMI (1949, 1951 **b** e **c**) correlacionar, histològicamente, os tipos de hormônios produzidos no pedúnculo ocular com células neurosecretoras. Observou, histològicamente, que em **Sesarma** a inervação da glândula do seio se faz exclusivamente pelo nervo da glândula do seio, não tendo sido

achadas outras vias, embora alguns dos mais finos ramos do nervo oculomotor, pareçam estar em contacto superficial com o tecido glandular. O tecido da glândula do seio é muito semelhante ao neurilema dos tecidos nervosos, em sua estrutura fundamental, segundo HANSTRÖM (1951) fato êste já muito conhecido pelos trabalhos dêste autor. A lamela da glândula é essencialmente de natureza sincicial; uma diferença marcante entre a glândula do seio e o neurilema está na quantidade e variabilidade das inclusões acidófilas que predominam na glândula.

Distinguem-se, na glândula do seio, quanto à configuração, à plasticidade e à estrutura microscópica, três tipos de colóides: I. Colóide A: massas ovóides ou elípticas de 4 a 8 micra de comprimento; coram-se mais intensamente com a fucsina ácida de Mallory. II. Colóide B: massas irregularmente formadas, parecendo originadas da coalescência de massas de colóide A e mostra fraca afinidade pela fucsina ácida. No colóide B ocorrem vacúolos que permanecem acromáticos em Susa-Mallory. Não se nota movimento browniano, o que sugere considerável viscosidade dentro do vacúolo. III. Colóide C: massas informes, que por coalescência conduzem a massas maiores e que contornam as divisões do tecido glandular. Têm baixa viscosidade e em contraste com o colóide A e B, rejeita a alizarina "in vitro".

Tudo indica (ENAMI 1951b) uma possível transformação do colóide A em C passando pelo colóide B. O neurilema de todos os tecidos ganglionares produz um tipo de colóide semelhante ao A da glândula do seio, devendo-se ainda assinalar que nesse órgão não foram encontrados colóides B e C. Nas investigações de elementos incretórios no tecido nervoso, fora do neurilema, foram distinguidos três tipos de células neurosecretoras: α, β e γ.

As células α foram vistas no cérebro, na medula terminal, nas medulas interna e externa, bem como no gânglio torácico; geralmente medem 30 μ de diâmetro com núcleo de 12 μ onde 1 ou mais nucléolos estão presentes, exceto nas encontradas no

gânglio torácico, que são células gigantes de cêrca de 60 μ de comprimento e incluem um grande núcleo vesicular de cêrca de 20 μ de diâmetro e também com 1 ou 2 nucléolos. Tôdas são ricas em citoplasma e vacúolos.

As células neurosecretoras de tipo β encontram-se no cérebro, medula terminal e na comissura glanglionar. No cérebro estão localizadas, exclusivamente, num agrupamento de células nervosas situadas anteriormente ao lobo olfatório. Células de 30 μ de comprimento, com núcleo vesicular de 10 μ, citoplasma bastante homogêneo e de natureza compacta. As células β pertencentes à medula terminal são representadas por um certo número de células gigantes unipolares que formam um aglomerado na parte ventral da porção proximal da neuropila da medula e enviam fibras nervosas para a glândula do seio. O conjunto de células β de ENAMI que se encontram no pedúnculo ocular corresponde ao chamado órgão X. Característica importante das células β é a sua forte afinidade para com os componentes azul de anilina do Mallory.

Enquanto as células α e β mostram secreções citoplasmáticas, as células do tipo γ, localizadas no cérebro e na medula terminal, distinguem-se pela secreção nuclear A ocorrência de células neurosecretoras fora do órgão X foi confirmada em outros Crustáceos por BLISS & WELSH (1952). Verificaram êles que pelo menos uma parte dêsses centros secretórios, envia axônios para a glândula do seio, a qual parece, portanto, acumular neurosecreção, não sòmente do órgão X, mas também de outros grupos celulares secretores do sistema nervoso central.

As células neurosecretoras segundo HANSTRÖM (1954) não formam sinapses numa cadeia neurônica e nem transmitem impulsos nervosos a qualquer efetuador. Pelo contrário, numerosos axônios de tais células terminam "cegamente" num lugar de armazenamento, para o qual a secreção é transportada ao longo dos axônios. Nesse local, a secreção pode ser reservada em quantidade considerável para necessidades futuras. Hoje não há mais dúvida de que as células neurosecretoras produzem, nos Invertebrados, uma substância biològicamente ativa

à qual se atribui natureza hormonal. Sabe-se que o pedúnculo dos crustáceos contém um ou mais hormônios e que sua remoção provoca mudança de pele (BROWN 1952). Os crustáceos Decapodes, no seu desenvolvimento, sofrem tìpicamente um certo número de mudas, passando por uma série de estágios larvais característicos e, mesmo no estado adulto, continuam a crescer por muda periódica do exoesqueleto. Pouco ou nada se sabe com relação aos fatôres integradores em atividade, no desenvolvimento larval. O ciclo de muda dos crustáceos pode ser dividido em 4 períodos.

Parece-nos interessante o caso de **Cambarus**, que durante o primeiro ano de vida muda em intervalos de 12 a 13 dias, provàvelmente sem intervenção de significante período de intermuda. Posteriormente ocorrem duas mudas por ano, durante o período de prémuda de 3 a 5 semanas, ocasião em que há uma gradual reabsorção de exoesqueleto e deposição de sais de Ca na forma de gastrolitos, e também um gradual aumento na taxa de consumo de oxigênio e do teor de água; essas modificações fisiológicas ocorrem uma semana antes da muda. Na pós-muda, essas alterações dão-se no sentido inverso e num tempo mais ou menos igual. Estudos dos quocientes respiratórios aparentes em **Cambarus** dão para a intermuda um valor cêrca de 0,8 e para os recemudados valores tão baixos como 0,1-0,2 durante as primeiras horas, em virtude da fixação de CO_2 durante o endurecimento da carapaça. Ao cabo da primeira semana, todavia, o QR já retorna ao nível normal. Tôdas essas alterações estão ìntimamente relacionadas com as células neurosecretoras. Remoção de pedúnculos oculares em **Astacus** (MEGUSAR 1912), **Eriocheir** (HANSTRÖM 1939), **Palaemonetes** (BROWN 1939), **Uca** (ABRAMOWITZ & ABRAMOWITZ 1940) e **Cambarus** (BROWN & CUNINGHAM 1939 e SMITH 1940) resulta num aparecimento mais rápido da muda seguinte. Em **Cambarus** jovens, a remoção do pedúnculo ocular encurta o período de intermuda (Smith, 1940). A remoção dos pedúnculos oculares em **Cambarus** maduros, surge a muda antes do tempo esperado, que é o dos controles. Todavia, implantações de glândulas do seio no abdomem de

animais apenduculados determinam, pelo contrário, a muda muito depois da dos animais testemunhos (BROWN 1939; BROWN & CUNNINGRAM 1939). A remoção de um só pedúnculo ocular resulta numa muda levemente acelerada sugerindo ser o efeito de caráter quantitativo. PYLE em 1943, mostrou que alterações histológicas da glândula do seio são correlacionadas com o ciclo da muda, aparecendo grânulos de secreção acidófilos e predominância dos grânulos basófilos depois de completada a muda. Parece provável que todos êsses processos, que ocorrem durante a muda, estão sob a influência de um único hormônio inibidor da glândula do seio (SCUDAMORE, 1947).

A relação entre o hormônio inibidor da muda, da glândula do seio e a reprodução pode ser verificada com a fêmea ovada de **Crangon** pelo fato dela não "mudar" até que a prole tenha eclodido (HESS 1941) e ocorrendo a muda diversas semanas mais tarde do que nos machos, e sòmente depois que os filhotes abandonaram os pleiópodes. Sòmente em 1951, graças ao trabalho de PASSANO, foi possível provar que a muda estava relacionada com o órgão X e não sòmente com a glândula do seio. O órgão X é a fonte do hormônio que impede a muda. Implantações de glândula do seio em animais sem pedúnculo têm um efeito retardante, por ser essa glândula o armazém do hormônio fabricado pelas células neurosecretoras do órgão X. O mesmo acontece com implantações de gânglios cerebrais e conetivos esofágicos em animais apenduculados, pois células neurosecretoras do tipo β de ENAMI ocorrem no cérebro e nas comissuras ganglionares. No pedúnculo encontramos também um fator que influencia o metabolismo. A ausência do órgão X, depois da extirpação do pedúnculo, causa aumento do consumo de oxigênio em **Gecarcinus** e **Astacus** (BLISS, 1951, FROST, SALOUM & KLEINHOLZ 1951) e em **Gecarcinus** causa baixa do quociente respiratório (BLISS, 1951). Fala também em favor de o efeito hormonal provir principalmente do órgão X, a observação de TRAVIS (1951) segundo a qual, depois da extirpação do pedúnculo, mas não depois de extirpação da glândula do seio, alterar-se o conteúdo de fósforo sangüíneo e fósforo inorgânico em **Panulirus**. O hormônio do órgão

X regula o metabolismo do fósforo. PYLE (1943) estudando a histogênese do pedúnculo, verificou já no embrião, que o órgão X, evolui antes do aparecimento da glândula do seio. Esta pode regenerar-se depois da extirpação (BLISS & WELSH 1952).

A função das células α no sistema nervoso central de camarões é ainda desconhecida (ENAMI 1951b). Semelhante a cromatóforos da pele dos crustáceos, as células distais do pigmento da retina estão sob o contrôle do hormônio existente nos extratos do sistema nervoso central e do pedúnculo ocular (WELSH, 1941; SMITH, 1948; BROWN, FINGERMAN & HINES, 1951; BROWN, HINES & FINGERMAN, 1952). Uma localização mais exata dêsses efeitos até agora não foi feita pormenorizadamente (SCHARRER & SCHARRER, 1954).

As considerações feitas acima sôbre os tipos de células neurosecretôras, baseadas no trabalho de ENAMI (1.c.) não se modificam deante do recente estudo publicado por MATSUMOTO (1958, p. 107) em que se faz a revisão dos referidos tipos. Todavia, julgo de conveniência lembrar que êste autor indíca as seguites vias de percurso da substância neurosecretora, a saber: para a glândula do seio, através do cérebro e dos pedúnculos opticos, para os espaços tissulares originando-se em cada gânglio e para o os nervos das extremidades proveniente do gânglio toráxico (p. 159). Sejá lembrado a este respeito que MATSUMOTO trabalhou apenas com Crustáceos marinhos.

Como se vê, o processo da neurosecreção nos Crustáceos ainda não se acha perfeitamente esclarecido. Inúmeros são os problemas abertos à discussão e resolução. Nos demais Invertebrados ainda menos se sabe sôbre êste assunto.

Afora os Insetos e outras classes de invertebrados, últimamente se vêm acentuando investigações sôbre determinados órgãos tidos como secretores de hormônios e relacionados com o sistema nervoso. Assim, na opinião de CARLISLE (1951, p. 468 e 1951a, p. 202) a glândula neural das Ascideas é uma glândula homóloga à pituitária dos Vertebrados. Segundo o mes-

mo autor, injeção de gonadotrofina corionica de Mamíferos em **Ciona** e **Phallusia** provoca a liberação de gametas.

A maturação dos gametas dos Poliquetos é também regulada por um fator oriundo do cérebro (DURCHON, 1951). Por outro lado, células neurosecretoras têm sido descritas também em **Chilopodes** e **Onychophoros.**

Os órgãos internefridiais dos Vermes Sipunculideos aproximadamente se assemelham aos órgãos interrenais dos Vertebrados (SCHARRER. 1941); as glândulas protorácicas podem ser comparadas ao tipo descrito (SCHARRER 1948).

A verificação das atividades dos hormônios aqui mencionados fêz-se até agora pela via biológica. Só recentemente é que BUTENANDT (1954) obteve, em forma pura cristalizada, um dos hormônios responsáveis pela metamorfose dos Insetos, utilizando pupas de **Bombyx mori.** Empregou pupas dêstes insetos para isolar o hormônio das glândulas protorácicas, o qual, como se sabe, é responsável pela muda da pele. Teve de empregar quantidade muito grande de Insetos (500 quilos) para se conseguirem os cristais puros. Para se obter uma reação no teste, foi necessária uma quantidade de 0,0075 gama por animal. O material cristalizado foi usado biològicamente por C. A. WILLIAMS em pupas de Lepidopteros, que estavam em hibernação (sono de inverno), sendo capaz de promover o desenvolvimento das pupas para o estado de imago, provando assim que se tratava de um hormônio das glândulas protorácicas. A análise química demonstrou que se trata de uma substância sem nitrogênio, cuja composição é $C_{4,5}H_{7,3}O$. E' òticamente ativa, funde-se numa temperatura de 235-237°C e demonstra um máximo de absorção de 244 milimicrons.

Como se vê, o estudo da neurosecreção em Invertebrados e em Vertebrados acha-se ìntimamente relacionado com o dos hormônios, especialmente da primeira destas divisões. Várias têm sido as revisões bibliográficas de ambos os temas. Nas páginas precedentes procuramos focalizar sòmente alguns aspectos mais interessantes para os objetivos que temos em mira.

Resta apenas lembrar, em resumo, o estado atual destas questões. Referindo-se o presente trabalho particularmente

aos Crustáceos, será digna de menção a mais recente resenha apresentada por KNOWLES & CARLISLE (1956) onde se encontra extensa revisão da bibliografia e judiciosos comentários a respeito. De acôrdo com êstes autores, há divergências quanto à função da glândula do seio. Assim, a ablação dêste órgão não importa numa completa cessação (p. 399) dos efeitos hormônicos normais, o que levou alguns pesquisadores a considerarem a possibilidade da produção de hormônios em outras regiões do pedúnculo ocular fora da "glândula". Entre êles contam-se KNOWLES (1951), que identificou os hormônios produzidos na região do tritocérebro e ENAMI (1951) que descreveu várias células neurosecretoras localizadas no cérebro de **Sesarma**. Os produtos da secreção são conduzidos ao longo das fibras nervosas até a glândula do seio. Conclusivas, neste particular, foram as pesquisas de PASSANO (1951, a, b) ao verificar que a secreção ocorre nos grupos de sélulas neurocretoras gigantes localizadas na **medulla terminalis** e que o material secretado é transportado ao longo dos axônios das fibras para a glândula do seio. Existe, mesmo, conforme BLISS & WELSH (1952) verdadeiros sistemas neurosecretores dos pedúnculos oculares nos Crustáceos, e que a glândula do seio recebe fibras axônicas de muitas partes dos gânglios do pedúnculo ocular e do cérebro e algumas de outras partes do sistema nervoso central. Conclusivas ainda foram as experiências de BLISS & WELSH sôbre a ablação da glândula do seio com conseqüente acúmulo de material neurosecretor após degeneração dos axônios. Em tais casos houve regeneração de material secretado aí depositado. Tais experiências conduziram ao resultado de se admitir a glândula do seio apenas como um repositório do material original das células neurosecretoras, e que a própria "glândula" não tomaria parte no processo de secreção. Cumpre notar, porém, que alguns autores (ENAMI 1951: GABE 1952, 1953) admitem que o tecido da "glândula do seio" também produz secreção hormonal.

Como quer que seja, fato indiscutível é o de estar a glândula do seio em conexão com as células neurosecretoras existentes nos órgãos nervosos. Algumas dessas células, localizadas no

pedúnculo ótico, constituem o que se denomina "órgão X". Vários foram os elementos rotulados com êste nome, antes que HANSTRÖM (1939) emitisse a hipótese de o "órgão X" representar células sensoriais transformadas, i. é, células de uma papila ocular rudimentar ou poro sensorial, e por isso tais estruturas habitualmente se designam por "órgão X sensorial de HANSTRÖM". Tal órgão caracteriza-se pela presença de concreções de forma concêntrica, tidas por HANSTRÖM como produtos acumulados de secreção, e por CARLISLE (1953b) interpretadas como terminações nervosas dos axônios provenientes da **medulla terminalis,** sendo que cada axônio se divide em muitos ramos cada um dos quais termina em um corpo claviforme de várias camadas (CARLISLE, 1953, a. b. c). Êsse conjunto assemelha-se à contextura de um bulbo de cebola visto em secção, e daí o nome que se lhe atribuiu de "corpos de cebola". Tal estrutura, sabe-se, caracteriza o "órgão X de Hanström" nos **Decapoda Natantia.**

O encontro de um órgão no pedúnculo ocular de **Cambarus** por WELSH (1941) semelhante ao órgão X de HANSTRÖM, e sua posterior descrição por BLISS & WELSH (1952), PASSANO (1951 a, b, 1952), PORTER (1954) e BLISS e col. (1954) como órgão X, levou à indicação da existência de mais de um órgão com essas características. Mais tarde, em 1953, CARLISLE & PASSANO verificaram não se tratar da mesma estrutura e designaram êste último órgão pelo nome de **pars ganglionaris X organi** que corresponde ao "órgão X" dos autores americanos, dando a designação de **paz distalis X organi** (PDX) ao "órgão X de HANSTRÖM". A ocorrência de ambas essas partes conjuntamente ou separadamente nos Crustáceos é variável, havendo vários graus de reunião de ambas. Por outro lado, como geralmente se aceita, o órgão X de HANSTRÖM acha-se associado à papila sensorial e devido a êste fato KNOWLES & CARLISLE (1956, p. 401) propuseram a designação de "papila sensorial do órgão X" (SPX) a esta estrutura que contém os "corpos cebola" e outros. Assim, o PDX de CARLISLE & PASSANO passaria a designar-se SPX de KNOWLES & CARLISLE. Em virtude do fato de os grupos celulares ocor-

rerem distintamente ou se espalharem pelos vários núcleos do sistema nervoso central, e principalmente por encontrar-se o maior número dêstes grupos na medula terminalis, KNOWLES & CARLISLE (1956, p. 402) sugeriram a substituição do PGX da nomenclatura há pouco citada pela designação MTGX que quer dizer "órgão X ganglionar da medula terminal". Descrições pormenorizadas do MTGX em **Sesarma** foram feitas por ENAMI (1951, b) e em **Lysmata** por CARLISLE (1953, d. e). No primeiro caranguejo êle é formado de um grupo de células unipolares gigantes de cêrca de 50 μ de diâmetro, que forma um agrupamento bem evidente na superfície ventral da porção proximal do neuropilo da medula. Em **Lysmata,** que se assemelha ao Camarão, as células do MTGX são um pouco menores que as de **Sesarma** (25-40 μ) mas semelhantes a elas. Assim, pode-se deduzir que o mesmo nome de "órgão X" foi dado a duas estruturas diferentes que funcionam diferentemente (KNOWLES & CARLISLE 1956, p. 403).

Pelo que acabamos de mencionar, além do "órgão X de Hanström" no pedúnculo ocular dos Crustáceos outras estruturas existem, com função incretória, descritas como êsse órgão e rotulados com o mesmo nome. A questão se torna mais complicada quando se procura examinar estas estruturas em conexão com a glândula do seio. Vários autores mostraram que tal "glândula" vem a ser o ponto de encontro de fibras neurosecretoras de vários grupos celulares que existem no pedúnculo ocular ou outras regiões (BLISS & WELSH, 1952; CARLISLE 1953a e POTTER, 1954; BLISS e col. 1954; KNOWLES, 1955). Há comum acôrdo em considerar que o material produzido nos corpos celulares neurosecretores é transportado ao longo dos axônios terminando as respectivas fibras na glândula do seio sob a forma de terminações claviformes. Ainda comum é o acôrdo quanto às propriedades corantes das inclusões secretadas que passam dos corpos celulares para a glândula do seio. Os corpos celulares são dotados de finos grânulos basófilos que se podem verificar também na parte proximal do axônio; a parte intermediária da fibra contém material homogêneo que se cora em lilás pela cromo-hematoxilina-floxina (BLISS

& WELSH, 1954); mais distalmente o material está sob a forma de gotículas maiores que são floxinofílicas e nas terminações bulbares há uma massa homogênea de material acidofílico secretado que contém grânulos basófilos (BLISS e col. 1954). Haveria pois evidência da transformação do material secretado quando passa do ponto de origem para o ponto de liberação, e que a condição acidófila é a condição de armazenamento. Todavia, como salientam KNOWLES & CARLISLE (1956, p. 403) não se pode ainda decidir se os corantes usados coram material hormônico ou substância transportadora.

Por outro lado, a verdadeira função da glândula do seio, segundo os autores acima citados não se acha elucidada com precisão. Como se viu, há autores que negam sua participação no processo secretor, e outros, como GABE (1952, 1953, 1954) que considera haver elementos histológicos evidentes que autorizam a admitir tal participação. Aventou-se ainda a idéia da existência de hormônio precursor que viria transformar-se no hormônio pròpriamente dito dentro da glândula. Observou-se ainda que a remoção da glândula do seio não afeta a muda, mas a remoção do complexo glandular do seio-órgão X a provoca (PASSANO 1953 b). Parece provável, concluem KNOWLES & CARLISLE (1955, p. 404) ocorrerem transformações químicas na glândula do seio que envolvem a atividade do material hormônico e seu transporte através da membrana limitante da glândula e que, possìvelmente, haverá elementos celulares na glândula do seio responsáveis pela produção das enzimas necessárias para as transformações químicas das matérias precursoras ou das substâncias envolvidas na passagem dos hormônios através da parede da glândula.

Minhas pesquisas não procuraram abordar muitos dos aspectos acima assinalados. Motivos vários levaram-me a restringir meu estudo aos seguintes pontos:

O sistema neurosecretor de **Tr. petr.**

a) atividade motora dos crustáceos considerados normais;
b) comportamento dos animais após remoção do pedúnculo ocular.

Neurosecreção e metabolismo respiratório do animal, avaliado pelo oxigênio consumido.
a) animais íntegros;
b) " monopedunculados;
c) " bipedunculados.
Influência da pedunculoectomia sôbre a atividade motora.
Influência da luz.
Propriedades do extrato de pedúnculos.
Influência do pedúnculo sôbre o funcionamento do escafognatito.

II — MATERIAL E MÉTODOS

A — A espécie, coleta e manutenção no laboratório

O animal usado no presente trabalho, como dissemos, foi **Trichodactylus petropolitanus** GOELDI, Crustacea, Decapoda. E' comum nas águas do sul do Brasil e ocorre em grande número no Rio Tietê e seus tributários, na vizinhança da cidade de São Paulo. Vivem em águas pobres de oxigênio e podem ser considerados verdadeiros "anfíbios" no sentido próprio do têrmo. E' de se supor que as características físico-químicas das águas dos rios que banham São Paulo, tenham induzido os **Trichodactylus** a conquistarem o meio aéreo de modo muito acentuado (VALENTE, 1948). O seu mecanismo regulador da respiração reside principalmente no funcionamento dos escafognatitos, que são um par de placas localizadas nas maxilas internas (2a. maxila). Estas placas são responsáveis pela direção da corrente de água que penetra pela abertura inalante e pela remoção da água empobrecida dentro da câmara branquial eliminando-a pela abertura exalante.

Por serem animais de hábitos noturnos, a coleta geralmente era feita durante a noite, com auxílio de armadilhas apropriadas. No laboratório eram mantidos em aquários de vidros em água de torneira, constantemente renovada e arejada. A temperatura do aquário era igual a do ambiente natural, isto é, aproximadamente 22°C.

B — Métodos utilizados

1. Histologia

Para o estudo histológico do tecido neurosecretor de **Trichodactylus** usamos vários fixadores, tais como, Susa, Bouin, Zenker e vários métodos de coloração, i. é, Mallory da tríplice coloração, GOMORI ou mesmo outras técnicas inclusive o exame com coloração vital. O método de Gomori, no qual os grânulos de secreção se coram seletivamente em azul escuro, foi o preferido com a simplificação indicada por SCHARRER & SCHARRER (1954, p. 957). O seu emprêgo fêz-se da seguinte maneira:

Peças fixadas em Susa, geralmente durante 14 horas, deshidratadas e incluídas em parafina. Cortes desparafinados e deshidratos permaneceram em Bouin a 37°C com ou sem ajuntar alumem de cromo a 3% durante 12 horas. Depois do mordente, os cortes, geralmente de 5 a 8 micra, foram lavados em água da torneira durante 5 minutos e em seguida sofreram um tratamento durante um minuto na seguinte solução: Permanganato de potássio a 2,5% — 20 cm^3; ácido sulfúrico a 5% — 20 cm^3; água — 160 cm^3.

Nessa solução os cortes adquirem a côr castanha, a qual deve ser retirada por imersão numa solução de bisulfito de sódio a 3%, a seguir lavados em água corrente durante 5 minutos e permanecendo depois uma hora na hematoxilina. A composição da hematoxilina é a seguinte: hematoxilina aquosa 1% mais alumem de cromo 3%, em parte iguais; ajuntar a 100 ml de hematoxilina 2 ml de bicromato de potássio a 5% e mais 2 ml de ácido sulfúrico a 2,5%. Esta solução deve amadurecer durante 48 horas antes de ser usada. Guardada na geladeira, a solução conserva suas propriedades até dois meses, mas antes do seu uso deve ser aquecida a 22°C. Em geral os cortes se hipercoram, mas pela lavagem rápida em água distilada, álcool 70% e ácido clorídrico a 1% durante 1-5 minutos diferenciam-se bem. Depois da diferenciação os cortes são lavados em água corrente até que fiquem azuis. Faz-se uma coloração durante 5 minutos numa solução de floxina (eritrosina BB) a 0,5%, lava-se ràpi-

damente e coloca-se um minuto numa solução de 5% de ácido fosfovolfrâmico. Finalmente, lava-se a preparação durante 5 minutos em água corrente. Procede-se à montagem como de hábito.

2 — Medida da atividade locomotora coordenada

Um dos meios mais simples para se medir a atividade locomotora é o registro dos movimentos locomotores coordenados (EDWARDS 1950) do animal localizado dentro de um disco plástico (Fig. 1) constituído de 2 discos de 8 cms de raio

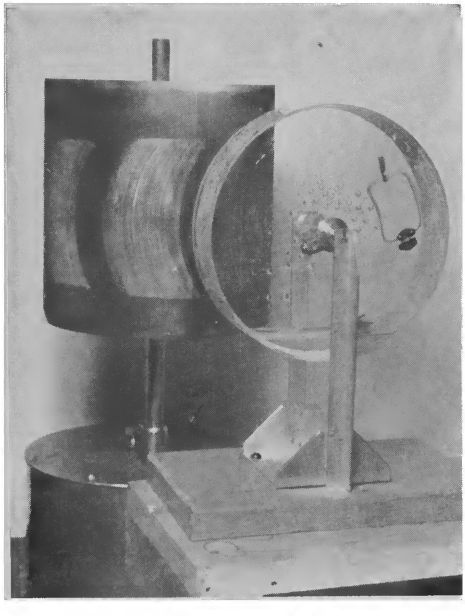

Fig. 1 — Aparelho para medida da atividade locomotora. (Edwards, 1950).

mantidos por uma lâmina de 4 cms de largura, cuja face interna é provida de grânulos de areia de modo a oferecer uma superfície áspera que possibilite o animal movimentar o disco quando anda. Nas faces do disco há orifícios para ventila-

ção e uma janela, pela qual se introduz o animal. O pêso da janela é equilibrado por pesos colocados no lado oposto. O disco gira em tôrno de um eixo suportado por dois braços de metal. Todo o aparelho é de tal forma equilibrado de modo a girar ao menor movimento de um pequeno animal, sendo assim apropriado para **Trichodactylus**. Colocou-se uma pequena quantidade de água no interior do disco para dar ao animal condições próximas do seu ambiente natural. Um fio de cabelo, fixo numa das bordas da roda e tangente à superfície de um quimógrafo, propiciou o registro dos movimentos do animal. A revolução do quimógrafo usado é de 8 dias de duração, o que possibilitou o registro gráfico diurno e noturno. Sendo de 50 cms a circunferência da roda, cada traço do registro correspondeu a essa extensão, isto é, uma certa distância percorrida pelo animal. Tal aparelho possibilita avaliar a atividade motora de **Trichodactylus** em diferentes condições.

3 — Remoção e preparação do extrato do pedúnculo ocular

Na remoção do pedúnculo ocular, os animais eram prèviamente submetidos, por alguns minutos, à baixa temperatura, isto é, ao redor de 4°C, com o que se evitaram hemorragias, pois com o frio aumenta-se a viscosidade do sangue. Os pedúnculos removidos eram depositados num dissecador e postos na geladeira a 0°C. Para preparação de extrato, os pedúnculos foram triturados e a massa resultante dissolvida em etanol aquecido a 60°. Pela evaporação do álcool obtêm-se cristais que, por três vêzes, foram dissolvidos e recristalizados. Finalmente, os cristais foram conservados na geladeira até o momento de serem usados, dissolvidos em Ringer para Crustáceos (VALENTE 1958 no prelo). Nos experimentos, ml 0,1 de uma solução equivalente a 20 pedúnculos por ml foi injetado numa equivalência final, pois, de 2 pedúnculos por animal.

4 — Medida do consumo de 0_2

Determinou-se o consumo de 0_2 dos animais no microrespirômetro volumétrico de SCHOLANDER (1942). O gás carbônico produzido foi absorvido por ascarite colocada na câ-

mara que continha o animal, o qual aí ficava, no fundo, em alguns ml de água. Fizeram-se as medidas em banhos com temperatura constante a 25°C. No fim de cada experimento secaram-se os animais com papel de filtro, procedendo-se à pesagem. Indicou-se o consumo de oxigênio em mm^3 consumidos por mgr. e por hora ($mm^3 0_2$/mgr/hr).

III — PARTE EXPERIMENTAL

A — O sistema neurosecretor localizado no pedúnculo ocular de Tr. petropolitanus

Como se viu anteriormente, no presente trabalho trata-se sòmente de alguns aspectos da atividade motora e do metabolismo do **Tr. petropolitanus** (**Tr. p.**), em relação com os elementos neurosecretores localizados no pedúnculo ocular.

Pela resenha bibliográfica já mencionada, viu-se que tais elementos neurosecretores variam quanto à estrutura, topografia e função, segundo os crustáceos considerados. Até agora, as pesquisas efetuaram-se com animais marinhos, principalmente **Decapoda Brachyura**. Quanto aos crustáceos de água doce, ùnicamente **Cambarus** e **Astacus** que são Decapoda macrura, foram investigados. Ora, **Tr. p.** é um **Decapoda Brachyura** de água doce, com a característica excepcional de ser desprovido de cromatóforos. Possìvelmente, na migração para o ambiente límnico houve interferência de fatôres que levaram à perda dos cromatóforos. Daí o interêsse em se procurar saber se, não obstante esta peculiaridade, no pedúnculo ocular de **Tr. p.** ainda subsistem elementos neurosecretores responsáveis pela elaboração de princípios cromatoforotrópicos como os existentes nos correspondentes marinhos, e, nesse caso, se guardam êles as mesmas características. Ainda mais, como se viu, tais elementos e seus produtos influem decididamente na atividade motora e no metabolismo do animal.

a) Pedúnculo ocular

O pedúnculo ocular de **Tr. p.** (Fig. 2), visto da parte externa para a interna, consta de uma carapaça quitínica, bastante grossa, calcificada, transparente apenas na região da retina.

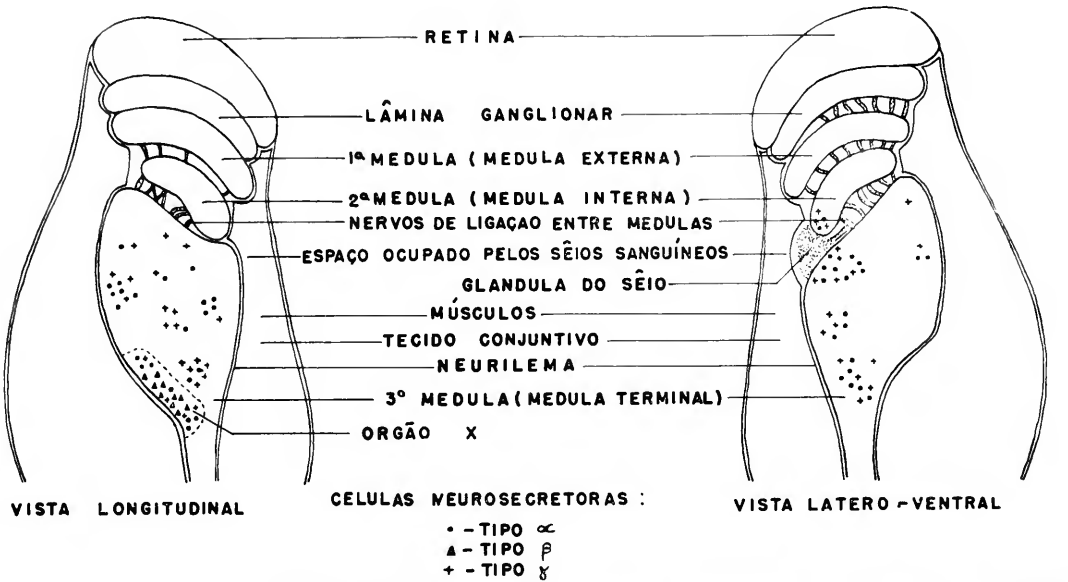

Fig. 2 — Localização esquemát'ca no pedúnculo ocular de Trichodactylus petropolitanus, da glândula do seio, do órgão X e da distribuiçãc das células neurosecretoras.

Removendo-se essa carapaça encontra-se uma membrana conjuntiva escura, bastante pigmentada, rica em melanina, por baixo da qual se encontram músculos estriados, em pacotes bastante grossos. O centro do pedúnculo é ocupado por um órgão sensorial nervoso formado pela retina, lâmina ganglionar da retina, medulas: externa, interna e terminal (Fig. 2). Esta última se continua pelo lobo ótico, que, saindo do pedúnculo vai até o gânglio cerebral do animal. As medulas e o lobo ótico são recobertos por uma membrana — o neurilema — que na parte externa é rica em tecido colágeno não pigmentado e na parte em contacto com o órgão nervoso é rica em tecido conjuntivo frouxo. O tecido colágeno separa o tecido nervoso dos grandes seios sangüíneos, dos quais êste se acha cercado.

b) Glândula do seio

Acha-se localizada, em **Tr. p.**, dentro do pedúnculo ocular, entre a medula interna e medula terminal, podendo, quando muito rica em grânulos, expandir-se atingindo região mais alta, até a medula externa. Vista em preparações frescas, é um órgão globular, de côr azulada leitosa, de contôrno às vêzes irregulares. Ocupa a posição dorso-lateral interna em relação ao eixo mediano do animal. Removendo-se a carapaça quitínica, abaixo da membrana conjuntiva pigmentada, encontra-se a glândula do seio que em material fresco, é fàcilmente distinguível.

Fazendo-se a dissecção do material fresco inicialmente, e depois fixando-o lentamente com formol, consegue-se isolar a glândula do seio que é ligada por um ramo nervoso ao lobo ótico e, por outros 2 ramos de 2 grupos de células neurosecretoras. Recebe a glândula também um filete nervoso vindo da lâmina ganglionar.

Examinando cortes em séries do pedúnculo ocular ainda não me foi possível traçar corretamente o caminho dos ramos nervosos dentro das medulas.

A glândula do seio apresenta-se em cortes transversais como uma formação triangular (Figs. 3 e 5) cheia de grânulos que se coram, pelo Susa-Mallory, em castanho-arroxeado, e ou-

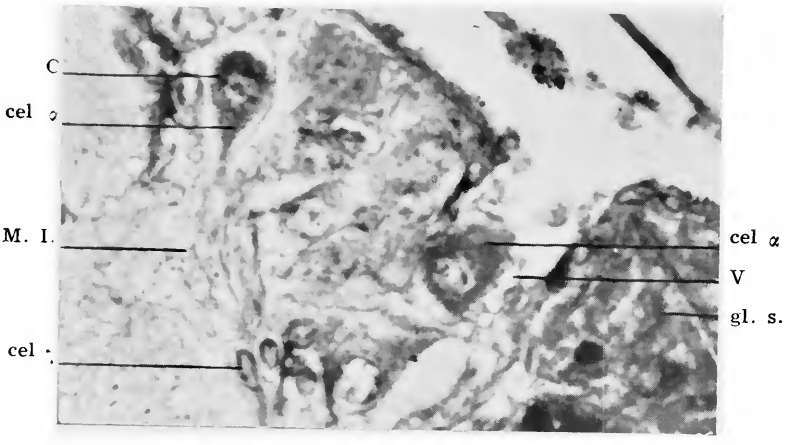

Fig. 3 — Corte transversal de pedúnculo ocular de *Trichodactylus* passando pela medula interna (M. I.), e glândula do seio (Gl. s.) mostrando vacúolos (V), citoplasma (C) de células neurosecretoras α e γ. Aum. 950 x. (Fotomicrografia).

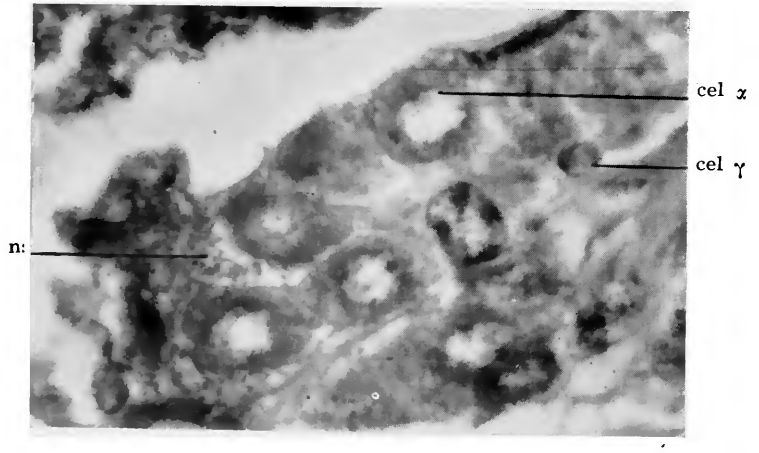

Fig. 4 — Corte transversal do órgão X onde notamos neurosecreção (ns) e células α e γ. Aum. 1.400 x. (Fotomicrografia).

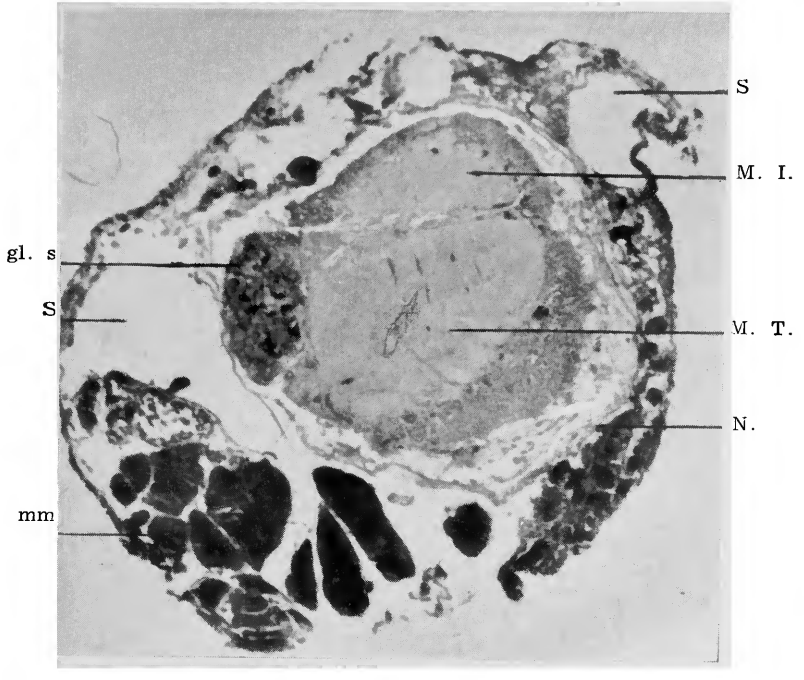

Fig. 5 — Corte transversal do pedúnculo na altura da medula terminal (M. T.) e medula interna (M. I.). Notar a glândula do seio (Gl. s.), seio sangüíneo (S), nervos (N) e musculatura (mm.). Aum. 150 x. (Fotomicrografia).

tros que se coram em vermelho. Examinando-se cuidadosamente a parte interna da glândula em contacto com o sistema nervoso, vê-se que parece ser formada por terminações nervosas que se dilatam muito ao chegar à glândula, as quais transportam os grânulos (Fgs. 5 e 8). Mas, em geral, a estrutura da glândula é, tôda ela, mascarada pela grande quantidade de grânulos. A glândula, na sua parte externa, é separada do grande seio sangüíneo por tecido conjuntivo — o neurilema (Figs. 3 e 5).

c) Células neurosecretoras

Existem vários tipos de células neurosecretoras, localizadas em grupos e isoladamente. A sua distribuição é, quase tô-

da ela confinada à **medula terminalis** (Fig. 2). Além do órgão X, há agrupamentos de células neurosecretoras bastante conspícuos, como se pode ver pela figura 2.

Encontramos os três tipos de células neurosecretoras descritas por ENAMI (1951), células α, β e γ distribuídas por vários grupos (Fig 2).

C_1) Células α — são células de tamanhos variáveis, indo 130 micra x 100 a 60 x 60 micra, com núcleos cujo tamanho é de 40 micra e com 6 nucléolos. Às vêzes apresentam vacúolos citoplasmáticos bastante grandes (Figs. 3 e 6)

As células são comumente piriformes e monopolares. Nelas se podem encontrar grânulos que se coram pelo Susa-Mallory em vermelho, podendo também a secreção tomar a côr

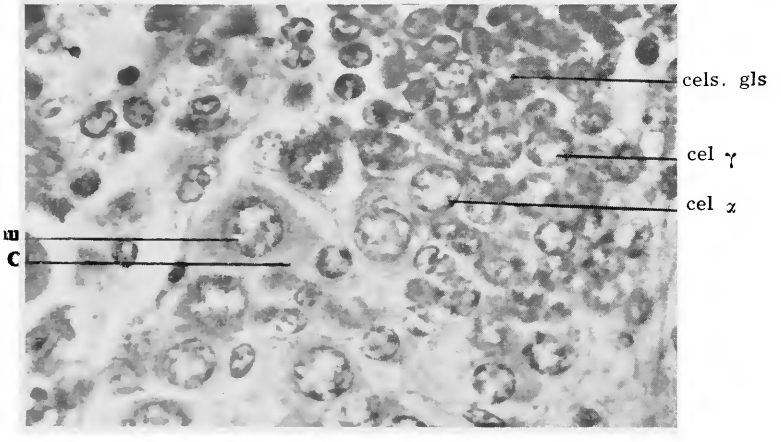

Fig. 6 — Medula terminal do pedúnculo ocular mostrando agrupamento de células neurosecretoras (α e γ) com os núcleos (nu), citoplasma (C) e Células gangliomares (cels. gls.). Aum. 1.300 x em Fotomicrografia.

arroxeada. Esta variação de côr talvez seja devida a estados funcionais diferentes. Em muitas células pude ver a secreção saindo pelo prolongamento celular (Fig. 7). Além de agrupadas, algumas são visíveis isoladamente.

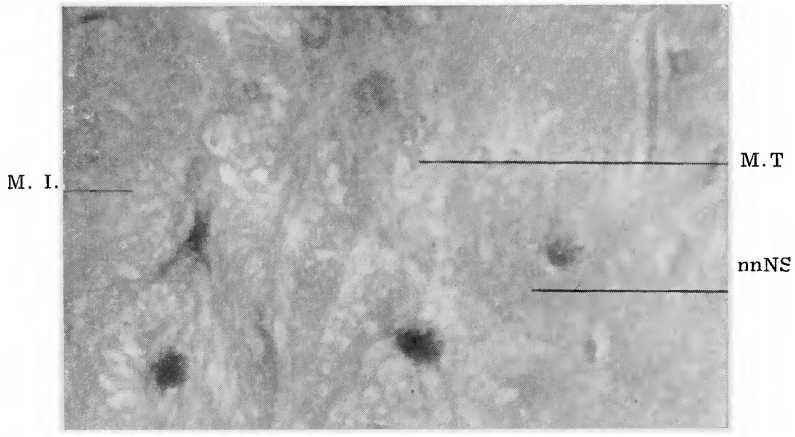

Fig. 7 — Corte longitudinal de nervo com neurosecreção (nnNS) passando pela medula terminal (M. T.), medula interna (M. I.) e nervos com neurosecreção (nnNS), dirigindo-se para a glândula do seio. Aum. 1.400 x. (Fotomicrografia).

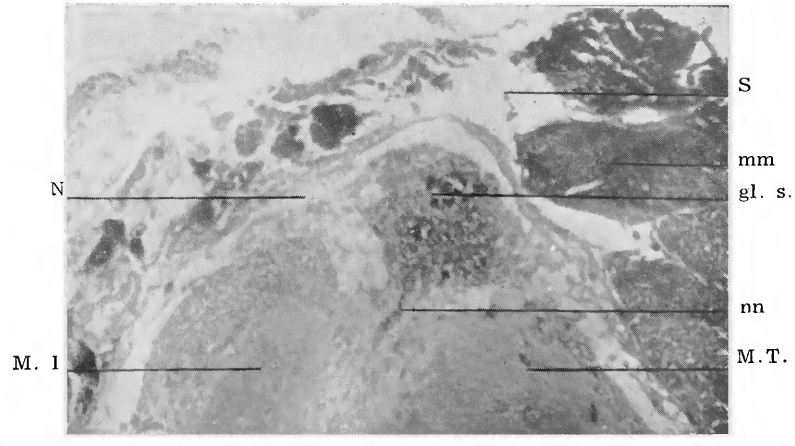

Fig. 8 — Nervo (nn) desembocando na glândula do seio na altura da medula terminal (M. T.). Medula interna (M. I.), musculatura (mm), nervos (nn), neurilema (N) e seios sangüíneos (S) são também observados. Corte transversal aumentado 320 x. (Fotomicrografia).

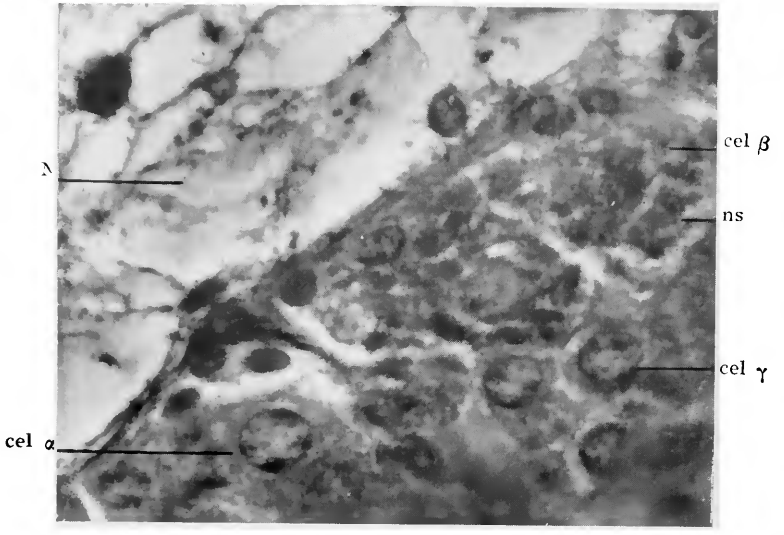

Fig. 9 — Células neurosecretoras dos tipos α, β e γ, neurilema (N) e neurosecreção (ns) num corte transversal do órgão X. Aum. 1.500 x. (Fotomicrografia).

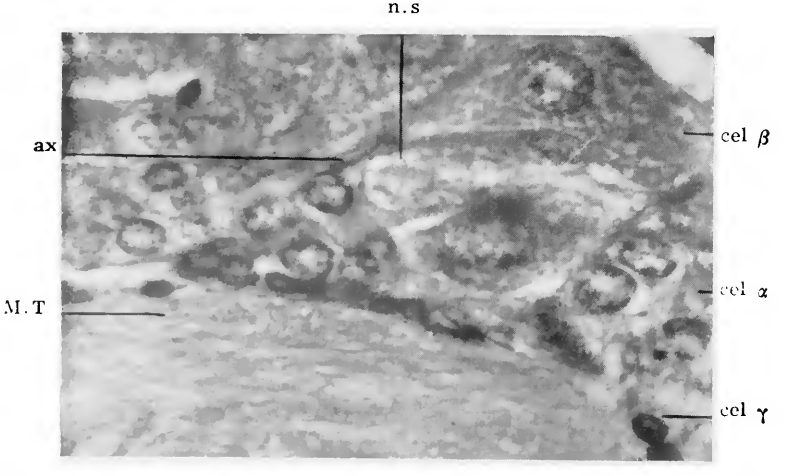

Fig. 10 — Corte transversal do órgão X notando-se medula terminal (M. T.), substâncias neurosecretoras (n. s.) ao longo do axônio (ax). Aum. 1.300 x. (Fotomicrografia).

C_2) Células β — Trata-se de elementos monopolares grandes (Fig. 11) cuja variação de tamanho é menor que no caso anterior (células α). O tamanho vai de 110 x 110 micra até 100 x 60 micra, com menor número de nucléolos e com núcleo de 40 a 30 micra. Têm grânulos que se coram pelo Susa-Mallory em roxo, e pelo Gomori em azul; a granulação lembra de

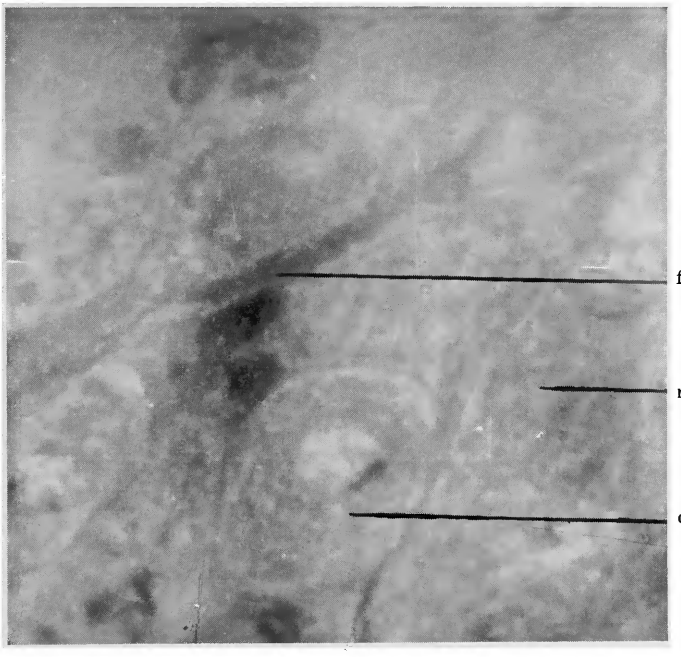

Fig. 11 — Substâncias neurosecretoras (n. s.) numa fibra nervosa (f. n. n. s.) saindo de uma célula β do órgão X. Aum. 1.850 x. (Fotomicrografia).

certo modo a substância tigróide ou de Nissl, do sistema nervoso central dos vertebrados (Fig. 11). A distribuição destas células (Figs. 8, 9, 10, 11 e 12) é confinada ao órgão X. Êste órgão, como se pode ver pela Fig. 2, possui além das células β, células α e γ.

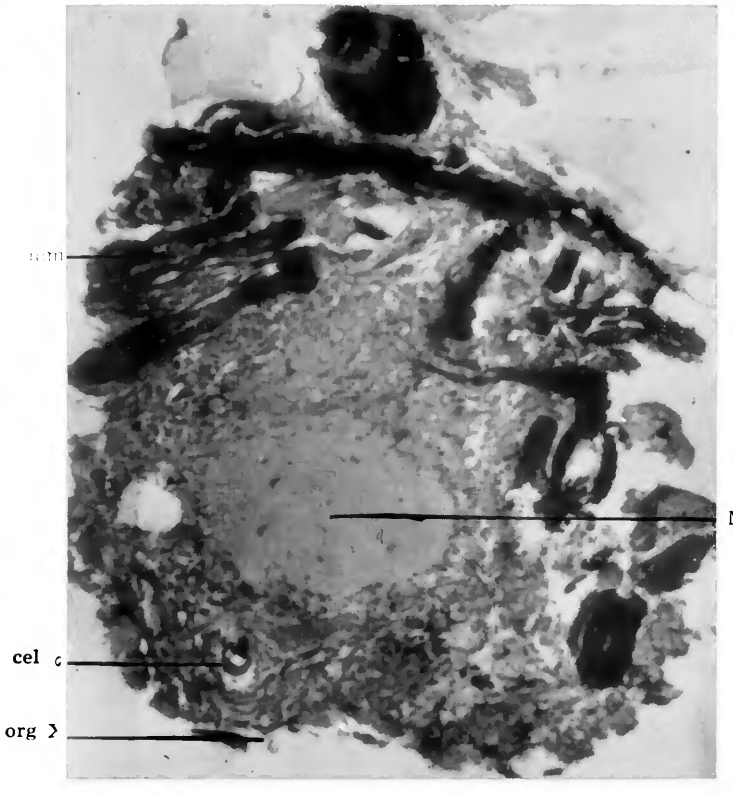

Fig. 12 — Topografia do órgão X (org X) e da medula terminal (M. T.) do pedúnculo ocular de *Trichodactylus petropolitanus* em corte transversal. Ainda vemos musculatura (mm) e célula alfa (cel $_{\alpha}$). Aum. 150 x. (Fotomicrografia).

C_3) Células γ — São células menores que as α e β, com menor conteúdo citoplasmático, porém maior que o das células ganglionares. Seus contornos são perfeitamente distinguíveis, ao contrário do das ganglionares. Existe em todos os agrupamentos de células neurosecretoras. Muitas vêzes não apresentam grânulos; êstes quando ocorrem são de difícil observação. O tamanho das células gama vai de 40 x 30 a 50 x

30 micra, com núcleo variando de 20 a 15 micra e com grande número de nucléolos (Fig. 6).

d. Seios sangüíneos

São grandes lagos sangüíneos ao redor do órgão nervoso (Fig. 8).

Com estas observações histológicas, verifiquei que no pedúnculo ocular de **Tr. p.** existem células e órgãos neurosecretores cuja distribuição foi indicada.

B — **Neurosecreção e respiração**

a) Consumo de Oxigênio pelos **Trichodactylus**

Sabe-se que o consumo do oxigênio pelos crustáceos pode apresentar variações se forem estirpados os pedúnculos oculares. Nos **Tr. p.** procurei primeiro determinar a taxa do oxigênio consumido por animais íntegros, recentemente capturados, com boa atividade locomotora. Fiz as medidas segundo a técnica já indicada, de 18 **Tr.** adultos (8 machos e 10 fêmeas) com máximo de 6 dias de captura, todos em jejum.

TABELA 1

Consumo de oxigênio pelos Trichodactylus petropolitanus íntegros e sem pedúnculos

Animais e sexo	Íntegros		Monopedunculados		Apedunculados	
	N.º de animais	mm^3O_2 mg/hr	N.º de animais	mm^3O_2 mg/hr	N.º de animais	mm^3O_2 mg/hr
Machos	8	0,069	5	0,045	10	0,131
Fêmeas	10	0,063	3	0,041	14	0,103

Os resultados das determinações encontram-se na tabela 1, pela qual se verifica não ter havido, pràticamente, diferenças de oxigênio consumido quanto ao sexo (0.069 pelos ♂ e 0.063 pelas ♀, mm^3O_2/mg/hr).

A seguir, determinei o oxigênio consumido por machos e fêmeas, mono-apedunculados. Os resultados das medidas de 6 ♂ e 3 ♀ mostraram ainda que a operação não ocasiona diferenças do consumo quanto ao sexo dos animais (0.045 e 0.041 de $mm^3 0_2$ mg h respectivamente pelos ♂ e ♀), mas indicam sensível diferença quando se comparam êstes resultados com os dos anormais (de 0.024 para os ♂ e 0.022 para as ♀).

De 10 machos e 14 fêmeas apedunculados, os primeiros consumiram em média 0.131 e as segundas 0.103 $mm^3 0_2$/mg/h, o que corresponde a uma diferença de 0.062 e de 0.040 compa-

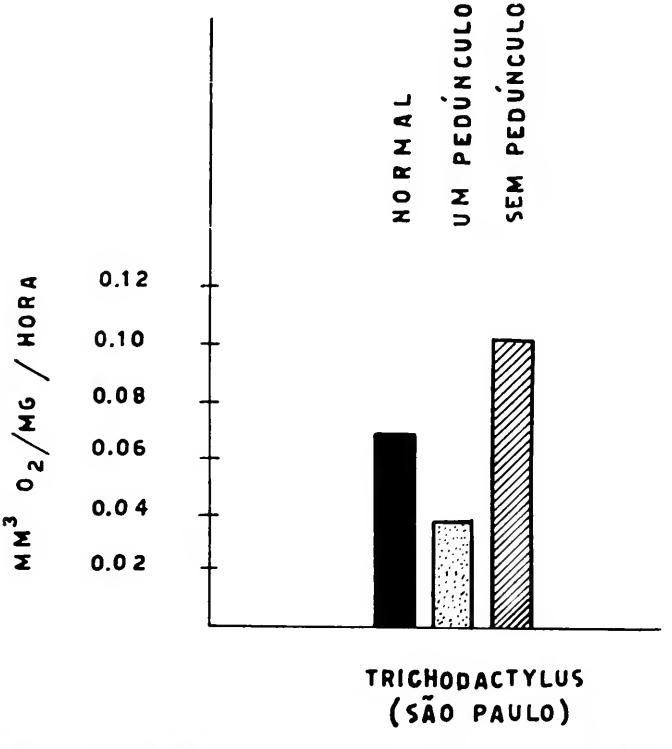

Fig. 13 — Influência da remoção do pedúnculo ocular sôbre o consumo de oxigênio de *Trichodactylus petropolitanus*.

rando-se com o consumo pelos **Tr.** normais, respectivamente machos e fêmeas e de 0.086 e 0.058 comparados com os machos e fêmeas mono-apedunculados.

Cumpre salientar, com relação ao último caso, que a pendunculectomia se fazia nos animais submetidos a 4°C durante 2-5 minutos, como foi dito, e que só eram utilizados 48 horas após a operação.

Como se vê, pois, pelos resultados da Tabela 1, os animais sem ambos os pedúnculos têm um consumo 54% maior que o dos normais, ao passo que **Tr.** monopedunculados mostraram um consumo 34% menor que o dos íntegros.

TABELA 2

Influência da remoção dos pedúnculos sôbre o consumo de oxigênio de uma fêmea de **Trichodactylus petropolitanus**

Dias	Pêso (gr)	Horas após operado	Q 0_2	Q 0_2 médio	% de variação diária
1.° dia	9,897	0	0,066	0,066	
2.° dia	"	30	0,094		
	"	31	0,090		+ 35%
	"	32	0,084	0,089	
3.° dia	"	46	0,132		
	"	47	0,123		+ 69%
	"	48	0,099		
	"	49	0,090	0,111	
4.° dia	"	72	0,070	0,070	+ 6%
6.° dia	"	144	0,122		
	"	145	0,122	0,122	+ 84%
7.° dia	9,603	168	0,052		
	"	169	0,053	0,052	— 20%

A Fig. 13 representa um gráfico dêstes resultados, no qual se verifica esta expressiva diferença entre o comportamento dos animais íntegros, mono- e bi-apedunculados.

Ainda mais, se compararmos os resultados das medidas do oxigênio consumido pelos **Tr.** mono- e bipedunculectomizados, verificamos que a diferença de consumo de O_2 é de 88%.

Os animais operados são relativamente resistentes, pois duram cêrca de um mês no aquário, se alimentados com fragmen-

tos de carne crúa (de vaca), de minhoca ou de peixes de água doce.

Depois de me certificar dessa resistência procurei conhecer o comportamento dos animais operados após período mais longo que o das experiências há pouco relatadas que foram feitas 30 horas depois da extirpação dos pedúnculos. Como se pode ver pelo gráfico da Fig. 14 o consumo, logo após a bipe-

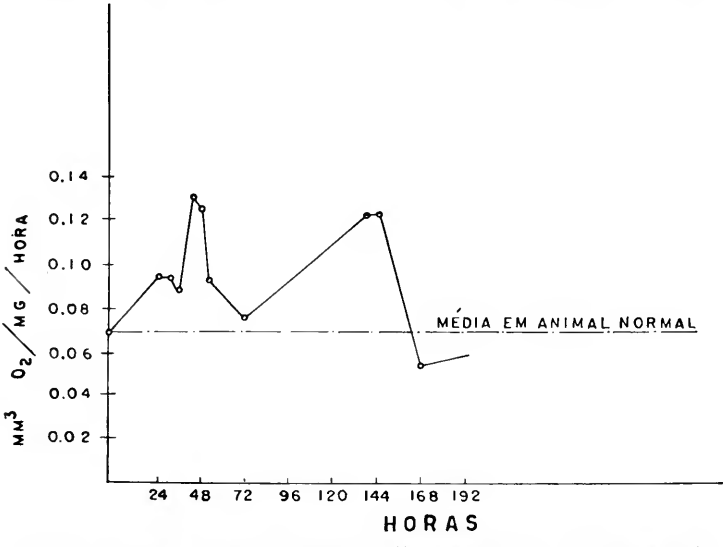

Fig. 14 — Variação do consumo de oxigênio de *Trichodactylus* após a pedunculoectomia.

dunculectomia, é normal (0.066 $mm^3O_2/gr/hr$). Trinta horas após a bipedunculectomia entram a sofrer oscilações conservando-se, não obstante, até cêrca de 168 horas acima do valor médio normal. A variação em percentagem oscilou de um mínimo de 6% até um máximo de 100% de aumento do consumo de oxigênio.

Interessante de se assinalar é a porcentagem de variação diária (tab. 2), enquanto que no segundo dia o consumo teve um aumento de 35% acima da média do consumo normal, no terceiro êsse aumento passou para 69%, decrescendo sensivel-

mente para 6% no quarto dia, com novo aumento para 84% ainda acima do consumo médio normal no sexto dia. Sòmente no sétimo dia foi que o consumo de oxigênio nesses animais sem pedúnculo acusou 20% abaixo do normal.

b) Influência da remoção do pedúnculo sôbre o escafognatito.

Uma vez que a pedunculectomia decididamente influi na taxa de consumo de O_2, pareceu-me importante verificar se tal se dá através de ação sôbre o mecanismo respiratório, antes que por meio de levantamento real do metabolismo.

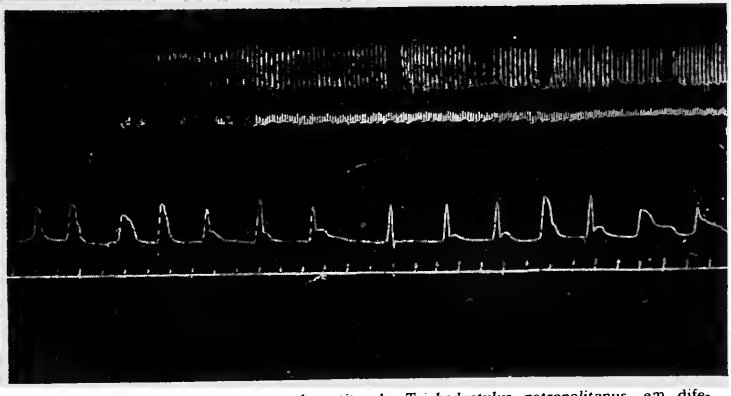

Fig. 15 — Batimentos de um escafognatito de *Trichodactylus petropolitanus*, em diferentes velocidades. Tempo em segundos.

Como se sabe, o tipo de respiração dos **Tr.** é o branquial, havendo nove brânquias e três epipoditos dentro da câmara branquial, de uma estrutura que lhe faculta longa permanência no ar, absorvendo diretamente da atmosfera o oxigênio (VALENTE 1948). A entrada da água para a câmara branquial dá-se pelo orifício inalante e saída pelo exalante localizado no rostro.

A movimentação do fluído dentro das câmaras branquial e pré-branquial, fenômeno a que se dá o nome de ventilação, faz-se com o auxílio do escafognatito. Êste ocorre bilateral-

mente e é formado por uma placa localizada na maxila interna (2a. maxila). Quando em posição normal, dispõe-se paralelamente à porção anterior do epipódio do primeiro maxilípede e fica situado entre êsse epipódio e a região pterigostômica. Está ligado ao coxopodito da segunda maxila, e nessa região é orientado para cima e para trás, alargando-se bastante na parte contida na câmara branquial. No animal adulto, o escafognatito mede ca. de cm 0,7 de diâmetro antero-posterior (VALENTE 1948). O escafognatito tem por função remover a água empobrecida de oxigênio, permitindo assim, a penetração de água fresca dentro da câmara branquial.

Em trabalho anterior verifiquei que os escafognatitos são responsáveis pela renovação do meio aquático dentro da câ-

Fig. 16 — Pausas nos batimentos de um escafognatito de *Trichodactylus* em animais íntegros. Tempo em segundos.

mara branquial, sendo a freqüência dos seus batimentos diretamente proporcional à queda da tensão do oxigênio contido na água. São êles também sensíveis ao gás carbônico, pois a freqüência dos batimentos é aumentada com a elevação do CO_2 contida na água, até um ponto em que, na água saturada com gás carbônico, os **Tr.** ficam narcotizados. A freqüência e a intensidade dos batimentos dos escafognatitos varia, de acôrdo com a temperatura, pH, a presença de água, excitações mecânicas diretas ou indiretas e as tensões de 02 e as de CO2 do meio aquático (VALENTE 1948).

Nas experiências que se seguem vali-me do método gráfico para registrar os movimentos do escafognatito. Removendo-se pequena porção da região pterigostômica da carapaça, os escafognatitos são expostos e com auxílio de gancho delicadissimo (alfinetes entomológicos n.º 000) foi sua borda an-

terior transfixada e o gancho ligado por um fio de cabelo a uma leve alavanca inscritora. Imobilizou-se o animal numa placa e, em seguida, foi o mesmo colocado num aquário em decúbito dorsal. Os registros gráficos fizeram-se com o auxílio de um quimógrafo.

Registrou-se, primeiramente (Fig. 15) os batimentos do escafognatito de um animal íntegro, isto é, com ambos os pedúnculos oculares. São características dos batimentos normais, as pausas de tempo em tempo, que correspondem a paradas do movimento do escafognatito. No mesmo gráfico temos, na parte inferior, os batimentos em uma velocidade maior (Figs. 15 e 16).

Em seguida operei um **Tr.** conforme a técnica já descrita para retirar um pedúnculo ocular. Vê-se que as pausas que, são consideradas normais, aqui também se apresentam indicando que a remoção de um pedúnculo não altera os batimentos dos escafognatitos.

Numa terceira série de experiências usaram-se animais bipedunculectomizados (Fig. 17) fazendo-se o registro gráfico du-

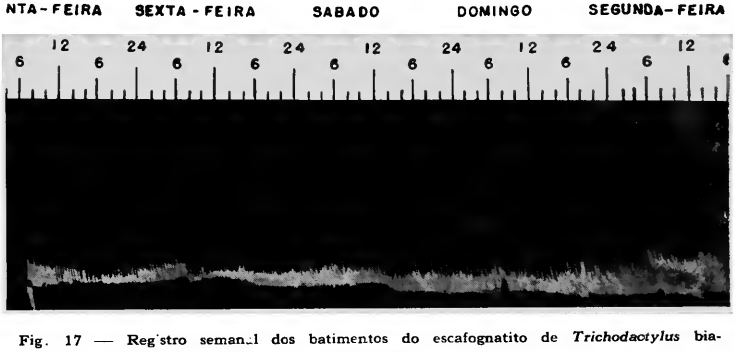

Fig. 17 — Registro semanal dos batimentos do escafognatito de *Trichodactylus* biapedunculado.

rante 6 dias. Sem ambos os pedúnculos oculares o escafognatito não deixou de funcionar durante êsse período.

Com êsses resultados podemos concluir que a ventilação da água dentro da câmara branquial, isto é, os batimentos dos es-

cafognatitos, não está relacionada com os hormônios localizados dentro do pedúnculo ocular.

C. RITMO DE ATIVIDADE

a. Consumo de Oxigênio e atividade locomotora

Os estudos dos fenômenos de coordenação e de integração nos Crustáceos têm sido objeto de muitas pesquisas, principalmente no tocante ao papel aí desempenhado pelos hormônios produzidos nas diferentes partes do corpo. Inúmeras analogias já se têm estabelecido entre funções endócrinas dos Vertebrados e as dos Crustáceos (BROWN, F. A. Jr., 1948; VALENTE 1958).

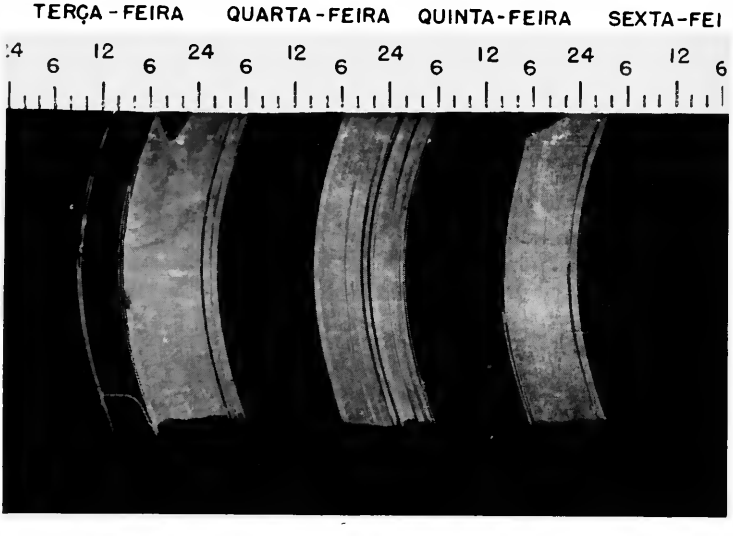

Fig. 18 — Atividade locomotora normal de *Trichodactylus petropolitanus*.

Os hormônios que interferem em tais funções e que se produzem no sistema nervoso central ou anexos, ainda não se acham bem estudados, faltando dados referentes à isolação ou purificação dos princípios ativos, para se ter uma idéia das verdadeiras funções dessas substâncias. Segundo BROWN (l.

c., p. 160), comparado com os nossos conhecimentos sôbre os mecanismos hormônicos dos Vertebrados, o que se sabe sôbre os hormônios dos Crustáceos, ainda está num estado muito elementar e fragmentário.

Nos estudos para o conhecimento dos hormônios dos Crustáceos como em geral dos Invertebrados, os processos até agora comumente usados são: 1. extirpação de tecido ou órgão contendo hormônios; 2. implantação de tecido ou de órgãos; 3. transfusões de sangue e 4. injeções de extratos de tecido glandular.

Nas condições em que consegui elaborar o presente trabalho sòmente me foi possível adotar as técnicas da extirpação dos pedúnculos e das injeções de extratos dos mesmos. Para poder avaliar o comportamento dos animais nas condições experimentais procurei registrar, segundo a técnica já mencionada à pág. 23, o ritmo da atividade motora e suas variações.

Observações dos animais nos aquários dispostos no laboratório, constantemente arejados com o auxílio de uma bomba compressora, indicaram-nos, desde logo, que os **Tr.** são animais de atividade noturna, pois raramente se movem durante o dia se não forem perturbados por ruídos. Apenas escurece, começam a locomover-se no fundo do aquário e passam a capturar os alimentos. Essa locomoção é relativamente lenta, mas contínua.

Os registros de animais íntegros obtidos no aparelho da Fig. 1 corroboraram a observação precedente (Fig. 18). Em média, percorrem os animais ca. de 210 m por noite. Geralmente iniciam a atividade com o crepúsculo, entre 18 e 19 horas na primavera e cessam-na pela manhã (nos registros 7.04 hs. em outubro) (VALENTE e EDWARDS, 1955).

O registro da atividade motora de um **Tr.** íntegro mostra, além disso, não ser ela contínua, durante a noite, pois há interrupção periódica como se pode verificar no respectivo gráfico (Fig. 19) relativo à locomoção durante uma noite, quando o movimento se iniciou às 20,30 hs. e cessou às 7 horas do

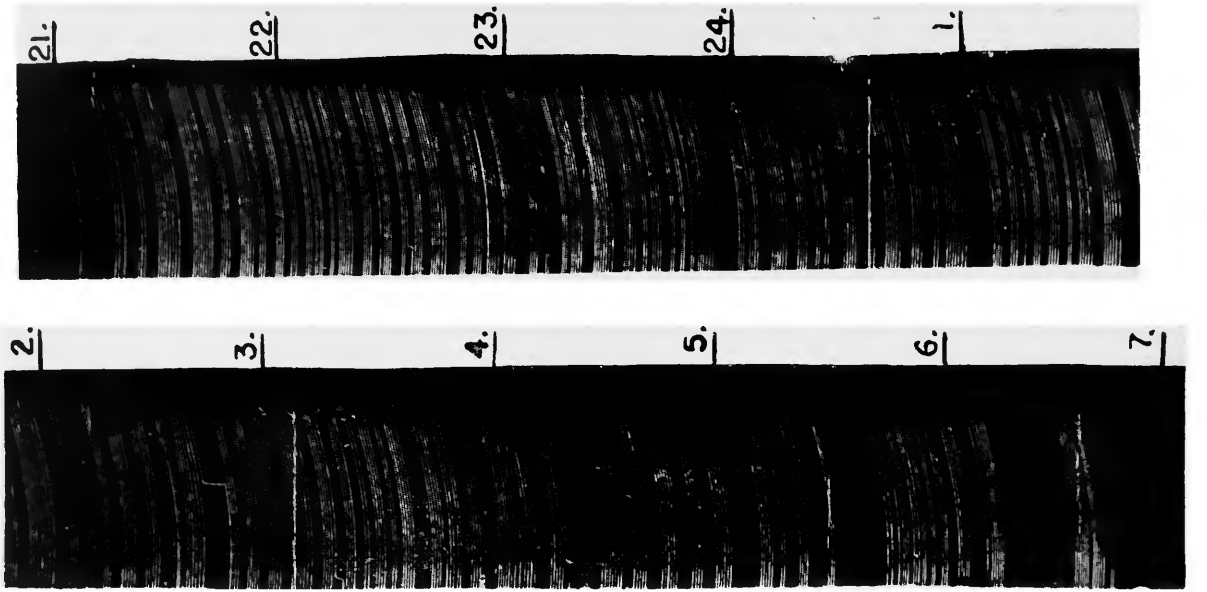

Fig. 19 — Atividade locomotora de *Trichodactylus petropolitanus* durante uma noite (das 21 às 7 horas).

dia seguinte. O percurso durante êsse período foi de 209 metros.

Dificuldades técnicas impediram-me de registrar concomitantemente a atividade locomotora e o consumo de 02. Todavia, não é supérfluo confrontar a velocidade locomotora com os dados obtidos pelo consumo de oxigênio de normais e mono ou bi-apedunctomizados durante o dia. **Tr.** normais percorrem 210 m durante um ciclo de 24 horas e têm um consumo de oxigênio de 0.066 mm3/03/mgr/hr, durante o dia.

O comportamento dos animais mono-pedunculados no aparelho foi o seguinte:

Nota-se desde logo que a locomoção é arítmica (Fig. 20) e caracteristicamente contínua embora desordenada. O animal caminha desordenadamente durante as 24 horas, numa média que não ultrapassa o registro respectivo (Fig. 20) não ultrapassa 9 m num ciclo de 24 horas para um consumo de oxigênio diurno também mais reduzido (em média de 0.045 ..... $mm^3/0_2/gr/h$).

Nos **Tr.** apedunculados, a locomoção apresenta características semelhantes às anteriores (Fig. 21), mas o percurso percorrido é maior, pois varia de 1 a 45 m no ciclo de 24 horas para um consumo de oxigênio diurno, todavia bem maior.

Os resultados, pois, indicam que a locomoção do **Tr.** íntegro é rítmica e dependente da luminosidade, cuja influência inibidora se dá logo que o animal está sob a influência da luz. A Fig. 19 representa o registro da atividade locomotora de um **Tr.** durante uma noite, com o quimógrafo de revolução mais rápida para mostrar as características dos movimentos. Neste registro vê-se que, numa hora, há cêrca de 46 movimentos do disco o que corresponde a 23m/h. Nota-se, todavia, que o movimento não é contínuo, havendo entre cada 2-6 movimentos parada do animal.

Os **Tr.** mono-pedunculados perdem, como se viu, a ritmicidade, mas não a capacidade de movimentar-se, sendo até impossibilitados de permanecer em repouso durante o dia, como os **Tr.** íntegros, o que pode significar que algo neles ocorre a impedir a inibição do movimento como se observa durante

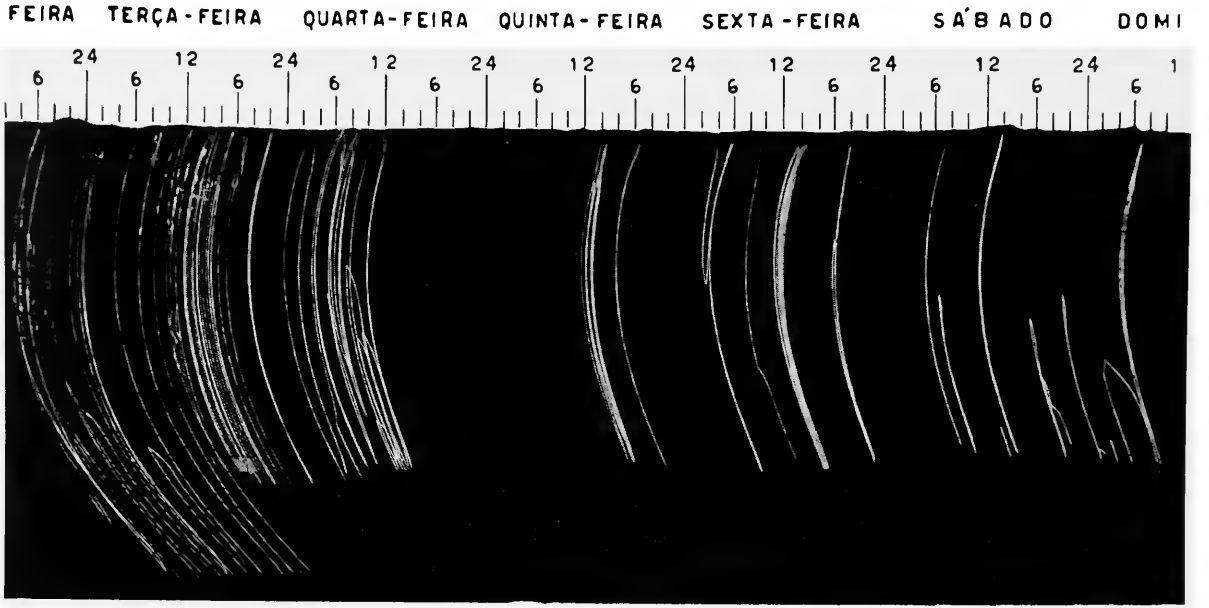

Fig. 20 — Atividade locomotora de *Trichodactylus petropolitanus* monopedunculados.

o período diurno. Por outro lado, como vimos, tais **Tr.** consomem em média 0.045 $mm^3 0_2/gr/hr$ durante o dia e percorrem no ciclo de 24 hs. uma distância bem menor, de ca. de 9 m por ciclo de 24 hs. E' difícil conciliar o menor consumo diurno registrado nos **Tr.** monopedunculados com a existente embora pequena atividade locomotora observada durante o dia também. Talvez em tais animais o fato de ainda subsistir os órgãos neurosecretores localizados nos pedúnculos se acha relacionado com o fenômeno.

Nos **Tr.** apedunculados há também perda de ritmo, sendo muito mais acentuada que nos monopedunculados a atividade locomotora contínua (Fig. 21). Os animais exibem todavia atividade locomotora bem menor que a dos animais íntegros, pois o percurso em um ciclo de 24 horas é de ca. de 45 m. Entende-se neste caso, porque o consumo de 02 diurno dêstes animais é muito maior que os dos normais (0.13 $mm^3 0_2/mg/hr$), o que se deve evidentemente à falsa atividade locomotora diurna, ao passo que nos **Tr.** íntegros a atividade é apenas noturna.

b. Ação da luz

Um dos fatôres que influencia a atividade dos Crustáceos é a luz. Viu-se (p. 22) que os **Tr.** são animais tìpicamente noturnos. A Fig. 22 registra os movimentos executados durante a noite e as fases de repouso diurno. Essas duas fases do ciclo de vinte quatro horas do animal coincidiam com os períodos de luminosidade e de obscuridade. Todavia, dever-se-ia ainda verificar se o fenômeno era dependente. na realidade, da interferência da luz ou de outros fatôres. A análise experimental do fenômeno iniciou-se com a manutenção dos animais durante 24 horas na obscuridade e depois, durante o igual período, sob a ação ininterrupta da luz artificial. A fim de verificar a influência tão sòmente dêsse fator: luz e falta de luz, procurei manter os **Tr.** em ambiente privado de outras fontes de estimulação, principalmente ruídos.

Assim o disco foi posto a funcionar durante uma semana sob a iluminação de lâmpada de 200 watts colocada a 50

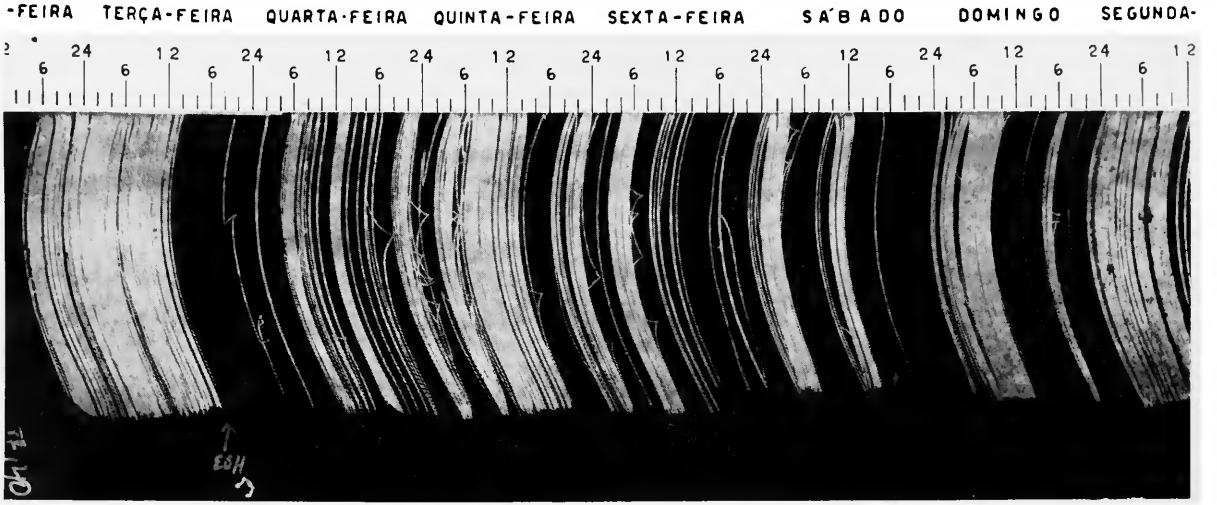

Fig. 21 — Atividade locomotora de um *Trichodactylus* pedunculectomisado e ação do extrato peduncular (ESH).

cms de distância. A quantidade de luz recebida pelo animal era de 400 Westons, medida com um fotômetro Weston.

A Fig. 22 mostra que a despeito da iluminação contínua, subsistem os ritmos, embora os períodos de atividade correspondente à noite sejam mais atenuados. Em conseqüência, a extensão percorrida foi menor, pois em média não ultrapassa de 10 ms por noite.

Numa segunda série de experiências os animais foram mantidos ininterruptamente no escuro e registrada a atividade. Os gráficos obtidos não diferem dos normais (Fig. 22). A alternância dos períodos de atividade e de repouso é a mesma, sendo idêntica a extensão dêsses períodos. Assim, a ausência de luz, durante o dia, não parece induzir nessa parte do ciclo de 24 hs.

Os resultados de tôdas estas experiências indicam que o comportamento dos **Tr.** nas referidas condições depende em certa medida também da luz, mas a ritmicidade está muito mais relacionada com fatôres internos, sendo pois, um fenômeno intrínseco do animal. Voltarei ao assunto na discussão.

c. Ação do extrato do pedúnculo

A remoção de ambos os pedúnculos oculares não afeta essencialmente o tipo de atividade exibido por animal que possui apenas um pedúnculo ocular, isto é, nos 2 casos a atividade contínua arítmica e contínua durante todo o tempo da experiência, de sete dias de duração.

Viu-se ainda que a ritmicidade deve achar-se relacionada com fatôres internos. O passo seguinte, pois, foi verificar a possível relação entre a presença ou ausência dos pedúnculos oculares com êsses fatôres. Para isso utilizamos animais íntegros, mono e bipedunculados, cuja atividade locomotora era registrada como acima se descreveu, e que em certa fase dos experimentos receberam injeções de extrato de pedúnculos oculares.

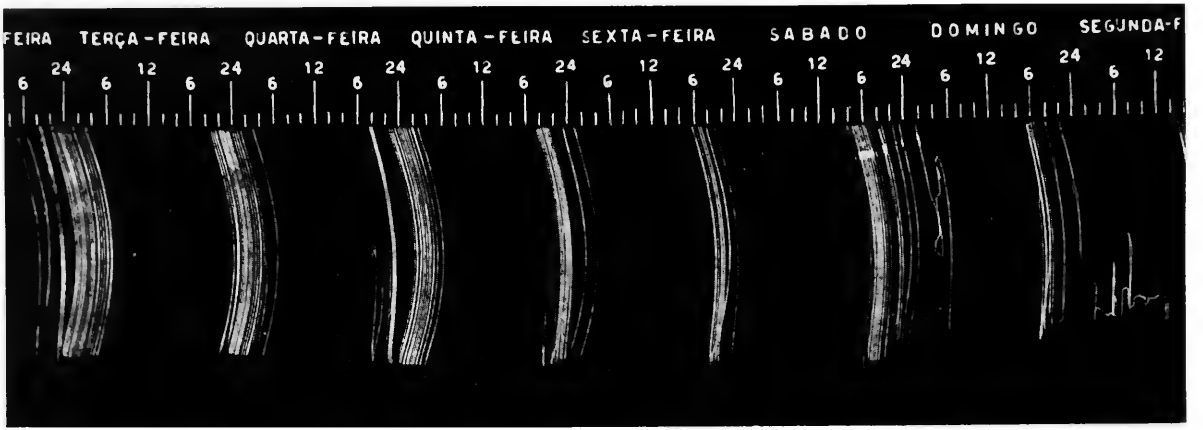

Fig. 22 — Ação da luz sôbre a atividade locomotora de um *Trichodactylus* íntegro.

D. ATIVIDADE LOCOMOTORA

a. Extração do princípio ativo

Para extração do princípio ativo, os pedúnculos foram triturados e dissolvidos em etanol quente; em seguida, o álcool foi evaporado naturalmente a 37°C e os cristais assim obtidos foram disolvidos em Ringer para Crustáceos segundo a seguinte fórmula (VALENTE, 1958):

Líquido para perfusão de **Trichodactylus** (Braquiuros de água doce). Valores em grs/litro. pH 5,5

NaCI	5,5788
KCI	0,6769
CaC12	1,6082
MgC12	0,6982
Na3PO4	0,1163

A solução usada equivale a 20 pedúnculos por ml, o que, na base do ml 0,1 injetados, corresponde finalmente, a uma concentração de 2 pedúnculos por animal.

Nos pedúnculos oculares encontramos a glândula do seio formada exclusivamente por terminações nervosas repletas de grânulos. Êstes estão envolvidos por uma membrana permeável à água e a eletrólitos e impermeáveis aos não eletrólitos como sacarose e albumem. Segundo PÉREZ GONZÁLEZ (1957) os homogenados da glândula do seio, em sacarose, libertam os hormônios contidos nos grânulos quando submetidos aos seguintes tratamentos: 1. Diminuição da tonicidade do meio; 2. resfriamento e aquecimento sucessivos; 3. aquecimento, durante 5 minutos em banho-maria; 4. ação de detergentes e outras substâncias; 5. em soluções eletrolíticas.

No nosso caso, fizemos a extração e rompimento da membrana que envolve os grânulos contenedores de hormônios à custa do resfriamento e aquecimento sucessivos e sua extração em banho-maria dada a sua solubidade em etanol quente.

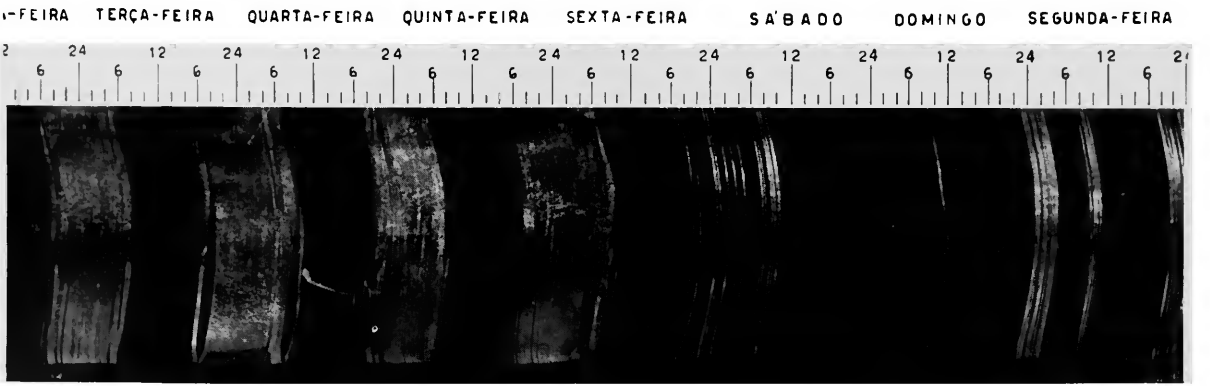

Fig. 23 — Influência da injeção do extrato peduncular, à noite, sôbre a atividade locomotora de animais íntegros

b. Ação do extrato de pedúnculo sôbre a atividade locomotora de **Trichodactylus** íntegros.

Para estudar a ação do hormônio (ou hormônios) do pedúnculo ocular, fiz inicialmente experimentos em animais normais. Colocado o caranguejo no disco plástico, após 5 dias e 4 noites sucessivos durante os quais se obtiveram gráficos normais de atividade locomotora (Fig. 23), injetei na quinta noite, às 19 horas, momento êsse que esteve para recomeçar a atividade locomotora, 0,2 ml de extrato dissolvido em Ringer para crustáceo. Nessa dose, o extrato inibiu parcialmente a atividade locomotora durante 3 noites voltando o animal à normalidade na 4a. noite. Êsse resultado sugere a existência, no extrato de pedúnculo ocular, de uma substância ativa que tem a propriedade de inibir a locomoção de **Tr.** que aos poucos se foi gastando.

c. Ação do extrato do pedúnculo sôbre a atividade locomotora:

I. **Trichodactylus** pedunculectomisados.

Numa segunda etapa fiz experiências com animais operados. Como vimos, os **Tr.** desprovidos de pedúnculos oculares têm uma atidade locomotora arítmica e contínua, sugerindo que na ausência da fonte da substância hormonal não cessa a atividade locomotora. Vários animais sem pedúnculo, foram colocados, 48 hs. após a operação no disco plástico, durante vários dias. Como se pode notar na Fig. 21, das 15 horas de segunda-feira até às 14 horas de terça-feira, o animal se locomoveu contìnuamente. Nesse momento injetou-se no abdomem 0,1 ml de extrato de pedúnculo em solução de Ringer. Observou-se completa parada da atividade locomotora durante as 18 horas subseqüentes, após o que a locomoção recomeçou de uma maneira mais lenta e desordenada.

Repetiu-se essa experiência várias vêzes com intervalos de 3 dias conseguindo-se o mesmo resultado, i. é, injeção de extrato sempre provocou uma parada do movimento do animal durante ca. 16 horas, sendo interessante observar que, no

período de 42 horas (terça-feira, 14 horas, até quinta-feira, às 8 horas) o animal se locomoveu apenas 3 metros. No mesmo gráfico pude verificar o retorno à atividade locomotora e a uma nova injeção de ml 0,1 de extrato houve paralisação de 10 horas. Essa experiência também vem confirmar a ação inibidora da atividade locomotora do extrato total do pedúnculo ocular.

II. Trichodactylus íntegros

Finalmente, procurei determinar a ação do extrato do pedúnculo sôbre o comportamento de **Tr.** não operados, à noite. O gráfico 23 mostra que num animal que vinha apresentando o ritmo normal, caracterizado por atividade noturna e pausa diurna já há 4 noites, a injeção, às 19 horas de extrato de pedúnculo, nitidamente induziu um esmorecimento da atividade locomotora nessa noite. O efeito acentuou-se poderosamente na noite subseqüente, sobrevindo afinal a recomposição da atividade noturna completamente na 8a. noite de experimentação.

d. Ação oxitocicomimética do extrato do pedúnculo.

São conhecidas as semelhanças entre muitos dos efeitos hormonais dos elementos incretórios localizados no pedúnculo ocular de **Tr.** e os da hipófise, o que me levou a estudar a possível analogia fisiológica entre o sistema hipotálamo-hipofi-

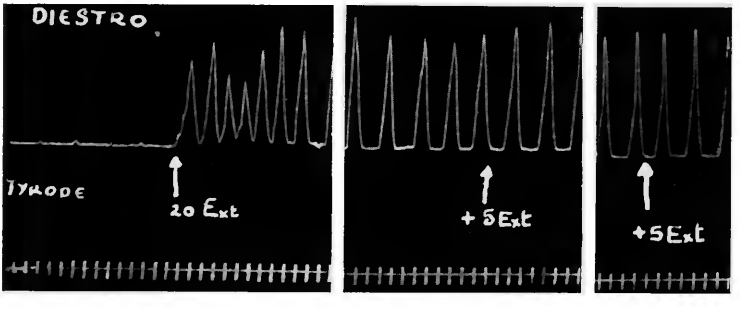

Fig. 24 — Ação de doses crescentes de extrato de pedúnculo sôbre o útero de rata em diestro. Tempo: 30 segundos.

sário dos Vertebrados e o complexo "órgão-X-glândula do seio" dos Crustáceos. Procurei saber se, de fato, o aparelho hipotalâmico dos Vertebrados tem seu correspondente no órgão X dos Crustáceos, pois que possuem ambos células neurosecretoras com fibras que inervam órgãos endócrinos complexos, como pituitária e glândula do seio de Crustáceos. Tanto em Vertebrados como em Invertebrados, tais fibras nervosas contêm o colóide, que pode ser seguido até o órgão de depósito.

Relativamente ao hormônio ou hormônios contidos na glândula do seio dos Crustáceos verificou-se até agora ter influência na regulação da mudança de côr, na adaptação dos olhos compostos a diferentes intensidade de luz, na ecdisis, no me-

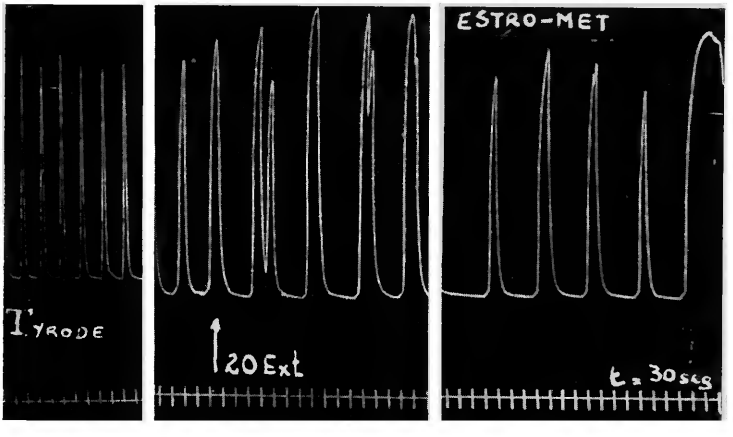

Fig. 25 — Contrações uterinas secundárias de ratas em estro-metestro provocadas pela ação do extrato pedunc ular de *Trichodactylus*. Tempo: 30 segundos.

tabolismo (consumo de oxigênio), na concentração de açúcar no sangue, na regulação do cálcio e do fósforo e na maturidade sexual. Pouco ou nada se tem feito no sentido de averiguar a ação de extrato de pedúnculo sôbre estruturas de Vertebrados.

De um extrato de 30 pedúnculos oculares consegui 0,118 mgrs. de substância sêca, as quais foram dissolvidas em Ty-

rode modificado, sem glicose e que considerei como padrão, pois que o músculo uterino reage bem em tal solução (GRINKRAUT & SAWAYA, 1957). A composição dêsse líquido é a seguinte: NaCl 9 grs; KCl 0,42; $CaC12Cl_2$ 0,06; MgC12 0,005; NaHCO3 0,5; Relação Na/K = 21,4; Relação Mg/Ca = 0,083; seu pH é 8.

Empregou-se o músculo uterino de ratas virgens, WISTAR, de 180 grs (± 20 gr) fornecidas pelo Instituto Butantã. Fêz-se a perfusão em aparelho Palmer para órgão isolado, a 37°C (± 1°C) em 40 cm^3 de Tyrode. O ciclo estral das ratas era verificado antes de cada experiência por um simples esfregaço vaginal corado por azul de metileno a 0,5%. Como se sabe, êsse ciclo repete-se cada 108 a 109 horas (LONG & EVANS 1922). O útero em proestro apresenta poucos movimentos espontâneos: em estro a contração é aumentada com duração de 18-25 hs; no metestro, regridem as contrações e, finalmente em diestro é a fase melhor para pesquisas com o extrato, os movimentos espontâneos são nulos ou quase nulos, tendo duração de cêrca de 57 a 60 hs.

Ação do extrato do pedúnculo ocular pode ser fàcilmente verificada pela Fig. 24, que corresponde ao gráfico obtido com útero em diestro, sob a influência de 20 ml de uma solução de 0,118 mgrs de extrato em 100 ml, contendo cada ml portanto, mgr. 0,00118. Assim que foram adicionados os 20 ml, o útero se contraiu com pequenos intervalos de repouso. Mesmo adicionando-se mais 5 ml por 2 vêzes, êle apresentou o mesmo tipo de contração. Num útero em estro-metestro (Fig. 25) repetiu-se a dose da experiência anterior (20 ml) e surgiram contrações duplas, secundárias com diminuição da freqüência das contrações espontâneas, aumentando de muito a amplitude das contrações.

Dada, assim, a semelhança de resposta com a obtida em ensaios da atividade oxitócica (LANGREBE, KETTERER & WARING, 1956) dos Mamíferos, fiz uma série de experiências de contrôle para ver qual o comportamento dos ratos à própria pitocina dos Mamíferos. A pitocina usada (Parke Davis Co. Ltda.) continha 5 unidades internacionais de oxitocina e

a sua ação em diestro (Fig. 26) revelou-se semelhante a do extrato do pedúnculo ocular.

Outra semelhança relativamente à oxitocina é concernente à empregada. Tanto por oxitocina como por extrato do pedúnculo ocular a amplitude é proporcional a concentração (Fig. 26). Na Fig. 27 vê-se que depois de um pequeno repouso das contrações uterinas espontâneas, quando se injetou 1 ml de extrato do pedúnculo e obtiveram-se pequenas contrações.

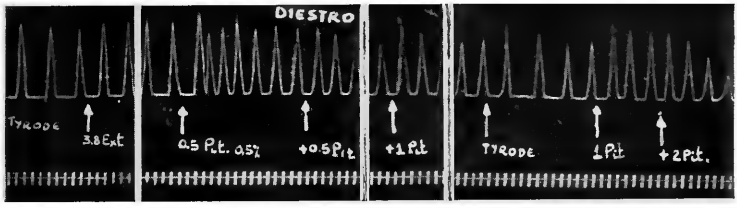

Fig. 26 — Ação do extrato peduncular de *Trichodactylus* e de pitocina sôbre útero de ratas em diestro. Tempo: 30 segundos.

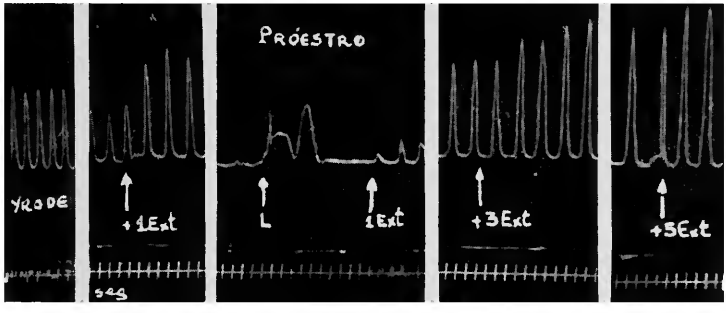

Fig. 27 — Contrações uterinas, de ratas em proestro, provocadas pelo extrato de pedúnculo de *Trichodactylus*. Tempo: 30 segundos.

Com mais 1 ml, a amplitude de contrações foi maior, aumentando sucessivamente com mais 3 ml e mais 5 ml. Portanto, com um total de 10 ml obtivemos, em proestro, contrações secundárias como podem ser vistas na parte terminal do gráfico 20.

IV — DISCUSSÃO

Os resultados das experiências relatadas sôbre o funcionamento dos órgãos incretórios, confirmam em parte os de outros autores e, estabelecem alguns novos pontos de vista sôbre o problema que poderão talvez contribuir para o maior entendimento do mecanismo da neurosecreção.

Discutirei a seguir os resultados obtidos tendo em vista os pontos acima indicados.

1. Relativamente ao consumo de oxigênio pelos **Tr.** verificou-se que a ablação de um dos pedúnculos oculares determina uma diminuição, ao passo que a extirpação dos dois pedúnculos ocasiona, pràticamente, uma duplicação do consumo de oxigênio (Fig. 28). Tal comportamento claramente sugere que

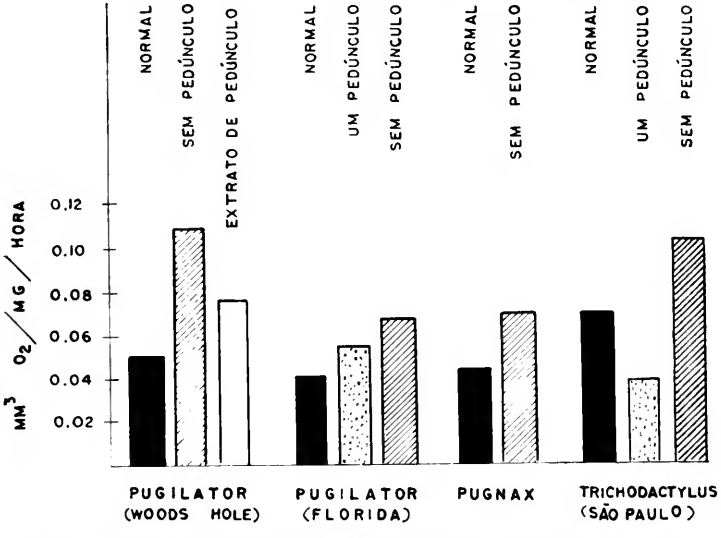

Fig. 28 — Gráfico comparativo da pedunculoectomia em diferentes crustáceos.

a falta parcial ou total dos elementos incretórios elaborados pelos órgãos localizados nos pedúnculos oculares do Crustáceo interfere no processo respiratório. Comparando os animais mo-

no- e bi-pedunculados com os íntegros, observa-se que os primeiros consomem, em média, 34% menos e os segundos 54% mais que os últimos. A bi-pedunculectomia dos animais determina assim um aumento de 88% no consumo de oxigênio em relação à monopedunculectomia.

Já foi dito que é difícil explicar porque a ablação de um só pedúnculo implicou essa diminuição do consumo, quando a extirpação de ambos redunda em aumento. EDWARDS (l. c., gráfico 22) observou em Uca aumento do consumo também no caso da monopedunculectomia. De acôrdo com os meus resultados poder-se-ia pensar em um desequilíbrio surgido pela supressão apenas parcial de hormônio causado pela monopedunculoectomia.

Tais resultados, não obstante, indicam que nos pedúnculos oculares do **Tr.** existem elementos que influem no processo respiratório, fato aliás já conhecido de numerosas pesquisas, ùltimamente sumariadas por SCHARRER & SCHARRER (1954, p. 233; 1955, p. 66). Aliás, sabe-se que o consumo do oxigênio se relaciona também com a fase da muda do animal (SCHARRER & SCHARRER, 1954, p. 425), mas no caso de nossas experiências, os **Tr.** estavam todos fora dêsse período.

2. A remoção de um ou de ambos os pedúnculos não ocasiona perturbação nos batimentos dos escafognatitos, o que sugere que êstes independem da presença de um ou dos dois pedúnculos. Comparados êstes resultados com os do item anterior, pode-se inferir que a ventilação das câmaras branquiais, que se dá pelos escafognatitos, não se modifica pela ausência da fonte de hormônios representada pelos pedúnculos oculares, e que a diminuição ou o aumento do consumo de oxigênio, mencionados há pouco não se acham relacionados com essa ventilação.

3. Visto que a presença ou ausência parcial ou total dos pedúnculos oculares influi acentuadamente no metabolismo respiratório dos Tr. estudaram-se os elementos neurosecretores que ocorrem nesses órgãos e que são bem conhecidos em outros Decápodes.

Revendo a bibliografia verifiquei serem muito escassas as referências sôbre a relação entre atividade locomotora e metabolismo respiratório. A maioria dos autores apenas aborda a questão do metabolismo respiratório relacionado com a presença ou ausência dos pedúnculos oculares. Assim, BAUCHAU (1948, p. 84)fêz a extirpação dos pedúnculos em **Ericheir sinensis** e verificou um rápido aumento do consumo do oxigênio que chega até a 60% relativamente ao gás consumido pelos crustáceos íntegros. Pelo processo dos enxertos de g. s. concluiu ser êste órgão responsável por estas modificações do metabolismo respiratório. Criticando SCUDAMORE (1947, p. 200), que trabalhou com **Cambarus** e encontrou grande variação das medidas de 02 consumido BAUCHAU (l. c., p. 73) atribui o fato às anomalias devidas aos movimentos do animal. Êstes trabalhos referem-se apenas à influência da presença e da ausência dos pedúnculos oculares sôbre o consumo do oxigênio. A relação entre essas duas condições e a atividade locomotora foi estudada por EDWARDS (1950, p. 38). De suas experiências efetuadas com Uca, conclui (p. 49) que a respiração dos monoapedunculados é intermediária entre a dos animais íntegros e os biapedunculados, e que a extirpação de ambos os pedúnculos faz aumentar a respiração do crustáceo. De acôrdo com a Fig. 2 que ilustra o seu trabalho, a falta dos pedúnculos oculares provoca diminuição da atividade locomotora coordenada.

Em minhas experiências, como se viu, essa diminuição também se verifica com a extirpação de um ou de ambos os pedúnculos.

No que se refere ao consumo de oxigênio relacionado com a atividade locomotora e ausência de um ou dos dois pedúnculos oculares, verificou-se que aquela atividade diminui e é menos coordenada quando falta um pedúnculo, ao passo que o consumo de O_2 em animais nessas condições é menor. Há pois, nestes casos, uma correlação entre a diminuição da coordenação da atividade locomotora e o consumo de oxigênio. No caso de animais completamente cegos, também ocorre uma diminuição e maior descoordenação da atividade

locomotora, mas em virtude da continuidade ininterrupta dessa atividade, o consumo de oxigênio é mais elevado que o dos animais íntegros. Compreende-se tal fato em virtude de nestes últimos haver um repouso de doze horas, que não existe nos apedunculados.

4. Um **Tr.** íntegro percorre ca. de 210m em 12 horas noturnas. Essa atividade locomotora se inicia ao crepúsculo (entre 18 e 19 horas no outono) e cessa pela manhã (ca. de 7 horas). Durante o dia os **Tr.** íntegros consomem em média 0.066 mm^3 O_2/mgr/hr. A mono-pedunculectomia determina modificação acentuada do ritmo dos movimentos noturnos, havendo pausas prolongadas e instala a locomoção diurna. Êsse decréscimo da atividade locomotora noturna (animais nestas condições percorrem 9 m em 24 horas) e a instalação da atividade diurna paralelamente coincide com a diminuição do consumo de O_2 medida durante o dia, já apontada. Os **Tr.** bi-pedunculectomizados apresentam um aumento acentuado da atividade locomotora contínua, relativamente a dos monoapedunculados (45 m de percurso em 24 horas). Aqui, concordantemente observa-se o aumento do oxigênio consumido durante o dia. Vê-se pois, que já a ausência de um pedúnculo determina aritmia locomotora (comparada com a dos íntegros), caracterizada pela incapacidade de permanecerem em repouso durante o dia. Essa aritmia caracteriza-se por longos períodos de repouso à noite. o que num ciclo de 24 horas, determina um decréscimo da atividade locomotora total. Nos animais cegos pela bi-pedunculectomia, a aritmia é muito maior, diminuindo acentuadamente os períodos de repouso, com conseqüente aumento da atividade locomotora total. Sobrevem, pois, incapacidade daquele repouso diurno que caracteriza os animais íntegros. Os resultados que aqui apresento concordam com os de EDWARDS (1950, p. 38) que, em Uca, verificou determinar a falta de ambos os pedúnculos oculares, uma diminuição da atividade locomotora coordenada. Nossos resultados indicam na bipedunculectomia uma correlação entre a diminuição de coordenação da atividade locomotora e o consumo de oxigênio.

Assim, pois a pedunculectomia parece claramente interferir com a produção de uma substância inibidora do metabolismo e da atividade locomotora do **Tr.**

Deixei de lado outros aspectos da interferência dos elementos neurosecretores no metabolismo do animal, como p. e., à muda, já exaustivamente estudada por vários autores entre outros, CARLISLE & DOHR (1953, p. 69).

5. Os **Tr.** têm atividade preponderantemente noturna. Êste fato induziu-me a verificar se a luz é um dos fatôres excitantes da neurosecreção. Meus resultados indicam que a atividade locomotora noturna dos **Tr.** íntegros independe de certo modo do fator luz, pois submetidos à iluminação ininterrupta durante 24 horas, apresentam êles o período "diurno de repouso". Apenas é mais atenuada a atividade noturna. A manutenção dos **Tr.** contìnuamente no escuro, todavia, não suprime os períodos diurnos de repouso. O ritmo normal, pois, não seria dependente da alternância diária de luz e escuro mas antes de fatôres internos constituindo o que os autores denominaram "cronômetro biológico". Com êsse nome designam-se os chamados "relógios e calendários" dos sêres vivos (BROWN JR., 1957, p. 129; 1957a, p. 302). Realmente, há nos animais e nas plantas um ciclo de atividades hoje bastante estudado. Por ex., nos animais que habitam a zona entre as marés. Possìvelmente o ritmo cíclico da atividade locomotora do **Tr.** segue essa chamada "cronometria biológica", a qual se acha, como nos outros animais, ìntimamente relacionada com fatôres hormonais.

6. Para comprovar essa asserção poderia indicar os resultados das experiências efetuadas com a injeção de extrato do pedúnculo, em animais apedunculados. De fato, depois de produzida uma substiuição do ritmo pela atividade contínua fazendo a ablação de ambos os pedúnculos oculares, a injeção de extrato de pedúnculo ocular, provocou uma inibição dessa atividade, e um certo retôrno do ritmo. Estas experiências, preliminares, sugerem que no extrato de pedúnculo ocular existe uma ou várias substâncias que interferem na coordenação da

atividade locomotora de **Tr.** Além disso, o fato verificado por vários autores, entre êles EDWARDS (1950) de que a injeção de extrato de pedúnculo ocular diminui o consumo do oxigênio em Uca cegos, corrobora também a asserção do final do item anterior e os resultados que obtive nos Tr. também cegos, quanto à atividade locomotora. Segundo KNOWLES & CARLISLE (1956, p. 445) acontece com o Leander o mesmo verificado em Uca. Acentui-se, afinal, que os meus resultados confirmam a hipótese de EDWARDS de que a ablação dos pedúnculos oculares levaria o animal a adotar um permanente hábito noturno, em que pese a opinião contrária de KNOWLES & CARLISLE (l. c., p. 446).

7. Levando em consideração a existência de uma certa semelhança de efeitos entre o material das células neurosecretoras dos Crustáceos e o da hipófise, consegui verificar que no extrato de Tr. existe um princípio oxitocicomimético. Os resultados da ação do extrato do pedúnculo ocular sôbre a musculatura uterina pareceu não deixar dúvidas a respeito. O comportamento das fibras musculares dêsse órgão em relação ao extrato é semelhante ao que exibem em presença da oxitocina. A ação do extrato, com a da oxitocina, pode ser avaliada quantitativamente, isto é, as contrações da fibra muscular uterina aumentam à medida que recebem maior quantidade de extrato de pedúnculo ocular. Êstes resultados são os primeiros de uma série de pesquisas que se acham em curso, e que constituem a nosso ver, um fato novo no estudo da endocrinologia dos invertebrados. Tais resultados também indicam a existência de uma analogia fisiológica entre o sistema — hipotálamo — hipofisário dos Vertebrados e o complexo "órgão-X-glândula do seio "dos Crustáceos, como foi lembrado recentemente por HANSTRÖM (1956, p. 29).

V — CONCLUSÕES

1. Submeteu-se o caranguejo dágua doce, **Tr. petropolitanus,** a vários experimentos a fim de estudar o funcionamento de órgãos endócrinos contidos no pedúnculo ocular.

2. Comparados com os animais íntegros, os **Tr.** com um só pedúnculo ou sem pedúnculo apresentam acentuadas modificações do metabolismo respiratório. Os **Tr.** mono-pedunculados consomem 34% menos oxigênio, e os apedunculados consomem mais 54% que os íntegros.

3. As modificações indicadas no item anterior devem-se considerar como resultantes de interferências na produção de princípios neurosecretores por elementos especializados existentes nos pedúnculos oculares.

4. A remoção de um ou de ambos os pedúnculos oculares não interfere na ventilação das câmaras branquiais, avaliada pelo funcionamento dos escafognatitos.

5. Os elementos neurosecretores existentes nos pedúnculos oculares dos **Tr.** apresentam aproximadamente as mesmas características que nos demais crustáceos Decápodes.

6. A ablação de um pedúnculo ocular provoca descoordenação da atividade locomotora, perda do ritmo e o aparecimento de atividade diurna.

7. **Tr.** cegos apresentam igualmente atividade locomotora contínua, com perda de ritmo e maior consumo de oxigênio pelos animais durante o dia.

8. A injeção do extrato de pedúnculo ocular de **Tr.** aplicada em animais cegos, nas condições indicadas no item anterior, provocam inibição da atividade locomotora, induzindo um certo retôrno ao ritmo normal.

9. Iluminação contínua dos animais apenas rarefaz a atividade noturna. Manutenção no escuro, não altera o ritmo normal.

10. Lembrou-se a hipótese de a atividade locomotora coordenada devida à influência da secreção pelos órgãos neurosetores, achar-se relacionada com fatôres intrínsecos. Dada a sua ritmicidade, a atividade relaciona-se com o que se chama "cronometria biológica" existente em várias plantas e animais.

11. E' evidente uma ação oxitòcicomimética do extrato de pedúnculo ocular do **Tr.**

VI — SUMMARY

CONTRIBUTION TO THE STUDY OF THE NEUROSECRETION ON CRUSTACEANS

This paper deals with the neurosecretion system of **Trichodactylus petropolitanus (Tr. petr.),** one of commonest freshwater Decapod living in the rivers of the outskirts of São Paulo.

The animals were caught usually at night and transferred to the laboratory. They were kept in small glass aquarium containing little tap water and fed by small pieces of beef or shrimps. Under these conditions the crustaceans can live several months. Some were mantained in running freshwater in order to keep them a longer time. In the laboratory the following points were studied:

a) Motor activity.

Recordings were obtained on the motor activity by using Fig. 18 shows the normal recordings of activity of the Crustacean, during one week. It is konown that **Tr. petr.** has nocturnal habits. According to the recording the animals walk ca. 210 m every night. Its activity initiates at dawn, between 6 to 7 p.m. and stops at 7 a.m. Fig. 19 shows that the activity at night is discontinued.

Removing of one eyestalk of **Tr. petr.** produces a disordered activity. The animal moves without interruption during 24 hours. Fig. 20 is characteristic of this situation.

When both eyestalk are removed similar behaviour is shown by **Tr. petr.,** but the animal walks faster than that with only one eyestalk.

It seems interesting to note that the removal of both eyes determine a continous walking of the crustacean. This means that in the eyestalk something exists responsible for the inhibition of the movement.

Blind **Tr. petr.** loses the rythm, but its activity is higher than that of the **Tr. petr.** provided with only one eyestalk.

Fig. 22 refers to a recording of night activity of the animal. It can be seen that resting phase and moving phase correspond to periods of light and darkness respectively. **Tr. petr.** mantained continuously under the influence of light during 24 hours, showed that the two phases persist (Fig. 22), but the movements are slower. **Tr. petr.** left in complete darkness during 24 hours has also both phases of activity and resting. In consequence, we can say that the lack of light during day time has no effect on the 24 hours cycle. This means that the behaviour of **Tr. petr.** under the conditions referred to, depends upon the light in a certain measure, but the rythmicity is much more related to some internal factors.

Analysis of these presumed factors were made by injecting an extract of the eyestalk in normal (not operated) **Tr. petr.**

For preparation of this extract both eyestalk were cut off and ground in a mortar. Hot ethanol was added and a little later the alcohol was evaporated at 37° C. Some crystals were obtained and then dissolved in a special Ringer for crustacean (formula see pp. 49).

The solution employed corresponds to 20 eyestalks per 1 ml. From this solution 1 ml was taken and injected into the animal, the concentration corresponding to two eyestalks per animal.

Fig. 23 shows the record from a normal **Tr. petr.** Four days later the animal was subjected to a second injection with 2 ml of extract. The movement was partially inhibited during the following three nights. At the 4th night the normal rythm was reestablished. This seems to indicate that in the eyestalk of the **Tr. petr.** exists some movement inhibiting substance: **Tr. petr.** without one or both eyestalks after receiving small quantity of extract, shows sigus of regulation of the movements as can be seen on Fig. 21.

b) Oxygen Consumption and Respiration.

The oxygen consumption by **Tr. petr.** either normal or without one or both eyestalks was determined. The animal in which one eyestalks was cut off shows a decreasing oxygen

consumption, and those without both eyestalks double the consumption of oxygen (Fig. 28). We can assume that incretory secretion produced by the structures existing in the eyestalks interfere with the respiratory metabolism. By comparing animals with only one eyestalk and eyeless with the normal ones it was seen that the former consume 34% less, and the second 54% more oxygen than the later. This means that the removal of both eyestalks is responsible for the 88% increase of the oxygen consumed. It is rather difficult to explain why the animals without only one eyestalk consume less oxygen, and why the absence of both eyestalks determine an increasing of the oxygen consumption. It seems that the supression of one eyestalks determines some disturbance in the production of the corresponding hormon.

Removing one, or both eyestalks does not affect the scaphognathite beatings. The movement of the organs of ventilation seems independent of the hormone produced by the neurosecretory organs of the eyestocks.

c) Neurosecretion and Pituitary.

The study of the neurosecretory structures of the eyestalk was made. The similarity of the effects determined by the secreted substance by such structures and by the secretion very well known from the pituitary, led us to perform some experiments in order to verify if the neurosecretory cells of the **Tr petr.** eyectocks have something similar to the pituitary hormones. It is shown that the extract of **Tr. petr.** eyestalk contains substances which act on the contraction of the Rat uterus. It seems that this oxytocic element of the eyestock extract has the same influence on the Rat myometrium as that of oxytocin of the Vertebrate pituitary. These results are preliminary and have induced a new serie of experiments in order to isolate the oxytocic principle from the Crustacean eyestalks, which will be published later elsewhere. For the moment, it is possible to say that some analogy exists between the hypothalamus-pituitary system of the Vertebrates and the "X-organ-sinus

gland" system of the Crustacean according to the indication of Hanström (1956, p. 29).

CONCLUSIONS

1. The eyeless or one eystalked animals show a great change in their respiratory metabolism. In animals with only one eyestalk the oxygen consumption is 34% less but in the eyeless ones it is 54% highter than the normal (not operated) animals.

2. The neurosecretion of the "X-organ" and the sinus glands is responsible for the modifications in the oxygen-consumption.

3. The neurosecretion does not change the activity of the scaphognathites of the animals.

4. The neurosecretion-cells of **Tr. petr.** do not differ from those known in other Decapods.

5. Removal of one or both eyestalks causes a decreasing in the total activity and breaks the normal 24 hours rhythm.

6. Injections of eyestalk extract into blind the animals provokes a return toward normal, but not rhythmic, activity.

7. Injection of eyestalk extract into normal animals during the period of evening activity blocks out the activity of the animal.

8. Animals in constant artificial light decreases the activity during the night, but not the rhythm of the movements.

9. The relation between the regulation of the activity rhythm and "biological cronometry" is considered.

10. Preliminary experiments were made on the action of the eyestalk extracts on the uterus of the rats in various oestrial periods. It is clear the existence of a oxytocimimetic action in the extract.

VII — BIBLIOGRAFIA

ABRAMOWITZ, R. K., & A. ABRAMOWITZ — 1940 — Molting, growth and survival after eyestalk removal in Uca pugilator. Biol. Bull., **78**: 179-188.

BAUCHAU, A. G. — 1948 — Intensité du metabolisme et glande sinusaire chez Eriocheir sinensis H. M. — Soc. Ann. Zool. Belg., **79**: 73-85.

BLISS, D. E. — 1951 — Metabolic effects of sinus gland or eyestalk removal in the land crab, Gecarcinus lateralis. Anat. Rec., **111**: 502-503.

BLISS, D. E. e J. H. WELSH — 1952 — The neurosecretory system of brachyuran Crustacea. Biol. Bull., **103**: 157-159.

BLISS, D. E., J. R. DURAND & J. H. WELSH — 1954 — Some decapod Crustacean neurosecretory systems. Pubbl. Staz. Zool. Napoli, **24**: suppl. 68-69.

BROWN Jr., F. A. — 1939 — Sinus gland and molting in Palaemonetes. Anat. Record (Suppl), **75**: 129.

—— 1948 — Hormones in Crustaceans, in "The Hormones" Pincus Thimann K. V., ed. Academic Press, N. York, pp. 159-199.

—— 1952 — The action of hormones in plants and invertebrates. K. V. Thimann. Academic Press, N. York, p. 171.

—— 1957a — Biological chronometry. Amer. Nat., **91**: 129-133.

—— 1957b — The Rhythmic Nature of Life. Rec. Adr. Inv. Physiology. Univ. Oregon Publ. Congress, n. 302.

BROWN Jr., F. A. & O. CUNNINGHAM — 1939 — Influence of the sinus gland of crustaceans on normal viability and ecdysis. Biol. Bull., **77**: 104-114.

BROWN Jr., F. A., M. FINGERMAN & M. N. HINES — 1951 — Hormones controlling the distal retinal pigment of Palaemonetes vulgaris. Biol. Bull., **101**: 217-218.

BROWN Jr., F. A., M. N. HINES & M. FINGERMAN — 1952 — Hormonal regulation of the distal retinal pigment of Palaemonetes vulgaris. Biol. Bull., **102**: 212-225.

BUTENANDT, A. — 1954 — Über das erste Kristallisierte Insektenhormon. Angewandte Chemie, **66**: 490.

CARLISLE, D. B. — 1951 — On the hormonal and neural control of the release of gamets on Ascidians. J. Exp. Biol., **28**: 463-472.

—— 1951a — Corpora lutea in an Ascidian Ciona intestinalis. Quart. J. Microscop. Sci., **92**: 201-203.

—— 1953 — Studies on Lysmata seticaudata Risso (Crustacea-Decapoda) — III. On the activity of the moult-accelerating principle when administrated by the oral route. IV — On

the site of origin of the moult-accelerating principle experimental evidence. Pubbl. Staz. Zool. Napoli, **24**: 278-291.

—— 1953 — Studies on Lysmata seticaudata Risso (Crustacea Decapoda) V. — The ovarian inhibiting hormone and the hormonal inhibition of sex-reversal. Pubbl. Staz. Zool. Napoli, **24**: 355-372.

—— 1953a — Studies on Lysmata seticaudata Risso (Crustacea Decapoda) VI. — Notes on the structure of the neurosecretory system of the eyestalk. Pubbl. Staz. Zool. Napoli, **24**: 434-446.

CARLISLE D. B. & P. F. R. DOHRN — 1953 — Studies on Lysmata seticaudata Risso (Crustacea-Decapoda). II. — Experimental evidence for a growth and moult-accelerating factor obtainable from eyestalk. Pubbl. Staz. Zool. Napoli, **24**: 69-83.

CARLISLE, D. B. & L. M. PASSANO — 1953 — The X-organ of Crustacea. Nature, **171**: 1070-1071, London.

DURCHON, M. — 1951 — L'ablation du prostomium provoque, chez Néréidiens la maturation précoce des produite génitaux mâles. C. r. Acad. Sci., Paris, **232**: 442-443.

EDWARDS, G. A. — 1950 — The influence of eyestalk removal on the metabolism of fiddler Crab. Physiol. Comp. Oecol., **2**: 34-50.

ENAMI, M. — 1949 — Studies on the controlling mechanism of black chromatophores in the young of a fresh-water crab, Sesarma haematocheir. I. On a humoral principle from several ganglionic tissues as concerned with the pigmentary activities. Physiol. a. Ecol. (Kyoto) Japan, 3: 23-31

—— 1951a — The sources and activities of two chromatophorotropic hormones in Crabs of the genus Sesarma. I. Experimental analysis. Biol. Bull., **100**: 28-43.

—— 1951b — The sources and activities of two chromatophorotropic hormones in crabs of the genus Sesarma. II. Histology of incretory elements. Biol. Bull., **101**: 241-258.

—— 1951c — Mechanism of control of the chromatophore responses of teleosts and crustaceans. J. Exp. Morph., **7**: 1-22,

FROST, R., R. SALOUM & L. H. KLEINHOLZ — 1951 — Effect of sinus gland and of eyestalk removal on rate of oxygen consumption in Astacus. Anat. Record, **111**: 572.

GABE, M. — 1952 — Particularités histochimiques de l'organe de Hanström (organe x) et de la glande du sinus chez quelques crustacés décapodes. C. r. Acad. Sci. Paris, **235**: 1430-1432.

—— 1953 — Sur l'existence, chez quelques crustacés malacontracés, d'un organe comparable à la guande de la mue des Insectes. C. r. Acad. Sci. Paris, **237**: 1111.

—— 1954 — La neuro-secretion chez les Invertebrés. Année biol., **30**: 1.

GRINKRAUT, C. N. e P. SAWAYA — 1957 — On the action of ions on the uterine mechanics. Bol. Fac. Fil., Ciênc. Letr. Univ. São Paulo. Zool. **21**: 123-152, São Paulo.

HANSTRÖM, B. — 1931 — Neue Untersuchungen über Sinnesorgane und Nervensystem der Crustaceen. I. Zeits. Morph. ökol. Tiere, **23**: 80-236.

—— 1933 — Neue Utersuchungen über Sinnesorgane und Nervensystem der Crustaceen. II. Zool. Zeits. Morph. Ökol. Tiere, **56**: 387-520.

—— 1938 — Zwei Probleme betreffs der hormonalen Likalisation im Insektenkopf. Kungl. Fysiogr. Sällsk. Handl. Lund. N. F. **49**.

—— 1939 — Hormones in invertebrates. Oxford Claredon Press, pp. 1-198.

—— 1954 — Neurosekretionen, ett nytt forsknongsfält inom endokrinologien (Neurosecretion, a New field in endocrinology). Statens naturvetenskapliga forskningsrads, Arsbok 1953-54, 34-64.

—— 1956 — The comparative aspect of neurosecretion with special reference to the hypothalamo-hypophysical system. Proc. English Symp. Research Soc., 8: 23-37, London.

HESS, W. N. — 1941 — Inhibition of molting in egg-bearing female crustaceans. Biol. Bull., **81**: 215-220.

KNOWLES, F. G. W. — 1951 — Hormone production within the nervous system of a crustacean. Nature, **167**: 564-565.

—— 1955 — Crustacean colour change and neurosecretion. Endeavour, **14**: 95-104.

KNOWLES, F. G. B. & D. B. CARLISLE — 1956 — Endocrine control in the crustacea. Biol. Rev. **31**: 396-473.

LANDGREBE, F. W., B. KETTERER & H. WARING — 1955 — Hormones on the Posterior Pituitary, pp. 389-431. in The Hormones de PINCUS, G. & K. V. THIMANN, vol. III, p. 1012, Academic Press. New York.

LONG, J. A. & H. M. EVANS — 1922 — The Oestrous Cycle in the Rat and its asociated phenomena. Mem. Univ. Calif. Berkeley, **6**: 1-148.

MATSUMOTO, K. — 1958 — Morphological studies on the neurosecretion in Crabs. Biol. Jour. Okayama Univ. **4**: (3-4): 103-176. Okayama, Japan.

MEGUSAR, F. — 1912 — Esperimente über die Farbwechsel der Crustacean. Arch. Entwicklungs Mech., **33**: 462-665.

MENDES, E. G. — 1942 — The "X-Organ" of Ocypoda albicans Bosc (Crustacea, Decapoda, Brachyura). Proc. 8th Amerc. Sci. Cong., **3**: 423-424.

PASSANO, L. M. — 1951a — The X-organ-sinus gland neurosecretory system in crabs. Anat. Record, **111**: p. 502.

—— 1951b — The X-organ, a neurosecretory gland controlling moulting in crabs. Anat. Record, **111**: 559.

—— 1952 — Phase contrast observations on living neurosecretory cells of Sesarma. Anat. Rec., **112**: 460-461.

—— 1953a — The neurosecretory control of moulting in crabs by the X-organ sinus gland complex. Physiol. comp. Ecol., **3**: 155-189.

—— 1953b — Phase microscopic observations of neurosecretory product of the crustaceans X-organ. Pubbl. Zool. Napoli, **23**: supp. 72.

PÉREZ-GONZÁLEZ, M. D. — 1957 — Evidence for hormone-containing granules in sinus glands of the fiddler crab Uca pugilator. Biol. Bull., **13**: 426-441.

POTTER, D. D. — 1954 — Histology of neurosecretory system of the blue crab Callinectes sapidus. Anat. Rec., **120**.

PYLE, R. W. — 1943 — The histogenesis and cyclic phenomena of the sinus gland and X-organ in crustacea. Biol. Bull., **85**: 87-102.

SAWAYA, P. — 1939 — Sôbre a mudança de côr nos Crustáceos. Contribuição para o estudo da fisiologia dos cromatóforos e dos hormônios dos invertebrados. Bol. Fac. Fil., Ciên. Letr. Univ. São Paulo. Zool. **3**: 3-109.

SCHARRER, B. — 1948 — Hormones in insects. The Hormones. Pincus Thimann, K. V. ed., Acad. Press. New York, pp. 121-158.

SCHARRER, E. — 1941 — Neurosecretion. I. The nucleus preopticus of Fundulus heteroclitus L. — J. Comp. Neur. **74**: 81-92.

SCHARRER, E. & B. SCHARRER — 1954 — Neurosekretion — Hand. Mikroskop. Anat. des Menschen, W. MÖLLENDORFF, part V. pp. 953-1066.

SCHOLANDER, P. F. — 1942 — Volumetric micro-respirometer. Rev. Sci. Inst., **13**: 32-33.

SCUDAMORE, H. H. — 1947 — The influence of the sinus gland upon molting and associated changes in the crayfish. Physiol. Zool., **20**: 187-208.

SMITH, R. I. — 1940 — Studies on the effect of eyestalk removal upon young crayfish (Cambarus clarkii GIRARD). Biol. Bull., **79**: 145-152.

—— 1948 — The role of the sinus glands in retinal pigment migration in grapsoid crabs. Biol. Bull., **95**: 169-185.

TRAVIS, D. F. — 1951 — The control of the sinus glands over certain aspects of calcium metabolism in Panulirus argus Latreille. Anat. Rec. 111: 503.

VALENTE, D. — 1945 — Consumo de oxigênio em diferentes tensões, pelo Trichodactylus petropolitanus Goeldi (Crustacea-Brachyura). Bol. Fac. Fil., Ciênc. Letr. Univ. S. Paulo, Zool. **9**: 87-98.

—— 1948 — Mecanismo da Respiração de Trichodactylus petropolitanus (Goeldi) (Decapoda Brachyura). Bol. Fac. Fil., Ciên. Letr. Univ. S. Paulo, Zool., **13**: 259-327.

—— 1958 — Solução fisiológica para Crustáceos de água doce. Ciênc. e Cultura, **10**, n. 3.

—— 1958 — Sôbre neurosecreção nos animais. Ciência e Cultura, **10**: n. 4.

VALENTE, D. e G. A. EDWARDS — 1955 — The regulation of the activity rhythm of the crab (Trichodactylus petropolitanus). Bol. Fac. Fil., Ciên. Letr. Univ. S. Paulo, Zool, **20**: 5-18.

WELSH, J. H. — 1941 — The sinus glands of 24-hour cycles of retinal pigment migration in the crayfish. J. Exper. Zool. 86, 35-49.

SÔBRE A FISIOLOGIA DOS MÚSCULOS LONGITUDINAIS DE **HOLOTHURIA GRISEA** *

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(10 Figuras)

I

INTRODUÇÃO

Muito variada é a distribuição da musculatura estriada e lisa nos Invertebrados e Vertebrados. Deixando de parte os últimos — os melhores conhecidos — em que a musculatura somática é estriada e a lisa é a visceral, excetuada a musculatura cardíaca, nos Invertebrados, um tal tipo de distribuição é pràticamente ausente.

Assim, animais há dotados sòmente de musculatura estriada, como p. ex., os Insetos e outros, que constituem a maioria dos Invertebrados, e os em que faltam os músculos estriados, como acontece, p. ex., com os Equinodermes.

As modernas técnicas utilizadas em farmacologia, muito contribuiram para o melhor conhecimento das reações de ambos os tipos de músculos (mm.) e, conseqüentemente, de suas particularidades tanto nos Vertebrados como nos Invertebrados. Como é sabido, a existência de uma placa motora na junção neuromuscular, nos músculos estriados, concorreu enormemente para o conhecimento da fisiologia dêsses músculos. A ausência dessa placa nos mm. lisos e a diversa reação dêles em face das mesmas drogas, está a exigir acurado estudo especialmente sôbre a fisiologia da fibra muscular lisa. Nos Vertebrados esta musculatura vem sendo intensamente estu-

(*) Trabalho efetuado com o auxílio da Fundação Rockefeller e do Conselho Nacional des Pesquisas.

dada, mas nos Invertebrados muitos pontos, principalmente de sua fisiologia, não foram ainda abordados.

O fato de existirem animais em que tôda a musculatura é do tipo liso, oferece campo para tais estudos que poderão ser de valia para a elucidação de vários fenômenos que ocorrem no comportamento da fibra muscular lisa.

Trabalhando já há algum tempo com os músculos de Equinodermes, especialmente de Holotúrias, procuramos verificar as reações dos músculos longitudinais sob diversas condições experimentais.

São êsses mm. de contração relativamente rápida, e constituem o principal elemento determinante das freqüentes modificações de forma do corpo dêsses animais. Assim, ao estabelecerem o volume do corpo de **Holothuria grisea,** Pantin & Sawaya (1953, p. 60) puderam verificar a influência intensa da musculatura longitudinal e a da anelar, que nas suas contrações provocam acentuadas diferenças da forma do corpo. Tal musculatura longitudinal, tôda constituída de mm. lisos, apresenta aspectos interessantes que serão relatados no decorrer do presente trabalho.

Utilizando êsses músculos, visamos primeiramente analisar o seu comportamento em face de drogas de conhecido efeito sôbre a musculatura lisa dos Vertebrados. A seguir, apresentaremos alguns aspectos da estrutura dos mm. longitudinais e, finalmente, tentaremos correlacionar os resultados obtidos.

De há tempos os músculos longitudinais de Holotúrias constituram objeto de estudos farmacológicos. Assim, em 1937 Bacq (p. 175) verificou serem tais músculos de **Stichopus regalis** e de **Holothuria nigra,** sensíveis à acetilcolina, e Moussatché (1949, p. 525) assinalou a sensibilidade de tais músculos ao mesmo éster, na concentração de 10^{-8}, e a potencialização do éster pela eserina a 5×10^{-7}. Êstes autores agora citados informam que a motilidade espontânea apresentada pelos referidos músculos não permitiria utilizá-los como método de ensaio rotineiro. Em 1951 Sawaya (p. 41) ao descrever o chamado "efeito de acetilcolina" apresentado pelo músculo longitudinal de **H. grisea,** em oposição àqueles autores, recomendou êsse músculo

como específico para a dosagem do éster nos extratos de tecidos. Por sua vez, Ambache & Sawaya (1953, p. 53) também verificaram poder êsse músculo servir perfeitamente para a determinação do teor de acetilcolina em extratos de tecidos, do mesmo modo que as preparações habitualmente empregadas, a saber o m. reto abdominal da rã ou o m. do dorso da sanguessuga. A verificação de Sawaya supra citada foi confirmada por Moussatché e Aronson (1951, p. 220) que indicam a sensibilidade do músculo ao aludido éster, na concentração de 1 x 10^{-8} até 1 x 10^{-9}, e que depois da potencialização pela eserina a reação poderá dar-se até a concentração de 1 x 10^{-11} do éster. Já nesse trabalho os autores mostraram (p. 221) que o músculo longitudinal dessa holotúria reagiu em presença de pequena quantidade de Ach e respondeu quantitativa e regularmente, permitindo a dosagem do éster no extrato.

Durante alguns anos (1951-1958) trabalhamos contìnuamente com os mm. longitudinais de **Holothuria grisea,** tendo os resultados parciais sido objeto de comunicação às Reuniões Anuais da S. B. P. C.* (1951, 1954), à Academia Brasileira de Ciências (1953) e a Sociedade de Biologia de São Paulo (1955). No corrente ano pudemos rever êsses resultados, e a sua exposição constitui a primeira parte do presente trabalho.

II

MATERIAL E MÉTODOS

As Holotúrias (**Holothuria grisea** Selenka 1867) provieram tôdas da baía de Santos e do Laboratório de Biologia Marinha de São Sebastião (L. B. M.). Tratando-se de pecilotermos, a perfusão do músculo fêz-se simplesmente imergindo-se no banho perfusor, constituído de água do mar filtrada, na qual se colocava a droga. Uma corrente de ar era injetada contìnuamente no banho de acôrdo a prover de oxigênio a preparação e misturar a droga. Utilizaram-se alavancas isotônicas tipo gimbal ou Shild modificada, e alavancas semi-isométricas. Fizeram-se as experiências à temperatura ambiente, a qual perma-

(*) — Sociedade Brasileira para o Progresso da Ciência.

neceu, em geral, entre 20 e 25°C, quer em São Paulo quer no litoral.

Quase sempre se utilizaram holotúrias recentemente capturadas. Nas séries de experiências no Aquário Municipal de Santos ou no L. B. M., os animais colhidos na zona entre as marés eram transportados para os aquários e logo operados. Nas experiências do Departamento de Fisiologia Geral e Animal em São Paulo, colocaram-se êstes Equinodermes em aquários apropriados, com água do mar constantemente filtrada e purificada por meio de dispositivo filtrador especial.

Empregamos clorhidrato de acetilcolina Roche, empolas de 0.1 grs., sulfato de atropina Roche e Merck; cloreto de d-tubocurarina dos Bios Laboratories Inc.; di-hidrocloreto de histamina do Pfanstiehl Chemical Co. e nicotina da Eastman Organic Chemicals. A d-tubo-curarina, a histamina e a nicotina foram obtidas graças ao auxílio da Fundação Rockefeller, à qual apresentamos nossos agradecimentos.

III

INFLUÊNCIA DA ACETILCOLINA

As holotúrias em repouso, completamente relaxadas, chegam a medir 40 cms de comprimento. Isto quer dizer que no animal intacto, em repouso, os músculos longitudinais podem atingir em **H. grisea,** aproximadamente, essa extensão. Ao serem retiradas do aquário, as holotúrias reduzem de mais de 50% o seu comprimento, devido, principalmente, à irritação produzida pelo contacto das mãos. Quando essa irritação é mais forte, o animal pode chegar até a expelir as vísceras. A redução em mais de 50% do seu comprimento deve-se em grande parte à intensa contração dos músculos longitudinais e dos transversais. A diminuição do comprimento é acompanhada de extraordinário aumento do turgor, o que se compreende em animal do grupos dos chamados "animais ocos" ("Hohlorganartige Tiere" de Jordan 1914, p. 365).

Essa capacidade de contração em tão grande amplitude nota-se perfeitamente quando se extraem os músculos longitudinais do animal.

Segundo a técnica utilizada, os animais retirados do aquário são imediatamente abertos expondo-se a cavidade do corpo, isolando-se tão ràpidamente quanto possível os músculos longitudinais ligados por fios nas duas extremidades. Em tais condições, o comprimento do animal, devido à excitação durante a operação, era em geral de 20 cms. Acontece que os músculos desligados da parede do corpo se contraem tão fortemente que o seu comprimento se reduz a 2 ou 3 cms. Em tais condições os mm. eram colocados numa placa de Petri contendo água do mar filtrada e, a seguir, utilizados imediatamente ou após 3 a 24 horas de repouso. Nos casos em que os músculos deviam ser empregados além de 2 horas após a retirada do corpo do animal, eram êles transferidos para a geladeira e aí mantidos à temperatura de cêrca de 2°C. Obtiveram-se melhores resultados com preparações frescas. Os mm. usados além de 12 horas, mesmo mantidos na geladeira, apresentavam particularidades diversas que serão assinaladas adiante. Os resultados que relataremos a seguir referem-se aos de preparações frescas ou no máximo usadas 2 horas após a dissecção.

Iniciamos as perfusões empregando primeiramente alavancas isotônicas. Verificamos que êsses músculos são sensíveis à Acetilcolina na concentração de até 1×10^{-12}, isto é, correspondente a um teor de 0.001 $\mu\gamma$ por ml (Fig. 1). Também foi possível determinar a proporção entre a intensidade da contração e a quantidade de droga adicionada. Os gráficos da Fig. 1 e 2 indicam as variações das contrações quando a Ach é empregada nas concentrações de 0.001 $\mu\gamma$ a 1 γ (1×10^{-12} a 1×10^{-6}). Nota-se ainda que à medida que aumenta a concentração do éster maior é a contração. O exame do gráfico referido indica haver uma relação entre a intensidade da contração e a concentração da Ach empregada. Graças à extrema sensibilidade do músculo, prescindiu-se da eserina para obtenção da resposta muscular.

Com o uso de alavancas semi-isométricas, avaliou-se aproximadamente o grau de tensão de tais músculos.

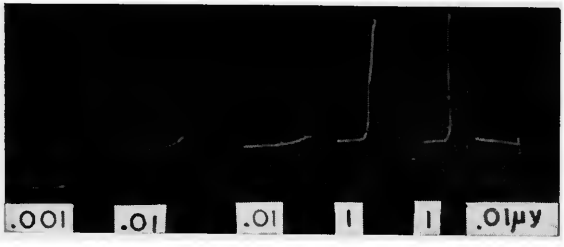

Fig. 1 — Ação da Ach sôbre o músculo longitudinal de *H. Grisea*. Concentração ester de .001 $\mu\gamma$ a .01 $\mu\gamma$.

Como sói acontecer em preparações biológicas, a contração dos músculos sob a influência da Ach não se repete exatamente quando se submete a preparação de novo à influência da mesma droga na mesma concentração. E' o que se pode ver ainda na Fig. 2, quando se comparam as curvas de contração provocadas por 1 γ de Ach (1×10^{-6}) O que se poderia dizer é que sob a influência de uma determinada concentração de

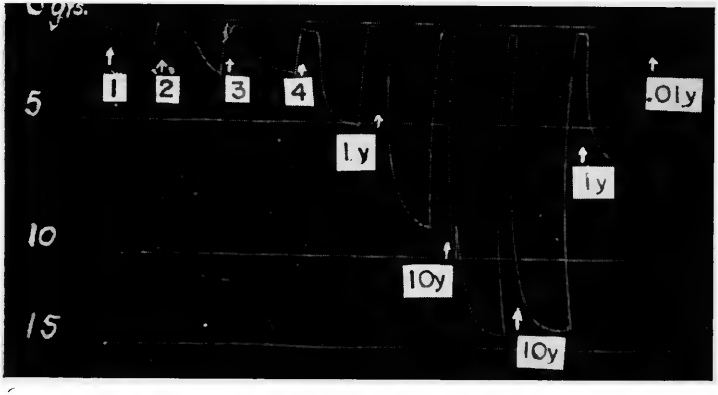

Fig. 2 — Ação da Ach sôbre o músculo longitudinal de *H. Grisea* Contração semi-isométrica. 1, 2, 3 e 4 = .01 $\mu\mu\gamma$, 1 $\mu\mu\gamma$, 1 $\mu\gamma$ e .01 γ de Ach. As outras indicações correspondem às concentrações da Ach.

Ach se obtém uma curva, e quando a concentração é menor, a curva de contração também é menor e vice-versa.

No músculo sob a ação da Ach a partir da concentração de 0.01 $\mu\mu\gamma$ (1 x 10^{-14}) as tensões vão aumentando, a partir de 0.33 grs até cêrca de 12 grs, quando se usa o éster na concentração de 10 γ (Fig. n. 2). Nesse mesmo gráfico ainda se nota mais que ao reduzir-se a concentração de Ach a tensão também diminui.

Calculando-se o comprimento médio das curvas e comparando-se com as concentrações utilizadas obter-se-á o seguinte gráfico:

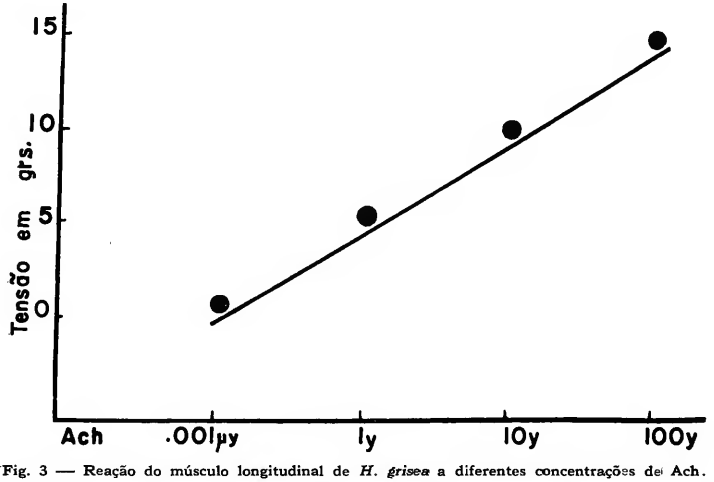

Fig. 3 — Reação do músculo longitudinal de *H. grisea* a diferentes concentrações de Ach.

Tais resultados podem justificar o emprêgo do músculo longitudinal de holotúria na determinação do teor do éster acetilcolínico em extratos de vários tecidos. Nem sempre se obtém curvas semi-isométricas correspondentes, com músculos de animais diferentes, mas, mesmo assim, verifica-se uma certa relação entre o teor da droga e a tensão do músculo. Como se pode notar no gráfico da Fig. 4, em que se empregaram concentrações cada vez maiores de acetilcolina de 2 $\mu\gamma$ até 25 $\mu\gamma$ as curvas foram cada vez mais longas.

Por outro lado, a tensão dos músculos é sensível a doses mínimas de Ach. Assim, no gráfico da Fig. 5, a variação das curvas se pode notar mesmo com diferenças de 0,5 μγ na concentração dêste éster.

Com as curvas semi-isométricas verificou-se também fenômeno interessante, que se poderia comparar talvez com o da facilitação. Assim, como se vê na Fig. 6, o músculo sob a influência de 1 γ de Ach mostrou-se sensível, apresentando uma tensão de 12 grs aproximadamente. Lavada a preparação e submetido o músculo à influência da Ach na concentração de 1 μγ, pràticamente não houve resposta, mas na terceira vez, o músculo reagiu a essa mesma concentração com uma tensão de 13 grs. Repetida a operação, obteve-se uma curva aproximadamente igual; reduzida a concentração do éster a 0.01 μγ obtiveram-se curvas de tensões cada vez menores.

Os gráficos da Fig. 7 mostram as variações das tensões do músculo quando submetido a doses mínimas de acetilcolina desde 0.5 μγ até 5 μγ. Como se vê, persiste uma proporção entre o grau de tensão e a concentração de éster; variando-se a concentração de 0.5 γμ até 5 γ, as tensões passaram de 2,5 gr até 11 grs.

O gráfico da Fig. 8 mostra as variações das tensões desde a dosagem diminuta de 0.0005 γμ até 0.1 μγ, indo de 1 gr até 18 grs respectivamente as tensões do músculo.

IV

INFLUÊNCIA DA ATROPINA, DO CURARE E DA HISTAMINA

O músculo longitudinal de **H. grisea** não apresenta reação alguma quando se utilizam essas substâncias, isto é, atropina, curare ou histamina em determinadas concentrações.

E' interessante, também, o fato de não se notar aqui a conhecida influência antagônica da atropina à Ach como ocorre nos músculos lisos de Vertebrados. O gráfico da Fig. 8 mostra falta de reação do músculo quando submetido à Atropina a

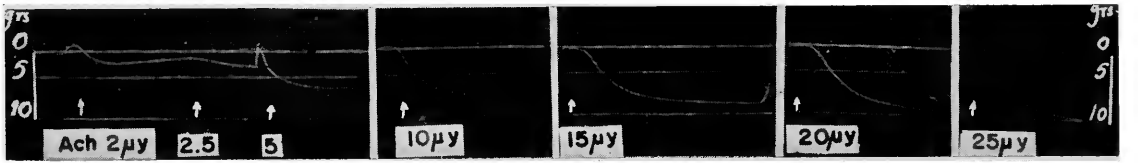

Fig. 4 — Ação da Ach sôbre o músculo longitudinal de *H. grisea*. Contrações semi-isométricas.

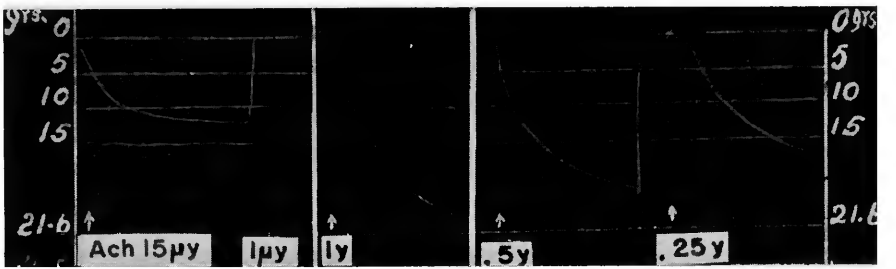

Fig. 5 — Ação da Ach sôbre o músculo longitudinal de *H. grisea*. Contrações semi-isométricas.

0.02γμ e reação sensível do músculo logo após a ação da acetilcolina a 0.1 μγ.

Como se vê ainda na Fig. 8 submetido o músculo ao Curare (100 mg de d-tubo-curarina) não se observa reação; introduzida a seguir, a Ach (0.1 μγ) no banho, o músculo contrai-se.

Em outras experiências pudemos verificar que qualquer que seja a concentração de atropina empregada nunca se verifica antagonismo ao éster.

Também o efeito da histamina sôbre o m. longitudinal é negativo, em qualquer concentração. Submetido o mm. a esta substância não se observa reação alguma. Lavada ou não a preparação, e fazendo-se agir a Ach (1 μγ) imediatamente êle se contrai.

V

INFLUÊNCIA DA NICOTINA

Se os mm. longitudinais de **H. grisea** se mostram insensíveis à Atropina, ao Curare e à Histamina, o mesmo não acontece quando em presença de nicotina.

A diferença com a contração determinada pela Ach está no fato de, sob a influência da Nicotina, o músculo entrar pràticamente em contratura. Como foi visto, uma das particularidades importantes dêste músculo é o seu rápido e imediato relaxamento após a lavagem da preparação em Ach. Neste particular está a principal diferença entre a influência da Ach e da Nicotina. A contração provocada pelo alcalóide permanece por 10 a 30 minutos ou mais não obstante as lavagens sucessivas da preparação.

VI

COMENTARIOS

Como se vê pelos resultados obtidos, é inegável a extrema sensibilidade dos músculos longitudinais de **H. grisea** à Ach. O

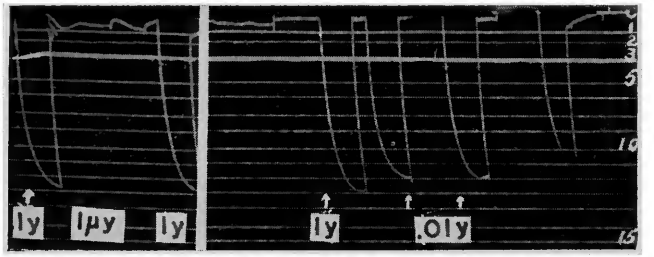

Fig. 6 — Ação da Ach sôbre o músculo longitudinal de *H. grisea*. Contrações semi-isométricas.

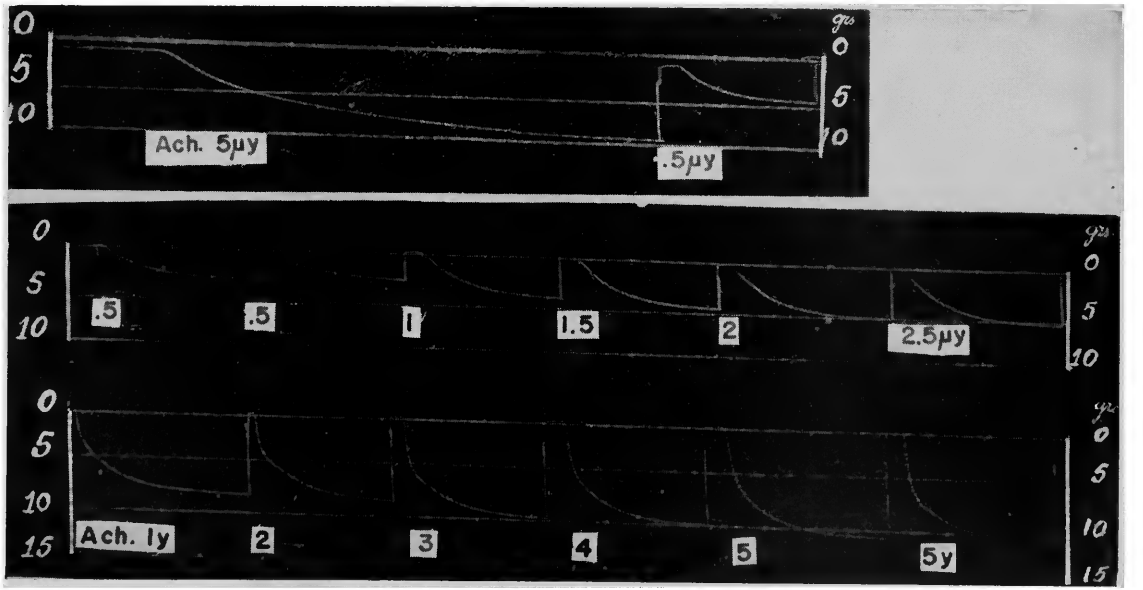

Fig. 7 — **Ação da Ach sôbre o músculo longitudinal de** *H. grisea*. Contrações semi-isométricas.

músculo reage intensamente ao éster, em doses ínfimas, ou seja até 0.01 $\mu\mu\gamma$ (1×10^{-14}). Conseguimos, assim, em nossas experiências registrar maior sensibilidade do referido músculo à Ach que as indicadas por outros autores (Bacq, 1937; p. 176: Moussatché, 1949, p. 525; Sawaya, 1951, p. 41; Moussatché & Aronson, 1951, p. 220; Ambache & Sawaya, 1953, p. 53; e Sawaya, 1954, p. 193).

Outro fato digno de especial menção vem a ser a ineficácia da Atropina, reconhecidamente antagonista da Ach e a ausência de reação do músculo à Histamina e ao Curare.

Parece da mais alta importância esta pronta reação do músculo à Ach e o seu rápido e imediato relaxamento. Esta reação é, como vimos, imediata, não obstante a atividade colinesterásica existente no músculo (Bacq & Nachmannson, 1937, p. 369; Sawaya & Mendes, 1953, p. 730). A relação entre a sensibilidade à Ach e a atividade colinesterásica é ponto ainda a ser elucidado. E' de se lembrar, porém, que o referido músculo possui quantidades apreciáveis de acetilcolina (Bacq, 1939, p. 29; 1947, p. 77) as quais agiriam talvez como elemento adjuvante durante a contração. Como se sabe, é um músculo de baixo consumo de oxigênio (0.215 mm^3 por miligrama de pêso sêco por hora, cf. Mendes, 1954, p. 178; 1954, p. 180) se comparado com músculos lisos de homeotermos.

Ainda no que se refere à Ach, é digno de nota o comportamento singular do músculo longitudinal, se cotejarmos com o que ocorre nos músculos estriados dos Vertebrados, não se verificando o efeito antagônico da atropina conhecido nestes últimos músculos.

Por outro lado, as únicas substâncias a que o músculo reage vêm a ser a Ach e a Nicotina, sendo bem distintas as diferenças do comportamento do mesmo em relação a ambas estas substâncias durante a fase de relaxamento. Como se viu, lavada a preparação após a contração sob a influência da Ach, o músculo volta rápida e imediatamente à posição primitiva, mas, no caso da Nicotina, a contração é mais duradoura dando-se o relaxamento sòmente em ca. de 10-30 minutos após a lavagem.

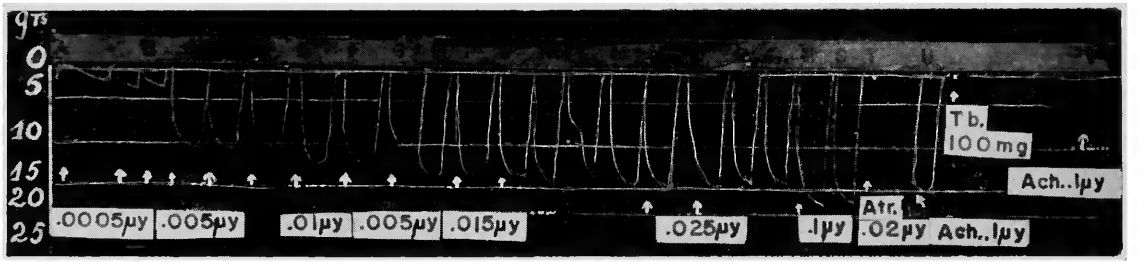

Fig. 8 — Ação da Ach. da Atropina (Atr.) e da d-tubocurarina (Tb.) sôbre o músculo longitudinal de *H. grisea*. Contrações semi-isométricas.

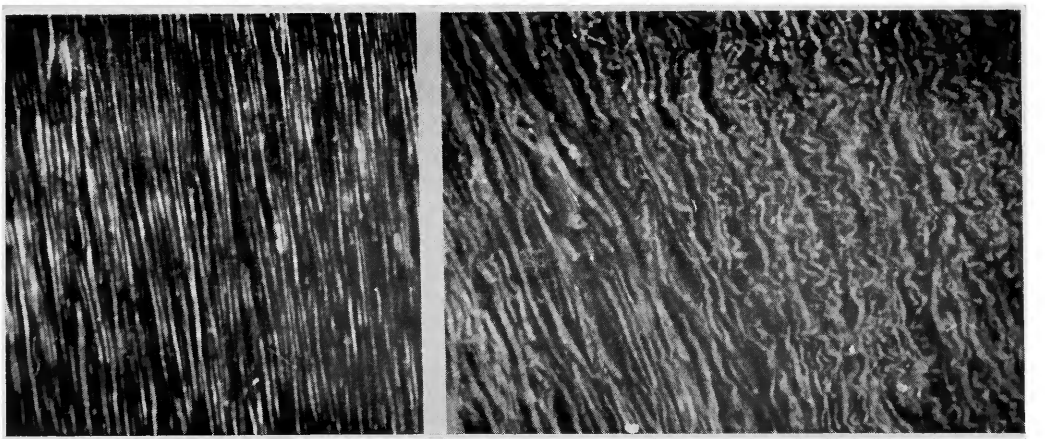

Fig. 9 — Secção longitudinal do músculo longitudinal de *H. grisea* em distensão. Fix. Bouin. Col. Hem. Eosina. x 10 vêzes.

Fig 10 — Secção longitudinal do músculo longitudinal de *H. grisea*, parte semi-distendida (à esquerda) e parte contraída (à direita). Fix. Bouin, Col. Hem. Eosina. x 10 vêzes

Cumpre lembrar, neste particular, que os resultados obtidos com o músculo longitudinal de **H. grisea** só em parte confirmam os de Bacq (1937, p. 175) com os de **H. stellata**. Realmente, em ambas, os músculos longitudinais são extremamente sensíveis à Ach, mas, enquanto que nos de **H. grisea** não oferecem reação à histamina, o contrário se dá com os de **H. stellata**. Devido a êste fato, Bacq (l. c.) diz que os mm. longitudinais desta holotúria são inadequados para identificação ou dosagem da acetilcolina em um perfusado de água do mar.

Talvez êsse comportamento diferente dos músculos longitudinais de **H. grisea** e de **H. stellata** corra por conta da diferença de espécie ou de qualquer modalidade técnica.

Outro fato interessante vem a ser a extrema redução do comprimento do músculo logo após separado do corpo do animal. Como foi dito, esta redução pode ser até da ordem de 80%. Além disso, músculos assim fortemente contraídos podem ser mecânicamente distendidos. Cessada a distensão, voltam depois ao grau de extrema contração, comportando-se como uma fita elástica.

As preparações histológicas de músculos completamente distendidos mostram as fibras musculares delgadíssimas, dispostas umas ao lado das outras, paralelamente (Fig. 9). Já nas preparações em que os músculos foram fixados em estado de máxima contração, tais fibras têem aspecto sinusoide, bem característico.

A Fig. 10 apresenta uma preparação em que em parte o músculo se mostra contraído e em parte distendido. Nesta preparação vêem-se perfeitamente os aspectos supra mencionados. Esta grande capacidade de contração do músculo pode ser explicada pelo fato de, na extensão máxima, as fibras musculares que se achavam contraídas e, portanto, em disposição sinusoide, distenderem-se e deslizarem-se umas sôbre as outras de modo a dar ao músculo o aspecto típico de fibras musculares paralelas.

Outro fenômeno para o qual pensamos se deva chamar a atenção vem a ser a progressiva distensão do músculo que se obtém principalmente em preparações que permaneceram vá-

rias horas na geladeira ou mesmo em preparações frescas usadas durante longo tempo no aparelho de perfusão.

E' muito comum, quando se toma um músculo já em contração, com cêrca de 3 cms de comprimento e se o submete à ação da Ach a várias concentrações, não retornar êle exatamente ao estado primitivo, mas apresentar em relação a êste, um certo grau de distensão, o qual vai aumentando pouco a pouco até um limite que ocorre quando o músculo se torna pràticamente insensível a doses mesmo altas de acetilcolina.

Como causa dêsse fenômeno foi lembrada a própria Ach que, além do efeito de determinar a contração rápida do músculo, também teria uma ação sôbre a miofibrila de tal modo a provocar o seu relaxamento gradativo com perda da elasticidade, ou induziria talvez uma certa disposição do sarcoplasma, fazendo-o perder o seu poder contrátil. A êste fenômeno deu-se o nome de "efeito de acetilcolina" (Sawaya, 1951, p. 41). Não obstante o grau de conhecimento que se tem da estrutura dos mm. longitudinais (Antunes 1954, p. 167; Mendes 1954, p. 145) e mesmo de sua ultraestrutura (G. A. Edwards & P. S. Santos, comunicação pessoal) ainda não se encontrou explicação para o chamado "efeito da acetilcolina".

Cumpre ainda chamar a atenção para o fato de tal efeito ocorrer apenas com a Ach, pois a nicotina não o determina.

Além destas observações deduzidas das experiências realizadas, vale a pena relembrar alguns fatos relacionados com o comportamento do animal vivo e o alto poder de contração dos músculos longitudinais.

Quando Pantin & Sawaya (1953, p. 51) efetuaram várias experiências para medir a variação do corpo do animal em diferentes condições, puderam correlacionar o alto poder de contração dos músculos longitudinais com aquela variação. Assim, uma holotúria depositada num aquário com água, periòdicamente suga o fluído pela cloaca, expulsando-o depois de algum tempo. Com isso faz variar o volume do corpo de 10%. Evidentemente, conforme verificaram aquêles autores, esta variação decorre principalmente da contração conjugada dos músculos longitudinais e circulares do animal.

VII

RESUMO

Demonstrou-se a grande sensibilidade do m. longitudinal de **H. grisea** à Ach. Os ensaios fazem-se com grande rapidez, se comparados com os outros métodos usualmente preconizados na determinação do teor da Ach em extratos de tecidos.

Confirmou-se a sensibilidade do m. longitudinal de **H. grisea** à acetilcolina na dose de 1 x 10^{-14} ou sejam 0.01 $\mu\mu\gamma$ por ml. Verificou-se ainda mais a estreita relação entre a concentração do éster e a intensidade de contração. Isto pôde ser bem avaliado com o uso de alavancas semi-isométricas, com as quais se obtiveram os registros gráficos que possibilitaram a avaliação da tensão dos músculos. Tomando-se os valores médios das tensões e comparando-os com as concentrações de Acetilcolina obteve-se um gráfico bastante significativo (Fig. n. 3).

Demonstrou-se ainda mais que o m. longitudinal de **H. grisea** não reage à atropina e nem à histamina. E', porém, sensível à nicotina. As diferenças das contrações decorrentes da ação da Ach e da nicotina, estão no fato de ser o referido m. muito mais sensível à primeira que à segunda substância. Além disso, o período de contração é muito mais longo quando o mesmo se acha sob a influência alcaloide que do éster colínico.

Visto ser muito rápida a volta do músculo ao estado normal, após a lavagem do mesmo quando se encontra sob a influência da acetilcolina é o mesmo recomendado nos métodos rápidos de ensaios do teor do éster nos extratos de tecidos.

Não se observaram em tôdas as experiências os movimentos espontâneos, assinalados por outros autores, em mm. longitudinais de holotúrias (Bacq 1926, p. 172 em **H. tubulosa** e **Stichopus badionotus**; Moussatché, 1941, em **H. grisea**) o que recomenda o uso dêsse músculo para as dosagens de Ach.

Atribuiu-se a ocorrência de movimentos espontâneos a precárias condições dos animais mantidos nos aquários. Chama-se a atenção neste particular, para o fato de, quando tais condições forem realmente precárias, a holotúria poder chegar a expulsar as vísceras.

Alude-se ainda ao fenômeno denominado por Sawaya (1951, p. 41) "efeito de acetilcolina" que consiste na ação extensora que o éster, ao fim de algum tempo, provoca no músculo. Êste fenômeno foi várias vêzes verificado nas experiências aqui realizadas.

Finalmente, nos comentários lembram-se vários dados da bibliografia recente em especial a relação entre o forte poder de contração dos mm. longitudinais e as variações de volume do corpo de **H. grisea** como Pantin & Sawaya tiveram oportunidade de determinar em 1953, aspectos que se podem também relacionar com a estrutura especial dêste mms, assunto êste também aqui, objeto de estudo.

Em conclusão:

1. O músculo longitudinal de **H. grisea** é extremamente sensível à influência da acetilcolina, contraindo-se quando sob a influência dêste éster na concentração de 0.01 $\mu\mu\gamma$ (1×10^{-14}). Lavada a preparação, o relaxamento do músculo é imediato.
2. O músculo longitudinal de **H. grisea** não reage em presença da atropina, da histamina e do curare.
3. A atropina não impede a ação da Ach.
4. Dada a grande sensibilidade do m. longitudinal de **H. grisea** à Ach e a relação aproximadamente constante entre a intensidade da contração e a concentração da droga, e ainda mais, à vista do imediato relaxamento do referido músculo quando subtraído à ação, pode ser recomendado como material excelente para a determinação do teor de acetilcolina nos extratos de tecidos.
5. O m. longitudinal de **H. grisea,** quando perfundido com água do mar filtrada, não apresenta contrações espontâneas.
6. Discutiram-se as relações entre o comportamento de **H. grisea,** as propriedades dos mm. longitudinais e a estrutura dos mesmos.
7. O músculo longitudinal de **H. grisea** reage também à nicotina. Lavada a preparação o relaxamento do músculo dá-se sòmente após 20-30 minutos.

VIII

SUMMARY

ON THE PHYSIOLOGY OF THE LONGITUDINAL MUSCLES OF **HOLOTHURIA GRISEA.**

Distribution of the striated and smooth muscles in Invertebrates and Vertebrates presents a great deal of variation. In the later the somatic muscles are always striated and the visceral ones are smooth, except the cardiac muscle. But in Invertebrates, there are some classes as, for exemple, the Insects, in which the musculature is exclusively striated, and in others, as the Echinoderms, all the muscles are smooth.

The physiology of such muscles of Invertebrates has developed in several directions by the use of modern techniques of research, chiefly those introduced in pharmacology.

This paper deals with some interesting aspects presented by the longitudinal muscles of one of the most common Holothurians found at the Brazilian coast — the **Holothuria grisea,** Selenka 1867.

The longitudinal muscles of several species of holothurians has been investigated before by different authors. Bacq (1937, p. 174), Moussatché (1949, p. 521), Sawaya (1951, p. 41), Ambache & Sawaya (1953, p. 53) and others have demonstrated the great sensitivity of those holothurian muscles to Acetylcholine, which was mentitioned by Sawaya (l. c.) as to about 1 x 10^{-14}, that is .01 $\mu\mu\gamma$ per ml of sea water.

In order to confirm the different results referred to in current litterature, and as an attempt to introduce a simple and inexpensive method for biological assay of Ach, several experiments were performed by using the longitudinal muscles of **Holothuria grisea,** captured at the Marine Biological Laboratory of São Sebastião (L. B. M.) and maintained in the laboratory of the Department of General and Animal Physiology, in São Paulo, where several animals were transferred to

in good conditions. These Echinoderms were kept alive for a long time at the laboratory in running filtered sea-water.

Resting holothurians frequently measure ca. 40 cm in length, but this figure falls to about 20 or 15 cm when the animals are irritated. If the stimulus is strong enough the holothurians expulse the intestines, as it is well known.

When the longitudinal muscles are taken off from the body the original length of 40 cm of resting animals can be reduced to about 2 cm. Some peculiarities of the structure of those muscles are responsible for this remarkable shortening as will be seen later.

For perfusing fluid filtered sea water was employed in the commun perfusing bath. A piece of 2 to 3 cm of the longitudinal muscle of recently captured holothurians was immersed in a filtered sea water bath of about 10 ml capacity and a rather strong stream of air is passed through the suspending fluid for securing prompt mixing and supplying of oxygen.

The muscle was attached to a gimbal or shield modified level and after resting for some time known doses of Ach (Acetylcholine Roche) either Atropine sulfate (Roche or Merck), Histamine (hi-hydrochloride of histamin from Pfanstiehl Chemical Co.), d-tubo-curarine (bios Laboratories) or Nicotine (Eastman Organic Chemicals) were added to the perfusing bath, and the contractions recorded according to the common pharmacological techniques.

The results are shown on the several recordings. Fig. n. 1 records the muscular contraction after addition of .001 to .01 $\mu\gamma$ of Ach. It is noted that after each dose of Ach the muscle contracts very quickly and after washing relaxation follows promptly (less than 3 minutes on average), so that within limited time many experiments can be done.

Other experiments were used with semi-isometric levels, in order to see the relationship between the doses of the drug and the muscular tension. Fig. n. 2, indicates several results by which a relation between the tension of the muscle and the concentration of Ach can be detected. Fig. n. 3 shows the mean

values obtained in several experiments by the use of different doses of Ach. These results permit us to recommend the use of the longitudinal muscles of **H. grisea** for biological assays. In Fig. n. 4 the intensity of the contraction of the muscles determined by 2 $\mu\gamma$ to 20 mg of Ach can be seen, and in Fig. n. 5 the effect of minimal doses of the ester (5 mcg) is clearly seen. By using a very small doses of Ach (.01 $\mu\gamma$) very often no reaction of the muscle is recorded, but by repetition of the experiment after a little later the muscle starts to react. This phenomenon is compared to that of facilitation.

Correlation between concentration of Ach and tension of the muscle are indicated in Fig. 7, when small doses of Ach (.5 to 5 $\mu\gamma$) are employed and the tensions vary intensively (from 2.5 gr to 11 gr.). If less concentrated solution of Ach is used in the bath (.0005 $\mu\gamma$ to .1 γ) the tensions vary from 1 gr to 18 gr.

No influence was observed when either atropine, histamine or d-tubo curarine were added to the bath. Also, the well known anatogonism between atropine and Ach is not noticed in the longitudinal muscle of **H. grisea**.

Besides Ach only Nicotine has a strong effect on that muscle. The difference between the two effects, those of Ach and those of Nicotine, is that the alcaloid provokes stronger and more stable contraction of the muscle. Another difference is that when the preparations is washed out, after Ach, the muscle relaxes immediately (3 minutes on the average) and after the action of Nicotine the relaxation does not occur in less than 20 to 30 minutes. Very often under Nicotine influence the muscle remains in contracture of a long time, in spite of several renewing of the filtered sea water of the bath. By toxic dose of Nicotine (1×10^{-2}) the muscle does not recover at all.

Ach acts not only by promoting the contraction of the longitudinal muscle of **H. grisea** but also after the addition of the drug to the bath the muscle presents, after washing, a continuous extension in the length. If the muscle stays for a long time in experiments, about 5 or 8 hours, or if the muscle used has been kept long enough in the ice-box, the extension after relaxation is progressively more and more intense. It

seems that either the Ach has some effect on the myofibers, or acts on the sarcoplasma in order to determine a lose of contractility, because under these conditions the preparation became less sensitive to Ach. This peculiarity of the longitudinal muscle is called "acetylcholine effect". Up to now no other explanation has been found for this "acetylcholine effect" of the longitudinal muscle of **H. grisea.**

The structure of these muscles was also studied. Out of the body the muscle presents very strong contraction, has been said, the length pass from 40 cm to 2 or 3 cm, but if an extension is made by pulling at both ends the primitive length can be reached. As a matter of fact this behaviour of the muscle is in relation to the disposition of its myofibers as can be seen in Fig. n. 9 and 10. In the extended muscle (Fig. 9) the myofibers have parallel disposition, but in contracted muscle (Fig. 10) they appear in sinusoidal form. The abundance of collagen also may take part in the strong elasticity of that muscle.

Finally, according to Pantin & Sawaya (1953, p. 51) the longitudinal muscles of **H. grisea** and the circular ones are resposible for the continuous changes of the body volume. The animals, as have been shown, alters the body volume from about 10% by expelling sea water through the cloaca.

CONCLUSIONS

1. The longitudinal muscles of **Holothuria grisea** are extremely sensitive to Ach. They contract immediately after adding .01 $\mu\mu\gamma$ (1×10^{-14}) of Ach to the perfusion bath. After washing, relaxation follows immediately.
2. The longitudinal muscles of **H. grisea** are insensitive to atropine, histamine or d-tubo-curarine.
3. Atropine sulfate does not block the effect of Ach.
4. In view of the great sensitivity of those muscles to Ach and to the close relation between the intensity of contraction and the concentration of the drug, those muscles are recommended for biological assays in order to determine the amount of Ach in tissues extracts.

5. The longitudinal muscles of **H. grisea** normally do not present spontaneous contractions.
6. The behaviour of those muscles is discussed according its microscopical structure.
7. The longitudinal muscules of **H. grisea** are also sensitive do nicotine. After washing they do not relax immediately, but only after 20-30 minutes.

IX

BIBLIOGRAFIA

AMBACHE, N. & SAWAYA, P. — 1953 — Use of Holothuria grisea for Acetylcholine assays of electric organ extracts from Narcine brasiliensis (Ölfers). Physiol. Comparata et Oecologia, v. 3, n. 1, pp. 53-56, Den Haag.

ANCONA LOPEZ, A. A. & SAWAYA, P. — 1955 — Holotúrias de Recife. Ciência e Cultura, v. 7, n. 3, p. 166, São Paulo.

ANTUNES, R. A. — 1954 — Histologia do Músculo longitudinal de Holothuria. Ciência e Cultura, v. 6, n. 4, p. 167, São Paulo.

BACQ, Z. M. — 1937 — Nouvelles Observations sur l'Acetylcholine et la cholinesterase chez les invertebrés. Archiv Int. Physiol. v. 44, pp. 174-188, Liège.

—— 1935 — Recherches sur la physiologie et la pharmacologie du systême nerveux autonome. XVII. Les esters de la choline dans le extraits des tissues des Invertebrés, Arch, Intern. Physiol. v. 42, pp. 24-42, Liège.

—— 1939 — Un test marin pour l'acetylcholine. Ibid. vol. 49, pp. 20-24, Liège.

—— 1947 — L'Acetylcholine et l'adrénaline chez les Invertebrés. Biol. Rev., v. 22, n. 1, pp. 73-91, Cambridge.

BACQ, Z. M. & NACHMANSON, D. — 1937 — Cholinesterase in Invertebrate muscles. J. Physiol. v. 89, pp. 368-371, London.

JORDAN, H. — 1914 — Die Holothurien als hohlorganatige Tiere und die Tonusfunktion ihres Muskulatur. Zool. Jahrb., Abt. Allg. Phys., 34, f. 3, pp. 365-436, Jena.

MENDES, E. G. — 1954 — Sôbre o metabolismo do músculo longitudinal de "Holothuria". Ciência e Cultura, v. 6, n. 4, p. 178, S. Paulo.

—— 1954a. — Sôbre o metabolismo e o Equipamento enzimático do músculo longitudinal de Holotúria. Bol. Fac. Fil., Ciênc. Letr. Univ. S. Paulo, Zool. n. 19, pp. 123-193, S. Paulo.

MOUSSATCHE', H. — 1949 — O músculo das Holotúrias ("Holothuria grisea"), como método de ensaio biológico, para verificar

a presença de acetilcolina. Rev. Bras. Biol., v. 9, n. 4, p. 525, Rio de Janeiro.

MOUSSATCHE', H. & ARONSON, M. — 1951 — Sur l'emploi des holothuries comme méthode d'essay biologique et de dosage de l'acetylcholine. Rev. Bras. Biol., v. 11, n. 2, pp. 219-221, Rio de Janeiro

PANTIN, C. F. A. & SAWAYA, P. — 1953 — Muscular action in Holothuria grisea. Bol. Fac. Fil., Ciênc. Letr. Univ. S. Paulo, Zoologia n. 18, pp. 51-61, São Paulo.

SAWAYA, P. — 1951 — Sensibilidade do músculo longitudinal Radial de Holotúria à Acetilcolina (efeito da acetilcolina). Ciência e Cultura v. 3, n. 1, pp. 41-42, São Paulo.

—— 1954 — Sôbre a contração do músculo longitudinal de "Holothuria grisea". Ciência e Cultura, v. 6, n. 4, p. 193, S. Paulo.

SAWAYA, P. & MENDES, E. G. — 1953 — Studies on the Action of Acetylcholine in Insects and Echinoderms XIX Int. Physiol. Congress, Montreal, pp. 130, Montreal, Canadá.

STUDIES ON OLIVIDAE

by Eveline and Ernesto Marcus

(with 11 plates)

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1. INTRODUCTION

We use the name Stenoglossa in the sense of Risbec's "Sténoglosses vrais" (1955, p. 76). Hence the Toxoglossa (Terebracea and Mitracea) are held apart, though the nervous system of the Mitracea is similar to that of the Stenoglossa (Ibid., p. 73). The Stenoglossa comprise a number of families related with one another, whose grouping in three "stirpes" (superfamilies) is somewhat artificial. This is shown by the terms of the diagnoses for these "stirpes" (Thiele 1931, p. 287, 301, 330). Nevertheless we continue to use the names Muricacea, Buccinacea, and Volutacea, because they are still useful in the present state of our knowledge. One of our purposes is to examine the position of the Olividae, a family of the Volutacea, in the Stenoglossa, principally by comparison with Muricacea and Buccinacea.

As the Volutacea are scarcely represented in European seas, they are little known, e. g. not treated in the modern comparative studies of Graham (1941; 1949), Fretter (1941), and Johansson (1957). Anatomical observations regarding the Volutacea were published by Woodward (1900), Bergh (1901), Pace (1902), and Eales (1923, p. 33-38). Also the classical works of Bouvier (1887), Perrier (1889), and Bernard (1890) deal with many anatomical details of several Volutacea. Their most important results are recorded by Simroth (1896-1907), and their informations concerning the olivids, as well as those of further contemporary authors were cited by Haller (1905) and Küttler (1913). Both studied **Oliva peruviana,** and specially Küttler's work was useful for us. Recently Risbec (1955, p. 50, 62) described the oesophageal glands and the central nervous system of **Oliva erythrostoma.**

Of the two other genera we studied, **Olivella** and **Olivancillaria,** the first lives nearer to the centres of malacological studies. Therefore certain aspects of its life were already recorded (P. Fischer 1881; Crosse & Fischer 1882) and some of the organs were figured (Dall 1889, t. 34, f. 1). The radular characters, also known of **Olivancillaria,** were combined with

the differences of the shells in the recent systematic monograph of **Olivella** (Olsson 1956).

The following study contains anatomical and other observations of **Olivella verreauxii** (Ducros 1857), **Oliva sayana** Ravenel 1834, **Olivancillaria (Lintricula) auricularia** (Lamarck 1810), and **Olivancillaria (Olivancillaria) brasiliensis** (Chemnitz 1788).

2. CLASSIFICATION OF THE MATERIAL

We established the name of our operculate **Olivella** on the basis of Olsson's monograph (1956). The key (p. 166-168) of shell characters leads to the subgenera with a separate pillar structure (CCCc), among which **Dactylidiella** (p. 187) was forgotten. The latter contains only one large recent species with a range from the Gulf of California to northern Peru. **Mansfieldella** (p. 194) is known by a single species from the Pliocene of Florida. In **Macgintiella** the inner side of the pillar wall is deeply excavated, and the rhachidian tooth of the radula (f. 19 on p. 162) differs considerably from our material. The two remaining subgenera with a separate pillar structure are **Dactylidia** (p. 183) and **Niteoliva** (p. 189). Their types have often been confused (p. 191), but the rhachidian teeth (f. 17, 15) are quite different. The present species agrees with **Niteoliva**. The two Atlantic species of this subgenus are **minuta** (Link 1807) and **verreauxii** (Ducros 1857). Fortunately Olsson reprinted (p. 191) the original description of Ducros whose "Revue critique du genre Oliva" is not at our disposal. This description separates **minuta** and **verreauxii** univocally. Also Reeve's pictures (1850) which we compared in accordance with Olsson's synonymy, lead to the name **verreauxii** for our species.

Lange (1949, p. 101) and Olsson (1956, p. 219) indicate only Bahia as Brazilian locality for **O. (N.) verreauxii**. The whitened shell of this species shows a lirated inner side of the outer lip in Olsson's photograph (t. 9, f. 3). In our material internal lirations of the outer lip were found only in 3 shells out of 50 examined for this structure. It is an inconstant character as in

O. (N.) minuta and **O. (N.) peterseni**. In **O. (N.) morrisoni** it is absent.

We have compared our material with the descriptions of all species of **Olivella** listed from Brazilian waters by Lange (1949), Gofferjé (1950) and Souza Lopes & Alvarenga (1955, p. 176) and considered their synonymy (E. A. Smith 1890, p. 487; Olsson 1956). Lange's "**Olivella mutica petiolita** Dall, 1889" (1949, p. 101) could not be located in any of Dall's numerous papers we consulted. However we did not have Dall's preliminary catalogue (Bull. U. S. Nat. Mus. 37) at our disposal. We only found "**Olivella mutica** Say var. **petiolita** Ducl." used by Ihering (1897, p. 170) for a species from the Island of São Sebastião. The name cannot be maintained, because **O. mutica** Say 1822 is the type of the operculate subgenus **Dactylidia,** while **O. petiolita** Duclos 1835 belongs to the subgenus **Olivella** without operculum.

The name of our **Oliva** is sufficiently justified by the agreement of our material with the descriptions and figures of Reeve (1850, t. 11, f. 18), Weinkauff (1878, p. 64, t. 15, f. 1-8) and Abbott (1955, p. 245, t. 12, fig. a). The bright yellow form **citrina** Johnson (1911, p. 123) is not represented in our material. As Dr. R. Tucker Abbott kindly informed by letter of April 21, 1958, **O. citrina** is a colour form, not a subspecies, and thus it is not correct to write **O. sayana sayana**. The species occurs from North Carolina (Johnson 1934, p. 133) southward to Sta. Catharina (Gofferjé 1950, p. 247). E. A. Smith (1890, p. 487) reported it from Fernando Noronha; Lange (1949, p. 101) from the Island of São Sebastião. The catalogue of Lange mentions **O. reticularis** Lm., which Ihering (1897, p. 170) listed from São Sebastião, only from Bahia.

The exact form of the name, the author's name, and year can only be settled for one of our two species of the genus **Olivancillaria** d'Orbigny (1841, p. 420) with the bibliography at our disposal. This is the type of the subgenus **Lintricula** H. & A. Adams (1853, p. 141, cited from Neave, Nomenclator Zoologicus), **O. (L.) auricularia** (Lamarck 1810, p. 323). The other species, the type of the genus **Olivancillaria,** has, as far as we

can see, to be called **O. (O.) brasiliensis** (Chemnitz 1788; paper not seen). Our names agree with those indicated by Lange de Morretes (1949, p. 100). We have compared our material with d'Orbigny (l. c.), Reeve (1850, t. 18, f. 13 a-b, f. 39) and Weinkauff (1878, p. 19. 50, 52). The geographic distribution of both species extends from Rio de Janeiro to the Gulf of San Matias, Lat. 42° S. (Carcelles 1944, p. 259). Carcelles found **O. (L.) auricularia** among mytilids, probably where the rocks were surrounded by sand.

In the following we refer to **Lintricula** and **Olivancillaria** with their subgeneric names only.

3. NOTES ON LIVING SNAILS

Of **Olivella verreauxii** 21 males and 73 females were found in December 1954, West of Ubatuba at the Enseada. At the same place Mr. Caio Del Rio Garcia collected some 50 specimens in August 1958 with the same sex-ratio. In November 1958 we found hundreds of these snails at the Enseada and neighbouring beaches (Praia do Lázaro, Praia Domingos Dias). The shells are about 11 mm. long and 5 mm. broad. The localities correspond to Bales' (1946, p. 47) and Olsson's descriptions of the places where they found **Oliva** and **Olivella**. The Enseada is a typical "Feinsandstrand" of the littoral of São Paulo, as Gerlach (1957, p. 416-419) described it. At high tide it receives the surf of the open ocean, though weakened by a broad gradually sloping shore of fine and clean sand. At a normal low tide (0,3 at 8,10 a. m.) on December 9, 1954 the snails crawled immediately beneath the surface of the sand exposed to air but still wet without emerging from the sand. The snails are found on the broader end of their trail (Fig. 1). They were active near the surface for about half an hour from 8,30-9 h. then all disappeared long before the tide re-covered the beach. Like **O. tehuelchana** observed by d'Orbigny (1841, p. 418) and other species (Olsson 1956, p. 161) also **O. verreauxii** swims by means of its wing-like metapodial flaps.

The stomach contains principally Bivalvia **(Donax hanleyanus)** and besides Foraminifera, Copepoda, Amphipoda, and Scaphopoda. As Dall (1889, p. 134) noted, clams one third as long as the snail's shell may be swallowed whole. When we offered crushed larger **Donax** to the snails in a dish, they tore particles of them, till the shells were emptied, leaving back only the occlusor muscles. This observation corresponds to Ankel's statement (1938, p. 276) that firm muscular elements cannot be torn off from a loose shell, if it is not held fast with the foot. Skeletons of diatoms occur in the faeces of the snail, probably as contents of the guts of their prey

We collected 26 living specimens of **Oliva sayana** in November 1957 in the upper littoral of the Island of São Sebastião, in a sheltered sandy bay near Ilhabela. The topography of the shore at this place differs from Bales' and Olsson's above cited ecological indications. The narrow strand shelves rather steeply to the bottom of the bay. This consists of middle fine, slightly muddy sand near the shore and seawards of mud. At normal low tides the trails of the olives forming two parallel ridges could be seen about 50 cm. below the surface, provided that the water was calm. The few empty shells of **O. sayana** washed ashore at Ilhabela are all corroded. This shows that the snails go deeper when the water becomes agitated and are not as easily dislodged by the waves as **Bulla striata,** in any case not in the rarely rough sea in the canal of São Sebastião.

Our material of **Oliva sayana** was composed of 21 males and 5 females, the former with an average length of the shells of 49,9 mm., the latter with one of 44,5 mm. Also Küttler (1913) had only 3 females of **Oliva peruviana** and evidently many more males. In our material the variation of the proportion between greatest diameter and length ranges from 1 : 2,36 to 1 : 2,17 without correlation with size or sexes. Our largest empty shell was 73 mm. long and 30 mm. in greatest diameter. Ten half-grown, 22-38 mm. long and only slightly worn shells were found on the surf-beaten sandy beach of the Ilha Comprida near Cananéia in July 1958.

The contents of the stomachs in our snails were small crustaceans and juice. Meat juice as food of **Oliva** was already supposed by Weinkauff (1878, p. 5). He cites Quoy & Gaimard's observation that on Mauritius **Oliva** is baited with meat. Generally the species of **Oliva** are called scavengers (Graham 1955, p. 151). The anatomy of the nerve ring of **Oliva sayana** impedes an **Oliva** really to swallow an **Olivella,** as Olsson (1956, p. 164) indicates. Probably it grasps its prey with the foot, scrapes particles off with the radula and sucks them in, as **Lintricula auricularia** does.

Our material of 72 living **Lintricula auricularia** (Fig. 3) was brought together with the efficient help of Dr. Claudio Gilberto Froehlich and Mr. Caio Del Rio Garcia. Their observations are included in our report. The snail is common on gradually sloping shores with fine an clean sand along the entire coast of the State of São Paulo, from Ubatuba in the Northeast to the Ilha Comprida near Cananéia in the Southwest. Near Ubatuba, on the Praia de Itaguá, the animal is so frequent that it is collected and boiled with the daily rice; the people call it "vitela" (veal), perhaps due to its white flesh, or "vaquinha" (little cow). The shells are sold by the litre for ornamental purposes. The shells of our collection were 8-56 mm. long, the largest empty conch was 60 mm., smaller than Goferjé's (1950, p. 246) biggest specimen of 68 mm. Both sexes were present in nearly equal numbers in July and August 1958, and without noticeable difference of size.

The adult snails were found at the water-line in fine sand and in shallow water in loose shelly sand, as well as rather high over the low tide-line in rather dry sand. It seems that the snails prefer somewhat loose sand, as occurs just below the high water-line. When caught, the snails expel water from their mantle cavity. Some half-grown animals were dredged in a depth of 4-5 m., together with many dead shells of bivalves. Several times the snails were frequent in the sand in the neighbourhood of the mouths of rivulets. Perhaps the inflow of fresh-water plankton which dies in the sea and sinks down favours the bivalves upon which the snails feed. Among

the bivalves **Donax hanleyanus** constitutes the common food of **L. auricularia** on the coast of São Paulo. The snails shove the sand aside and leave a trail. Sometimes the shell stands partly out from the sand. Anaesthetized animals become well stretched.

When specimens were brought together with **Donax,** they grasped the clams, as Hirsch (1915, p. 382) described it for **Natica**. The snail drags one or two clams about in the concavity of its metapodium. A dish with snails was put on a tripod for observing the act of ingestion with help of a mirror placed under the dish. In order to intensify the chemical stimulus for the snails exposed to artificial conditions out of the sand, we offered them clams with cracked valves. Immediately the snails everted their proboscis towards the prey in front of them and tore off particles of the flesh with their radula (Fig. 4-7). If the clam is seized with the metapodium, the head is curved downwards and the proboscis everted along the median furrow of the propodium to reach the food on the shortest possible way.

When the proboscis is everted (Fig. 8) the red pharyngeal region and the white salivary glands shine through the proboscidean wall, and brownish food particles are pumped upwards through the white oesophagus by peristalsis. The brilliant radula comes out at the orifice of the proboscis and tears small pieces of the mantle from the valve of **Donax**. During feeding the pharynx moves intensely and changes its position within the proboscis turning to the dorsal or ventral side. Feeding went on for at least 2 hours. The snails succeeded to tear particles from **Donax** lying loosely in the dish (Fig. 3), also without fixing them with their foot. In a bowl with a layer of sand so high that the **Lintricula** were covered, they fed regularly on large living **Donax** as under natural conditions and cleaned the shells completely with their proboscis.

When the foot encloses prey with its sharp posterior margin it appears, in dorsal view, rounded and thick, as if it was swollen, though it is really rather flat. This aspect together with the backward bent proboscis might explain Olsson's des-

cription (1956, p. 164) of an **Oliva undatella** swallowing an **Olivella** whole and swelling into a large, rounded, ball-like mass. Olsson thought that the mouth of olivids opens on the ventral surface of the metapodium near its forward margin (p. 161).

Of **Olivancillaria brasiliensis** (Fig. 2) we found only 3 middle-sized individuals alive with 34-43 mm. long shells, 1 male and 2 females, near the water-line of the Ilha Comprida, though empty conchs (19-62 mm.) were much more numerous there than those of **L. auricularia**. The shells of **brasiliensis** are much more solid, but this difference hardly explains satisfactorily why they were far more frequent than those of **auricularia**. We still ignore the regular habitat of **O. brasiliensis**. According to d'Orbigny (1841, p. 420) the species lives in depths of 4-5 m. (Rio de Janeiro), but Gofferjé (1950, p. 246) found it half burried in the sand and following the tide-line (coast of Paraná) in the same manner as **auricularia**. Our biggest empty shells surpass Gofferjé's of 55 mm.

4. EXTERNAL FEATURES

In **Olivella verreauxii** (Fig. 10-12) the zigzagged lines of the shell vary very much, 5-12 being visible from one side on the body whorl. Exceptionally these lines are wanting, and the entire shell is uniformly yellow. The brown pigment of the lines belongs to one of the layers under the uppermost one. It evidently lies in an organic substance between the calcareous layers, because it remains coherent, forming ribbons when the shell is decalcified. The same applies to the brown pigment of **Oliva sayana**. The shells of L. **auricularia** found on the Ilha Comprida had a bluish hue, while those from a beach West of Ubatuba (Praia Domingos Dias) were alive more yellow without the bluish tone. The first collector of the snails near Ubatuba, Mr. Caio Del Rio Garcia, noted this difference and also observed the more yellow shade of the sand on the mentioned beach.

The resorption of the inner walls and the columella in **Olivella** was described by P. Fischer (1881) and Crosse & Fis-

cher (1882). The apex of **O. verreauxii** is strongly calcified. The inner walls of the apical whorls of **Oliva sayana** are thin and transparent like glass. Part of their thickness is resorbed. This is visible where they pass into the thick outer walls. Here the various layers of the thick shell are laid free one beside the other as in an oblique polish. The thinning of the inner walls of the upper whorls is less conspicuous in our two species of **Olivancillaria**. The dropping angle of the apical whorls of **Oliva sayana** is rather variable; in many shells it is about 70°, in others up to 105°. Also in **Olivancillaria brasiliensis** this angle varies. The larval shell is distinct in our **Olivella** and **Oliva**; in **Olivancillaria brasiliensis** it is still just visible, also in the biggest specimens; in **Lintricula auricularia** it is often completely concealed by the callus. The periostracum of **Oliva, Lintricula** and **Olivancillaria** contains dark pigment, and therefore the slightly worn shells which are found on the beach are lighter than those of living snails and reddish brown.

Of our studied olivids **Oliva,** externally of almost bilateral symmetry (Morton 1958, p. 7, f. 3), has the most complete appendages. Two vertical flaps which flank the mouth bear tentacles with eyes above the middle of their height. The other appendages are an anterior and a posterior mantle tentacle and a short posterior mantle lobe. Simroth (1897, p. 153) approached the posterior mantle tentacle to the pallial caecum of **Acteon**. But these structures are quite different. The hollow caecum of **Acteon** (Fretter & Graham 1954, p. 568) contains two ciliated ridges and lies inside the shell; the posterior mantle tentacle of the olivids is solid, glandular and lies outside the shell in the channel of the suture. A chondroid support of the posterior mantle tentacle as described for **Voluta musica** (Pace 1902, p. 23) does not occur in our olivids. As Olsson (1956, p. 164) reports for **Oliva** also **O. sayana** lifts the posterior mantle tentacle out of the suture and pulls it into the shell, when the soft parts are withdrawn. In **Olivella verreauxii,** however, it frequently remains outside the shell, when the animal retracts due to preservation. Evidently the small operculum of our species does not close the aperture completely.

The posterior mantle tentacle of **Olivella** is longer than that of the other studied species and surpasses the length of the shell. The posterior mantle lobe is broad and produces a thick callus in **O. verreauxii**. An anterior mantle tentacle is present, but no tentacles nor eyes. The vertical flaps on both sides of the mouth which bear the tentacles in **Oliva** are developed also in **Olivella,** but are not innervated and thus do not substitute the tentacles. In **Lintricula** and **Olivancillaria,** also without tentacles and eyes, these flaps are supplied with nerves. Both species have no anterior mantle tentacle; Weinkauff's figures of **O. brasiliensis** (1878, t. B, f. 4-5) with long tentacles, eyes, and anterior mantle tentacle are incorrect. The posterior mantle lobe is rather thick in **Lintricula** and **Olivancillaria,** the posterior mantle tentacle a little longer than in **Oliva sayana**. It extends nearly around the body whorl to the point where the callus closes the channel of the suture.

The siphon has smooth borders in **Olivella** and frilled ones in **Oliva.** In **Lintricula** and **Olivancillaria** (Fig. 9) they bear branched papillae, evidently functioning as filter (Clark 1958, p. 58, 62). The foot of **Olivella** and **Oliva** measures about two thirds of the length of the shell and can be completely withdrawn. In **Lintricula** and **Olivancillaria** it has twice the length of the shell and cannot be entirely retracted, even when the snails are stimulated mechanically. The expanded foot of an **L. auricularia** with a 42 mm. long shell is 80-90 mm. long and 40-45 mm. broad.

5. MANTLE APPENDAGES

Anterior and posterior mantle tentacles, posterior mantle lobe and siphon are similar to one another and alike in the examined species. The dorsal columnar epithelium of the anterior mantle tentacle of **Olivella verreauxii** contrasts with the flat cells of the ventral surface. Several bundles of longitudinal muscle fibres lie under the epidermis together with erythrophilous and cyanophilous glands. Nerves run in the connective tissue. Blood lacunae are scarce.

The posterior mantle tentacle of **Olivella** and **Oliva sayana** has cyanophilous glands specially under the high and ciliated dorsal epithelium. In **Oliva** the glands form a voluminous concentrated mass, while **Olivella** possesses also a ventral accumulation. The nerve of **Olivella** courses between a dorsal and a ventral blood sinus. In **Oliva** the several nerves, in part of considerable diameter, are ventral; a large blood sinus is dorsal. The muscles in the connective tissue are dorsally and ventrally concentrated in **Olivella,** while those of **Oliva** fill up almost the whole ventral parenchyma around the mass of glands and the large sinus.

The posterior mantle tentacle of **Lintricula auricularia** shows many basophilous intra-epithelial glands in the high and ciliated dorsal epidermis and a few subepithelial ones under the flat epithelium of the sides. Under the dorsal epithelium acidophilous glands constitute a thick layer. Ventrally to them the longitudinal muscles form a compact stratum too. Still farther ventral lie two large blood lacunae. Between them run several nerves of different calibre. The same elements appear in the posterior mantle lobe of the mentioned species. Its muscles are more uniformly distributed and consist mainly of longitudinal and dorso-ventral fibres. The blood lacunae lie in the middle of the lobe; the nerves run near the ventral epidermis.

The siphons of **Olivella** and **Lintricula** were examined. Their ciliated borders are as said, smooth in **Olivella,** in **Lintricula** branched. In the latter species a larger blood sinus runs near each border. The connective tissue contains numerous radial muscles besides longitudinal and dorso-ventral fibres in **Lintricula,** while in **Olivella** these as well as the other muscles are weakly developed, and the interior of the siphon is vacuolized by numerous blood lacunae. Both species have a higher dorsal and a lower ventral epidermis, few basophilous intra-epithelial glands, and many nerves in the ventral half of the parenchyma. Part of these is evidently sensory and supplies the subepithelial cells whose terminations form sensory, probably chemoreceptive (Yonge 1947, p. 509; Morton 1958, p. 5),

pads on the surface of the siphonal papillae of **Lintricula**. The cilia of a narrow strip along the outer borders of the siphon evidently beat to the exterior. They produce the current by which heavy particles are removed from the inhalant opening (Yonge 1938, f. 1, on p. 454, "current A") described by Yonge (1947, p. 495), Fretter (1948, p. 619), and Clark (1958, p. 58). Further cilia inside the siphon or between its root on the mantle border and the ctenidium are not developed. Hence the branchial cilia produce the inhalant current as generally in pectinibranchs. Under the gill a longitudinal ciliated strip extends backwards on the floor of the mantle cavity. It is continued into a right-sided field and corresponds to the streak that leads medium-sized particles out in **Buccinum** (Yonge 1938, f .1, on p. 454, "current B"). The right-sided field is mentioned later in connexion with the reproductive organs of **Olivella**.

6. PEDAL GLANDS

Olivella

Scattered blue staining glands occur below the ciliated epithelium of the propodium and the parapodial flaps. A dorsal and a ventral unciliated transverse furrow separate propodium and metapodium. In the female the right half of the dorsal furrow bears cilia. The anterior border of the propodium has two shallow horizontal clefts, the so-called labial clefts. Clusters of blue staining glands (ae), the anterior pedal mucous glands (Graham 1957, p. 141) lie among the connective tissue and muscles of the propodium and discharge into the clefts (Fig. 15). The ridge between the clefts is underlain with clusters of sensory cells. The clefts disappear in the middle, because here a median furrow passes from the back of the propodium to its sole. In the female the ventral propodial furrow is indistinct. Both sexes have an unciliated metapodial sole which flattens backwards. A small group of blue staining gland cells (ra) is located at the crossing point of the ventro-median and the transverse furrows. This posterior pedal gland (Graham

1957, p. 142) occurs in both sexes. A little behind lies the much more conspicuous ventral pedal gland of Graham's terminology, "la glande pédieuse ventrale" of Touraine (1952, p. 242), whose aperture is longer than broad. Only females have this gland (Fig. 13, 15, v), as Pace (1902, p. 22) first noted in **Voluta musica**. Intra-epithelial and subepithelial sole glands (Graham 1957, p. 141-142) occur in **Olivella, Oliva** and **Lintricula**. The rim of the ventral pedal gland projects outwardly in living and preserved snails (Fig. 11), while Simroth (1899, p. 262) expressly indicated such salient lips as a consequence of preservation. The shape of this pit with prominent circumference evidently led Olsson (1956, p. 161) to take it for the mouth. The surrounding sole has 12-14 micra high epithelial cells with 8-10 micra long cilia; the ventral gland is lined by an 8-12 micra high epithelium with 5 micra long cilia. This difference is common among the prosobranchs (Simroth 1898, p. 264-265). As in the posterior gland also in the ventral one the blue staining secretory cells lie below the uniformly ciliated epithelium which is not glandular. The same disposition of the glandular elements was observed in the only existing pedal gland, the ventral or female one, of **Nassa** (Abbott 1955: **Nassarius) reticulata** (Fretter 1941, p. 195). The thickness of the gland cells in **Olivella** is different in accordance with the reproductive activity of the individual. The musculature is composed of chiefly radial fibres. Particles are sometimes entangled in the cilia of the female pit and the sole.

Perhaps this material came from the storage vesicle of faecal matter (Fig. 45), when the egg capsule entered the pit for being moulded and hardened. Moulding of the egg capsule was presumed as function of the ventral pedal gland by Simroth (1907, p. 993), and observed by Ankel (1929), Fretter (1941, p. 199 ff.; 1946, p. 337) and others (see Ankel 1936, p. 172; P.-H. Fischer 1950, p. 204). Besides the egg capsule is fixed to the substratum by the secretion of the gland cells; the egg capsule itself is produced by the glandular oviduct, not by the pedal gland (Graham 1957, p. 142), as still indicated by Thiele (1935, p. 1033).

Oliva

Intra-epithelial blue staining gland cells are abundant in the ciliated cylindrical epithelium of the propodium and the lateral flaps. The dorsal and ventral transverse furrows separating propodium and metapodium are present. The ventral one is not straight as in **Olivella** but forms an obtuse angle opened backwards, as described by Brock (1889, p. 74-75) and drawn by Küttler (1913, f. B on p. 480). Hence it is parallel to the anterior border of the propodium. The labial cleft is very shallow. Its epithelium consists of 25 micra high ciliated cells with cilia of the same height. It is underlain with groups of sensory cells, identical with those under the ridge in **Olivella.** The subepidermal glands of the anterior pedal border open above and below the sensorial furrow; they are more or less distinctly stained in different individuals.

The dorsal median propodial furrow is always present, while the occurrence of the ventral one varies. The ventral metapodial furrow is allusively developed. Both sexes possess glands that correspond to the posterior pedal cluster of **Olivella**. However these sub-epidermal gland cells accompany the entire angulated furrow between propodium and metapodium in **Oliva**.

The ventral pedal gland (Fig. 16) exists, as in **Olivella,** only in females. It is located a little behind the point of the angle formed by the transverse furrow. Its aperture is a nearly 2 mm. long, longitudinal slit whose rim projects slightly. The glandular mass is 0,9 mm deep and broad, the lumen 0,7 mm. deep. The walls of the cavity are thrown into folds and consist of ciliated cells and unciliated gland cells as in Fretter's muricids. The epithelium and the cilia vary in height; the cilia are longest on the crests of the folds. Subepithelial glands about 0,2 mm. in length open among the epithelium and in 0,2 mm. breadth on both sides of the aperture. The metapodial musculature composed of longitudinal, dorso-ventral, and transverse bundles is not much modified by the pedal gland. Some transverse fibres curve around the gland, thus function-

ing as circular constrictors, and dorso-ventral fibres are radially disposed as divaricators.

Küttler (1913, p. 479, 485) discovered the female pedal gland in **Oliva**. The terms of his summary (p. 539) are vague: "In some individuals there is the rudiment of a pedal gland". Unfortunately he drew the ventral view of a snail (fig. B) with glandular slit (pap) and penis (pe). The "rudimentary" stage of the pedal gland of Küttler's species (p. 485) evidently corresponds to the non-reproductive phase of his material (p. 516), also revealed by the absence of sperms in the testis and the empty bursa copulatrix.

Among the female pedal glands of Fretter's four Stenoglossa (1941, p. 194 ff.) that of **Ocenebra erinacea** is similar to that of **Oliva sayana**.

Lintricula

Lintricula and **Olivancillaria** have the same transverse dorsal and ventral furrows which separate propodium and metapodium as the other examined olivids. The propodium of both has a dorso-median furrow in males and females. Also a metapodial groove occurs in both species and both sexes (Fig. 14). The crescent-shaped propodium of **Lintricula** is short in comparison with the metapodium and its dorsal extension is about twice as long as the ventral one. The anterior border of the propodium bears numerous sensory cells, but has no horizontal furrow. The anterior pedal glands open dorsally to the gland-free foremost projection of the propodium. The posterior pedal gland accompanies the dorsal and ventral transverse furrows as in **Oliva**. About 3 mm. behind the ventral transverse furrow the above mentioned groove extends for a length of 6-12 mm. It is 2-4 mm. broad and 1 mm. deep in the middle. In front of and behind this groove the sole glands are 50 micra long. Those in the groove differ considerably from them, as they are crowded and up to 200 micra long. The dorso-ventral muscles of the sole are strengthened in the area of the groove which may be used when the snail holds on its prey In the

anterior half of the groove the gland cells attain a height of 0.4 mm. in the middle. In adult males only few are so long and are pale, but in adult females these high cells are densely disposed and, in part, stain dark blue. Moreover some of the adult females have two shallow transverse furrows going out from the area of the central high cells. Evidently this region corresponds to the ventral pedal glands of **Olivella, Oliva** and other Stenoglossa, but is not so distinctly set off as in these and differs from that in the male only by the quantity of the high cells and their colourability. The ventral groove does not have the structure of a sucker as the pit in front of the ventral gland Fretter (1946a, p. 126) discovered in males and females of certain muricaceans.

7. CENTRAL NERVOUS SYSTEM

Olivella

The perioesophageal nerve collar lies behind the everted and below the inverted proboscis. The nerve ring is rather concentrated, but all ganglia are distinctly separated (Fig. 19). The pedal ganglia are the most voluminous centres as in **Buccinum** (Dakin 1912, p. 67), **Harpa** (Bergh 1901, p. 614), **Voluta** (Pace 1902, t. 2, f. 3) and other Stenoglossa (Simroth 1899, p. 412-425, t. 29). The large pedal ganglia of the olivids correspond to the size and the biological importance of the foot as in the naticids (Risbec 1956, p. 28, 29). By means of this powerful locomotor organ the snail which lives in the dense medium of fine sand reaches its prey, its mate and the substratum to which it attaches its eggs. To the locomotor function of the foot must be added the sensory activity of its anterior border. Risbec (1955, p. 62) found the nerve ring of **Oliva erythrostoma** so much deflected that the flattened undersides of the pedal ganglia which are directed ventrally in other prosobranchs are turned towards the right side. In some specimens of **Olivella verreauxii** we have seen it so, when the nerve ring was isolated, but not in all cases, and never in the sections where the natural position of the nerve collar is maintained.

Short connectives unite the pedal and the propodial ganglia (Fig. 15). The occurrence of the latter is a primitive feature (Krull 1935, p. 462). A propodial commissure is not developed, nor are there metapodial ganglia, as, e. g. in the primitive hydrobiids (Bregenzer 1916, p. 250-51, 271).

The cerebral and the pleural ganglion of the left side lie farther in front than the corresponding right centres. Hence the connectives between the right dorsal ganglia and the pedal and buccal ones are longer than those of the left side. The right cerebro-pedal connective is especially long (0,15-0,2 mm.), and some nerve cells were found on them in two of the sectioned nerve rings (Fig. 18). Of the other connectives only the right pleuro-pedal one showed some ganglion cells in one series of sections. No nerve cells were found on the commissures.

The connective which unites the subintestinal ganglion with the left pleural ganglion, the left root of the visceral loop, is strong. Also the dextral zygoneurous connection, the connective between the subintestinal ganglion and the right pleural ganglion, is thick. As in the two following olivids there are two ganglia in the posterior course of the visceral loop. The right and bigger one is located near the hind border of the mantle cavity, a little to the right of the oesophagus, the left or accessory one (Giese 1915, f. 7 on p. 180) 0,4 mm. farther in front, to the left of the oesophagus.

The nerve cells which form the lateral caps of the pleural ganglia are similar to those of **Dolium** (Abbott 1955: **Tonna) galea** figured by Schreiber (1930, t. 4, f. 11, t. 5, f. 20, 25). These cells contain pigment and are surrounded by pigment which is, according to Schreiber, stored by amoeboid cells. The peculiar aspect in stained preparations or sections of the nerve ring is due to the red staining supporting fibres of the mighty neuroglia tissue which accompanies connectives and commissures (Fig. 15, 17). The rich development of this tissue is correlated with the feeding habits of **Olivella**. The snail engulfs lamellibranchs one third as long as its own shell, has small salivary glands and no crop (pharynx of Leiblein) nor oesophageal gland (gland of Leiblein). Hence the prey enters the stomach undi-

vided and undigested, and on its way extends the oesophagus and the nerve collar. The distention of the latter is made possible, as in the tentacle nerve of **Arion** (Jakubski 1913, p. 114), by the neuroglia fibres. Peculiar are the neuroglia cells situated axially among the nerve fibres (Fig. 17). The processes of these cells ramify towards the periphery, where they join the external neuroglia coat (ibid., p. 109, 113). Axial neuroglia cells are frequent in the pedal commissure, in all connectives, and in the propodial nerves.

Each propodial ganglion gives origin to 4 nerves. The two thinner ones run towards the sides, the two thicker nerves form the propodial nerve net with its transverse rows of secondary ganglia discovered by Brock (1889, p. 70-71, t. 6, f. 2) in **Oliva maura**. The sensory cells below the anterior border of the propodium are connected with the propodial nerve net. Of the numerous nerves going out from the pedal ganglia only the anterior ones were drawn; the thick penial nerve leaves the right pedal ganglion. Though tentacles and eyes are not present, the innermost cerebral nerve can be called tentacle nerve in conformity with Küttler (1913, p. 520, n. opt.). As in his species, **Oliva peruviana,** the nerves of the proboscis and the proboscis sheath of **Olivella** are more conspicuous on the left cerebral ganglion than on the right, where they, however, also exist. The static nerve is short, and the statocyst (s) lies near the brain, viz. to the side of the posterior border of the pedal ganglia (l. c., p. 524-25). The lumen of the statocyst is 66 micra in diameter, the statolith 60 micra. The low epithelium of the vesicle consists of fungiform sensory cells and vesicular cells. The capsule of the statocyst is composed of an inner layer of homogeneous supporting substance and an outer one of vesicular cells of Leydig which form a tissue similar to Schaffer's chordoid tissue (1913, text-fig. B on p. 328).

The short buccal connectives arise antero-ventrally from the cerebral ganglia. The buccal commissure is about as long as the diameter of each buccal ganglion (ca). The radular

nerve leaves the commissure, and more nerves than were drawn go out from the ganglia. The columellar (cv) and the pallial-siphonal (sn) nerve (Bouvier 1887, p. 310) as well as one innervating the body wall come from the left pleural ganglion. The peripheral left zygosis (ez) between the pallial--siphonal and the osphradial-branchial nerve known of **Buccinum** (Dakin 1912, p. 70) and **Oliva** (Küttler 1913, p. 521) is present. Also the nerve of the subintestinal ganglion (un) which innervates the right mantle and the posterior mantle appendage, and the nerve from the supra-intestinal (va) ganglion to the floor of the mantle cavity are the same as in **Oliva**.

The nervous system of **Olivella verreauxii** resembles that of two Volutidae, **Harpovoluta charcoti** (Eales 1923, f. 37) and **Cymbiola** (Powell 1951: **Adelomelon**) **ancilla** (Woodward 1900, t. 10, f. 9).

Oliva

The central nervous system of **Oliva** is well known by the studies of Bouvier (1887, p. 309-312), Haller (1905, p. 653-658) and Küttler (1913, p. 517-523). We give a combined drawing of six successive sections (Fig. 22) and dorsal and right-side views (Fig. 20, 21). The spongy mass of connective tissue which surrounds the nerve collar is stippled with brown pigment. The torsion that Risbec (1955, p. 62) described for **Oliva erythrostoma** was mentioned as present in some isolated nerve rings of **Olivella**. In **Oliva sayana** the deflection exists in a similar degree as in Risbec's species. The pedal ganglia which are antero-ventral to the cerebral ganglia are enormous, as big as all others together (Bouvier 1887, p. 310). All connectives are so short that the nerve cells of the ganglia touch over them. Bouvier (l. c.) evidently meant this when he stated that all connectives are covered with nerve cells, and the ganglia are only separated by superficial constrictions. This nerve ring is too narrow for the passage of an entire snail or clam of the size of an **Olivella** or **Donax** (Olsson 1956, p. 164).

Morphologically the nerve ring of **Olivella** with distinct connectives and separate ganglia is more primitive than that

of **Oliva** with its extreme concentration and fusion of the ganglia. But considered in relation to the alimentary possibilities the dilatable central nervous system of **Olivella** appears more advanced than the rigid collar of **Oliva,** who must feed on small prey or break its food up outside its body, exposing foot and prey to ennemies for a long time.

As Bouvier (1887, p. 311) found the nerve ring of the Marginellidae almost as concentrated as that of **Oliva,** he considered the marginellids nearer related with olivids than with volutids (p. 312). Fused and indistinct ganglia, except the cerebral ones, were also described for **Marginella hyalina** (Eales 1923, p. 38).

As in other Stenoglossa (Bouvier 1887, p. 417, 487) the statocysts lie at different distances from the nerve ring. The capsule of the statocyst has the same struture as in **Olivella;** its cells of Leydig contain some pigment.

The tentacles are dorso-ventrally compressed continuations of the laterally compressed oral flaps (see: alimentary tract). Their lengths vary considerably in the examined specimens. The epidermis of the tentacles bears the same vertically striped cuticle as the outer side of the flaps. The tip of the tentacle is formed by a short flagellum set off from its proximal base. The eye measures 0,18 × 0,2 mm., the lens 0,12 × 0,14 mm.; the pigment lies in the supporting cells. Though the tentacle nerve runs through the entire length of the tentacles and ramifies in the flagellum, the much richer innervation of the propodial border suggests that also in **Oliva** this border is the most important sense organ as in **Natica** (see: Ankel 1936, p. 140).

Lintricula

The central nervous system of this species (Fig. 23-25) is brick red, due to pigment in its outer neuroglia and the nerve cells that cover a great extension of the cerebral (cr) and pleural (ua) ganglia. These centres are broadly coalesced. The remaining connectives and commissures are distinct, shorter than in **Olivella,** but much more distinctly set off than in **Oliva**. The supporting neuroglia fibres are chiefly developed in the con-

nectives and occur also in the propodium. This highly extensile and contractile organ whose function substitutes the wanting tentacles is supplied by eight nerves, four originating from each propodial ganglion. These nerves ramify and anastomose and form about 8 series of consecutive secondary ganglia. The longitudinal conectives of these ganglia are enveloped in a neuroglia containing the above mentioned fibres which allow for rapid movements of the propodium. As in the other examined olivids the pedal ganglia (en) are the most voluminous centres; including the primary propodial ganglia (se) their mass is about the same as that of the other ganglia together. The flat underside of the pedal ganglia is parallel to the foot, not deflected as in **Oliva**. The left cerebro-pleural ganglion lies farther in front than the right one as in **Olivella**.

Also the position of the statocysts (s) is asymmetrical, as the right is farther distant from the nerve collar than the left. The capsule of the statocyst is formed by a dense fibrillar connective tissue with scarce nuclei whose periphery is intermingled with cells of Leydig. The lining of the statocyst corresponds to Küttler's description (1913, p. 525-526) of **Oliva**. There are two types of cells, dark staining ones and between them others which appear empty in the sections. The dark staining cells broaden distally and partly overlap the adjacent pale cells. Every dark cell bears one long sensory hair. Küttler considers the pale cells as supporting cells, but they look rather like secretory cells. The description of the epithelium of the statocyst applies also to our **Olivella** and **Oliva sayana**. All our olivids have one big spherical statolith.

8. ALIMENTARY TRACT

Olivella

The oral flaps (Fig. 30, mv) are small cutaneous projections whose inner surface is ciliated. The interior of the flaps contains muscle fibres and some loose connective tissue, a blood vessel and some lacunae. The mouth (mo) lies under the right flap. A short narrow oral tube connects this outer opening of the gut with the proboscis sheath (sz). The sheath is almost

completely turned inside out, when the proboscis is everted (Fig. 15). The introverted sheath extends dorsally farther backwards than ventrally and attains the level of the gill. The proboscis is often turned round, hence the pharynx is dorsal in Fig. 15, and the oesophageal loop may occupy any position when the proboscis is introverted.

The two lateral radular cartilages (rs), better cushions of supporting tissue (Schaffer 1913, p. 372), are separate on their entire length. Over them the cuticular radular membrane (Fig. 31, ru) passes on to the lateral walls of the pharyngeal cavity. The radula (Fig. 26, 32) consists of about 35 rows of 2.1.2 plates. Thiele (1931, p. 372) considers the 2 lateral plates as one medial, curved and pointed cusp (q) jointed with a lateral rectangular base (az). We prefer to follow Fischer (1887, p. 599) and Olsson (1956, p. 169) who speak of two lateral plates, calling Thiele's base an accessory plate. In some subgenera of **Nassa (Nassarius)** a plate without cusp is known (Simroth 1901, f. 122e on p. 476). It lies between rhachidian and lateral plate, not outside or under the latter as in **Olivella**. The middle plate is 0,12 mm. broad and bears 20-35 denticles of different size which vary individually. Among nine radulae two medial denticles were the biggest in eight cases, only once a single median denticle was strongest. Between the two medial denticles 1-3 minute ones occur.

Contrary to the rule in Stenoglossa the primary salivary, better pharyngeal, glands (ss) are short ramified tubes which come from both sides and open unusually far in front into the beginning of the pharyngeal cavity. Tubular salivary glands were found in **Marginella hyalina** (Eales 1923, p. 38). The glands of **Olivella verreauxii** are formed of big cells whose number is 4-6 in a transverse section. Their secretion is basophilous. The cells of the quite short duct are smaller, more numerous and ciliated. As in some other Stenoglossa, e. g. **Buccinum** and **Harpa,** there are no secondary (accessory) salivary glands. The mentioned snails feed in a quite different manner from our **Olivella**.

The oesophagus (o) whose foremost part is called proboscis gut (Küttler 1913, fig. L, RD), is continuous with the dorsal channel of the pharyngeal cavity. When the proboscis is retracted, the anterior oesophagus runs forwards from its inner end. This anterior oesophagus is lined with columnar ciliated cells, and contains some mucous glands and smaller ones with red staining secretion. This epithelium forms about eight rather uniform longitudinal ridges, so that the transverse section appears stellate. The three stars drawn in the ascending limb of the oesophagus in Fig. 30 show the torsion in which this part of the alimentary tract is involved by the torsion of the visceral mass (Graham 1939, p. 76 and elsewhere), though as in **Buccinum** (id. 1941, p. 12) distinct dorsal folds cannot be defined.

In some specimens the anterior oesophagus suddenly enlarges in front of the nerve ring. The epithelium of this expansion (Fig. 33) contains more numerous gland cells, deep folds are developed on the topographically ventral side, and the musculature thickens. The folded, phylogenetically dorsal side has more red staining gland cells than the smooth ventral side, but the histological difference between both sides is insignificant. This little differentiated and individually inconstant dilatation of the oesophagus corresponds to the pharynx of Leiblein (pyriform organ) known in Muricacea, Buccinacea (absent in Fasciolariidae Simroth 1901, p. 515) and **Oliva**. According to Bergh's figures (1901, t. 47, f. 2, 3) of the outer aspect of the gut an only allusive pharynx of Leiblein seems to occur in **Harpa** too.

In the oesophagus behind the nerve ring a mid-oesophagus and a posterior oesophagus (Graham 1941) cannot be distinguished, because there is no oesophageal gland (gland of Leiblein). The eight oesophageal folds of the backward course are lower than those of the anterior limb and flatten in the middle: farther backwards they increase again. The epithelium of the postneural oesophagus containing two types of gland cells is the same as in front. The absence of an oesophageal gland **Olivella verreauxii** has evidently in common with the species of **Harpa** studied by Bouvier (1887, p. 311) and Bergh (1901).

As in the higher mesogastropods (Taenioglossa) and Stenoglossa (Graham 1949, p. 755) the oesophagus opens into the topographically anterior end of the stomach, near the intestine (Fig. 34, 36). The short oesophageal region of the stomach bears longitudinal ridges which are prolongations of those running along the hind end of the oesophagus. Medially the area of these ridges is limited by a thick fold (os) which leads the food to the fundus. This large triturating area (ie) occupies about two thirds of the entire stomach. Its lining cuticle (uc) and longitudinal and annular muscle layers (mz) characterize this region as gizzard. Cuticle and annular muscles are especially thick in a broad ectal belt of the gizzard (Fig. 35). The crushed food passes along the medial side of the thick fold to the posterior sorting area (re), a small field of transverse folds which, at least in part, bear cilia. This area communicates with the common opening of the ducts (d) from the digestive gland and with the intestinal groove (ir), the latter flanked by the major (mi) and minor (mu) typhlosoles. The epithelium of the hepatic ducts is similar to that of the stomach (Küttler 1913, p. 506), not to that of the acini (ui). The epithelium of the latter consists of digestive cells and very rare lime cells (Graham 1938, p. 279).

The peri-intestinal connective tissue (Fig. 37, rc) forms a thick pad around the region of the sorting area, intestinal groove and duct of the digestive gland. It consists of indifferent cells and scattered clusters of cells with intensely red staining plasma. The same type of cells occurs as free amoebocytes in the body cavity, and some similar elements were seen in the epithelium of the gut in this region. They are evidently the wandering phagocytic cells mentioned by Morton (1953, p. 243, f. 1, 2, PH). Storage and conduction of alimentary substances by the peri-intestinal connective tissue was indicated by Haller (Simroth 1899, p. 295).

Of the stomachs examined by Graham (1949) that of **Nassa (Nassarius) reticulata** (p. 749, f. 22) is the least different from that of **Olivella verreauxii**. The great extension of the sclerosed surface of the stomach in **Olivella** is certainly secondary.

The anus lies on the topographically right side of the pallial cavity, behind the hypobranchial gland and in front of the female pore. An anal gland is not developed.

Oliva

As in the other examined species there are two vertical dextro-sinistrally compressed oral flaps. Their epidermis is ciliated on the inner surface and coated with a striped cuticle ("Bürstensaum") on the outer side This cuticle, the thicker epidermis and its more numerous glands, the stronger musculature and the innervation distinguish the flaps of **Oliva** from those of **Olivella**. The blood vessel and the lacunae are the same. Both flaps are grown together at their bases, and the mouth lies in the middle under this connection.

The following description contains principally additions to Küttler's good study (1913, p. 494-511) or mentions differences, possibly of specific character. The oral tube of **Oliva sayana** (Fig. 38) is short and wide; the proboscis sheath (sz) may be almost completely everted. The backward extension of the inverted sheath is the same on all sides. The retractors of the proboscis (vo) originate with branching bundles on the body wall and insert, ramifying again, on the anterior part of the sheath (l. c., p. 495). The wall of the sheath is in front thinner than that of the proboscis. The latter is thin below the pharynx. Sheath and proboscis wall thicken towards the base. The mucous glands of the proboscis dwindle towards the tip, and are dense in the region where the wall of the proboscis passes to that of the sheath, and where lubrification is especially needed. As in **Olivella verreauxii** also in **Oliva sayana** the everted proboscis is much shorter than the shell.

The radula (Fig. 27) is 3,3 mm. long, 0,36 mm. broad, and contains about 120 rows. The 0,15 mm. broad rhachidian plate bears two big lateral and one smaller median cusp; the lateral plates are broad at the base and pointed at the tip. The radular membrane spreads over the separte cushions of supporting tissue as in **Olivella**. Different from this fast cuticle is the

thick and soft one that coats the tip of the proboscis and covers also the high columnar epithelium of the odontophore (rotella). The dorsal food channel and the lateral walls of the pharynx are free from cuticle.

The foremost opening (oa) of a salivary (pharyngeal) gland is that of the unpaired, accessory gland which discharges into the bottom of the proboscis tube near the tip. The paired, primary glands (ss) open laterally (ov), 0,16 mm. the one and 0,46 mm. the other behind the orifice of the accessory gland. The thin duct of the latter runs backwards to the root of the proboscis along its floor and ventrally to its artery. Farther behind this duct lies between the oesophagus and the artery, flanked by the ducts of the primary glands. At the level of the pyriform organ the duct of the unpaired gland is connected with the simple acinus (as), the "coecum glandulaire impaire" (Risbec 1955, p. 50), whose length is individually different The primary glands are branched and coiled tubules; their ducts contain a thick bundle of fine threads of secretion.

The dorsal folds are already marked by the salivary ducts and continue distinct to the sides of the dorsal food channel approximately to the end of the proboscis. The ciliated columnar epithelium of the anterior oesophagus contains only very few mucous glands. Light blue staining vacuolized ovoid cells with small nuclei, possibly phagocytic cells, occur in the lumen of the anterior oesophagus and in its epithelium. The same cells were also seen between the perioesophageal muscle fibres. Millott (1937, p. 185, 187) and Fretter (1939, p. 608, 614) found wandering amoebocytes in the oesophagus of opisthobranchs.

Behind the root of the proboscis the oesophagus bends forwards and its epithelium is thrown into a number of equal folds, so that the dorsal folds are no longer recognizable. This course of the anterior oesophagus has several dilatations with more or less thinned walls. It widens suddenly (no) into the pharynx of Leiblein (pyriform organ) which is really pyriform, thick in front and thin behind. A yellow transparent spiral around its anterior surface is the mucous pad (Graham 1941,

p. 6) with a ventral interruption. The pad surrounds the base of the glossy cone which projects into the cavity of the pyriform organ and is formed by a dorsal reduplication of the anterior oesophagus. The high epithelium of the pharnx of Leiblein has basal and apical nuclei (Küttler 1913, p. 499) pertaining to unciliated columnar glands and slender ciliated cells respectively (Graham, l. c.). The dorsal folds reappear in the pyriform organ, flanking a cleft at the topographically ventral side. The latter wanders over the right to the dorsal side, attaining the median plane at the end of the pyriform organ. As in the Muricacea (Graham 1941, p. 4-5) the ventral cleft is seen from the outside.

Behind the nerve ring or in other specimens even at the hind end of the pyriform organ the short mid-oesophagus shows a whitish thickening (io) of the dorsal folds. The epithelium is so high that it restricts the oesophageal lumen to a dorsoventral slit. The cells are ciliated and glandular, as in the pyriform organ, but the glands stain pink in the latter and blue in the mid-oesophagus. The flat, not glandular, epithelium of the ventral wall is bordered by two ridges. The high epithelium enters the duct of the oesophageal gland (gland of Leiblein), where it was found in **O. peruviana** by Haller (1905, p. 660), not by Küttler (1913, p. 503). From the duct the glandular epithelium spreads far into the ramifications of the oesophageal gland (oe). The latter is similar to that of **Nucella** (Graham 1941, p. 10). It is subdivided internally into many small lobes. In each lobe the club-shaped secretory cells are in the same phase of secretion, high and almost obliterating the lumen, or medium-sized with few granules, or quite short with detached ends. These fill the centre of the lobes as spherules containing granular secretion and are also found in the posterior oesophagus on their backward course.

At the level of the posterior border of the mantle cavity the posterior oesophagus is approximately in its middel fastened to the ventral wall of the body cavity by a ring of muscle fibres (om). At this point the efferent duct passes over the

oesophagus. High pink staining glands, single blue ones, and ciliated cells with brown pigment constitute the epithelium of the posterior oesophagus, whose hindmost part shows about 16 high longitudinal folds. In the summits of the largest of these runs an artery. The oesophagus enters the stomach through a constriction a little behind the pyloric region.

The very much modified stomach of **Patella vulgata** (Graham 1949, p. 749) confirms Simroth's statement (1901, p. 529) of the discrepancy between the usual systematic arrangement and the anatomy of the stomach. This statement applies also to **Olivella** and **Oliva**. In the latter one would expect a simplified stomach in correlation with the complexity of the secretory pharyngeal and oesophageal glands (Fretter 1939, p. 640; Morton 1953, p. 244), but actually the stomach of **Oliva** is not simple. It contains even more of the landmarks known by Graham's studies (1939; 1940) than that of **Olivella**.

The stomach of **Oliva sayana** is longer than that of **O. peruviana** (Küttler 1913, fig. N), and its form varies individually (Fig. 39, 41). When it is collapsed in hungry animals, the stomach forms a long caecal extension (ce) as in **Littorina littorea** (Graham 1949, p. 747). A fold extends from the entrance of the oesophagus to the common opening of the liver ducts. The big median longitudinal fold (os) originates near the major typhlosole (mi). The fundus is longitudinally folded, and irregularities of these folds may produce small villosities. Backwards the folds flatten out if the stomach is distended by food. Dorsal to the cardia a more strongly folded field corresponds to the posterior sorting area (re). The origin of the minor typhlosole (mu) is connected with the major typhlosole. The latter borders a small cuticularized gastric shield (si). The intestinal groove (ir) begins in the stomach with a soft and smooth concavity which corresponds to a style sac (wi). In one of the opened stomachs this region contained a rod of mucus studded with faecal particles, a protostyle (Morton 1953, p. 251). There are some transverse folds in the course of the style sac. On the outside the pyloric region is surrounded by clusters of peri-intestinal connective tissue.

In the terminal part of the intestine its epithelium is rich in red staining glands, and the cilia of the high columnar cells are long. An extremely small tubular anal gland (an) 1 mm. long and 40 micra in diameter was found only in serial sections of the anal region. The gland opens beside the anus into the mantle cavity as in **Oliva peruviana,** where it is much bigger (Küttler 1913, p. 506-07). Behind the anus the mantle cavity forms a tubular prolongation.

Lintricula and **Olivancillaria**

The oral flaps agree with those of **Oliva sayana** regarding the thick, glandular epidermis, strong muscles, innervation, blood vessel and blood lacunae. The cuticle of the outer surface described in **Oliva** is not developed in **Lintricula, Olivancillaria** and **Olivella,** but as in the latter and **Oliva** the inner side is ciliated. The mouth lies under the right flap as in **Olivella.**

The alimentary tract has the same subdivisions and glands as in **Oliva.** The retractors of the proboscis are simpler, not so much branched. The posterior end of the proboscis is broader open than in **Oliva** (Fig. 40) As in the previously treated species the proboscis is much shorter than the shell.

The radula (Fig. 28) contains 136-140 rows. The rhachidian plate has a smaller central cusp and two bigger lateral ones and besides an outermost small cusp on each side. This is a simple point in **Olivancillaria** (Fig. 29) and a tricuspidate saw whose denticles diminish outwards in **Lintricula.** In the latter the inner borders of the big lateral cusps have occasionally a minute point near their base. The lateral plates of both species are similar to those of **Oliva.**

Pharynx, pharyngeal glands and oesophagus of **Lintricula** are not so tightly wrapped in connective tissue as in **Oliva.** Specially the ducts of the paired glands run free, not connected with the oesophagus, and also the duct of the unpaired gland (as) courses independently. The glandular pharynx of Leiblein (no) has the same pyriform aspect as in **Oliva.** The whitish glandular thickening (io) of the dorsal oesophageal folds be-

gins behind the nerve ring and extends into the duct of the oesophageal gland (oe), and also for a certain distance into the oesophagus behind the entry of that duct. In other specimens there is no posterior extension, but the thickening goes farther into the oesophageal gland. The latter is a tube winding around the cephalic aorta. The posterior oesophagus is fastened to the ventral body wall by a muscle ring as in **Oliva**.

The stomach is characterized by a ciliated smooth furrow which leads from the entrance of the oesophagus to the liver ducts and to the pyloric region. The liver ducts branch immediately outside the stomach. A food string was seen in the intestinal groove. A gastric shield is not developed. The intestine contains sand and blackish faecal masses. About 0,4 mm. from the anus a 0,9 mm. long and 40 micra thick anal gland opens into the rectum.

The mantle cavity is prolonged at its right posterior corner. This pouch subdivides into three finger-like tubes which border the rectum. **Oliva** and **Olivancillaria** have only one pouch. In **Oliva, Lintricula** and **Olivancillaria** a small papilla occurs beside the anus.

The alimentary tract of **Olivancillaria** is similar to that of **Lintricula**. The posterior end of the proboscis is even broader open than in that species. The radula comprises 134-136 rows. Particularities of the stomach are: the strong longitudinal fold descending from the opening of the oesophagus into the caecum is dorsal to the openings of liver and oesophagus; a rather shallow furrow runs from the caecum to the hepatic opening and a deeper one from there to the intestinal groove. Both furrows embrace the posterior sorting area. The intestinal groove begins with a broad, smooth and deepened area. This region is outwards surrounded by a belt of peri-intestinal connective tissue. As gastric shield is absent as in **Lintricula**.

9. REPRODUCTIVE ORGANS

Our species contain mature germ-cells; nevertheless animals collected in only one season cannot inform about the cy-

cle of the generative organs. Of **Olivella verreauxii** we had material from spring (November) and winter (August), and based the description on the former. As the winter material might be affected by the season, we compared the receptaculum seminis and the internal vesicle with their state in November, but did not find any differences.

Olivella, male

The testis (Fig. 42, t) begins near the apex, behind the liver and extends into the penultimate whorl. The acini of the testis shine through the tunica. The coiled testicular duct (ns) is distended with sperm and functions as vesicula seminalis (Fretter 1941, p. 175, 178, 179). A great part of the sperms is atypical and similar to the atypical spermatozoa of the conids (Bergh 1896, p. 95-97, t. 10, f. 220; Ankel 1930, textfig. 47, a). The cubical epithelium of the seminal vesicle bears scattered cilia. The following region, the short renal spermiduct, is narrow, straight and courses along the columellar muscle. The renal spermiduct is regularly ciliated. As a remnant of a gonopericardial duct (Fretter 1941, p. 175) a short strand of connective tissue passes from the spermiduct to a long and narrow diverticulum (g) of the pericardium (er); the wall of the spermiduct is not modified. The cilia become stronger and more numerous in the pallial portion of the spermiduct. This part begins with a broad, densely ciliated pouch (so) which is in open communication (sm) with the mantle cavity. The strong cilia of the pouch continue on to the floor of the pallial cavity. These cilia constitute an efferent strip which comprises the anus and ends at the outer right border of the pallial cavity. Under the beginning of this ciliated area lies the bigger of the two visceral ganglia; the smaller or accessory visceral ganglion lies farther in front and to the left. The cylindrical pallial spermiduct (sa) continues under the floor of the mantle cavity. The high epithelium of this prostatic part consists of cells with eosinophilous granular secretion and basal nuclei and slender supporting cells with distal nuclei and with cilia. The prostatic duct courses nearly straight forwards. At the level of the nerve ring it cur-

ves to the right and enters the long penis (vr), where it continues secretory nearly to the tip. In the penial spermiduct (te) the muscles are stronger than farther entally. The male opening is a little subterminal, it lies 20 micra below the tip. The penis (Fig. 43) is dorso-ventrally flattened and 7 mm. long in a snail with a 10 mm. long shell. Tucked into the mantle cavity the penis lies behind the ctenidium (k), parallel to the border of the latter and attains the posterior angle of the cavity. In the retracted penis the spermiduct is located on the left side, the nerve (vn) and the blood spaces (oc) are situated to the right. In the distal half of the penis the epidermal cells of the dorsal side are ciliated. Blue staining epidermal glands reach under the subepidermal muscles and between the latter lie numerous branches of the penial nerve.

Olivella, female

The ovary is located in the two uppermost coils behind the liver. The first part of the oviduct, the ovarian duct (ou), is lined with a low unciliated epithelium and runs beside the columellar muscle. It is very dilatable, as is shown by the voluminous eggs (Fig. 44, c) in it. The following portion, the renal oviduct (rv), is folded and ciliated. Farther ectally the gonopericardial duct (g), which begins with a sphincter (vs), leaves the renal oviduct. The gonopericardial duct is unciliated and passes to the low epithelium of the pericardium (er) without limit (cf. Krull 1935, p. 436).

The pallial oviduct differs from that of **Oliva** by the external separation between albumen (aa) and capsule gland (c), both of which located in the body whorl. Also the receptaculum seminis (rn), whose position between albumen and capsule gland is identical in both species, differs by shape of that of **Oliva**. In **Olivella** it is a ciliated duct without a terminal dilatation. Sometimes its epithelium is slightly undulated by variations in the depth of the underlying connective tissue. The sperms lie principally in its blind end, some of them also in the duct. The disposition of their heads towards the wall, the tails towards the lumen, is typical for a sperm-storing or-

gan. Ingestion of sperm by the wall cells of the receptaculum was not seen. The capsule gland has a transversely flattened lumen as that of **Oliva,** but its direction is dorso-ventral only in the middle part of the organ. In its ental and ectal parts the lumen extends from the right to the left side. This torsion of the capsule gland is not correlated with the coiling of the body. The sperm channel (Fig. 46, sr) lies entally on the right, in the middle on the ventral, and ectally on the left side. The angle lodging the sperm channel is characterized by longer cilia and lower cells, and separated from the lumen of the capsule gland not by folds as in **Oliva,** but only by stripes of unciliated low cells on both sides. These stripes are distinct from the high and ciliated epithelium which lines the capsule gland and is pierced by strands of secretion of the underlying glands disposed in rows. These secretory cells are cyanophilous in the smaller ectal section of the capsule gland and acidophilous in the larger ental part. In the middle of the latter there are two antero-posterior stripes of blue staining glands opposite to one another.

The outermost part of the capsule gland (we) has no secretory cells. This vestibule (Fretter 1941, p. 281) is wide and strongly ciliated. It is connected by a narrow passage with the likewise ciliated outer part. The latter has a posterior 0,15 mm. deep pouch (vc) with a little fewer cilia. Opposite to this pouch and farther ectally, immediately behind the female aperture, begins a long and narrow ciliated duct (uv). It runs inwards connected by its coat of connective tissue with that of the capsule gland, whose tubules sometimes enclose the duct. The duct leads to a vesicle (iv) located behind and to the left of the capsule gland. This vesicle is 0,5 mm. long and broad and 0,4 mm. high. Its wall (Fig. 45) consists of a high cylindrical unciliated epithelium with basal nuclei surrounded by a thin musculature. The vesicle appears as a brown lump through the skin of the snail after its shell is removed. The contents of the vesicle are identical with amorphous masses of faeces which form a grumose ball. Broken skeletons and small entire diatoms, as well as fragments of sponge spicules occur in this mass.

but not any sand grains, though such are frequent in the faeces. Probably the heavy sand grains ejected from the anus sink down on to the cilia which produce the efferent current. Some particles of the contents of the internal vesicle lie in vacuoles of the epithelial cells. The blind end of the receptaculum seminis is apposed to the vesicle, but the lumina do not communicate. The female aperture (eo) lies in the posterior angle of the mantle cavity behind the anus. A ciliated streak begins at the female aperture, encloses the anus and continues to the opening of the mantle cavity on the right side. It is the same as the efferent streak in the male where it leads out faeces and possibly an excess of sperm and prostatic secretion. Only in females the ciliated strip continues outside the pallial cavity extending to the ciliated right half of the dorsal furrow between pro and metapodium and to the mouth. Presumably these cilia lead the egg capsule to the neighbourhood of the ventral pedal gland.

The unciliated and the ciliated section of the oviduct are the ovarian and the renal oviduct of many gastropods. Linke (1933, p. 27) called them pseudoviduct and oviduct in **Littorina obtusata**. Often the renal oviduct is the site of fertilization (Fretter 1956, p. 377), hence the folded part of **Olivella** may serve as fertilization chamber. However sperm did not lie in this part in our sectioned females, though the ovarian duct already contains eggs near its end. The oviduco-coelomic funnel (Bourne 1908, p. 840), since Giese (1913; 1915, p. 180) called gonopericardial duct, **Olivella** has in common with the four stenoglossan species studied by Fretter (1941). The sphincter of the gonoducal side of this duct may prevent the passage of sperm into the pericardium. The homologies and functions of albumen gland, receptaculum seminis and capsule gland with Fretter's Stenoglossa and with **Oliva** need not be discussed. Also the homology of the pouch (vc) with the corresponding organ of **Oliva,** where it is smaller (Fig. 48), and the copulatory bursae of Fretter's species is obvious. In **Olivella** it might be a sperm-receiving organ, perhaps together with the vestibule.

The internal vesicle stores faeces, and these are evidently led to it from the neighbouring anus by means of the cilia in its duct. We cannot explain the biological significance of the incorporation of faecal particles by the epithelium of the vesicle. The occurrence of faecal matter in the pedal gland was mentioned in our description of this gland. We suppose the function of the vesicle of **Olivella** to be the same as that of the crystal sac (Bourne 1908) in the Neritidae. This is the storage of particles to be spread over the egg capsule (Risbec 1932, p. 361, 363, f. 3, 4) as described by Andrews (1933; 1935; 1937) and Risbec (1937; 1942, p. 24-25). Hence the vesicle of **Olivella** would be analogous with the reinforcement sac of the neritids. In this organ of **Theodoxus fluviatilis** Fretter (1946, p. 319) distinguished an anterior, ciliated conducting part, and a muscular, unciliated storing fundus.

The internal vesicle of **Olivella** and its duct are homologous with the bursa copulatrix and its canal in **Oliva** (Fig. 48) and **Lintricula** (Fig. 54). The tubular distal part in the olivids dealt with here, and the opening independent of the female pore in **Olivella** differ from the distal bursae in the Stenoglossa studied by Fretter (1941) and Johansson (1957, f. 4, 7), and in the other species mentioned in our introduction. As an exact homology of these organs cannot be established between the olivids on one side and the other examined Stenoglossa on the other, it is perhaps imprudent to extend the discussion to Haller's "Uterus-Enddrüse" (see Simroth 1904, p. 615) or to that of **Bithynia tentaculata** (Ankel 1924, p. 4), which Krull (1935, p. 448) homologized with the crystal sac. Bourne (1911, p. 804-805) warned against phylogenetic combinations based on similarities of reproductive appendages in gastropods, whose other organs, e. g. nerves and radulae, are widely different. Also Johansson (1947, p. 107) searches for homologies of accessory genital organs only in systematically related families.

Oliva, male

The testis (Fig. 47, t) which begins apically and extends into the penultimate whorl contains typical and atypical sperms

mingled. The latter are similar to those of **Olivella**. The testicular duct is a coiled vesicula seminalis (ns) as in **Olivella**. This part is muscular and its unciliated epithelium encloses brownish yellow granules as the kidney and other organs of the species. Unrolled the testicular duct is 12-15 cm. long; it contains the typical sperms mostly with their heads nearer to the wall and the atypical ones in the middle. The outer portion of the seminal vesicle is near to the pericardium (er), and the straight and short renal spermiduct goes on in the same position. A remnant of a gonopericardial duct is developed in the form of a pericardial diverticulum (g) which ends blindly at the renal spermiduct.

The renal spermiduct passes into the pallial spermiduct which begins with a pouch-like dilatation (so). The broad anterior opening (sm) of this pouch communicates with the mantle cavity under the broad aperture of the kidney. As in **Olivella** this communication does not show vestiges of its origin from an open groove. Farther forwards the closed prostatic pallial spermiduct (sa) meanders under the floor of the mantle cavity. Its musculature is much thicker than in **Olivella**. Its cylindrical epithelium with basal nuclei and evidently unciliated, stores pink staining granular secretion produced by glands which lie outside the muscles. The dorso-ventrally flattened penis (vr) is similar to that of **O. peruviana** (Haller 1905, textfig. 3 B; Küttler 1913, fig. D, on p. 512), though the winding spermiduct of **O. sayana** is located somewhat to the left side. The duct is lined by an epithelium storing pink granules. Blue staining glands send their strands of secretion through the very strong musculature. The number of these glands increases outwards, where the very high epithelium stores blue and pink secretion. The penial nerve lies to the right as in **Olivella**; blood lacunae are developed on both sides of the spermiduct. The outermost part of the duct is ciliated; its opening is terminal, contrary to **O. peruviana,** whose opening lies on a subterminal papilla (Küttler 1913, p. 511). The cutaneous glands of the penis end 1,5 mm. from the tip; the entire skin of the penis is unciliated; its muscularis is strong.

Oliva, female

The ovary lies as in **Olivella**. As there and in many other prosobranchs (Linke 1933, p. 28) the structure of the ental or ovarian duct (Fig. 48, ou) resembles that of the gonad. The ectal portion (rv) which corresponds to the post-torsionally right kidney (l. c., t. 8, f. 81) has a vacuolized epithelium similar to that of the kidney. Cilia appear only where the renal oviduct enters the albumen gland (aa). The gonopericardial duct (g) is unciliated. Albumen and capsule glands (c) stain differently in the sections, but are not separated externally The receptaculum seminis (rn) consists of a cluster formed by about four vesicles with short stalks surrounded by a common coat of connective tissue (Fig. 49). Each vesicle has its own musculature. The epithelium is low and ciliated. The heads of the numerous sperms are attached to the wall. Incorporation of sperm by the epithelium was not seen. The common orifice of the ducts is located in the dorsal angle of the pallial oviduct, at the limit between albumen and capsule gland. The latter has the same compressed transverse section as in **Olivella** and Fretter's Stenoglossa (1941, f. 5), and the same ciliated epithelium pierced by the strands of secretion of the underlying gland-tubules. Küttler's section of the "oviducal gland" of **O. peruviana** (1913, f. J, on p. 513) shows essentially the same structure. Glandular tubules occur in the albumen and the capsule gland; the ventral gutter or sperm channel runs only along the capsule gland. There it is set off from the lumen as a T-shaped fold on the side opposite to the orifice of the receptaculum. Some single sperms were seen in the ventral channel, whose position is, contrary to **Olivella,** the same along the whole capsule gland. The cilia become scarce towards the outlet of the glandular oviduct, where also the glands end. This part, the vestibule, forms a shallow backward pouch containing unorientated sperm. The narrow canal between the vestibule and the mantle cavity bears strong cilia.

Opposite to the pouch a ciliated canal (b) goes out from the vestibule. This duct is enveloped in a thick mantle of an-

nular and longitudinal muscles and projects into the lumen of a vesicle (cc). This opening lies on the left side of the vesicle and far inwards, not at the bottom. The ample vesicle is located in the thickness of the posterior wall of the mantle cavity, the rectum being dorsal to and a little in front of it. The wall of the pallial cavity beside the female aperture is bulged outwards by the vesicle. Its epithelium is unciliated; in the region of the entrance of the canal it is underlain by vesicular connective tissue. The cells that line the vesicle are secretory with distal vacuoles and basal nuclei. Some glands are depressed and reach beyond the musculature. The latter is evidently stretched by the massy contents of the vesicle, which consist of sperm and eosinophilous secretion. Sperms were not seen within the epithelial cells.

According to the position of the sperms (Johansson 1939, p. 336-337) the sperm-storing organ of **O. sayana** and **Olivella verreauxii** was called a receptaculum seminis. Fretter (1941, p. 206) said that the ingesting gland of her four stenoglossan species is homologous with a receptaculum. Later on (1951, p. 575-576) she found signs of sperm digestion in the double receptaculum of **Cerithiopsis tubercularis**. Johansson (1957, p. 89-90) ponders the possibility that the ingesting gland of the Muricacea and Buccinacea is a proximal bursa, and that these Stenoglossa have no receptaculum. But Fretter's view is supported by **Olivella** and **Oliva,** whose receptacula lie exactly as the ingesting gland of Fretter's species. The here following **Lintricula** shows the morphological identity of receptaculum seminis and ingesting gland clearly.

Unoriented spermatozoa and secretion, in part evidently produced by the prostatic pallial spermiduct, characterize the voluminous vesicle of **O. sayana** as bursa copulatrix in the sense of a sperm-receiving organ. We suppose that the penis is inserted into the long and muscular bursal canal which functions as a vagina. The vestibular pouch of **O. sayana,** though homologous with that of **Olivella verreauxii** and the distal copulatory bursae of the Muricacea and Buccinacea (Fretter 1941), is much too small to receive the voluminous penis. The sperms found

in this pouch may have come from the passage of sperms out of the bursal canal to the ventral channel of the capsule gland. The function of the vestibular pouch of **O. sayana** is not known; possibly the egg capsule lies here before it is transferred to the pedal gland. The bursa copulatrix of **O. sayana** is analogous with the distal bursae of the Muricacea and Buccinacea. The long bursal canal might be correlated with the narrowness of the mantle cavity in **Oliva** (cf. Johansson 1953, p. 2).

The shape of the receptaculum seminis of **O. sayana** differs from that of **O. peruviana** (Küttler 1913, p. 512). Also two species of **Trivia** have different receptacula (Fretter 1946, p. 328). We do, however, not think that the receptaculum of **peruviana** really opens into the renal oviduct, as must be inferred from Küttler's description (1913, p. 512 514, fig. E). The "uterus" and the "uterus gland" of **O. peruviana** correspond topographically to the bursal canal and the bursal vesicle of **O. sayana**. "Uterus" and bursal canal also agree histologically. In Küttler's diagram (fig. E., on p. 513) the diameter of the bursal canal (ut) differs from the much narrower of our species. This might be a specific difference, as such are known for the female organs of many species (cf. Johansson 1939, p. 334. 386). The much smaller bursal vesicle of **O. peruviana** (Küttler 1913, p. 513: fig. E, ut. dr.) compared with our **sayana** seems to be functionally conditioned. The secretory activity of the epithelium (l. c., fig. H) is much less intense than in our material, and no sperm was seen in the bursal vesicle of **peruviana**. Our females were evidently preserved a short time after copulation and have the bursal vesicle mightily distended. As in the Hydrobiidae (Krull 1935, p. 439) the secretion in the bursa is on its height in the period of reproduction also in **Oliva**. Secretory bursae are known in many mesogastropds (e. g. Fretter 1956, p. 377; Johansson 1953, p. 2) and the neritid **Theodoxus fluviatilis** (Fretter 1946, p. 319). In the stenoglossan **Nucella** (Abbott 1955: **Thais) lapillus,** whose receptaculum acts as ingesting gland, the bursa copulatrix seems to combine the functions of a sperm-receiving and sperm-storing organ (Fretter 1941, f. 6 d).

Haller (1905, t. 27, f. 6) did not see the receptaculum of **O. peruviana** and observed the bursa only in part. As be found the structure of the "uterus gland" consistent with the "uterus" (capsule gland) of other prosobranchs, he evidently had sectioned the bursal vesicle, not the bursal canal. This provided, the bursal vesicle of **O. peruviana** seems in certain stages to assume the same position as in our **O. sayana**.

Lintricula, male

The testis (Fig. 51, t) extends from the apex to the end of the body whorl. Atypical sperms were not seen, neither in the testis, seminal vesicle and male duct nor in the bursa copulatrix and in the receptaculum seminis. The coiled testicular duct (ns) functions as vesicula seminalis; it is full of sperm. The ciliated renal spermiduct is straight and approached to the pericardium whose blind ending diverticulum (g) attains the ectal end of the renal spermiduct as in **Oliva sayana**.

The pallial spermiduct (sa) is muscular and densely ciliated. As in the preceding olivids it begins with a 3 mm. long, dilated pouch (so) which communicates with the mantle cavity. It receives the renal spermiduct 0,7 mm. to the right of its proximal end. The pouch is thin-walled and has few intra-epithelial red staining glands. Its cilia extend on to the floor of the pallial cavity forming a folded area underlain by a big visceral glanglion. Acidophilous prostatic glands occur also in the epithelium of the next, tubular part of the pallial spermiduct, but become scarce farther in front and are no longer present in the part of the pallial spermiduct that passes to the penial spermiduct (Fig. 52). Both the pallial and the penial spermiduct (Fig. 53) show a suture consisting of two joined strips of epithelium which lead from the lumen of the duct to the epithelium of the mantle cavity and to that of the penis respectively. The same feature was observed in the two Muricacea studied by Fretter (1941, p. 177-178).

The course of the pallial spermiduct is straight and continues straight through the penis. The outer opening lies subterminally. The penis is dorso-ventrally flattened as in the other

examined olivids, but contrary to these it ends with a blunt, not pointed, tip. In a young male (shell: 17 mm.) the penis is about 3 mm. long, in a little longer snail (shell: 19 mm.) it is 12 mm. long, and in an adult male (shell: 45 mm.) its length is 20 mm. The penial epidermis bears small cilia and blue staining glands near the tip. Subepithelial glands are not developed in the penis. The muscular spermiduct without glands runs on the left side; there are blood lacunae to the right and left of the duct, while the nerves are principally on the right side.

The ancestral type of gonoducts, the open groove (Moore 1899, p. 160-166; Johansson 1953. p. 15-17), constitutes the historical basis for all kinds of spermiducal pores (id. 1939, p. 384). As these occur in Muricacea, Buccinacea (Fretter 1941) and the here examined **Olividae,** and even open spermiducal grooves are known in **Adelomelon ancilla** (Woodward 1900, p. 118), **Harpovoluta charcoti** (Eales 1923, p. 34), **Harpa ventricosa** and **H. nablium** (Bergh 1901, p. 618, 623), one must state that this primitive feature is frequent among the highly specialized (Fretter 1941, p. 208) Stenoglossa.

The vestigial male gonopericardial duct in the present olivids is similar to that in **Ocenebra erinacea** (Fretter 1941, p. 175); one of Fretter's buccinacean species (p. 178) has only a slight trace of such a duct. Fretter (p. 175, 206) mentioned **Littorina** in this connection, but Linke (1933, p. 14, 3rd paragraph) stated that there are no remnants of a male gonopericardial duct in his three examined species of **Littorina.**

Lintricula, female

The ovary (Fig. 54, zo) extends from the apex almost to the end of the body whorl. The grown ovocytes in the ovary contain up to 30 micra big yolk granules. The ovarian oviduct (ou) runs along the columellar muscle as in the other olivids dealt with here. Its wall is thick, muscular and provided with a high unciliated epithelium. Also the following narrower renal oviduct (ro) has a high (60 micra) unciliated epithelium whose vacuolized cells resemble those of the kidney as in **Oliva.** The renal oviduct communicates (g) with the pericar-

dium (re) near its ectal end. The gonopericardial duct is wide; its outer section is lined with the kidney-like epithelium of the renal oviduct. Its inner section is continuous with the low pericardial epithelium. A sphincter is not developed, neither at the pericardial nor at the oviducal end of the gonopericardial duct. The angle between it and the oviduct makes the passage of eggs into the pericardium improbable, but the lumen of the communication would not hinder it. The terminal section between the entrance of the gonopericardial duct and the beginning of the albumen gland has thick circular muscles. The epithelium is thrown into longitudinal folds and about 40 micra high with short (4 micra) cilia.

In the albumen gland (aa) the height of the epithelial cells is 25 micra, the length of the cilia 10 micra. The epithelium is underlain by 0,4 mm. long clusters of glands staining light violet and dark blue. The slit-like lumen of the albumen gland is straight from the inner to the outer end and is continued into the likewise broad lumen of the capsule gland (c). At the junction of the two glands a cylindrical muscular duct (w) originates and runs along the furrow between albumen and capsule glands. It enters an ingesting gland (rn) located dorsally over the glands of the pallial oviduct. The duct of the ingesting gland contains sperms attached to the wall with their heads and functions as recepetaculum seminis as in the Stenoglossa studied by Fretter.

Also the ingesting gland (Fig. 55) is very similar. It is a brown organ composed of ramified tubules with many amoebocytes and loose connective tissue between them. In a young female the epithelium contains only red granular secretion. In the adult female the 80-100 micra high cells lodge brown masses near their bases. Their free edges engulf tufts of sperm and are detached from the cell bodies by constriction together with their contents. Probably part of the sperm is actually digested and transformed into the brown granular masses. Amoebocytes enter the basal portions of the ingesting cells. As in two of Fretter's species (1941, p. 189, 192), also in **Lintricula** yolk granules evidently proceeding from disintegrated eggs occur in

the ingesting gland. Dorsally tubules of the right renal lobe enter the connective tissue of the ingesting gland.

The capsule gland (c) is a massive organ, hard to be sectioned, which forms 3-4 folded lobes. These stain differently in their various parts. Specially the glands of the dorsal and those of the ventral wall have secretion of different colourability, and in between a third type occurs (Fretter 1941, p. 184). A broad gland-free ventral zone of the capsule gland might correspond to the sperm channel but is not limited by folds. The location of the receptaculum seminis and the bursa copulatrix makes it improbable that the spermatozoa of **Lintricula** pass through the capsule gland.

At the junction of albumen and capsule glands a 0,4 mm. wide muscular ciliated canal (b) goes to the ventral side. This is the bursal canal which enlarges into the copulatory bursa (cc), a curved vesicle situated under the antero-ventral wall of the capsule gland. For the intromission of the penis the bursal canal must be somewhat dislocated, or it receives only the tip of the penis, and the sperms move to the vesicle assisted by the beat of the cilia of the duct. The 0,12-0,14 mm. high epithelium of the bursa is ciliated. The spermatozoa lie unorientated in the bursa, frequently in groups which appear to have originated from one spermatogonium each. The size of these groups corresponds to the portions incorporated by the epithelial cells of the ingesting gland. There is no prostatic secretion in the bursa in accordance with the scarcity of such secretion in the spermiduct.

A short vagina unites the mantle cavity with the point where the glands of the pallial oviduct meet and where the ducts of bursa and ingesting gland begin. Histologically the vagina belongs to the mantle cavity; it forms a pouch which corresponds to a vestibule. The female aperture (eo) lies level with the anus, but far to the left of the mouth of the pallial cavity, in the same position as the opening of the pallial spermiduct. Hence its location differs considerably from that of other Stenoglossa and resembles that of Johansson's hypothetical stage 5 (1942, p. 9) with the difference that there is no

anterior opening in **Lintricula.** The egg capsules will probably be directed through the mantle cavity by the exhalant pallial current as are sperm and ova in archaeogastropods (Fretter 1946, p. 334). But as a complete pallial oviduct is developed in **Lintricula,** the posterior position of the female aperture cannot be considered as an ancient character. It is the consequence of a rotation of the capsule gland which entrains the bursa.

The homology of the ingesting gland with the receptaculum seminis of the mesogastropods (Fretter 1941, p. 206) and the other olivids dealt with here is, in our opinion, certain. In **Lintricula** part of the organ, viz. its ectal duct (w), is a receptaculum, and only the ental tubules (rn) have acquired a new function. To Fretter's examples (p. 204) of sperm digestion in other animals than molluscs Cernosvitov's four papers may be added (Zool. Jahrb. Anat. v. 52, 54, 55).

Olivancillaria, female

O. brasiliensis shows some differences from **L. auricularia** in the female reproductive system. The terminal part of the renal oviduct between the entrance of the gonopericardial duct and the beginning of the albumen gland is only 0,15 mm. long against about 0,5 mm. in **Lintricula.** Its epithelium is 15 micra high with 5 micra long cilia. The duct of the ingesting gland runs between the complexes of the albumen and capsule glands, not in the outer furrow between them. In the sectioned specimen the duct does not lodge any sperm; it is very narrow, 50 × 250 micra in transverse section against 400 × 600 micra in **Lintricula.**

The spermatozoa in the lumen of the ingesting gland (Fig. 50) lie singly, not in bundles as in the copulatory bursa. Their blue heads are distinct; the pink tails disintegrate. Between the sperm detached apices of epithelial cells are plentiful, most of them containing many heads of sperms. The epithelial cells of the ingesting gland are up to 60 micra high. Their vacuoles which contain several to 20 sperms occupy the distal half of the cells. Within the vacuoles the heads are generally arranged in bundles. Basally to these vacuoles there are empty vacuoles

and such with brown contents, farther basally lie the nuclei, and between them and the basement membrane the sockets of the cells which consist of dense homogeneous plasm. In this zone nuclei of interepithelial amoebocytes are conspicuous.

Two kinds of amoebocytes are distinguishable, smaller, 8 micra big ones with pink cytoplasma and larger ones, 12 micra in diameter, containing dark brown granules. Both types occur in the connective tissue of ingesting gland and kidney; evidently the bigger ones also enter the lumen of the ingesting gland.

The bursal epithelium is much lower (70 micra) than in **Lintricula,** whilst the epithelium of the bursal canal (0,1 mm.) and its diameter are the same in both species. An area of subepidermal blue staining glands is located to the left of the female aperture both in **Olivancillaria** and **Lintricula**. In the former its diameter is twice (1 mm.) that of the latter (0,5 mm.), and the glands are 0,3 mm. long against 30 micra in **Lintricula**. The heads of the sperms of **Olivancillaria** are 12 micra long and half as thick as the 6 micra long ones of **Lintricula**.

In the studied female of **Olivancillaria** a functioning receptaculum seminis is wanting. The outer opening of the bursal canal immediately opposite to the junction of albumen and capsule glands enables the sperm to fertilize the ova when they pass from the albumen to the capsule gland. Hence storage of sperm in a proximal oviducal appendage is unnecessary.

10. RENAL ORGAN

Perrier's description of the kidney of **Oliva** (1889, p. 246-49) quoted by Simroth (1902, p. 569-70) is better than Haller's (1905, p. 661) and Küttler's (1913, p. 53-54) whose material was not sufficiently preserved. In our olivids the excretory organ is a dorso-ventrally flattened, simple not lobed, sac which tapers towards the left and lies at the posterior end of the mantle cavity. The anterior part of the kidney belongs to the roof of the pallial cavity and bears the slit-like renal aperture (x) on its ventral wall. Beside and to the left of this external opening of the kidney is localized the reno-pericardial aperture (y) which is smaller than the external opening. The posterior extra-

-pallial part of the kidney is situated in the hind border of the body whorl. On the right side the renal organ lies between the integument and the pallial oviducal glands, on the left side between digestive gland and pericardium (er). The kidney of **Lintricula** extends farther over the pallial complex of the oviduct than that of **Olivancillaria** where both organs are separated by an interspace filled with spongy tissue. In the tapering fundus of the renal sac a dorsal stripe of the wall side by side with the pericardium is thickened; this is the nephridial gland (vv).

Two different structures of the renal organ can be distinguished, folds (Fig. 56, zc) and villosities (zn). In the olivids these structures do not characterize a right and a left renal lobe. This was already stated by Perrier (1889, p. 247), and we will not apply these terms. In comparison with the Naticidae, Cypraeidae and others with a bilobed kidney the folds of the Olividae correspond to the right and the villosities to the left lobe. The folds occur chiefly on the bottom of the renal sac, and the villosities only on the roof. The circulatory system shows that the folds are analogous to the principal renal system of Perrier's Pycnonephridia (Simroth 1902, p. 571-572; Cuénot 1914, p. 279-80), and the villosities to the accessory system. The nephridial gland (Fig. 60) is similar to the villous part (Fig. 57) and can hardly be separated from it in sections of **Olivella**. Also in young specimens of **Lintricula** both organs are alike. The folded part at the bottom covers the rectum (i) and its accompanying blood sinus. In **Oliva** the folded part is brown and the villous part white. **Lintricula** and **Olivancillaria** show the same difference less distinctly. In **Olivella** both parts are white.

The folds extend from the bottom to the roof in the right major part of the renal sac (Fig. 58). Their epithelium consists of acidophilous cells 3-5 times as high as their nuclei (Fig. 59). Most of these cells bear a striped cuticle ("plateau strié" Cuénot, l. c.; "Bürstensaum"), some of them are ciliated. Scattered blue staining gland cells lie in the epithelium of the folds in **Lintricula** and **Olivancillaria**. The cells on the combs

of the folds in **Olivancillaria** detach their apices (Fig. 62) as the merocrine cells of the ingesting gland (cf. also Ankel 1936, p. 114). The connective tissue of the folds is most developed in **Oliva** where it contains much brown pigment. The connective tissue of **Lintricula** is less developed, and in **Olivella** it is scarce. Therefore the branching of the epithelial tubes is most clearly visible in Olivella (Fig. 56) where the ramification is less complicated than in our bigger olivids.

In **Oliva** the whole bottom of the left minor part of the kidney contains folds, but here they do not extend to the roof. On the latter the villosities are developed along the vessels. In front the villosities pass on to the nephridial gland. Between this and the folds there is a smooth stripe, the roof of the pallial cavity, which contains the reno-pericardial (y) and the outer renal (x) apertures. The posterior wall of the renal sac between folds and villosities is also smooth and apposed to the digestive gland. In **Lintricula** (Fig. 61) this smooth area is more extense, because the folds occupy only two thirds of the bottom. The epithelium of the villosities is quite low (Fig. 57), an the nuclei bulge the cells. Small blue staining glands lie between the epithelial cells. The richly developed connective tissue with amoebocytes and blood lacunae contains acidophilous protein crystalloids (Cuénot 1914, p. 281), plentiful in adults and few in young snails.

The nephridial gland (vv), as we summarily call nephridial and blood glands (Ankel 1936, p. 116) together, has the structure known of **Buccinum** (Dakin 1912, p. 87) and other prosobranchs. It consists of ramified epithelial tubes, outgrowths of the renal wall, which penetrate into a spongy connective tissue and are fastened by muscles. The epithelium is low as that of the villosities and ciliated; the connective tissue contains many blood lacunae and amoebocytes.

The following description of the circulation in the kidney refers mainly to **Olivella** (Fig. 56), sections of which comprise the entire topography. The strongly muscular afferent renal vessel (uz) emerges from the abdominal blood sinus (oc), gives off vessels to the folds at the bottom of the kidney and

continues through the renal cavity (h) to the roof. Its ascending course is contained in the innermost of the folds which extend to the roof. Here the afferent vessel ramifies and supplies the villosities (zn) which are disposed along its branches. The blood from the folds is collected in peripheral coalescing lacunae which communicate with the afferent branchial sinus. The blood from the villosities passes into the nephridial gland, and on the right side the sinus of the latter opens into the auricle (au).

Haller's pericardial gland of **Oliva** (1905, t. 27, f. 7) is, as already Küttler had observed (1913, p. 534), the gonadial duct visible through the pericardium.

11. SYSTEMATIC DISCUSSION

Authoritative malacologists agree to derive the Stenoglossa from the taenioglossan Doliacea (Thiele 1935, p. 1095; Graham 1941, p. 15-16; Risbec 1955, p. 71), perhaps from the Cassididae (Graham, p. 17). Two stenoglossan superfamilies, the Muricacea and the Buccinacea, are separated by differences in radula, central nervous system, foot and anterior gut (Thiele 1931, p. 287, 301; Graham 1941). Graham observed that in the Muricacea the effect of torsion is shown anterior to the nerve ring, whilst in the Buccinacea it is the posterior half of the mid-oesophagus in which the rotation occurs. Hence the two superfamilies represent parallel lines of evolution and cannot be derived one from the other (l. c., p. 17).

In Thiele's system the rest of the Stenoglossa constitutes the superfamily Volutacea. It is indistinctly characterized, because its most important character, the lack of lateral radular plates, develops gradually by thinning (Harpidae) and disappearing in most, not all, genera of the family Volutidae. This process is not correlated with the concentration of the central nervous system. The latter is most advanced in the Olividae with the radular formula 1-2.1.1-2, and in the Marginellidae with 0.1.0. In the present state of knowledge the Volutacea are distinguished from the two other stenoglossan su-

perfamilies only by their columellar folds, and even these are sometimes missing.

One can understand that Olsson (1956, p. 168) tries to dissolve the Volutacea establishing an own superfamily, Olivacea, for the Olividae, due to the sharp separation of the propodium. The three "stirpes" of the mesogastropods in Thiele with a single family each (Valvatidae, Naticidae, Cypraeidae) have more significant and more numerous disjunctive characters than the Olivacea as defined by Olsson. It is possible with Bergh (1901, p. 610) to join the Harpidae with the Olividae in one and the same superfamily with the diagnosis: Foot large, divided into propodium and metapodium by lateral incisions or a complete transverse furrow. Evolutionally the paleocene harpids can be traced from the upper cretacean olivids, provided the general stenoglossan trend to reduce the lateral plates of the radula and suppress the accessory pharyngeal and oesophageal glands. This suppression occurs also within the olivids (**Olivella**).

Thiele (1935, p. 1095) derived the Olividae from the Muricidae due to radular plates, accessory salivary glands, and anal gland. The last character however does not weigh phylogenetically, as Thiele (p. 1051) said. Anal glands occur sporadically among meso and neogastropods (Simroth 1901-02, p. 535, 548). It is improbable that they are remnants of a right kidney (ibid., p. 590) though Fretter (1946a, p. 128-130) proved their excretory function in some muricaceans. **Olivella** lacks this gland, and in **Oliva sayana** and **Lintricula** it is so small that it could be verified only in sections. Secondary salivary glands, paired with unpaired duct, or unpaired on their whole length, are certainly homologous organs. Risbec (1955, p. 50) goes too far back to the remote taenioglossan Naticidae, when he compares the precerebral part of their oesophageal gland (1956, p. 25-26) with the unpaired pharyngeal gland of the Olividae. The radular characters of the olivids are, on the whole, more muricidan than buccinacean, but some comments are necessary. The first refers to the accessory plate of **Olivella** whose parallel is found among the Buccinacea

(Nassidae). Also the peculiar rhachidian plate of **Olivella** with its great number of denticles occurs in several Buccinacea (Thiele 1935, p. 1044).

Finally the radula of **Pseudoliva** is typically buccinacean (cf. Thiele 1931, figs. 343, 383). It is significant that this relatively old genus (Moore, Lalicker, Fischer 1952, p. 318) is either classified among the Buccinacea (Olsson 1956, p. 169) or among the Olividae (Thiele). Also its foot is remarkable by its primordial propodium (Thiele 1935, p. 1095). The mighty foot of the Olividae (and Harpidae) is a buccinacean not a muricacean feature. The same applies to the concentrated central nervous system of the olivids. Muricids with concentrated ganglia occur (Simroth 1899, p. 416), but they are exceptional. Also among the Volutidae different nervous systems are found in one and the same subfamily (**Harpovoluta charcoti** Eales 1923, fig. 37; **Voluta musica** Pace 1902, t. 2, f. 3) and even within one genus (**Cymbium**: Thiele 1935, p. 1096). Apart from these cases which prove that the position of the supra-intestinal ganglion, contiguous with the right pleural ganglion or close to the osphradium, should not be over-rated, the high concentration of the nerve ring of the Olividae is rather buccinacean than muricacean. The anterior lobes of the pedal ganglia in nassids (Risbec 1952, p. 494) which are nearly set off as accessory ganglia resemble the propodial ganglia of olivids.

The remnants of a male gonopericardial duct in the here examined olivids are muricacean features. The same applies to the pallial spermiduct of **Lintricula** with the remainder of its origin from an open groove. On the other hand this duct is a closed canal without any suture in **Olivella** and **Oliva,** hence their male ducts are buccinacean.

Our discussion suggests an origin of the Olividae neither directly from the Muricacea nor from the Buccinacea, but from the common root of these. Most of the other volutacean families are too little known to settle their position. The Harpidae and the Marginellidae may descend from the Olividae, the first as an on the whole reduced group, the second as an advanced one. The Volutidae combine rather advanced radular charac-

ters with relatively primitive nervous ones. Possibly they stand at the root of the Volutacea, together with the Olividae, forming an own line of evolution.

In a discussion of the mutual relations between the here studied olivids the various organs must be considered. **Lintricula** and **Olivancillaria,** for example, are the most perfect diggers due to their enormous feet. Evidently advanced regarding this locomotor organ **Lintricula** has central ganglia less fused than **Oliva,** and its male duct with a suture extending from the beginning of the pallial spermiduct to the penis is the most ancestral of the forms examined here. **Oliva** and **Lintricula** have obvious muricacean features in their fore gut, viz. primary and secondary pharyngeal glands; a pyriform organ showing the effect of torsion; a mid-oesophagus with convolutions of the dorsal folds which enter the oesophageal gland and its lobes; and these lobes having a highly secretory surface. The fore gut of **Olivella** is an extreme case of reduction characteristic of the Volutacea (Graham 1941, p. 17). Correlated with this reduction, these engulfers of voluminous entire prey have distensible neuroglia fibres in their central nervous system, and a gizzard-like stomach. All these features are specializations of the relatively young (Olsson 1956, p 165) genus **Olivella,** and its systematic separation from the Olivinae (ibid., p. 169) appears justified. Contrary to the mentioned specializations several species of **Olivella,** including the present, maintain the ancestral character of an operculum. **Oliva** appears primitive in the possession of tentacles with eyes, but its central nervous system attains the highest degree of concentration among Stenoglossa. Maybe that the hitherto anatomically unknown olivids will connect these loose and dissimilar facts in future and permit a phylogenetical arrangement of the genera which appears impossible at present.

12. SUMMARY

3. The olivids dealt with here live in sand. **Olivella verreauxii** engulfs its prey entire, chiefly **Donax hanleyanus**. **Oliva sayana** swallows particles rasped by the radula. **Lintricula**

auricularia feeds in the same way, grasping and securing its prey, chiefly **Donax,** with the foot.

4-5. The mentioned species and **Olivancillaria brasiliensis** have a posterior mantle tentacle which lies in the channeled suture and a posterior mantle lobe which produces the callus and is specially strong in **Olivancillaria brasiliensis**. An anterior mantle tentacle occurs in **Olivella** and **Oliva,** and paired tentacles with eyes in **Oliva**. The resorption of the inner walls of the upper whorls known of **Olivella** is evident also in **Oliva, Lintricula** and **Olivancillaria**. The borders of the siphon of the two latter are beset with branched papillae which bear sensory cells. Their big foot does not enter into the shell completely. Also the posterior mantle tentacle of **Olivella** frequently remains outside the shell, though **O. verreauxii** is operculate. The various mantle appendages are similar to one another and alike in the examined species.

6. Glands and clusters of sensory cells lie under the epidermis of the anterior border of the propodium in the here studied species. The sensory cells are connected with a propodial nerve net. The posterior pedal gland associated with the transverse ventral furrow between pro and metapodium is concentrated in **Olivella** and accompanies the furrow in **Oliva** and **Lintricula**. The ventral pedal gland of the females is independent in **Olivella** and **Oliva,** whilst it is only a differentiated part of a glandular metapodial groove developed in both sexes of **Lintricula**.

7. The central nervous system of **Olivella** shows distinct separation of the ganglia, even short pedal-propodial connectives are developed Neuroglia fibres accompany all central connections. They make it possible that together with the oesophagus the nerve ring can be distended when the snail swallows entire bivalves. The ganglia of **Oliva** are only separated by superficial constrictions; their nerve cells touch over the quite short connectives. This rigid collar allows only for feeding on rasped particles, small animals, or juice of meat. The nerve collar in **Lintricula** is more concentrated than in **Olivella**

and less than in **Oliva**. Neuroglia fibres are specially developed between the secondary propodial ganglia. The extensile and contractile propodium is the most richly innervated sense organ of the present olivids, also in **Oliva** whose oral flaps bear tentacles with well developed eyes.

8. **Olivella** has only primary pharyngeal glands, no pyriform organ nor oesophageal gland. A great part of its stomach is cuticularized and acts as gizzard. The fore gut in **Oliva** and **Lintricula** is similar to that of the Muricacea (Graham 1941). The stomach of **Oliva** is highly differentiated; that of **Lintricula** and **Olivancillaria** is similar but without gastric shield. The anal glands of **Oliva** and **Lintricula** could be seen only in sections; that of **Oliva** opens beside the anus, that of **Lintricula** into the rectum. **Olivella** has no anal gland.

9. Atypical sperms of the **Conus** type occur in **Olivella** and **Oliva**. **Lintricula** has no atypical sperms. The examined olivids have remains of a male gonopericardial duct in form of a diverticulum of the pericardium ending blindly at the renal spermiduct, and in all the pallial spermiduct communicates with the mantle cavity. The origin of this duct from an open groove is indicated by its suture in **Lintricula**. In the latter few prostatic glands exist only in the epithelium of the pallial spermiduct. A female gonopericardial duct is present in all species. As in the Muricacea and Buccinacea (Fretter 1941) there is a proximal appendage of the pallial oviduct. It functions as receptaculum seminis in **Olivella** and **Oliva** and as ingesting gland in **Olivancillaria brasiliensis** and **Lintricula auricularia**. In the last species its duct stores orientated sperm. Voluminous copulatory bursae with long ducts are developed in **Oliva, Lintricula** and **Olivancillaria**. A vestibular pouch functions as bursa copulatrix in **Olivella**. The vesicular organ (internal vesicle) with long duct of **Olivella** stores faecal particles; it probably functions like the crystal or reinforcement sac of the Neritidae. Capsule gland and bursa of **Lintricula** and **Olivancillaria** are rotated inwards so that the female aperture lies at the same place where in the male the pallial spermiduct opens into the mantle cavity.

10. The simple, not lobed, renal organ comprises two structures: folds and villosities. The first correspond to the right renal lobe, the second to the left, or to the principal and accessory systems respectively. Merocrine excretion by detached apices of the epithelial cells was observed in the folds of **Olivancillaria**. Blood from the abdominal sinus enters folds and villosities. From the first it passes to the branchial sinus, from the latter through the nephridial gland to the auricle.

11. Muricacea and Buccinacea are parallel lines of evolution (Graham 1941). The Volutacea are a loosely united superfamily. Olsson (1956) dissolved it and established a superfamily Olivacea with one family Olividae. The Harpidae can be included in the same superfamily. The Olividae have several muricacean and some buccinacean characters, hence they cannot be derived from one of these lines, but evidently descend from its common root. Of the genera treated here **Oliva** has the most concentrated, central nervous system, that of **Olivella** is the least concentrated, and that of **Lintricula** is intermediate. Also the operculum of the studied **Olivella** is a primitive character. The alimentary tract of **Olivella** is the most advanced by reduction (oesophagus) and specialization (stomach). **Lintricula** whose foot is most highly developed has the most ancestral pallial and penial spermiduct. With Olsson (1956) we consider **Oliva** and **Lintricula** nearer related with one another than one of them with **Olivella,** the geologically youngest genus.

13. SUMÁRIO

3. As Olividae aqui estudadas vivem na areia. **Olivella verreauxii** engole a presa inteira, especialmente **Donax hanleyanus**. **Oliva sayana** deglute partículas raspadas pela rádula. **Olivancillaria (Lintricula) auricularia** come do mesmo modo segurando a presa, principalmente **Donax** com o pé.

4-5. As espécies mencionadas e **Olivancillaria (Olivancillaria) brasiliensis** possuem tentáculo palial posterior situado na sutura canaliculada e um lóbulo palial posterior, produtor do calo, especialmente forte em **O. brasiliensis**. Tentáculo palial anterior ocorre em **Olivella** e **Oliva**; tentáculos pares com

olhos, em **Oliva**. Reabsorção das paredes internas das circunvoluções superiores conhecida de **Olivella** é evidente também em **Oliva, Lintricula** e **Olivancillaria**. Os bordos do sifão das duas últimas são providos de papilas ramificadas que contêm células sensoriais. O grande pé delas não entra completamente na concha. Também o tentáculo palial posterior de **Olivella** permanece freqüentemente fora da concha apesar de ser operculada a espécie estudada. Os vários apêndices do manto são semelhantes entre si e nas espécies examinadas.

6. Glândulas e grupos de células sensoriais situam-se sob a epiderme do bordo anterior do propódio das espécies aqui tratadas. As células sensoriais ligam-se à rêde nervosa do propódio. A glândula pedal posterior, associada ao sulco transversal ventral, é concentrada em **Olivella** e acompanha o sulco em **Oliva** e **Lintricula**. A glândula pedal ventral das fêmeas é independente em **Olivella** e **Oliva,** ao passo que é sòmente uma parte diferenciada do sulco glandular longitudinal do metapódio presente nos dois sexos em **Lintricula.**

7. No sistema nervoso central de **Olivella** os gânglios são distintamente separados, havendo até curtos conetivos pedal-propodiais. Fibras de neuroglia acompanham tôdas as conexões centrais e possibilitam a distenção do anel nervoso juntamente com o esófago, quando o caramujo deglute biválvios inteiros. Os gânglios de **Oliva** são separados apenas por constrições superficiais; as células nervosas tocam-se sôbre os conetivos muito curtos. Êste colar rígido permite sòmente comer partículas raspadas, pequenos animais e suco de carne. O anel nervoso de **Lintricula** é mais concentrado que o de **Olivella** e menos que o de **Oliva**. Fibras de neuroglia são especialmente conspícuas entre os gânglios secundários do propódio. O propódio extensível e contráctil é o órgão sensorial mais ricamente inervado das Olividae presentes, também de **Oliva** cujos lóbulos orais têm tentáculos com olhos bem desenvolvidos.

8. **Olivella** possui sòmente glândulas faríngeas primárias, não porém órgão piriforme ou glândula esofágica. Grande parte do seu estômago é cuticularizada e atua como moela. O intestino de **Oliva** e **Lintricula** é semelhante ao dos Muricacea

(Graham 1941). O estômago de **Oliva** é altamente diferenciado; o de **Lintricula** e o de **Olivancillaria** são semelhantes, mas desprovidos de escudo gástrico. As glândulas anais de **Oliva** e **Lintricula** puderam ser verificadas em cortes; a de **Oliva** abre-se ao lado do anus; a de **Lintricula,** no reto. **Olivella** não tem glândula anal.

9. Espérmios atípicos, semelhantes aos de **Conus,** ocorrem em **Olivella** e **Oliva**. **Lintricula** não tem espérmios atípicos. As Olividae examinadas têm vestígios do duto gonopericardial masculino em forma dum divertículo do pericárdio que termina cegamente no espermioduto renal. Em tôdas, o espermioduto palial comunica-se com a cavidade palial. A origem dêste duto dum sulco aberto é indicada pela sutura dêle em **Lintricula**. Na última há poucas glândulas prostáticas no epitélio do espermioduto palial. Duto gonopericardial feminino existe em tôdas as espécies. Apêndice proximal do oviduto palial ocorre como nos Muricacea e Buccinacea (Fretter 1941). Funciona como receptáculo seminal em **Olivella** e **Oliva** e como glândula absorvente em **Olivancillaria** e **Lintricula**. Na última, o duto da glândula armazena espérmios. Bursas copuladoras volumosas com dutos compridos existem em **Oliva, Lintricula** e **Olivancillaria**. Em **Olivella,** uma bolsa do vestíbulo funciona como bursa copuladora. O órgão vesicular (vesícula interna) com duto comprido de **Olivella** armazena partículas fecais; provàvelmente funciona como o saco de cristais ou de refôrço das Neritidae. Glândula de casulo e bursa de **Lintricula** e **Olivancillaria** são de tal modo voltadas para dentro que a abertura feminina se localiza no mesmo lugar em que o espérmioduto palial do macho se abre na cavidade do manto.

10. No órgão renal que é simples, não lobado, há duas estruturas: dobras e vilosidades. As primeiras correspondem ao lóbulo direito; as segundas ao esquerdo, ou aos sistemas primário e acessório, respectivamente. Excreção merócrina por separação dos ápices das células epiteliais foi vista nas dobras de **Olivancillaria**. O sangue do seio abdominal entra nas dobras e nas vilosidades, saindo das primeiras para a brânquia e das segundas, através da glândula nefridial, para o átrio.

11. Muricacea e Buccinacea são linhas evolutivas paralelas (Graham 1941). Os Volutacea constituem uma entidade frouxamente ajuntada. Olsson (1956) dissolveu-a estabelecendo a superfamília Olivacea com a família Olividae. As Harpidae poderiam ser incluídas nos Olivacea. As Olividae têm vários caracteres dos Muricacea e alguns dos Buccinacea. Por isso não podem ser derivadas de uma destas linhas, mas descendem evidentemente da raiz comum das duas. Dos gêneros aqui estudados, **Oliva** tem o sistema nervoso central mais concentrado; **Olivella,** o menos concentrado, sendo o de **Lintricula** intermediário. Também o opérculo da **Olivella** estudada é caráter primitivo. O tracto alimentar de **Olivella** é o mais adiantado por redução (esôfago) e especialização (estômago). **Lintricula** cujo pé é o mais altamente desenvolvido tem o espermiduto palial e penial mais ancestral. Com Olsson (1956) consideramos **Oliva** e **Lintricula** mais relacionadas uma com outra que uma delas com Olivella, o gênero geològicamente mais jovem.

14. REFERENCES

ABBOTT, R. T. 1955, American Seashells. XIV + 541 p., 40 t. New York (D. van Nostrand Company, Inc.).

ANDREWS, E. A. 1933, The storage sac for capsule reinforcement in the Neritidae. Science (n. ser.) v. 78, n.º 2011, p. 39-41. Washington, D. C.

—— 1935, The egg capsules of certain Neritidae. J. Morphol. v. 57, p. 31-59, 3 t. Philadelphia, Pa.

—— 1937, Certain reproductive organs in the Neritidae. J. Morphol. v. 61, n.º 3, p. 525-561, 6 t. Philadelphia, Pa.

ANKEL, W. E. 1924, Spermatozoendimorphismus und Befruchtung bei Bythinia tentaculata L. Senckenbergiana v. 6, fasc. 1-2, p. 1-12, text-figs. 1-6. Frankfurt a. M.

—— 1929, Ueber die Bildung der Eikapseln bei Nassa-Arten. Verh. D. Zool. Ges. p. 219-230, 6 text-figs. Leipzig. (Suppl. v. 4, Zool. Anz.).

—— 1930, Die atypische Spermatogenese von Janthina (Prosobranchia, Ptenoglossa). Zeitschr. Zellforsch. & mikr. Anat. v. 11, fasc. 3-4, p. 491-608, 61 text-figs., t. 6. Berlin.

—— 1947, Ueber den offenen Uterus bei einigen Monotocardiern ohne Kopulationsorgane. Zool. Bidr. v. 25, p. 102-110, 11 text-figs. Uppsala.

—— 1953, On the genital organs of some mesogastropods... Contributions to the phylogeny of the pallial gonoducts of the Prosobranchia. Zool. Bidr. v. 30, p. 1-23, 10 text-figs., t. 1. Uppsala.

—— 1957, Notes on the littorinacean and stenoglossan genital organs, and a comparison with the Rissoacea. Zool. Bidr. v. 32, p. 81-91, 8 text-figs. Uppsala.

JOHNSON, C. W. 1911, Some notes on the Olividae — 3. Nautilus v. 24, p. 121-124. Philadelphia, Pa.

—— 1934, List of marine Mollusca of the Atlantic coast from Labrador to Texas. Proc. Boston Soc. Nat. Hist. v. 40 (1934-35), p. 1-204. Boston.

KRULL, H. 1935, Anatomische Untersuchungen an einheimischen Prosobranchiern und Beiträge zur Phylogenie der Gastropoden. Zool. Jahrb. Anat. v. 60, p. 399-464, 22 text-figs. Jena.

KÜTTLER, A. 1913, Die Anatomie von Oliva peruviana Lamarck. Zool. Jahrb. Suppl. v. 13 (Fauna Chil. v. 4), p. 477-544, 51 text-figs. Jena.

LANGE DE MORRETES, F. 1949, Ensaio de catálogo dos Moluscos do Brasil. Arq. Mus. Paranaense v. 7, p. 5-216. Curitiba (Brazil).

LINKE, O. 1933, Morphologie und Physiologie des Genitalapparates der Nordseelittorinen. Wiss. Meeresunters. N. F. Abtlg. Helgol. v. 19, fasc. 2, n.° 5, p. 1-60, t. 1-8. Kiel & Leipzig.

MILLOTT, N. 1937, On the morphology of the alimentary canal, process of feeding, and physiology of digestion of the nudibranch mollusc Jorunna tomentosa (Cuvier). Phil. Transact. R. Soc. Lond. ser. B. — Biol. Sci. n.° 551, v. 228, p. 173-218. London.

MOORE, J. E. S. 1899, Molluscs of the Great African Lakes. III. Tanganyikia rufofilosa and the genus Spekia. Qu. Journ. micr. Sci. n. ser. v. 42, n.° 166, p. 155-185, t. 14-19. London.

MOORE, R. C., LALICKER, C. G. and FISCHER, A. G., 1952, Invertebrate Fossils. 766 pp. McGraw Hill Book Co., New York.

MORTON, J. E. 1953, The functions of the gastropod stomach. Pr. Linn. Soc., session 164 (1951-52), part 3, p. 240-246, 3 text-figs. London.

—— 1958, Torsion and the adult snail; a re-evaluation. Proc. Malacol. Soc. v. 33, part 1, p. 2-10, 4 text-figs. London.

EALES, N. B. 1923, Mollusca. Part V. Anatomy of Gastropoda (except the Nudibranchia). Brit. Antarct. ("Terra Nova") Exped. 1910. Zool. v. 7, n.º 1 ,p. 1-45, 42 text-figs. London.

FISCHER, P. 1881, Note sur le genre Olivella. Journ. Conchyliol. v. 29, p. 31-35. Paris.

FISCHER, P.-H. 1950, Vie et Moeurs des Mollusques. Bibl. Scient., 312 p., 180 text-figs. Paris (Payot, 106, Boulevard Saint-Germain).

FRETTER, V. 1939, The structure and function of the alimentary canal of some tectibranch molluscs, with a note on excretion. Transact. R. Soc. Edinb. v. 59, part 3 (n.º 22), p. 599-646, 22 text-figs. Edinburgh.

—— 1941, The genital ducts of some British stenoglossan prosobranchs. Journ. mar. biol. assoc. Unit. Kingd. v. 25, n.º 1, p. 173-211, 6 text-figs. Cambridge.

—— 1946, The genital ducts of Theodoxus, Lamellaria and Trivia, and a discussion on their evolution in the prosobranchs. Journ. mar. biol. assoc. Unit. Kingd. v. 26 (1947), n.º 3 (1946), p. 312-351, 7 text-figs. Cambridge.

—— 1946a, The pedal sucker and anal gland of some British Stenoglossa. Proc. Malacol. Soc. v. 27, part 3, p. 126-130, 2 text-figs. London.

—— 1948, The structure and life history of some minute prosobranchs of rock pools, etc. Journ. mar. biol. assoc. Unit. Kingd. v. 27, p. 597-632, 6 text-figs., t. 4. Cambridge.

—— 1951, Observations on the life history and functional morphology of Cerithiopsis tuberculata (Montagu) and Triphora perversa (L.). Ibid. v. 29, p. 567-586, 6 text-figs. Cambridge.

—— 1956, The anatomy of the prosobranch Circulus striatus (Philippi) and a review of its systematic position. Pr. Zool. Soc. London v. 126, part 3, p. 369-381, 5 text-figs. London.

FRETTER, V. & GRAHAM, A. 1954, Observations on the opisthobranch mollusc Acteon tornatilis (L.). Journ. mar. biol. assoc. Unit. Kingd. v. 33, p. 565-585, 9 text-figs. Cambridge.

GERLACH, S. A. 1957, Die Nematodenfauna des Sandstrandes an der Küste von Mittelbrasilien. Mitteil. Zool. Mus. Berlin v. 33, fasc. 2, p. 411-459, 16 text-figs., 4 t. Berlin.

GIESE, M. 1913, Gonopericardialgang und Umbildung der Geschlechtswege im Zusammenhang mit Protandrie bei Calyptraea sinensis. Zool. Anz. v. 42, p. 433-440. Leipzig.

—— 1915, Der Genitalapparat von Calyptraea sinensis Lin., Crepidula unguiformis Lam. und Capulus hungaricus Lam. Zeitschr. wiss. Zool. v. 114, p. 169-231, t. 5-8. Leipzig.

GOFFERJE', C. N. 1950, Contribuição à zoogeografia da malacofauna do litoral do Estado do Paraná. Arq. Mus. Paranaense v. 8, p. 221-282, t. 31-35. Curitiba (Brazil).

GRAHAM, A. 1938, The structure and function of the alimentary canal of aeolid molluscs, etc. Transact. R. Soc. Edinb. v. 59, part 2, n.º 9, p. 267-307, 14 text-figs. Edinburgh.

—— 1939, On the structure of the alimentary canal of style-bearing prosobranchs. Pr. Zool. Soc. London, Ser. B, v. 109, p. 75-112, 10 text-figs. London.

GRAHAM, A. 1941, The oesophagus of the stenoglossan prosobranchs. Pr. R. Soc. Edinb. v. 61, part 1, p. 1-23, 5 text-figs. Edinburgh & London.

—— 1949, The molluscan stomach. Transact. R. Soc. Edinb. v. 61, pt. 3 (n.º 27), p. 737-778, 24 figs. Edinburgh.

—— 1955, Molluscan Diets. Proc. Malacol. Soc. v. 31, p. 144-159. London.

—— 1957, The molluscan skin with special reference to prosobranchs. Proc. Malacol. Soc. v. 32, part 4, p. 135-144. London.

HALLER, B. 1905, Ueber Oliva peruviana Lamarck. Jen. Zeitschr. Naturw. v. 40, p. 647-664, t. 27. Jena.

HIRSCH, G. Chr. 1915, Die Ernährungsbiologie fleischfressender Gastropoden. I. Teil. Zool. Jahrb. Allg. Zool. Physiol. v. 35, fasc. 4, p. 357-504, 44 text-figs. Jena.

HULBERT, G. S. E. B. & YONGE, C. M. 1937, A possible function of the osphradium in the Gastropoda. Nature v. 139, n.º 3524, p. 840-841. London.

IHERING, H. v. 1897, A Ilha de São Sebastião. Rev. Mus. Paulista v. 2, p. 129-171. São Paulo.

JAKUBSKI, A. 1913, Studien über das Gliagewebe der Mollusken, I. Teil. Lamellibranchiata und Gastropoda. Zeitschr. wiss. Zool. v. 104, p. 81-118, t. 3-5. Leipzig.

JOHANSSON, J. 1939, Anatomische Studien über die Gastropodenfamilien Rissoidae und Littorinidae. Zool. Bidr. v. 18 (1938-40), p. 287-396, t. 1-11. Uppsala.

—— 1942, Von diaulen Geschlechtsapparaten bei den Prosobranchiern. Ark. Zool. v. 34 A, n.º 12, p. 1-10, 8 text-figs., t. 1. Stockholm.

—— 1936, Prosobranchia. Grimpe & Wagler, Tierw. Nord- & Ostsee, part IX b 1, 240 p., 222 text-figs. Leipzig (Akadem. Verlagsges.).

—— 1938, Erwerb und Aufnahme der Nahrung der Gastropoden. Zool. Anz. Suppl. v. 11 (Verh. D. Zool. Ges. 1938), p. 223-295, 68 text-figs. Leipzig.

BALES, BLENN R. 1946, The Bar. Nautilus v. 59, n.º 3, p. 73-76. Philadelphia, Pa.

BERGH, R. 1896, Beiträge zur Kenntnis der Coniden. Nov. Act. Leopold. v. 65, n.º 2, p. 67-214, t. 1-13. Halle a. S.

—— 1901, Beitrag zur Kenntnis der Gattung Harpa. Zool. Jahrb. Anat. v. 14, p. 609-629, t. 47. Jena.

BERNARD, F. 1890, Recherches sur les organs palléaux des Gastéropodes prosobranches. Ann. Sci. nat. Zool. sér. 7, v. 9, p. 89-404, t. 6-13. Paris.

BOURNE, G. C. 1908, Contributions to the morphology of the group Neritacea of aspidobranch gastropods. Part 1. The Neritidae. Proc. Zool. Soc. London 1908, p. 810-887, t. 46-66. London.

—— 1911, Id. Part 2. The Helicinidae. Ibid. 1911, p. 759-809, t. 30-42. London.

BOUVIER, B. L. 1887, Système nerveux, morphologie générale et classification des Gastéropodes prosobranches. Ann. Sci. nat. Zool. sér. 7, v. 3, p. 1-510, t. 1-19. Paris.

CARCELLES, A. 1944, Catalogo de los moluscos marinos de Puerto Quequén. Rev. Mus. La Plata (n. ser.), Zool. v. 3, p. 233-309, t. 1-15. La Plata.

CLARK, W. C. 1958, Notes on the mantle cavity of some Trochid and Turbinid Gastropoda. Proc. Malacol. Soc. v. 33, part 2, p. 57-64, 7 text-figs. London.

CROSSE, H. & FISCHER, P. 1882, Note complémentaire sur la résorption des parois internes chez les Olivella. Journ. Conchyliol. v. 30, p. 181-183, t. 8, f. 12, 15-16. Paris.

CUÉNOT, L. 1914, Les organes phagocytaires des Mollusques. Arch. Zool. expér. génér. v. 54 (1914-15), fasc. 9 (6. VII. 1914), p. 267-305, t. 10-13. Paris.

DAKIN, W. J. 1912, Buccinum (The Whelk). L. M. B. C. Mem. XX, VIII + 115 p., 8 t. London.

DALL, W. H. 1889, Report on the Mollusca ("Blake") II. Gastropoda and Scaphopoda. Bull. Mus. Comp. Zool. v. 18, p. 1-492, t. 10-40. Cambridge, Mass.

OLSSON, A. A. 1956, Studies on the genus Olivella. Proc. Acad. Philad. v. 108, p. 155-225, t. 8-16. Philadelphia, Pa.

ORBIGNY, A. d' 1835-1846, Voyage dans l'Amérique Méridionale v. 5, 3e partie Mollusques. Atlas 1846. Paris & Strasbourg.

PACE, S. 1902, On the anatomy and relationships of Voluta musica, Linn.; with notes on certain other supposed members of the Volutidae. Proc. Malacol. Soc. v. 5 (1903), p. 21-31, t. 2. London.

PERRIER, R. 1889, Recherches sur l'anatomie et l'histologie des Gastéropodes prosobranches. Ann. Sci. nat. Zool. sér. 7, v. 8, p. 61-315, t. 5-13. Paris.

POWELL, A. W. B. 1951, Antarctic and Subantarctic Mollusca: Pelecypoda and Gastropoda. Discovery Reports, v. 26, p. 47-196, 130 figs., t. 5-10. Cambridge.

REEVE, A. L. 1850, Monograph of the genus Oliva. Conchologia Iconica, v. 6 (1851), 29 t. London.

RISBEC, J. 1928, Contribution à l'étude anatomique de quelques espèces de Mitres de la presqu'île de Nouméa. Bull. Mus. Hist. Nat. v. 34, p. 105-112, t. 1; p. 173-180, t. 2; p. 225-227. Paris.

—— 1932, Note sur la ponte et le développement de Mollusques Gastéropodes de Nouvelle-Calédonie. Bull. Soc. Zool. France v. 57, n.° 4, p. 358-374. Paris.

RISBEC, J. 1937, Signification de la glande du calcaire des Neritina. C. R. Acad. Sci. v. 204, p. 374-376. Paris.

—— 1942, Recherches anatomiques sur les prosobranches de la Nouvelle Calédonie (troisième partie). Ann. sci. nat. Zool. sér. 11, v. 4, p. 21-83, 8 t. Paris.

—— 1952, Observations sur l'anatomie des Nassidae de Nouvelle-Calédonie. Bull. Soc. Zool. France v. 77, n.° 5-6, p. 487-495, 11 text-figs. Paris.

—— 1955, Considérations sur l'anatomie comparée et la classification des Gastéropodes Prosobranches. Journ. Conchyliol. v. 95, p. 45-82, 22 figs. Paris.

—— 1956, Étude anatomique de Naticidae de Nouvelle-Calédonie. Journ. Conchyliol. v. 96, p. 12-45, t. 1-6. Paris.

SCHAFFER, J. 1913, Ueber den feineren Bau und die Entwicklung des Knorpelgewebes, etc. IV. Teil. Zeitschr. wiss. Zool. v. 105, p. 280-347, t. 18-19. Leipzig.

SCHREIBER, G. 1930, Studi sul pigmento cromolipoide, l'apparato fenestrato e la respirazione di supplemento del sistema nervoso. Pubbl. Staz. Zool. Napoli, v. 10, fasc. 2, p. 151-195, t. 3-6. Roma & Berlin.

SIMROTH, H. 1896-1907, Gastropoda Prosobranchia. Bronn, Kl. Ordn. v. 3, 2. Abtlg., VII + 1056 p., 63 t. Leipzig (C. F. Winter).

SMITH, E. A. 1890, Mollusca in: H. N. Ridley, Notes on the Zoology of Fernando Noronha. J. Linn. Soc. Zool. v. 20 (1886-90), p. 483-503, t. 30. London.

SOUZA LOPES, H. DE & ALVARENGA, M. 1955, Contribuição ao conhecimento dos Moluscos da ilha Fernando de Noronha-Brasil. Bol. Inst. Oceanogr. v. 6, fasc. 1-2, p. 157-196, 3 t., 1 map. São Paulo.

THIELE, J. 1931; 1935, Handbuch der systematischen Weichtierkunde. VI & V, 1154 pp., 897 text-figs. Jena (G. Fischer).

TOURAINE, J. 1952, Les glandes pédieuses des Gastéropodes Prosobranches Monotocardes. Bull. Soc. Zool. France. v. 77, p. 240-241. Paris.

WEINKAUFF, H. C. 1878, Die Gattung Oliva. In: Martini & Chemnitz, Systematisches Conchylien-Cabinet, v. 5, Abthlg. 1, 172 pp., t. A-C, 1-39. Nürnberg (Bauer & Raspe).

WOODWARD, M. F. 1900, Note on the anatomy of Voluta ancilla (Sol.), Neptuneopsis gilchristi Sby., and Volutilithes abyssicola (Ad. & Rve.). Proc. Malacol. Soc. v. 4 (1901), p. 117-125, t. 10. London.

YONGE, C. M. 1938, Evolution of ciliary feeding in the Prosobranchia, with an account of feeding in Capulus ungaricus. Journ. mar. biol. assoc. Unit. Kingd. v. 22 (1937-38), n.º 2 (1938), p 453-468, 6 text-figs. Plymouth.

—— 1947, The pallial organs in the aspidobranch Gastropoda and their evolution throughout the Mollusca. Phil. Transact. R. Soc. Lond. ser. B. Biol. Sci. n.º 591, v. 232, p. 443-518, 40 text-figs., t. 18. London.

15. EXPLANATION OF LETTERS

a — anterior mouth tentacle.
aa — albumen gland.
ae — anterior pedal gland.
am — amoebocyte.
an — anal gland.
ao — anterior aorta.
ar — anus.
as — accessory salivary gland.
au — auricle.
az — accessory radular plate.
b — bursal canal.
c — capsule gland.
ca — buccal ganglion.
cc — bursal vesicle.
cc — caecum.
cm — callus-forming mantle flap.
cn — cerebro-pedal connective.
co — cerebral commissure.
cr — cerebral ganglion.
cu — buccal glands.

cv — columellar nerve.
cw — callus.
cz — columellar muscle.
d — ducts of digestive gland.
e — egg.
ea — left posterior visceral ganglion.
em — pedal commissure.
en — pedal ganglion.
eo — female aperture.
er — pericardium.
ez — left zygosis.
f — fold behind propodium.
g — gonopericardial duct or strand.
h — kidney.
i — intestine.
ia — orifice of proboscis.
ie — gizzard region of stomach.
io — gland in duct of oesophageal gland.
iv — intestinal groove.
iu — propodium.
iv — internal vesicle.
j — stomach.
k — ctenidium.
l — labial cleft.
m — metapodium.
ma — mantle border.
mi — major typhlosole.
mo — mouth.
mr — middle plate of radula.
mu — minor typhlosole.
mv — mouth flaps.
mw — posterior mantle tentacle.
mz — muscular belt of stomach.
n — neuroglia nuclei.
ne — nerve fibres.
ni — neuroglia fibres.
no — pharynx of Leiblein.
nr — parapodium.
ns — vesicula seminalis.
nu — neuroglia coat.
o — oesophagus.
oa — opening of accessory salivary gland.
oc — blood lacuna.
oe — oesophageal gland.
om — oesophageal muscle ring.
oo — odontoblasts.
or — operculum.
os — longitudinal fold of stomach.
ou — ovarian duct.
ov — opening of primary salivary gland.
ow — osphradium.
p — proboscis.
q — lateral radular plate.
r — radula.
ra — posterior pedal gland.
rc — peri-intestinal connective tissue.
re — posterior sorting area.
rn — receptaculum seminis or ingesting gland.
ro — secondary propodial ganglia.
rs — radular support.
ru — radular cuticle.
rv — renal oviduct.
rw — right posterior visceral ganglion.
s — statocyst.
sa — pallial spermiduct.
se — propodial ganglion.
si — gastric shield.
sm — communication between spermiduct and mantle cavity.
sn — pallial-siphonal nerve
so — pouch of pallial spermiduct.
sr — sperm channel.
ss — primary salivary gland.
su — testicular duct.
sz — proboscis sheath.
t — testis.
te — spermiduct.
ua — pleural ganglion.
uc — gastric cuticle.

ue — pleuro-pedal connective.
ui — digestive gland.
un — subintestinal ganglion.
uo — **Donax.**
us — suture.
uv — duct of internal vesicle.
uz — afferent renal vessel.
v — ventral pedal gland.
va — supra-intestinal ganglion.
vc — pouch outside capsule gland.
ve — ventricle.
vi — siphon.
vn — penial nerve.
vo — retractor of proboscis.
vr — penis.
vs — sphincter.
vv — nephridial gland.
vz — ventricle.
w — duct of ingesting gland.
we — vestibule.
wi — style sac.
wn — visceral loop.
x — renal aperture.
xo — aorta.
y — renopericardial duct.
z — nerve cells.
zc — folded part of kidney.
ze — epithelium of mantle cavity.
zn — villous part of kidney.
zo — ovary.

PLATES

PLATE 1

Fig. 1 — Trail of **Olivella**.
Fig. 2 — **Olivancillaria** gliding.
Fig. 3 — **Lintricula** feeding on a clam in front of it.
Fig. 4 — **Lintricula** feeding on a clam held in its foot; dorsal view.
Fig. 5 — **Lintricula** feeding as in Fig. 4; ventral view.
Fig. 6 — **Lintricula,** different feeding position; ventral view.
Fig. 7 — **Lintricula** feeding as in Fig. 6; lateral view.
Fig. 8 — **Lintricula,** everted proboscis.
Fig. 9 — **Lintricula,** tip of siphon of preserved specimen.

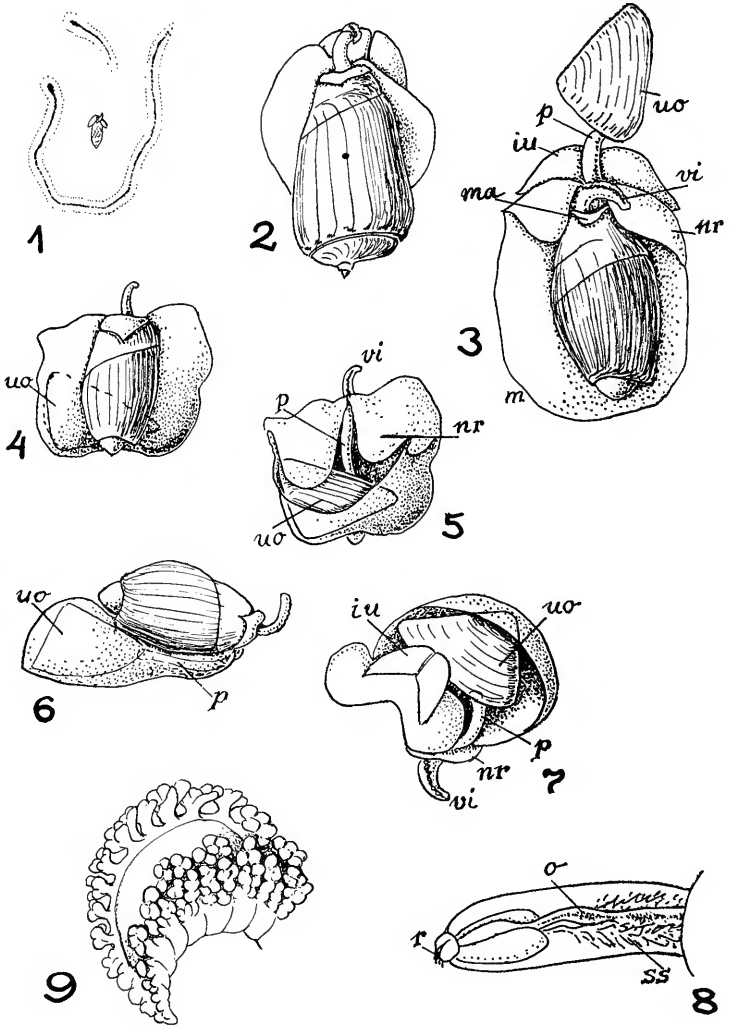
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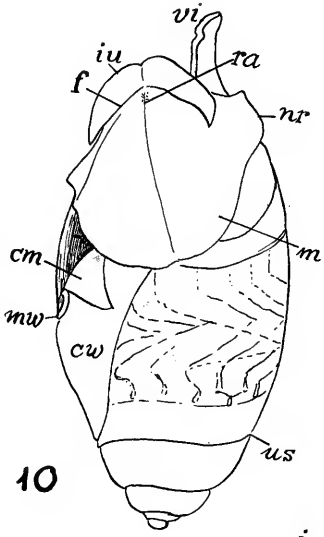
PLATE 2

Fig. 10 — **Olivella,** ventral view of preserved male.
Fig. 11 — **Olivella,** ventral view of preserved female.
Fig. 12 — **Olivella,** dorsal view of anterior part.
Fig. 13 — **Olivella,** diagram of anterior skin glands.
Fig. 14 — **Lintricula,** sole of preserved male.



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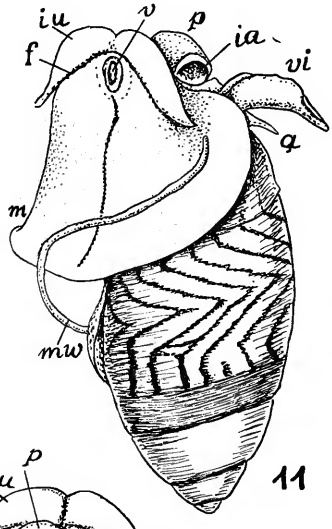

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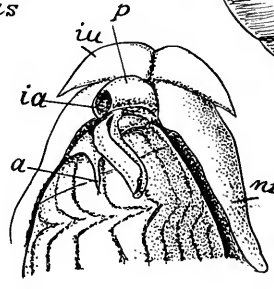

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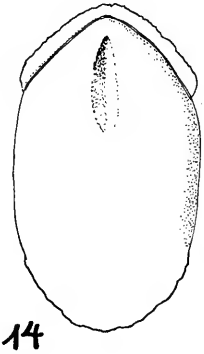

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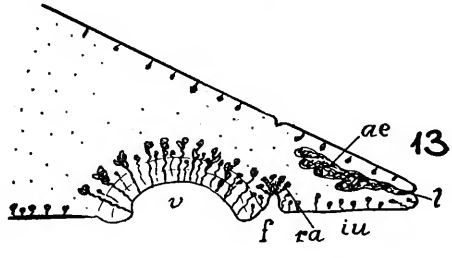

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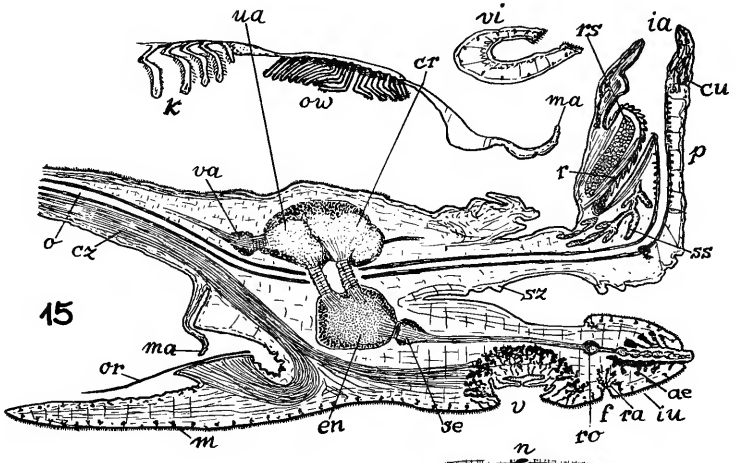
PLATE 3

Fig. 15 — **Olivella,** combined sagittal section of anterior region.
Fig. 16 — **Oliva,** transverse section of ventral pedal gland.
Fig. 17 — **Olivella,** longitudinal section of pleuro-pedal connective.
Fig. 18 — **Olivella,** transverse section of cerebro-pedal connective.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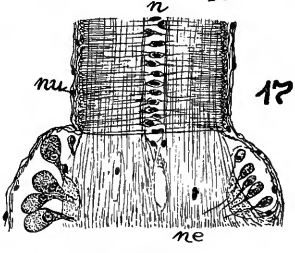
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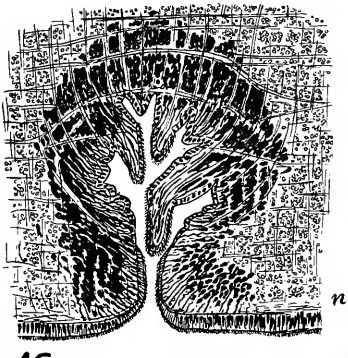
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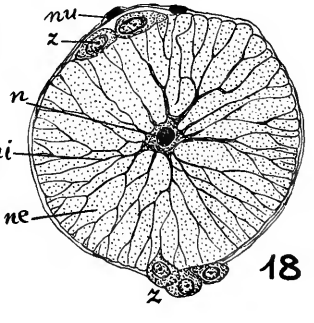
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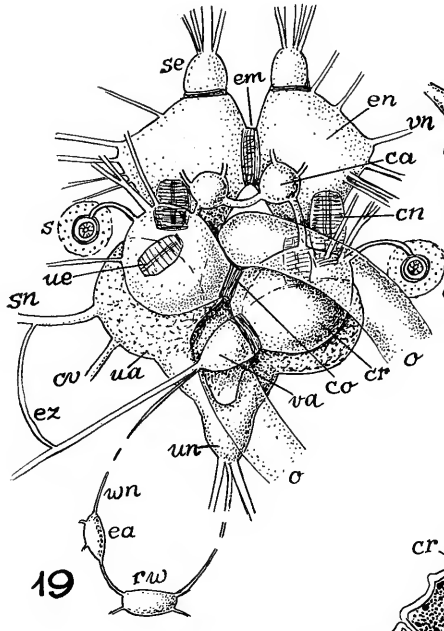
PLATE 4

Fig. 19 — **Olivella,** dorsal view of central nervous system.
Fig. 20 — **Oliva,** dorsal view of central nervous system.
Fig. 21 — **Oliva,** right-side view of central nervous system.
Fig. 22 — **Oliva,** combined transverse section of nerve ring.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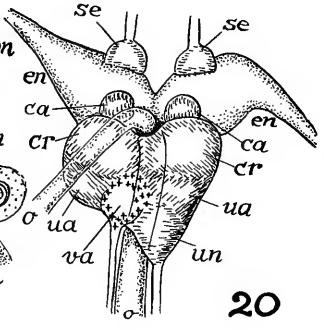
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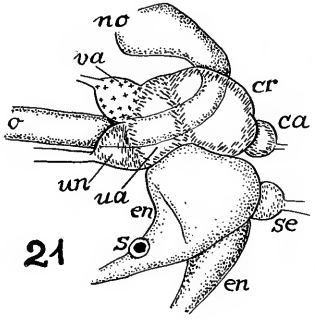
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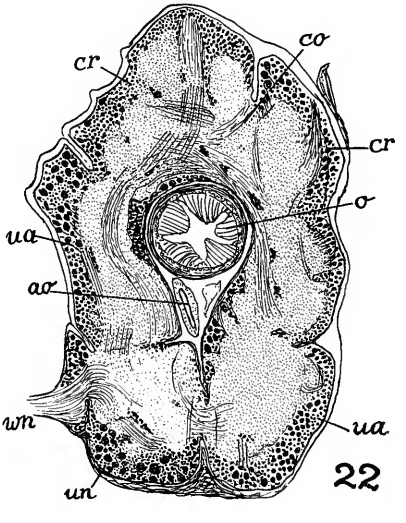
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PLATE 5

Fig. 23 — **Lintricula,** dorsal view of central nervous system.
Fig. 24 — **Lintricula,** right-side view of central nervous system.
Fig. 25 — **Lintricula,** a sagittal section of the central nervous system.
Fig. 26 — **Clivella,** plates of radula.
Fig. 27 — **Oliva,** plates of radula.
Fig. 28 — **Lintricula,** plates of radula. The scale applies to Figs. 27-29.
Fig. 29 — **Olivancillaria,** plates of radula.

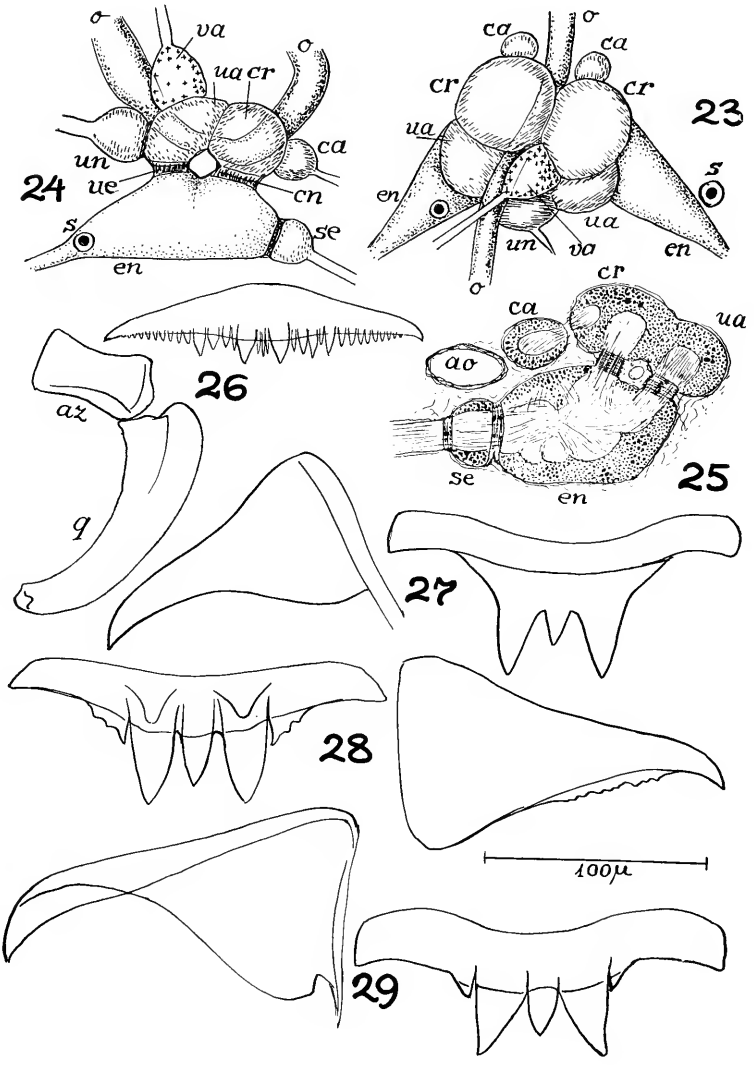
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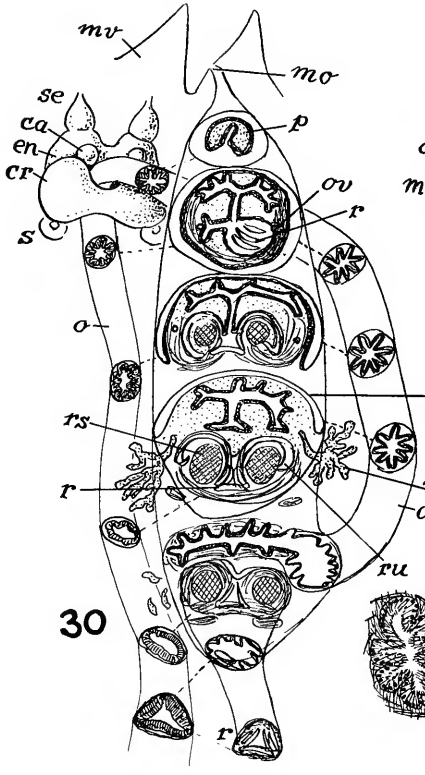
PLATE 6

Fig. 30 — **Olivella,** diagram of proboscis and oesophagus.
Fig. 31 — **Olivella,** transverse section of radula and support.
Fig. 32 — **Olivella,** transverse section of radular sac.
Fig. 33 — **Olivella,** transverse section of pre- and post-cerebral limb of oesophagus; the former with allusive pharynx of Leiblein.
Fig. 34 — **Olivella,** stomach.
Fig. 35 — **Olivella,** transverse section of sclerosed part of stomach.



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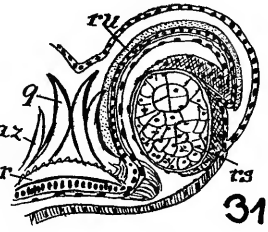

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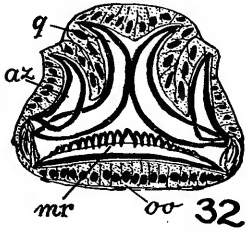

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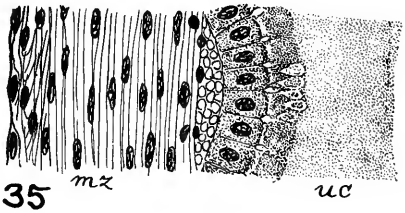

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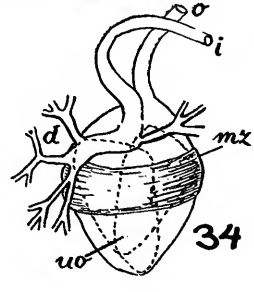

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PLATE 7

Fig. 36 — **Olivella,** opened stomach.
Fig. 37 — **Olivella,** section of pyloric region.
Fig. 38 — **Oliva,** alimentary tract.
Fig. 39 — **Oliva,** stomach distended by food.
Fig. 40 — **Lintricula,** anterior part of alimentary tract.

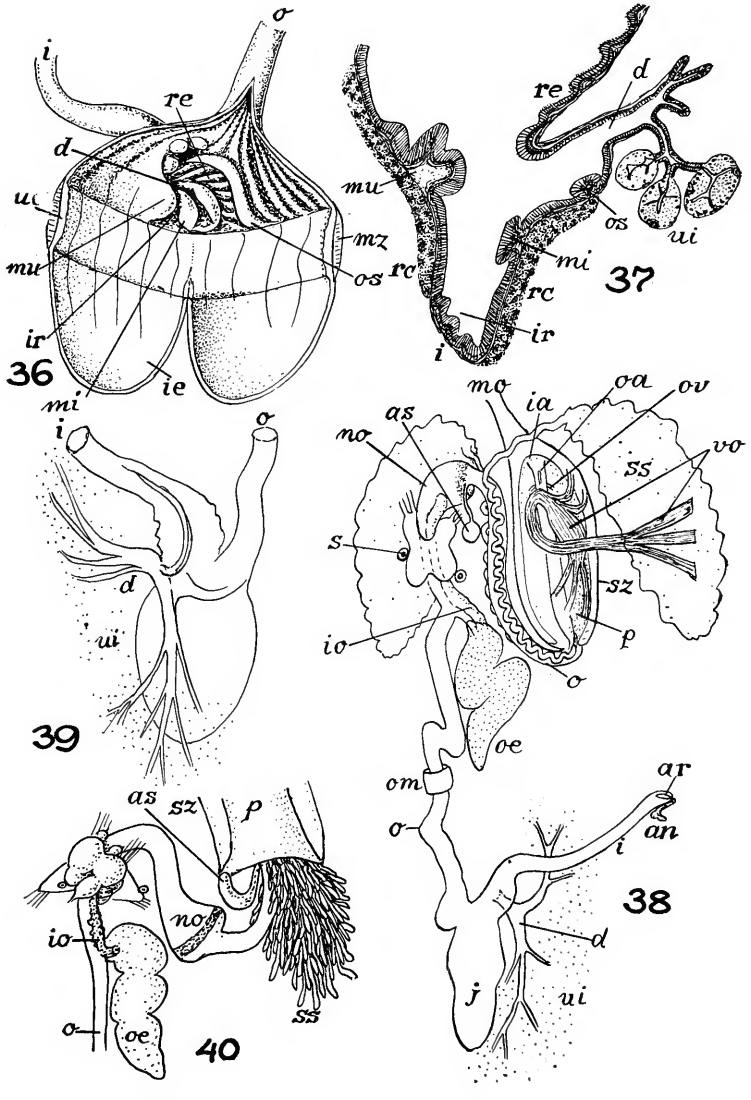
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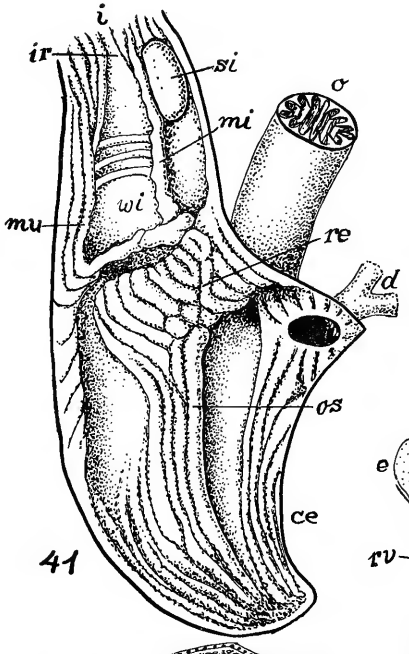
PLATE 8

Fig. 41 — **Oliva,** stomach opened by median dorsal cut.
Fig. 42 — **Olivella,** male organs in situ.
Fig. 43 — **Olivella,** transverse section of outer part of penis.
Fig. 44 — **Olivella,** diagram of female organs.
Fig. 45 — **Olivella,** section of internal vesicle.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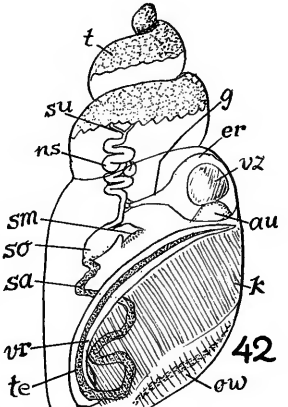
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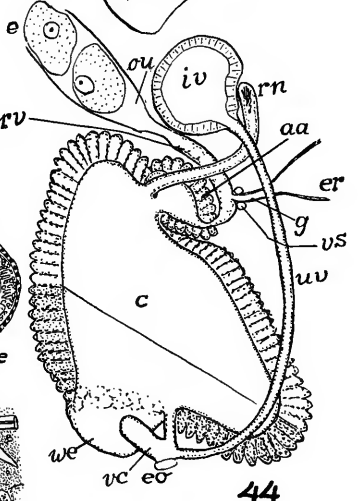
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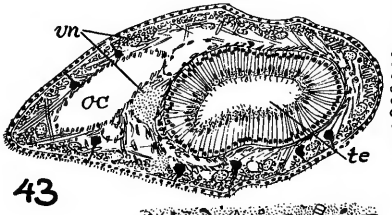
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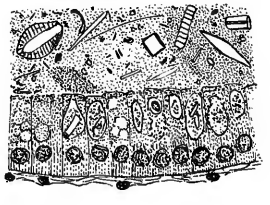

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PLATE 9

Fig. 46 — **Olivella,** section of capsule gland.
Fig. 47 — **Oliva,** male organs in situ.
Fig. 48 — **Oliva,** diagram of female organs.
Fig. 49 — **Oliva,** transverse section of female organs.
Fig. 50 — **Olivancillaria,** section of ingesting gland.

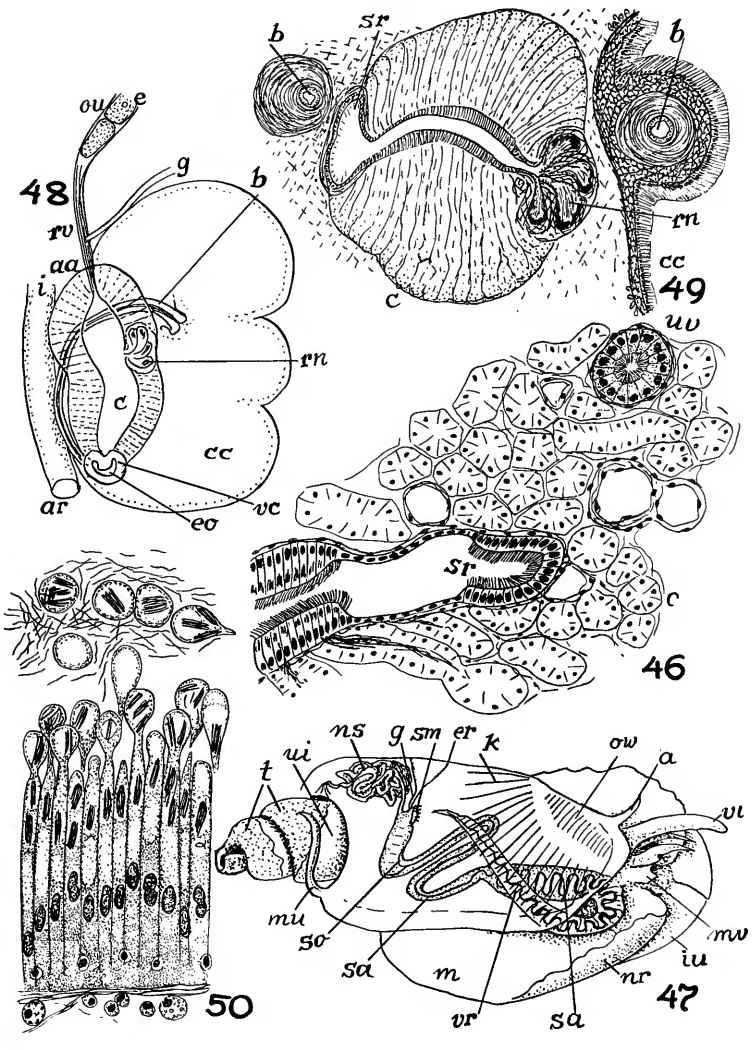
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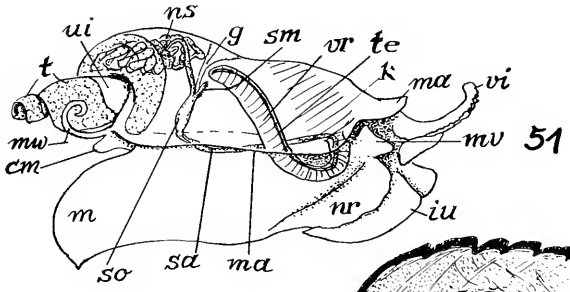
PLATE 10

Fig. 51 — **Lintricula,** male organs in situ.
Fig. 52 — **Lintricula,** transverse section of base of penis.
Fig. 53 — **Lintricula,** transverse section of penis.
Fig. 54 — **Lintricula,** diagram of female organs.
Fig. 55 — **Lintricula,** section of ingesting gland.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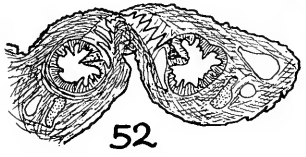
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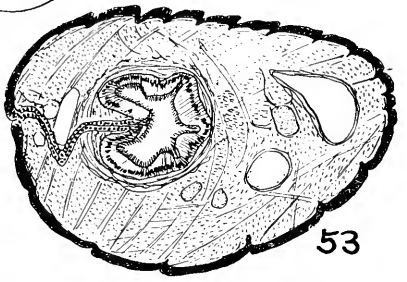
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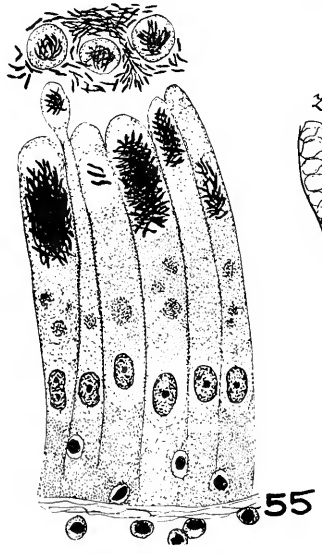
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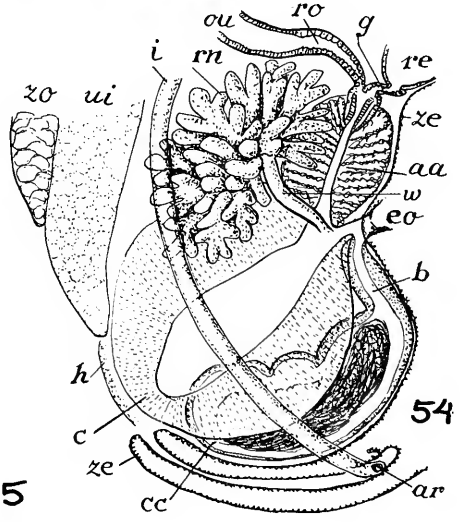
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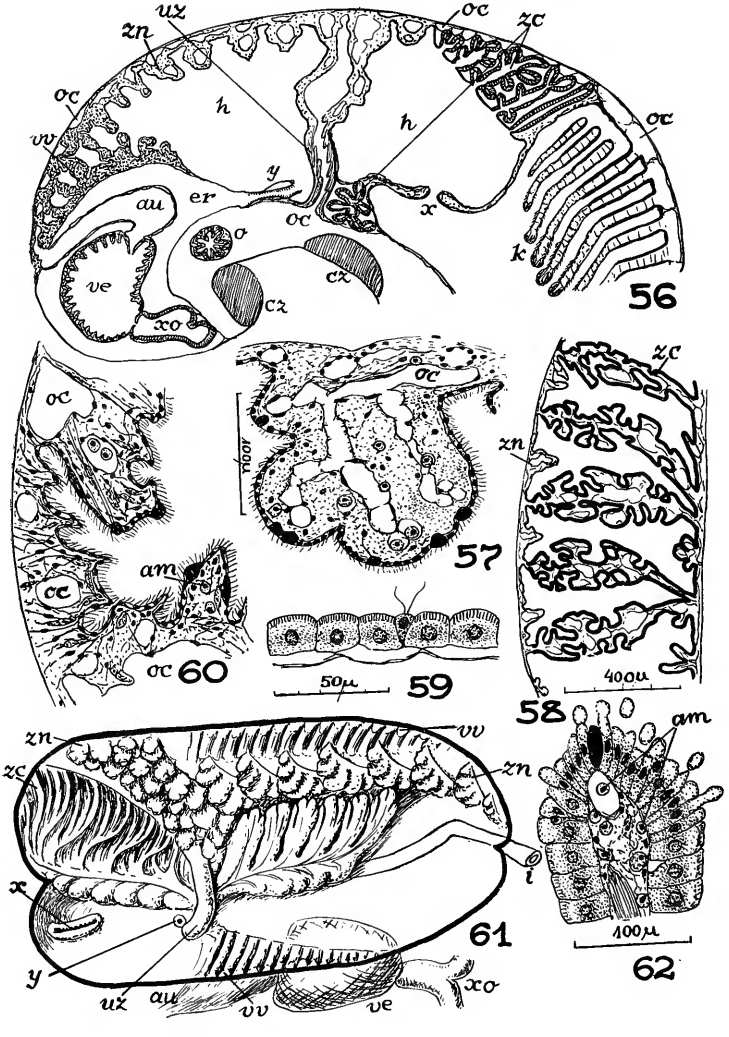
PLATE 11

Fig. 56 — **Olivella,** combined transverse section of renal organ.
Fig. 57 — **Lintricula,** villosity of kidney of young snail.
Fig. 58 — **Lintricula,** folded part of kidney of same.
Fig. 59 — **Lintricula,** epithelium of folded part of same.
Fig. 60 — **Lintricula,** nephridial gland of same; to same scale as Fig. 57.
Fig. 61 — **Lintricula,** renal organ opened at anterior border.
Fig. 62 — **Olivancillaria,** merocrine secretion in renal epithelium of folded part.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ON THE REPRODUCTION OF OLIVELLA

by Eveline and Ernesto Marcus
(with 1 plate)

Thanks to the Oceanographic Institute of the University of São Paulo (Professor Wladimir Besnard) we could observe the reproduction of **Olivella** whose female organs are the most peculiar of our formerly studied olivids. The "Northern Base" (Dr. Edmundo Nonato) of the mentioned Institute put its installations at our disposal in the most generous way.

In the fine sand of the Enseada near Ubatuba (23°27' S. 45°6' W) **O. verreauxii** (Ducros 1857) lives in the zone of the neap tides. On october 31st, 1958 there were about 30 snails per sq. m. on the surface of the sand or a little under it at low tide-time. As the population is dense and the animals crawl rapidly, about 10 cm. a minute at 24° C., the mates meet easily. We maintained about 300 snails in an enamel tray of 60 × 40 cm. with 1-2 cm. of sand and equal height of water without aerification. They were fed with living young **Donax hanleyanus** and crushed larger ones and observed for 9 days nearly without losses. One or other snail which crawled out of the tray during the night, and was found on the dry in the morning, recovered after a short time in water. The accumulation of the animals evidently favoured copulation, because joining began in the afternoon, about 1 hour after the installation of the snails.

The position of the mates of **Olivella** during the act of coupling (Fig. 2) is not recorded from other prosobranchs in the compendia of Simroth (1904), Meisenheimer (1921), Ankel (1936) and Fischer (1950); in some tectibranchs the slug that functions as male is situated behind the female.

The male stretches its propodium forward like a snout (Fig. 1, o) and grasps the uppermost whorl of the female's shell in its median propodial furrow (Fig. 2). We remember that

only in males the ventral propodial furrow is distinct. The anterior pedal mucous gland does not seem to take part in the attachment, to judge from the fact that the mates separate easily after copulation, and threads or traces of mucus with adhering sediments were not left on the shell of the female. A reaction of the female when the tip of the shell was seized by the male was not observed; both snails continue to crawl at the same rate of motion. Evidently the male recognizes the female by chemoreceptors, perhaps the propodial sensorial cells, before it puts forth its propodium; this movement was never undertaken tentatively but always followed by seizing the shell of the snail crawling in front. As in the previously counted samples the females outnumbered the males greatly, so that there was no competition among the latter. While the couple crawls, the penis (Fig. 2, p) is protruded, extended forward on the right side, and enters the mantle cavity of the female. Sometimes a male was seen attached to a feeding female. Rarely the male curved its penis to the left and did not succeed to enter the mantle cavity; usually an initial curving to the left was corrected quickly.

When the male begins to ejaculate, the female stops moving. Both may be on the surface or half burried in the sand, or only the female is partially concealed and the male remains visible. The male jerks vividly so that the ejaculations can be counted; we observed up to 16 effected with intervals of a few seconds. The white pellets of sperm are seen passing through the transparent penis. Sometimes the ejaculating male twitches so violently that the female is turned around without loss of the penial and propodial contact. After the first 3 hours of observation, during which copulation was frequent, the males seemed to be rather tired, to judge from the long duration, up to 10 minutes, of the pre-copulatory attachment.

Females preserved immediately after copulation, dissected or sectioned, showed pellets of sperm with prostatic secretion between the leaflets of the ctenidium. While studying the female organs we had already found sperm in the gill of snails preserved immediately after capture. Therefore we conclude

that the penis does not enter the opening of the capsule gland nor that of the internal vesicle (Marcus 1959, f. 44, iv), but only the mantle cavity. **O. verreauxii** does not have any sperm-receiving organ in the outer part of its female system. The receptaculum seminis (ibid, rn) lies far inward between albumen and capsule glands. It contains sperm in the sectioned females that were preserved immediately after copulation. Problably these sperms do not come from the last but from previous copulations. This is indicated by the stage of the internal vesicle which is in the phase of oviposition to be described in the following. Though spermatozoa were not seen in the sperm channel of the capsule gland in females preserved immediately after copulation or during and after deposition of egg capsules, they probably make their way actively from the gill to the receptaculum.

Paired mates separated as softly as they join; the male withdraws the penis and loosens his propodium from the female's shell. The male moves away immediately, while the female frequently rests for a short time, perhaps due to a momentary difficulty of respiration. When the eggs are excluded from the opening of the mantle cavity they are already fertilized, and each (Fig. 3, ei) is encased in a capsule (k). The capsule passes through the ciliated furrow on the right side that only females have into the ventral pedal gland as in **Nassa mutabilis** (Ankel 1929, f. 1). There the capsule is moulded for about 3 minutes. With help of a mirror one can see how the spherical capsule is kneaded rapidly in the cavity of the gland (Fig. 3).

The internal vesicle contributes to the formation of the capsule with its contents and with secretion. The faecal particles stored in the vesicle which were described in our first study of olivids appear in a fine layer on the outer surface of the deposited capsule (Fig. 5). These particles are spread so sparsely that they cannot afford any mechanical protection to the capsule nor conceal the embryo which is white and completely visible through the transparent capsule. Hence the internal vesicle of **O. verreauxii** cannot be called a "reinforcement sac" as the similar appendage of the neritids. nor

do its contents constitute an "armature" (Andrews 1937, p. 531). Capsules brought from the beach on a shell were quite clean without sand-grains sticking to them, and to those obtained in the laboratory only occasionally a few grains adhered. In egg-laying females the cells of the internal vesicle emit a secretion of pink staining spherules. This secretion forms a layer between the epithelium and the faecal particles. The quantity of the latter diminishes measurably during oviposition. In a female which had laid 10 eggs the internal vesicle had one quarter of the volume of others preserved after copulation before depositing eggs.

In our opinion the faecal particles and the secretion of the internal vesicle supply the egg capsule with a mark. We think that the secretion fastens the particles to the capsule when this leaves the pallial oviduct or capsule gland. The face that receives the particles will be the upper part or lid of the deposited capsule. The latter does not have its definitive form when it passes from the opening of the mantle cavity through the furrow on the right side to the moulding gland. But it has a finely granular and a smooth sticky face. Thus differentiated it is attached to the substratum with the sticky face and pressed into the moulding gland with the face containing the particles.

The folds of the ventral pedal gland shape the ridges of the capsule which is soft when it enters the gland. As Ankel (1929, p. 224) exposed, the gland functions as a mould and its secretion probably hardens the capsule (p. 230). Each capsule is attached separately and with irregular distances from the others (Fig. 4) to living or empty **Donax**, to the shells of other Bivalvia (Veneridae), to **Bulla striata** or to the glass dish. One female isolated after copulation produced 6 egg-capsules in 3 hours, another 13 in 6 hours.

The egg capsule (Fig. 5), a vitreous hemisphere of conchiolin, consists of a right and a left half, firmly coalesced in a median suture as in all capsules of prosobranchs (Ankel 1936, p. 169). In many capsules the median suture corresponds to the greater diameter. It extends like a meridian over the capsule and corresponds to the sperm channel which separates the

two halves of the capsule gland. Functionally more important is the future opening of the capsule, a circular, latitudinal layer which divides the capsule into an upper opercular and a lower part (Fig. 6). The first is 0,18, the second 0,12 mm. high. The capsule is broadest at the bottom, viz. about 0,8 mm. The wall of the bottom, Ankel's foot plate (1937, p. 77), is 5 micra thick, that of the upper part 20-40 micra, and the lumen 0,5-0,6 mm. wide. The latter contains one egg or embryo without "nurse eggs" but floating in albuminous liquid evidently furnished by the albumen gland.

The wall of the capsule (Fig. 6) is composed of the same layers as in **Nucella lapillus** (Ankel 1937, p. 79), an innermost fibrous one, a homogeneous layer, one with longitudinal and one with radial fibres. The innermost and the homogeneous layers are contiguous, while they are separated from one another in **Nucella**. As both stain with light-green and the homogeneous layer is thick, we suppose that they derive from the acidophilous secretion of the greater, inner region of the capsule gland. Certainly they correspond to Ankel's inner pellicle and inner layer. These layers can be peeled off from the outer ones. These are the middel and the outer layer of Ankel's terminology. The longitudinal fibres of the first, which is firm and thin, and the radial fibres of the second are recognizable in clarified empty capsules. Middle and outer layer are basophilous. Therefore their origin might be traced from the basophilous secretion of the glands in the smaller, outer region of the capsule gland. Also the cement which attaches the capsule to the substratum may be a product of this region. The faecal particles from the internal vesicle are embedded in the outer layer of the operculum.

The opercular border is probably produced by the blue staining glands which are disposed in two antero-posterior stripes opposite to one another in the middel of the inner region of the capsule gland. The limiting layer between lid and lower part of the capsule is gradually dissolved and the entire lid falls off (Fig. 4) when the veliger hatches. The surface of the operculum is sculptured with about 5 micra high raised

lines which are 20-30 micra distant from one another (Fig. 4-6). They run more or less parallely to the median suture and their course is wrinkled by the radial fibres of the outer layer disposed transversely to the ridges. The latter are united at their ends generally forming a right angle with the suture. But if the capsule had lain obliquely in the moulding pedal gland, the direction of the ridges is not correlated with that of the median suture. This suture continues over the lower part of the capsule (Fig. 6). Here the ridges are circular, parallel to those of the lid, but straight not wrinkled, though radial fibres are present also here in the outer layer of the wall. The bottom of the capsule is smooth and consists of inner fibrous layer, homogeneous layer, and cement.

We were not interested in the cleavage and the early development in the present study. The eggs are rich in yolk as those of other Stenoglossa. Hence the micromeres occupy a limited and concentrated area over the macromeres (cf. Pelseneer 1906, p. 24). The two and four blastomeres resulting from the first and second cleavage respectively are of equal size. Thus segmentation corresponds to the **Crepidula**-type; a yolk sac (Ankel 1936, p. 182) or polar lobe (Korschelt 1936, p. 869) does not occur.

In order to observe the embryos and obtain the larvae the egg capsules were isolated in small petri dishes. The temperature will not have deviated much from the natural condition, where it is colder at night and warmer when low tides coincide with sunny days. The oxygen supply in the dishes however differed widely from the thoroughly aerified superficial layer of the sand in the zone of the neap tides where the snails live. Therefore our data concerning the duration of the embryo's life in the capsule and that between hatching and metamorphosis only illustrate the plasticity of these processes and cannot be considered averages. The embryonal development lasted 8-9 days. The embryo began to rotate on the 3rd day and had a small bipartite velum on the 4th. Some veligers metamorphosized 2 hours after hatching (Fig. 14), others had a free living stage of about a week. To judge from its behaviour in our

dishes the veliger is not pelagic, but swims at the bottom. It lives upon its yolk (Fig. 13, y) and evidently does not feed.

The newly hatched veliger (Fig. 9) has a quite colourless shell (Fig. 7; 9, x) without any sculpture as it occurs in **Buccinum** (Dakin 1912, f. 64; Portmann 1925, f. 7) and many other prosobranchs (Vestergaard 1935, f. 2, 5, 7A; Thorson 1946, f. 104 A, 130 C, 137 C-F; Rasmussen 1951, f. 8, and others). The width of the shell is 0,4 mm., its height 0,32 mm. The large velar area bears two short tentacles and black eye spots (Fig. 11, z); the cells of the velum (v) contain brown pigment. The statocysts (Fig. 13, t) lie in the region behind the velum. Coloured larval kidneys are not developed nor could we find such organs (Portmann 1930) in the sections. The nuchal sinus (Pelseneer 1906, p. 135) or larval heart (Dakin, l. c., f. 64, Pul) is recognizable (Fig. 9, e) dorsally to the velum at the mantle border (Fig. 13, mi). Its muscle fibres appear in the sections, but the organ that visibly beats in the veliger is the definitive heart (Fig. 9, h) which lies beside the kidney (Fig. 13, n). Also the organs of the pallial cavity (l), a voluminous osphradium (r), a ctenidium (b) with 6 or more leaflets, and a hypobranchial gland are developed. The ganglia of the central nervous system (c, d) are already connected with one another, even the roots of the visceral loop (su, u) are united with the pleural ganglia. The latter do not lie in the plane of the section drawn in Fig. 13. The propodial ganglia (ro) begin to emit nerves into the foot, whose propodium (o) is delimited by a constriction and provided with an anterior gland (g). Three days after hatching the parapodial flaps (Fig. 11, q) are distinct. The stomach (Fig. 13, so) and the two sacs of the digestive gland are in open communication with the yolk (y) which in the living veliger conceals the columellar muscle (w). The fore-gut (i) and the intestine are simple tubes.

RÉSUMO

Olivella verreauxii (Ducros 1857) foi criada no laboratório da Base Norte (Dr. Edmundo Nonato) do Instituto Oceanográfi-

co. Cópula, ovipostura, e larva foram descritas. Os espermatozóides ejaculados foram encontrados entre os folhetos branquiais, de onde sobem para o receptáculo seminal. Os grumos fecais armazenados na vesícula interna da fêmea são grudados no opérculo da cápsula ovular por secreção da vesícula. Está, destarte, marcada a face dirigida para a água por finos grânulos e diferenciada da face lisa cimentada ao substrato.

REFERENCES

For literature see our paper in this Bulletin (Marcus, E. & E. 1959) and add:

ANKEL, W. E. 1937, Der feinere Bau des Kokons der Purpurschnecke Nucella lapillus (L.) und seine Bedeutung für das Laichleben. Zool. Anz. Suppl. v. 10 (Verh. D. Zool. Ges. 1937), p. 77-86, 12 text-figs. Leipzig.

KORSCHELT, E. 1936, Vergleichende Entwicklungsgeschichte der Tiere (Korschelt & Heider, new ed.), v. 2, p. 537-1314, f. 561-1312. Jena (G. Fischer).

MEISENHEIMER, J. 1921, Geschlecht und Geschlechter im Tierreiche, v. 1. XIV + 896 p., 737 text-figs. Jena (G. Fischer).

PELSENEER, P. 1906, Mollusca. E. Ray Lankester, A Treatise on Zoology, pt. 5, 355 p., 301 figs. London (Adam & Charles Black).

PORTMANN, A. 1925, Der Einfluss der Nähreier auf die Larvenentwicklung von Buccinum und Purpura. Zeitschr. Morphol. Oekol. v. 3, fasc. 4, p. 526-541. 18 text-figs. Berlin.

PORTMANN, A. 1930, Die Larvennieren von Buccinum undatum. Zeitschr. Zellforsch. mikr. Anat. v. 10, fasc. 2, p. 401-410, text-figs. 1-10. Berlin.

RASMUSSEN, E. 1951, Faunistic and Biological Notes on Marine Invertebrates. II. Vidensk. Meddel. Dansk Naturh. Foren. v. 113, p. 201-249, 29 text-figs. Köbenhavn.

THORSON, G. 1946, Reproduction and larval development of Danish marine bottom invertebrates. Medd. Komm. Danm. Fisk. & Havunders. ser. Plankton v. 4, n.º 1, p. 1-523, 196 figs. Köbenhavn.

VESTERGAARD, K. 1935, Ueber den Laich und die Larven von Scalaria communis (Lam.), Nassarius pygmaeus (Lam.) und Bela turricola (Mont.). Zool. Anz. v. 109, fasc. 7-8, p. 217-222, 7 text-figs. Leipzig.

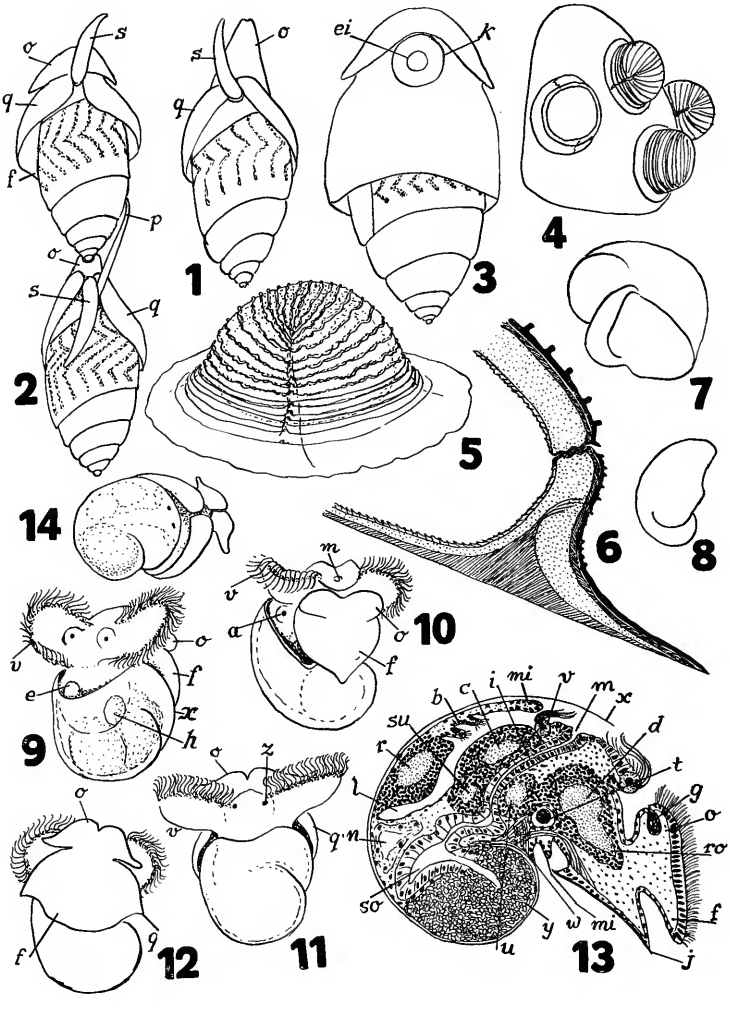
PLATE

PLATE 1

Olivella verreauxii

Fig. 1 — Male scenting female.
Fig. 2 — Mating couple.
Fig. 3 — Female moulding capsule.
Fig. 4 — Shell of **Donax** with 3 capsules and an empty lower part.
Fig. 5 — Egg capsule.
Fig. 6 — Section of same.
Fig. 7 — Shell of veliger.
Fig. 8 — Operculum of same.
Fig. 9 — Newly hatched veliger, dorsal view.
Fig. 10 — Ventral view of same.
Fig. 11 — Three days old veliger, dorsal view.
Fig. 12 — Ventral view of same.
Fig. 13 — Sagittal section of veliger.
Fig. 14 — Recently metamorphosized snail.

a — anus. b — ctenidium. c — cerebral ganglion. d — pedal ganglion. e — larval heart. ei — egg. f — foot. g — foot gland. h — definitive heart. i — fore-gut. j — operculum. k — egg capsule. l — mantle cavity. m — mouth. mi — mantle border. n — kidney. o — propodium. p — penis. q — parapodium. ro — propodial ganglion. s — siphon. so — stomach. su — supra-intestinal ganglion. t — statocyst. u — subintestinal ganglion. v — velum. w — columellar muscle. x — shell. y — yolk. z — eye.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ON GEOPLANIDS FROM BRAZIL

by Claudio G. Froehlich

(with 11 plates)

In June-July, 1953 my wife and I made an excursion to the southern states of Brazil to collect land planarians and, especially, to try to find again some of the species of **Geoplana** described by Fritz Müller, 1856, and by Graff, 1899. As regards the latter aim, our success was mediocre, for at Blumenau and nearby localities we found only five **(G. mülleri, G. schultzei, G. atra, G. marmorata,** and **G. pulchella)** of Müller's 13 species, and two **(G. ladislavii** and **G. polyophthalma)** of Graff's. In Taquara, State of Rio Grande do Sul, where Hermann von Ihering collected several species he sent to Graff, the land planarian fauna was very scarce, perhaps on account of the very cold winter of that year, and we succeeded in collecting only 3 specimens of **G. ladislavii** Graff. In this paper are also included land planarians collected at Blumenau by Lic. Natalia Gabrusewycz and her father, Mr. Oleh Gabrusewycz, and in the State of Rio Grande do Sul by Prof. Dr. J. Hauser, S. J., and Prof. Dr. R. Gliesch, to all of whom we are grateful. The last species described in this paper was collected by Mr. Johann Becker in the environs of Salvador, State of Bahia. It is the second species known from that State, the first being **G. flava** Moseley. At the end of the systematic part are found some remarks on the status of Fritz Müller's species of **Geoplana.**

To the National Research Council (Conselho Nacional de Pesquisas) we are specially thankful, for its grants have made possible our excursion.

List of the species appearing in this paper:

1. **Geoplana marmorata** Fritz Müller
2. **G. mülleri** Diesing
3. **G. abundans** Graff

4. **G. carrièrei** Graff
5. **G. ladislavii** Graff
6. **G. pseudorhynchodemus** Riester
7. **G. quagga** Marcus
8. **G. tapetilla** Marcus
9. **G. velina** C. G. Froehlich
10. **G. apeva,** n. sp.
11. **G. assu,** n. sp.
12. **G. fita,** n. sp.
13. **G. gaucha,** n. sp.
14. **G. glieschi,** n. sp.
15. **G. hauseri,** n. sp.
16. **G. nataliae,** n. sp.
17. **G. suva,** n. sp.
18. **Choeradoplana iheringi** Graff
19. **Geoplana beckeri,** n. sp.

The types of the new species are deposited at the Departamento de Zoologia da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo.

GEOPLANA MARMORATA **Fritz Müller**

Geoplana marmorata Fritz Müller, 1856, p. 25 [Blumenau, S. C., Brazil].

Geoplana rufiventris, Graff, 1899, p. 294 (part.).

Localities: Rio do Testo, 2 specimens, June 28, 1953.
Blumenau, 3 specimens, July 2, 1953.

Measures, in mm., of three sectioned worms:

Length	Width	Mouth	Gonopore
84	9.5	61	73
50	9	35.5	42
50	7	35.0	43

Our larger specimens attained, creeping (Fig. 1), a length of 100 mm. by a width of 7 mm. A smaller specimen was 60 mm. long by 5 mm. broad. At rest (Fig. 2) they are shorter and broader, with wavy margins.

In the creeping worms, the body broadens gradually from the rounded anterior tip backwards, attaining its maximal width shortly in front of the pharynx. From this point, the margins are almost parallel down to the abrupt posterior narrowing.

The dorsal ground is light brown with a pinkish tint; this tint increases towards the cephalic end, which is reddish, both dorsally and ventrally. On the ground there are numerous dark brown spots, aggregated into irregular strips, which give the worms a marbled appearance (Fig. 3). At the cephalic region the strips are prevailingly longitudinal, on the rest of the back they are more or less oblique. The more mesial spots are commonly smaller but more crowded, especially in the region of the pharynx and copulatory apparatus. A spot-free median line may be present or not. The ventral side is light grey with pinkish-brown borders.

In the largest worm, the small eyes are marginal and crowded in the first 20 mm. (Fig. 8); backwards (Fig. 9) they spread on the dorsal side to a maximum of 1/4 of the body width on each side but commonly less; from about 60 mm. from the anterior tip backwards they get gradually more thinly scattered.

The pharynx (Fig. 4) is cylindrical, with the ventral insertion more anteriorly placed than the dorsal, and with richly folded border.

The copulatory apparatus of the three measured specimens were sectioned (Figs. 5-7). The two smaller worms present both the male and the female genital organs mature; in the longest, however, the female organs are fully mature, but the male are not: the testes, e. g., present almost only the first stages of spermatogenesis, and there are only a few ripe sperms in the ectal part of the efferent ducts.

The seminal vesicle (s) has the form of an inverted U with the anterior arm longer and forked entally. Each branch of the fork receives one of the efferent ducts (d). The larger part of the vesicle is situated outside the main muscle coat of the penis bulb (b) but it is, nevertheless, encircled by some

fibres from the latter. The vesicle is not much dilated; it receives eosinophilous glands, and is lined by an epithelium provided with long cilia. The lining of the narrow ejaculatory duct (e) is similar. The penis papilla (p) is large, asymmetrical, irregular, and variable. In all the three sectioned specimens it is bent to the left in such a way that the atrium forms a depression or recess that extends from the left to the right. In two specimens (Figs. 5, 7) the ejaculatory duct opens into this recess; in the third (Fig. 6) it opens into the outer part of the atrium.

The oviducts (o) rise behind the gonopore (g). Shell glands (z) open into the ectal ascending and into the transverse portions of the oviducts, and into the common glandular duct (q). The latter is directed backwards and downwards and opens into a short vagina. The female atrium (f) is ample, with folded lateral walls. The whole genital atrium receives eosinophilous and cyanophilous glands, and is lined by a non-ciliated epithelium that is higher, pluriserial to pluristratified, in the female part.

Remarks: Our specimens of **Geoplana marmorata** are in part from the original locality and fit perfectly to the short description given by Fritz Müller. Graff (1899, p. 294) considered **G. marmorata** to be a synonym of **G. rufiventris** Fr. Müll. but his opinion cannot be held, because the two species present different colour patterns, **G. rufiventris** presenting a dark brown dorsal side and a brick red ventral side. By its external features, **G. marmorata** stands near the group of the large, broad and flat species, but it cannot be included in that group because of the irregular and asymmetrical form of the penis.

GEOPLANA MÜLLERI **Diesing**

Geoplana elegans Fritz Müller, 1856, p. 23 (non Darwin, 1844, p. 244) [Blumenau, S. C., Brazil].

Geoplana pallida Fritz Müller, 1856, p. 24 (non Darwin, 1844, p. 245) [Blumenau, S. C., Brazil].

Geoplana mülleri Diesing, 1862, p. 511; Graff, 1899, p. 333.

Geoplana schultzei Diesing, 1862, p. 512; Graff, 1899, p. 327.

Localities: Paranapiacaba, 40 km. SE from the city of São Paulo: 1 young specimen, possibly of the present species, Nov. 1, 1954.
Blumenau (type locality): 24 specimens, collected between June 23 and July 2, 1953.

Rio do Testo (formerly Pommerode), ca. 35 km. NNW from Blumenau: 1 specimen June 28, and one July 1, 1953.

Itajaí: 6 specimens, June 25, 1953.

Brusque: 5 specimens, June 26, 1953.

The specimens were found under fallen logs and in the leaf rosettes of fallen epiphytic Bromeliaceae.

Measures, in mm., of three preserved worms:

Locality	Length	Width	Mouth	Gonopore
Blumenau	51.0	2.3	30.0	38.2
Itajaí	53	2.5	30	38
Brusque	53.0	2.5	30.0	38.7

This is a lively species, presenting quick reactions when stimulated. Creeping (Figs. 11, 12), the body is long and slender, narrowing gently to both ends, more so to the anterior. The dorsal ground colour is light-yellow; the cephalic end, and commonly also the posterior, although to a lesser extent, are darker, orange to ferruginous. The ventral side is white bordered by the dorsal colour. The specimens from Blumenau and Rio do Testo (Figs. 12, 14) present on the back a black median longitudinal stripe and, on each side, a ferruginous one. At the cephalic end all the stripes merge into the ground colour; at the posterior, the stripes unite shortly before the tip. The specimens from the remaining localities lack the lateral reddish stripes. The worms from Itajaí (Figs. 11, 13) present a narrow median black line, those from Brusque (Fig. 10) a median stripe, broader than that of the worms from Blumenau. The young worm from Paranapiacaba is similar to the specimens from Brusque, but the stripe is relatively even broader.

Around the anterior end the eyes are uniserial; backward they spread on the dorsal side to about a third of the body width (Fig. 13). At about 2 cm. from the tip they get sparser, and from ca. 3 cm. on they are restricted to a marginal row (Fig. 14).

The pharynx (Fig. 15) is bell-shaped (glockenförmig).

The three sectioned specimens are mature. The efferent ducts (Figs. 16-18, d) are full of spermatozoa. Their final portions bend forward, upward and mesially to enter separately into the ental, upturned end of the large seminal vesicle (s). The walls of the approximately S-shaped vesicle are folded; the lining is a columnar epithelium provided with long cilia and traversed by numerous ducts of granular eosinophilous glands. On entering the muscle coat of the male atrium, the vesicle narrows to the short ejaculatory duct (e), which is lined by a ciliated cubical epithelium. The ejaculatory duct opens directly into the male atrium (a), a penis being absent. The male atrium presents a small number of large folds. Both its subepithelial muscles (muscularis) and the outer muscle coat are strong. The male atrium is lined by a low columnar, nonciliated epithelium. At its ental half (or less) the epithelium is irregular, presenting small projections into the atrial lumen; in this region open fine-grained eosinophilous glands. At the ectal half, where the epithelium is more regular, open more intensely staining eosinophilous glands together with some cyanophilous ones. In the specimen from Brusque (Fig. 18) the male atrium presents a ventral protuberance to which a large cluster of spermatozoa is attached. The epithelium has disappeared at the place of attachment, and the sperms, oriented at right angles to the surface, are in direct contact with a mass of eosinophilous secretion; the same secretion also covers partially the whole cluster. The same phenomenon has already been observed in **G. sexstriata** Graff (C. G. Froehlich, 1956, p. 317 fig. 7, p. 319, and p. 341), another species in which a penis is lacking. The mass of sperms should be considered a spermatophore, for it probably is deposited as a packet into the fe-

male atrium or vagina of the other individual during copulation.

The vitellaria are mature in the three sectioned worms. The oviducts (o) begin to rise in front of the gonopore, ascend slanting backwards, and bend mesially to unite into the common oviduct (q). Shell glands open into the final portions of the paired oviducts and, excepting a very short ectal portion, into the common oviduct (common glandular duct). The common oviduct is directed backward and downward; ectally it is continuous with the slightly wider, nonciliated vagina. The vagina bends forward to open into the small female atrium (f). The vagina and female atrium are lined by an epithelium similar to that of the ectal part of the male atrium but both, the vagina chiefly, receive a greater number of cyanophilous glands. The male and female atria are not sharply delimited, the gonopore canal, which presents much folded walls, issuing between the two.

Remarks: Fritz Müller (1856) described as different species the specimens with a median black stripe and a pair of lateral ferruginous ones **(Geoplana elegans)**, and those provided with a narrow median stripe and lacking the lateral ones **(G. pallida)**. These species became homonyms of **Planaria elegans** and **P. pallida** Darwin, 1844. Diesing, 1862, renamed them **G. mülleri** and **G. schultzei,** respectively. Our specimens from Blumenau and Rio do Testo fit perfectly to Müller's description of **G. elegans,** while those from the environs of the harbour of Itajaí fit to **G. pallida**. The specimens from Brusque are similar to those from Itajaí, but the median stripe is much broader. The anatomical uniformity of the three forms led us to consider them a single species, **G. mülleri,** which has priority over **G. schultzei** because it preceded the latter in the same paper. To ascertain wether the different forms represent subspecies or varieties would require an intensive investigation of the region where they occur, not possible during our brief stay there. As regards the young worm from Paranapiacaba, its definitive identification must await the finding of mature specimens.

G. mülleri, concerning the general topography of the copulatory apparatus, stands near **G. marginata,** Graff (not Fr. Müll.), but none of our specimens showed the formation of an atrial copulatory papilla as in the latter. The shape of the body and of the pharynx are also similar in both; the coloration, however, is different.

GEOPLANA ABUNDANS **Graff**

Geoplana marginata, var. **abundans** Graff, 1899, p. 306 ["Taguara do mundo nuovo, Prov. Rio Grande do Sul", Brazil].

Locality: São Leopoldo, R. G. S.: 2 specimens; Prof. Dr. J. Hauser, S. J., col.

What Graff called **G. marginata** cannot be Fritz Müller's species, as will be discussed later. Among the specimens H. von Ihering sent him, however, there was one with seven stripes, which Graff named **G. marginata,** var. **abundans.** Through the kindness of Prof. Hauser we received two specimens of a seven-striped species which agree very well with Graff's variety. This species should, therefore, be called **G. abundans** Graff. Its anatomy, as well as its relations to the species Graff, and after him several authors, called **G. marginata,** will be presented and discussed in a future paper.

GEOPLANA CARRIÈREI **Graff**

Geoplana carrièrei Graff, 1897, p. 2; 1899, p. 315 [Missión d'Aguairenda, Chaco Boliviano].
Geoplana carrièrei Marcus, 1951, p. 62.

Locality: São Leopoldo, State of Rio Grande do Sul, 5 specimens; Prof. Dr. J. Hauser, S. J., col.

Measures, in mm., of three sectioned specimens:

Specimen	Length	Width	Mouth	Gonopore
a	53.7	5.0	37.1	45.1
b	57.7	6.6	37.6	45.9
c	54.6	7.0	34.5	42.5

Only specimen **a** presents the typical coloration: reddish cephalic region and dark brown dorsal side. In the remaining worms, the anterior end, although usually lighter than the brown, olive-brown, or brownish-black dorsal side, lacks the conspicuous reddish hue. The ventral side is grey or brownish-grey.

The distribution of the eyes agrees with the description of Marcus, 1951. In specimen **a,** however, the maximum spread of the eyes is only about one tenth of the width of the body on each side, whereas usually the eyes spread to one third or one fourth. The diameter of the larger pigment cups varies from 50 to 60 μ in different specimens.

The anatomy of the pharynx and of the copulatory apparatus are also in agreement with Marcus's (1951, p. 62) description. The penis is asymmetrical, the insertion of the papilla being displaced to the right, a condition easily seen in the cleared worms. The degree of the asymmetry varies in the three examined specimens. Specimen **b,** which presents spermatozoa in the efferent ducts, but in which vitellaria are still undeveloped, is the most strongly asymmetrical, the penis papilla being almost transverse. Specimens **a** and **c,** of which **a** is younger than **b,** and **c** is fully mature, are much less asymmetrical, an indication that the degree of asymmetry is not related to age. In both these specimens the ejaculatory duct opens into a bowl-shaped depression on the ventral surface of the penis papilla.

GEOPLANA LADISLAVII **Graff**

Geoplana ladislavii Graff, 1899, p. 300 ["Taguara do Mundo nuovo, Prov. Rio Grande do Sul", Brazil].

Localities: Blumenau, S. C.: 6 specimens between June 23 and July 2, 1953.
1 specimen, Oct. 3, 1955; Gabrusewycz col.
Taquara, R. G. S. (Type locality): 3 specimens, July 7, 1953.
São Leopoldo, R. G. S.: 1 specimen, Sept. 1955; Prof. Dr. J. Hauser, S. J., col.

Creeping, medium-sized worms are 50 mm. long by 5 mm. broad. The measures, in mm., of four preserved worms are:

Length	Width	Mouth	Gonopore
50	8	33.5	41
39	9	21.5	27.5
38	8	25	31
36	7.5	20	26

The specimens from Blumenau presented an olive-green dorsal side with darker spots over the testes and pharynx (Fig. 19). The broad creeping sole was translucent white, through which could be seen the gut diverticula and the efferent ducts. The dorsal side of the worms from Taquara was brownish-green.

Distribution of the eyes according to Graff's description.

The copulatory apparatus of two worms were sectioned (Figs. 24-25). The efferent ducts (d) open into paired portions of the seminal vesicle (s). These are directed upward and mesially, and unite into the common vesicle, which shortly on turn backwards, and penetrate into the penis bulb (b). The extrabulbar portion of the vesicle has a relatively weak muscular coat, independent from the bulb musculature. Within the bulb, the vesicle changes gradually into the ejaculatory duct, which presents a narrower lumen. Both vesicle and ejaculatory duct receive eosinophilous glands and are lined by an epithelium provided with long cilia. The penis papilla (p) is muscular, massive, and traversed centrally by the ejaculatory duct, which opens at its tip. Both papilla and genital atrium are lined by a columnar nonciliated glandular epithelium, especially high in the female atrium, where it may attain 200 μ. The male atrium presents a small dorsal fold which partly separates it from the female.

Vitellaria mature in both specimens. The oviducts (o) rise caudally to the gonopore (g), and unite into a short common oviduct (q) directed ventrally, which opens into the vagina. Shell glands (z) open into paired ectal portions and into common portion of oviducts. The female atrium (f) is ampler

in one specimen (Fig. 24) than in the other (Fig. 25); the vagina turns dorsally in both. The gonopore canal issues from the anterior part of the female atrium.

Remarks: This species, easily recognized by its uncommon green colour, is one of the best studied by Graff, 1899. Our specimens were collected at the same localities as Graff's. Graff had larger specimens than we did, some reaching 100 mm. when creeping, and being up to 65 by 7 mm., preserved. On the whole Graff's description applies well to our material, the chief differences following:

The numerous granules, 1-2 μ in size, which Graff (l. c., p. 21, footnote) observed on the epidermis, in the peripheral parenchyma, and some also inside the epidermal cells, and which he suggested could be symbiotic green algae, were not present in our material. Only some secretion granules of the size indicated by Graff are present in the peripheral parenchyma; no algal cells could be seen. Graff's suggestion should, on these grounds, be dismissed. The granules he saw are probably only secretion granules. The nature of the pigments which produce the green colour remain unknown.

In Graff's drawing of the copulatory apparatus, the seminal vesicle is shown as being totally intrabulbar, and the efferent ducts as having a longer ascending portion. The female atrium is smaller than in our sectioned specimens, but in one of ours it is also smaller than in the other.

G. ladislavii belongs to group B of Brazilian Geoplanas (E. M. Froehlich, 1955, p. 328), distinguishing itself from the other species of the group by its green colour.

GEOPLANA PSEUDORHYNCHODEMUS **Riester**

Geoplana pseudorhynchodemus Riester, 1938, p. 32 [Teresópolis, R. J., Brazil].

Geoplana pseudorhynchodemus, Marcus, 1951, p. 76.

Locality: Blumenau, S. C.: 8 specimens, June 23 — July 2, 1953.

GEOPLANA QUAGGA Marcus

Geoplana quagga Marcus, 1951, p. 97 [São Paulo, S. P., Brazil].

Locality: Blumenau, common in vacant lots in the town. We collected several specimens in June, 1953; N. Gabrusewycz, 2 specimens in July, 1955.

GEOPLANA TAPETILLA Marcus

Geoplana tapetilla Marcus, 1951, p. 98 [Piraçununga, S. P., Brazil].

Localities: Blumenau and Itajaí, S. C.: a common species in vacant lots, under bricks, boards, etc.

GEOPLANA VELINA C. G. Froehlich

Geoplana pulchella, du Bois-Reymond Marcus, 1951, p. 234 (non Fritz Müller, 1856, p. 25) [Brusque, S. C., Brazil].
Geoplana velina C. G. Froehlich, 1955b, p. 190.

Locality: Blumenau, S. C.: one immature specimen, July 22, 1955; N. Gabrusewycz col.

GEOPLANA APEVA, n. sp.

Localities: Blumenau, S. C. (type locality): 2 specimens, June 24, 1953.
1 specimen, Oct. 3, and 1, Dec. 1, 1955; Gabrusewycz col.
Brusque, S. C.: 1 specimen, June 26, 1953.

Measures, in mm., of three specimens:

Specimen	Locality	Length	Width	Mouth	Gonopore
a	Blumenau (1953)	53.5	10	36.5	43.5
b	Blumenau (1955)	77	9.5	50	61
c	Brusque	67	7.5	47	54.5

Creeping, specimen **a** was 75 mm. long by 9 mm. broad; specimen **c**, 85 mm. by 6 mm., respectively.

Creeping (Figs. 26, 30), the worms present a slightly carinate dorsal side, and the body tapers very gradually to the anterior end, less so to the posterior; both ends are sharp. At rest, the body may be kept as in Fig. 28 or much shortened (Fig. 30).

The dorsal side varies from brown to black in different specimens, and is provided with a light longitudinal stripe. The dark colour is darker at the limit with the median stripe, lighter at the margins of the body. The median stripe varies from a deep yellow to a dirty white. The youngest specimen is lighter, umber in colour, what could be an indication that the worm darkens with age. The ventral side (Fig. 27) is light orange to brick red with bluish-grey margins. The anterior end, and, in some specimens, a zone around the orifices of the body are also grey.

In the cephalic region (Fig. 31) the eyes lie crowded at the margins; at ca. 2 cm. from the tip they spread, in one specimen, to ca. one ninth of the body width on each side (Fig. 33); in the other specimens, to about a fourth or a fifth (Fig. 32). The eyes are surrounded by small light halos. The diameter of the larger pigment cups is 55 μ.

The pharynx (Fig. 34) is collar-shaped, with a small number of primary folds.

All the three sectioned worms are incipiently mature. Only specimen **c** has some spermatozoa inside the efferent ducts (Fig. 36, d). These ducts open into a pair of short lateral processes of the extrabulbar seminal vesicle. The vesicle (s) is tubular; in specimens **a** and **c** it presents a loop directed dorsally, in specimen **b** (Fig. 35) it is straighter. The ejaculatory duct (e) is somewhat narrower than the vesicle. The penis has a strong musculature, the bulb (b) is sharply delimited, and the papilla (p) is conical, rather long, filling up the greater part of the genital atrium. The length of the papilla in the sectioned specimens is, respectively: **a**, 0.7 mm.; **b**, ca. 2 mm.; **c**, ca. 1.2 mm.. The gonopore (g) lies before the middle of the atrium.

Vitellaria still absent in all specimens. The oviducts (o) rise behind the gonopore and unite into a short common oviduct

(q) that opens into the short vagina (v). Shell glands, present in an incipient stage only in specimen **b**, open into the final portions of the paired, and into the common oviducts. The vagina, located at the ental part of the female portion of the genital atrium, is directed upwards.

Remarks: The colour pattern of the ventral surface of the specimen of **Geoplana apeva** from Brusque is identical to one of Graff's figures of **G. rufiventris** (Graff, 1899, pl. 1 fig. 22; also, 1913, pl. 33 fig. 3). **G. rufiventris** Fr. Müll. is a uniformly brown species which occurs in the same region. **G. rufiventris,** Graff, is an heterogeneous assortment of worms from various localities. **G. apeva** distinguishes itself from all of them by its longitudinal light stripe on the back.

Within the group of the large, broad and flat species, the colour pattern of the dorsal surface of **G. apeva** is similar to those of **G. maximiliani** Fr. Müll. and **G. catharina** Hyman. The former was described by Müll. as presenting, among other characters, a yellowish longitudinal stripe ("Längsbinde"), and a nearly spherical penis. The colour pattern alone would indicate the identification of our material to Müller's species, but as the penis in **G. apeva** is an elongated cone and, besides, as **G. apeva** seems to be a broader species than is indicated by Müller, we think it wiser to consider **G. apeva** distinct from **G. maximiliani**. The copulatory apparatus of **G. apeva** is not inconsistent with that of **G. catharina** Hyman but the pharynx of the latter was described as "simple tubular", whereas that of **G. apeva** is collar-shaped.

GEOPLANA ASSU, n. sp.

Localities: Blumenau, S. C. (type locality): 4 specimens, June 23 — July 2, 1953.

Rio do Testo, S. C.: 1 specimen, July 1, 1953.

Measures, in mm., of three sectioned worms:

Specimen	Length	Width	Mouth	Gonopore	Penis Papilla: Length	Diameter
a	104	9	66	80	7.3	1.2
b	100	10	60	75	1.5	1.8
c	84	10	56	69	4.6	1.2

Specimen **b** had an egg capsule inside the genital atrium (Fig. 49). The larger specimens were 110 mm. long by 9 mm. broad, creeping (Fig. 38).

A large, flat and broad species. Creeping, there appears along the middle of the back a low keel, the margins of the body are subparallel, and the body tapers gradually to the anterior end, more abruptly to the posterior end. At rest, the body becomes much shorter and broader (Fig. 37). To the naked eye, the dorsal side is dark grey with lighter margins and with a darker line along the median keel; the ventral side is brown with dark grey border. Magnified (Fig. 41), the dorsal colour pattern appears as numerous close-set small dark grey spots on a light brown ground; the ventral, as fine dark brown pigment dots evenly distributed.

The eyes circle the anterior tip in an irregular row (Fig. 40). Backwards they increase in number and spread progressively on each side to about one fourth of the dorsal surface (Fig. 41). Shortly in front of the pharynx they begin to get sparser. The eyes are surrounded by small light halos.

The pharynx (Fig. 42) is collar-shaped but the pharynx pocket extends well beyond the caudal insertion of the pharynx.

A pair of ventrolateral processes of the seminal vesicle receive each an effernt duct (Figs. 43, 44, and 49, d). The ental part of the seminal vesicle is slightly dilated and vertical. The ectal, tubular and bent, enters the penis bulb (b) and continues as the ejaculatory duct (e). Both the seminal vesicle and the penis bulb are small in relation to the size of the penis papilla (p). The vesicle, as well as the ejaculatory duct, receive eosinophilous glands. The penis papilla is long, filling up the genital atrium (a), and is traversed by the sinuous ejaculatory duct. In specimen **a** (Fig. 43) the papilla is much

extended, pushing before it the posterior wall of the genital atrium, and displacing the vagina (v), which normally issues from the posterior end of the atrium, to a dorsal position. In specimen **b** (Fig. 49), due to the presence of an egg capsule inside the greatly enlarged genital atrium, the papilla is strongly contracted. The penis is highly muscular, the muscularis of the seminal vesicle, of the ejaculatory duct, and of the papilla being particularly well developed. The epithelium of the penis papilla is nonciliated, irregular, and traversed by numerous ducts of weakly eosinophilous glands and of some cyanophilous ones; around the root of the papilla open more strongly eosinophilous glands. The atrial epithelium is also nonciliated, higher and more irregular on the female side; both eosinophilous and cyanophilous glands, not numerous, discharge into the atrium. The gonopore is located approximately at the end of the first third of the atrium.

Under a fallen log, near one of the collected specimens, we found an egg capsule 7-8 mm. in diameter. Nine days later hatched from it 5 young worms (Fig. 39), almost certainly of the present species. Creeping, the largest was 18 by 3 mm., the smallest, 12 by 2.5 mm. Along the middle of the back ran a deep yellow stripe; the rest of the back was pink to wine-coloured, with dark grey margins in some specimens.

The vitellaria are mature in specimens **b** and **c**, spent in specimen **a**. The oviducts (o) rise obliquely well beyond the gonopore, at the sides of the last third of the genital atrium, then run mesially to unite into the common glandular duct (q). Shell glands (z) open also at the ectal ascending and at the transverse portions of the oviducts. The common glandular duct is long, directed backwards and downwards, and is continuous with the vagina. The latter is a tubular extension of the genital atrium.

Remarks: Geoplana assu is similar, as regards the size, shape, and colour pattern, to **G. carinata** Riester and **G. olivae** Marcus. It differs from these two species in its more homogeneous coloration, especially of the ventral side (it is true, however, that in some rare specimens of **G. carinata** the ventral

side is not spotted). Also, the young of **G. carinata** are more drab coloured (those of **G. divae** are still unknown). As regards the copulatory apparatus, **G. assu** must be distinguished from the two named species because the penis papilla is, normally, two to more than three times longer than in both **G. carinata** or **G. divae**.

G. catharina Hyman, 1955, also from the State of Santa Catarina, agrees well in the distribution of the eyes, the colour pattern (except the light median line), and in other external characters with **G. assu**. The copulatory organs are also similar. However, the pharynx, examined by Hyman only in the cleared worm, is described as simple tubular, whereas that of **G. assu** is collar-shaped.

G. catharina, G. apeva, and **G. assu,** as regards size and shape of the body, belong undoubtedly to the group of the large, broad and flat species (Group B of E. M. Froehlich, 1955, p. 328). The copulatory apparatus also conforms to the general types of the group, excepting, particularly in **G. assu,** the large size of the penis papilla. The inclusion of these species in group B makes natural the inclusion of **G. burmeisteri** M. Schultze too, whose remaining characters agree with those of this group.

GEOPLANA FITA, n. sp.

Locality: Blumenau, S. C.: 5 specimens, June 23 — July 2, 1953.

Measures, in mm., of two sectioned specimens:

Length	Width	Mouth	Gonopore
100	2.3	64	80
64	1.6	40	50

A very long and slender species (Fig. 45), the bigger of our specimens being 110 mm. long by 2 mm. broad, creeping. The margins are parallel along the greater part of the body; the anterior tapering is gradual, the posterior more rapid; both ends are blunt. The dorsal ground colour is light straw-

yellow, a little darker at the margins; at the cephalic region it acquires a ferruginous tint, also darker at the margins, and at the anterior tip it darkens even more, becoming brownish. On the back, there are two pairs of narrow longitudinal ferruginous stripes, a darker mesial pair and a lighter submarginal one. At the cephalic region (Fig. 46) there is a fine median dark line. All the stripes end, without fusion with the others, near to the extremities of the body, except the submarginal, which, one specimen excepted, unite at the posterior end (Fig. 46). The ventral side is white.

The eyes (Fig. 47) are marginal, in one row. The pigment cups present commonly a diameter of 21-26 μ.

The pharynx (Fig. 48) approaches the campanuliform (glockenförmig) type of Graff. Its border is richly folded.

The efferent ducts (Figs. 50, 51, d) full of spermatozoa in both sectioned specimens, turn mesially near to the copulatory complex and open at the lateral walls of the seminal vesicle (s). The vesicle is very long, extending, in one specimen (Figs. 51-52) for 4.2 mm. in front of the openings of the efferent ducts. The lining epithelium of the vesicle is cubical, ciliated, and traversed by ducts of numerous eosinophilous and sparse cyanophilous glands. On entering the muscle coat of the male atrium, the seminal vesicle narrows to the ejaculatory duct. The ectal half of this duct is narrower, and more regular in form than the ental half. A penis papilla is absent, the ejaculatory duct opening dorsally into an atrial recess bounded by folds which take the shape of a copulatory papilla. Numerous cyanophilous glands (y), interspersed with eosinophilous ones, open on these atrial folds. The male atrium (am) is rather elongated, with folded walls, the more ectal dorsal fold (x) separating it from the common genital atrium. This fold runs diagonally, so that to one side the gonopore canal leads directly to the male, to the other side to the common atrium. The male atrium is lined by a nonciliated cubical to low columnar epithelium. Besides the special glands already referred to, it receives eosinophilous and, ectally, cyanophilous glands.

The yolk glands are mature in both specimens. The oviducts (o) rise at the sides of the gonopore (g), turn mesially, and unite into the common glandular duct (q). The transverse glandular duct is long, dorsally situated, and directed backwards; it opens dorso-posteriorly into the female atrium (f). The female atrium, separated from the common by one or by a pair of lateral slanting flaps (fl), is lined by an irregular pluristratified epithelium provided with lacunae into which accumulates the secretion of numerous subepithelial cyanophilous glands. The common atrium receives sparser cyanophilous glands and is lined by a simple, nonciliated epithelium with irregular border toward the female atrium.

Remarks: The species that stands closest to **Geoplana fita** is **G. caissara** E. M. Froehlich, but the former is much longer and more slender than **G. caissara,** and has four dorsal longitudinal dark stripes, against five on the latter. As regards the copulatory organs, the seminal vesicle of **G. caissara** is longer, provided with numerous irregular projections, and it forks at the anterior portion into two branches, which may lie at the sides of the pharynx pocket; in **G. fita** the vesicle presents no projections and its anterior end, not forked, stands at a distance from the pharynx pocket.

GEOPLANA GAUCHA, n. sp.

Locality: São Salvador, Montenegro municipality, R. G. S.: 2 specimens, Sept. 1955; Prof. Dr. J. Hauser, S. J., col.. According to the collector, a common species at that locality, and also at Pôrto Alegre, capital of the State.

Measures, in mm., of the preserved specimens:

Length	Width	Mouth	Gonopore
26.0	4.0	16.5	20.5
22.5	3.2	15.3	18.2

The ventral side is flat; the dorsal arched, but not high. A broad light-grey band, bordered by black, runs along the back. The width of the band is ca. 1/4 to 1/3 of the width of

the body. Inside the band there is a pair of brownish-grey stripes. The black border fades laterally to the colour of the lateromarginal portions of the back, which in the larger specimen is dark violet-grey, in the smaller, brownish-grey with a violet tinge. The cephalic end (Fig. 54) is grey like the margins, the stripes merging into the grey at a distance from the tip. At the posterior end, the stripes fade out before the tip, but the light zone reaches it. A light spot is present over the pharynx. The ventral side is greyish-cream with a narrow border of the dorsal colour; the anterior end is grey.

The cephalic eyes (Fig. 54) are marginal, in one row. At 3.5 to 4 mm. from the tip the eyes begin to spread onto the dorsal side; the maximum spread (Fig. 55) is slightly over one fourth of the body width on each side. The dorsal eyes are located in the centre of small light halos, almost invisible to the naked eye. The larger eye-cups are ca. 40 μ across.

The pharynx (Fig. 56) is typically cylindrical. The pharynx pocket is relatively small, with the mouth at its posterior part.

In the larger specimen, which is more mature than the smaller, the male genital system is well developed, but the female is still unripe. Except for some minor differences due to age, the anatomy of the copulatory apparatus of both specimens agree entirely. The description is based on the more mature specimen.

The seminal vesicle forks anteriorly into two branches (Fig. 57, s_1), and each receives laterally the corresponding efferent duct (d). The branches extend anteriorly to the vicinity of the pharynx pocket (t). The vesicle (s_1, s_2) receives eosinophilous and purple glands, is lined by a columnar nonciliated epithelium, and has a rather strong muscularis. On entering the weak penis bulb (b), the vesicle narrows to the ejaculatory duct (e), which has a weak muscularis, and which presents two portions. The ental one receives fine-grained purple glands and is lined by a nonciliated epithelium; the ectal one receives rare glands and its lining epithelium is ciliated. The ejaculatory duct

traverses the penis in a simple course, slightly bent downwards, and opens at the tip of the papilla (p). The penis papilla is small, cylindroid, and weakly muscular; it almost fills up the male atrium (a). The epithelium of the apical half of the papilla is low and insunk; toward the base it becomes normal, columnar, similar to that of the atrium. On the surface of the papilla open numerous eosinophilous glands of two kinds, one of coarser granulation and bright red, the other finer and with a bluish tint. At the base, chiefly dorsally, there are also cyanophilous glands. The male atrium narrows ectally and is distinct from the female atrium. It is lined by a nonciliated columnar epithelium and receives eosinophilous glands.

Vitellaria are still absent. The oviducts (o) rise at the level of the gonopore (g), turn mesially, and unite into the common glandular duct (q). Shell glands (z), still in early stages of development, open into the oviducts from the middle of the ascending portion on, and into the common glandular duct. This duct curves gently backward and downward, and opens into the female atrium (f). The lumen of the female atrium is restricted to a central narrow passage, all the rest of the atrium being filled by a compact mass of small cells (r). Some eosinophilous and cyanophilous glands traverse this mass of cells to open into the lumen. The gonopore canal issues between the two atria. It is asymmetrical, one side leading to the male atrium, the other to the female, the median part being common.

Remarks: The colour pattern of **Geoplana gaucha** is distinctive. Only **G. doederleini** Schirch presents a similar pattern, but it is much lighter, the median light zone is broader, and the latero-marginal zone is spotted.

The copulatory apparatus is very similar to that of **G. multicolor** Graff as regards the female part, the asymmetry of the gonopore canal, and the shape and size of the penis. The long paired extensions of the seminal vesicle, into which open the efferent ducts, is, besides the colour pattern, a disjunctive character.

GEOPLANA GLIESCHI, n. sp.

Locality: Iraí, R. G. S.: 1 specimen, Oct. 1953; Prof. Dr. Rudolf Gliesch col.

The preserved worm (Fig. 60) is 120 mm. long and ca. 10 mm. broad. The body is broad and flat, tapering at both ends; the margins are subparallel, and, along the greater part of the length of the body, they have rolled to the ventral side. The anterior tip is damaged. The mouth is located at 70 mm., the gonopore at 88 mm. from the anterior end.

The dorsal surface is black. In front of the pharynx the pigment had been rubbed off along two bands (Fig. 58), and in the cleared worm the testes could be seen in these regions as light spherules. The ventral side is light brownish-orange with dark borders.

The small eyes (Fig. 58) are located in the centre of small light halos. The eyes spread on the dorsal surface to a maximum of one seventh of the body width, on each side.

The pharynx (Fig. 59) is cylindrical, with caudally displaced dorsal insertion and folded border. The pharynx pocket is long, extending to the vicinity of the seminal vesicle.

The seminal vesicle (Fig. 61, s) is irregular, not much dilated, and lined by an epithelium provided with long cilia; it receives lightly stained purple glands. The ental part of the seminal vesicle extends each side as short transverse processes that receive the efferent ducts (d). Inside this transverse portion of the vesicle is found a mass of spermatozoa. On entering the small, muscular penis bulb (b), the diameter of the vesicle decreases as it becomes the ejaculatory duct (e). This duct, histologically similar to the vesicle but with few glands, traverses longitudinally the penis papilla, following the curve of the latter. The penis papilla (p) is rather long, muscular, and is bent to the ventral side; its covering epithelium is nonciliated, columnar, and is traversed by ducts of cyanophilous and of slightly stained glands, the latter occurring chiefly near the tip. The genital atrium (a) is lined by a nonciliated co-

lumnar epithelium higher in its female part, and it receives cyanophilous glands.

Vitellaria wholly mature. Shell glands (z) open into the ectal transverse portion of the oviducts (o) and into the common glandular duct (q); the latter is dorsal, directed backwards and downwards, and opens into the vagina, a narrow upturned portion of the female atrium (f). The strong muscle coat of the female atrium is independent from that of the male.

Remarks: Geoplana glieschi belongs to the group of the large, broad and flat species of **Geoplana** (Group B, E. M. Froehlich, 1955, p. 328). It presents, like the heterogeneous material classified as **G. rufiventris** by Graff, a rufous ventral side and a dark dorsal side. In **G. glieschi,** however, the dorsal coloration is a homogeneous deep black, against a spotted, marbled or brown one in Graff's **G. rufiventris**. The topography of the copulatory apparatus of **G. glieschi** conforms to that of Graff's description and figure of **G. rufiventris,** but the pharynges are different, cylindrical in **G. glieschi** and collar-shaped in **G. rufiventris.**

G. burmeisteri M. Sch., **G. applanata** Graff, **G. dictyonota** Riest., **G. itatiayana** Schirch, **G. apeva,** n. sp., and, in part, **G. assu,** n. sp. are species with a reddish ventral side and belong to the same group as **G. glieschi**. None of them has, however, a uniform black back. Besides, **G. burmeisteri** has a shorter and more cylindrical pharynx, **G. applanata** and **G. itatiayana** shorter and more massive penis papillae, **G. dictyonota** intrabulbar seminal vesicle, and **G. apeva** and **G. assu** collar-shaped pharynges.

GEOPLANA HAUSERI, n. sp.

Locality: São Leopoldo, R. G. S.: 1 specimen, Jan. 1955; 3 specimens, Oct. 1956; Prof. Dr. J. Hauser, S. J., col.

Measures, in mm., of the preserved specimens:

Specimen	Length	Width	Mouth	Gonopore
a	73	4	49	62
b	84.4	4.0	54.4	71.8
c	42.6	3.4	27.6	36.0
d	61.3	3.5	46.4	56.0

Specimen **a** collected Jan. 1955; specimens **a, b,** and **c** were sectioned. Creeping, specimen **c** was 80 mm. long by 5 mm. broad; the body was flattened, with almost parallel margins, narrowing rather abruptly at both ends.

The dorsal surface (Fig. 67) presents a greyish-brown pigment on a light yellow ground. Along the back runs a lighter stripe, ca. 1 mm. broad, due to a thinning of the dark pigment. At the borders of the light stripe the pigment is darker than on the rest of the back. The ventral side is ivory.

Beginning at the anterior end, the small and numerous eyes (Fig. 68) spread onto the dorsal surface, leaving free only the median stripe.

The pharynx (Fig. 69) is long, collar-shaped, with richly folded border.

The efferent ducts (Fig. 70, d), full of spermatozoa in all specimens, enter the common muscular coat (mc) of the copulatory apparatus and turn forward before opening into the ental paired portions of the seminal vesicle. These portions run forward and unite (at x) into the interrogation mark- or S-shaped common part (s). The paired portions and the ental part of the common receive fine-grained eosinophilous glands; the glands of the ectal are separated by a short narrow portion with rare glands. The lining of the vesicle is folded and ciliated. The rather short ejaculatory duct (e) presents a narrow lumen, is lined by a regular ciliated epithelium, and receives sparse fine-grained eosinophilous glands. A penis is absent. The male atrium (a), separated from the female by oblique folds, is ample, with folded walls. Near its ental end there are two conspicuous glandular rings, of which the first (ys) is made up of heavily stained eosinophilous glands, the second (yw), of lightly-stained eosinophilous glands interspersed with

sparse cyanophilous ones. Ectally to these rings, the male atrium receives both eosinophilous and cyanophilous glands. The male atrium is lined by a low columnar epithelium, ciliated in its ental part to a different extent in the various specimens, nonciliated in the rest. The muscle coat of the male copulatory complex is strong.

The vitellaria are fully mature in the two bigger specimens. The oviducts (o) turn mesially ca. 2 mm. behind the gonopore without rising and unite into the ascending common glandular duct (q), which is continuous with the horizontal vagina. Shell glands (z) open also into the transverse portions of the oviducts. The vagina is lined by a pluriserial ciliated epithelium, and receives eosinophilous and sparse cyanophilous glands. The female atrium (f) is simple, rather long, and is lined by an epithelium similar to that of the vagina. The muscle coat of the female atrium and vagina is much weaker than that of the male.

Remarks: Geoplana hauseri belongs to Group A (E. M. Froehlich, 1955, p. 327), a very homogeneous group of species of **Geoplana**. Within the group, **G. hauseri** stands very near to **G. rosea** E. M. Froehlich. Externally, the chief difference is the broader median stripe, a character that may be subject to individual variation and, therefore, not of much weight. Some differences in the copulatory apparatus of these two species, however, force their separation. The principal are: 1. **G. rosea** presents two seminal vesicles connected by a narrow canal, and the ental vesicle is not forked, whereas **G. hauseri** presents one vesicle forked entally; 2. in **G. rosea** does not occur the glandular rings in the ental part of the male atrium; 3. the lining of the vesicle is folded in **G. hauseri,** smooth in **G. rosea;** and 4. the muscularis of the male atrium is much stronger in **G. hauseri.**

The worms Graff (1899, p. 299) classified as **G. maximiliani** present a great external similarity to **G. hauseri,** but in Graff's species the pharynx is cylindrical with caudally displaced dorsal insertion (l. c., p. 101).

GEOPLANA NATALIAE, n. sp.

Locality: Blumenau, S. C.: 1 specimen, July 31, 1955; Natalia Gabrusewycz col.

The preserved worm (Fig. 62) is 52 mm. long by 6 mm. broad; the mouth is at 30 mm., the gonopore at 40 mm. from the anterior end. The body is rather thick with rounded borders. It tapers more gently to the anterior end than to the posterior. The anterior third is coiled up. The dorsal side is deep black. The ventral is brownish-grey, darker toward the margins and at the extremities. Ventro-marginally runs a light line, light-grey to orange, that corresponds to the sensory border.

The eyes are marginal and located at the centre of small light halos. In the cephalic region (Fig. 63) they are pluriserial and closely set, backwards they get progressively sparser.

The pharynx (Fig. 64) is short and broad, of the cylindrical type with caudally displaced dorsal insertion, approaching the bell-type of Graff.

The efferent ducts (Fig. 65, d), full of spermatozoa, bend mesially and forward before entering each into a branch of the entally forked seminal vesicle (s). The paired and the ental common portions of the seminal vesicle are outside the penis bulb (b) proper but are enclosed by some muscle fibres derived from the latter. The seminal vesicle receives abundant secretion from extrabulbar eosinophilous and cyanophilous glands. It is lined by a columnar epithelium, much folded in the bulbar portion; cilia were not ascertained. The bulbar portion is much dilated and its lumen is nearly filled up with secretion (se). The muscularis of the vesicle is rather weak. The vesicle opens into the ejaculatory duct by a narrow passage located at a small papilla-like projection into that cavity. The penis bulb is strongly muscular. The penis papilla (p) is short and broad. The ejaculatory duct is very ample, forming an ejaculatory cavity (ce); it opens into the male atrium by a vertical slit. Both the ejaculatory cavity (except the small dorsal recess, n) and the penis papilla are covered by a

low columnar, nonciliated epithelium whose cells store the secretion of eosinophilous subepithelial glands; cyanophilous glands are also present. The basement membrane (Figs. 65, 66, mb) is very thick (ca. 30 μ, locally), thicker even than the epithelium. It is also thick in the ental part of the male atrium (Fig. 65, a), but ectally thins out progressively to a tenuous layer before the middle of the atrium. The muscularis of the ejaculatory cavity and of the papilla are strong. The dorsal recess is connected to the ejaculatory cavity by a narrow horizontal slit. The principal histological peculiarities of the recess are: 1. it receives only cyanophilous glands, 2. its lining epithelium is more irregular, 3. the basement membrane is thin, and 4. the muscularis is weak.

The male atrium is long and ample. It is lined by a high, irregular, nonciliated epithelium, except in the immediate neighbourhood of the papilla, were its epithelium is similar to that of the latter. The epithelial cells of the ental half store cyanophilous and some eosinophilous secretion from subepithelial glands at its apical half. The ectal, narrower half of the atrium receives numerous eosinophilous and some cyanophilous glands. The apical ends of the epithelial cells release secretion-containing globules. The musculature (mc) of the male atrium is strong.

The vitellaria are fully mature. The oviducts (o) begin to rise shortly in front of the gonopore (g). Shell glands (z) open into the final part of the oviducts, and into the common glandular duct (q); this duct is short, directed downward and backward, and is continuous with the rather short vagina. A female atrium is lacking, the vagina opening directly into the male atrium close to the wide open gonopore. The vagina is lined by a high columnar, irregular epithelium, and receives the same glands as the adjacent atrium, but it does not produce the secretion globules. The whole copulatory apparatus is enveloped by a common muscular coat.

Remarks: The homogeneous black dorsal surface is shared by **Geoplana nataliae** with several other species of the genus: **G. atra** Fr. Müll., **G. preta** Riest., **G. astraea** Marc., **G. plumbea**

C. G. Froeh., and **G. glieschi,** n. sp., from Brazil; **G. talpa** du B.-R. Marc. and **G. idaia** du B.-R. Marc. from Peru; and **G. eugeniae** Graff from Paraguay. The first four are small species, **G. glieschi** is very large, **G. talpa** and **G. idaia** are shorter and relatively much broader, and **G. eugeniae** has a similar shape but is much smaller. As regards the anatomy of the copulatory organs, **G. nataliae** readily distinguishes itself from all the referred species and presents an isolated position within the genus.

GEOPLANA SUVA, n. sp.

Locality: Blumenau, S. C.: 3 ripe specimens, June 22 — July 2, 1953.

Measures, in mm., of two sectioned specimens:

Length	Width	Mouth	Gonopore
20.5	2.5	14.7	17.7
16.0	2.7	12.0	13.8

This is a small species. The body (Fig. 71) tapers gradually to the blunt anterior end, more abruptly to the pointed posterior one.

The margins excepted, there are on the back, over a milky ground, irregular black strips which may fork or anastomose (Fig. 73). The borders of the strips are not sharp, fading to the ground colour. Toward the margins, the strips are shorter, even reduced to small rounded spots. The margins of the body are orange, turning to grey at the cephalic end. The broad creeping sole is light-grey, acquiring a dark tint at the anterior end.

The small eyes (Fig. 75) are restricted to the margins. The pigment cups are commonly 20-25 μ across, the largest attaining 32 μ.

The pharynx (Fig. 74) is a long tube; the ventral insertion is more anterior than the dorsal, and the border has few folds. The mouth opens near the posterior end of the pharynx pocket.

The efferent ducts (Figs. 76, 77, d) overpass the first seminal vesicle (s_1), then loop forward to enter into it. The first vesicle is connected to the second (s_2) by a narrow canal. Both vesicles are irregular and lined by a ciliated columnar epithelium; the first receives a homogeneous, weakly-stained secretion; the second, a granular secretion (y) that takes both eosin and haematoxylin in one specimen, almost only eosin in the other. The outer part of the muscular coat is common to both vesicles. The inner is separate. Both vesicles are extrabulbar. The ciliated ejaculatory duct (e) traverses almost straightly the small, slightly elongate to globular penis papilla (p). Both papilla and male atrium are lined by a low, irregular epithelium provided with a cyanophilous border. The male atrium is comparatively ample.

Vitellaria mature in both specimens. The oviducts (o) begin to rise shortly in front of the gonopore (g), and unite dorsally into the common glandular duct (q). The ectal part of the oviducts also receives shell glands (z). The common glandular duct, directed caudally, opens into the dorsal part of the female atrium (f). The ental portion of the female atrium is globular, the ectal tubular, between them occurring a small constriction. The globular and part of the tubular portions of the female atrium are lined by a high pluristratified mass of cells (r). In this mass, the cells are closely packed but leave between them small reticulate spaces. There are also some larger lacunae, commonly containing degenerating cells. In one specimen there are spermatozoa inside the female atrium, the common glandular duct, and the oviducts, what indicates recent copulation. Those sperms inside the female atrium are mixed in part with eosinophilous secretion and many of them are directed to the cell mass, some even penetrating into lacunae of the same. The reticulate spaces contain, in this worm, cyanophilous granules. The ciliated gonopore canal issues between the two atria; the female atrium is ciliated ectally and partially also where the lining is pluristratified.

Remarks: Among the species of **Geoplana** provided with a mass of cells in the female atrium, **G. multicolor** Graff, **G. phocaica** Marc., **G. preta** Riest., and **G. incognita** Riest. probably constitute a natural group (E. M. Froehlich, 1955, p. 329), and it is in this group that **G. suva** and, also **G. gaucha,** n. sp., should be placed. As regards the colour pattern, **G. suva** is quite distinct from the referred species, for only **G. phocaica** is spotted, but it has not the orange margins, and presents a spotted creeping sole and dorsal eyes. As regards the copulatory apparatus, **G. suva** stands closer to **G. preta,** but in the latter there is no canal separating two seminal vesicles; the seminal vesicle being simple or at most provided with a constriction (Riester, 1938, p. 37, fig. 38). **G. goettei** Schirch presents, like **G. suva,** a mass of cells in the female atrium, and two vesicles connected by a narrow canal, but **G. goettei** has a larger and more muscular penis papilla than **G. suva,** besides the differences in size, shape, and colour pattern.

CHOERADOPLANA IHERINGI **Graff**

Choeradoplana iheringi Graff, 1899, p. 395 ["Taguara do mundo nuovo, Prov. Rio Grande do Sul", Brazil].

Choeradoplana iheringi, Riester, 1938, p. 75.

Choeradoplana iheringi, Marcus, 1951, p. 103.

Locality: São Leopoldo, R. G. S.: one specimen, Prof. Dr. J. Hauser, S. J., col.

GEOPLANA BECKERI, **n. sp.**

Locality: Chapada, environs of Salvador, Bahia: 3 specimens, July 20, 1955; J. Becker col.. The worms were found under a gneiss stone lying on lateritic soil. The locality was relatively dry, with a cover of loose shrubby vegetation. The worms were preying on small snails.

Measures, in mm., of the preserved worms:

Specimen	Length	Width	Mouth-Gonopore	Gonopore-Posterior tip
a	22	3.8	3.7	7.0
b	26	3.2	3.5	8.2
c	20	3.0	3.8	6.7

Specimen **b** had lost the tip of the fore end; specimen **c**, a larger piece, probably 2-3 mm. long, of the same end. The mouth lies, therefore, at the beginning of the second half of the body, and the genital opening at the beginning of the last third of the body.

The body (Fig. 78) is flattened. The colour of the live worms was described as presenting orange margins and a pair of stripes of the same colour along the middle of the body, the rest of the back being bright yellow. The worms were fixed in hot formalin in a tin can, and an ensuing reaction turned the colour of the worms to black. Fortunately the histology was in good condition.

The eyes spread onto the whole dorsal surface, beginning at the anterior end (Fig. 79). The diameter of the larger eye cups is about 35 μ.

The pharynx (Fig. 80) is a long tube, 2.1 mm. long in a sectioned worm. The dorsal insertion is more posteriorly located than the ventral. The lumen of the pharynx presents longitudinal folds. The mouth lies shortly in front of the middle of the pharynx pocket.

Two worms were sectioned. Both are mature, and agree perfectly in the structure of the copulatory apparatus. The efferent ducts (Fig. 81, d), full of spermatozoa, curve mesially and forwards to enter into the short paired ental portions of the seminal vesicle. The final portions of the efferent ducts have few or no spermatozoa. The tubular vesicle (s) lies outside the penis bulb (b), but is surrounded by sparse fibres derived from the latter. The ental part of the common portion of the vesicle loops forward and dorsally; the ectal part is contorted. The epithelium of the vesicle is columnar ciliated an is traversed by ducts of strongly eosinophilous granular glands and of weakly stained glands. The ducts of the latter

form a mass of alveolar appearance between the epithelium and the weak muscularis; its cell bodies lie nearby. The musculature of the penis bulb is not strong, and the bulb is not sharply delimited against the surrounding parenchyma. The ectal part of the vesicle narrows gradually, but on entering the penis bulb it narrows more abruptly to become the ejaculatory duct. The epithelium of the latter is nonciliated; in the ental half of the duct (e_1) it is irregular and cubical to low columnar, in the ectal half (e_2), flattened, what is probably due to stretching resulting from a great accummulation of glandular secretion (u) at the tip of the penis papilla. The alveolar coat of gland ducts of the vesicle continues around the ejaculatory duct but decreases progressively in thickness, disappearing where the epithelium becomes flattened. The ejaculatory duct is nearly straight and opens at the tip of the penis papilla. The latter (p) is conical, but its apical part is dilated by the accummulation of secretion referred above. This secretion is strongly eosinophilous and is produced by extrabulbar glands. The male atrium (a) is simple, lined by a columnar epithelium higher ectally, and receives the secretion of eosinophilous glands; dorsally open the ducts of numerous fine-grained neutrophilous (purple) glands (w).

The yolk glands are mature in both specimens. The oviducts (o) rise approximately at the level of the gonopore (g). Shell glands (z) open into the transverse portions of the oviducts and into the common glandular duct (q). The latter runs backward and opens dorsally into the ental, globular half of the female atrium (f). The ectal half of the same is more tubular, and is separated from the ental by a constriction. The lining epithelium of the female atrium is high, pluriserial in the ectal half to pluristratified in the ental. The female atrium receives sparse eosinophilous glands. Its muscularis is relatively strong, chiefly at the globular portion Male and female atria are not sharply separated from each other, the gonopore canal issuing between the two.

Remarks: Geoplana beckeri cannot be identified with the previously known species from Bahia, **G. flava** Mos., 1877, for

it lacks the glistening white longitudinal stripes on the back present in the latter. Moreover, in **G. beckeri** the eyes spread onto the whole of the dorsal surface, whereas in **G flava** they spread but little from the margins (Moseley, 1877, p. 283; Graff, 1899, p. 345, pl. 3 fig. 31). The copulatory apparatus of **G. flava** has not been described.

G. beckeri distinguishes itself also from the other species of the genus by the colour pattern and by the anatomy of the copulatory organs.

REMARKS ON THE STATUS OF FRITZ MÜLLER'S SPECIES OF LAND PLANARIANS

Fritz Müller, in a letter to Max Schultze published together with observations by the latter in 1857, described 13 species of **Geoplana**. This letter seems to be the first scientific paper Müller got published about the Brazilian fauna, and it is his only contribution on land planarians. His descriptions are very short, and commonly insufficient to define clearly a species, but in general also clear enough to show what worms cannot be of any of his species. Graff (1894, 1899) tried to make good use of Müller's species but he was on the whole very unfortunate in his identifications, as we shall see presently Seven of Müller's species remain obscure. They are **G. tristriata, G. octostriata, G. marginata, G. rufiventris, G. olivacea, G. nephelis,** and **G. maximiliani**. A clear redescription of them will only be possible by a thorough field work at the type locality (Blumenau, State of Santa Catarina).

1. GEOPLANA TRISTRIATA **Fritz Müller**

In Müller's time this was a common species at Blumenau, but we couldn't find any during our two-week stay at that locality in the winter of 1953.

Hermann v. Ihering sent to Graff a three-striped worm he collected at Taquara, State of Rio Grande do Sul, and suggested it could be **G. tristriata,** a suggestion Graff accepted. There are,

however, several differences that make this identity improbable: a) the shape of the body is different, Müller's species being broader; b) in Müller's species the greatest width of the body, and there also the mouth, are situated behind the second third of the body; in Graff's, the mouth is at about three fifths of the length of the body; c) Müller's species presents three longitudinal narrow dark lines; in Graff's, at least the lateral stripes are much broader. However, as neither Müller's, nor Graff's species have been found again, both remain obscure.

2. GEOPLANA OCTOSTRIATA **Fritz Müller**

Geoplana sexstriata Graff could be a synonym of this species, but only the study of eight-striped specimens from the type locality can settle this problem. The eight-striped worm Graff had from Rödersburg, State of Rio Grande do Sul, probably was correctly identified. Schirch's **G. octolineata** is a synonym of **G. sexstriata** (du B.-R. Marcus, 1951, p. 236), and the same may be the case with his **G. octostriata.**

GEOPLANA ELEGANS **Fritz Müller**

A homonym of **G. elegans** (Darwin), Diesing, 1862, renamed it **G. mülleri**. During our stay in Blumenau this was a common species, and we collected a rich material. We have analysed it in this paper.

GEOPLANA PALLIDA **Fritz Müller**

Diesing, 1862, renamed this species **G. schultzei,** because it was a homonym of G. pallida (Darwin). We have had several specimens of this form, and its study led us to consider it a synonym of the preceding species.

GEOPLANA ATRA **Fritz Müller**

In a former paper (C. G. Froehlich, 1957) we described a small black species, of which we had specimens from the State

of Paraná, and from Blumenau and nearby localities. As it differed in its internal anatomy from the worm Graff considered to be **G. atra,** we named it **G. nana**. As our species, however, agrees perfectly with Müller's description and occurs in the type locality, we conclude it to be a synonym of **G. atra** Fr. Müll., and Graff's **G. atra** from Taquara, Rio Grande do Sul, to be another species. We rename it **G. nigra,** nom. nov..

6. GEOPLANA MARGINATA **Fritz Müller**

It is clear from Müller's description that **G. marginata** is a dark ("Rücken und Bauch dunkelschwarzbraun glänzend"), large ("3-4 Zoll lang") and broad ("einige Linien breite") species provided with one pair of mesial and one of marginal yellow stripes. Graff (1894, 1899) considered an elongate, light-coloured, five-striped species to belong to **G. marginata,** and he even employs the term "typical" to the five-striped worms. Graff's identification is surely mistaken, but unfortunately it has been accepted by several authors, the present writer included. In a future paper we shall discuss Graff's species, which evidently cannot stay under Müller's name. As regards **G. marginata** var. **abundans** Graff, which we have raised to specific status, see above.

G. marginata, Schirch, 1929, and **G. marginata,** Riester, 1938, are synonyms of **G. caissara** E. M. Froeh. (E. M. Froehlich, 1955, p. 295). In Riester's paper, where he refers to the external features of **G. marginata** (l. c., p. 30), the remarks he attributes to Müller are really H. von Ihering's (cf. Graff, 1899, p. 306).

7. GEOPLANA RUFIVENTRIS **Fritz Müller**

Fr. Müller's **G. rufiventris** presented a dark brown back and a brick-red ventral side. Several "large, broad, and flat" species of **Geoplana** have a dark back and a reddish ventral side, and Graff, misguided by these characters, lumped a heteroge-

nous material into **G. rufiventris** (cf., e. g., Riester, 1938, p. 53). Only one of Graff's specimens can, with reasonable certainty, be considered as **G. rufiventris**: it is the worm collected at Blumenau by G. W. Müller, for both the locality and the colour are the same as those of Fr. Müller's specimen (Graff, 1899, p. 296, pl. I figs. 26-27). Graff's anatomical analysis of **G. rufiventris** is also impaired by the fact that he doesn't indicate which specimens he studied.

G. rufiventris, Schirch, 1929, is a synonym of **G. applanata** Graff (C. G. Froehlich, 1955b, p. 192).

8. GEOPLANA OLIVACEA **Fritz Müller**

G. olivacea is a long and slender species, as can be inferred by comparison with the description of **G. nephelis**. Here Graff lumped also at least two species, one represented by some fragments collected at Blumenau by G. W. Müller, the other (or others) by several specimens from Argentina and Chile. The fragments from Blumenau present a slender shape and the same colour pattern as Fr. Müller's, the difference in hue being probably due to the preservation. The specimens from the other localities are much broader and flatter, despite the similarity in colour pattern. These should be removed from **G. olivacea,** but as their status is obscure, it is better not to rename them as yet. The name **G. olivacea** must be restricted, therefore, to the fragments from Blumenau.

G. olivacea, Busson, from Colombia, is also a large, broad and flat species, and must also be removed from **G. olivacea** Fr. Müll.. At it is well analyzed anatomically, we rename it **Geoplana bussoni,** nom. nov..

9. GEOPLANA NEPHELIS **Fritz Müller**

G. nephelis is also an elongate species, with uniform brown back and a lighter ventral side. In colour and shape it reminds one of a **Nephelis,** says Müller. Graff (1899, p. 337) put into this species a material from the Berlin Museum that had no

data about its provenance, and a material collected by Michaelsen in Chile. The material from the Berlin Museum was analysed anatomically by Graff. It presents ventral testes, part of the male efferent system ventral to the nerve plate, and longitudinal parenchymal muscles in the peripheral parenchyma (Graff, l. c., pl. 26 figs. 1-2). Besides, the copulatory apparatus presents a general resemblance to that of **?Coenoplana munda** (Graff, l. c., p. 191 fig. 41 and 42). As we have already indicated (C. G. Froehlich, 1955a, p. 200), the provenance of the material from the Berlin Museum must be sought in the Indo-Pacific region, and it cannot be **G. nephelis** Fr. Müll. because it isn't even a **Geoplana**. It may be put tentatively into the genus **Coenoplana** Moseley, 1877 (cf. C. G. Froehlich, l. c., pp. 200, 246), and we rename it **?Coenoplana graffi**, nom. nov.. As regards the material from Chile, without an anatomical study it cannot be called **G. nephelis** with any certainty, for the land planarian fauna of Chile differs considerably from that of Brazil. **G. nephelis,** Graff is, in conclusion, heterogeneous, and the possibility that at least the Chilean material could remain in Müller's species is remote.

10. GEOPLANA MAXIMILIANI **Fritz Müller**

Almost as the preceding, says Müller, with a lighter yellowish longitudinal stripe. The penis is nearly spherical, the pharynx deeply 5-lobed. Being similar to **G. nephelis, G. maximiliani** must also be elongate, but less so than **G. olivacea**. Graff (1899, p. 299) classified as **G. maximiliani** some specimens H. von Ihering sent him from Taquara, but his justification for doing so is not stated with much conviction (l. c.). We cannot agree with Graff's identification, for his specimens belong to a very elongate species, the colour has a violet shade not referred to by Müller, and, chiefly, because the unripe copulatory apparatus (similar to that of his "**G. burmeisteri**") lacks a penis. In those species that possess one, the penis differentiates very early in the development of the copulatory apparatus, long before the gonopore is open. If the young copu-

latory apparatus had already an ejaculatory duct and a vesicle, as Graff (l. c., p. 16 fig. 15, p. 167, and p. 187) indicates, it should present also a penis, which Müller's species has. Graff (l. c., p. 299) states also that the distribution of the eyes of his specimens agrees with that of Müller's, but Müller says nothing about the eyes of **G. maximiliani**. Graff's species agrees very well with **G. hauseri,** the pharynx excepted, being collar-shaped in the latter, and cylindrical (type c) in Graff's material.

The shape and colour pattern of **G. maximiliani,** Schirch (1929, p. 2 fig. 10) from Teresópolis, State of Rio de Janeiro, seem to be compatible with Müller's data, but as its anatomy is unknown, and as it is from a locality far from Blumenau, nothing can be advanced about its real identity.

There is a possibility that **G. apeva,** n. sp., is a synonym of **G. maximiliani** Fr. Müll.. The reasons we considered them distinct are given in the remarks on **G. apeva.**

11. GEOPLANA MARMORATA **Fritz Müller**

We have had material from the original locality (Blumenau) and environs coinciding with Müller's description. We have redescribed it in detail, and also removed it from the synonym of **G. rufiventris** into which Graff had put it.

12. GEOPLANA PULCHELLA **Fritz Müller**

We collected an immature specimen of this species at the original locality. Although we could not describe its copulatory apparatus, the colour pattern is distinctive enough to avoid any misidentification (C. G. Froehlich, 1955b, pp. 189-90, pã 191 fig. 1).

13. GEOPLANA SUBTERRANEA **Fritz Müller**

Diesing, 1862, transferred it to a new genus, **Geobia.**

Although we had no material from the original locality, we think there can be no doubt as regards the identity of the worms classified by Riester (1938, p. 28) and Marcus (1951, p.

106). The reasons why we have kept the genus **Geobia** are given in a former paper (C. G. Froehlich, 1955a, p. 216).

RESUMO

O trabalho presente refere-se principalmente a planárias terrestres coligidas nos Estados de Santa Catarina e do Rio Grande do Sul. Entre as espécies já conhecidas são estudadas anatômicamente **Geoplana marmorata** Fr. Müll., **G. mülleri** Dies., **G. carrièrei** Graff e **G. ladislavii** Graff. **G. marginata** var. **abundans** Graff é considerada como espécie distinta, **G. abundans**. A ocorrência de **G. pseudorhynchodemus** Riest, de **G. quagga** Marc. e de **G. tapetilla** Marc. no Estado de Santa Catarina, e novos achados de **G. velina** C. G. Froeh. em Santa Catarina e de **Choeradoplana iheringi** no Rio Grande do Sul são registrados. São descritas as seguintes espécies novas: **Geoplana apeva, G. assu, G. fita, G. gaucha, G. glieschi, G. hauseri, G. nataliae** e **G. suva**. Do Estado da Bahia é descrita **G. beckeri,** a segunda espécie descrita dêsse Estado.

Na parte final é discutido o status taxonômico das espécies de **Geoplana** descritas por Fritz Müller, 1856, cujas conclusões seguem:

G. tristriata, que Müller diz ter sido comum em Blumenau, não foi mais reencontrada. Graff considerou como pertencentes a esta espécie alguns vermes tristriados coligidos por H. v. Ihering em Taquara, mas provàvelmente não o são. A situação de ambas é obscura.

G. octostriata também permanece obscura. **G. sexstriata** Graff poderia ser sinônimo dela.

G. elegans e **G. pallida,** por serem homônimas de espécies de Darwin, foram redenominadas **G. mülleri** e **G. schultzei,** respectivamente, por Diesing. O estudo anatômico de ambas levou-nos a considerá-las sinônimas.

G. atra. Por coincidir com a descrição de Müller e por ocorrer na mesma localidade, consideramos **G. nana** C. G. Froeh.

como sinônimo de **G. atra** e redenominamos a espécie chamada de **G. atra** por Graff **G. nigra,** nom. nov., pois esta não pertence à espécie por nós reencontrada.

G. marginata permanece obscura. A espécie que Graff e, seguindo-o, outros autores chamaram de **G. marginata,** seguramente não o é.

G. rufiventris também permanece obscura. Do material de Graff, apenas o verme de dorso castanho escuro coligido em Blumenau pode ser **G. rufiventris,** o resto é material heterogêneo.

Com **G. olivacea** acontece caso semelhante, pois, do material de Graff, só o verme de Blumenau é compatível com a descrição de Müller. **G. olivacea,** Busson, da Colômbia, é outra espécie e redenominamo-la **G. bussoni,** nom. nov.

O que Graff considerou como **G. nephelis** é material heterogêneo e mesmo a possibilidade de que parte pertença à espécie de Müller é remota. O material sem proveniência do Museu de Berlim colocamos tentativamente no gênero **Coenoplana** Mos. e redenominamos **Coenoplana graffi,** nom. nov.. **G. nephelis** também permanece obscura.

G. maximiliani. A identificação de Graff é também aqui impossível. Quanto ao verme de Schirch, nada se pode adiantar, pois é de região diferente e de anatomia desconhecida. **G. apeva** C. G. Froeh. poderia ser sinônimo de **G. maximiliani,** o que ainda não pode ser resolvido com os dados em mãos.

G. marmorata não é sinônimo de **G. rufiventris,** como pensou Graff. Foi reencontrada e redescrita por nós.

De **G. pulchella** encontramos um exemplar imaturo. O colorido da espécie é invulgar e permite determinação segura.

G. subterranea, transferida por Diesing para o gênero **Geobia,** é espécie bem conhecida.

REFERENCES

du BOIS-REYMOND MARCUS, E., 1951: On South American Geoplanids. Bol. Fac. Fil., Ci. Letr. Zoologia n. 16, pp. 217-255, pls. 1-8. São Paulo.

BUSSON, B., 1903: Über einige Landplanarien. S. Ber. Akad. Wiss. Naturw. Kl. v. 112 pt. 1, pp. 375-429, 2 pls. Wien.

DARWIN, Ch., 1844: Brief Descriptions of several Terrestrial Planaria and of some remarkable Marine Species, etc. Ann. Mag. Nat. Hist. v. 14, pp. 241-251, pl. 5 figs. 1-4. London.

DIESING, K. M., 1862: Revision der Turbellarien. Abtheilung: Dendrocoelen. S. Ber. math.-naturwiss. Cl. Akad. wiss. Wien, v. 44, pp. 485-578.

FROEHLICH, C. G., 1955a: Sôbre morfologia e taxonomia das Geoplanidae. Bol. Fac. Fil., Ci. Letr. Zoologia n. 19, pp. 195-279, 14 pls. São Paulo.

—— 1955b: Notas sôbre geoplanas brasileiras: Pap. Av. Dep. Zool. Secr. Agric. São Paulo, v. 12 n. 7, pp. 189-198, 6 figs. S. Paulo.

—— 1956: Tricladida Terricola das regiões de Teresópolis e Ubatuba. Pap. Av. Dep. Zool. Secr. Agric. São Paulo, v. 12 n. 16, pp. 313-343, 56 figs. São Paulo.

—— 1957: Planárias Terrestres do Paraná. Dusenia v. 7 n. 4, pp. 173-191, pls. 4-8. Curitiba.

FROEHLICH, E. M., 1955: Sôbre espécies brasileiras do gênero **Geoplana**. Bol. Fac. Fil., Ci. Letr. Zoologia n. 19, pp. 289-369, 16 pls. São Paulo.

GRAFF, L. von, 1894: Viaggio del Dott. Alfredo Borelli nella Repubblica Argentina e nel Paraguay. V. Landplanarien. Boll. Mus. Zool. Anat. Comp. Univ. Torino v. 9 n. 182, pp. 1-4. Torino.

—— 1897: Neue Landplanarien (Viaggio Borelli IX). Boll. Mus. Zool. Anat. Comp. Torino v. 12 n. 288, 4 pp., 1 fig. Torino.

—— 1899: Monographie der Turbellarien II. Tricladida Terricola. v. 1, xiii + 574 pp.; v. 2, 58 pls. Leipzig (Engelmann). — 1912-1917: Turbellaria. II. Tricladida. H. G. Bronn, Klass. Ordn. Tier-Reichs v. 4 Abt. Ic, xxxviii + pp. 2601-3370, pls. 31-64. Leipzig.

HYMAN, L. H., 1955: Miscellaneous Marine and Terrestrial Flatworms from South America. Am. Mus. Novitates n. 1742, 33 pp., 42 figs. New York.

—— 1957: A Few Turbellarians from Trinidad and the Canal Zone, with Corrective Remarks. Am. Mus. Novitates n. 1862, 8 pp., 6 figs. New York.

MARCUS, E., 1951: Turbellaria Brasileiros (IX). Bol. Fac. Fil., Ci. Letr. Zoologia n. 16, pp. 5-215, 40 pls. São Paulo.

MOSELEY, H. N., 1877: Notes on the Structure of Several Forms of Land Planarians, with a Description of Two New Genera and several New Species, etc. Quart. J. Micr. Sci. v. 117 N. S., pp. 273-292, pl. 20.

MÜLLER, Fr. — see Schultze, Max, and Fritz Müller.

RIESTER, A., 1938: Beiträge zur Geoplaniden-Fauna Brasiliens. Abh. Senckenb. Naturf. Ges. Abh. 441, 88 pp., 2 pls. Frankfurt a.M.

SCHIRCH, P., 1929: Sôbre as planárias terrestres do Brasil. Bol. Mus. Nacional v. 5, pp. 27-38, pls. 1-4. Rio de Janeiro.

SCHULTZE, Max, and Fritz Müller, 1856: Beiträge zur Kenntnis der Landplanarien, etc. Abh. Naturf. Ges. Halle v. 4, pp. 19-38 Halle a. S.

PLATES

PLATE 1 (Figs. 1-7)

Geoplana marmorata Fritz Müller
(also Plate 2, Figs. 8-9)

Fig. 1 — Creeping worm, dorsal side.
Fig. 2 — Resting worm, dorsal side.
Fig. 3 — Magnified portion of the dorsal side.
Fig. 4 — Pharynx, median section.
Fig. 5-7 — Copulatory apparatus, combined sagittal sections of three specimens.

a, male atrium; b, penis bulb; c, mouth; d, efferent ducts; e, ejaculatory duct; f, female atrium; g, gonopore; i, intestine; k, subepithelial muscles (muscularis) of pharynx; mc, common muscle coat of copulatory apparatus; o, oviduct; p, penis papilla; q, common glandular duct; s, seminal vesicle; z, shell glands.

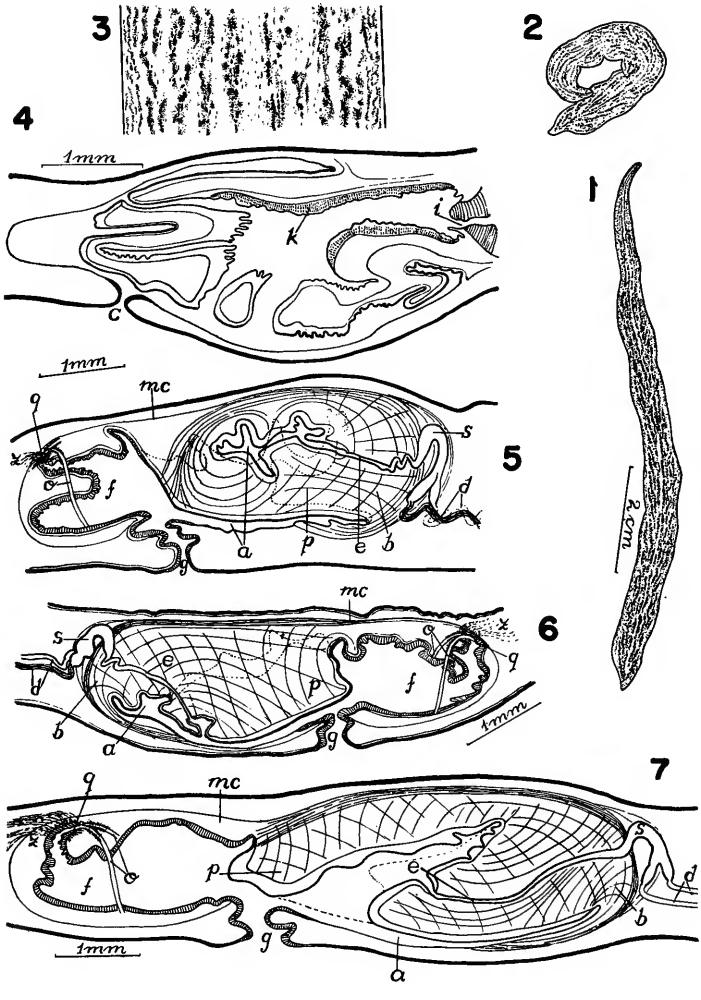
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PLATE 2 (Figs. 8-16)

Geoplana marmorata Fritz Müller
(also Plate 1, Figs. 1-7)

Fig. 8 — Cephalic region, distribution of the eyes.
Fig. 9 — Maximum spread of the eyes.

Geoplana mülleri Diesing
(also Plate 3, Figs. 17-18)

Fig. 10 — Specimen from Brusque, resting position.
Fig. 11 — Specimen from Itajaí, creeping.
Fig. 12 — Specimen from Blumenau, creeping.
Fig. 13 — Distribution of the eyes in the cephalic region.
Fig. 14 — Distribution of the eyes in front of the pharynx.
Fig. 15 — Pharynx, median section.
Fig. 16 — Copulatory apparatus, combined sagittal sections; Specimen from Itajaí.

a, male atrium; c, mouth; d, efferent ducts; e, ejaculatory duct; f, female atrium; g, gonopore; i, intestine; m, muscularis of male atrium; mc, muscle coat of male atrium; o, oviducts; q, common glandular duct; s, seminal vesicle; w, glands of pharynx; y, eosinophilous glands; z, shell glands.

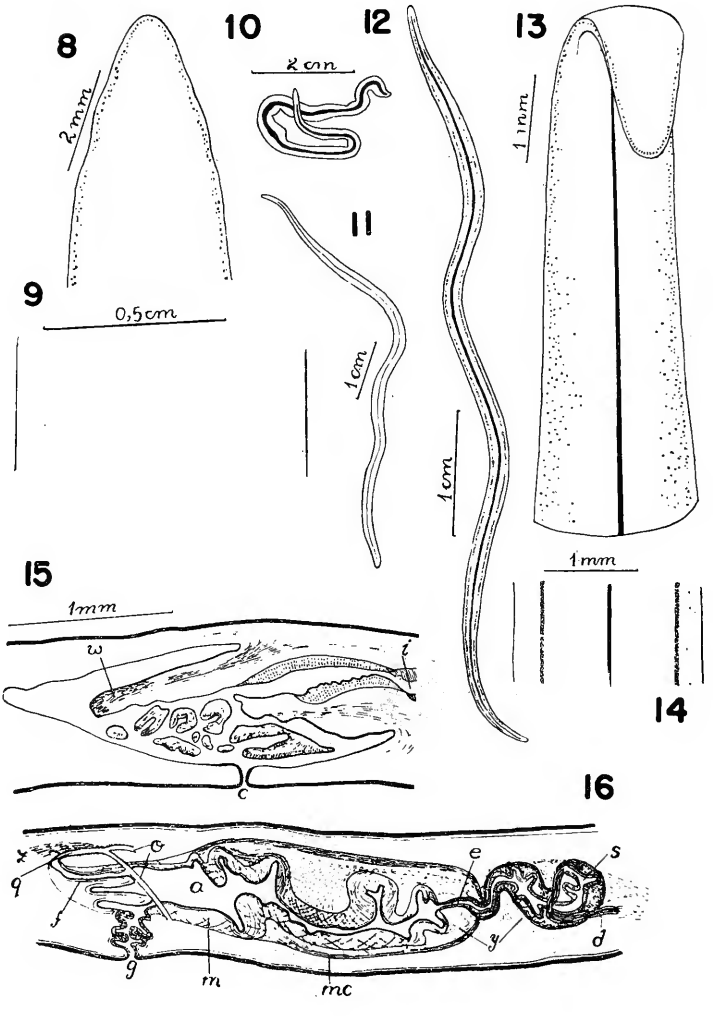
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PLATE 3 (Figs. 17-23)

Geoplana mülleri Diesing
(also Plate 2, Figs. 10-16)

Fig. 17 — Copulatory apparatus, combined sagittal sections; specimen from Blumenau.
Fig. 18 — Copulatory apparatus, combined sagittal sections; specimen from Brusque.

Geoplana ladislavii Graff
(also Plate 4, Figs. 24-25)

Fig. 19 — Creeping worm, specimen from Blumenau.
Fig. 20 — Outline of a resting worm.
Fig. 21 — Distribution of the eyes in the cephalic region.
Fig. 22 — Maximum spread of the eyes.
Fig. 23 — Pharynx, median section.

a, male atrium; c, mouth; d, efferent ducts; e, ejaculatory duct; f, female atrium; g, gonopore; i, intestine; k, muscularis of pharynx; mc, muscle coat of male atrium; o, oviducts; q, common glandular duct; s, seminal vesicle; sp, cluster of spermatozoa attached to wall of male atrium; t, pharynx pocket; z, shell glands.

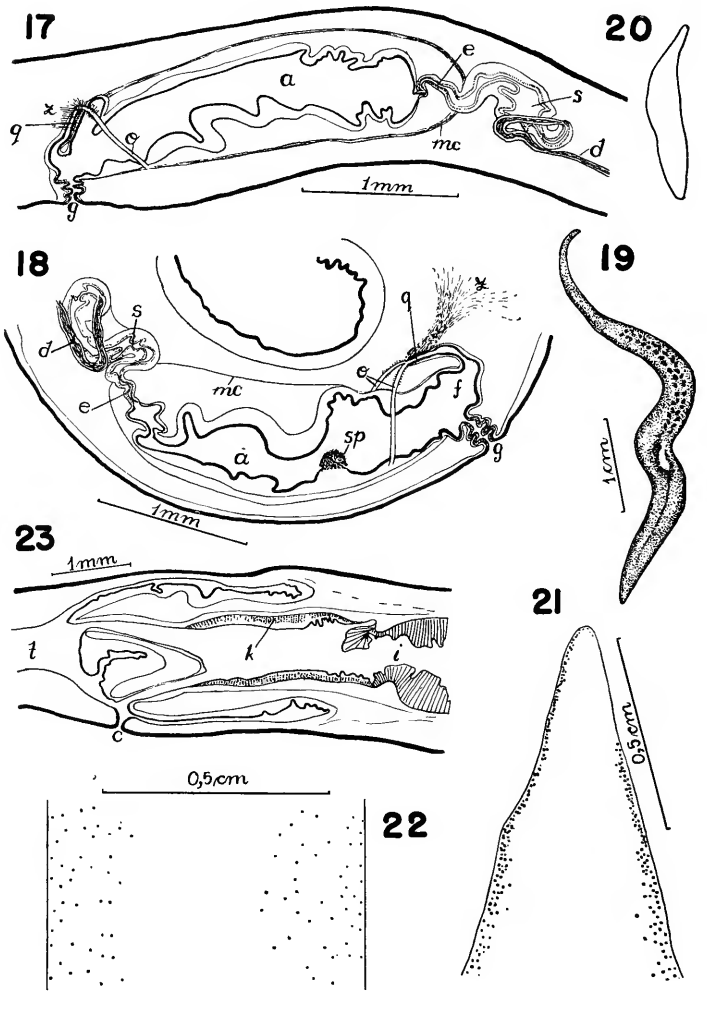
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PLATE 4 (Figs. 24-34)

Geoplana ladislavii Graff
(also Plate 3, Figs. 19-23)

Figs. 24-25 — Copulatory apparatus, combined sagittal sections.

Geoplana apeva, n. sp.
(also Plate 5, Figs. 35-36)

Fig. 26 — Specimen from Brusque, creeping.
Fig. 27 — The same, ventral side.
Fig. 28 — The same, resting.
Fig. 29 — Specimen from Blumenau, creeping.
Fig. 30 — The same, resting.
Fig. 31 — Distribution of the eyes at the anterior end.
Figs. 32-33 — Maximum spread of the eyes in two specimens.
Fig. 34 — Pharynx, median section.

a, male atrium; b, penis bulb; c, mouth; d, efferent ducts; e, ejaculatory duct; f, female atrium; g, gonopore; i, intestine; k, muscularis of pharynx; o, oviduct; p, penis papilla; q, common glandular duct; s, seminal vesicle; z, shell glands.

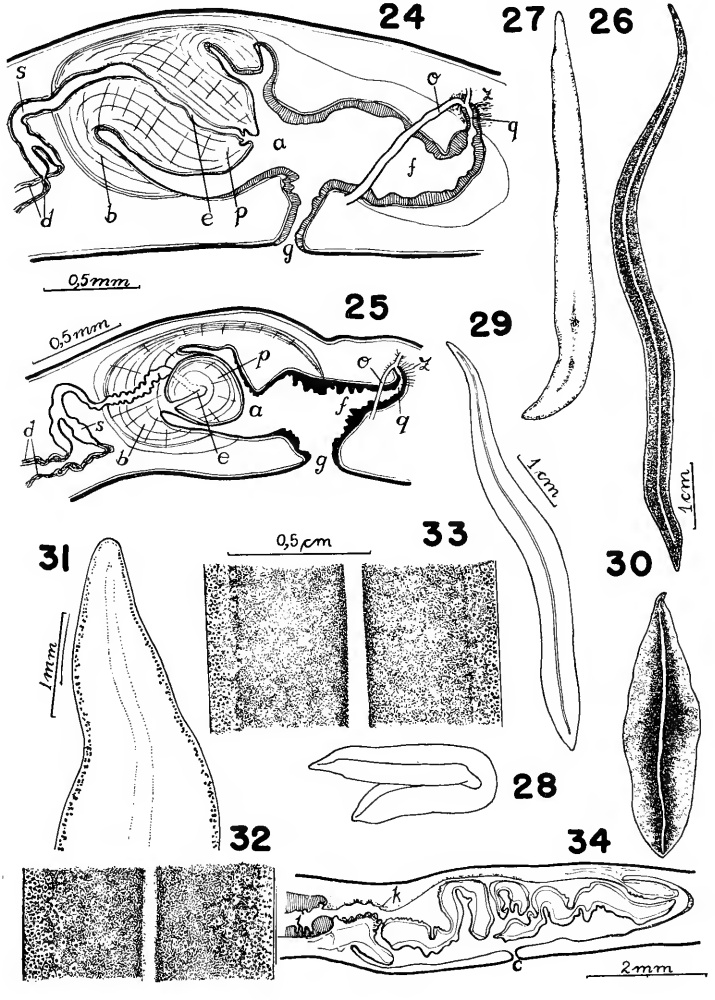
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PLATE 5 (Figs. 35-42)

Geoplana apeva, n. sp.
(also Plate 4, Figs. 26-34)

Figs. 35-36 — Copulatory apparatus, combined sagittal sections of two specimens.

Geoplana assu, n. sp.
(also Plate 6, Figs. 43-44; Plate 7, Fig. 49)

Fig. 37 — Resting worm, showing dorsal colour pattern.
Fig. 38 — Creeping worm, dorsal view.
Fig. 39 — Newly hatched young, dorsal view.
Fig. 40 — Distribution of the eyes at the anterior end.
Fig. 41 — Colour pattern of the back and maximum spread of the eyes.
Fig. 42 — Pharynx, median section.

ac, genital atrium; b, penis bulb; c, mouth; d, efferent ducts; e, ejaculatory duct; g, gonopore; p, penis papilla; q, common glandular duct; s, seminal vesicle; t, posterior extension of pharynx pocket; v, vagina; y, glands of pharynx; z, shell glands.

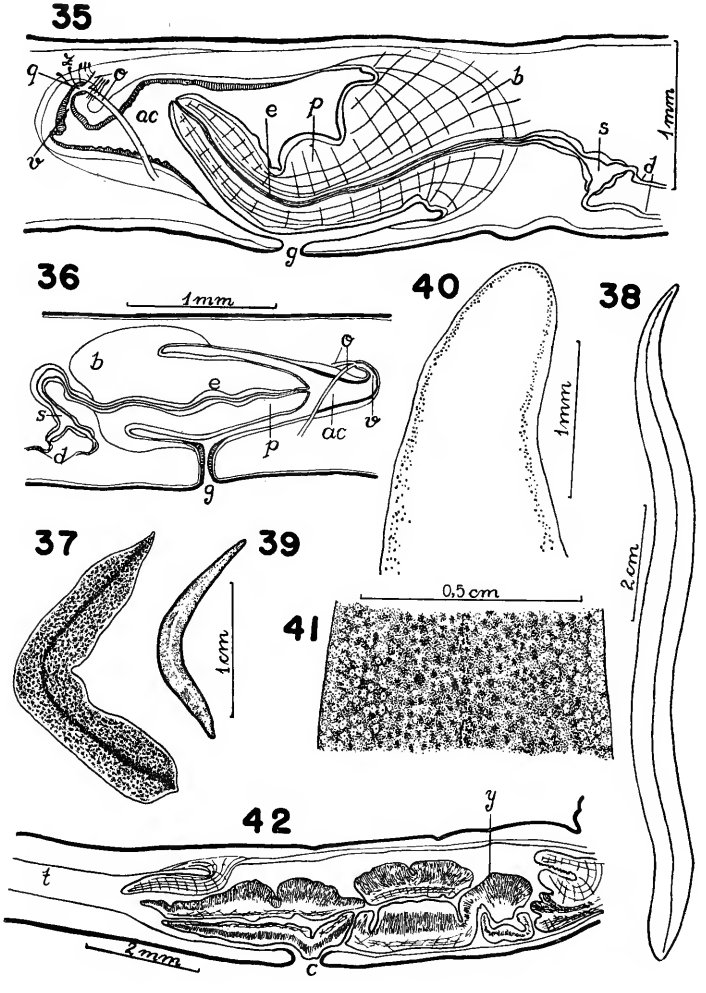
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PLATE 6 (Figs. 43-48)

Geoplana assu, n. sp.

Fig. 43 — Copulatory apparatus of specimen **a,** combined sagittal sections.
Fig. 44 — Copulatory apparatus of specimen **c,** combined sagittal sections.

Geoplana fita, n. sp.
(also Plate 7, Figs. 50-52)

Fig. 45 — Outline of a worm.
Fig. 46 — Colour pattern of the back, various regions.
Fig. 47 — Distribution of the eyes, anterior end.
Fig. 48 — Pharynx, median section.

a, genital atrium; b, penis bulb; c, mouth; d, efferent ducts; e, ejaculatory duct; g, gonopore; i, intestine; k, muscularis of pharynx; m, muscularis of penis papilla; o, oviducts; p, penis papilla; q, common glandular duct; s, seminal vesicle; v, vagina; z, shell glands.

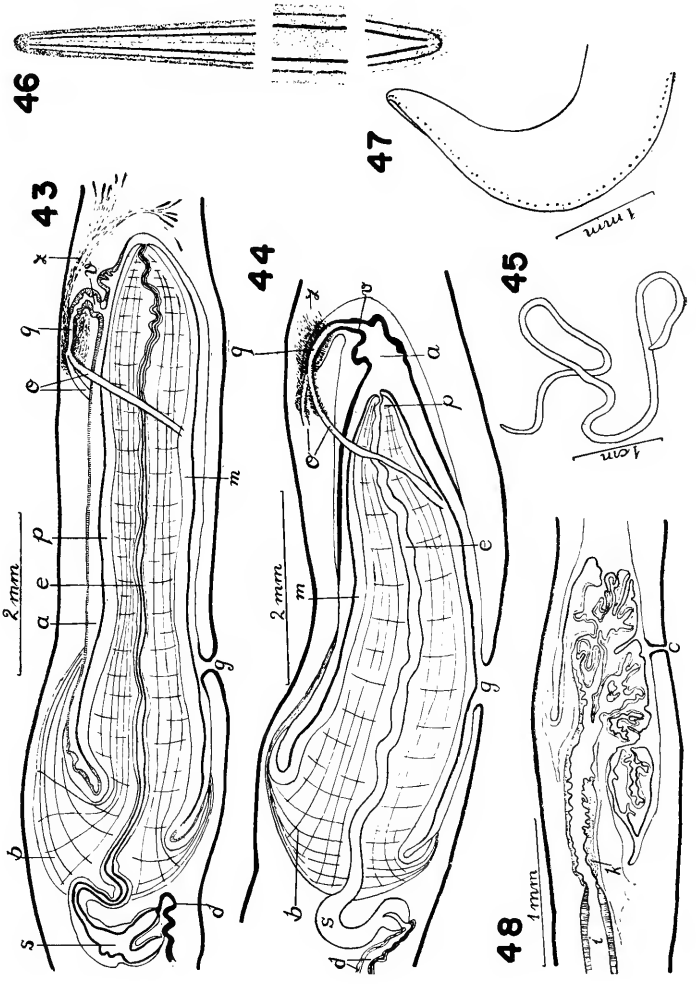
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PLATE 7 (Figs. 49-52)

Geoplana assu, n. sp.
(also Plate 5, Figs. 37-42; Plate 6, Figs. 43, 44)

Fig. 49 — Copulatory apparatus of specimen **b,** with egg capsule inside genital atrium; combined sagittal sections.

Geoplana fita, n. sp.
(also Plate 6, Figs. 45-48)

Fig. 50 — Copulatory apparatus of one specimen, combined sagittal sections.

Figs. 51-52 — Copulatory apparatus of another specimen, showing the extension of the seminal vesicle; combined sagittal sections.

a, genital atrium; am, male atrium; b, penis bulb; d, efferent ducts; e, ejaculatory duct; eg, egg capsule; f, female atrium; fl, flap separating female from common atrium; g, gonopore; m, muscularis of penis papilla; o, oviducts; p, penis papilla; q, common glandular duct; r, pluristratified epithelium of female atrium; s, seminal vesicle; x, dorsal fold separating male from common atrium; y, cyanophilous and eosinophilous glands; z, shell glands.

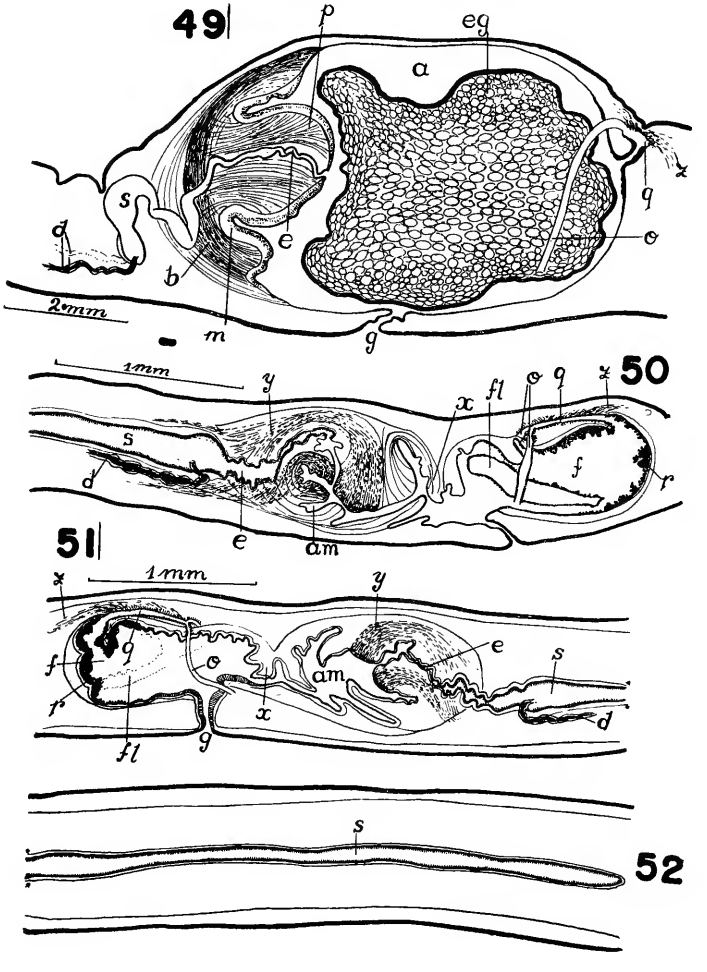
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PLATE 8 (Figs. 53-59)

Geoplana gaucha, n. sp.

Fig. 53 — Colour pattern of the back.
Fig. 54 — Cephalic region: colour pattern and distribution of the eyes. Pigment not drawn in margins to show eyes.
Fig. 55 — Maximum spread of the eyes.
Fig. 56 — Pharynx, median section.
Fig. 57 — Copulatory apparatus, combined sagittal sections.

Geoplana glieschi, n. sp.
(also Plate 9, Figs. 60-61)

Fig. 58 — Portion of dorsal side in front of the pharynx, showing the eyes and, where the pigment has been rubbed off, testes follicles.
Fig. 59 — Pharynx, median section.

a, male atrium; b, penis bulb; c, mouth; d, efferent ducts; e, ejaculatory duct; f, female atrium; g, gonopore; i, intestine; o, oviducts; p, penis papilla; q, common glandular duct; r, mass of cells of female atrium; s_1, paired portions of seminal vesicle; s_2, common portion of seminal vesicle; t, caudal extension of pharynx pocket; y, eosinophilous glands; z, shell glands.

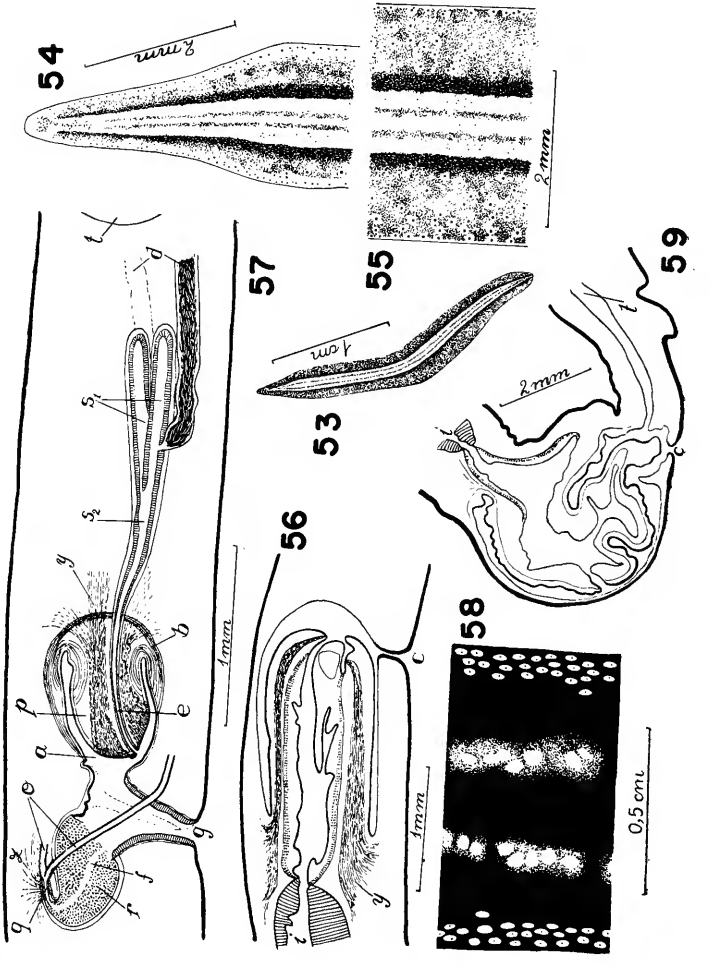
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PLATE 9 (Figs. 60-66)

Geoplana glieschi, n. sp.
(also Plate 8, Figs. 58-59)

Fig. 60 — Ventral view of the preserved worm, showing rolled sides.
Fig. 61 — Copulatory apparatus, combined sagittal sections.

Geoplana nataliae, n. sp.

Fig. 62 — Dorsal view of preserved worm, anterior end rolled up.
Fig. 63 — Lateral view of the body, showing distribution of eyes.
Fig. 64 — Pharynx, median section.
Fig. 65 — Copulatory apparatus, combined sagittal section.
Fig. 66 — Magnified view of wall of ejaculatory cavity (at 1).

a, male atrium; b, penis bulb; c, mouth; ce, ejaculatory cavity; d, efferent ducts; e, ejaculatory duct; f, female atrium; g, gonopore; i, intestine; m, muscularis of penis papilla; mb, basement membrane; mc, musculature of male atrium; n, dorsal recess of ejaculatory cavity; o, oviducts; p, penis papilla; q, common glandular duct; s, seminal vesicle; se, secretion inside seminal vesicle; v, vagina; ve, ventral side; y, eosinophilous glands; z, shell glands.

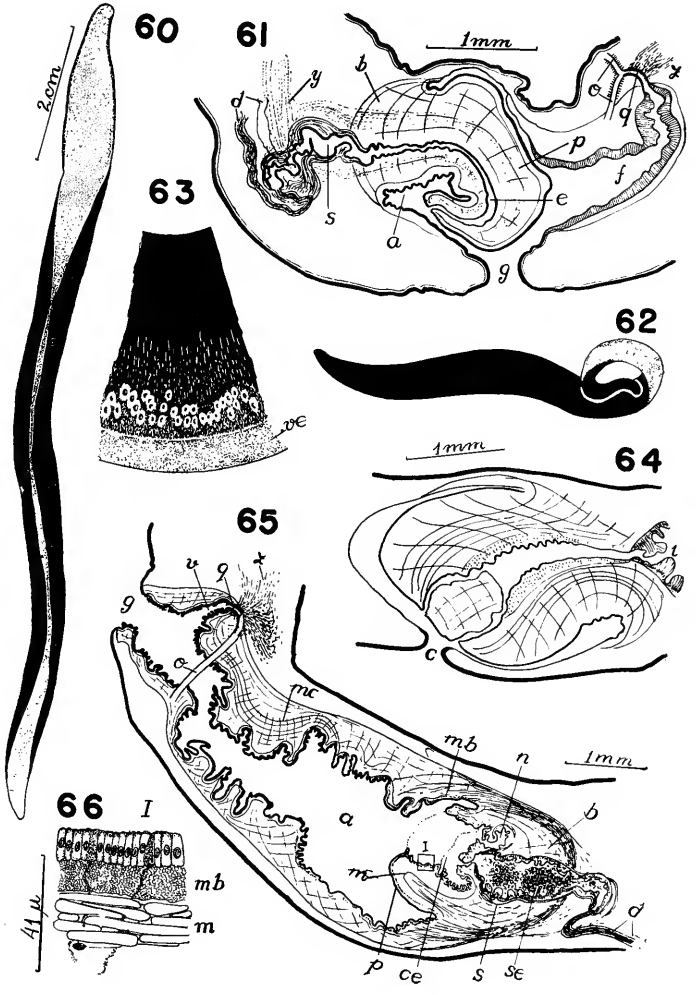
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Plate 10 (Figs. 67-74)

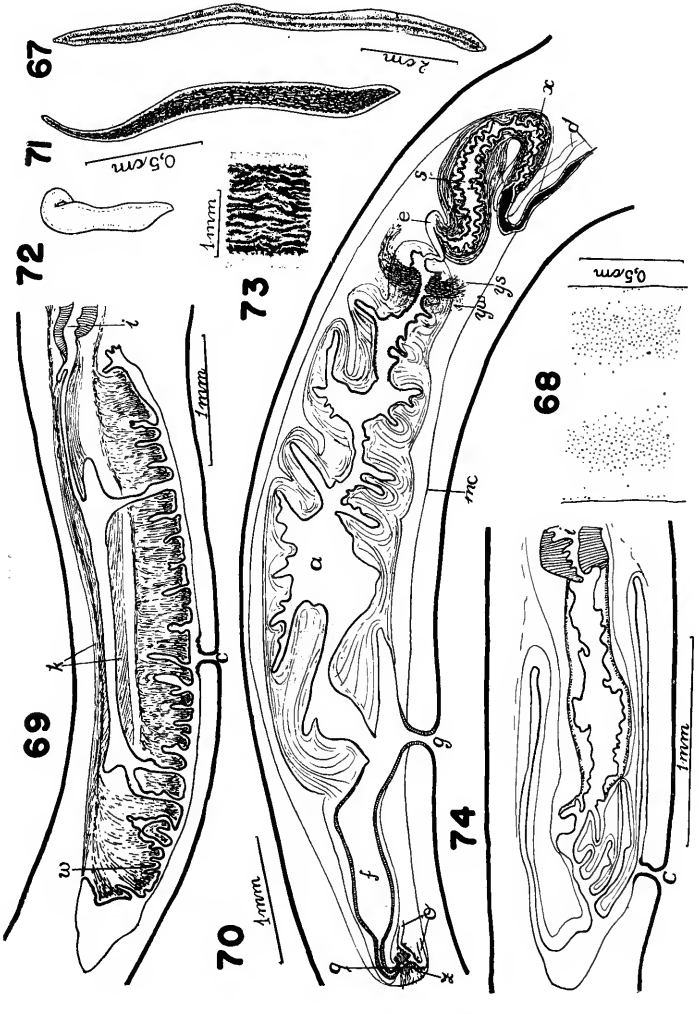
Geoplana hauseri, n. sp.

Fig. 67 — Dorsal view, colour pattern.
Fig. 68 — Maximum spread of the eyes.
Fig. 69 — Pharynx, median section.
Fig. 70 — Copulatory apparatus, combined sagittal sections.

Geoplana suva
(also Plate 11, Figs. 75-77)

Fig. 71 — Dorsal view, colour pattern, creeping worm.
Fig. 72 — Dorsal view, resting worm.
Fig. 73 — Magnified portion of dorsal side, colour pattern.
Fig. 74 — Pharynx, median section.

a, male atrium; c, mouth; d, efferent ducts; e, ejaculatory duct; f, female atrium; g, gonopore; i, intestine; k, muscularis of pharynx; mc, common muscle coat of copulatory apparatus; o, oviducts; q, common glandular duct; s, seminal vesicle; w, glands of pharynx; x, point of union of the two ental rami of seminal vesicle; ys, heavily stained eosinophilous glands, yw, lightly stained eosinophilous and some cyanophilous glands; z, shell glands.


67
2cm
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74
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68
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a
b
c
d
e
f
g
gd
gs
mc
o
p
s
x

PLATE 11 (Figs. 75-81)

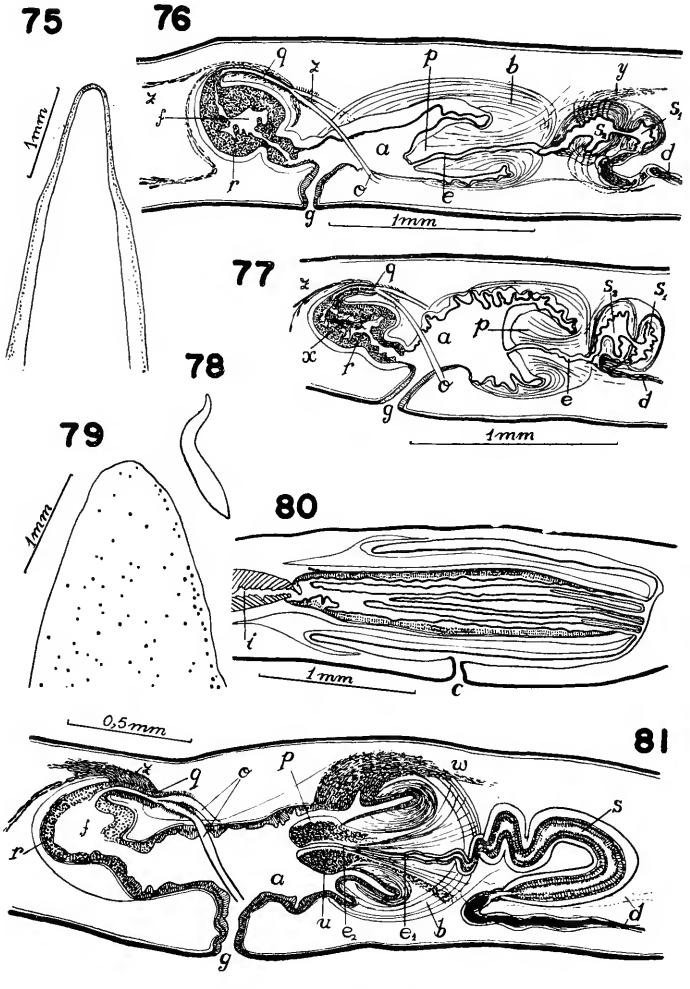
Geoplana suva, n. sp.
(also Plate 10, Figs. 71-74)

Fig. 75 — Anterior end, distribution of the eyes.
Figs. 76-77 — Copulatory apparatus of two specimens, combined sagittal sections.

Geoplana beckeri, n. sp.

Fig. 78 — Outline of a preserved worm.
Fig. 79 — Anterior end, distribution of the eyes.
Fig. 80 — Pharynx, median section.
Fig. 81 — Copulatory apparatus, combined sagittal sections.

a, male atrium; b, penis bulb; c, mouth; d, efferent duct; e, ejaculatory duct; e_1, ental part of ejaculatory duct; e_2, ectal part of ejaculatory duct; f, female atrium; g, gonopore; i, intestine; o, oviducts; p, penis papilla; q, common glandular duct; r, pluristratified lining of female atrium; s, seminal vesicle; s_1, first seminal vesicle; s_2, second seminal vesicle; u, accummulation of eosinophilous secretion; w, purple glands of male atrium; x, spermatozoa inside female atrium; y, granular glands of seminal vesicle; z, shell glands.


75
1mm
76
q
z
p
b
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f
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S_2
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S_2
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78
79
1mm
80
i
1mm
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81
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e_2
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