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FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS

BOLETIM N.° 260 **ZOOLOGIA N.° 23**

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SÔBRE A PRESENÇA DE CLOACA E RESPIRAÇÃO INTESTINAL NO CASCUDO *

[*Loricariidae*: *Plecostomus plecostomus* (Linn.)]

PAULO SAWAYA e LINA MARIA DE PETRINI
Departamento de Fisiologia Geral e Animal — Universidade de São Paulo.

(1 Est.)

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1.

INTRODUÇÃO

Os estudos sôbre a respiração aérea de peixes das águas doces tropicais intensificaram-se com as investigações de Carter e Beadle (1931) no Gran Chaco Paraguaio, de Carter (1932-34) na Guiana Britânica e de Beadle (1932-34) na África do Norte. Rauther (1911, p. 510) e Carter (1935, p. 229) assinalaram a utilização do estômago como órgão de respiração aérea em *Loricariidae* (*Ancistrus* e *Plecostomus*). Êste último autor juntamente com Beadle (l. c.) descreveu também a respiração intestinal em *Haplosternum* e *Callichthys*.

Bertin (1958) sumaria os trabalhos dos autores inglêses aduzindo as particularidades de utilização do intestino como órgão respiratório em peixes das famílias *Cobitidae* (*Cobitis, Misgurnus*) e *Callichthyidae* (*Haplosternum* e *Callichthys*), dizendo que o ar é introduzido pela bôca e evacuado pelo ânus depois de deixar certa parte do oxigênio, que contém, nos capilares da mucosa intestinal. O mes-

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mo autor (p. 1386) dá numerosas informações sôbre a utilização dos intestinos pelas *Cobitidae* como órgão respiratório, mencionando os conhecidos estudos de Lupu (1910, 1935) em *Misgurnus* e os de Wu & Chang (1945) em *M. anguillicaudatus* que mostra alternância das fases respiratória e digestiva.

Quanto aos peixes das regiões tropicais da América do Sul, além das antigas referências de Jobert (1877, 1878), as mais recentes restringem-se às investigações de Rauther (l. c., p. 479) e de Carter & Beadle (1931, p. 346). O primeiro estudou *Plecostomus* e *Otocinclus,* e os segundos referem-se a *Haplosternum* e a *Callichthys,* dizendo que as substâncias alimentares não são encontradas a não ser no estômago e nas porções duodenal e retal. Todo o resto do intestino é uma vasta bôlsa de ar de paredes delgadas, desprovidas de glândulas e com pouca musculatura, mas ricamente vascularizada. Um problema não resolvido, dizem, é o da progressão do alimento desde o duodeno até o reto, na ausência de musculatura e de cílios vibráteis.

Associam os aludidos autores a existência dêste modo singular de respiração, ao baixo teor de oxigênio das águas dos charcos das regiões tropicais. A observação é repetida por Willmer (1934, p. 283) que teve oportunidade de estagiar também na Guiana Britânica, ao notar que "em muitos charcos investigados, verificou-se ser o teor de oxigênio da água extremamente baixo, tão baixo de modo a tornar a respiração aquática, como naturalmente se compreende, inadequada para a respiração do peixe, a menos que o sangue se tenha modificado estranhamente para funcionar em tensões mínimas de oxigênio". As observações e experiências de Carter & Beadle (l. c.) em que mediram no Gran Chaco Paraguaio, na Guiana Britânica (Carter 1933, 1935a) e também as de Beadle na África Oriental (1932a) o teor do oxigênio nos charcos serviram de base a vários estudos ecológicos, especialmente no que se refere à fauna ictiológica como se pode notar no bem elaborado trabalho de Johnels (1953, p. 338) sôbre os peixes do Rio Gambia, na África.

Dentre os peixes tropicais estudados, figuraram, na família das *Loricariidae,* o *Ancistrus anisitsi* e o *Plecostomus plecostomus,* que ocorrem em muitas regiões de tôda a América do Sul.

Os Cascudos já foram objeto de investigação, a pele e o trato digestivo por Rauther (1911, p. 497), v. Ihering (1930, p. 96) es-

tudou-lhes os hábitos de reprodução, Carter (1935, p. 229) pesquisou a respiração e Azevedo (1938, p. 211), no Nordeste do Brasil, investigou a desova natural e a fecundação artificial do *Plecostomus plecostomus*. No decorrer de seu trabalho, Azevedo (l. c., p. 214, 215) dá informações sôbre o regime alimentar, o aparelho digestivo, os órgãos genitais, etc. Quanto à respiração limita-se a resumir as observações de Carter. Uma boa resenha destas observações com outros informes úteis se encontra nos trabalhos básicos de Carter (1931, p. 1), de Leiner (1938, p. 75) e no de Bertin (1958, p. 1763).

O *Plecostomus,* sendo um dos peixes muito comuns nos rios e ribeirões que circundam ou atravessam a cidade de São Paulo, onde se conhecem pelo nome de Cascudo, dada a carapaça de escamas ósseas que os caracteriza, foi-nos possível efetuar uma série de experiências e observações sôbre a respiração aérea intestinal, e especialmente sôbre a estrutura e funcionamento da região terminal dos intestinos. Resolvemos abordar êste tema não só por ser pràticamente desconhecida a estrutura da região urogenital destas *Loricariidae,* como por serem as poucas informações existentes sôbre a respiração (Rauther, l. c., p. 521; Carter, 1935, p. 229) restritas principalmente ao estômago.

Apresentaremos agora os principais resultados de nossas observações e experiências.

2.

MATERIAL E MÉTODOS

Colhemos vários Cascudos dos arredores da Capital, principalmente dos ribeirões Pirajussára e Jaguaré, êste último, atravessa os terrenos onde se localiza a Cidade Universitária, no Butantã. Servimo-nos também de peixes provindos dos rios Atibaia e Jaguarí, das fazendas Atibaia e Santa Úrsula. Todos êstes Cascudos foram classificados como *Plecostomus plecostomus*. Conseguimos ainda inúmeros peixes do rio Piracicaba, que são diferentes no porte e no colorido, mas que apresentam caracteres concordantes com os observados nos *Plecostomus*. Por se tratar de espécie e talvez gênero

diferente, deixamos de lado, por enquanto, nossas observações feitas nos exemplares de Piracicaba.

Os peixes foram todos pescados com tarrafa e trazidos imediatamente para o Laboratório e colocados em grandes tanques de água corrente da torneira. Logo a seguir eram operados sob anestesia com solução a 5‰ de uretana. Na dissecção expunha-se a cavidade abdominal pela retirada da parede do abdomem, inclusive as duas nadadeiras pélvicas. Enquanto se faziam as observações ou as experiências, os animais eram mantidos anestesiados, para o que a solução de uretana era gotejada na bôca do animal que jazia em decúbito dorsal numa placa com um leito de cêra do formato do peixe. Fêz-se, assim, o estudo sob a lupa Greenough.

Algumas observações foram feitas pela Lic. Maria Aparecida Esquibel, colaboradora do Departamento, que nô-las cedeu. Registramos aqui o nosso agradecimento. Os desenhos foram da autoria da Srta. Lúcia Rocha Bastos. Aos srs. Octavio Camargo Morais e Alberto A. N. Morais, proprietários das Fazendas referidas, nossos agradecimentos pelos peixes fornecidos.

3.

OBSERVAÇÕES E EXPERIÊNCIAS

Nos tanques de água dôce corrente, os peixes permaneciam a maior parte do tempo (observações feitas durante o dia e à noite) fixos firmemente pela bôca ao substrato: paredes e fundo do tanque, tijolos ou pedras. Muito raramente eram vistos nadando na superfície, e neste caso, de vez em quando expunham a bôca na atmosfera. Para conseguir manter os Cascudos nos aquários durante longo tempo, é indispensável provê-los de água corrente. Retirados do aquário sempre expulsavam bôlhas de ar pelo orifício anal. Ao chegar nova remessa de peixes, era necessário deixá-los em tanques separados, pois, do contrário, os antigos cascudos matavam os recém-chegados. Isto se deve à predileção pelas algas, pois, os que se mantinham há muito nos aquários raspavam com intensidade o corpo dos recém-chegados para retirar-lhes as algas aderentes à pele, e êstes morriam pouco depois. Nossas observações confirmam, assim, as idênticas de Azevedo (1938, p. 212) feitas no Nordeste.

Ao examinarmos os peixes anestesiados, tivemos a atenção voltada para a região posterior, pela presença de uma papila (Fig. 1, P) que se salienta na região anal, situada a mms 7,5 da inserção da nadadeira anal. Essa papila tem a forma de um cône em cujo ápice se acha o ânus. A altura do cône varia de 2 a 4 mms e habitualmente quando o peixe está em decúbito dorsal ela se dobra de modo a ter o ápice do cône na pele da região. Apresenta-se de tempos em tempos erectil, executando movimentos de retração e propulsão, durante os quais, o ar ou a água são sugados para dentro do intestino. Êsses movimentos são rítmicos, e no peixe anestesiado têm a freqüência de 16 a 20 por minuto. São movimentos sincrônicos com os da ventosa bucal, que se têm por movimentos respiratórios.

Aberto o abdomem e observado à lupa, notam-se logo os intestinos enrolados em espiral, interpondo-se entre as alças do tecido gorduroso, o qual assim estabelece a conexão entre elas. Desenroladas as alças, o intestino tem de comprimento de ms 2,5 num peixe de 20 cms.

As paredes dos intestinos mostram estrutura típica, bem visível à lupa, sendo os feixes de fibras musculares dispostos em forma de ogivas agudas, acompanhadas de densa rêde de capilares sangüíneos. Chama logo a atenção o local de onde partem as espirais dos intestinos, que é constituído pelo fígado — massa castanho-clara tendo no centro um tufo de vasos que se irradiam sôbre a superfície das espirais dos intestinos, dirigindo-se os mais calibrosos para a região caudal do abdomem. Retirados os intestinos, nota-se que as espirais se volteiam ao redor do fígado, o qual apresenta, assim, várias chanfraduras que abrigam as alças intestinais. Afastando-se a massa intestinal, nota-se, à direita, o estômago sempre cheio de ar, aparecendo como uma grande vesícula. Deve ter sido principalmente êste aspecto do estômago que levou Rauther (1911, p. 521) e Carter (l. c.) a admitirem a utilização do estômago como órgão respiratório por êstes peixes.

a. *Aspiração retal* — A primeira experiência consistiu em depositar algumas gôtas de solução concentrada de tinta nanquim sôbre a papila anal. Em contacto com essa papila, a solução de nanquim formou logo nítida corrente que se orientou para o orifício anal, onde penetrou de modo intermitente, acompanhando os movimentos rítmi-

cos da papila. Dissecado o intestino terminal, viu-se o mesmo repleto da solução de nanquim, espalhando-se os grânulos pretos por tôda a superfície da mucosa. Esta experiência foi repetidas vêzes, sempre com idêntico resultado. Nas condições experimentais, i. é, o peixe anestesiado, recoberto d'água ou fora dela, mostra contínua sucção de água ou de ar pelo orifício anal.

b. *Vascularização do intestino terminal*. — Exposto o intestino terminal, logo se notou a nítida diferença entre a musculatura dêste trato e o do restante das alças, pois, no intestino terminal os feixes musculares não se dispõem em forma de ogivas bastante típicas como se vê nas alças do restante dos intestinos. Além disso, os feixes musculares do intestino terminal formam linhas longitudinais providas de capilares abundantíssimos, que circundam o órgão constituindo uma rêde bastante concentrada, na qual se percebe, com muita facilidade, a circulação do sangue, podendo-se mesmo distinguir o colorido vermelho vivo de alguns capilares, do vermelho escuro de outros, e, além disso, notar a diferença de direção da corrente sangüínea da e para a parede intestinal. As duas correntes partem ou confluem do centro circulatório já apontado no fígado.

c. *Região uro-genital* (Fig. 1). — Para expor o intestino terminal houve necessidade de afastar a massa de espirais intestinais e retirar a membrana peritoneal dorsal, escura, que recobre tôda a face do abdomem. Continuando a ablação dessa membrana, deparamos com uma vesícula claviforme, situada à direita do intestino terminal (Fig. 1). Logo percebemos ser essa vesícula septada no sentido dorso-ventral, sendo nítida a reentrância na face ventral, a qual se prolonga pela base da clava e prossegue pela face dorsal (S). Essa reentrância corresponde à inserção do septo que divide a vesícula em duas metades, uma direita e outra esquerda. Aberta a vesícula, nota-se não ser o septo completo em tôda extensão, mas cessa a 1 ou 2 mms do ponto de conexão da vesícula com o intestino, o que se dá pela face dorsal dêste. A extensão do septo é muito variável, de modo que, nos casos em que é reduzido, a bexiga se apresenta dupla, com as duas porções paralelas. Encontramos a vesícula conexa com os rins por meio de dois cordões tubulares (U) que se inserem em cada uma das metades da base da clava e se prolongam craneal-

mente, aprofundando-se nas massas renais que se encontram de cada lado da coluna vertebral. Certificamo-nos assim tratar-se bexiga urinária (B) que recebe os uretéres (U) pela base e se conjuga com o intestino terminal, que aí forma uma bôlsa, constituindo assim uma verdadeira cloaca (C). Essa bexiga (B), em vários Cascudos, encontrava-se cheia de gás e em outros continha ca. de ml 1,5 a 2 de um líquido claro, transparente. De cada lado da clava vesicular acham-se as gônadas. Nos machos, os dois testículos (T) são cilíndricos, alongados e se estendem à ca. de 5 cm acima da base da bexiga prolongando-se caudalmente, margeando o órgão e contornam-no passando para a face ventral onde, a 1-2 mms de desembocadura na cloaca, se conjugam com a bexiga formando o seio uro-genital (SU). Êsses dutos deferentes são, no início numerosos, contando-se até dez, depois se fundem uns com os outros até se reduzirem a um par de cada lado (Fig. 2, D), para se abrirem na cavidade não septada da bexiga urinária. Os ovários são também em número par, um de cada lado da bexiga urinária, formando, nos exemplares em época de postura, duas grandes massas amareladas, com as eminências dos ovos bem salientes, dando assim, ao órgão um aspecto crenelado típico. A desembocadura dos ovidutos na bexiga urinária faz-se na mesma altura que os dutos deferentes.

d. *Cloaca* (Figs. 1, 2 e 3). — Diante destas observações interessantes, procuramos verificar as relações entre os órgãos acima citados. Dissecamos alguns exemplares, abrindo o intestino terminal com um corte longitudinal, partindo do orifício anal. Afastados os lábios da pele divisa-se imediatamente uma empola (C) relativamente ampla, com extensão de ca. de 1 cm, a partir do qual se inicia o intestino pròpriamente dito, com o aspecto característico das ogivas formadas pelos feixes musculares. A empola é forrada por uma mucosa pregueada longitudinalmente. Cateterizando a bexiga urinária em sentido retrógrado, pudemos distinguir o orifício de abertura na empola cloacal. Repetida a operação com os ovidutos notamos que os mesmos se abrem na bexiga pela face ventral a ca. de 1 mm do ponto de conexão com a cloaca. Não foi possível canular os dutos deferentes. A fim de verificar a exatidão destas conexões, fizemos uma série de preparações microscópicas, de 5 a 25 micra de espessura, com material fixado em Bouin — acético e co-

rado pela hematoxilina e eosina. Tais preparações, que compreendem a região que vai da abertura anal, inclusive, até o início do tubo intestinal que se segue à empola intestinal, mostraram, na papila cutânea epitélio pavimentoso pluriestratificado corneificado, sem inclusões ósseas. Numerosos botões do gôsto são evidentes na espessura do epitélio. A mucosa da cloaca é provida de um epitélio pavimentoso pluriestratificado (Fig. 2, E) sem corneificação evidente. Ao redor do orifício anal nota-se um anel muscular espêsso (M), circundado por fibras musculares longitudinais esparsas (Ml). A 2-3 cms do orifício anal encontra-se o ponto de desembocadura da bexiga urinária na cloaca. A Fig. 2, B, mostra claramente a origem dêsse orifício caracterizada pela presença de duas reentrâncias guarnecidas de epitélio cilíndrico simples. Quase não hã transição entre o epitélio da cloaca e o da bexiga urinária, mas o anel muscular acima citado, aí se interrompe para continuar completo depois da conexão da bexiga com a cloaca. A 1-2 cms dêsse ponto nota-se a desembocadura dos quatro dutos gonadais na face ventral da bexiga urinária. Distinguem-se os mesmos pelo epitélio cúbico que os guarnece interiormente (Fig. 2, D). Em certos cortes seriados da bexiga urinária foi possível ver o início do septo que divide a bexiga em duas porções, direita e esquerda. Tanto no cório do septo, como no das paredes da bexiga, assim como no da cloaca e do reto são evidentes as rêdes de capilares sangüíneos (V). Os métodos de coloração utilizados permitiram apenas divisar na espessura do epitélio do intestino e da cloaca, e também no da bexiga urinária, esparsos capilares sangüíneos. Tôda região uro-genital e intestinal aparece assim densamente irrigada.

e. *Respiração intestinal*. — Outra observação que julgamos de interêsse registrar, vem a ser a presença, em tôda extensão do intestino, de numerosas bôlhas de gás. Após termos verificado que a cloaca exerce sucção rítmica, mesmo quando o peixe se acha exposto ao ar, nos animais anestesiados, com o abdomem aberto, notamos a presença de numerosas bôlhas gasosas movimentando-se dentro das alças intestinais. O aspecto vesicular do intestino descrito por Carter & Beadle (1931) em *Haplosternum* e em *Callichthys* não observamos nos nossos Cascudos. Na realidade as bôlhas de gás misturavam-se com o conteúdo intestinal esverdeado típico dêstes ani-

mais que são grandes comedores de algas (Azevedo 1938, p. 212). Os movimentos das bôlhas gasosas acompanhavam a ritmicidade dos movimentos da cloaca, havendo, pode-se dizer, uma concentração de bôlhas gasosas nas zonas mais densamente irrigadas do intestino.

4.

DISCUSSÃO

A disposição dos órgãos verificada na região uro-genital do Cascudo é, sem dúvida, digna de registro. A ocorrência de uma cloaca nos Teleósteos, tem sido assinalada em *Anguilla vulgaris* e em alguns outros peixes (Audigé 1910, p. 379; v. d. Brock, v. Oordt & Hirsch 1938, p. 840). Como se sabe a existência de um poro uro-genital e de um ânus é a disposição mais freqüente nos Teleósteos, como Lickteig (1913, p. 17) figura no tipo 7 de seus esquemas. A presença de uma cloaca nos Teleósteos é fato inusitado (Rauther 1911, p. 522). No que se refere aos *Plecostomus* parece-nos ser esta a primeira vez que esta estrutura é estudada com pormenores. O próprio Rauther (l. c., p. 522) que dispôs apenas de alguns exemplares de *Plecostomus* diz serem fragmentárias as suas observações e descreve sumàriamente a cloaca dos mesmos. O autor, mais tarde (1940, p. 977), assinala a formação em outras *Loricariidae*. Não há dúvida ser a vesícula disposta à direita do reto uma verdadeira bexiga urinária. Suas conexões com ambos os rins são bastante evidentes, e a sua desembocadura na cloaca pode ser evidenciada com pormenores tanto da estrutura como do funcionamento. O fato de ter sido encontrada ora com conteúdo gasoso, ora com líquido, indica ser a mesma um depósito dos excreta do organismo. Sòmente pesquisas ulteriores poderão demonstrar o teor dos gases e das substâncias existentes no líquido encontrados na bexiga. Chama a atenção a riqueza de vascularização do órgão, o que não exclui sua participação na função respiratória do animal. Além disso, sendo o Cascudo um peixe desprovido de bexiga natatória e utilizando o estômago como reservatório de ar ou como órgão respiratório é possível que a bexiga urinária se encha de gás para auxiliar o animal durante a natação, mantendo o necessário equilíbrio hidrostático. São êstes pontos dignos

de investigação para esclarecer esta insólita disposição dos órgãos na região uro-genital.

Segundo Carter (1931, p. 7; 1957, p. 68) as condições para se estabelecer a função respiratória de um órgão seriam:

1. O epitélio do órgão deveria ser suprido por rica vascularização, e o sangue deveria achar-se mais oxigenado depois de atravessar os capilares do órgão;

2. Deveria haver troca regular de ar entre as cavidades do órgão e o exterior;

3. O gás contido e o excretado do órgão deveria possuir menos oxigênio e mais dióxido de carbono que o ar atmosférico.

Até o momento, as observações efetuadas no Cascudo, referentes à respiração intestinal, atendem apenas em parte às condições indicadas por Carter. E' fora de dúvida que os intestinos são dotados de densíssima rêde vascular sangüínea, há regular troca de gases entre a cavidade intestinal e o meio ambiente. Restaria verificar o teor do oxigênio e o do CO2 no sangue que vai para e no que provém dos intestinos. Dificuldades técnicas, principalmente devido à extrema exigüidade do calibre dêsses vasos impediram, até agora, essa verificação.

Como bem acentua Carter (l. c.), o fato de um peixe ter respiração aérea não significa necessàriamente ser êste incapaz de respirar água. Parece ser êste o caso de *Plecostomus*. Lembra ainda Carter (l. c., p. 69) o caso dos peixes que respiram ar e não podem viver com respiração aquática mesmo com água bem oxigenada, como sejam as *Lepidosiren* (Fullarton 1931; Sawaya 1946); *Haplosternum* (Carter e Beadle 1931), *Electrophorus* (Carter 1935), etc. As observações efetuadas até agora em *Plecostomus* indicam pertencer o mesmo ao primeiro grupo e não ao segundo.

Não temos dúvidas em afirmar as relações dos intestinos do *Plecostomus* com a respiração. A presença de bôlhas de gás na cavidade intestinal, em tôda a sua extensão e os movimentos rítmicos das mesmas, acompanhando os de sucção exercidos pela cloaca são índices certos de uma utilização do intestino como órgão respiratório. Aliás, nossas observações confirmam as asserções de Marlier (1938, p. 164) ao indicar que "em todos os peixes (inclusive as *Loricariidae*) o in-

testino posterior não serve mais à digestão, mas seu epitélio adelgaçado é finamente vascularizado por capilares de artéria celíaca ou de aórta dorsal, retornando o sangue à circulação pela veia porta-hepática ou veia inter-renal". A diferença de nossas observações está em que no *Plecostomus* o ar que se encontra nos intestinos é sugado pela cloaca e não só pela bôca como diz Marlier (l. c.).

A informação de Rauther (1911, p. 521) e a de Carter (1935, p. 229) sôbre a respiração de *Plecostomus,* pelo estômago, merece reparo. Realmente, todos os peixes que estudamos apresentavam o estômago repleto de gás. Vivendo, porém, seguros a diversos substratos, para o que utilizam a bôca em forma de ventosa, e raramente vindo à superfície para absorver o ar, parece que o mecanismo do enchimento do estômago deveria ser mais complexo, ou por outras palavras, a origem do gás do estômago não seria só diretamente do ar atmosférico. Hora (1932, 1933) fêz interessantes observações em *Misgurnus,* que apresenta idêntico aparelho para se firmar nos substratos, i. é, uma bôca em forma de ventosa. Para explicar a utilização do oxigênio dissolvido na água, lembra a possibilidade de correntes retrógradas de água que penetrariam assim na cavidade branquial. E' possível que o mesmo aconteça nos *Plecostomus,* mas a julgar pelo que até agora pudemos observar, não sòmente quanto à riquíssima vascularização intestinal como aos movimentos rítmicos da cloaca, quer-nos parecer que neste peixe predomine a respiração do tipo intestinal, sendo o estômago utilizado como reservatório de ar para as ocasiões de emergência. Aliás sendo peixe comestível, é levado para as bancas do mercado, como vimos em São Paulo, raramente, mais habitualmente em Piracicaba onde se empilham os Cascudos aos montes, todos êles permanecendo vivos por mais de 8 horas em condições realmente precárias. Examinados os peixes nestas condições notam-se movimentos acelerados da membrana bucal e aumentada a freqüência dos movimentos rítmicos da cloaca.

Finalmente, um outro ponto parece-nos de interêsse referir e vem a ser o do uso do sangue que banha os intestinos como vetor do oxigênio e do dióxido de carbono, o qual segundo Krogh & Leitch (1919, p. 288) deve apresentar-se bastante modificado para utilizar o oxigênio a tensões bastante baixas. Willmer (1934, p. 306) trabalhando com peixes habitantes dos charcos da Guiana Britânica,

cujas águas são muito pobres em oxigênio, verificou que em nenhum dêles haveria maior afinidade da hemoglobina pelo oxigênio, como seria de esperar. O Cascudo embora habite os charcos é, reconhecidamente, peixe de correnteza, e daí o poder adiantar não ser sua hemoglobina provàvelmente dotada de particularidades especiais, pois, o teor do oxigênio nas águas em que vive não deve ser tão baixo. E' ponto ainda a elucidar o do comportamento da hemoglobina que atravessa as paredes do intestino e conduz os gases respiratórios.

5.

RESUMO

As experiências e observações realizadas em Cascudos (*Plecostomus plecostomus*) sôbre o trato intestinal terminal e suas relações com a respiração permitem as seguintes considerações:

1. Os *Plecostomus* são providos de uma cloaca formada por dilatação do reto, na qual se abre o seio urogenital. A cloaca se comunica com o exterior por meio do orifício anal.

2. A bexiga urinária dupla septada, recebe os dutos gonadais formando o seio urogenital.

3. O orifício anal localiza-se numa papila que constitui a papila anal, distante 8-10 mms da base da nadadeira anal.

4. A penetração dos gases ou da água nos intestinos faz-se por movimentos rítmicos da papila anal perceptíveis externamente.

5. Os *Plecostomus* utilizam provàvelmente os intestinos para troca dos gases da respiração, dissolvidos na água ou existentes no ar.

6. Os intestinos de *Plecostomus* são providos de densa rêde de capilares sangüíneos, que provém da artéria celíaca e, depois de penetrarem na parede intestinal voltando em sentido retrógrado ao ponto de origem dessa artéria.

7. Na luz do intestino são evidentes numerosas bôlhas gasosas que se movimentam rìtmicamente acompanhando as pulsações rítmicas da papila anal.

6.

SUMMARY

ON THE PRESENCE OF A CLOACA AND INTESTINAL RESPIRATION IN THE CASCUDO FISH [*LORICARIIDAE*: *PLECOSTOMUS PLECOSTOMUS* (Linn.)].

Aerial respiration in a species of *Plecostomus* has been known since the publications of Jobert (1877-8), and later of Rauther (1911) and Carter (1935). These authors restricted their study to the ability of *Plecostomus* to catch air bubbles from the open air.

This fish is very common in the small rivers which run across the outskirts of São Paulo, where they were caught and brought up to the laboratory and deposited in large tanks supplied with running fresh water. Fishes captured in the rivers Atibaia and Jaguary were also used. With this abundant material, some observations and experiments have been made in order to study the terminal region of the intestine, and also the respiration.

In the opened body cavity, the typical curled spiral arrangement of the intestine is seen. This animal is provided with a characteristic cloaca (Fig. 1), which communicates with the exterior by an anal papilla, and by an urogenital sinus to the urinary bladder and the gonads. The bladder is divided in two portions by one thin septum, and each half bladder is in connection with the corresponding kidney through a very narrow ureter inserted on the top of that organ. The genital ducts open into the ventral side of the urinary bladder, through the urogenital sinus. This sinus is connected with the dorsal side of the cloaca.

The anal papilla in fishes lying in the open air, or observed under water, pulses rythmically (16-20 beatings per minute). Drops of a solution of Indian ink were placed over the anal papilla and immediately the ink was sucked into the cloaca. Dissection of the terminal intestine has shown the cloacal cavity full of the ink, and ink granules which have passed into the intestine are also observed. The intestines have some bundles of muscles disposed as ogives. Between the ogives a dense net of capillary blood vessels can be seen. The

same muscles in the cloaca from longitudinal bundles emerging on the inner surface of the mucosa.

Depending on the phase of the pulses of the anal papilla the bubbles may either run in the direction to the stomach or the cloaca

The blood supply of the intestines consists of branches of the coeliac artery and of the hepatic portal vein. Both vessels are divided into numerous capillaries. Under the microscope the arterial (red bright) and the venous (dark red) blood streams my be observed running from and to the intestines walls. Technical dificulties have so far prevented the collection of the blood from the minute capillaries, a procedure which would be required in order to determine the amount of O_2 and Co_2 in the respective blood currents.

The fine structure of the cloaca, urinary bladder and gonadal ducts were studied in microscopical preparations. The stratified epithelium of the cloaca and also the columnar epithelium of the intestine contain a rich supply of capillaries. Undoubtely the fish uses not only the stomach but also the intestine as an organ for respiration.

On the basis of the experiments and observations made, we can summarise:

1. *Plecostomus* in spite of being a Teleostean fish has a typical cloaca.
2. An anal papilla is present in which the anus opens. The cloaca communicates to the exterior by this papilla.
3. The anal papilla undergoes rythmic movements by means of which water and air are sucked into the cloaca and passed to the cavity of the intestine.
4. The urinary bladder is divided into two portions by one thin septum, and receives the genital ducts in the urogenital sinus.
5. The fish inspires and expels the gases of respiration during those movements of the anal papilla.
6. The presence of an intestinal respiration in *Plecostomus* is thus clearly indicated.

7.

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8.

ESTAMPA

FIGURA 1

Intestino terminal, bexiga e testículos de Cascudo (*Plecostomus plecostomus* L.).

B = bexiga urinária.

C = cloaca.

I = Intestino.

P = papila anal.

S = Septo da bexiga urinária.

SU = Seio urogenital.

T = Testículos.

FIGURA 2

Secção esquemática transversal da região terminal intestinal do Cascudo (*Plecostomus plecostomus* L.). Indicações da figura anterior, mais D = ductos deferentes, E = epitélio pavimentoso pluriestratificado da cloaca, M e Ml = músculos anelares e longitudinais; V = capilares intraepiteliais. Bouin acético — Hemt. eosina.

FIGURA 3

Hemisecção esquemática transversal da cloaca, no ponto de desembocadura da bexiga urinária, em que se nota a diferença do tipo de epitélio da bexiga urinária (cilíndrica simples) e da cloaca (pavimentoso pluriestratificado). Indicações como nas figuras anteriores. Bouin acético — Hemat. eosina.

Fig. n.º 1

Fig. nº 2

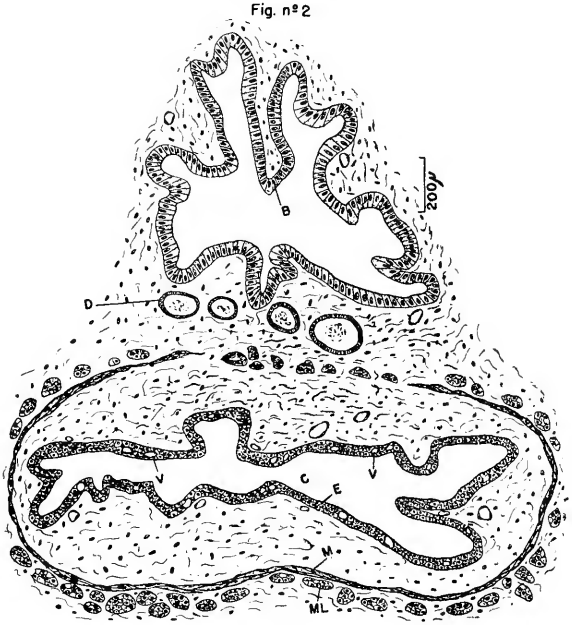
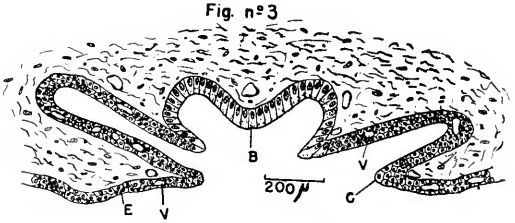


Fig. nº 3



ON HASTULA CINEREA

by EVELINE and ERNST MARCUS

(with 5 plates)

During our studies of sand-burrowing olivids a terebrid called our attention by its ability to move in the sand. In July 1958 we began to study this snail in order to become acquainted with the anatomy of the Toxoglossa, the most highly specialized prosobranchs. Though the material obtained near Cananéia and Ubatuba was abundant, and the snails collected in November copulated in a laboratory dish, they did not lay eggs. Nor did we succeed to observe other biological aspects. The snails endured in the aquarium, but lived there with reduced functions. Also the biology of another toxoglossan family, the Conidae, of general interest because some are poisonous to man, has only been worked out recently.

Our acknowledgments are due to the former and the present Director of the Oceanographic Institute of the University of São Paulo, the late Professor Wladimir Bernard and Dr. Ingvar Emilsson, who granted us the permission to work at the Research Stations, of the Institute, where we found the obliging and comprehensive help of Dr. Edmundo Nonato (Ubatuba), Dr. Victor Sadowsky, and Mr. Caio del Rio Garcia (Cananéia).

SYSTEMATIC NOTE

The auger shell common in the area of Santos is called *Hastula cinerea* (Born, 1780) by the South American conchologists v. Ihering (1897, p. 170), Lange de Morretes (1949, p. 110), Gofferjé (1950, p. 250), Carcelles (1953, p. 14), and L. and E. H. Buckup (1957, p. 33). According to Smith (1877, p. 229-230) Lamarck was right when he united *cinerea* Born and *aciculina* Lamarck (1822, p. 290; 1844, p. 250), but not every material called *aciculina* Lm. is *cinerea* (Born) (see Smith, l. c.). Bouvier's (1887, p. 324) *Terebra aci-*

culina Lm., for example, differs in details of the outer oral region and the central nervous system from our species. The latter agrees, on the other hand, with Bergh's *Hastula aciculina* Lm. (1908, p. 124) from Santos. We are, however, not quite certain that our species can be called *cinerea*. This species described, among others, by Reeve (1860, pl. 9, spec. 35) and Abbott (1955, p. 266) has 45-50 riblets per whorl against 28-36 in our shells and in *Terebra salleana* Deshayes (1859, p. 287). As the latter species differs from ours by size, colour, and punctuations, we call our material *cinerea* till further conchological studies.

According to Carcelles and Abbott the range of *cinerea* extends from SE Florida and the West Indies to S Brazil, Sta. Catarina. Coomans (1958, p. 101) reported it from Margarita Island on the Caribbean coast of Venezuela. Some indications of *cinerea* from the coast of Texas (Johnston 1934, p. 134; Behre 1950, p. 36) possibly refer to *salleana*.

The conchologically characterized subgenus *Hastula* H. and A. Adams, 1853, was considered as a genus by Troschel (1866, p. 29-35) because of particularities of its fore-gut. Among others, Bergh (1908, p. 124), Thiele (1931, p. 375), Joyce Allan (1950, p. 195), and A. Myra Keen (1958, p. 494) follow Troschel. By its shell *Hastula cinerea* belongs to the subgenus, in Thiele "sectio", *Impages* E. A. Smith (1873, p. 263).

OCCURRENCE

The snails live burrowing in clean and fine sand of the gradually sloping beaches of the State of São Paulo, from Ubatuba to Cananéia. Near the Station of Ubatuba they live near the low waterline in the zone that Gerlach (1957, p. 418) calls "Nerine-Zone". Here the polychaete *Nerinides agilis* (Verrill 1873, p. 346, 600) widely distributed along the American Atlantic coast (Hartman 1956, p. 291) occurs. Its vertical burrows extend farther down than the worms are long. In our material their length corresponds to that of *N. minuta,* according to Hartman (1951, p. 81) identical with *agilis.* Miner (1950, p. 336) applies another synonym, *heteropoda*. As we found typical setae in the gut of *Hastula cinerea,* we conclude that the snail regularly feeds on this spionid. On the sandy beaches of

the islands in front of Cananéia the snails are most frequent in the zone that Gerlach (l. c.) calls "Prallhang" and feed principally on an opheliid.

Hastula cinerea occurs chiefly in the intertidal zone, but was sometimes obtained also over the mean high water-line. It can be dug up from about 3-5 cm depth in the sand. It is easier to collect the animals at low tide, when their trail is visible on the sand, or when they are washed out by a wave and glide straight against the refluent water, "with the spire at the posterior mid point" (Morton 1958a, p. 7). When attained by the next wave they generally have dug the pedunculate foot sufficiently deep into the sand to maintain their place. For burrowing the shape of the shell is perhaps less fit than the torpedo-form of the genus *Olivella* (G. E. and N. MacGinitie 1949, p. 355), but the efficiency of the foot of *Hastula cinerea* compensates a possibly not quite as perfect adaptation of the shell-shape.

SHELL

The shell is medium-sized, with subulate spire. The whorls are nearly plane; 11-13 adult ones are topped by 3-4 of the protoconch (Fig. 1), frequently broken off, even in living snails. The protoconch of the species that Yen (1935, p. 263, pl. 11, f. 19) calls *Hastula lepida* (possibly *strigilata*) is much shorter. In *Hastula cinerea* the larval shell is 1 mm long, hence of considerable size. Due to its thick conchiolinous coat it is brown. It is filled up by secondary calcification as are the 3-4 following whitish whorls. The remaining whorls of living snails are bluish grey, sometimes olive grey. These colours are brought about by colourless layers of calcium carbonate alternating with dark brown conchiolinous ones. The periostracum is extremely thin. The inside of the shell and the strong columella are brown. Around the body whorl, a little below its middle, runs a white zone which is also distinctly seen within the brown aperture. Wrigley (1942, f. 21) figured this band in *H. anomala*. Dead shells whose outer calcareous layer is worn are light brown.

As in many sand-burrowers the surface of the shell is smooth, shining, though under a lens minute pricks appear which form more than 100 fine spiral rows. The apical half of the whorls bears flat axial riblets whose number is 28-36 per whorl. As calcareous thi-

ckenings the ribs are whiter than the furrows between them, and sometimes the light and dark pattern is even more pronounced than could be expected from the sculpture. The sutures are concealed by the apical borders of the whorls and only recognizable where these, as frequently, are broken.

The aperture is broadened in front (below). A siphonal notch separates the thin outer lip from the columella. The latter projects in front and bears two quite low folds. The parietal wall (inner lip) is dark brown and contiguous with a white parietal callus on the body whorl. The length of our shells is up to 55 mm, the breadth up to 11 mm; the aperture of our longest shells is 11 mm high, 6 mm broad.

We obtained the biggest living snails in April, August, November, and the last week of January (greatest number of them) near Ubatuba. In July and the last week of January specimens from Canaéia did not surpass 32 mm in length.

In many shells local breaks are somewhat irregularly repaired. In July 1958 we frequently found eggs of Turbellaria Proseriata densely set in a single layer on the shell of living snails, several colonies of the membraniporid *Conopeum commensale* (Kirkpatrick and Metzelaar, 1922), and *Hydractinia*.

HEAD AND FOOT

Foot and siphon are the principal organs which come out of the shell; in the laboratory dish also the head with everted labial tube and once the proboscis were observed outside the shell. When preserved most of our anaesthetized motionless snails retracted foot, siphon and anterior tube, but when we crushed the shell of narcotized animals with a vise, many more remained stretched.

Siphon (vi) and head are richly pigmented. Only the folds around the outer mouth (m) are light as well as a stripe on the left side of the head which leads into the mantle cavity. The eyes are 40μ in diameter, their lens 25μ; they lie in the connective tissue of the bases of the tentacles (t). These are low, blunt cones. Bergh's statement "no tentacles" (1908, p. 124) must have been occasioned by macerated specimens.

The borders of the muscular siphon are papillate (Bergh 1908, pl. 10, f. 5) and close the canaliculated organ to form a tube. The

outer opening bears some cilia, the lumen is nonciliate. Six principal longitudinal nerves whose origin is described in the chapter on the central nervous system occur in the siphon. At the base the borders of the siphon are thickened and produce a funnel leading to the tip of the osphradium (ov.). The lobate appendage which can close the siphon of *Conus lividus* (Alpers 1931, p. 591-592) is not developed.

The foot is 25 mm long in a snail with a 48 mm long shell; it is slightly pointed in front and rounded behind. Its most contractile zone lies in front of two slight folds which appear transitorily on both sides under the head. These folds allusively separate propodium and metapodium and are better marked in the living than in the preserved snail. Other pedal folds do not occur, also the anterior border is entire, not bilabiate and notched as in *Conus* (Bergh 1896, p. 74-75). Except for this border which is light, sole and back of the foot are richly pigmented. As the columellar and nervous connection between visceral hump and foot is thin, peduncle-like, the amplitude of the movements of the foot is wide. In the laboratory dish we often saw the foot moving in direction of the spire, and the shell swinging into normal position only after some time.

Behind the columellar connection the operculum (or) is attached on about half its length to the back of the foot. The operculum is oval, light yellow, horny, and thick; 3 mm long, 1,6 mm broad. Its border is convex, smooth; its nucleus, terminal. The operculum does not seal the aperture. Even in animals preserved without anaesthetization, hence maximally withdrawn, it does not close the shell. In these snails the frilled rim of the foot and the tip of the siphon stand out around the operculum.

The origin of the columellar muscle (cz) lies on the level of the posterior border of the anterior digestive diverticulum (l). In adult full-grown snails whose 4 larval and 4 first definitive whorls are filled up by calcification, the visceral hump ascends to the ninth whorl. Five to six further whorls contain the viscera. The retracted "Kopffuss" (Ankel 1936, p. 10, 11) occupies the two foremost whorls.

The sole of the foot is densely ciliated, the back is ciliated only in front under the mouth and in the stripe of the egg-guide (zo) described later on. The anterior border of the foot is amply supplied

with sensory cells connected by a nerve net which contains accessory ganglia. As in other burrowing prosobranchs, e. g. *Natica* and the olivids, the anterior border of the foot is an important sense organ. The glandular equipment of the foot is however weak. Scattered unicellular blue-staining glands occur in the epidermis of the foot and chiefly in that of the sole, but subepidermal insunk glands are restricted to the pedal borders mainly in front, in the middle of the fore end. They correspond to the anterior pedal mucus gland (Graham 1957, p. 141) and discharge to the anterior border and the dorsal surface of the foot, not to the sole. *Conus* has a concentrated anterior pedal gland (Houssay 1884, p. 266).

Penis, egg-guide, and ventral female gland are described with the reproductive organs.

PALLIO-PERICARDIAL COMPLEX

The pallial cavity is deep and narrow; the half apical angle which indicates its breadth (Fretter 1951, p. 584) is small, 7-7,5°. The border of the mantle cavity is light, the anterior region of the roof deep black, except for osphradium and gill; the floor is dark in some places, light in others. When the shell is removed, a sutural fold (so) appears as a sharp edge on the right side between pallial roof and bottom. This fold, Bergh's "Kapuze des Mantelgebrämes" in *Conus* (1896, p. 75-76, pl. 1, f. 5, c; explanation: p. 200), is thickened by vesicular tissue. Also in the visceral cavity this interstitial tissue is richly developed, wrapping the coils of the poison gland, salivary gland, radula-sac, central nervous system, and other organs.

The inhalant current produced by the branchial cilia passes over the osphradium. Big particles were seen to be retained by the papillae of the outer opening of the siphon. The exhalant current was observed on the roof of the pallial cavity on the right side, on some parts of the hypobranchial gland and on a ciliated streak on the floor, which is underlain by the pallial spermiduct in the male.

As generally in the Stenoglossa (Simroth 1907, p. 1029) the slightly pigmented light brownish osphradium (ov) is large, 8 mm long, 2 mm broad, bipectinate, and has about 50 filaments on either side. The great size of this presumed chemoreceptor is evidently associated with carnivorous life (Morton 1958b, p. 72); whether besides

it is correlated with much sediment in the respiratory water (Hulbert and Yonge 1937; Yonge 1942, p. 200; 1947, p. 510) is discussed (Morton, l. c.; Clark 1958, p. 58-59). Of course only systematically related groups should be compared. Within the Toxoglossa certain conids show an osphradial-sedimental correlation. *Conus lividus* found on reefs (Alpers 1931, p. 588), hence in clean water, is about the same length (25-50 mm) as *Hastula cinerea;* its osphradium comprises only about 15 filaments on either side (l. c., f. 2). *Conus mediterraneus* which burrows in sand, is about 20 mm (Alpers 1932, p. 439), maximally 38 mm long (Bergh 1896, p. 166). Its osphradium is 6-8 mm long, 3-4 mm broad and has 50-60 filaments on either side.

The enlarged surface of the osphradium can be understood if the function is tactile (Yonge 1947, p. 512), but just as well if the snail is "macrosmatic". The sedimentary particles might exercise a mechanical stimulus to which the osphradial cells could react with accelerated divisions and therewith increase the size of the osphradium, even if its sensory cells are chemoperceptive as hitherto supposed by Crofts (1929, p. 44), Ankel (1936, p. 143), Kohn (1956, p. 170), Morton (1958a, p. 4-5) and others, though not proven (Bernard 1890, p. 128; Stork 1934, p. 98).

In *Hastula cinerea* the osphradium lies at the extreme left of the pallial roof. It is nearly symmetrical as in *Conus* (Bergh 1896, f. 30), contrary to *Buccinum* (Dakin 1912, p. 78). The leaflets are flattened in antero-posterior direction (Fig. 3) nearly rectangular, and fastened to the roof along their entire upper border which is very thin. The lower border hanging free into the mantle cavity is almost straight and covered with mantle epithelium containing some blue-staining gland cells. The same epithelium coats the outer border of the leaflet.

The surface of each leaflet is subdivided into 3 bulges by furrows (ur) which run from the outer border inwards on the anterior and posterior side. The dorsal bulge is longest, the ventral one shortest. The epithelium of the furrows is low, while the faces of the bulges are occupied by the sensory areae. The question whether the principal elements are epithelial sensory cells (Bernard 1890, p. 150-151) of indifferent cells with free nerve endings between them (Dakin 1912, p. 181) has been decided in favour of Bernard (Yonge 1947,

p. 511). We did not analyze the sensory areae, only verified that they contain some ciliated but no pigment-bearing cells. The border of the bulges bears a broad band of ciliated cells (cs). Beside these cells, on their inner side, courses a stripe of lower cells containing brown pigment (mo). Cilia and pigment continue over the ventral border of the leaflet which is covered with the already mentioned mantle epithelium with mucus glands (xc). A special layer of glands dorsally to the ciliated cells as in *Buccinum* (Dakin 1912, p. 79) is not developed; the epithelium beside the attachment of the leaflets is quite low mantle epithelium with scattered blue-staining glands.

A well delimited blood space (oc) runs along the axis of the osphradium. According to Bernard's injections (1890, p. 156-157) this vessel-like space communicates with the efferent branchial vessel only by lacunae. It is accompanied by 2 cartilage-like rods (ca) which consist of vesicular cells enveloped in muscle fibres; Bernard (p. 171-172) found comparable thickenings in trochids. These rods contain pigment which in life seen from the upper side appears blood red. The central osphradial blood space supplies blood lacunae on the upper border of the leaflet, and these are connected with blood lacunae of the cavity of the leaflet as well as with such on the outer border. The latter penetrate between the furrows of the leaflet.

Ventrally the central blood space extends the enormous osphradial ganglion (ow) whose mass, about 0,16 mm^3, surpasses by far that of all the central ganglia together. The osphradial nerve enters this ganglion in its middle. From the ganglion a nerve (n) coated with glia cells goes into each leaflet and gives one branch to each bulge as in *Conus mediterraneus* (Bernard, p. 205, pl. 8, f. 31). The secondary nerves which supply the sensory cells on the anterior and posterior surface of the leaflet are paired.

The white gill (k) lies to the right of the osphradium, begins at about the same level and extends farther inwards. It is 15 mm long, 2 mm broad, and contains about 200 lamellae. Separated from the ctenidium by a smooth interspace the light yellow, opacous hypobranchial gland (xi) attains the intestine. It belongs to the right half of the pallial roof, from whose low epithelium the 0,2 mm thick cushion is sharply set off. In some preserved specimens the secretion was reddish violet as in *Terebra muscaria* Lm. (Risbec, 1953, p. 577).

RENAL ORGAN

Inwards to the ctenidium the pigmentfree kidney (h) lies over the pallial roof as a dorso-ventrally flattened sac. In front it is extended to the posterior end of the gill on the left, and to the anterior border of the hypobranchial gland to the right side; behind it ends over the stomach. Contrary to Bergh's statement for *Hastula coerulescens* (1908, p. 128) the intestine does not run through the cavity of the kidney, though the renal sac overlies the intestine (i) intimately. The transverse section of the kidney is triangular with the smallest side left, where the renal gland (vv) is apposed to the pericardium. In the anterior part the inner surface of the ventral wall is smooth. Here the renal aperture (x) is located, a long slit between thick muscular lips. A little behind passes the ciliated funnel of the reno-pericardial duct (y) into the pericardium (er).

In the region behind the mantle cavity the ventral wall of the kidney bears transverse folds which are, contrary to *Lintricula auricularia* (E. and E. Marcus 1959, f. 58), restricted to the renal bottom. From the abdominal blood sinus emerges the muscular afferent renal vessel and branches into the folds. Besides there are also direct communications between sinus and folds, not developed in *Olivella* (l. c., f. 56). Of the blood spaces in the folds that in the summit is largest. Here the high epithelial cells of the folds were sometimes seen to detach their apices as in *Olivancillaria brasiliensis* (l. c., f. 62). The blood from the folds is collected in peripheral coalescing lacunae and passes to the afferent branchial sinus.

Beside the reno-pericardial duct (y) the afferent vessel (uz) ascends through the renal cavity to the roof. Its outer wall is smooth, the inner bears muscular projections. Its ramifications on the roof supply the villosities (zn); then the blood flows to the nephridial gland (vv), whose sinus opens into the auricle (ic). In conids the vascular axis of the villous part, Perrier's left lobe (1889, p. 249), runs on the left side, near the border of the renal gland. In *Hastula cinerea* it is situated (va) in the middle, so that its branches to right and left are of equal length.

The low epithelium of the villosities differs widely from that of the folds. It contains some blue-staining gland cells. Also the con-

nective tissue is quite different from that of the folds. It leaves only quite narrow blood spaces free, and is in some places, in one case nearly everywhere, stuffed with the protein crystalloids described by Cuénot (1914, p. 281). Amoebocytes occur principally in the villosities, but also in the blood lacunae and in the folds.

Perrier (1889, p. 262) called attention to the minuteness of the blood spaces in the accessory system, the villosities, of his Pycnonephridia (Muricacea, Buccinacea, and others). In this system, the so-called left lobe, circulation is slow and irregular (p. 256). The same certainly applies to *Hastula cinerea*. This system is principally absorbent (Cuénot 1914, p. 281-282). The nephridial gland (vv) differs from the adjacent villous region (zn) by a much higher epithelium and less richly developed connective tissue.

CENTRAL NERVOUS SYSTEM

The nervous system of the Toxoglossa is best known by Bouvier's (1887), Bergh's (1896), and Shaw's (1914) studies of various species of *Conus*. The terebrids were less completely described by Bouvier (p. 316-325); Risbec added some observations (1953, p. 582-583). Bouvier's (p. 341) and Shaw's (p. 36) difficulty in removing the connective tissue (Fig. 9, 10, co) which surrounds and binds firmly together nerves, oesophagus and coils of poison gland in *Conus* was also experienced in *Hastula cinerea*. Therefore our results obtained by dissection were controlled and completed by microtomic sections.

Together with radula-sac (rs) and salivary gland (sa) the central nervous system (b) shows the effect of torsion, though less pronounced than in *Conus* and *Terebra cancellata* (Risbec, l. c.). In *Hastula cinerea* the position is somewhat oblique, as the anterior border is more ventral than the posterior. The cerebro-pedal and pleuro-pedal connectives of the right side are longer than the corresponding left connections. The pleuro-pedal connectives are a little thicker than the cerebro-pedal ones. Neuroglia cells as described of *Olivella verreauxii* (E. and E. Marcus 1959, f. 17, n) are developed in the connectives.

The longish pedal ganglia (1) lie far in front of the cerebral ganglia (2) and the radula-sac, approximately over the peduncle of the foot. The volume of the pedal ganglia surpasses a little that of the

cerebral ganglia. In some cleared total mounts the pedal ganglia were topped by propodial ganglia (1a), not recognizable in other preparations. The accessory pedal ganglia of *Terebra cancellata* (Risbec 1953, f. 11, pda) are dorsal to the pedal ganglia, not terminal as in our species. On their adjacent sides the pedal ganglia are fused together. Each ganglion gives 2 nerves (10) off on its anterior border which subdivide short after their beginning. They enter the foot through the peduncle and form a net of branching nerves with accessory ganglia which supply chiefly the anterior pedal border as in the likewise burrowing olivids. In the male the right pedal ganglion is a little bigger than the left and gives rise to a penial nerve (11) near the cerebro-pedal connective.

The statocysts lie about 1 mm in front of the pedal ganglia under the anterior evaginable tube. Both are located in the same transverse plane. The diameter of the statocyst including its capsule is 0,2 mm, that of the statolith 0,08 mm. The extremely thin static nerves (12) can be followed backwards into the capsule of the pedal ganglia and farther to the pleuro-pedal connectives. Hence the static nerve reaches the cerebral ganglion as in *Buccinum* (Bouvier, p. 261), *Nassa* (p. 277), *Purpura* (p. 284), and *Conus virgo* (p. 333); in *C. tulipa* and *textile* (Shaw 1914, p. 33, 49) the static nerve runs through the cerebro-pedal connectives.

The broad cerebral ganglia (2) are separated from one another by a furrow; a true cerebral commissure is not developed. They cover the slightly smaller pleural ganglia (3), against which they are delimited by constrictions. The short buccal connectives of Bouvier's *Terebra aciculina* Lm. ("les ganglions buccaux sont très rapprochés des ganglions cérébroides", p. 325) contrast with those of *Hastula cinerea*. Also the long anterior tube (Bouvier, pl. 17, f. 81, X) and the short pleuro-subintestinal connective (f. 81, Cg, Sb) make it probable that Bouvier studied a different species.

Intimately connected with the static nerve (12) a nerve courses from the cerebral ganglia through the pleuro-pedal connectives forwards (13). Its twigs can be followed into the thick and loose layer of crossing muscle fibres under the outer mouth. Besides this cephalic integumentary nerve there are 3 labial nerves (14) on either side which issue from the cerebral ganglia farther backwards and outwards.

They enter the anterior tube (au) through its ventral attachment (xa) and spread into its wall. Behind these 3 labial nerves we only found 2 further cerebral nerves on either side, the thicker proboscidean nerve (16) and the thinner common nerve (15) for tentacle and eye. The proboscidean nerves trifurcate at the base of the proboscis, so that 6 principal nerves run in the inner muscle layer of the proboscis, chiefly on the ventral side. In correspondence with the smallness of tentacles and eyes and their insignificance for orientation of the burrowing snail tentacular and optic nerves are not separated, and their common trunk is tenuous.

As in *Conus* and the previously studied terebrids the buccal commissure is long. It is single as in *Terebra dimidiata* (Bouvier, p. 320), not double as in *Conus* (id., p. 340; Bergh 1896, f. 29, g, f. 31; Shaw 1914, p. 35), but also suboesophageal. As far as the 2 nerves (17) from each buccal ganglion (6) can be traced, they supply radula-sac and salivary gland; an unpaired nerve (18) from the right buccal ganglion enters between the coils of the poison gland (vo) where it was followed to the muscular bulb (q).

While there is no nerve given off from the right pleural ganglion in *Conus* (Bouvier, p. 337; Bergh, p. 80; Shaw, p. 44), such exists in *Buccinum* (Bouvier, p. 268: "grand nerf latéral droit"; Dakin 1912, p. 70). It goes out from the right pleuro-pedal connective and has its counterpart on the left side. The same disposition occurs in *Hastula cinerea*. The left nerve bifurcates soon into an anterior parietal (19) and a columellar (20) nerve. Also in the muricid *Concholepas peruvianus* Lm. the corresponding parietal nerve sends a branch to the columellar muscle. The right anterior parietal nerve (21) of *H. cinerea* does not divide for the length that was followed. From the left pleural ganglion the strong siphonal nerve (22) originates and divides into 6 branches in the root of the siphon. The dialyneurous connection (8) with the osphradial nerve (24) is distinct. Bouvier's indication of a columellar nerve coming from the "ganglion palléal droit" (p. 321) in *Terebra dimidiata* Lm. is a lapsus for "gauche", as his figure 82 shows (nerve l). The same columellar nerve (23) exists in *Hastula cinerea*.

The left pleuro-subintestinal connective of *H. cinerea* is a little longer than that between right pleural and supra-intestinal ganglion.

This feature reminds of *Turris nodifera* Lm. (Bouvier, pl. 17, f. 78). The short right, stout zygosis (7) of *H. cinerea* and the beginning of the supra-intestinal branch of the visceral loop agree with Bouvier's figure of *Terebra aciculina* (f. 80).

The osphradial nerve (24) and the anterior branchial nerve (25) originate from the supra-intestinal ganglion (4); a posterior branchial nerve (26), together with the exit of the visceral loop from this ganglion. These nerves correspond to Bouvier's description of *Terebra dimidiata* (p. 321).

Three right posterior parietal nerves (27) course towards the right from the subintestinal ganglion (5), while the nerve (28) that goes out from the left and ventral surface of this ganglion seems to correspond to the parieto-columellar nerve in *Conus* (Shaw 1914, p. 43, pl. 4, f. 19, e).

The visceral loop contains 3 ganglia (9) as in the Conidae (Bouvier 1887, p. 332) and, according to Haller (quoted from Simroth 1901, p. 416, 418), the Muricidae. In the retracted snail of Figure 5 the visceral loop is twisted.

ALIMENTARY TRACT

On the level of the tentacles the integument of the head passes through the outer mouth (m) into the anterior tube (au), Bouvier's "gaine proboscidienne". According to its innervation by labial nerves this tube corresponds to lips. In many of the preserved snails the labial tube is introverted. Annular muscles around the outer mouth are distinct, but no special glands besides the epidermal ones. The tube has thick muscular walls and can be extroverted through the outer mouth which in this condition constitutes the base of the tube.

Bouvier's two specimens classified as *Terebra aciculina* Lm. can hardly be our species, because their movable anterior tube is of approximately the same length as the proboscis (1887, p. 324, pl. 17, f. 81, X).

In living and preserved animals the tube was seen turned out. The snails possibly seize their food, polychaetes, with it, as Alpers (1932, p. 428 ff.) observed in *Conus mediterraneus*. Inner and outer epithelium of the tube contain more numerous gland cells than the

epidermis. The anterior tube bears a strong sphincter (r), dorsally less developed than ventrally. So a dorsal slit-like communication appears between the lumen of the tube and the spacious rhynchodaeum (ri), as Oswald (1893) called the cavity delimited by the proboscidean sheath. This slit is the "fente dorsale" of Bouvier (1888, quoted from Simroth 1897, p. 128-129), but its position varies according to the contraction of the sphincter. When the tube is everted, its opening is longer ventrally (Bergh 1908, pl. 10, f. 7), because the ventral wall of the tube is fastened to the bottom of the rhynchodaeum for a certain extent (xa). When the tube is evaginated, the inside of its dorsal wall is turned out completely, while the excursion of the ventral wall is restricted.

A tubular fold of the proboscidean sheath containing the buccal tube (uc) forms the proboscis (p), its anterior opening is the pharyngostome (Oswald 1893). As it functions as entrance of the gut, we call it inner mouth (im). The epithelium lining the rhynchodaeum is high in the portions apposed to the body wall. This epithelium contains numerous mucus gland cells; the subjacent muscle layer is thin. Retractors (s) of the proboscis originate on the lateral body walls and insert on the circle where the proboscis rises from the bottom of the rhynchodaeum.

Inner and outer epithelia of the proboscis are underlain by thick layers of longitudinal and diagonal muscles. These layers are connected by single radial fibres. Due to the longitudinal and diagonal fibres the retracted proboscis is bent and folded, not much contracted. Unfolded it is 10 mm long in adult snails. Annular muscles, principally in the outer layer, produce the lengthening of the proboscis. In our preserved snails the proboscis is never protruded beyond the evaginated anterior tube. In living animals we only once saw it project beyond this tube, when a polychaete was placed in front of the snail. We did not succeed to feed the snails, perhaps they prey only on worms that stick deep in the sand and maybe only at night as *Conus* (Kohn 1959, p. 69).

Six longitudinal nerves (Fig. 7, n.) run in the muscle layer of the buccal tube (uc). Near the inner mouth (im) these muscles form a sphincter (r), Hermitte's muscular collar (1946, p. 497) in *Conus*. Farther inwards the buccal tube is richly folded. At the level of the

proboscidean base the muscles around the buccal tube are thickened mightily forming a pharyngeal bulb (f).

The right ventral wall of the bulb is connected with the radula-sac (rs) by a short tube. The sac contains about 30 teeth fastened in two rows on the wall. The basal flange of every tooth is connected with the wall by a hyaline, acellular ligament (see Fig. 8). This band corresponds to a basement membrane (Pruvot-Fol 1926, p. 306). The tops of the older teeth are directed towards the lumen of the sac, those of the latest teeth point into the radular papilla. The rows of teeth begin proximally on the inner wall and pass to the outer wall in an S-curve. The hollow teeth are 0,54 mm long and 0,08 mm thick at the base or 0,11 mm when the flange is measured. The tooth bears a lancet-shaped tip and is rolled in along its whole length. The so-called pores are not holes, but bridges between the inner border and the outer wall of the tooth. These bridges produce the illusion of an inner spiral (Fig. 8). The shape of the tip is possibly correlated with the habit of feeding on burrowing polychaetes as Kohn (1959, p. 79) supposed for the terminal knob of the tooth in *Conus distans*.

Pruvot-Fol (1926, p. 306) compared the rolled up toxoglossan tooth with basally coalesced marginal rhipidoglossan teeth, e. g. of the Potamiasidae, but of course without phylogenetic claims.

Most of our snails show a tooth (za) in the buccal tube (uc) where its flange is fastened on an epithelial plug between inner mouth (im) and sphincter (r). We consider this position as normal and definitive, as long as the tooth functions. Its tip attains the inner mouth. In some of our sections the tooth lies proximally to the sphincter. We presume that such a tooth is being moved into place by peristalsis of the buccal tube. Hermitte (1946, p. 497) suggests that in *Conus* the transference of a fresh tooth from the radula-sac to the place where it functions is brought about by invagination of the proboscis, whose mouth grasps a tooth from the sac. Such is unlikely in *Hastula cinerea,* because we have seen normally directed teeth between pharyngeal bulb (f) and sphincter (r). Sometimes loose teeth with backwards directed tips were seen in the oesophagus. Probably such teeth had been engulfed together with the prey, as Kohn (1956, p. 170; 1959, p. 68) observed in piscivorous and certain

vermivorous cones. Alpers (1931, p. 650) found fragments of teeth mingled with the digested contents of the gut in *Conus lividus*. Though we did not observe a tooth right in the inner mouth like a stylet as in Amaudrut's figure of a *Terebra*-species (1898, pl. 3, f. 20) reproduced by Hescheler (1900, f. 265) and Simroth (1901, pl. 39, f. 6) we do not doubt its correctness for Amaudrut's species. In *Conus* it has been confirmed by Kohn (1956, f. 2).

Kohn's admirable photographs of feeding cones contradict Alpers' view (1931, p. 603, 650) that every occurrence of a tooth in the buccal tube of a toxoglossan (e. g., Bergh 1896, pl. 4, f. 70) is due to the violent contraction of the snail during preservation. According to Risbec (1953, p. 581) Alpers is possibly right to presume a normal function of the radula of *Conus lividus* in the way of his figure 28, VIII (1931). The anterior teeth which lie in the distal part of the radula-sac, the "quiver" (Alpers's "Köcher"), would act upon a worm while it passes through the pharyngeal bulb. In *Hastula cinerea,* however, the function of the radula must be similar to those conids that protrude the proboscis and harpoon their prey (Hermitte 1946; Kohn 1955; 1956). The fore-gut of the Toxoglossa varies in form and function and does not permit generalization. In *Conus mediterraneus* no function of the radula could be verified (Alpers 1932, p. 433, 445). Among the terebrids there are species without radula and without poison gland, others with radula and without gland, and still others, the genus *Hastula,* with radula and poison gland. Of course the various types feed differently.

Isolation of the numerous sympatric species of *Conus* in Hawaii is brought about, besides by the different habitats, subtidal reefs and marine benches, by the specifity of the preferred prey (Kohn 1959, p. 82 ff.).

A single salivary gland (Fig. 6, 9, sa) lies on the topographically left side. In some snails it is constricted in the middle, and its originally paired structure is evident in the sections. Its two ciliated ducts (wi) course above and below the gut, without passing through the nerve collar (b). In some snails the duct that emerges from the right half of the salivary gland runs under the oesophagus (e), and in others the left. The suboesophageal duct either goes around the radula-sac (Fig. 9, rs) or courses between it and the poison gland

(Fig. 6, vo). The salivary ducts open from both sides into the communication between pharyngeal bulb and radula-sac. The convolutions of the so-called poison duct (vo) lie principally on the topographically right side. The secretory part of this gland is the contorted long tube which passes through the nerve collar (b) and opens into the gut close to the radula-sac, immediately behind the pharyngeal bulb (f). The blind end of the gland is the bulb (q) composed of 4 muscle layers as in *Conus* (Shaw 1914, p. 24) and lined with a non-secretory epithelium (Fig. 10).

In the bulb of the poison gland the thick outer layer of spiral fibres is followed by a thin layer of annular ones. The third layer is also thick and spiral with parallel fibres as those of the outer layer, but crossing them. The fourth layer is annular and thicker than the middle annular one. The tubular part of the gland (vo), the poison duct of the literature, begins with a short muscular portion. The high cells of the tube contain rather big granules of secretion. From the structure we presume that the product of the gland cells is pumped into the proboscis by the muscular bulb (q). The same idea of the so-called poison gland as propulsive organ for the ejection of poison secreted in the so-called duct was for *Conus* first conceived by Hermitte (1946, p. 499) and independently by Kohn (1955).

Bouvier (1887, p. 323, 324, 329, f. 81, 83, 84), Bergh (1896, p. 89; 1908, p. 128), Amaudrut (1898, pl. 3, f. 20), and Shaw (1914, p. 14, 19) found a more or less distinctly bipartite salivary gland and two salivary ducts in *Terebra* and *Conus*. Alpers (1931) considered all these statements as erroneous, because he found only a single salivary duct in *Conus lividus* (f. 24). He homologized the poison gland with the second salivary gland, and Thiele (1935, p. 1049-1050) adopted this forced view. If the anterior limit of the oesophagus (e) is the point at which the radula-sac (rs) separates from the gut proper (Graham 1939, p. 76), the outlet of the poison gland (vo) lies in the foremost part of the oesophagus. Here the cilia begin and continue along the whole alimentary tract. The gland is comparable with the gland of Leiblein in the Stenoglossa, as Bouvier (1887, p. 330, 430, 473), Amaudrut (1898, p. 98), Dakin (1912, p. 32), Shaw (1914, p. 12) and Risbec (1955, p. 52) said. It is true that it opens into the anterior oesophagus, not into the mid-oesophagus as in

the Stenoglossa. This difference is brought about by the different formation of the preneural proboscides in Stenoglossa and Toxoglossa: in the first by a lengthening of the anterior oesophagus, in the second by that of the buccal tube, Hermitte's "prepharynx" (1946, p. 495).

The oesophagus is indistinctly separated from the stomach and about 15 mm long. Stomach and intestine together are of the same length. The oesophagus runs on the ventral side of the body cavity. At its origin from the pharyngeal bulb it forms a forward loop which is more or less straightened according to the state of protrusion or retraction of the proboscis. The oesophageal epithelium is thrown into 20-25 rather uniform longitudinal folds, particularly high in the region under the pericardium. Stronger dorsal folds or stretches with distinct accumulation of gland cells are not developed; the structure of the oesophagus is alike in front of the nerve collar and behind it. A dilatation was verified in many of the dissected specimens. Bergh (1908, p. 125) observed it in *H. coerulescens* (Lm.). It occurs on quite different levels of the oesophagus and is histologically not peculiar, hence only a temporary widening, not a true crop. Close in front of the diaphragm between cephalic and visceral body cavity the oesophagus is fastened to the ventral wall by a ring of muscle fibres as in *Oliva sayana* and *Lintricula auricularia* (E. and E. Marcus 1959, p. 126, 129).

Some transverse gastric folds between the longitudinal ones of the oesophagus (e) indicate the region of the cardia. The thin-walled stomach is only a little wider than the oesophagus, tubular and quite without caecum, somewhat similar to that of the muricids studied by Graham, *Nucella, Ocenebra,* and *Urosalpinx* (1949, p. 748-749). The gastric portion of the mid-gut of *Hastula cinerea* (j) loops around the anterior, morphologically left liver (l), a spherical organ whose anterior border lies level with the pylorus. The hepatic ducts unite just before they open into the cardiac part of the stomach. The anterior duct (l) comes from the small anterior digestive diverticulum. The posterior duct (os) runs backwards under the stomach to the long posterior, morphologically right liver. This extends to the hindmost whorls, where it is topped by a knob of vesicular connective tissue. This liver is accompanied by the gonad and the latter's pigmented duct on its columellar side. Both digestive diverticula are brittle organs with smooth surface and very few nuclei. The volume of the visceral

hump varies considerably in our snails in correspondence with the size of the ovary before and after egg laying.

The longitudinal folds that run on the concave side of the oesophagus curve into the common hepatic duct and continue into the separate ducts. Some longitudinal folds course along the convex side of the stomach. Going out from the aperture of the digestive diverticula some folds follow the concave side of the stomach and constitute a kind of excurrent or intestinal groove (in). The upper or pallial wall of the stomach and the under or columellar wall are clothed with arched transverse folds. The 30-50 frequently bifurcate folds of the pallial wall may correspond to a major typhlosole (oa), those of the columellar wall which are straighter and less numerous (about 20) to a minor typhlosole (oi). Towards the intestine (i) the folds of the typhlosoles converge in some, not all examined snails on the convex side, but a true sorting area is not developed. This character, the anterior entrance of the oesophagus, and the absence of caecum and gastric shield are the same in *Hastula cinerea* and the above mentioned muricids.

Partly decomposed pieces of polychaetes were found in the stomach.

The beginning of the intestine (i) is characterized by a slight narrowing of the alimentary tube and the conversion of the transverse folds into longitudinal ones. The cilia of the epithelium continue; red-staining gland cells increase in number outwards, but rarify and finally disappear near the middle of the anal gland. This black gland (Fig. 13, 14, 17, an) is an up to 6 mm long, richly branched organ with narrow lumen and resembles that of the muricids (Fretter 1946, p. 129) and *Oliva peruviana* (Küttler 1913, p. 506-507). Contrary to the latter it is of equal size in males and females. Its ciliated epithelium contains brown concrements. As in the muricids studied by Fretter (l. c.) the anal gland of *Hastula cinerea* is combined with a histologically extremely simple digestive gland, and possibly assumes the function of an accessory excretory organ, as in the muricids. In one case the lumen of the gland contained a patch of brown granules on a lump of secretion. The epithelium on the side facing the granules was low and free of granules, thus proving recent excretion. A blood lacuna (oc) runs between gland and rectum. The latter projects into

the mantle cavity on the right side apposed to its roof, and the anus (ar) lies in the anterior third of the cavity. The anal gland opens into the anus or immediately beside it. As in *Oliva, Lintricula,* and *Olivancillaria* the wall of the mantle cavity forms a tubular papilla (z) containing a nerve over the anus.

Masses of setae of polychaetes were observed in the rectum.

MALE ORGANS

The follicular testis (me) extends from the apical whorls forward on the columellar side of the posterior liver (os) and is continued into the coiled, nonciliate testicular duct (su) which functions as vesicula seminalis as in Muricidae, Buccinidae, and Olividae. There are no atypical sperms, as such are known from *Conus, Olivella,* and *Oliva,* neither in the testis and seminal vesicle, nor in the female organs. The straight and ciliated renal spermiduct (re) runs so closely approached to the pericardium that the presence or absence of a gonopericardial strand of connective tissue could not be verified.

The orange yellow pallial spermiduct (d) begins as a muscular pouch whose ciliate epithelium is surrounded by clusters of closely set subepithelial prostatic glands as drawn by Fretter (1941, f. 1). At its origin this pouch opens by a 0,5 mm long slit (sn) into the pallial cavity as in *Ocenebra lapillus* (l. c., p. 175), *Olivella, Oliva,* and *Lintricula* (E. and E. Marcus 1959). In buccinids (Fretter 1941, p. 178, 180) this communication beween prostate and mantle cavity is established by a duct. The dilated stretch of the pallial spermiduct in the roof of the mantle cavity is parallel to the sutural fold. The following thin tube curves from the roof to the floor, where its course produces a slight ridge.

After its entrance into the penis (vr) the spermiduct (nv) becomes sinuous and continues so along the centre of the flattened copulatory organ. The hatchet-shaped, solid and muscular penis (Fig. 14, 15) is not as long as that of *Terebra* (*Subula*) *dimidiata* (Bouvier 1887, p. 319), nor dentate as in *T.* (*Oxymeris*) *maculata* (p. 322). The penial spermiduct opens on a small papilla which lies in the middle of a terminal concavity of the penis. The penis enters the terminal pouch in the mantle cavity of the female, where we found sperm. The muscular coat of the spermiduct (nv) can project the narrow

papilla and attain the bursa copulatrix and the ventral sperm channel through the pore-like outer opening of the capsule gland. At rest the penis is tucked into the mantle cavity, as Bouvier (1887) and Bergh (1908) already observed.

FEMALE ORGANS

The ovary lies on the columellar side of the posterior digestive diverticulum. The straight ovarian portion of the oviduct (ou) is about 5 mm long and lined with the same low epithelium as the ovary. The eggs in the oviduct measure 300 × 150 × 100 μ. As about 6 of these ovocytes were found descending the gonadial duct of one sectioned female, it is probable that several eggs are included in one capsule. In *Conus jaspideus* Gm. there are 3-7 eggs per capsule (Perry and Schwengel 1955, p. 180); in other conids many more (Risbec 1932, p. 366).

The renal oviduct (rv) curves in an almost right angle from the ovarian duct. A short gonopericardial duct (g) connects this angle with the pericardial cavity (er). This duct is ciliated, but the cilia are less developed than in the renal oviduct. Its opening into the pericardium is a pore, not a funnel. A sphincter is not present.

The beginning of the subepithelial glands marks the start of the pallial oviduct. Its innermost portion, the albumen gland (aa), is thinner along the ventral side of the duct. From the bound between albumen gland and capsule gland a canal (se) with high longitudinal folds leads to a wide, lobed pouch (rn). In the folds of the canal there are often sperms fastened with their heads to the low ciliate epithelium. The histological limit is sharp between this and the epithelium of the pouch whose reddish brown colour resembles that of the foliaceous organ of *Conus* (Bergh 1896, p. 98). The sperm masses included in it are disorderly, and the high nonciliate cells engulf sperms. The sperm pouch is obviously homologous to the receptaculum seminis of muricids, buccinids (Fretter 1941) and *Lintricula auricularia* (E. and E. Marcus 1959, p. 143). In the last species as well as in *Nucella lapillus* and *Buccinum undatum* the duct of the receptaculum stores orientated sperm, while blind tubules going out from the duct function as ingesting gland.

Also the orange red capsule gland (c) of *Hastula cinerea* agrees with that of the Stenoglossa studied by Fretter (1941, f. 5). The different colourability of the glands in the dorsal, middle and ventral areae is indicated by different stippling in our Figure 18. The lumen of the capsule gland and its ventral sperm channel (sr) is lined by ciliate epithelium. The channel contains sperms; its epithelium is surrounded by muscles. Of the two longitudinal folds of the sperm channel present in Fretter's Stenoglossa only the left is developed in *Hastula cinerea*. This fold becomes higher towards the distal end of the capsule gland. The latter opens through a narrow pore (ec) into the outermost portion of the pallial oviduct.

Over the passage of the capsule gland into this outermost portion the lumen of the capsule gland forms an about 0,5 mm deep pouch, the bursa copulatrix (u). Its epithelium is glandular where it is continuous with the capsule gland and towards the fundus thrown into narrow folds of short ciliate cells without glands. Sperms were seen in the bursa of a snail preserved immediately after copulation. They lay as a small lump in the lumen.

The about 4 mm long outermost portion of the pallial oviduct is a colourless pouch (wo) open against the pallial cavity. Its walls form oblique folds; the epithelium is ciliate without glands. Its slit is bounded by thickened muscular lips. This terminal pouch of the oviduct ends behind the anal papilla (z). Its folds indicate that it is distensible by the egg capsule and also by the penis. Snails preserved after copulation had a loose mass of sperm lying in the terminal pouch without contact with its epithelium. The nutritive and protective coats of the eggs are already furnished by the glands of the pallial oviduct before the capsule enters the terminal pouch. Its slit may be correlated with the definitive size and firmness of the egg capsule as in *Pomatias elegans* (Creek 1951, p. 635). Topographically the terminal pouch corresponds to the jelly gland and the brood pouch in *Littorina* (Linke 1933, p. 33-35).

Except for the terminal pouch which has no counterpart in the known Stenoglossa the female organs of *Hastula cinerea* are very similar to those of this Order . A comparison with the Conidae was not possible, because we did not succeed to understand Bergh's descriptions (1896, p. 97-100, 175-177). It is possible that the elements

in *Hastula* and in *Conus* ( ) are the same: terminal pouch (vulva), bursa copulatrix (Samenblase), capsule gland (Schleimdrüse), receptaculum seminis and ingesting gland (blättriges Organ), albumen gland (Fortsetzung der Schleimdrüse an der Hinterseite der Niere). This interpretation is tentative.

On the right side of females of *Hastula cinerea,* whose shell is at least 31-32 mm long, a nearly white 0,9 mm broad stripe (Fig. 2, zo) runs from the floor of the mantle cavity along the pedal peduncle and ends at the small marginal pedal fold that marks the limit between propodium and metapodium. This egg-guide is characterized by its much less intense pigmentation, a little higher epithelium, and principally by its 3 μ long cilia. The adjacent areae of the back of the foot are nonciliate.

In middle-sized and adult females a pigmentfree slit or circle about 1,3 mm in diameter, the ventral pedal or nidamental gland, lies about 2 mm behind the anterior border of the foot. This gland appears already in snails with 29 mm long shells. The blue staining gland cells extend 0,3-0,5 mm inwards. Among the strong muscles of the foot no special arrangement of fibres could be recognized related with this gland, nor a tract on the sole between it and the fold on the pedal border where the egg-guide (zo) ends.

The figures of ventral pedal glands of conids (Bergh 1896, pl. 1, f. 12, b; 22, a) refer to species of which the author had only females. In one case he mentioned a "posterior pedal gland" of a male lying "uncommonly far in front" (p. 108). This gland can hardly be the posterior pedal gland of Graham (1957, p. 142), because Bergh in other cases always mentions two glands, the anterior pedal mucus gland and the ventral pedal gland.

CONCLUSIONS

Among the Terebridae the genus *Hastula* with both radula and poison gland is less specialized than the other genera without gland or without gland and without radula. The proboscis of the Terebridae is highly specialized. The central nervous system attains a higher degree of specialization in conids than in any terebrids. It seems plausible to derive Terebridae and Conidae from the Turridae (Pleurotomidae), as Bouvier did (1887, p. 474) and not to consider the

last as a subfamily of the Conidae (Thiele 1931). The Turridae ar better maintained as a separate family (Abbott 1955; Risbec 195 A. Myra Keen 1958). Then the Toxoglossa would comprise Ter bracea (Turridae, Conidae, Terebridae) and Mitracea, i. e., the M tridae in their restricted range (Risbec 1928; 1955, p. 71-74, 76) The Turridae are anatomically and also systematically (e. g., M MacGinitie 1959, p. 65) little known. Hence one cannot decide fro which other stenoglossan family they should be derived, Volutid (Bouvier 1887, p. 305-6, 315-316) or Muricidae (Thiele 1935, 1095). Perrier (1889, p. 241) considered the Conidae as Meron phridia and therewith nearer to the Volutidae, but the difference be ween Meronephridia and Pycnonephridia is gradual. A system base exclusively upon the anatomy of the kidney would lead to artifici results, viz. approach Cypraeidae to Volutidae (l. c., p. 239, 281 and Harpidae to Buccinidae (p. 264, 281).

RESUMO

Para a espécie das Terebridae comum nas costas de São Paul na areia fina das praias de declive gradual, usamos o nome *Hastu cinerea* (Born, 1780), apesar de nela ocorrerem 28-36 não 45-5 costelas axiais na margem apical de cada volta.

Os animais comem poliquetos. Tentáculos e olhos são pequ nos; a margem anterior do pé e o grande osfrádio (Fig. 3), intens mente inervados. Como nos Pycnonephridia de Perrier os espaç sangüíneos do sistema renal acessório são diminutos.

O volume dos gânglios pedais (1) ultrapassa algo o dos cer brais (2). O pé é como que pedunculado, extraordinàriamente m vel. Corresponde à separação indistinta entre propódio e metap dio a irregularidade de gânglios propodiais (1a) separados. O ner penial (11) sai do gânglio pedal perto do conectivo cérebro-pedal Pelo mesmo conectivo o nervo estático (12) sai do gânglio cer bral. Os conectivos cérebro-bucais são compridos. Cada gângl pleural (3) emite um nervo. A alça visceral contém 3 gânglios (9)

Pela inervação o tubo anterior evaginável (Fig. 6, au) é um formação labial. Um dente da rádula (Fig. 7, za) no tubo buc (uc), perto da bôca interna (im), serve evidentemente para arpo a prêsa. O chamado duto da glândula de veneno (vo) é secretor;

bulbo (Figs. 6, 10, q), propulsor. A glândula salivar (sa) tem 2 dutos (wi). A glândula de veneno não corresponde a uma glândula salivar (Alpers; Thiele), mas à de Leiblein dos Stenoglossa. O estômago (Figs. 11, 12) é muito simples; a grande glândula anal (Figs. 13, 14, 17, an) é excretora.

O penis (Figs. 14, 15) entra na bolsa terminal (Figs. 16, 17, wo) do oviduto, podendo a papila penial chegar à bursa (u). Entre as glândulas albuminógena (Fig. 16, aa) e capsulígena (c) o órgão lobulado (rn) ingere espermatozóides alheios em excesso, servindo o canal (se) como receptáculo seminal. Os ovos passam pela faixa ciliada (Fig. 2, zo) da cavidade palial à margem da sola. Nesta, ocorre glândula nidamental nas fêmeas.

Conidae e Terebridae parecem ter evoluído das Turridae cuja ligação às Volutidae (Bouvier 1887) ou Muricidae (Thiele 1935) ainda não é possível definir.

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EXPLANATION OF LETTERS IN FIGS. 1-19

(Hastula cinerea Born)

aa — albumen gland
am — amoebocyte
an — anal gland
ar — anus
au — anterior tube
av — afferent penial vessel
b — nerve collar
c — capsule gland
ca — cartilage-like rod
co — connective tissue
cs — ciliate band
cz — columellar muscle
d — pallial spermiduct
e — oesophagus
ei — egg
eo — aperture of capsule gland
er — pericardium
ev — efferent penial vessel
f — pharyngeal bulb
g — gono-pericardial duct
h — kidney
i — intestine
ic — auricle
im — inner mouth
iu — intestinal groove
j — stomach
k — gill
l — anterior liver duct
m — outer mouth
me — testis
mo — pigment
mr — mantle border
n — nerve
nv — penial spermiduct
oa — folds of pallial wall of stomach
oc — blood lacuna
oi — folds of columellar wall of stomach
or — operculum
os — posterior liver duct
ou — ovarian duct
ov — osphradium
ow — osphradial ganglion
p — proboscis
q — poison bulb
r — sphincter
re — renal spermiduct
ri — rhynchodaeum
rn — ingesting gland
rs — radula-sac
rv — renal oviduct

s — retractor
sa — salivary gland
se — receptaculum seminis
sm — communication between spermiduct and mantle cavity
so — sutural fold
sr — sperm channel
su — testicular duct
t — tentacle
u — bursa copulatrix
uc — buccal tube
ur — osphradial furrows
uz — afferent renal vessel
va — renal vascular axis
ve — ventricle
vi — siphon
vo — poison gland
vr — penis
vv — nephridial gland
wi — salivary ducts
wo — terminal pouch
x — renal aperture
xa — ventral attachment of anterior tube
xc — gland cells
xi — hypobranchial gland
y — reno-pericardial duct
z — anal papilla
za — tooth
zn — villous part of kidney
zo — egg-guide
1 — pedal ganglia
1a — propodial ganglia
2 — cerebral ganglia
3 — pleural ganglia
4 — supra-intestinal ganglion
5 — subintestinal ganglion
6 — buccal ganglia
7 — right zygosis
8 — dialyneurous connection
9 — visceral ganglia
10 — pedal nerves
11 — penial nerve
12 — static nerve
13 — cephalic integumentary nerve
14 — labial nerves
15 — tentacular and optic nerve
16 — proboscidean nerve
17 — radular nerves
18 — poison gland nerve
19 — left anterior parietal nerve
20 — columellar nerve
21 — right anterior parietal nerve
22 — siphonal nerve
23 — columellar nerve
24 — osphradial nerve
25 — anterior branchial nerve
26 — posterior branchial nerve
27 — right posterior parietal nerves
28 — right parieto-columellar nerve

PLATES

PLATE 1

Hastula cinerea

Fig. 1 — Tip of shell with protoconch.
Fig. 2 — Anterior part of preserved snail extracted from shell an unrolled.
Fig. 3 — Diagram of 4 pairs of osphradial filaments; on the le side, surface view; on the right side, cut in the middle.
Fig. 4 — Transverse section of two osphradial filaments.

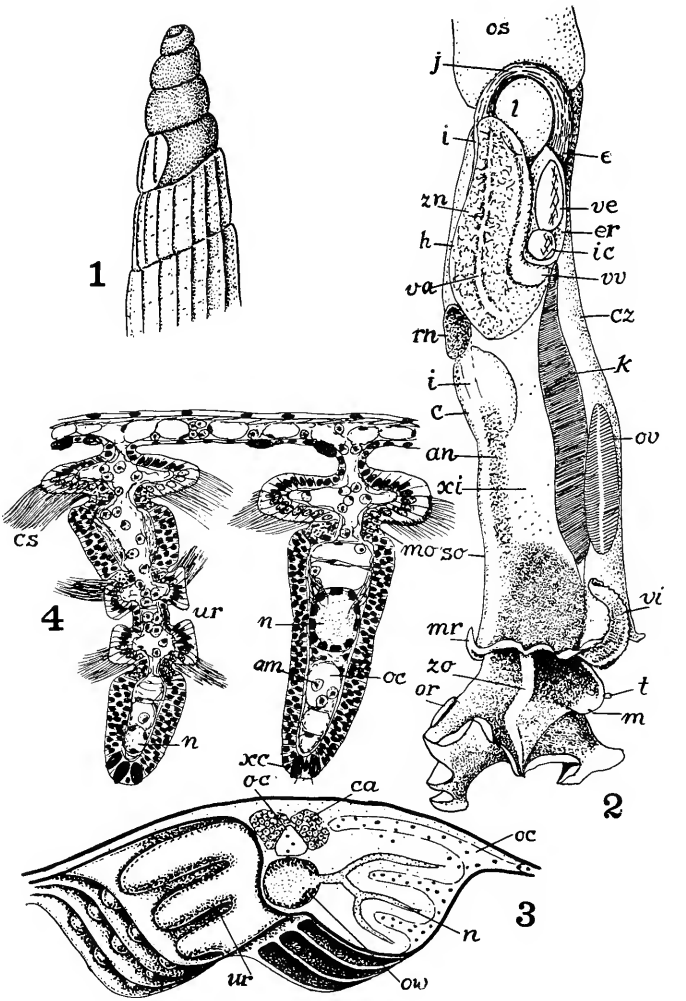
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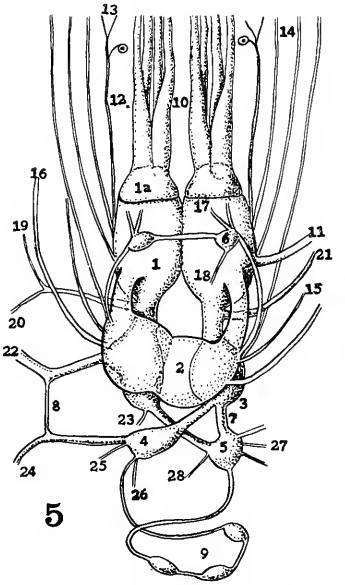
PLATE 2

Hastula cinerea

Fig. 5 — Central nervous system.
Fig. 6 — Diagram of fore gut.
Fig. 7 — Section of tip of proboscis.
Fig. 8 — Radular tooth with ligament.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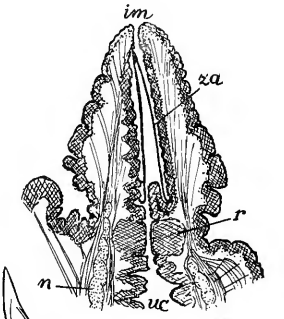
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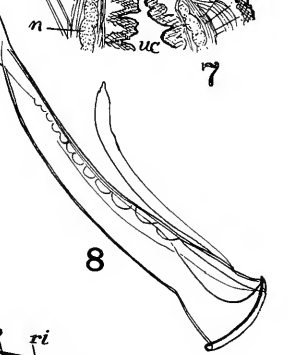

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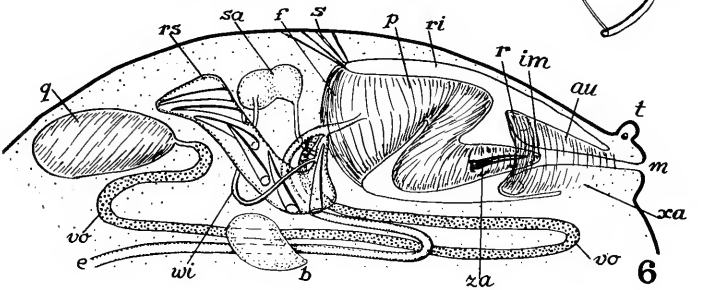
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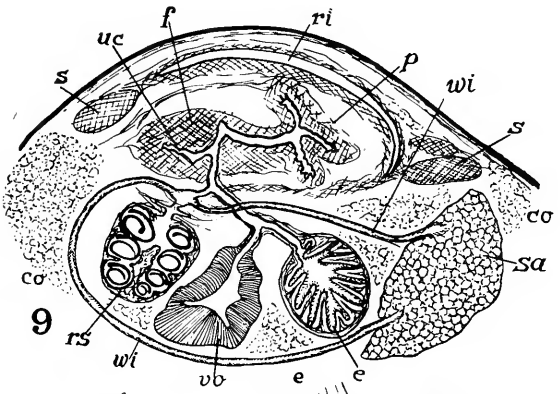
PLATE 3

Hastula cinerea

Fig. 9 — Combined transverse section at level of salivary gland.
Fig. 10 — Section of bulb and beginning of poison gland.
Fig. 11 — Dorsal view of stomach.
Fig. 12 — Ventral view of stomach.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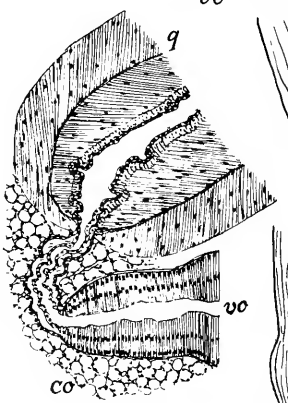
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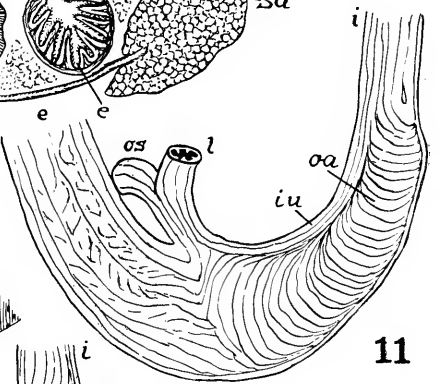
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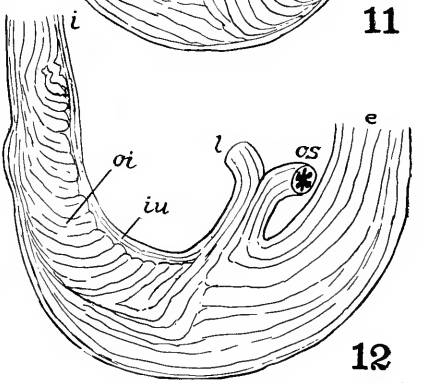
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PLATE 4

Hastula cinerea

Fig. 13 — Rectum and anal gland.
Fig. 14 — Diagram of male organs.
Fig. 15 — Transverse section of penis.

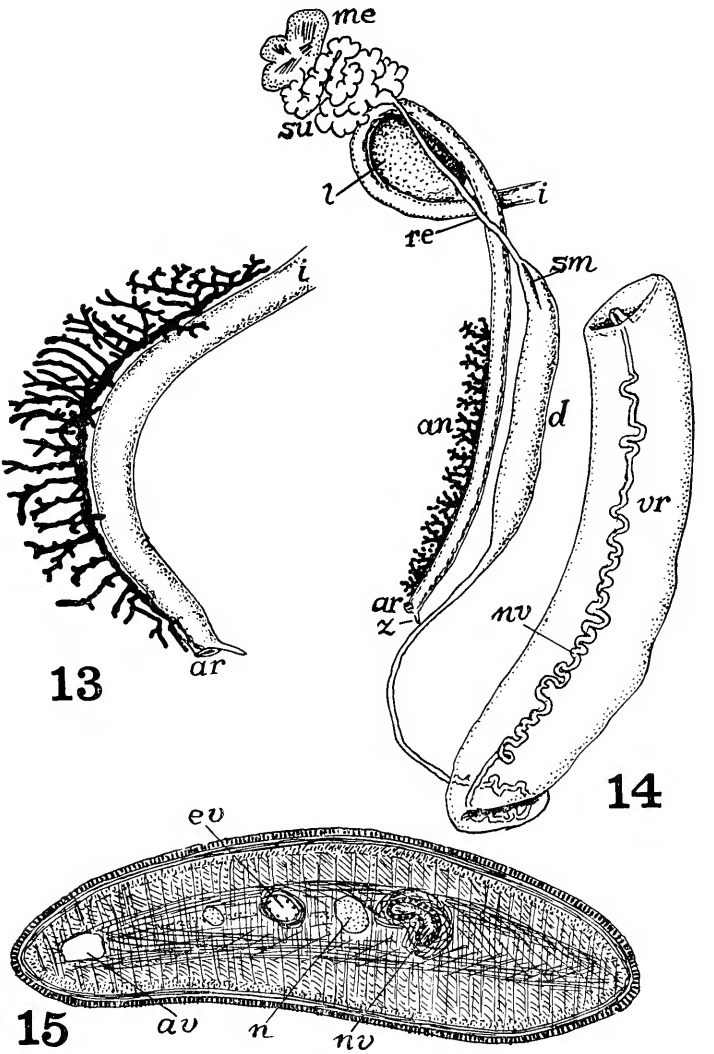
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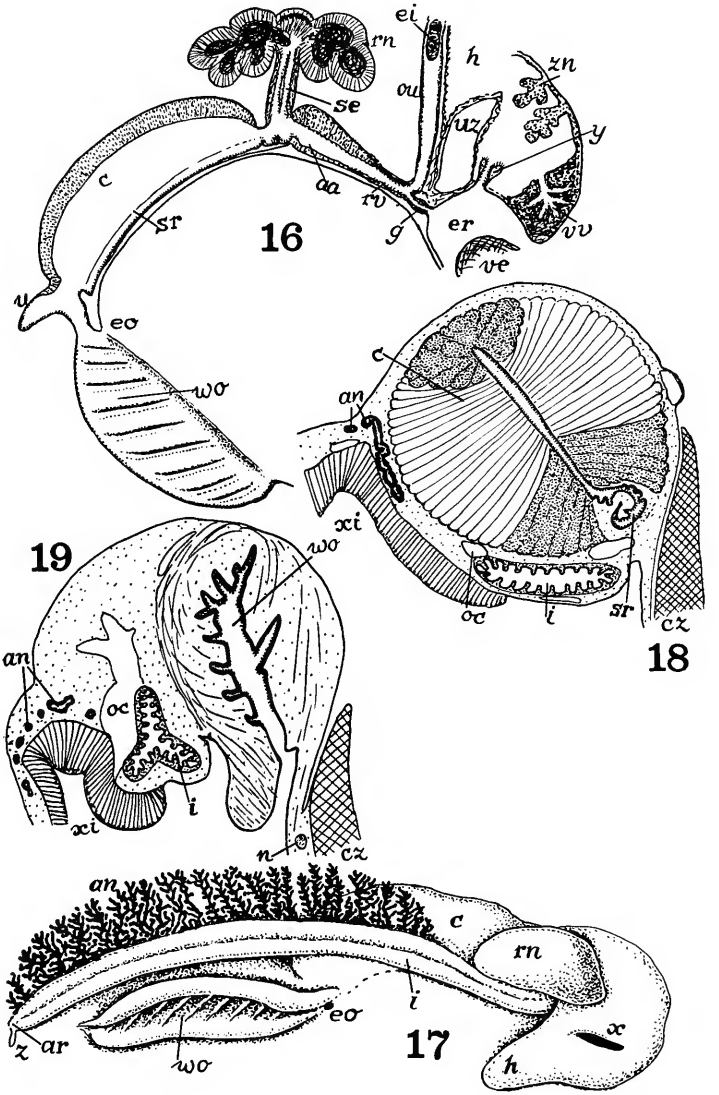
PLATE 5

Hastula cinerea

Fig. 16 — Diagram of female organs.
Fig. 17 — Organs of right suture of mantle cavity of female.
Fig. 18 — Transverse section of capsule gland.
Fig. 19 — Transverse section of terminal pouch.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ÉTUDE AU MICROSCOPE ELECTRONIQUE DE JONCTIONS NEUROMUSCULAIRES DU CRABE BLEU, ("CALLINECTES DANAE", SMITH) *

par ROGER LAVALLARD

Département de Physiologie Générale et Animale, et Section de Microscopie Electronique de l'Université de São Paulo.
SÃO PAULO — BRÉSIL

(7 Planches)

INTRODUCTION

L'utilisation du microscope électronique pour observer l'ultrastructure de la jonction neuromusculaire, entreprise pour la première fois par BEAMS et EVAN (1), fut d'abord consacrée au cas de la plaque motrice des Vertébrés. Une série de travaux (2, 3, 4, 5), effectués avec des techniques en cours de développement, est bientôt suivie par l'importante publication de ROBERTSON (6) sur la jonction myoneurale d'un Reptile, et par le travail de REGER (7) sur le gastrocnémien de la Souris. Des jonctions neuromusculaires d'Invertébrés sont à leur tour examinées en microscopie électronique par EDWARDS, RUSKA et DE HARVEN (8, 9) qui ont choisi des muscles d'Insectes.

Dans le cas des Vertébrés, grâce à l'utilisation de méthodes plus fines que les imprégnations argentiques, comme les colorations postvitales au vert Janus ou au violet de méthyle (10), comme les localisations histochimiques de la cholinestérase (11), la cytologie avait mis en évidence des caractères essentiels de la jonction neuromusculaire: nature épilemmale de la terminaison nerveuse, existence de l'appareil sous-neural. Dans le cas des Arthropodes, la plupart des connaissances a été obtenu avec des imprégnations argentiques, en général de moins bon rendement que chez les Vertébrés, ou avec le

(*) Reçu pour publication: Avril 1960.

bleu de méthylène en colorations post-vitales, ce qui permet surtout des observations topographiques de l'innervation. Il en résulte, pour les Crustacés, des données fragmentaires, souvent contradictoires, sur l'existence de plaques motrices (18), sur le nombre de jonctions myoneurales par fibre musculaire (12, 13), sur la position épi- ou hypolemmale des terminaisons nerveuses par rapport au sarcolemme (17, 18).

Comme le démontrent les deux premiers travaux (8, 9) déja publiés dans ce domaine pour les Insectes [1], le microscope électronique semble être un instrument favorable d'informations sur les structures des jonctions myoneurales des Arthropodes, à propos desquelles les techniques de la cytologie classique n'ont pas été jusqu'à present très fructueuses.

Cette publication rapporte les premières observations de zones de jonctions neuromusculaires que nous avons rencontrées au cours d'une étude de muscles de Crustacés en microscopie électronique. En plus de quelques données sur la structure des nerfs périphériques, des renseignements sont apportés sur la morphologie d'ensemble de jonctions myoneurales du Crabe Bleu, sur la disposition des gaines du nerf, des membranes basales, de l'axolemme et du sarcolemme au niveau de cette jonction, sur l'ultrastructure de l'axoplasme et du sarcoplasme terminaux.

MATERIEL ET METHODES

Les muscles utilisés proviennent des pièces buccales du Crabe Bleu (*Callinectes Danae,* Smith), animal très commun sur le littoral brésilien. Ce sont les deux antagonistes qui provoquent les battements du fouet de l'exopodite des pattes-machoires. L'article qui les contient, est isolé au niveau de son articulation sur le basipodite, et placé dans le fixateur après ouverture au scalpel d'une fenêtre dans la cuticule de sa face interne. Le fixateur est une solution de tétroxyde

(1). — Deux travaux récents, qui sont parvenus à notre connaissance après l'élaboration de ce manuscrit, devraient être inclus dans le commentaire bibliographique: — The Fine Structure of a Multiterminal Innervation of an Insect Muscle, by G. A. EDWARDS, J. Biophysic. and Biochem. Cytol., 1959, 5, 241.

— The Fine Structure and Morphological Organisation of the Peripheral Nerve-fibres and Trunks of the Cockroach, by A. HESS, Quart. J. Micr. Sc., 1958, 99, 333.

d'osmium à 3%, tamponnée au pH: 7,4-7,6 selon la méthode de PALADE, agissant pendant une heure à 18°C. Après rinçage et prélèvement hors du tégument, les muscles sont découpés en pièces pour la déshydratation et l'inclusion dans un mélange de 9 parties de méthacrylate de n-butyle avec une partie de méthacrylate de n-méthyle, dont la polymérisation est assurée par 1% de catalyseur Luperco CDB à 45°C. Les coupes, effectuées avec un microtome Porter-Blum, sont observées aux grossissements originaux de 2000 à 10500x, avec un microscope RCA, modèle EMU, à ouverture de l'objectif de 25 μ de diamètre.

RESULTATS

Les nerfs juxtaterminaux. Les principaux caractères de l'axone et de la gaine de Schwann d'un nerf de Crustacé, examinés en microscopie électronique, ont été rapportés par GEREN et SCHMITT (14, 15). Ces deux auteurs ont utilisé les nerfs de la patte locomotrice du Homard, c'est à dire des fibres nerveuses probablement coupées à un niveau assez éloigné de leurs terminaisons. Les micrographies des Figs. 2 à 7 présentent ici des nerfs ramifiés parmi les fibres musculaires où ils aboutissent et qui sont donc coupés au voisinage des synapses. D'autre part, obtenues à de faibles grossissements, elles permettent d'observer l'ensemble de la structure de ces nerfs juxtaterminaux d'un Crustacé.

Les premières études de l'organisation des nerfs périphériques des Crustacés semblent remonter à BIEDERMANN (16) qui a montré la présence, dans l'innervation d'un muscle, de deux fibres nerveuses suivant des trajets parallèles et se ramifiant de façon synchrone. Il estime, en rapport avec les phénomènes d'inhibition qu'il a mis en évidence sur le même matériel, que l'une des deux fibres est inhibitrice et l'autre motrice. Il suppose que toutes deux vont innerver chaque fibre musculaire du muscle.

MANGOLD (17) retrouve ces deux fibres nerveuses parallèles avec leurs ramifications simultanées qu'il désigne par le terme de "ramifications diplotomiques". Il semble généraliser cette double innervation à tous les Arthropodes, à tous leurs muscles et même à toutes les fibres musculaires, puisqu'il voit pénétrer ensemble les deux fibres

nerveuses sous le sarcolemme, où elles peuvent encore se ramifier avant de prendre fin.

Ces deux auteurs (16, 17) avaient utilisé des colorations post-vitales au bleu de méthylène. D'ANCONA (18) emploie en outre une méthode d'imprégnation argentique dérivée de la technique de CAJAL au nitrate d'argent réduit. Il retrouve des nerfs à deux fibres nerveuses, mais il montre que beaucoup de nerfs contiennent trois fibres parallèles dans une même gaine, qui se divisent synchroniquement quand le nerf se bifurque. Il représente même le dessin d'un nerf à quatre fibres parallèles rencontré chez Palinurus vulgaris. Cependant, au niveau des terminaisons sur les fibres musculaires, il ne voit que deux fibres nerveuses parallèles, ou même une seule fibre.

La notion de triple innervation est introduite par HARREVELD et WIERSMA (19) qui montrent la présence constante, dans des muscles de l'Ecrevisse, de trois fibres nerveuses parallèles, de diamètres différents, dont ils désignent les ramifications simultanées par l'expression "ramifications triplotomiques". En excitant séparément chacun de ces axones, ils observent que le plus fin possède un rôle inhibiteur, que l'intermédiaire produit une contraction du type "slow", et que le plus épais donne une contraction du type "fast".

Ultérieurement, HARREVELD (12), par une étude histologique, conclut que dans le cas de nerfs à deux fibres (duplet) comme dans celui des nerfs à trois fibres (triplet), chacun des axones innerve chacune des fibres musculaires du muscle correspondant. Le même auteur (21) met aussi en évidence des nerfs contenant quatre ou cinq fibres nerveuses (quadruple et quintuple innervations) chez les Crustacés. A ces données fait suite toute une série de travaux par différents auteurs sur la physiologie neuromusculaire des Crustacés (cf. HOYLE, 22).

Des colorations avec des solutions très diluées de bleu de méthylène et des essais d'imprégnations argentiques, nous ont montré que les muscles utilisés dans ce travail, étaient innervés seulement par deux axones de diamètre différent (A_1 et A_2, Fig. 1), présentant le même parcours parallèle et les ramifications diplotomiques décrits dans d'autres types de muscles, par les auteurs cités ci-dessus. L'axone fin est probablement l'inhibiteur, le plus gros correspondant à une innervation motrice simple.

Nous avons retrouvé cette double innervation sur de nombreuses micrographies électroniques. Dans la majorité des cas, on observe sur coupe, deux axones de taille inégale, au voisinage l'un de l'autre: A_1 et A_2, Figs. 2 et 7. Comme cela avait déjà été remarqué en microscopie optique (18), les deux axones sont contenus dans une même enveloppe, l'ensemble constituant un nerf à deux fibres nerveuses (Figs. 2 et 7). Il y a également des nerfs juxtaterminaux ne comportant qu'un seul axone (Fig. 5), mais D'ANCONA avait signalé, et nous l'avons quelquefois observé avec le bleu de méthylène, qu'au niveau des dernières ramifications, les deux fibres nerveuses parallèles peuvent s'éloigner l'une de l'autre avant de se terminer. La coupe de la Fig. 5 représente sans doute un de ces ultimes rameaux solitaires. La Fig. 6 ne montre aussi qu'un seul axone, mais il est pour sa plus grande part en coupe sublongitudinale, à l'exception de son extrêmité droite où il s'incurve; on ne peut donc savoir s'il s'agit d'un nerf à un ou deux axones, puisqu'il peut exister une autre fibre nerveuse parallèle à celle de la Fig. 6, dans les coupes précédentes ou suivantes. Le diamètre des nerfs juxtaterminaux rencontrés dans les muscles est assez constant et se situe aux environs de 7 μ.

Un double système de gaines entoure les axones. Un premier type de gaine est particulier à chaque axone: g. S., Figs. 2 à 7. Il correspond à la formation que GEREN et SCHMITT (14, 15) ont appelée cellule de Schwann chez le Homard. Cette gaine forme une enveloppe de cytoplasme nucléé autour de l'axone, sa limite interne étant marquée par sa juxtaposition avec l'axoplasme, sa limite externe par une membrane basale assez épaisse qui le circonscrit. Un complexe de membranes relie la surface externe à la surface interne de cette gaine tubulaire, à travers son cytoplasme; il est l'équivalent du mésaxone de GASSER (23). Comme dans les nerfs de la patte locomotrice du Homard (14, 15), l'épaisseur de cette gaine est faible, de l'ordre de 0,1 à 0,5 μ, sauf aux endroits où sont localisés les noyaux. Une seconde gaine plus externe (Pn, Figs. 2 à 7), est commune aux deux axones et à leur gaine de Schwann respective dont elle enveloppe l'ensemble. Elle est limitée extérieurement par une membrane basale épaisse qui forme aussi la limite du nerf vis-à-vis des espaces extracellulaires. Du côté interne, elle se termine au contact de la membrane basale qui entoure chaque gaine de Schwann.

La distance entre les deux membranes basales de cette gaine est très variable, ne serait-ce que par la position fréquemment excentrique des axones; elle atteint quelquefois 3,5 μ. Cette gaine peut être assimilée à l'enveloppe conjonctive = périlemme, décrite en microscopie optique chez les Insectes, par SCHARRER (24) pour les centres nerveux et par HOYLE (25) pour les nerfs. Ce périlemme comporte une partie nucléée = périneurium, surmontée d'une membrane épaisse, la "neural lamella", qui correspond ici à la membrane basale du nerf[2]. Cette gaine conjonctive peut être considérée également comme l'équivalent de la gaine endoneurale des Vertébrés (= gaine de Henlé ou gaine de Key-Retzius). Cette dualité des gaines existe aussi dans le cas de nerf juxtaterminal à un seul axone comme celui de la Fig. 5.

Le mésaxone décrit à travers le cytoplasme schwannien un parcours remarquablement réduit. Dans les nerfs myéliniques des Vertébrés, il peut s'enrouler autour de l'axone une vingtaine de fois ou davantage (26). Chez les Insectes (8), il ne s'enroule pas régulièrement autour de l'axone, mais il fait un trajet prolongé avant de rejoindre la surface interne de la gaine de Schwann. Dans les nerfs de la patte locomotrice du Homard (14, 15), le mésaxone passe une ou deux fois autour de l'axone. Dans le cas présent des nerfs juxtaterminaux, le mésaxone ne tourne pas même une fois autour de l'axone, mais effectue tout son parcours dans une zone qui correspond à peu près au quart de la circonférence de la gaine de Schwann (Mx, Figs. 3, 4, 6 et 7). Dans la Fig. 5, le trajet du mésaxone semble plus long, mais il faut tenir compte de la forte obliquité du plan de coupe par rapport à l'axe du nerf. Dans la Fig. 6, le mésaxone est visible deux fois selon deux orientations de coupe différentes, l'une sublongitudinale, dans la moitié gauche de la micrographie, l'autre transversale dans la zone où le nerf s'incurve.

Les deux sortes de gaines sont nucléées. La Fig. 2 montre un noyau schwannien N. S. à proximité de l'axone A_1. Il est toujours facile de distinguer dans un nerf, un noyau schwannien d'un noyau du périneurium (N. Pn., Fig. 7) grâce à sa position par rapport à la membrane basale qui limite ce périneurium intérieurement. La pré-

(2). — Pour Scharrer, l'ensemble de la gaine conjonctive = périlemme, comporte une partie cytoplasmique = périneurium, revêtue d'une membrane épaisse, sans structure = neural lamella. Hoyle n'utilise pas le terme périneurium; il nomme périlemme la partie cytoplasmique pour la distinguer de la neural lamella.

sence d'un noyau entraine une augmentation importante de l'épaisseur de la gaine de Schwann dont la surface externe est repoussée dans le périneurium; la distance entre l'axone et la paroi externe de la gaine de Schwann peut alors atteindre 3,5 μ. Dans le cas de la Fig. 2, le contour de l'axone n'est pas modifié, mais c'est le noyau qui se déforme pour se loger en partie entre l'axone et la limite externe de la gaine de Schwann. HOLMES (27) signale que dans le cas des nerfs myéliniques des Crustacés, au contraire des nerfs myéliniques des Vertébrés, les noyaux se trouvent à l'intérieur de la couche de myéline. Pour les nerfs amyéliniques juxtaterminaux, il est impossible de situer le noyau schwannien par rapport au mésaxone; en raison même de son parcours réduit, le mésaxone ne passe généralement pas au voisinage du noyau (Fig. 3). Les noyaux de la gaine de Schwann sont globuleux, plus on moins ovoides (3 x 2 μ); les noyaux du périneurium sont aplatis (6-7 x 1,5 μ) et leur présence ne déforme pas les contours de la gaine périneuriale.

Parmi les autres inclusions cytoplasmiques de la gaine de Schwann, des profils d'endomembranes peuvent être assez abondants au voisinage du noyau (r. e., Fig. 3) et au contraire rares et dispersés dans les autres zones. Nous n'avons pas observé de mitochondries. Il n'est pas possible de préciser si la gaine de Schwann est ici syncitiale ou cellulaire. Le périneurium contient quelques mitochondries de petite taille (1 x 0,3 μ), de distribution éparse, apparamment sans relations régulières avec un autre constituant de la gaine périneuriale. Deux ou trois strates d'une substance homogène analogue à celle des membranes basales, peuvent s'intercaler entre la neural lamella et la membrane basale interne du périneurium. Elles sont séparées par des zones de cytoplasme périneurial; elles sont reliées par les tractus de même nature qui décrivent un trajet oblique à travers le cytoplasme d'une strate à l'autre (Fig. 5, 6, 7). C'est en général la neural lamella la plus épaisse (50-130 mμ), les autres strates étant d'autant plus fines qu'elles sont plus proches de l'axone. Parfois ces strates sont absentes, mais on retrouve toujours la trace de un ou deux tractus de même substance à trajet oblique, entre la neural lamella et la membrane basale interne du périneurium (Fig. 5).

Les dimensions des axones juxtaterminaux sont de l'ordre de 2,5 à 3 μ de diamètre pour A_1 et de 1,5 à 1,8 μ pour A_2. La surface

de l'axoplasme est relativement régulière et sans relief, en comparaison avec les exemples donnés d'un axolemme hautement contourné chez le Homard (15). L'axoplasme contient de petites mitochondries de 0,25 μ environ de diamètre et d'une longueur variable qui peut atteindre 3 μ. Elles sont en position périphérique, sous l'axolemme, et leur plus grande dimension est orientée parallèlement à l'axe de la fibre nerveuse, comme cela est visible sur les coupes longitudinales de l'axone (Fig. 6). D'ANCONA (18) n'avait pas obtenu la mise en évidence des neurofibrilles; il signale que MANGOLD, qui attribuait les différences de diamètre des axones à des différences dans l'abondance des neurofibrilles, prétend les avoir vues, sans en donner cependant d'illustration. D'ANCONA semble donc mettre en doute leur existence dans les nerfs des Crustacés. Sur toutes nos micrographies électroniques de nerfs juxtaterminaux, les neurofilaments sont toujours bien visibles, parallèles à l'axe de la fibre nerveuse, distribués uniformément sur toute la surface de coupe de l'axone. Leur longeur, variable, peut atteindre 0,7 μ; leur diamètre est de l'ordre de 100 à 150 A. Une autre inclusion axoplasmique est représentée par des granulations fortement osmiophiles, de 150 à 200 A de diamètre (gr, Fig. 4). Elles sont réparties régulièrement dans tout l'axoplasme, mais ne sont pas toujours présentes dans toutes les préparations.

Les zones de jonction myoneurale. Les connaissances sur la distribution et la structure des jonctions neuromusculaires des Crustacés manquent encore de précisions. Un premier problème concerne le nombre de terminaisons nerveuses par fibre musculaire. HARREVELD (12) estime que pour une fibre musculaire de la pince de l'Ecrevisse, ce nombre se situe aux environs de quarante, peut-être plus en tenant compte que la technique utilisée, une imprégnation argntique, n'est pas parfaitement élective pour toutes les terminaisons. Il décrit autour de chaque fibre musculaire, un réseau nerveux d'où se détachent les rameaux les plus fins qui formeront les terminaisons nerveuses. Ce sont donc des fibres musculaires à "innervation multiple", terme qui désigne un grand nombre de jonctions myoneurales sur une même fibre musculaire, à ne pas confondre avec "innervation polyneuronale" plus appropriée pour l'innervation d'une même fibre musculaire par plusieurs axones. Ultérieurement, HOLMES (13) a

cru devoir réfuter cette notion d'innervation multiple. Il fait d'abord remarquer que D'ANCONA ne décrit qu'une ou deux terminaisons par fibre musculaire. Après des essais personnels d'imprégnation argentique, il conclut que le réseau mis en évidence par HARREVELD à la surface des fibres musculaires provient seulement de l'imprégnation des fibres de collagène et de réticuline enveloppant la fibre musculaire. Selon HOLMES, il n'existe qu'une ou deux terminaisons nerveuses par fibre musculaire chez les Crustacés.

Un autre aspect de la jonction neuromusculaire qui, chez les Vertébrés, a provoqué jadis de nombreuses discussions, intervient à propos de la position de l'extrêmité de l'axone par rapport au sarcolemme. Chez les Crustacés, MANGOLD (17) voit les extrêmités des fibres nerveuses pénétrer sous le sarcolemme et même s'y ramifier encore avant de se terminer. D'ANCONA (18) insiste sur la variété des terminaisons nerveuses selon les espèces de Crustacés envisagées, mais il pense que toutes sont hypolemmales, suivant plusieurs modalités: les unes accompagnent des cloisons de sarcolemme qui pénètrent entre les myofibrilles; d'autres s'alignent parallèlement aux stries Z sur les faisceaux de myofibrilles; d'autres sont simplement visibles dans le sarcoplasme, entre le sarcolemme et les myofibrilles. Donc dans le cas des Crustacés, la microscopie optique semble avoir toujours conclu en faveur d'une jonction neuromusculaire hypolemmale.

Le sujet de l'innervation multiple sera traité plus en détails dans la discussion. Nous rapportons seulement ici, parmi les résultats, deux observations qui sont en faveur de sa réalité. D'une part, les colorations au bleu de méthylène montrent que les rameaux nerveux les plus fins sont répartis sur toute la surface de la fibre musculaire; il est donc permis de penser que les terminaisons nerveuses présentent la même distribution. D'autre part, nous n'avons pas choisi de zones particulières dans les fibres musculaires pour faire les préparations, les régions de jonction myoneurale ayant été rencontrées dans des coupes provenant de niveaux très divers de la longueur des fibres musculaires.

Parmi les différentes micrographies électroniques de jonctions neuromusculaires que nous avons obtenues, quatre exemples ont été retenus pour figurer dans ce travail. Les trois premiers correspondent à trois plans de coupe perpendiculaires entre eux, leur orientation

étant déterminée par rapport à l'axe de la fibre musculaire de la jonction myoneurale considérée: Figs. 8 et 9, coupe transversale; Fig. 10, coupe radiale; Figs. 11 et 12, coupe tangentielle. Le quatrième exemple, Fig. 13, montre aussi une coupe tangentielle, mais qui contient deux terminaisons nerveuses au contact d'une même fibre musculaire. La Fig. 8, effectuée à faible grossissement, permet de retracer brièvement les principaux caractères structuraux des fibres musculaires de l'exopodite des maxillipèdes, déjà décrits ailleurs (20, 28): grande abondance de sarcoplasme; myofibrilles groupées en colonnettes musculaires; mitochondries nombreuses et volumineuses, réparties en deux groupes, l'un faisant une couche continue de mitochondries sous le sarcolemme, l'autre situé auprès des faisceaux de myofibrilles; reticulum endoplasmique localisé entre les myofibrilles; granulations abondantes de glycogène dans le sarcoplasme.

D'après les coupes transversales et radiales, la zone synaptique est représentée par une dépression de la surface de la fibre musculaire dans laquelle s'engage l'extrêmité de la fibre nerveuse. Le contour de cette dépression, examiné sur les coupes tangentielles, est de forme elliptique, probablement parce que l'extrêmité de l'axone pénètre obliquement dans la dépression synaptique. Le fond de la dépression n'est pas toujours régulier, mais peut être recoupé par une cloison partielle (Cl, Fig. 11), comme si l'extrêmité de l'axone présentait une ébauche de ramification, d'ailleurs très inégale. En réalité, il sera montré plus loin que la juxtaposition du sarcoplasme et de l'axoplasme offre des caractères de grande complexité, auxquels on pourra rapporter cet aspect. Sur la Fig. 13, deux dépressions synaptiques, de dimensions différentes, sont au voisinage l'une de l'autre, sur une même fibre musculaire, comme si deux fibres nerveuses de diamètre inégal prenaient contact avec la fibre musculaire. La profondeur des dépressions synaptiques, de l'ordre de 3,5 μ, est inférieure à l'épaisseur (jusqu'à 8 μ) de la couche de sarcoplasme subsarcolemmale séparant les premières myofibrilles du sarcolemme. En conséquence, sur coupe tangentielle, il n'y a généralement pas de myofibrilles dans de champ d'observation, parce que le plan de coupe est passé seulement dans la couche subsarcolemmale de sarcoplasme, parmi les mitochondries groupées contre la membrane de la fibre musculaire; la terminaison nerveuse se trouve donc entourée seulement par des mitochondries

dans un cytoplasme dont on pourrait discuter la nature sarcoplasmique. Cependant, les mitochondries du sarcoplasme ont des dimensions et une structure particulières qui, ajoutées à la présence de granulations de glycogène, permettent de reconnaître avec certitude ce qui appartient à la fibre musculaire dans une telle coupe tangentielle. Ces données sont confirmées par la Fig. 13, où dans l'angle inférieur gauche, la coupe tangentielle, légèrement oblique, est passés par quelques myofibrilles; les grandes mitochondries, les granulations de glycogène et les myofibrilles y font bien partie d'une même organisation cellulaire correspondant à la fibre musculaire.

La membrane basale de la fibre musculaire ne participe pas à la constitution de la dépression synaptique. La Fig. 9 montre sa brusque interruption au bord de la zone où la surface de la fibre musculaire se déprime; seule subsiste de la fibre musculaire, la membrane plasmique [3] qui forme le fond de la dépression. La coupe radiale de la Fig. 10 présente un cas particulier où la membrane basale de la fibre musculaire est ininterrompue et passe par dessus la terminaison nerveuse; mais la dépression synaptique y est aussi d'une profondeur plus faible en comparaison avec les autres exemples. Il est probable que la coupe soit passée dans une partie très latérale de la dépression, par un renfoncement situé sous la membrane basale. Cette interprétation peut être concrétisée à l'aide de la Fig. 9, en imaginant le passage d'une coupe radiale dans la partie pauche de la dépressions synaptique. A propos de la partie nerveuse de la synapse, la majorité de la gaine de Schwann et toute la gaine périneuriale restent à l'extérieur de la dépression synaptique, au-dessus de l'extrémité de l'axone qu'elles recouvrent et séparent d'un contact direct avec le milieu extracellulaire. Quelques prolongements de la gaine de Schwann peuvent cependant s'insinuer dans la dépression, entre l'axone et la fibre musculaire (g. S., Fig. 9). La membrane basale de la fibre musculaire peut être en continuité avec une membrane basale des enveloppes de l'axone (Fig. 8), mais il n'a pas été possible d'observer le devenir de l'ensemble de la gaine périneuriale et de ses structures au niveau de la terminaison nerveuse. Sur coupe tangen-

(3). — La définition du sarcolemme étant un sujet de discussion, ce terme est utilisé dans ce travail au sens large de limite de la fibre musculaire. Quand des précisions de structure sont nécessaires, les deux constituants principaux de cette limite sont désignés par membrane basale et membrane plasmique (= membrane sarcoplasmique) de la fibre musculaire.

tielle, les gaines de l'axone ne sont pratiquement pas détectables, ayant été emportées dans les coupes précédant celles qui passent par la dépression synaptique.

En résumé, dans ces exemples de jonctions neuromusculaires d'un Crustacé, l'extrêmité de l'axone privé de ses gaines, limitée seulement par l'axolemme, se trouve engagée dans une dépression de la surface de la fibre musculaire où la membrane basale a disparu, et où seule demeure la membrane sarcoplasmique. En contradiction avec les observations de la microscopie optique, il y a donc une séparation continue entre l'axoplasme et le sarcoplasme, ce qui rejette ici la possibilité d'une jonction de l'axone avec l'une quelconque des zones transversales des myofibrilles et d'une terminaison nerveuse hypolemmale.

La juxtaposition des membranes plasmiques de l'axone et de la fibre musculaire est visible sur la coupe radiale de la Fig. 10, sur la coupe tangentielle de la Fig. 12 et dans certaines régions des Figs. 14, 15, 16 qui présentent des agrandissements partiels de la Fig. 13. La surface de contact entre l'axoplasme et le sarcoplasme se traduit, sur coupe, par des contours complexes qu'il est nécessaire de décomposer pour examiner les diverses possibilités de disposition des deux sortes de membranes plasmiques participant à la synapse myoneurale. Le cas le plus simple est celui des zones désignées par I sur les Figs. 10, 12, 14, 15. L'axolemme est juxtaposé à la membrane sarcoplasmique suivant une surface régulière, relativement sans relief. L'espace séparant les deux membranes est de l'ordre de 100 à 150 A; il ne subit pas de grandes variations dans les différentes parties de la synapse. Une première complication se produit avec la différenciation vers le sarcoplasme, d'expansions d'axoplasme de formes et de dimensions très diverses. Ces formations sont particulièrement développées sur la Fig. 13. Le cas de la dépression synaptique partiellement cloisonnée de la Fig. 11 peut en être rapproché, la portion isolée de l'axoplasme étant considére comme une expansion très volumineuse. Le plus souvent, du sarcoplasme se trouve interposé entre une telle expansion et l'axoplasme principal: IIa, Fig. 16, au point que parfois, du fait de la coupe, elle paraît complètement isolée au milieu du sarcoplasme: IIb, Fig. 15. Dans d'autres cas, l'expansion d'axoplasme est juxtaposée à l'axoplasme principal, sans inter-

position de sarcoplasme: IIc, Fig. 12; les deux membranes plasmiques en vis-à-vis sont alors de même nature et font partie de l'axolemme. Enfin, une zone de sarcoplasme peut être plus ou moins enveloppée par les contours irréguliers d'une expansion, au point de sembler, sur coupe, complètement encerclée d'axoplasme: IId, Fig. 16. Un autre type de complication se traduit par l'existence d'invaginations de l'axolemme dans l'axoplasme, qui peuvent rester simples, s'avançant seulement dans l'axoplasme comme une cloison incomplète: IIIa, Fig. 10; ou bien, compliquées par des courbures, des angles, des bifurcations, elles peuvent déterminer un compartimentage plus avancé et même paraître isoler, sur coupe, une zone d'axoplasme, quand par exemple, deux extrêmités d'une invagination bifurquée se sont rejointes: IIIb, Fig. 10; dans certaines micrographies, on remarque bien, à leur contour arrondi, ces formations d'axoplasme isolées par les invaginations de l'axolemme: IIIb, Fig. 11, mais la partie de l'invagination qui les relie à l'axolemme principal périphérique n'est pas toujours visible, en raison du passage latéral de la coupe. La membrane sarcoplasmique peut aussi différencier des replis profonds et sinueux vers le sarcoplasme, où ne pénètre pas d'axoplasme: IV, Fig. 12; dans ce dernier cas, les deux membranes plasmiques juxtaposées sont toutes deux de nature sarcoplasmique.

Du fait de ces invaginations, la jonction neuromusculaire ne consite donc pas ici, en une simple juxtaposition des membranes plasmiques de l'axone et de la fibre musculaire. Il semble qu'il se produise, au niveau de la terminaison nerveuse, un système complexe d'augmentation des surfaces de l'axolemme et de la membrane sarcoplasmique. D'autres études, en cours, sont nécessaires avant de pouvoir donner un schéma d'ensemble de ce type de jonction myoneurale.

Sarcoplasme et axoplasme terminaux. Il existe également peu de données précises au sujet de l'influence de la terminaison nerveuse sur l'organisation du sarcoplasme. Dans les muscles de trois espèces de Macroures, D'ANCONA (18) ne constate aucune différenciation particulière du sarcoplasme au voisinage de la synapse; par contre, chez *Carcinus maenas,* les terminaisons seraient du type à plaque motrice, avec une sole granuleuse sarcoplasmique. Les travaux ultérieurs n'abordent pas cet aspect de la jonction myoneurale des Crustacés.

Dans les fibres musculaires des maxillipèdes du Crabe Bleu, aucun épaississement particulier du sarcoplasme périphérique n'a été remarqué au voisinage de la synapse. Il a d'ailleurs été signalé plus haut que l'épaisseur du sarcoplasme subsarcolemmal était supérieure à la profondeur de la dépression synaptique; elle peut atteindre 8 μ, alors que le maximum observé de la profondeur des dépressions synaptiques, n'excède pas 4 μ.

Chez les Vertébrés (10,6) et chez les Insectes (8, 9), la jonction neuromusculaire est caractérisée par une accumulation de mitochondries dans le sarcoplasme et dans l'axoplasme. Dans le cas présent, seul l'axoplasme terminal présente une abondance plus grande de mitochondries. Alors que dans les nerfs juxtaterminaux, les mitochondries sont seulement localisées contre l'axolemme, au niveau de la terminaison nerveuse, elles ont au contraire tendance à occuper tout le volume de l'axoplasme, ou même à se grouper au centre. Les mitochondries de l'axoplasme terminal sont d'aspect très varié, arrondies, ovales, allongées, présentant des étranglements ou des angles qui leur donnent une forme en V (Fig. 13). De petit diamètre (0,2 à 0,5 μ), elles peuvent dépasser 2 μ de longueur. Elles ne montrent aucune orientation prédominante de leur plus grande dimension par rapport à une orientation quelconque de l'axone, au contraire des mitochondries de l'axoplasme juxtaterminal. Leur structure est du type classique à lamelles. Les mitochondries du sarcoplasme n'ont pas une distribution modifiée au voisinage de la synapse. Leur répartition demeure selon deux groupes, l'un sous la membrane limitante de la fibre musculaire, l'autre auprès des faisceaux de myofibrilles. Elles sont bien plus volumineuses que les mitochondries de l'axoplasme, avec un diamètre moyen de 1,5 μ et une longuer atteignant 6 μ. Leur structure est aussi différente et présente un réseau complexe de membranes à travers la matrice mitochondriale. Il n'y a pas non plus augmentation du nombre des noyaux du sarcoplasme auprès de la jonction myoneurale; dans les micrographies présentées, un seul noyau est partiellement visible sur la Fig. 13, montrant une structure typique avec des amas périphériques de chromatine et une double membrane interrompue par les pores (Fig. 14).

Les formations vésiculaires caractéristiques des zones synaptiques, décrites par DE ROBERTIS et BENNETT (29), se retrouvent

ici dans l'axoplasme terminal, avec leur aspect habituel, comportant une limite externe dense, de coutour subcirculaire, autour d'une partie centrale plus claire. D'un diamètre allant de 250 à 400 A, elles sont distribuées irrégulièrement par groupes qui en renferment un nombre variable; les groupes les plus importants sont en général à proximité d'une zone de juxtaposition de l'axolemme avec la membrane sarcoplasmique, les petits groupes de quelques vésicules ou les vésicules isolées se rencontrant dans tout le volume de l'axoplasme terminal. Cependant, de larges zones de contact de l'axoplasme avec le sarcoplasme sont quelquefois dépourvues de vésicules synaptiques. Les vésicules sont aussi absentes en face du cytoplasme schwannien, quand des parties de la gaine de Schwann sont engagées dans la dépression synaptique; elles sont souvent accumulées autour des ilôts d'axoplasme délimités par les invaginations de l'axolemme (Figs. 11, IIIb). A proximité d'un groupe de vésicules, les membranes plasmiques juxtaposées sont plus denses (Fig. 12), comme s'il avait accumulation d'une substance plus osmiophile à cet endroit, fait déjà remarqué par PALAY (30) dans le cas de synapses interneuronales. Les granules denses de l'axoplasme des nerfs juxtaterminaux sont aussi présents dans l'axoplasme terminal ave les mêmes dimensions et la même distribution.

Toujours bien individualisés dans les axones juxtaterminaux, les neurofilaments disparaissent de l'axoplasme terminal, comme cela a déjà été mis en évidence dans d'autres groupes d'animaux (6,8). Cependant, sur la Fig. 13, la surface de coupe de l'axone A_1 est recouverte, entre les mitochondries, les vésicules synaptiques et les granules denses, par des structures très fines, évoquant des neurofilaments moins nettement figurés, à contours et à densité atténuées, comme si la disparition des neurofilaments était progressive. Dans l'axoplasme, il y a encore quelques profils de membranes à rapprocher probablement d'un reticulum endoplasmique qui serait très diffus. Dans le sarcoplasme, le reticulum endoplasmique est très développé et seulement localisé dans les colonnettes musculaires, entre les myofibrilles. N'existant pratiquement pas dans les larges zones de sarcoplasme subsarcolemmal, il ne montre aucun développement particulier au niveau de la jonction myoneurale, au contraire de ce qui a été vu chez les Insectes (8, 9). On constate la présence de reticulum

sarcoplasmique au voisinage de la synapse seulement si un faisceau de myofibrilles est lui-même auprès de la dépression synaptique. Même dans ce cas, le reticulum demeure entre les myofibrilles, sans émettre de prolongements spéciaux vers la zone de jonction et sans présenter une quelconque modification (Fig. 10). Dans le sarcoplasme, on remarque encore la présence de nombreuses granulations qui facilitent la distinction entre la fibre musculaire et l'axone, dans la zone d'intrication complexe de l'axoplasme et du sarcoplasme bordant la dépression synaptique. Ces granulations ne sont pourtant pas caractéristiques de la jonction myoneurale; elles existent par amas irréguliers dans tout le volume de la fibre musculaire (Fig. 8). Il a été montré par la réactions de Mac Manus que ces granulations sont constituées par du glycogène (28).

En plus de l'inégalité des diamètres des deux terminaisons nerveuses de la Fig. 13, il y a aussi des différences dans l'aspect des constituants de l'axoplasme. Les vésicules synaptiques, nombreuses en A_1, sont beaucoup plus rares en A_2; les structures fines, rappelant des neurofilaments "atténués", sont de même abondantes en A_1 et très dispersées en A_2; les mitochondries de A_2 ont des dimensions inférieures à celles de A_1; elles sont groupées au centre de l'axoplasme en A_2; elles sont réparties sur toute la coupe de A_1, où leurs formes sont aussi plus variées; enfin, les granulations osmiophiles énigmatiques sont moins nombreuses par unité de surface en A_2 qu'en A_1. Ces différences d'ultrastructures ne sont pas perceptibles quand deux terminaisons, qui ne sont pas totalement séparées, proviennent d'une ramification terminale incomplète de l'axone.

DISCUSSION

En comparant la structure des nerfs des pattes locomotrices du Homard, décrite par GEREN et SCHMITT (14, 15), avec celle des nerfs juxtaterminaux du Crabe Bleu, il apparaît un certain nombre de différences. Certaines sont peut-être seulement des différences de terminologie, comme par exemple à propos des enveloppes conjonctives. En effet, les micrographies de ces deux auteurs montrent, autour des gaines de Schwann des axones, un tissu comportant des strates successives de substante d'aspect homogène = "connective tissue", séparées par un cytoplasme nucléé = "connective tissue cell". Il est

probable que la gaine périneuriale des nerfs juxtaterminaux du Crabe Bleu soit l'équivalent de ces formations, bien que les micrographies des nerfs du Homard, effectuées à fort grossissement, ne permettent pas de voir si le tissu conjonctif forme une gaine commune aux axones et à leur gaine de Schwann respective, limitée à l'extérieur par une membrane basale du type neural lamella. Il y a cependant des différences dans l'organisation même des deux sortes de nerfs. On ne retrouve pas dans les nerfs juxtaterminaux, cette disposition des fibres nerveuses du type "C" des Vertébrés (23), partageant à plusieurs une gaine de Schwann commune, telle que GEREN et SCHMITT l'ont rencontrée pour les fibres nerveuses fines du nerf du Homard. Chaque axone possède ici sa propre gaine de Schwann dont l'individualité est soulignée par la membrane basale interne de la gaine périneuriale. Mais il se peut également que les fibres nerveuses à gaine de Schwann commune des nerfs du Homard n'appartiennent pas à l'innervation motrice des fibres musculaires des pattes locomotrices.

Les parties équivalentes des gaines des axones sont plus développées dans les nerfs des pattes du Homard que dans les nerfs juxtaterminaux étudiés ici: strates du tissu homogène plus épaisses, plus nombreuses, plus serrées dans la gaine périneuriale; mesaxone à parcours plus long dans la gaine de Schwann. Ces variations pourraient s'attribuer à des différences dans l'organisation du nerf selon les espèces ou selon les appendices considérés, mais elles peuvent dépendre aussi du niveau de la coupe par rapport au trajet du nerf entre la chaîne et les terminaisons nerveuses. Dans ce dernier cas, la proximité de la terminaison se traduirait par une simplification des gaines de l'axone. D'autre part, il a été signalé qu'à un même niveau juxtaterminal, les nerfs présentent des gaines périneuriales inégalement développées, notamment vis-à-vis du nombre des strates de substance homogène. Du fait de l'innervation multiple et de la distribution des potentiels de jonction (31), on peut admettre une constance relative du nombre de terminaisons nerveuses par unité de surface de la fibre musculaire. Cela implique, avec la croissance de la fibre musculaire après chaque mue du Crabe, la formation de ramifications nouvelles de nerfs juxtaterminaux pour accroître le nombre des terminaisons nerveuses. Il y aurait ainsi, à un même niveau juxtaterminal de l'in-

nervation, des rameaux nerveux d'age différent pour lesquels le faible développement des gaines de l'axone serait un caractère de "jeunesse". Ces dernières données, pour le moment hypothétiques, sont en cours d'étude pour tenter d'interpréter les différences, quelquefois importantes, qui apparaissent dans le développement des gaines des nerfs d'un même niveau. Pour SCHARRER (24) et HOYLE (25), la neural lamella est secrétée par le périneurium au niveau de la membrane plasmique sous-jacente. Ceci suggère que la membrane basale, qui circonscrit les gaines de Schwann, est un produit d'élaboration de la face interne du périneurium, son origine étant sans rapport avec la gaine de Schwann. De même, les strates intercalaires du périneurium sont doublées d'une membrane de 12 mμ d'épaisseur (inset 5' de la Fig. 5), limitant le cytoplasme périneurial. Il est possible que ces strates intermédiaires correspondent à une subdivision de la gaine périneuriale, en unités qui seraient cellulaires.

Au sujet des différences entre les résultats de HOLMES (13) et de HARREVELD (12), plusieurs arguments interviennent en faveur de l'innervation multiple des fibres musculaires des Crustacés. I) — Les observations de D'ANCONA ne sont pas en opposition avec l'existence d'une innervation multiple; chez Astacus, il décrit, autour des fibres musculaires, un réseau de fines fibres nerveuses à partir duquel prennent naissance les ultimes rameaux qui forment les terminaisons nerveuses; ceci est très proche des données de HARREVELD. 2) — Il y a certainement autour de chaque fibre musculaire, un revêtement important de fibres collagènes qui, comme nous l'avons aussi constaté, s'imprègne plus facilement à l'argent que les fibres nerveuses; cela n'exclut cependant pas la présence de fibres nerveuses, réparties sur toute la surface de la fibre musculaire, camouflées en quelque sorte par une imprégnation argentique du collagène semblable à celles effectuées par HOLMES. 3) — Avec des solutions très diluées de bleu de méthylène, seules sont colorées les fibres nerveuses; nous avons pu remonter la succession des ramifications nerveuses depuis les branches les plus fines, distribuées à la surface des fibres musculaires, jusqu'aux axones principaux qui, à la surface des muscles, sont en provenance directe du tronc nerveux de l'appendice; l'électivité du bleu de méthylène en coloration postvitale pour les fibres nerveuses et la polarité du réseau nerveux, per-

mettent de distinguer ce dernier du réseau de fibres collagènes. 4) — Il a été déjà remarqué que les zones synaptiques ont été rencontrées dans des coupes provenant de diverses régions des fibres musculaires, sans recherche de niveaux préférentiels comme cela est nécessaire dans le cas de la plaque motrice des Vertébrés (6); ce simple fait de technique est également en faveur d'une répartition diffuse des terminaisons nerveuses sur toute la surface de la fibre musculaire. 5) — FATT et KATZ (31) ont montré, par des enregistrements intracellulaires dans des fibres musculaires de Crustacés, que les potentiels de plaque motrice, ou mieux selon HOYLE et WIERSMA (32), les potentiels de jonction, sont distribués sur toute la longueur de la fibre musculaire, avec de faibles variations de leur amplitude locale. D'autre part, au contraire de ce qui fut observé chez les Vertébrés (10, 33, 6), les terminaisons nerveuses ne modifient pas la distribution des mitochondries dans le sarcoplasme. Ce caractère est peut-être en rapport avec l'innervation multiple, étant données les fonctions importantes des mitochondries dans les réactions du métabolisme cellulaire. En effet, dans le cas d'une plaque motrice, l'origine de l'excitation provoquant la contraction de toute la fibre musculaire se localise en un seul point, siège d'une activité enzymatique intense qui se traduit par une accumulation de mitochondries. Dans le cas de l'innervation multiple, il y a partage de cette activité entre les nombreuses terminaisons nerveuses, cette dispersion n'impliquant pas de modification dans la répartition des mitochondries de la fibre musculaire au niveau des synapses.

Les terminaisons nerveuses sont épilemmales par rapport à la limite de la fibre musculaire dans les muscles des maxillipèdes du Crabe Bleu. En raison des observations antérieures de la microscopie optique, faut-il restreindre cette donnée au seul cas de ces muscles, ou bien certaines particularités des fibres musculaires ou des jonctions myoneurales des Crustacés sont-elles responsables d'une description inexacte de terminaisons hypolemmales? Une première cause d'erreur est possible avec l'interruption de la membrane basale de la fibre musculaire au niveau de la dépression synaptique; c'est principalement cette membrane qui, en microscopie optique, représente la limite de la fibre musculaire, et sont interruption peut être à l'origine des descriptions du passage de la terminaison nerveuse à travers le

sarcolemme. D'autre part, le revêtement de fibres conjonctives autour des fibres musculaires a pu être considéré également comme faisant partie du sarcolemme; or très souvent, des fibres nerveuses sont engagées sous cette couche, pouvant même s'y ramifier avant de prendre fin. Il existe aussi, dans les fibres des appendices locomoteurs, des cloisons partielles insinuées entre les myofibrilles et contenant de fins rameaux nerveux; l'étude de leur développement en fonction de la croissance (34) a montré qu'il s'agit de replis invaginés de la membrane limitante de la fibre musculaire, c'est-à-dire que les fibres nerveuses correspondantes demeurent à l'extérieur de la membrane sarcoplasmique; mais en l'absence de coloration mettant en évidence ces formations, il est facile d'imaginer comment, sur des coupes d'histologie, des fibres nerveuses peuvent être localisées plus ou moins profondément à l'intérieur de la fibre musculaire selon l'ampleur des replis. Enfin, nous avons obtenu plusieurs fois, avec les imprégnations argentiques, les aspects de fines fibres disposées contre les faisceaux de myofibrilles, parallèlement aux lignes Z; ces structures sont en réalité présentes dans tout l'ensemble du faisceau de myofibrilles, puisqu'apparaîssant sur toute une série de coupes longitudinales successives; comme D'ANCONA l'avait lui-même remarqué, elles sont sans relation avec des fibres nerveuses situées à l'extérieur de la fibre musculaire; il est plus vraissemblable qu'elles soient le produit de dépôts d'argent sur les formations périodiques du reticulum endoplasmique localisé entre les myofibrilles. La majorité des observations de terminaisons nerveuses intrasarcoplasmiques en microscopie optique est donc passible de réserves.

L'innervation multiple et la complexité des contours des deux membranes plasmiques juxtaposées au niveau de la jonction neuromusculaire, doivent avoir pour conséquence une augmentation importante de la surface de la membrane de la fibre musculaire. FATT et KATZ (35), mesurant la capacité de membrane de fibres musculaires de Crustacés, ont obtenu des valeurs anormalement élevées, de l'ordre de 40 $\mu F/cm^2$. Ces deux auteurs ont envisagé une explication possible dans l'existence de replis et de circonvolutions au niveau des terminaisons nerveuses, augmentant considérablement la surface de la membrane. Dans des fibres musculaires d'appendices locomoteurs, il nous avait semblé (34) qu'un système de replis invaginés

du sarcolemme, indépendant de l'innervation de la fibre musculaire, pouvait rendre compte en partie, de la valeur élevée de la capacité de membrane. Dans les fibres musculaires des maxillipèdes, ce système de replis n'existe pratiquement pas. Il y a donc seulement, comme processus d'augmentation de surface de la fibre musculaire, que les circonvolutions complexes de la membrane sarcoplasmique dans les dépressions synaptiques. Si la capacité de membrane est ici aussi forte que dans le cas des fibres musculaires des appendices locomoteurs, l'hypothèse de FATT et KATZ se trouverait confirmée.

Dans les fibres musculaires d'autres groupes d'animaux, il y a des relations, d'une part entre les tubules du reticulum endoplasmique situés en face des lignes Z et M des myofibrilles et la membrane sarcoplasmique (36, 37), d'autre part entre le reticulum et les replis digitaux de la synapse myoneurale (39); chez les Insectes (9), le reticulum présente en outre une ramification profuse de tubules au voisinage de la jonction neuromusculaire. Sur ces observations, une hypothèse a été avancée (38, 39) à propos de la contraction musculaire, selon laquelle le reticulum endoplasmique serait le support d'une conduction intracellulaire de l'onde de dépolarisation depuis la membrane sarcoplasmique jusqu'aux différents sarcomères des myofibrilles. Dans le cas des fibres musculaires des maxillipèdes du Crabe Bleu, le reticulum endoplasmique est bien développé, mais seulement localisé dans les colonnettes musculaires, entre les myofibrilles; il n'est pratiquement pas perceptible dans les larges zones de sarcoplasme séparant les colonnettes musculaires les unes des autres, ou séparant les myofibrilles de la membrane sarcoplasmique; il ne montre pas de relations spéciales avec la jonction neuromusculaire et n'affecte aucune disposition particulière au niveau de cette dernière. L'organisation du reticulum endoplasmique de ce type de fibre musculaire ne semble donc pas permettre de le considérer comme le support morphologique possible d'une conduction intrasarcoplasmique de l'onde de dépolarisation.

Un dernier aspect à commenter parmi les observations de ce travail, est celui de la Fig. 13, où deux terminaisons nerveuses se présentent en jonction avec une même fibre musculaire coupée tangentiellement. Ces deux terminaisons correspondent-elles à l'extrémité de deux rameaux d'un même axone, ou sont-elles chacune la

terminaison d'une axone différent? Une réponse indiscutable à cette question ne peut pas être apportée d'après de simples données morphologiques. Cependant, un certain nombre de différences dans l'ultrastructure de l'axoplame des deux terminaisons a déjà été signalé. D'autre part, la différence de diamètre des deux jonctions neuromusculaires rappelle l'inégalité de taille des deux axones des nerfs juxtaterminaux, si toutefois elle n'est pas dûe au passage de la coupe à un niveau différent dans les deux dépressions synaptiques. Enfin, sur des coupes de même matériel examinées au microscope à contraste de phase, nous avons rencontré plusieurs fois des fibres nerveuses distinctes, à parcours parallèles, se terminant au voisinage l'une de l'autre sur une même fibre musculaire. Il est donc permis d'envisager que sur la Fig. 13, la zone de synapse myoneurale la plus grande corresponde à l'axone A_1 et la plus petite à l'axone A_2, les deux axones différents faisant jonction avec une même fibre musculaire, selon les données de l'innervation double.

RÉSUMÉ

Ce travail rapporte les premières observations de jonctions neuromusculaires rencontrées au cours d'une étude au microscope électronique sur les muscles des maxillipèdes du Crabe Bleu (*Callinectes Danae*, Smith). Quelques données concernent la structure des nerfs juxtaterminaux, principalement au sujet des constituants de l'axoplasme et des gaines des deux axones inégaux qui forment l'innervation double de ces muscles. Au niveau de la jonction neuromusculaire, l'extrêmité de l'axone, dépourvue de ses gaines, est engagée dans une dépression de la surface de la fibre musculaire où la membrane basale a disparu et où seule subsiste la membrane sarcoplasmique. Des expansions d'axoplasme vers le sarcoplasme, des replis de l'axolemme dans l'axoplasme et de la membrane sarcoplasmique dans le sarcoplasme, déterminent au niveau de la jonction neuromusculaire, un système complexe d'augmentation de la surface des membranes axo- et sarco-plasmiques. Dans l'axoplasme terminal, la distribution des mitochondries est modifiée, les neurofilaments s'estompent et les vésicules synaptiques sont réparties par groupes irréguliers; par contre, le sarcoplasme n'offre pas de changement sensible dans l'organisation de ses constituants auprès de la synapse. Un exemple de deux ter-

minaisons nerveuses au contact d'une même fibre musculaire est décrit et discuté.

Pour l'aide reçue sous de multiples formes au cours de ce travail, nous exprimons toute notre reconnaissance au Docteur Helena LOPES DE SOUZA-SANTOS, au Docteur Persio DE SOUZA-SANTOS, au Professour Paulo SAWAYA et au Professeur Paulo RIBEIRO DE ARRUDA.

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RESUMO

Êste trabalho relata as primeiras observações de junções neuromusculares encontradas durante um estudo com o microscópio eletrônico sôbre os músculos dos maxilípodos do Sirí Azul (*Callinectes Danae,* Smith). Alguns dados tratam da estrutura dos nervos juxtaterminais, principalmente a respeito dos constituintes do axoplasma e das bainhas dos dois axônios desiguais, que formam a inervação dupla dêsses músculos. Ao nível da junção neuromuscular, a extremidade do axônio, desprovida das suas bainhas, se introduz numa depressão da superfície da fibra muscular onde desapareceu a membrana basal e sòmente subsiste a membrana sarcoplásmica. Expansões de axoplasma em direção do sarcoplasma, dobras do axolema dentro do axoplasma e da membrana sarcoplásmica dentro do sarcoplasma, determinaram, ao nível da junção neuromuscular, um sistema complexo de aumento da superfície das membranas axo- e sarco-plásmicas. Dentro do axoplasma terminal, a distribuição das mitocôndrias é modificada, os neurofilamentos desaparecem e as vesículas sinapticas repartem-se por grupos irregulares; ao contrário, o sarcoplasma não apresenta modificações sensíveis da organização dos seus constituintes na proximidade da sinapse. Um exemplo de duas terminações nervosas ao contacto de uma mesma fibra muscular é descrito e discutido.

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EXPLICATION DES PLANCHES

PLANCHE I

Fig. 1 — Micrographie optique d'une préparation de muscle des maxillipèdes du Crabe Bleu, traitée par du nitrate d'argent. Les deux axones, A_1 et A_2, de l'innervation double, présentent un cas de ramification diplotomique; cependant seule est visible la ramification de A_1, en raison du recouvrement de celle de A_2 par le fond noir d'un noyau. En Nf sont perceptibles les contours du nerf qui contient les deux axones A_1 désigne l'axone le plus épais, et Nf, l'ensemble du nerf. N = noyaux, (x 1800).

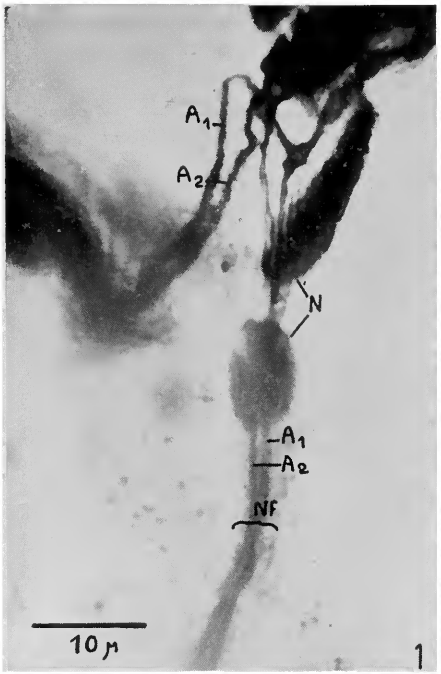
Fig. 2 — Micrographie électronique d'une coupe de nerf juxtaterminal dans un muscle des maxillipèdes du Crabe Bleu. Cette micrographie doit être comparée avec celle de la Fig. 7. Un tel nerf comporte deux axones A_1 et A_2 d'inégal diamètre, A_1 représentant le plus épais. Chaque axone est entouré d'une gaine de Schwann (g. S.) particulière, l'ensemble étant enveloppé par une gaine périneuriale commune. Cette dernière est limitée, intérieurement par une membrane basal (m. b.') qui circonscrit chaque gaine de Schwann, extérieurement par une membrane basale épaisse, la neural lamella (n. l.) qui est aussi la paroi du nerf vis-à-vis des espaces extracellulaires (ex); la partie cytoplasmique, ou périneurium (Pn), est traversée obliquement par un tractus (tr) de substance homogène semblable à celle des membranes basales.

Bien séparées l'une de l'autre dans la Fig. 7, les deux gaines de Schwann sont ici mitoyennes. La coupe est passée au niveau d'un noyau de la gaine de Schwann de A_1 (N. S.). Normalement de faible épaisseur, la gaine de Schwann se distend considérablement vers le périneurium au niveau d'un noyau; le contour de l'axone reste régulier; c'est le noyau qui se déforme pour se loger entre l'axone et la paroi externe de la gaine de Schwann.

Mt_1 = mitochondries de l'axoplasme; nf = neurofilaments; st = strate intermédiaire dans le périneurium; Sp = sarcoplasma. (x 9200).

Fig. 3 — Même préparation que dans la Fig. 2, photographiée avec un grandissement supérieur pour montrer la gaine de Schwann au niveau d'un noyau. La limite interne de la gaine de Schwann est marquée par la juxtaposition de son cytoplasme avec l'axoplasme: S-A; sa limite externe est située contre la membrane basale (m. b.') du périneurium. Le mésaxone (Mx) décrit un trajet réduit qui ne passe pas auprès du noyau schwannien; autour de ce dernier, des profils d'endomembranes (r. e.) sont assez abondants. Autres légendes comme précédenment (x 20000).

Fig. 4 — Micrographie électronique d'une portion de fibre nerveuse avec les constituants de l'axoplasme: mitochondries (Mt_1) localisées à la périphérie; neurofilaments (nf) et granulations osmiophiles énigmatiques (gr) distribuées dans tout l'axoplasme. Le trajet du mesaxone entre les surfaces externe et interne de la gaine de Schwann est notablement court, surtout s'il est tenu compte de l'obliquité de la coupe. Autres légendes comme précédemment. (x 19200).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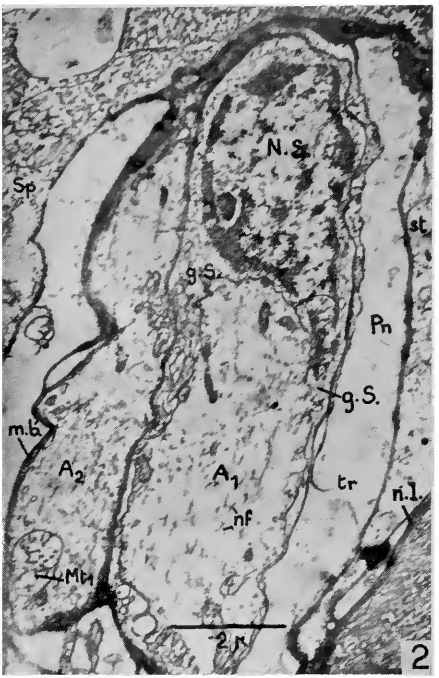
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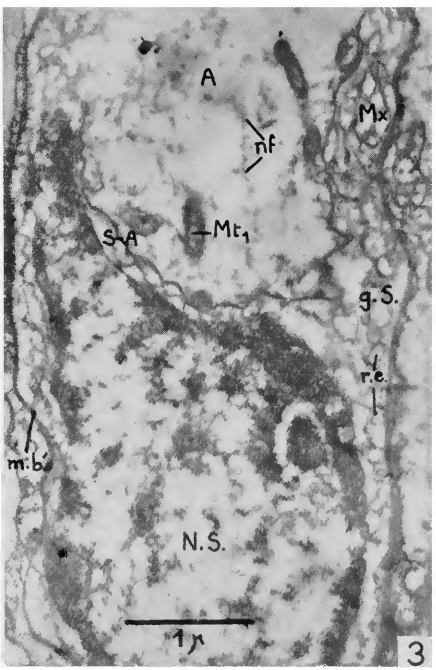
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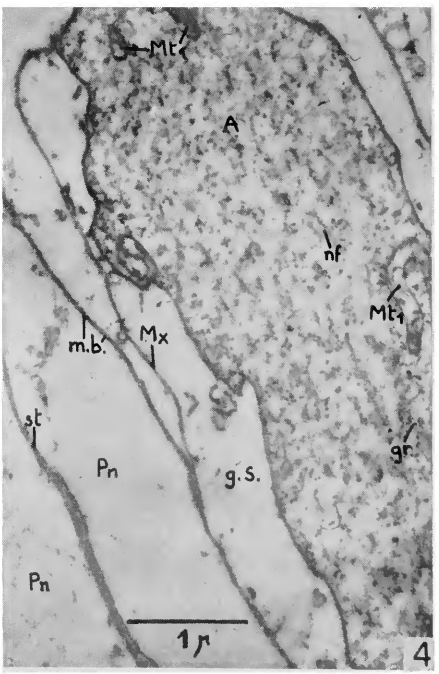
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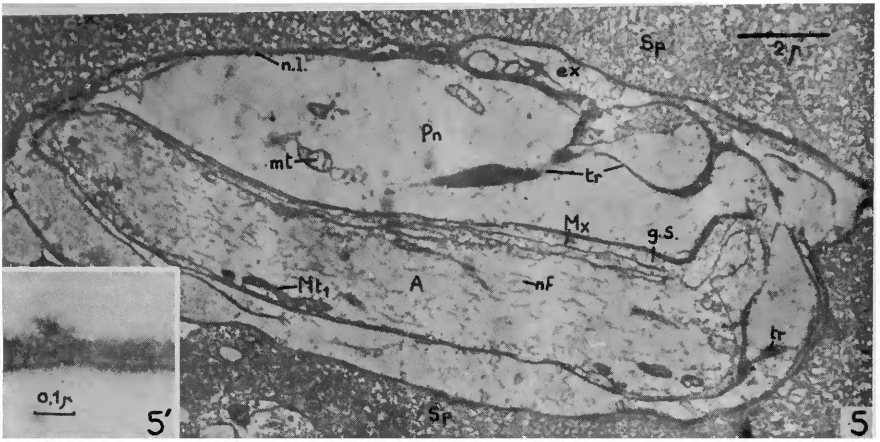
PLANCHE II

Fig. 5 — Coupe d'un nerf juxtaterminal comportant un seul axone. L'organisation des gaines est la même que dans un nerf à deux axones; la gaine périneuriale, limitée extérieurement par une neural lamella, montre des tractus obliques (tr) de substance d'aspect homogène reliant ses deux faces, en l'absence de strates intermédiaires du périneurium. Quelques mitochondries (mt) sont visibles dans le périneurium, sans relations apparentes avec d'autres constituants. Le mesaxone paraît plus allongé du fait de la forte obliquité de la coupe. Légendes comme précédemment. (x 7300).

Inset 5' — Agrandissement local d'une strate intermédiaire du périneurium. La membrane épaisse de substance d'aspect homogène semblable à une membrane basale est doublée par une membrane plus fine, probablement cytoplasmique. (x 62000).

Fig. 6 — La coupe passe longitudinalement par un nerf juxtaterminal qui s'infléchit brusquement dans la partie droite de la Fig. Un seul axone est visible dans le nerf, mais du fait de l'orientation sublongitudinale, un autre axone parallèle peut exister dans les coupes précédant ou suivant celle de la micrographie. Le périneurium présente plusieurs strates de substance d'aspect homogène interposées entre sa limite interne (m. b.') et la neural lamella (n. l.). Le mesaxone apparaît en deux endroits, d'une part très allongé en Mx' parce qu'en coupe longitudinale, d'autre part en coupe transversale en Mx" où il est très court. Dans l'axoplasme, l'orientation de la plus grande dimension des mitochondries et des neurofilaments est parallèle à l'axe de la fibre nerveuse, ce qui se vérifie au niveau de la courbure de l'axone par le raccourcissement de ces structures. (x 13500).

Fig. 7 — Coupe d'un nerf à deux axones, montrant un noyau de la gaine périneuriale (N. Pn). La position d'un tel noyau, par rapport à la membrane basale interne du périneurium, permet de le distinguer immédiatement d'un noyau schwannien. De forme aplatie, le noyau du périneurium n'entraîne pas de modifications sensibles dans les contours des différentes parties du nerf. Les autres caractères de structure du nerf sont déjà donnés à propos de la Fig. 2. (x 6800).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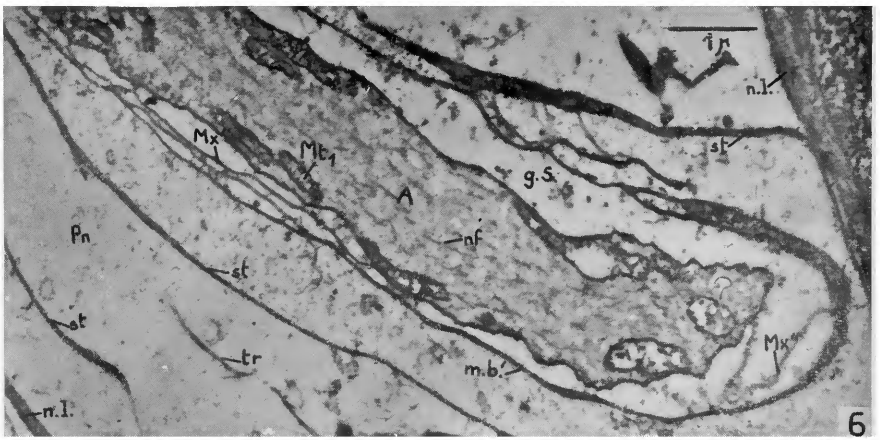
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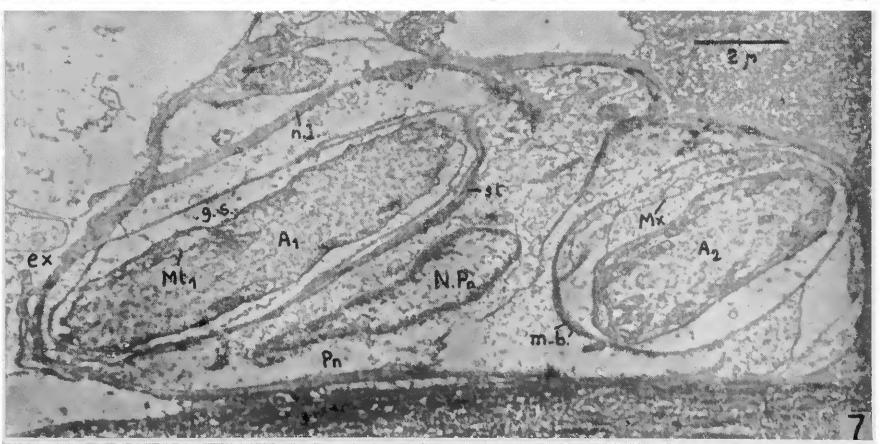
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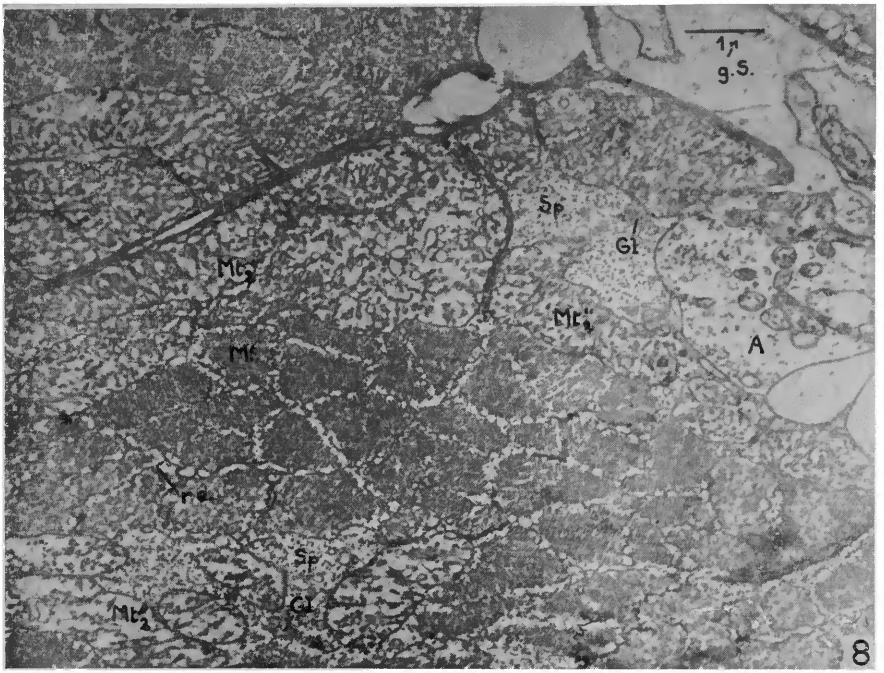
PLANCHE III

Fig. 8 — Coupe transversale d'une fibre musculaire passant par une zone de jonction myoneurale. Cette micrographie, effectuée à faible grossissement, résume les principaux caractères de la structure des fibres musculaires des maxillipèdes du Crabe Bleu: grande abondance de sarcoplasme (Sp); myofibrilles (Mf) groupées en colonnettes musculaires; mitochondries nombreuses et volumineuses, réparties en deux groupes, l'un (Mt_2') formant une couche continue sous la limite de la fibre musculaire, l'autre (Mt_2") situé près des faisceaux de myofibrilles; reticulum endoplasmique (r. e.) localisé entre les myofibrilles; nombreuses granulations de glycogène (Gl) par amas irréguliers dans tout l'ensemble du sarcoplasme.

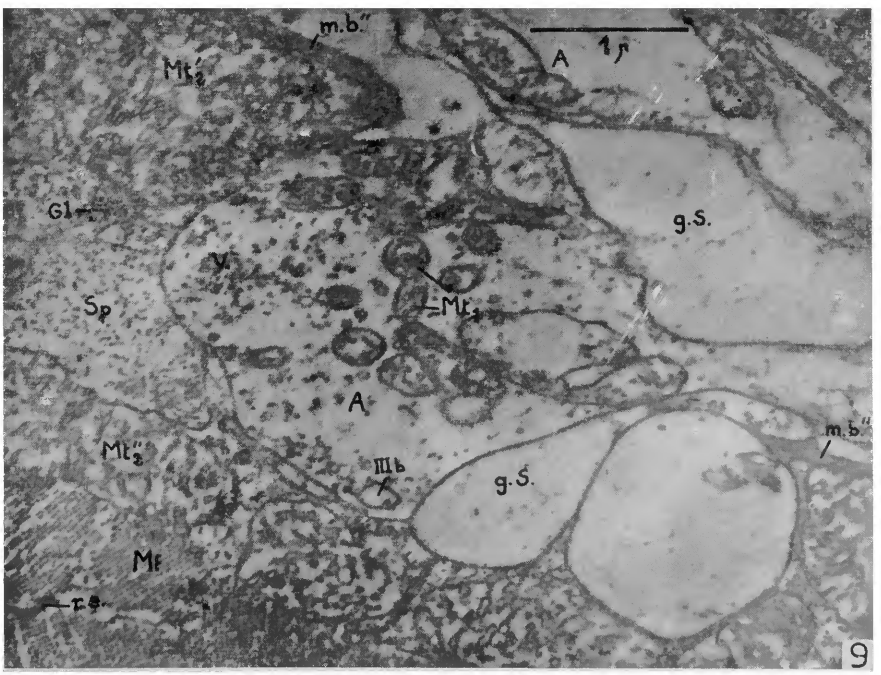
La zone synaptique est visible dans l'angle supérieur droit de la Fig.; elle est représentée par une dépression de la surface de la fibre musculaire dans laquelle est engagée l'extrêmité de la fibre nerveuse (A). La profondeur de cette dépression est inférieure à l'épaisser de la couche de sarcoplasme séparant les premières myofibrilles du sarcolemme. La majorité de la gaine de Schwann et toute la gaine périneuriale restent à l'extérieur de la dépression synaptique. La distribution des mitochondries du sarcoplasme n'est pas modifiée au niveau de la synapse de même que le reticulum n'y montre aucun développement particulier. (x 12000).

Fig. 9 — Micrographie électronique d'une jonction myoneurale sur une fibre musculaire en coupe transversale (même préparation que la Fig. 8). La membrane basale de la fibre musculaire (m. b.") ne participe pas à la constitution de la dépression synaptique; on remarque sa brusque interruption au bord de la zone où la surface de la fibre musculaire se déprime; seule subsiste la membrane sarcoplasmique qui forme le fond de la dépression. Quelques prolongements de la gaine de Schwann (g. S.) sont engagés dans la partie latérale de la dépression synaptique, entre l'axone et la fibre musculaire. Dans l'axoplasme terminal, les mitochondries sont plus nombreuses et ont tendance à se grouper au centre, mais les neurofilaments ne sont plus discernables. Les vésicules synaptiques (V.), distribuées par groupes irréguliers, sont absentes auprès de la juxtaposition de l'axoplasme avec les parties de la gaine de Schwann engagées dans la dépression synaptique.

— IIIb: cf. légendes de la Fig. 10; autres légendes comme précédemment. (x 25000).


1μ
g.s.
Sp
Gl
Mt'2
Mt''2
Mf
A
r.e.
Sp
Gl
Mt'2

8


m.b''
Mt'2
A
1μ
Gl
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V
Sp
Mt2
A
m.b''
Mt''2
III b
g.s.
Mf
r.e.

9

PLANCHE IV

Fig. 10 — Zone de jonction myoneurale sur une fibre musculaire en coupe radiale. La coupe est passée très latéralement dans la dépression synaptique, par un renfoncement situé sous les bords de la membrane basale (m. b.") de la fibre musculaire, de telle sorte que cette dernière semble ininterrompue au-dessus de la terminaison nerveuse; cette interprétation peut être rendue concrète à l'aide de la Fig. 9, en imaginant le passage d'une coupe radiale dans l'extrémité gauche de la dépression synaptique. En outre, les dimensions réduites de la zone synaptique de la Fig. 10 et l'importance du compartimentage de la partie axoplasmique par les invaginations de l'axoplemme confirment que seulement une portion réduite, parce que très latérale, de la dépression synaptique se trouve intéressée par la coupe et représentée sur la micrographie.

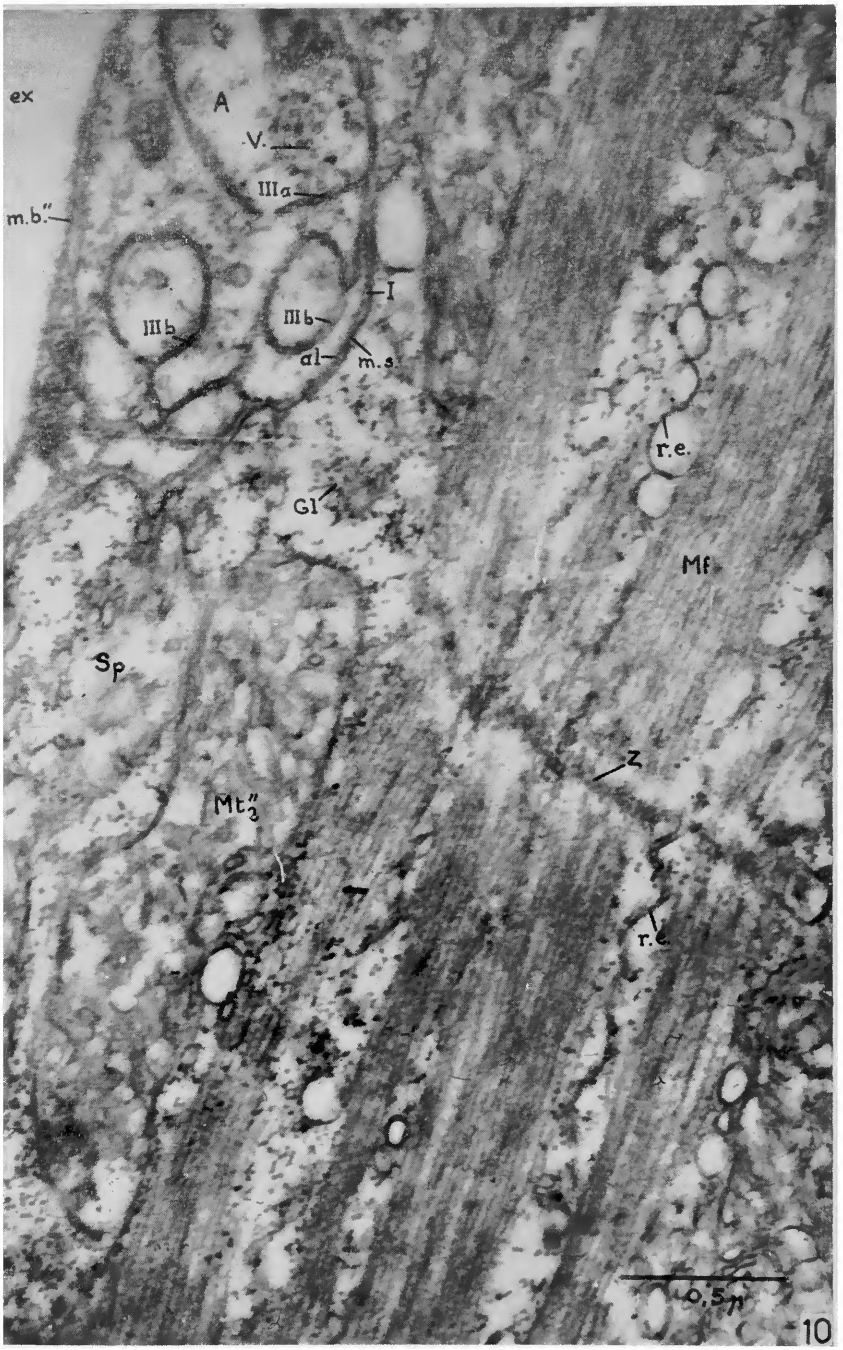
La juxtaposition des membranes plasmiques de la fibre musculaire et de la fibre nerveuse montre les dispositions suivantes:

— I = juxtaposition de l'axolemme (al) et de la membrane sarcoplasmique (m. s.) selon une surface régulière.

— IIIa = invagination de type simple de l'axolemme dans l'axoplasme.

— IIIb = invaginations de l'axolemme compliquées par des courbures, des angles, des bifurcations, déterminant un compartimentage de l'axoplasme terminal qui peut, sur coupe, isoler une fraction d'axoplasme, quando deux extrêmités d'une bifurcation se sont rejointes.

Aucune modification particulière du reticulum endoplasmique n'est perceptible, bien qu'une ligne Z de la striation transversale des myofibrilles soit en évidence à proximité de la synapse. (x 52000).



10

PLANCHE V

Fig. 11 — Micrographie électronique d'une zone de jonction myoneurale sur une fibre musculaire en coupe tangentielle. La dépression synaptique présente un contour elliptique, également visible sur la Fig. 13; entièrement comprise dans l'épaisse couche subsarcolemmale de sarcoplasme, elle est seulement entourée des mitochondries situées sous le sarcolemme; il n'y a pas de myofibrilles dans le champ de la micrographie. Cependant, la structure et les dimensions particulières des mitochondries du sarcoplasme et la présence des granulations de glycogène, permettent de reconnaître ce qui représente la fibre musculaire. Le contour de la dépression synaptique montre souvent des irrégularités de forme, recoupé par des cloisons incomplètes (Cl), ou même présentant à son voisinage comme des ébauches de ramifications de la terminaison nerveuse (A').

Les vésicules synaptiques sont distribuées inégalement par groupes d'importance variable; elles sont en général abondantes à la périphérie, à proximité de la juxtaposition de l'axolemme avec la membrane sarcoplasmique, le reste de l'axoplasme ne comportant que des vésicules isolées ou en petit nombre. En IIIb, une zone d'axoplasme délimitée par une invagination de l'axolemme présente une accumulation particulière de vésicules synaptiques. Aucune structure ne semble ici correspondre à des neurofilaments. (x 24000).

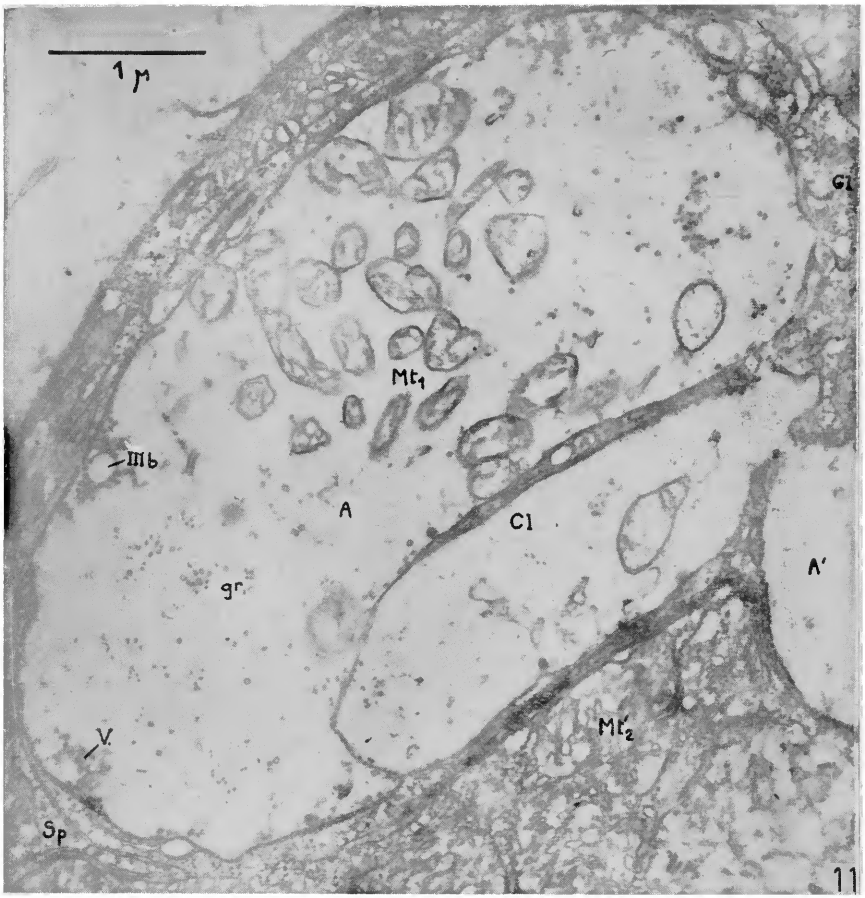
Fig. 12 — Agrandissement de la partie inférieure droite de la Fig. 11, montant la juxtaposition des membranes plasmiques de l'axone et de la fibre musculaire:

— I = juxtaposition des deux types de membrane plasmique selon une surface régulière.

— IIc = une expansion d'axoplasme qui ne comporte pas d'interposition de sarcoplasme; les deux membranes en vis-à-vis sont axolemmiques.

— IV = replis profonds et sinueux de la membrane sarcoplasmique vers le sarcoplasme; les deux membranes juxtaposées son sarcoplasmiques.

Dans l'axoplasme, les vésicules synaptiques offrent l'aspect habituel, avec une limite dense encerclant une zone centrale plus claire. A proximité d'un groupe de vésicules (flèche), les membranes plasmiqus juxtaposées apparaîssent plus denses. Légendes comme précédemment. (x 60000).


1 μ
Gl
Mt1
IIIb
A
Cl
A'
gr
Mt'2
V.
Sp
11

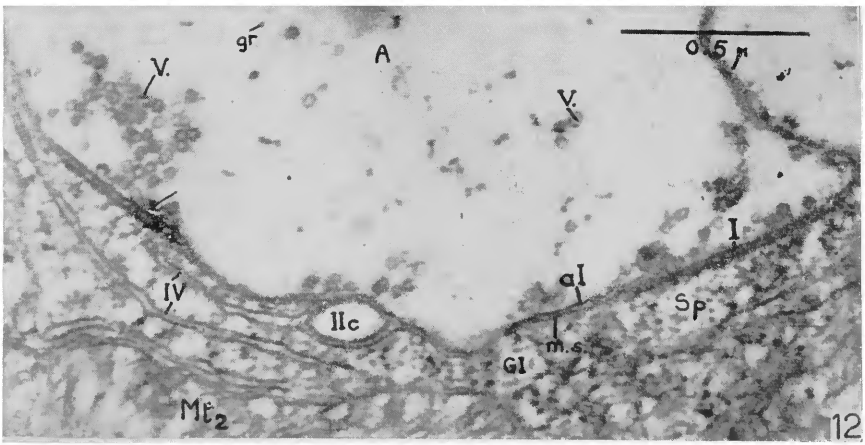

gr
A
0.5 μ
V.
V.
I
aI
IV
Sp
IIc
m.s.
Gl
Mt'2
12

PLANCHE VI

Fig. 13 — Micrographie électronique de deux terminaisons nerveuses au contact d'une même fibre musculaire coupée tangentiellement. Les deux dépressions synaptiques ont des dimensions inégales, ce qui peut provenir soit d'une différence dans les diamètres des deux fibres nerveuses A_1 et A_2, soit d'une différence dans le niveau du passage de la coupe à travers chaque dépression synaptique. La préparation comprend quelques myofibrilles (Mf) dans l'angle inférieur gauche; cela permet de confirmer que les grandes mitochondries (Mt_2), les granulations de glycogène (Gl) et les myofibrilles font partie d'une même organisation cellulaire correspondant à la fibre musculaire. Quelques prolongements de la gaine de Schwann (g. S.) sont insinués à certains endroits entre l'axone et la fibre musculaire.

Les mitochondries (Mt_1) de l'axoplasme terminal, de structure classique à lamelles, sont de formes très variées. Plus nombreuses que dans les nerfs juxtaterminaux, elles occupent tout le volume de l'axoplasme terminal ou peuvent même se grouper dans la partie centrale. La surface de coupe de la terminaison A_1 est couverte de structures très fines évoquant des neurofilaments de faible densité; elle comporte aussi les granules osmiophiles (gr) déjà vus dans l'axoplasme juxtaterminal. Les mitochondries du sarcoplasme (Mt_2) sont bien plus volumineuses que celles de l'axoplasme et présentent aussi une structure différente avec un réseau complexe de membranes à travers la matrice mitochondriale. Dans l'angle inférieur droit de la micrographie, un noyau du sarcoplasme (N. Sp) est visible avec des amas périphériques de cromatine et une double membrane percée de pores. (x 19500). Autres légendes comme précédemment.

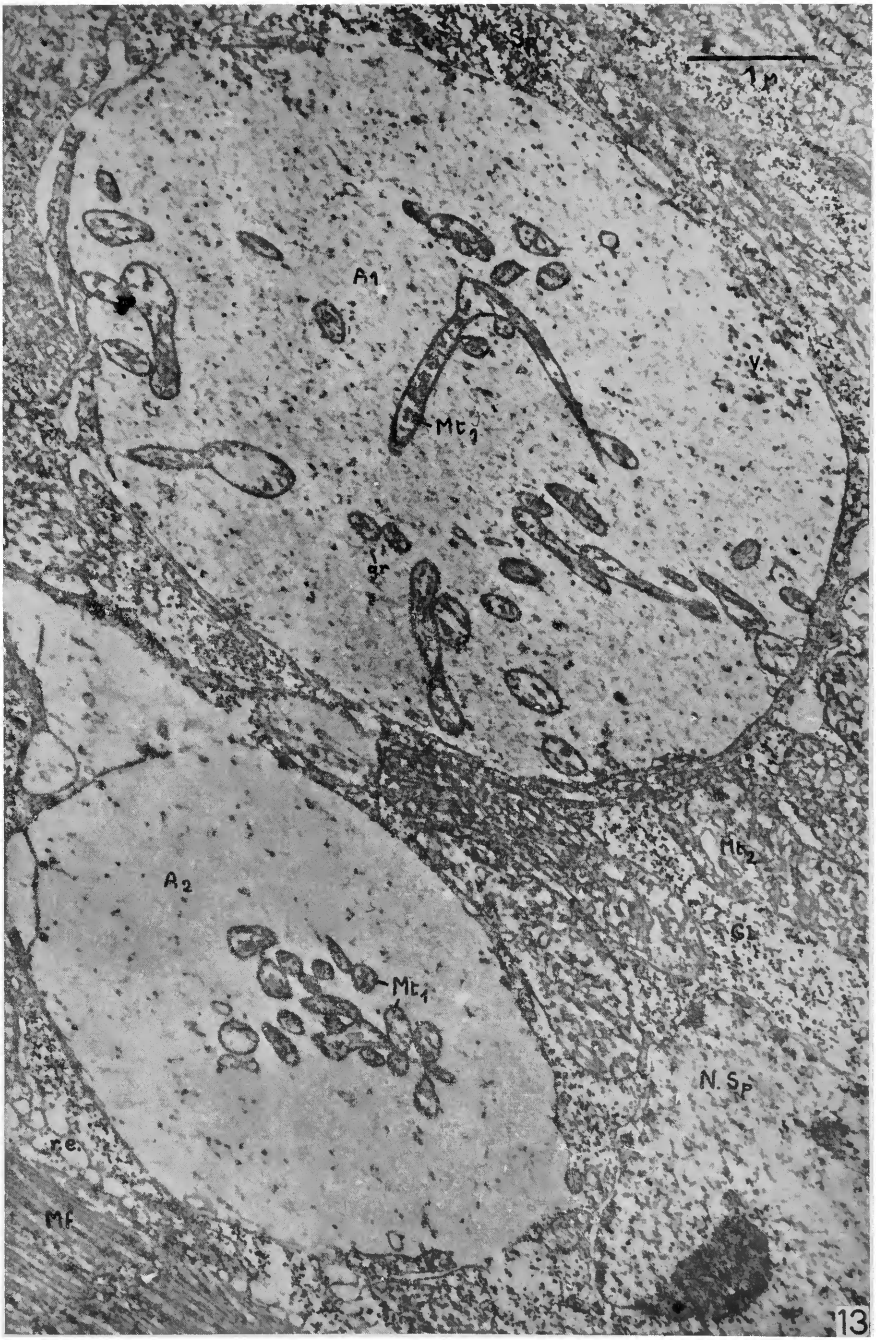
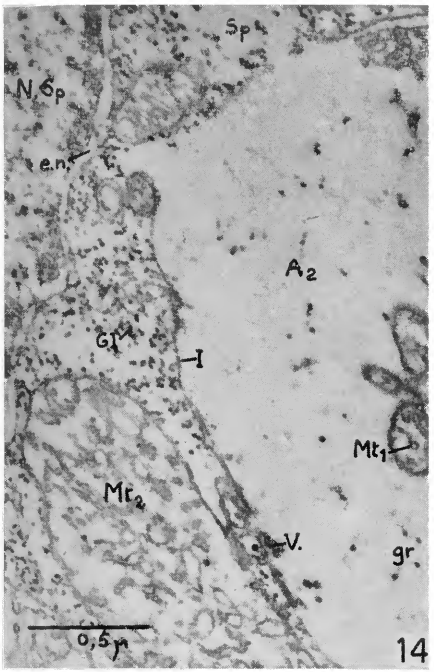

1 µ
A1
Mt1
gr
A2
Mt1
Mt2
Gl
N. Sp
re
Mf

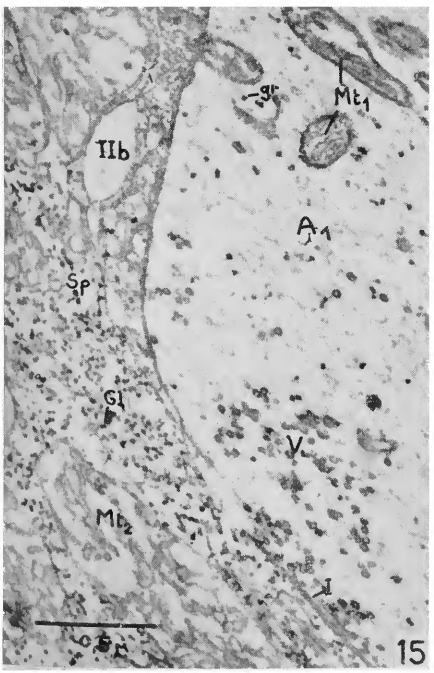
PLANCHE VII

Fig. 14 — Micrographie qui provient de l'agrandissement d'une région de la terminaison nerveuse A_2 de la Fig. 13. Elle présente en I une zone relativemente développée où la juxtaposition des membranes plasmiques de la synapse conserve des contours simples et réguliers. N. Sp = noyau du sarcoplasme avec l'espace périnucléaire (e. n.). (x 39000).

Fig. 15 — Agrandissement d'une partie de la terminaison A_1 de la Fig. 13 pour montrer en IIb une expansion de l'axoplasme qui, du fait de la coupe, semble complètement isolée au milieu du sarcoplasme. Dans l'axoplasme, des structures fines de nature inconnue, ayant peut-être des relations avec la disparition des neurofilaments, sont abondantes entre les vésicules synaptiques (V.) et les granules osmiophiles (gr). (x 39000).

Fig. 16 — Agrandissement provenant également de la terminaison A_1 de la Fig. 13. En IIa, une zone de sarcoplasme s'interpose entre la terminaison nerveuse et une expansion d'axoplasme. En IId, les contours irréguliers d'une expansion d'axoplasme paraissent, sur coupe, avoir complètement encerclé une zone de sarcoplasme. (x 43000). Les autres légendes comme précédemment.


Sp
N.6p
e.n.
A2
Gl
I
Mt1
Mt2
V.
gr
0,5 µ
14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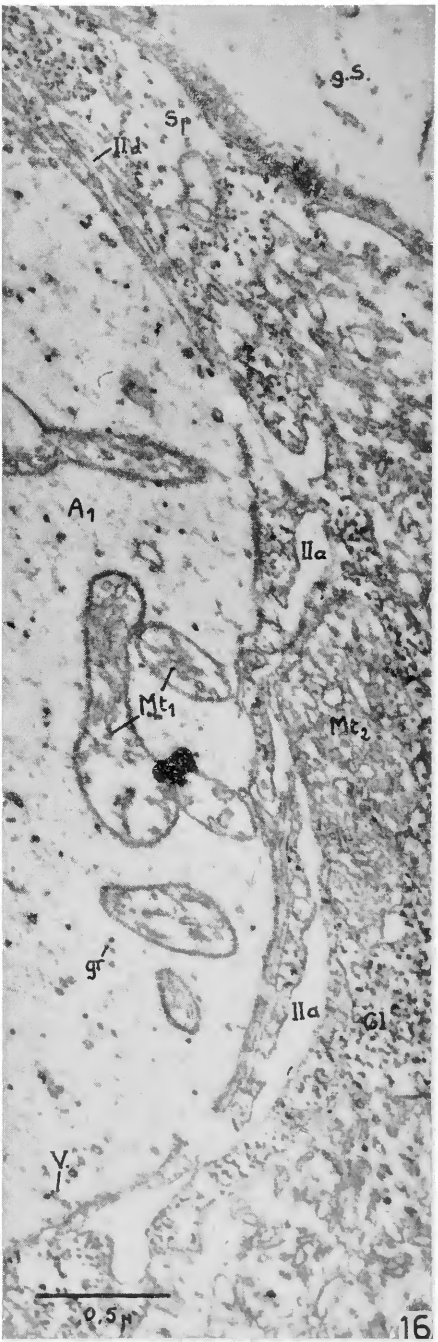
Mt1
IIb
A1
Sp
Gl
V
Mt2
I
0,5 µ
15


g.s.
Sp
IId
A1
IIa
Mt1
Mt2
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IIa
Gl
V
0.5 µ
16

ON SIPHONARIA HISPIDA

by EVELINE and ERNST MARCUS

(with 4 plates)

SYSTEMATIC NOTE

Hubendick (1946, p. 46) established the correct name for a species of *Siphonaria* which is evidently common on the Brazilian coast. Sure localities of this species are: Fernando de Noronha (Smith 1890, p. 497; Souza Lopes & Alvarenga 1955, p. 179); Bahia (ibid.; Hubendick, l. c.); Espírito Santo; State of Rio (Souza Lopes & Alvarenga); northern littoral of the State of São Paulo (present collection). To the last region refer also Ihering's (1915, p. 140) and Lange's (1949, p. 122) indications of *S. picta* d'Orbigny, so that it is probable that also these authors have seen *hispida*.

By its anatomy *Siphonaria hispida* Smith, 1890, is defined as belonging to the Subgenus *Siphonaria,* Section *Siphonaria* s. str. (Hubendick 1946, p. 44). Beside *S. alternata* (Say, 1826) it is the only Westatlantic species of this Section, whose numerous other species are Indo-Westpacific. This is a Tethyan distribution, as in many other cases interrupted on the African West coast and in the Mediterranean (Ekman 1935, p. 80 ff., 98-102).

OCCURRENCE AND LIFE

The locality where we found the species abundant, the Northern Base of the Oceanographic Institute, lies about 14 km west of Ubatuba (23° 27' S). The snails live on the granitic boulders resting on sand. The surf is seldom strong in front of the Station. The part west of the Station is very sheltered, and the inflow of terrigenous sediments is intense on rainy days. Here *Onchidella indolens* (Gould) is more frequent than *Siphonaria hispida*. Also *S. pectinata* (L.) occurs (G. L. & N. A. Voss 1955, p. 226) in Biscayne Bay, Florida, in an

area with little exchange of water. East of the Station where *S. hispida* is more numerous the snails live in the midlittoral zone (T. A. and A. Stephenson 1950, p. 372-376), and occupy higher and lower levels, to a certain degree comparable with the upper and lower yellow zones of the Florida Keys.

The accompanying sessile or semi-sessile fauna of *S. hispida* is constituted principally of barnacles. Oysters, limpets, and *Onchidella* are also numerous; littorinids occur higher up. Where the number of oysters increases, also that of their predator, *Thais haemastoma* (L.) does. On some boulders east of the Station *Siphonaria* is the widely preponderant element. *Drupa* (*Morula*) *nodulosa* (C. B. Ad.) of the Muricidae Purpurinae was observed preying on *Siphonaria*. It had drilled the shell, emptied the body cavity, and rasped a hole into the foot when it was disturbed.

At the examined locality of mixed tidal pattern the snails are about as long out of the water as covered. In the highest levels of the habitat big snails are frequent, while middle sized and small ones only occur farther down, as those of *S.* (*Benhamina*) *obliquata* (Borland 1950, p. 387). The mantle cavity is open, when the snail is at rest and surrounded by air, at least as long as the air on the rock is damp. We saw that snails taken out of the water open their pneumostome widely and take air into the pallial cavity. When they are immersed again, a bubble of air is expelled from the anterior, inhalant, part of the pallial opening, or, if they are lowered gradually into the water, one can see the water slowly running along the wall into the mantle cavity to substitute the air. When the lowest level of the tide coincides with a bright day, and the rock becomes dry and hot, the snails are so firmy apposed to the substratum, that we do not think they continue to breathe air during these hours.

In July 1960 we exposed some adult snails to the air in the laboratory. The dish was not exposed to the sun, and the temperature varied from 15 to 20°C. Adhering to a dry slide or fallen down and lying with the sole upwards the animals had their pneumostome constantly open and continued alive for 24 hours. Upon mechanical stimulus they closed the inhalant aperture with the anal lobe, but opened it again after some seconds. On the boulders the uppermost

snails may perhaps exceptionally be uncovered for a so long time during neap-tides combined with strong wind blowing offshore. When the animals in our experience were returned to the water they released two or three big bubbles of air and voided their excrements. As long as they were exposed to the air they did not move away or defecate.

Parts of the body most exposed to desiccation are mantle skirt, head, and upper surface of pedal border, because the edge of the shell does not fit quite tightly into all the unevennesses of the rock surface. The secretion of the big glands (g), well figured by Fretter and Graham (1954, f. 8), may protect these zones against drying out. The proportionally larger surface of a small snail loses much more water than that of a big one, and probably therefore the small snails are restricted to lower levels.

The animals crawl and feed while they are covered by water and also out of it, as long as the rock is wet. Uncovered *Onchidella indolens* is active longer than *Siphonaria hispida;* but the pulmonate remains uncovered for a longer time.

After browsing *S. hispida* generally returns to the place where it has settled, like *S. alternata, S.* (*Benhamina*) *obliquata,* and others (Hubendick 1947, p. 63; Borland 1950, p. 388). This is evidenced by the exact conformity between the outline of the shell with each of its peripherally projecting ribs and the contour left on the boulder. This was already observed by Boettger (1933, p. 343: also references to *Patella* and *Crepidula*). On an empty oyster shell with green algae in a dish a snail cleans its place in few hours by feeding. Uncovered the animals adhere as firmly to the rock as resting snails under water; true limpets cling much faster. Snails that fall upside down try to right themselves bending their head downwards.

The egg strings, similar to those of *Amphibola crenata* (Farnie 1924, f. 1), were found all the year round. They are 2 cm long, 4 mm thick, curved (Fig. 1), often forming a complete circle, and contain about 2.000 egg capsules with one egg each. In transverse section the string is as high as broad; it is attached to the rock with a plane underside (Fig. 2). The strings are fastened to the bare boulder, not in water-filled concavities ("cuvettes") as does *S. atra* (Risbec 1935, p. 414). At first the strings are colourless with ivory

eggs, after some days they become yellow and later on greenish by growing algae. The eggs are poor in yolk. The embryo has a big, bilobed velum (Fig. 3, ve), more prominent than that of *Amphibola crenata,* and a large transparent operculum (ou) which stands out over the foot as in *S. atra* (Risbec, l. c.).

The embryonic shell (s) is yellow and similar to that of *Otina* (Morton 1955a, f. 12), in that the first half whorl is at right angles to the rest of the apical bulb which has a dextral direction. Reduced heterostrophy occurs in several archaeopulmonates (Harry 1951, p. 13). As a character not subjected to adaptive influence this reduced heterostrophy, according to Morton (1955b, p. 151, 161), indicates a phyletic relation between basal cephalaspids and pulmonates. The larval shell of *S. hispida* has not quite one and a half whorls. In the hatching veliger the shell is 300-340 μ long, 200 μ broad. Embryonic malformations are frequent in the egg strings, especially unrolled shells, similar to those that Risbec described (1935, p. 414) and figured.

The hatching stage is ambivalent swimming-crawling, but while swimming it remains near the bottom of the dish. The eyes and the foot grow gradually; the foot is at first developed posteriorly, and its fore end enlarges later. The velum decreases, but is still ciliated on the third day of free life. On the fourth day, the last of our metamorphosing animal's life, the velum was reduced to unciliated knobs; the eyes were big; the foot was prominent in front and behind, and was the only organ of locomotion. However, movement was not accomplished in the definitive manner of even gliding, but by jerks, the stretched anterior border of the foot fixed itself and drew the body with the larval shell forward. The operculum was still present. Free-swimming veligers were described for *S.* (*Benhamina*) *obliquata* (Borland 1950, p. 391).

Like in most species of *Siphonaria* (Hubendick 1947, p. 63-64) the larva of *S. hispida* is not pelagic. Nevertheless it can be a means for gradual dispersal of the species. As many gastropod larvae can postpone their final settlement (Morton 1958, p. 143), even broad barriers, as sandy or muddy shores, can occasionally be overcome. For example the jetties on the coast of Texas were colonized in the last 50 years by *S.* (*Patellopsis*) *pectinata* (Hedgpeth 1953, p. 189).

SHELL

The shell is, in Hubendick's terms (1946, p. 7), small (under 15 mm) to medium large (15-30 mm), and low to medium high, that is, higher than one fourth of the length but not more than half the length. Among 500 of our shells the length varies from 2 (youngest found snails) to 20 mm. The last shell is 17 mm broad, so that its ratio of length to breadth is 1,25:1. This ratio varies from 1,06 to 1,50:1.

In our youngest, 2 mm long shells the apex occupies a distinctly posterior position. By later growth of the posterior ribs of the shell (Fig. 4) it is removed forward. Also in adult shells (Fig. 5) the apex lies a little to the rear. The upper side of the shell has 9-20 dominant white ribs, one or two over the siphon. Between the dominant ribs there are smaller ones or only a radial striation. The interspaces between the ribs are dark brown or black. Generally the dominant ribs project over the margin. The circumference is irregular, especially on the right side and behind. The colour of the underside is dark brown is living snails; the furrows corresponding to the ribs are white. The smooth centre occupies from one sixth to one half of the shell breadth, and its colour varies from brown over yellow and grey to greenish.

The narrow outermost part of the apex is bent backwards (Fig. 5). On its under surface the protoconch is exceptionally preserved.

The upper side of the shells, even of quite young ones, is densely covered with a felt of algae and sometimes fungi. On the larger shells there are often tufts of *Enteromorpha* or other leaf-shaped algae, small barnacles, oysters, and mussels.

EXTERNAL CHARACTERS

Corresponding to the dark furrows between the light ribs of the shell (Fig. 6, 7, s) the mantle skirt (ms) is pigmented with black. Round, frequently confluent black spots occur on the head and the upper surface of the foot. Two folds (ce) which begin on the sides of the head unite over the mouth (mo). Their anterior border is richly innervated and bears sensory cells; laterally the folds contain the subepithelial eyes. These oral lobes (Simroth 1909, p. 89) or

sensory head lobes (Borland 1950, p. 387) constitute what is called a frontal veil in opisthobranchs. The conspicuous cephalic lobes of *Trimusculus* (*Gadinia*) (Schumann 1911, p. 4) and the small tentacles of *Chilina* (Haeckel 1911, p. 95) correspond to the cephalic folds of *Siphonaria*.

Hidden in the deep furrow between head and foot (f) lies a pair of small white folds (Fig. 6, x). Their epithelium contains sensory cells and is underlain by nerve fibres, but these were not found converging to a common nerve, as such are distinct in the cephalic lobes and under the sensory epithelium of the anterior border of the foot. Topographically the much bigger labial palps of *Chilina* (Haeckel 1911, p. 94-95) can be compared with these sense organs of *Siphonaria hispida,* which are also very similar to those of *Philine* (Brown 1934, f. 5, 21, pa).

In many of the narcotized snails the walls of the buccal tube are everted, so that the unpaired jaw (Fig. 8, j) appears over the vertical mouth slit. Between head and mantle skirt is a deep furrow which ends a certain distance in front of the shell adductor. The latter's fore ends are united by muscles which irradiate into head and mantle skirt. Also dorsally to the respiratory opening muscle fibres bridge over this gap of the adductor. The pluricellular glands (Fig. 11, g) whose secretion may be repugnatorial (Fretter and Graham 1954, p. 578) or protective against desiccation are numerous on the front of the head, the upper surface of the foot, and the underside of the anal lobe (az). Unicellular, deeply insunk mucus glands are scattered over the sole, more numerous in front, but not concentrated.

PALLIAL ORGANS

Hubendick's proportion between width of pallial opening and body length (1947, p. 38, 88) was not found in *S. hispida*. In adult 14,5-15,5 mm long snails, preserved and removed from the shell, the width of this opening is 20%-39%. Nearly the same extremes, 22%-40%, occur in 4,5 and 5 mm long animals. As in other limpet-like gastropods the pneumostome is rich in glands (Graham in Morton 1955a, p. 116). The anal lobe (Köhler 1893, p. 3), inferior pallial or respiratory lobe (Pelseneer 1894, p. 73, 81), or siphon

(Hubendick 1947; Yonge 1952), is actually a modified anal papilla, provided with longitudinal muscles. It is tripartite as is a similar cutaneous appendage of *Viviparus* and *Planorbis* (Simroth 1876, pl. 20, f. 20-22, b). The pallial walls are more or less pigmented, except over the gill, where the roof is white. In a 15 mm long and 11 mm broad snail the pallial cavity is 7 mm long. Backwards it does not attain the adductor.

Along the posterior margin of the gill runs the dorsal ciliated ridge, and below this, on the floor of the pallial cavity, the higher ventral ridge (ci). These organs were well described by Köhler (1893, p. 5, 55), whose figures 38 and 39 show their continuity on the left side behind the pericardium.

In his postscript (p. 84) Köhler homologized them with the ciliated ridges in the Cephalaspidea. Among the opisthobranchs they are also known from the Pyramidellidae (Fretter and Graham 1949, p. 499) and young Anaspidea (E. and E. Marcus 1957, p. 11), among the pulmonates from *Chilina, Latia,* and *Amphibola* (Haeckel 1911, p. 100-101). Perrier and Fischer (1909) called the ridges "raphé supérieur" and "raphé inférieur". They understood (1910), as already Pelseneer (1894, p. 14) had, the respiratory function of these ridges. In *Siphonaria hispida* their cilia beat towards the exhalant opening (ne) of the siphon and so draw a compensating inhalant flow between the branchial lamellae. The cilia of the gill are too sparce in *Siphonaria* to have much effect on the flow of the water (Yonge 1952, p. 194). It is of high morphological interest to see how the pallial cavity diminishes inwards, where the ridges pass into one another. Here its wall even forms a short diverticulum, a very small pallial caecum, fixed with a bundle of the adductor muscle. Also *Chilina* and *Latia* have a caecum. As in *Acteon* (Fretter and Graham 1954, p. 568) a blood space (Fig. 10, am), partly with own walls, lies between the mantle (ia) and the dorsal ridge of *Siphonaria*.

The rejection of sediment carried into the pallial cavity with the respiratory current was described by Yonge (1952, p. 195 ff.). It does not work very efficiently at the place where we found our snails. In the shallow bay in front of the boulders the bottom is sand and mud, so that even the generally light surf carries sediments at

every flood tide. Not all, but many of our dissected specimens had a surprising amount of sand grains within their pallial cavity. Possibly they can get rid of them from time to time by muscular action as *Patella* does (Yonge 1947, p. 471).

The osphradium curves around the hind surface of the right anterior pillar of the adductor. While it is furrowed longitudinally in the species examined by Hubendick (1947, p. 192), it is even in *S. hispida*. The histological limit between middle and sides is sharp. The columnar cells in the middle are unciliate and contain brown pigment. To the sides the cells are ciliate, but the cilia are low, shorter than in Hubendick's figure 107. The sensory cells of the two lateral bands are connected with the osphradial ganglion by bundles of fibres with nerve cells between them.

The kidney (k) belongs to Hubendick's type c (1947, p. 35). The nephropore (rv) goes out from the outer part of the dorsal lobe, the reno-pericardial duct from the inner. Covering the surface of the pericardium (r) the ventral lobe of the kidney extends onto the floor of the mantle cavity. The ventral portion is smaller than the dorsal one, which is prolonged anteriorly beyond the efferent vein (ev) of the right part of the gill (b). This vein receives also vessels (vr) from the anterior part of the roof of the mantle cavity. This vascular net is generally called pulmonary (Hubendick 1947, p. 95), in the presupposition that the snails are air-breathing during low tide, e. g. Hutton (1882, p. 342-343); Pelseneer (1894, p. 81; 1895); Plate (1894, quoted from Simroth and Hoffmann 1927, p. 983); Cooke (1895, p. 151); and Cottrell (1911, p. 593).

Yonge (1952, p. 198; 1958, p. 36) thought that aerial respiration is improbable in *Siphonaria alternata*. In *S. hispida,* however, we observed, as mentioned above, the intake of air into the mantle cavity, when the snails are out of the water. In this species the pallial cavity has the same dual function of a lung and of a branchial chamber that was verified by Yonge (1958, p. 35) in *Trimusculus reticulatus* (Sowerby). Our species lives higher on the shore than *T. reticulatus* which occurs only if well protected in a damp atmosphere.

In a without shell 2,2 mm long *Siphonaria* (*Kerguelenia*) *lateralis* Köhler (1893, p. 29) found the left part of the gill incom-

pletely developed with no efferent branchio-renal vessel. The adult snails of this species are 16 mm long. An 1,8 mm long animal of *S.* (*S.*) *hispida* had this vessel and a gill (b) whose left part was only less folded than the right. In large snails right and left branchial portion have equal structure and correspond to the plicate type of tectibranchs. Whether such branchiae can be derived from pectinate ctenidia with filaments is an open question. Köhler (1893) and Gilchrist (1894) whose views are reported by Hoffmann (1940, p. 14-16) and Hubendick (p. 120) consider plicate and pectinate gills as modifications of a common primordium; Yonge (1947, p. 498; 1952, p. 197-198) stresses the differences.

The afferent renal vessel of the tectibranchs originates from the afferent branchial vessel. Topographically it corresponds to the anterior afferent renal vessl (ra) in *Siphonaria*. The posterior afferent renal vessel (or) of *Siphonaria* has no counterpart in tectibranchs. The efferent renal vessel of tectibranchs opens into the auricle together with the left or inner efferent branchial vessel; in *Siphonaria* it (nv) opens farther to the right into the efferent branchial vessel (vz). Therefore Hubendick considers the left part of the gill of *Siphonaria*, from the anterior afferent renal vessel inwards, as secondary and not comparable to the gill of tectibranchs. But as the entire gill of *Siphonaria* has one and the same structure and only shows slight differences in its development, we think it is homologous with the cephalaspidean gill and compare the two efferent branchial vessels (ev, vz) with the corresponding ones of the Cephalaspidea.

As Köhler observed (1893, p. 77) a muscle (mx) extends from the auricle (rc) into the principal branchial vein (ev). Hubendick (1947, p. 41, 95, 110) found one or two such muscles in *Siphonaria, Williamia,* and *Amphibola,* not in *Gadinia* and *Chilina*. Köhler's phylogenetic interpretation of this muscle was not accepted by Hubendick (p. 170). Possibly the heart is distended by the contraction of this muscle and circulation accelerated by its action.

CENTRAL NERVOUS SYSTEM (Fig. 13)

The cerebral ganglia (cn) lie ventrally to the oesophagus and are united by a long commissure which runs dorsally over the oesophagus. There is no subcerebral commissure. Hubendick (1947, p. 101) was

the first to find the lateral lobes (Fig. 12, xo) in sections of *Siphonaria*. They are more lateral than in *Trimusculus* (*Gadinia*) (Schumann 1911, p. 53-54). In young snails of *S. hispida* the cerebral tubes (t) which originate the lateral lobes of the cerebral ganglia are connected with the epidermis. On the right side the cerebral tube begins at the genital aperture (Fig. 21). These primordia pass through the thick body musculature as epithelial tubes (Fig. 12, t). They begin with a diameter of 20 μ, diminish abruptly to 6 μ half way through the muscular wall, and pass into the cavity of the head, where they run backwards to the brain. Here the tube becomes thicker and solid by increase of the size of its cells. A lumen between these cells was not seen, even in our smallest specimens, and in full grown snails also the cerebral tubes could no longer be distinguished, contrary to *Trimusculus peruvianus* and *garnoti*. The membrane of the cerebral capsule is interrupted between lobe and ganglion by several tracts of nerve fibres.

The buccal ganglia lie on the pharynx under the beginning of the oesophagus, and correspondingly the cerebro-buccal connectives are rather long. The length of the buccal commissure approximately equals the diameter of one of the ganglia.

In correlation with the importance of the foot as fastening organ the pedal ganglia (q) are voluminous, as big as the cerebral ones. The pedal ganglia lie behind the cerebral ganglia and a little farther ventral. The cerebro-pedal connectives are short but distinct. The statocysts (so) lie under the lateral surface of the pedal ganglia. There are two pedal connexions, the thick anterior pedal commissure and the thin posterior, still longer parapedal one. The genital nerve (na) goes out from the right pedal ganglion, accompanies the cerebro-pedal connective and becomes gradually independent from it. Perhaps therefore Dieuzeide (1935, quoted from Hubendick 1947, p. 91) indicated a cerebral innervation of the genital atrium, while Köhler (1893, p. 20) observed the origin of the genital nerve (f. 9, ng) correctly.

A big ganglion (wu) constitutes the root of the visceral loop on the left side. It is connected with the cerebral and the pedal ganglion by short connectives. As in the species examined by Hubendick (1947, p. 117) three nerves go out from this ganglion, two anterior ones to the left, and one which bifurcates soon after its origin, to the adductor.

One of the anterior nerves supplies the mantle skirt. By its nerves this ganglion is characterized as pleuro-parietal ganglion.

The corresponding right ganglion (eu) is small, simple, and without nerves. It is apposed to the right cerebral ganglion, while its connective to the pedal ganglion is distinct. It is a pleural ganglion. Though always much smaller than the left pleuro-parietal ganglion its size varies individually. In some snails there are only some nerve cells corresponding to the right pleural ganglion at the root of the pleuro-pedal connective. Cottrell's figure of the central nervous system of *S.* (*Benhamina*) *obliquata* (1911, f. 5) also shows two ganglia on the right side, but the anterior (proximal) is bigger than the posterior (distal) one.

Köhler (1893) and Hubendick (1947) found ganglia of equal quality on both sides of the visceral loop; Köhler called them pleuro-intestinal, Hubendick pleuro-parietal ganglia. As there are two separate ganglia on the right root in *S. hispida,* the name of the posterior one will in the following be proposed in conformity with its nerves and with the nature of the neighbouring ganglia.

We agree with Hubendick (1947, p. 115-116) who does not consider Köhler's qualification "abdominal" ganglion as sufficient for the single ganglion (as) of the visceral loop in *Siphonaria*. Its at least four nerves supply not only the ental (proximal) reproductive organs but also adductor, kidney, pericardium, siphon, and rectum. Hence this ganglion must be understood as a coalesced abdominal-subintestinal ganglion. As in the other species of the *Siphonaria* (*Siphonaria*) *sipho*-group (Hubendick 1947, p. 40) this ganglion lies nearly straight to the right of the nerve ring. A short connective connects it with the next right ganglion (zr).

This is a big ganglion and emits two nerves. One of them runs to the right body wall. The other supplies the border of the mantle and the osphradium. The osphradial branch is sometimes so short that the osphradial ganglion (zo) is almost continuous with the central ganglion. According to Hubendick's point of view this ganglion should be called right parietal ganglion. Hubendick (1947, f. 106) adopts the zygosis theory (Krull 1934) as best explanation for the phyletic origin of the central nervous system in the Pulmonata. This theory postulates a loss of the supra-intestinal portion of the visceral loop

including the ganglion. Consequently the innervation of the osphradium would have passed from the supra-intestinal to the parietal ganglion, or the osphradium of these "basically primitive" (Morton 1955b, p. 163) pulmonata must be considered as a novelty, heterologous with that of the Cephalaspidea. A discussion of the theories concerned with the evolution of the central nervous system in the Pulmonata lies beyond the limits of the present paper. In *Siphonaria* we suppose a dislocation of the left-sided osphradium of *Acteon* towards the right as in *Chilina*. Therefore we call the centre in question "parieto-supra-intestinal" ganglion, as it was named in some species of *Chilina* (Haeckel 1911, p. 127, f. 38, 46).

ALIMENTARY TRACT (Figs. 16, 17)

The radula (Figs. 14, 15) was examined in 25 snails. It comprises about 120 rows of 18-34.1.18-34 teeth. The rows are procoelous, i. e. concave in front. The minimum, 18 lateral teeth, refers to a 3 mm long animal. An outer denticle (ectocone) may appear already on the first lateral tooth, or on one of the following, once even on the 8th, independently from the size of the snails. Malformations generally extend over an entire longitudinal row, e. g. coalescence of 2 neighbouring teeth. On the whole the radula is rather multiform. The rhachidian tooth is narrow and bears a single cusp. The inner lateral teeth have a bicuspidate edge and the outer denticle. From about the 11th tooth outwards an inner denticle (entocone) appears, and the two principal cusps (mesocones) coalesce to a rectangular plate. In the lateral region of the radula there are two outer denticles. The outermost (marginal) teeth are low and simplified. The various regions of the radula pass gradually from one type of teeth to the next.

Alive the pharynx is dark red, perhaps due to haemoglobin, as frequently pharyngeal and radular muscles are, which need much oxygen (Pelseneer 1935, p. 156; Ankel 1936, p. 124).

The flattened salivary glands are composed of branched white tubes with a narrow lumen joined to a wider duct which opens dorso-laterally into the pharyngeal cavity. In this region the dorsal wall ot the alimentary channel assumes its folded oesophageal character. A little farther behind the brown oesophagus is already closed, and also

its ventral wall is folded. Here the buccal ganglia lie between the floor of the oesophagus and the pharyngeal bulb. Clusters of blue staining gland cells open into the ventral wall of the anterior oesophagus. The median buccal gland of *Otina otis* (Morton 1955a, p. 122, f. 5A, m bg) lies dorsal and does not open into the oesophagus, so that it is not comparable with the present structure.

The oesophagus (o) goes out from the dorsal side of the pharyngeal cavity near the middle of its length, hence relatively far in front. Its low epithelium and slight longitudinal folds become gradually taller and stronger (eo) backwards, where blue staining glands open on the crest of the folds. The epithelial cells contain brown pigment. Already in the oesophagus the algal particles scraped off from the rock by the radula are pasted together to a mucous food string (os). When stuffed with food, nearly the entire gut, viz. oesophagus behind nerve ring, stomach (sm), intestine (i), and rectum (re) are distended to smooth-walled sacs.

As in cephalaspids and pulmonates (Graham 1949, p. 755; Fretter and Graham 1954, p. 582) oesophagus and intestinal gastric apertures are approached to one another (Fig. 16). A tubular, not saccate, stomach, whose oesophageal and intestinal apertures lie on opposite ends (Hubendick 1947, f. 67, 68), is only obtained by wrenching off the hepatic appendages. There are 3 lobulate anterior digestive diverticula, two dorsal (l) and one ventral (ni). They communicate with the stomach by the corresponding dorsal (aa) and ventral (av) openings. As the liver ducts branch immediately at their roots, they appear to have subsidiary apertures as *Patella vulgata* (Graham, 1949, f. 23).

A broad fold (vm) connects the anterior ventral liver aperture with the opening (ao) of the posterior digestive diverticulum (um). The position of the posterior aperture in the middle of the gastric fundus (or apex) is a primitive character (Morton 1955b f. 3A, 3B). The fold is continuous with the major typhlosole (y) and is a landmark in the ventro-median line of the stomach. It approximately separates a ciliated right from a cuticulate left (cu) zone of the stomach.

The pigmented epithelium and the high folds (eo) of the posterior oesophagus end abruptly at the entrance into the stomach. The

ciliated part receives the food string (os), and here the latter forms a compact mass (oo) in living and preserved snails. Thence the string is continued backwards, curves to the left, and is drawn forward again into the intestine by the protostyle (ro).

The fundus of the stomach is coated with a silky transverse muscle layer and fewer longitudinal fibres. By the contraction of these muscles a terminal pocket (mu) may be jutted out which disappears when the muscles relax. This pocket is comparable to the "gizzard of the simplest form" in *Otina otis* (Morton 1955a, p. 126). The apical musculature is continuous with that of the cuticularized area (cu). This rather strong development of gastric musculature is a pulmonate feature.

Dense folds occur around the apertures of the anterior digestive diverticula and enter them. About 8 short longitudinal folds (sa) converge on the quite narrow intestinal groove (io) which is bounded by the typhlosoles (y, in). The folds are densely ciliated and constitute a posterior sorting area. Beside the groove lies the "style sac", a longitudinally folded zone lodging the protostyle (ro). The latter is continuous with the food string (os) as well as with the faecal rod (er) in living and preserved snails.

Cilia and peristalsis carry the faecal rod through the intestine (i). The two intestinal loops correspond to the type known in the subgenera *Siphonaria* (Hubendick 1947, f. 66) and *Patellopsis* (Köhler 1893, f. A). The cephalic artery (wa) whose wall contains black pigment passes through the first loop (Fig. 17). The rectum is coated with thick annular muscles. Their action discharges long faecal strings. As Yonge (1952, p. 196) observed in *Siphonaria alternata,* the anus opens and closes frequently. This activity is independent from releasing and nipping off a faecal pellet.

In sections of a 5 mm long snail we found a radula with incompletely digested musculature in the region where the protostyle passes into the faecal rod. The radula may have been that of a young *S. hispida*.

Young snails as well as hungry ones are darker than those well nourished.

REPRODUCTIVE ORGANS

The generative organs (Fig. 18) of an adult snail with full-grown ovocytes are characterized by an orange gonad (wn) and light orange glands (we, me) of the spermoviduct. The ovocytes are 150 μ in diameter, their nuclei 60 μ. The small ovotestis lies under the stomach between the digestive diverticula in the body cavity. The species is protandrous, but male and female phases are not separated. Spermatogenesis is never completely interrupted. Egg laying is followed by more intense production of sperm. The snails evidently live for more than one year, as Borland (1950) observed in *S. obliquata*. In the lobules of the hermaphrodite gland the male germ cells are central, the female ones peripheral.

The slightly pigmented ciliate hermaphrodite duct (h) is either principally straight or more coiled. Ectally it is somewhat dilated into a longish ampulla containing sperm. The so-called seminal vesicle distally to this ampulla might function as seminal receptacle (rs) (Simroth 1912, p. 491) and fertilization chamber.

Thence the spermoviduct becomes glandular, much convoluted, and sacculate. Its inner course is called albumen gland (we), the outer one mucus gland (me). In the latter a spermoviducal fold begins which separates a ciliate seminal and a glandular oviducal groove. Ectally to the mucus gland portion the spermoviduct (vi) rises and runs quite near the floor of the mantle cavity. Its fold disappears gradually and is substituted by longitudinal folds of a ciliate epithelium. The wall of the duct is strongly muscular and it passes through the right anterior pillar of the adductor. Before the duct enters the common atrium, it forms a dorsal glandular pouch (is). The spermoviduct (vi) opens inside the genital aperture (z) and at a certain distance from it.

Immediately beside the spermoviducal opening lies that of the bursa whose long duct (ur) is parallel to the spermoviduct. As in most species of the section *Siphonaria* (s. str.) it runs through the adductor near its outer edge (Hubendick's figure 63b). The retractor (e) of the bursal canal is contracted in some of our specimens, relaxed in others. Correspondingly the duct is distended into a loop close to the epiphallus (d) or not. Hence an extension of the bursal canal in front

of the epiphallus seems not to be a systematic character (Hubendick 1947, p. 30).

Farther inwards than bursa and spermoviduct the epiphallus duct (d) and the male copulatory organ (p), Hubendick's "muskulöse Scheide", enter the common genital outlet. The epiphallus duct becomes gradually thinner in its ental portion and ends with the flagellum (ae). Conspicuous glandular sacculations of the duct, the epiphallus gland (ze) or prostate, are in many species known to secrete the spermatophores. We did however not find any spermatophore in more than 50 mature animals examined in all seasons.

The copulatory organ (p), also provided with a retractor (e), is 1,05 to 0,77 mm in transverse sections of the invaginated phase and 2,5 mm long. The corresponding measurements of evaginated stages are 3,0 to 1,4 mm, and 7 mm. The organ is distended by liquid of the body cavity, and therewith its size increases enormously (Fig. 19). Its outward movement turns the entire common atrium inside out (Fig. 20). Epiphallic (ma), spermoviducal (ea) and bursal (ua) openings are separated in this condition; the epiphallus duct debouches at the root of the evaginated penis.

In one specimen Hubendick (1947, p. 9) observed a bipartite penis of *S. elegans*. This case may be explainable as an incomplete evagination.

DEVELOPMENTAL STAGES OF REPRODUCTIVE ORGANS

In without shell 1,8 mm long snails, the smallest we found, a 1,03 mm long common primordium of genital organs (Fig. 21) extends uninterrupted from the single aperture (z) inwards. The copulatory organ is not developed yet, nor the bursa canal. The epiphallus gland (ze) is already indicated by a thickening with a fold of high epithelium on either side of the lumen. Contrary to what Hubendick (1947, p. 32) expected, the development of the epiphallus duct precedes that of the bursa. The tubular spermoviduct (vi) is continuous with the incipient thickening of the glandular duct (w). The latter is connected with the ovotestis (wm) by the strand of the future hermaphrodite duct (h).

In the next, without shell 2 mm long snails a thin primordium of the bursa canal (Fig. 22, ur) appears. With a body length of 2,7 mm

the genital primordium from aperture to gonad is comparable with Köhler's developmental stage of a 2,2 mm long specimen (1893, p. 34, fig. D). Hubendick's stage (1947, f. 83) is more advanced. Köhler figured the opening of the epiphallus and the distal end of the spermoviduct not connected, but said (p. 33) that this separation could not be verified with certainty. As in his species *S.* (*Kerguelenia*) *lateralis* the spermoviduct of the adult opens into the outermost part of the common atrium (Hubendick 1947, f. 15), one can hardly infer from Köhler's single specimen that epiphallus and spermoviduct develop separately.

In a 3 mm long snail the glands of the spermoviduct begin to become differentiated; in a 4 mm long animal duct, gland and flagellum of the epiphallus are defined. In a snail of 5 mm the copulatory organ begins to grow out and sperms are accumulated in the hermaphrodite duct. The first quite small ovocytes were seen in a 7 mm long snail whose copulatory organ is well developed.

The indications of the lengths give merely an approach to the successive developmental stages, as sometimes snails of equal size are in different phases of their reproductive organs.

CONCLUSIONS

Siphonaria hispida belongs to the subgenus *Siphonaria* Hubendick (1946, p. 35), section *Siphonaria* s. str. (ibid., p. 44; 1947, p. 29-32). This section is the highest developed group of the genus (1947, p. 52, 75, 83).

The Siphonariidae are not wholly primitive nor geologically old. The oldest finds are Cretaceous (p. 74, 16: *Anisomyon*). The Silurian and Devonian *Hercyonella* to which Morton (1955b, p. 165) evidently alludes is not a sure siphonariid (Hubendick 1947, p. 76).

The following evaluation of primitive and specialized characters of *Siphonaria* is orientated by Hubendick's (1947, f. 105) and Morton's (1955b, f. 15) diagrams. The first shows two principal basommatophorous branches gradually diverging from a common root. One of these comprises Ellobiidae and Otinidae and gives origin to the Stylommatophora. The second comprehends Patelliformia (Siphonariidae, Trimusculidae), Amphibolidae, Chilinidae and Latiidae as more ancestral, and Thiele's (1931) families 3-6 as more advanced

groups. Morton's figure corresponds to Fretter's and Graham's concept (1954, p. 582-583) of the relationship of Prosobranchia, Opisthobranchia, and Pulmonata. The two latter originated side by side from the Trochacea, viz. unibranchiate archaeogastropods; the mesogastropods and their descendants, the neogastropods, from the same stock, but apart from the two other subclasses. Cox (1960, p. 246) suggests to consider Mesogastropoda and Neogastropoda as a single order, Caenogastropoda.

Primitive characters of *Siphonaria* are: larvae with big velum and operculum, cephalaspidean gill, osphradium immediately inside the pallial opening, ciliated pallial ridges, and pallial caecum. It the stomach primitive characters predominate. There is no pulmonate gizzard (e. g., Carriker 1946, p. 53, f. 17 on p. 30). The muscular pocket (Morton 1955a, f. 7, mp), a primordial gizzard, is inconstant. Anterior and posterior digestive diverticula remain in their primitive positions. A posterior sorting area, though small, an intestinal groove, though narrow, and a style sac with protostyle are developed. In a quite primitive stomach a posterior caecum might be expected, but this is absent.

Specialized characters of *Siphonaria* are: the short visceral hump with a correspondingly limpet-like shell, the absence of a hypobranchial gland which occurs in *Acteon* and the Ellobiidae, the spermatophores, the opening of the epiphallus together with spermoviduct and bursa, and in comparison with *Acteon* the radula. The nervous system of *Chilina* (Pelseneer 1894, f. 210; Haeckel 1911, pl. 10, f. 38; Hubendick 1947, f. 100a, b), *Amphibola* (Farnie 1919, p. 78, f. 5; Bargmann 1930, pl. 1, f. 5; Hubendick 1947, f. 88), and primitive ellobiids as *Pythia* (Plate 1897, p. 122-123) and *Ovatella* (Pelseneer 1894, f. 205) is less concentrated and has a much longer visceral loop than that of *Siphonaria* with short connectives, coalesced left, sometimes also right, pleural and parietal ganglia and only one "synthetic" (Hubendick 1947, p. 116) ganglion in the short visceral loop.

Yonge (1952, p. 196) and Morton (1955a, p. 148; 1958, p. 75) consider the Siphonariidae as derived from terrestrial pulmonates and re-adapted to submerged life. This opinion is the consequence of placing the Ellobiidae at the root of the pulmonates as archaeopul-

monata (Morton 1955b, p. 163) "par excellence", "acteonids of the pulmonates" (p. 160). The spermatophores of *Siphonaria* do not necessarily indicate a terrestrial origin; this advanced type of sperm transmission may as well have originated during aquatic life. We do not approach, as Boettger (1954, p. 267, f. 1) did, the Siphonariidae more to the basis of the Pulmonata than the Ellobiidae, but point to signs of a primary aquatic life in Hubendick's second branch of the Basommatophora to which *Siphonaria* belongs. Such are: the free-swimming veligers of certain species, gill, osphradium, pallial ridges, and renal pore within the mantle cavity. A new acquisition of these characters by originally terrestrial snails, especially the larvae, is hard to be imagined. A monophyletic origin of the Pulmonata is not questioned, if one assumes that the appearance of a lung in the marine ancestors enabled their descendants to breathe air periodically or constantly.

RESUMO

Da Ilha Fernando de Noronha até o litoral de São Paulo (aí como *picta* d'Orb.) conhece-se *Siphonaria hispida* Smith, 1890, pulmonado pateliforme com brânquia. Pertence à secção *Siphonaria* (s. str.) da qual é a 2a. espécie atlântica. Vive nas rochas, na zona das marés, raspa algas enquanto está coberta pela água e retorna ao lugar ocupado durante a vasante. Do ovo (Fig. 3) sai veliger com opérculo (ou); a concha larval (s) é ligeiramente heterostrófica.

Lóbulos cefálicos (ce), pequenas dobras (x), e bordo anterior do pé (f) são sensoriais. Na cavidade palial, os cílios das crestas dorsal e ventral (ci) produzem entrada e saída dágua. Nesta cavidade, abre-se o poro (rv) do rim (k). A brânquia dobrada (b) corresponde à dos Cephalaspidea. O osfrádio situa-se internamente ao orifício inalante (no); seu gânglio (zo) está ligado a um gânglio (zr) na raiz direita da alça visceral. Êste gânglio, separado do pequeno gânglio pleural direito (eu), chamamos de parieto-supra-intestinal.

A rádula (Fig. 15) é mais diferenciada que a de *Acteon*. Glândulas unicelulares desembocam na parede ventral do esôfago anterior. O estômago (sm) comunica-se com 3 divertículos digestivos anteriores, 2 dorsais (l) e 1 ventral (ni), e com 1 posterior (um). Pequena área escolhedora (sa), estreito sulco intestinal (io), e pro-

tostilo (ro) ocorrem no estômago; ceco posterior, não. O alimento já se forma em cordão (os) no esôfago, passa ao protostilo e, daí, ao cordão fecal.

A espécie é proterândrica, mas não há fases sexualmente separadas. O órgão copulador (p) evaginável pelo líquido do corpo aumenta de 2,5 a 7 mm de comprimento quando evertido (Figs. 7, 19). Espermatóforos não foram encontrados em mais de 50 animais maduros examinados; possìvelmente não ocorrem em *S. hispida*.

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EXPLANATION OF LETTERS OF SIPHONARIA HISPIDA

aa — apertures of anterior dorsal livers.
ae — flagellum.
am — amoebocytes.
ao — aperture of posterior liver.
ar — anus.
as — abdominal-subintestinal ganglion.
av — aperture of anterior ventral liver.
az — anal lobe.
b — gill.
ce — cephalic folds.
ci — ciliate ridges.
cn — cerebral ganglia.
cu — cuticulate gastric area.
d — epiphallus duct.
e — retractor.
ea — opening of spermoviduct.
ee — eye.
eo — oesophageal folds.
er — faecal rod.
eu — right pleural ganglion.
ev — efferent branchial vessel.
f — foot.
g — pluricellular skin glands.
h — hermaphrodite duct.
i — intestine.
ia — mantle.
in — minor typhlosole.
io — intestinal groove.
is — glandular pouch.
j — jaw.
k — kidney.
l — anterior dorsal livers.
m — adductor.
ma — opening of epiphallus.
me — mucus gland.
mo — mouth.
ms — mantle skirt.
mu — muscular pocket.
mx — intra-auricular muscle.
n — nerve.
na — genital nerve.
ne — exhalant opening.
ni — anterior ventral liver.
no — inhalant opening.
nv — efferent renal vessel.
o — mass of food.
or — posterior afferent renal vessel.
os — food string.
ou — operculum.
p — copulatory organ.
q — pedal ganglia.
r — pericardium.
ra — anterior afferent renal vessel.
rc — auricle.
re — rectum.
ri — ventricle.
ro — protostyle.
rs — seminal receptacle.
rv — renal pore.
s — shell.
sa — sorting area.
sm — stomach.
so — statocyst.
sz — shell glands.
t — cerebral tube.
ua — opening of bursa.
um — posterior liver.
ur — bursa duct.
us — bursa.
va — afferent branchial vessel.
ve — velum.
vi — spermoviduct.
vm — ventro-median fold.
vr — vessels from anterior part of pallial roof.

vz — efferent branchio-renal vessel.
w — glandular primordium in spermoviduct.
wa — cephalic artery.
we — albumen gland.
wm — hermaphrodite gland.
wu — pleuro-parietal ganglion.
x — sense organ.
xm — annular muscles of rectum.
xo — lateral lobe of cerebral ganglion.
y — major typhlosole.
z — genital aperture.
ze — epiphallus gland.
zo — osphradial ganglion.
zr — parieto-supra-intestinal ganglion.
zz — glands of copulatory organ.

PLATES

PLATE 1

Siphonaria hispida

Fig. 1 — Egg string.
Fig. 2 — Part of egg string.
Fig. 3 — Three egg capsules; two with veligers, one with empty shell.
Fig. 4 — Shells of 3, 4, 5, 6, and 11 mm length.
Fig. 5 — Left side view of 11 mm shell with larval shell.
Fig. 6 — Ventral view of fore end; anterior border of foot bent downwards.
Fig. 7 — Same with evaginated copulatory organ.
Fig. 8 — Same with everted buccal cavity and partly evaginated penis.

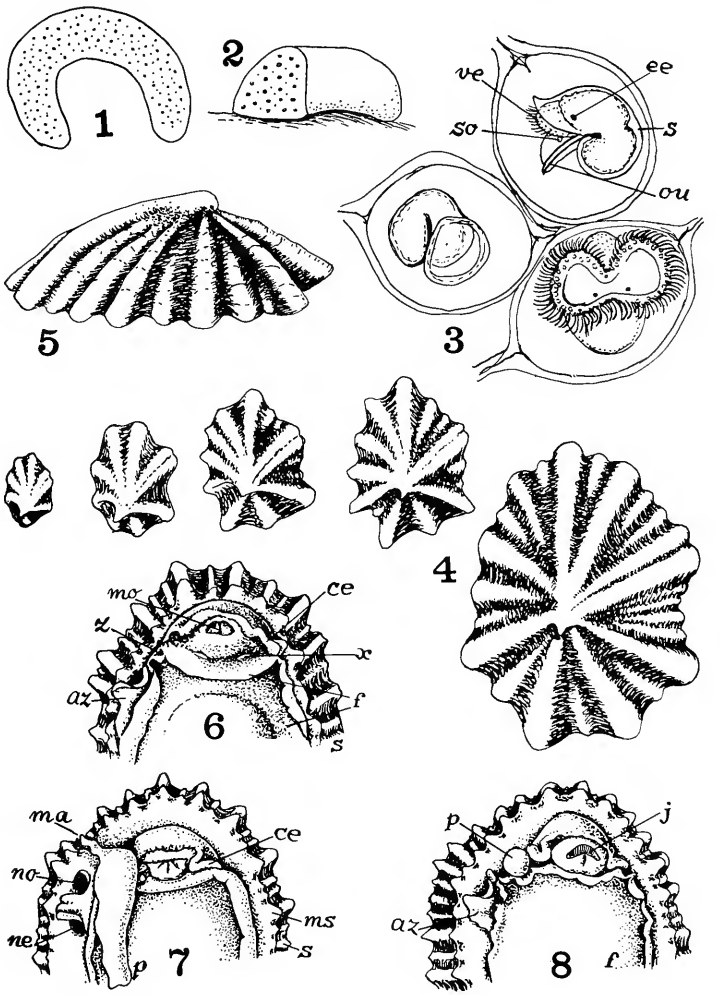
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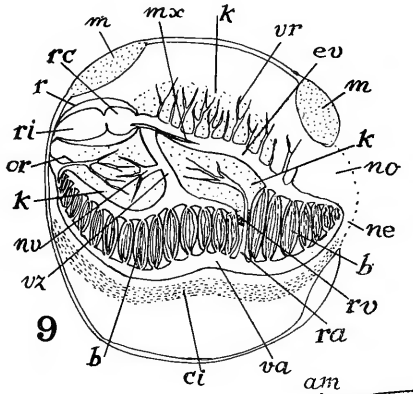
PLATE 2

Siphonaria hispida

Fig. 9 — Roof of pallial cavity.
Fig. 10 — Tangential section of pallial cavity.
Fig. 11 — Right side of transverse section of snail.
Fig. 12 — Transverse section of left cerebral tube.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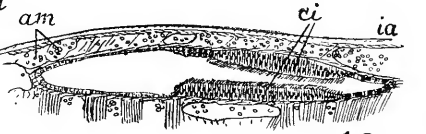
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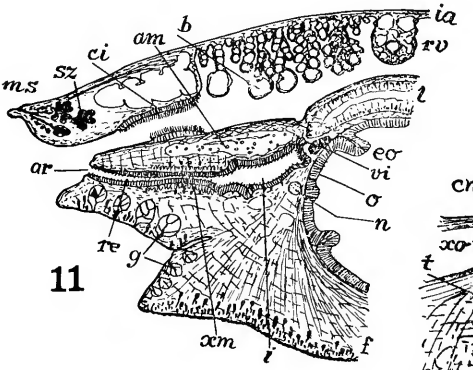
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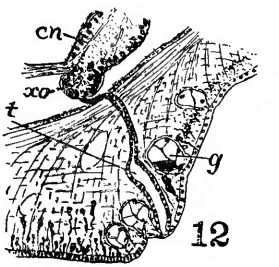
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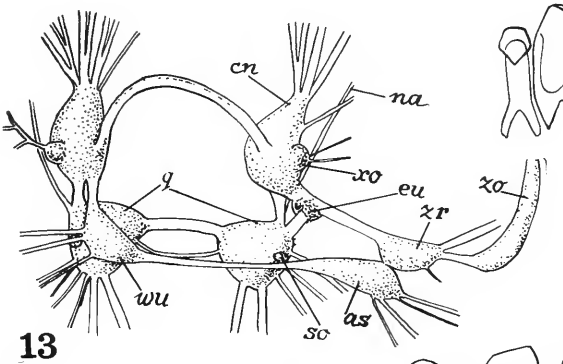
PLATE 3

Siphonaria hispida

Fig. 13 — Central nervous system.
Fig. 14 — Radula of 4 mm long snail.
Fig. 15 — Same of adult snail.
Fig. 16 — Alimentary organs.
Fig. 17 — Stomach of preserved snail opened from the dorsal side; broad trace indicates cuts. Food string and protostyle from life.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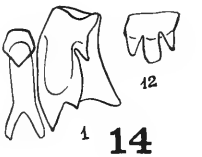
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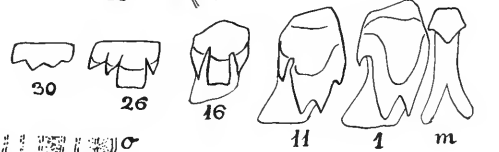
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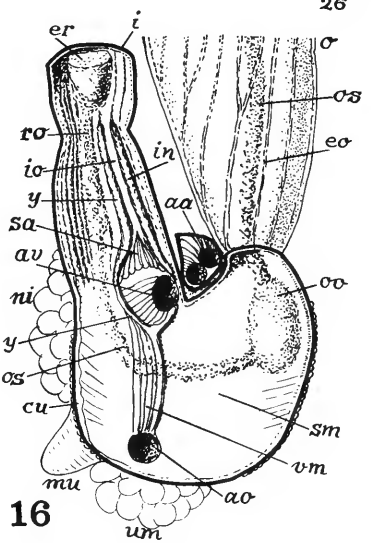
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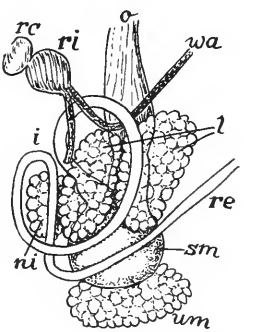
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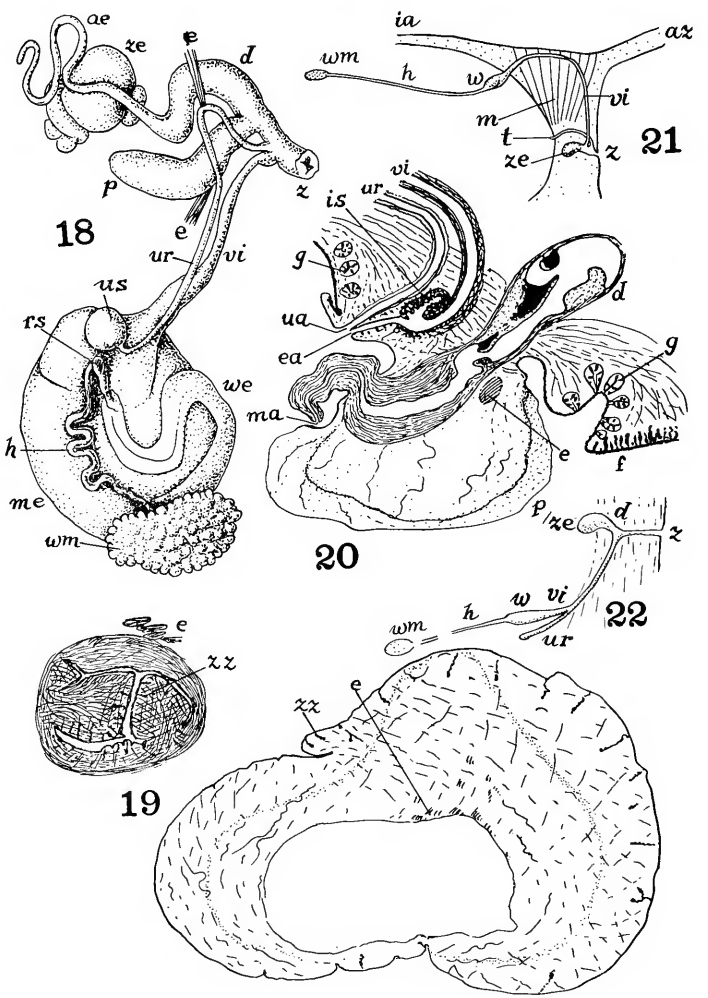
PLATE 4

Siphonaria hispida

Fig. 18 — Reproductive organs.
Fig. 19 — Transverse sections of invaginated and evaginated copulatory organ; same scale.
Fig. 20 — Combined transverse section of evaginated distal genital organs.
Fig. 21 — Primordial genital duct of 1,8 mm long snail.
Fig. 22 — Same of 2 mm long snail.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ESTUDO COM O MICROSCÓPIO ELETRÔNICO DO RETÍCULO ENDOPLASMÁTICO EM FIBRAS MUSCULARES DE CARANGUEIJOS *

ROGER LAVALLARD
(Departamento de Fisiologia Geral e Animal e Secção de Microscopia Eletrônica da Universidade de São Paulo)

(6 Estampas)

INTRODUÇÃO

Desde as primeiras observações, com o microscópio eletrônico, de uma rêde de membranas intracelulares nas células de culturas de tecidos (1, 2), uma série de trabalhos tem estabelecido a existência do "retículo endoplasmático" (3, 4, 5) no citoplasma da maior parte dos tipos de células animais (6, 7, 8). Esta estrutura é interpretada como uma rêde tridimensional de cavidades tubulosas e vesiculosas, de conteúdo homogêneo, limitadas por uma membrana simples e contínua, podendo apresentar diferenciações locais (9, 10, 11, 12), variáveis com as categorias celulares; seria contínua através do citoplasma desde a membrana celular (c. F. 13) até o núcleo (14, 8). Uma publicação de PALADE (13) recapitula o progresso dos conhecimentos a respeito do retículo endoplasmático, desde a sua descoberta.

Ao estudarem as fibras musculares estriadas, BENNETT e PORTER (15) evidenciaram, entre as miofibrilas, um sistema de membranas intrasarcoplasmicas, que identificaram com o retículo endoplasmático dos outros tipos celulares. Êste sistema é igualmente comparável (15, 16) às formações reticulares já observadas com o microscópio óptico, entre as miofibrilas, pelos histologistas (17, 18) do fim do século XIX. EDWARDS e colaboradores (19, 20) mostraram que o retículo endoplasmático faz parte dos constituintes das células musculares estriadas, cujas estrutura e distribuição variam

(*) Recebido para publicação em Abril de 1960.

com as espécies de animais e a especialização das funções da fibra muscular. PORTER e PALADE (21) analisaram com grande precisão, as relações morfológicas do retículo endoplasmático com as miofibrilas em três tipos diferentes de fibras musculares estriadas.

O tecido muscular estriado dos Crustáceos foi até o momento pouco estudado em microscopia eletrônica. O presente trabalho relata as principais observações feitas sôbre o retículo endoplasmático no decorrer do exame de numerosas micrografias eletrônicas de cortes longitudinais e transversais, em músculos das peças bucais de duas espécies de carangueijos. Depois de uma descrição dos principais caracteres morfológicos da fibra muscular em estudo, algumas observações novas são relatadas a respeito da estrutura do retículo endoplasmático, notadamente sôbre as formações vesiculares localizadas ao nível das zonas I e sôbre o retículo em frente ao disco H. Algumas particularidades, que não estão de acôrdo com as hipóteses já formuladas sôbre as funções do retículo endoplasmático da célula muscular estriada, também são discutidas.

MATERIAL E MÉTODO

Os músculos utilizados provêm das peças bucais de duas espécies de carangueijos, uma *Callinectes danae*. Smith (22), abundante nas praias do Estado de São Paulo, outra *Carcinus maenas,* Penn. (23), muito comum no litoral atlântico da França. São os dois músculos antagonistas que provocam os batimentos do flagelo no exopodito do terceiro maxilípodo. O exopodito é removido por ruptura da sua articulação com o basipodito, para evitar tôda lesão das fibras musculares, notadamente ao nível das suas inserções proximais. Para descobrir os músculos, uma janela é recortada na cutícula, do lado interno do artículo, menos calcificado do que o externo. Depois, o exopodito é imerso no fixador; portanto, os seus músculos são fixados *in situ,* mantidos em suas inserções naturais, sem ter sofrido nenhum dano.

O fixador utilizado é uma solução de O_s O_4 a 3%, tamponada a pH 7,4 — 7,6, que age durante uma hora à temperatura de 18°C. Depois de uma lavagem rápida com água distilada, os músculos são extraídos do tegumento, recortados em peças de menos de 1 mm3 que

são em seguida desidratadas, de maneira contínua, num aparelho de Bernhard (24) modificado (25). Em seguida são incluídas numa mistura de 9 partes de metacrilato de n-butilo com uma parte de metacrilado de n-metilo, polimerisada por 1% de Luperco CDB numa estufa de 45°C, durante 24-36 horas. Os cortes são efetuados com uma navalha de vidro num microtomo Porter-Blum. As micrografias são obtidas com ampliações originais de 2000 a 10500, usando-se um microscópio RCA, modêlo EMU.

RESULTADOS

1 — *ESTRUTURA DAS FIBRAS MUSCULARES*

Como no caso da maior parte dos músculos em Artrópodes, cada fibra muscular do exopodito dos maxilípedes está inserida pelas suas duas extremidades sôbre a cutícula: a extremidade proximal sôbre a parede tegumentar do artículo; a extremidade distal sôbre um apodema que provém da base do flagelo. O comprimento da fibra é assim função da dimensão do exopodito e, portanto, do tamanho do animal. Êste pode atingir 12 mm em carangueijos de grandes dimensões. Ao contrário, o diâmetro, sempre inferior a 100μ, é pequeno em comparação com os apêndices locomotores, onde as fibras musculares podem ter dimensões transversais consideráveis em certos carangueijos (26).

A descrição da estrutura das fibras musculares do exopodito, observada com o microscópio eletrônico, já foi dada numa publicação anterior (27); relatam-se aqui sòmente os principais caracteres com a terminologia usada por BENNETT e PORTER (15) a respeito dos músculos do frango. Com exceção do grande desenvolvimento do retículo endoplasmático, êstes caracteres correspondem aos das fibras musculares de alta freqüência de contração, como foram estabelecidos por EDWARDS e colaboradores (19, 20): grande abundância de sarcoplasma (Sp, Figs. 1 e 2), de que resulta um valor pequeno da relação miofibrilas/sarcoplasma; mitocôndrias (Mt, Figs. 1 e 2) muito numerosas; período curto da estriação transversal das miofibrilas (Fig. 3).

As miofibrilas estão agrupadas em colunetas musculares (= colunetas de Leydig, cl, Figs. 1 e 2), de importância variável (de 10

até 200 miofibrilas). As colunetas musculares são amplamente separadas umas das outras por sarcoplasma intercolunar (Sp_2, Fig. 2), cuja espessura pode exceder 6μ; são também separadas do sarcoplasma por uma camada espêssa (8μ e mais) de sarcoplasma subsarcolêmico (Sp, Fig. 1 e Sp_1, Figs. 2 e 4). Numa coluneta, o sarcoplasma interfibrilar (Sp_3, Fig. 3) ultrapassa raramente 0,4μ de espessura entre duas miofibrilas. A única diferença bem marcada entre as duas espécies utilizadas, do ponto de vista da estrutura da fibra muscular do exopodito, se traduz por uma abundância maior do sarcoplasma em relação às miofibrilas em *Carcinus;* as colunetas musculares são menos numerosas do que em *Callinectes,* e cada uma contém, em média, um número inferior de miofibrilas. Como mostra a Fig. 1, micrografia de um corte transversal de fibras musculares em *Carcinus maenas,* o sarcoplasma é mais importante do que as miofibrilas quanto ao volume ocupado na célula muscular.

As mitocôndrias estão repartidas em dois grupos, um aplicado contra o sarcolema: mitocôndrias subsarcolêmicas (Mt_1, Figs. 1, 2 e 4), outro situado ao nível das colunetas musculares, seja na periferia destas: mitocôndrias pericolunares (Mt_2, Figs. 1 e 2), seja no meio: mitocôndrias centrocolunares (Mt, Fig. 5). O diâmetro destas varia de 0,6 a 1,2μ; o comprimento pode ser grande, até 7μ.

As miofibrilas (Mf, Figs. 1, 2 e 5) apresentam, em corte transversal, um contôrno irregular, muitas vêzes de aspecto poligonal. As dimensões da superfície de secção destas são muito inconstantes de uma miofibrila para outra: existem miofibrilas grossas e finas, cuja espessura, considerada cada um no seu menor valor (para excluir o êrro ligado a uma obliquidade eventual do corte) varia de 0,2 a 1μ. A estriação transversal das miofibrilas comporta a linha Z, as zonas I e A e o disco H, exibindo muitas vêzes medianamente a estria M (Fig. 7). Os miofilamentos, sempre bem individualizados em A, H e M, não são discerníveis na zona I e na linha Z. Isto é bem visível na Fig. 10. Aqui, o corte transversal, ligeiramente oblíquo, passou pela zona I no caso de algumas miofibrilas que contrastam, pelo seu aspecto relativamente homogêneo, com a superfície pontuada de miofilamentos das miofibrilas apanhadas na zona A. Será mostrado também mais adiante, que as miofibrilas têm uma aparência diferente

em corte transversal no disco H em comparação com o resto da zona A.

No sarcoplasma ocorrem, ainda em certos lugares, acumulações irregulares de uma substância densa (GI, Figs. 1 e 15) que foi interpretada (28) como glicogênio, e pequenos grânulos densos (gr, Figs. 4 a 15) de 150 A de diâmetro, distribuídos uniformemente por tôda a massa sarcoplasmática.

2 — *ESTRUTURA DO RETÍCULO ENDOPLASMÁTICO*

a. *Generalidades*

O retículo endoplasmático das fibras musculares aqui estudadas está principalmente localizado entre as miofibrilas. Desenvolvido no sarcoplasma interfibrilar, êle não se prolonga nas outras categorias de sarcoplasma (r. e., Figs. 1 e 15) onde sòmente se encontram alguns raros perfis de endomembranas, aparentemente sem relação com o sistema interfibrilar. Qualquer que seja o nível considerado de um sarcômero, o retículo endoplasmático está sempre abundantemente representado entre as miofibrilas. A importância dêste é tal que contribui, de algum modo, para sublinhar a individualidade das miofibrilas, em corte transversal, envolvendo cada uma de um envoltório de membranas que a separa das suas vizinhas (r. e., Figs. 3, 10 e 12).

A Fig. 5 é destinada a dar uma vista geral dêste retículo em corte transversal, no interior de um mesmo sarcômero. O corte, ligeiramente oblíquo, passa por duas linhas Z sucessivas, que aparecem nas miofibrilas das extremidades superior e inferior da micrografia, como duas faixas densas (Z); portanto, entre estas duas linhas limitantes, são evidenciados cortes transversais das várias zonas do sarcômero. Êstes diferentes níveis podem ser identificados, de um lado, pela sua posição em relação às linhas Z, de outro, pelos caracteres da estrutura das miofibrilas, que é variável ao longo do sarcômero. Tôdas as miofibrilas que, na proximidade de Z, não mostram miofilamentos, são cortadas na zona I; as outras miofibrilas, com miofilamentos bem diferenciados, são cortadas na zona A; na parte mediana da Fig. 5, miofilamentos de contôrno mais nítido e de diâmetro maior, representam um corte transversal pelo disco H.

Ao primeiro exame, o retículo endoplasmático apresenta-se como um conjunto de perfis de membranas endocelulares de tamanho, forma, densidade e distribuição muito heterogêneos, no qual parece difícil reconhecer-se uma organização. Deve-se notar, porém, uma oposição quanto à morfologia do retículo entre as zonas terminais e a região mediana do sarcômero. Na proximidade da linha Z, o retículo é uma formação quase sem interrupção, feita de uma dupla membrana densa, interposta como uma parede contínua em volta das miofibrilas. Na parte central, ao contrário, o retículo é mais descontínuo transversalmente e, com exceção de uma zona particular designada por Y que corresponde ao disco H, é representado ùnicamente por túbulos e vesículas em orientação longitudinal, limitados por uma membrana de baixa densidade. O nível em que o retículo torna-se tubuloso e vesiculoso, está situado aproximadamente no meio do segmento de A incluído entre I e H; isto se constata, de um lado, sôbre a Fig. 5, onde miofibrilas cortadas em A são envolvidas por retículo contínuo transversalmente, de outro, nos cortes longitudinais das Figs. 6 e 7. Para a comodidade da descrição, serão distinguidas duas partes no segmento da zona A situado entre I e H, uma zona A distal do lado de I, uma zona A proximal do lado de H, a separação entre as duas zonas sendo demarcada pela passagem de um retículo contínuo para um retículo descontínuo no sentido transversal da fibra muscular.

Existe assim heterogeneidade de aspecto do retículo quando se considera níveis diferentes de um sarcômero, mas para um mesmo nível, a organização dêste retículo apresenta uma certa homogeneidade. Esta noção é ilustrada pelas Figs. 10 e 11; o aspecto do retículo é muito diferente de um corte para outro, porque o corte passa, na Fig. 10, pela zona I, de cada lado de Z, e pela zona A distal, enquanto que, na Fig. 11, as miofibrilas estão cortadas na zona A proximal e no disco H, mas, em cada Fig., o retículo endoplasmático é bastante uniforme em tôda a superfície da micrografia.

Portanto ressalta desta consideração geral que, em Crustáceos, como no caso das fibras musculares do frango (15), de Anfíbios (21, 28), de Répteis (23), de Insetos (20) e de Rato (21), o retículo endoplasmático apresenta uma organização definida que se repete, em cada sarcômero, segundo um sistema cuja segmentação está

em fase com a estriação das miofibrilas. Por outra parte, o estudo mais pormenorizado da estrutura dêste retículo pode subdividir-se em três partes, que correspondem aos três grandes aspectos diferentes que êle apresenta:

1) — Nível da linha Z + zona I + zona A distal, ou zona de continuidade transversal do retículo.
2) — Nível da zona A proximal, ou zona do retículo tubuloso e vesiculoso.
3) — Nível do disco H, onde o retículo exibe um aspecto de três membranas duplas contíguas.

b. *Linha Z + Zona I + Zona A distal*

Esta região inclui a parte do sarcômero situada entre a linha Z e o meio do segmento A que separa I de H. Nos cortes transversais das fibras musculares, a linha Z é fàcilmente identificada; aparece como uma linha escura, que atravessa a superfície de secção da coluneta muscular, tendo em geral um percurso bastante sinuoso de uma miofibrila a outra. No interior de uma mesma miofibrila, a linha Z forma uma faixa densa sôbre o fundo mais claro da zona I (Z, Fig. 5). Afora sua densidade maior, a linha Z é como a zona I, sem estruturas reconhecíveis. A zona I comporta, nos cortes transversais (I, Figs. 5, 10 e 12), tôdas as miofibrilas nas quais é impossível distinguir uma estrutura que poderá corresponder aos prolongamentos dos miofilamentos, tão nìtidamente diferenciados na zona A. Uma miofibrila, cortada na zona I (I, Fig. 10), aparece como uma mancha de substância de uma densidade semelhante à do material localizado entre os miofilamentos da zona A. Nesta última, ao contrário, os miofilamentos são bem individualizados, com um diâmetro de 120 a 150 A, separados uns dos outros por uma distância de 250 a 300 A. A passagem da zona I à zona A pode-se ver no interior de uma mesma miofibrila, se o corte transversal fôr um pouco oblíquo (A-I, Fig. 10).

Como já foi assinalado em outros tipos de músculos (21, 30), o retículo endoplasmático de dois sarcômeros vizinhos está interrompido ao nível da linha Z, na maior parte dos casos observados. Em c. t., Fig. 4, o retículo parece todavia, atravessar a zona interfibrilar em frente das linhas Z, mas êste aspecto excepcional pode

ser também ligado a uma ruptura ocasionada por retrações no curso da fixação. Existe, entre as miofibrilas, no local da passagem da linha Z, uma zona densa onde não é possível definir-se uma estrutura (Figs. 5 e 10); sòmente se verifica, neste nível, o desaparecimento progressivo dos contornos do retículo, que afinal não se percebem mais em frente da linha Z. A espessura da zona que separa o retículo de dois sarcômeros vizinhos ao nível das linhas Z é da ordem de 500 A.

O caráter mais notável do retículo endoplasmático em frente da zona I e da zona A distal é, sem dúvida, a sua continuidade, tanto transversal (r. e., Fig. 10) como longitudinal (Figs. 5, 6, 7 e 8) em todo o conjunto do sarcoplasma interfibrilar. Tudo se passa como se o sarcoplasma estivesse, neste nível, completamente dividido em compartimentos, no sentido transversal, por uma membrana dupla e contínua que delimitasse assim uma série de divisões, em cada uma das quais estivessem enclausuradas a zona I e a zona A distal de uma miofibrila. Esta dupla membrana tem uma espessura total de cêrca de 250 A, representando o espaço interno de 100 A. Trata-se aqui de valores médios, pois como se pode notar na Fig. 3, esta membrana dupla mostra, em numerosos lugares, constrições que se fazem à custa do espaço interno, às vêzes atingindo mais ou menos as bordas densas da formação, o que pode determinar pequenas rupturas. Estas irregularidades no aspecto da dupla membrana são provàvelmente alterações ligadas à fixação; produzem-se, de fato, com freqüência completamente desiguais segundo as preparações; em geral são mais freqüentes quando também as outras estruturas se apresentam defeituosas. Quanto às variações de densidades da dupla membrana, com exceção das que se produzem ao nível da linha Z, devem ser atribuídas principalmente às orientações mais ou menos oblíquas desta membrana em relação ao plano do corte.

Outros caráter interessante dêste retículo endoplasmático refere-se à existência de cavidades de grande tamanho que são designadas aqui pelo têrmo "cavidades terminais" por causa da sua localização em frente das zonas I, nas duas extremidades do sarcômero (c. t., Figs. 5, 6, 9, 10 e 12). Um certo número dos caracteres postos em evidência por PORTER e PALADE (21) a respeito das vesículas da zona I (= terminal cisternae), são aplicáveis a estas for-

mações. Tais cavidades não envolvem completamente as miofibrilas, mas se encontram sòmente em contiguidade sôbre um dos seus lados; sua maior dimensão é transversal; a cada unidade dêste tipo de um sarcômero corresponde uma formação equivalente do outro lado da linha Z, no sarcômero seguinte; as faces opostas destas duas cavidades são muitas vêzes achatadas contra a estrutura densa de 500 A de espessura situada ao nível das linhas Z. Mas na constituição da parede destas cavidades terminais, intervém uma diferença permitindo pensar que se trata de formação de natureza diversa da das "terminal cisternae" descrita por PORTER e PALADE, em músculos de Vertebrados. As Figs. 6, 9, 10 e 12 mostram em c. t. que estas não são uma simples dilatação local do espaço interno da dupla membrana do retículo, de tal maneira que a parede das vesículas seja uma membrana simples, como é o caso clássico. A parede das cavidades terminais é aqui uma membrana dupla, que tem a mesma estrutura que o retículo endoplasmático entre as cavidades. O conteúdo não é também a substância homogênea, característica do meio interno do retículo. E' uma substância que tem a mesma densidade que o sarcoplasma e que, como êste, compreende os pequenos grânulos osmiofílicos (gr) de 150 A de diâmetro, dispersos em todos os lugares no sarcoplasma. Nunca êstes grânulos são visíveis no interior das membranas do retículo. O conteúdo das cavidades terminais, é, portanto, constituído por sarcoplasma.

c. *Zona A proximal*

Esta zona corresponde à outra metade do segmento A compreendido entre I e H, a que está em contacto com o disco H, na direção da parte central da Zona A, donde sua designação como "proximal". Não é necessário insistir sôbre a determinação desta zona num corte transversal; o aspecto dos miofilamentos é o mesmo do que em A distal, pois é sòmente a mudança de forma do retículo que introduz a distinção. A proximidade do disco H e o aspecto disperso do retículo bastam para situar esta zona.

Já foi indicado mais acima que a zona A proximal se opõe à zona A distal e à zona I pela descontinuidade transversal do retículo endoplasmático. Êste último se apresenta, então, essencialmente sob a forma de túbulos e vesículas (t. c.p., Figs. 3 a 9, Figs. 11 e 13;

com orientação sobretudo longitudinal. Uma outra diferença nota-se na densidade das membranas do retículo, aqui muito mais fraca do que na zona precedente. Há predominância dos túbulos do lado da zona I e predominâncias de vesículas do lado de H. Em direção de H, os túbulos e as vesículas convertem-se em "cisternae", que têm aparentemente a significação clássica, quer dizer, são o produto da simples dilatação dos túbulos e das pequenas vesículas, provocando um aumento local de volume da fase interna do retículo. Estas cisternas proximais, como os túbulos, possuem uma parede constituída por uma membrana simples; o seu conteúdo é homogêneo; corresponde de fato, ao espaço compreendido no interior das membranas do retículo. Em corte longitudinal (Figs. 3, 4 e 6), as "cisternas" proximais prolongam os túbulos que vão conectar-se com a dupla membrana contínua da zona A distal; isto explica a predominância, em corte transversal, dos túbulos nos níveis próximos de I, de vesículas e de "cisternae" nos níveis próximos de H.

O têrmo de "central cisternae" utilizado por PORTER e PALADE (21) no caso dos músculos da larva de *Amblystoma,* não pode ser conservado aqui. De fato, as "central cisternae" estão localizadas em frente do disco H; são estruturas ímpares, no sentido em que são representadas uma vez só em cada sarcômero. De outro lado, caracterizam uma zona de continuidade transversal do retículo endoplasmático. No caso presente, a região do disco H está ocupada por uma estrutura particular, que separa duas metades dentro do retículo de um sarcômero; as cisternae proximais, de cada lado desta estrutura, são portanto formações pares. Além disso, já se evidenciou que estas caracterizam uma zona de descontinuidade transversal do retículo endoplasmático. O têrmo de "cisternae proximais" foi assim adotado em função destas diferenças.

A forma e a dimensão das "cisternae" proximais são variáveis; mais frcqüentemente, têm um contôrno arredondado ou ovóide (c. p., Figs. 11 e 13), mas algumas são muito deformadas. A membrana simples que as envolve, cuja osmiofilia é muito fraca, se acha muitas vêzes cortada oblìquamente e assim a sua densidade enfraquece-se ainda mais até o ponto em que é, às vêzes, difícil delimitar a parede dos túbulos e das vesículas. Como dimensão destas "cisternae" proximais, em virtude das irregularidades do contôrno, sòmente se pode

informar que, para "cisternae" de perfil sub-circular, o diâmetro pode atingir 0,4μ.

d. *Disco H*

Em corte longitudinal, o disco H é geralmente distinto no meio de cada sarcômero; possui um comprimento médio de 150 mμ (H, Figs. 3 e 6). No meio de H, uma faixa mais densa de cêrca de 70 mμ representa a estria M. Em corte transversal, a borda dos miofilamentos no disco H parece mais nìtidamente delimitada do que na zona A, em razão da densidade menor da substância interposta, do que resulta um contraste maior. O diâmetro dos miofilamentos é também superior no disco H, atingindo 200 A (H, Figs. 5, 11, 14 e 14'). O nível da estria M está marcado por uma zona menos densa no centro de cada miofilamento; todavia, nas várias Figs. correspondentes, H designa o conjunto do disco H, sem distinguir a estria M, pois não existe nenhuma diferenciação particular do retículo em frente desta última.

Ao nível do disco H, o retículo assume uma forma particular, designada por Y nas micrografias, que se resume a três duplas membranas contíguas, com a maior dimensão transversal em relação às miofibrilas. (Figs. 5, 6, 9, 11, 14 e 14'). Esta dimensão não é eqüivalente para as três formações; a formação central é a mais desenvolvida (Fig. 14 e 14'). Muitas vêzes, as duas formações laterais menores não estão em frente uma da outra; isto explica porque freqüentemente, em corte longitudinal, há sòmente duas ou uma formação em frente de H (y, Fig. 8), segundo a passagem do corte respectivamente pela formação central e uma das duas laterais, ou pela única formação central. No sentido longitudinal, as duplas membranas estão estritamente localizadas em frente do disco H, ou seja as suas dimensões não podem exceder 150 mμ. As suas espessuras variam de 200 a 250 A, com um espaço intermediário de 80 a 120 A.

As três duplas membranas formam uma zona de separação para o retículo endoplasmático de cada meio sarcômero; nenhuma das formações do retículo de um lado de H insinua-se entre essas e as miofibrilas adjacentes, para prolongar-se na outra metade do sarcômero. As cisternas proximais da zona A estão assim opostas de cada lado destas três formações da mesma maneira do que as cavidades

terminais de cada lado da linha Z. A interpretação destas três formações, em relação a outras partes do retículo, torna-se difícil. Algumas micrografias de cortes longitudinais sugerem que a formação mediana estaria em relação com uma das cisternas proximais e viria intercalar-se entre as duas formações laterais ligadas com o fundo da cisterna proximal oposta. Assim, poderia explicar-se, em corte transversal, a presença indiferentemente de três, duas, ou uma duplas membranas, segundo o corte tenha passado, seja a distância igual das cisternas, pelas três membranas duplas contíguas, seja mais em direção de uma ou outra das cisternas, pelas duas duplas membranas laterais ou, ainda, pela única central (y, Figs. 5 e 11). Ademais, também em corte transversal, aparentemente nos lugares os mais vizinhos de A, a formação mediana ou as formações laterais podem ser substituídas por uma fila de pequenas cavidades circulares (y', Fig. 5) que poderiam corresponder à zona de comunicação entre as cisternas e as duplas membranas; esta se estabeleceria por intermédio de túbulos muito curtos, de orientação longitudinal. Todavia, a diferença de osmiofilia entre as duplas membranas densas e as membranas claras das cisternas proximais é tal que não é possível confirmar estas relações de continuidade entre as duas formações. Talvez a membrana de pequena densidade das cisternas sòmente se apóie contra a membrana densa das três formações e, neste caso, existiria ùnicamente contiguidade. De todo modo, é preciso conceber que a presença das três duplas membranas corresponde a uma zona de discontinuidade longitudinal dentro do retículo endoplasmático ao nível do disco H.

3 — *RELAÇÕES DO RETÍCULO ENDOPLASMÁTICO COM OS OUTROS CONSTITUINTES CELULARES*

Uma observação freqüentemente descrita pelos histologistas (c. F. 15) refere-se ao aspecto em festão, que pode apresentar, num corte longitudinal, a bordadura de fibras musculares fixadas em estado de encurtamento, quando o sarcolema está com chanfraduras periódicas ao nível de cada linha Z, como se estivesse ligado ao feixe de miofibrilas neste ponto. Êste aspecto, que foi atribuído pelos histologistas a uma junção direta entre a linha Z e o sarcolema, foi encontrado de novo em microscopia eletrônica (13) e certas estruturas,

notadas sôbre sarcolema isolado, foram interpretadas (32) como vestígio da inserção da linha Z. Todavia, várias observações, no sarcolema periférico, de túbulos e de vesículas acumuladas em frente de Z e I, e mesmo de membranas ligando o retículo das miofibrilas ao sarcolema, fazem admitir agora que exista lá uma conexão entre, de um lado, não a linha Z mas o retículo endoplasmático situado em redor da região Z — I e, do outro, a membrana plásmica da fibra muscular (15, 16, 19, 20, 29, 21, 28); esta conexão seria assegurada por intermédio de membranas pertencentes ao retículo endoplasmático.

Sob êste prisma, as fibras musculares estudadas aqui parecem fazer exceção. Em virtude do grande desenvolvimento da camada do sarcoplasma subsarcolêmico, tais fibras constituem um material de observação favorável. De fato, para vencer uma tal distância entre as miofibrilas e o sarcolema, o retículo deve desenvolver-se notadamente e não pode escapar à observação numa zona tão largamente desenvolvida (Fig. 1). Na realidade, em todo êste conjunto de sarcoplasma periférico, os perfis de membranas são muito raros e muito distantes uns dos outros, sem relação com o retículo das colunetas musculares. A Fig. 15 representa uma parte da periferia de uma fibra muscular, cortada transversalmente ao nível da zona I, portanto na zona onde o retículo apresenta o seu desenvolvimento máximo entre as miofibrilas. Há uma desproporção enorme entre o retículo interfibrilar (r. e. 1) e alguns perfis (r. e. 2) no sarcoplasma periférico (Sp). O retículo interfibrilar desaparece de repente na zona fronteira entre a coluneta muscular e o sarcoplasma subsarcolêmico, sem apresentar prolongamentos em direção do sarcolema. Êste fato, ilustrado aqui na zona I, foi encontrado também em numerosos cortes longitudinais em todos os níveis dos sarcômeros (Fig. 4). O retículo endoplasmático é pràticamente inexistente no sarcoplasma periférico; êste não apresenta acumulação particular de túbulos e vesículas em frente das zonas I e da linha Z. Por outro lado, existe (27) aplicado ao sarcolema, uma camada de mitocôndrias contíguas (Mt, Fig. 4), tão apertadas umas contra as outras que muitas vêzes, estas se deformam recìprocamente, e não deixam entre elas nenhum espaço disponível que possa servir de passagem às junções entre o sarcolema e o retículo interfibrilar. Estas mitocôndrias formam, portanto, um

obstáculo importante entre o retículo e o sarcoplasma. O limite da fibra muscular (m. l., Figs. 1, 2 e 4) comporta uma membrana basal (m. b., Fig. 15) de 40 a 60 mμ de espessura, talvez equivalente à camada cuticular do sarcolema de PORTER (33), uma membrana mais fina, de cêrca de 10 mμ, às vêzes difícil de distinguir, considerada como a membrana plásmica (m. p., Fig. 15) e, entre as duas, um espaço de 15 a 20 mμ. Contra esta membrana, as mitocôndrias subsarcolêmicas estão aplicadas tão estreitamente que é muitas vêzes difícil discernir a membrana mitocondrial externa da membrana plásmica. Do mesmo modo, no sarcoplasma intercolunar, não aparecem membranas suscetíveis de conectar os sarcômeros de duas colunetas musculares vizinhas, nem acumulações de túbulos e de vesículas em certos níveis. Estas fibras musculares de carangueijos representam, portanto, um caso particular, onde o retículo não se estende através de tôda a célula muscular, mas sòmente no interior de cada coluneta muscular. Êstes resultados são obtidos com ainda mais evidência em *Carcinus maenas,* porque o sarcoplasma é muito mais abundante em relação às colunetas musculares.

Os núcleos são pouco numerosos nestas fibras musculares, e, em geral, localizados na periferia da célula. Exibindo acúmulos de cromatina principalmente contra a membrana nuclear e um envelope duplo limitando um espaço de 15 a 50 mμ de espessura, interrompido localmente pelos "pores canals", a sua estrutura é conforme ao que foi evidenciado em numerosos tipos celulares (14). Em vista da grande raridade dos perfis de membrana fora das colunetas musculares, não existe muito retículo na vizinhança do núcleo e não foi possível constatar com certeza uma junção qualquer do espaço perinuclear com o espaço interno do retículo endoplasmático. A respeito dos mitocôndrios, algumas micrografias (Flecha, Fig. 15) indicam a possibilidade de uma junção entre a membrana mitocondrial externa e o retículo, principalmente no caso de mitocôndrias pericolunares. Os pequenos grânulos são, sem dúvida, equivalentes àqueles observados em fibras musculares de Vertebrados e comparáveis aos grânulos presumidos de RNP evidenciados em outros tipos celulares (10, 11, 12). Êsses grânulos estão distribuídos indiferentemente em todo o conjunto do sarcoplasma e podem assim encontrar-se tanto aplicados contra as membranas do retículo, ou mesmo no interior

das cavidades terminais como contra as mitocôndrias, as miofibrilas e as membranas nucleares.

DISCUSSÃO

Examinando a ultraestrutura de fibras musculares de funções diversas e provindas de espécies variadas, EDWARDS e colaboradores (20) observaram que o retículo endoplasmático, bem desenvolvido nas fibras de baixa freqüência de contração, estava relativamente reduzido nas fibras de alta freqüência. No caso presente de fibras musculares das peças bucais de carangueijos, com exceção do grande desenvolvimento do retículo, a estrutura é característica de fibras de alta freqüência: diâmetro pequeno, valor baixo da relação miofibrilas/sarcoplasma, abundância e distribuição ordenada das mitocôndrias, curto período da estriação, reservas figuradas do glicogênio. Tal se evidencia ainda mais fortemente da comparação com os músculos dos apêndices locomotores, cujas fibras musculares oferecem um tipo de estrutura completamente oposto (27). Todavia, a freqüência de batimento do flagelo do exopodito é baixa; medida por PAINLEVÉ e HAMON (34) em *Galathea strigosa,* por meio de um registro cinematográfico, corresponde nesta espécie a 8 batimentos por segundo. Estimando que numa espécie de comportamento mais rápido como *Callinectes danae,* êste valor seja maior, êle não excederá provàvelmente o duplo, logo ainda é muito pequeno em relação às fibras dos músculos do vôo dos Insetos que, com uma estrutura análoga, têm uma freqüência de várias centenas por segundo. Como explicar, então a existência de uma estrutura de alta freqüência em fibras musculares, tendo de fato, uma baixa freqüência de contração? Na realidade, é provável que a estrutura de uma fibra muscular seja mais característica da intensidade do seu metabolismo do que da sua freqüência de contração. Naturalmente, de uma alta freqüência resulta um metabolismo intenso com uma estrutura correlativa, mas uma fibra de baixa freqüência pode igualmente apresentar um metabolismo elevado e uma estrutura análoga. De fato, de acôrdo com a equação de HILL (35, 36): $E = A + ax + W$, a energia E liberada pela fibra muscular no curso de um "twitch" aparece com três formas independentes umas das outras: A, calor

de ativação, ax, calor de encurtamento, c (onde x é a taxa de encurtamento, e a, uma constante) e W o trabalho mecânico. No caso de um batimento, pode ocorrer compensação da baixa freqüência por um encurtamento importante e um trabalho mecânico elevado em cada contração da fibra. O valor de W aumenta muito com a carga encontrada pelo músculo; no caso presente, a carga corresponde à resistência oposta pela água aos batimentos do flagelo do exopodito; é evidente que esta é muito maior do que para batimentos efetuados no ar por um animal não aquático. Por outro lado, o flagelo, ao bater desloca-se no interior de um ângulo que pode atingir 130°, o que implica igualmente uma taxa elevada de encurtamento x das duas fibras musculares. Para estimar a validade desta hipótese, medidas do metabolismo das fibras musculares do exopodito estão em andamento e os resultados serão dados ulteriormente. Ainda que tais resultados forem em favor da hipótese, restará não obstante o fato de que, conforme os dados de EDWARDS, o retículo apresenta uma característica de baixa freqüência que não está em relação com os outros fatos de estrutura. Seria necessário, nesse caso, considerar que talvez apenas o desenvolvimento do retículo endoplasmático depende diretamente da freqüência de contração da fibra muscular, independentemente da intensidade do metabolismo.

Nas fibras musculares de Vertebrados estudados por PORTER e PALADE (21), o retículo é um sistema que comporta sòmente túbulos, vesículas e cisternas, formando como regais muito fenestrados em redor das miofibrilas. No caso presente, o retículo comporta além dos túbulos, vesículas e cisternas, outro tipo de estrutura que não se pode referir a estas formações. A parte do retículo que se estende em volta das miofibrilas em frente das zonas I e A distal é de fato uma parede contínua, formada de duas membranas justapostas, separadas por um espaço intermediário correspondendo ao meio interno do retículo. Não existem soluções de continuidade se se admitir que as pequenas rupturas ocasionais são alterações, como sugere a falta de regularidade da presença nas preparações. Trata-se, porém, de uma estrutura com "duplas membranas" que evoca a terminologia utilizada por SJÖSTRAND e colaboradores a respeito de "membranas intracelulares citoplasmáticas" (37, 38, 39 e 40) e de "citomembranas" (40). Mas aqui não são duplas mem-

branas independentes; estão em conexão com as outras partes do retículo endoplasmático, no conjunto do qual se incorporam. A ausência de fenestração nestas duplas membranas em frente da zona I e da zona A distal, modifica a noção de rêde introduzida pelo têrmo "retículo". Assim os regais de membranas dispostos ao redor das miofibrilas não são constituídos por uma rêde no sentido longitudinal, ao nível destas duas zonas. Todavia, apesar desta objeção, o têrmo de retículo pode ser conservado considerando-se que, no sentido transversal, as duplas membranas formam também uma rêde numa outra escala, contendo em cada malha, uma miofibrila.

Segundo os dados de PORTER e PALADE, os elementos tubulosos e vesiculosos do retículo são limitados por uma membrana simples, encerrando um conteúdo homogêneo sem estruturas. Não é o caso aqui presente para as formações em frente das zonas I. As cavidades terminais têm de fato uma parede formada de uma dupla membrana e o seu conteúdo é feito de sarcoplasma com pequenos grânulos densos. O têrmo de "terminal cisternae" não pode ser conservado para designar estas formações, apesar de que elas possuem um certo número de caracteres comuns com as vesículas da faixa I assim designadas por PORTER; sua localização por exemplo é a mesma e justifica o uso da palavra "terminal". Mas o têrmo "cisternae" implica numa noção de reservatório contendo um fluído e limitado por uma membrana simples, quer dizer, o fluido só pode ser a fase compreendida no interior do retículo. No caso presente, nos níveis terminais em frente da zona I, não há nenhuma dilatação das membranas do retículo determinando uma acumulação do meio interno dêste último. O que está encerrado aí nas cavidades limitadas por uma dupla membrana é outra substância, externa em relação à fase contida no retículo e para qual o têrmo cisterna não convém. Isto é ainda mais evidente considerando-se que existe neste mesmo retículo perto do disco H, "cisternae" proximais que correspondem à acepção clássica desta palavra. E precisa, portanto, distinguir as cavidades terminais como duas estruturas de natureza bem diferente, apesar de uma localização semelhante.

As formações que representam o retículo em frente do disco H são igualmente estruturas com duplas membranas, que não fazem parte dos tipos túbulos, vesículas e cisternas do retículo. Não há

nenhuma comunicação direta entre as três formações. Se existem relações entre elas, pelo menos, no caso das formações laterais, será pelo intermédio das cisternas proximais, com as quais estas podem ser conetadas. A questão desta junção é de um certo interêsse e é lastimável que ainda não esteja elucidada; com efeito, se esta junção não existe, se as cisternas proximais só se apoiam sem anastomoses contra as membranas duplas do disco H, estas últimas devem então ser consideradas como duplas membranas independentes, não incorporadas ao conjunto do retículo. Observações mais completas são necessárias para saber se há descontinuidade do retículo. No caso presente, os raros perfis de membranas das zonas largas de sarcoplasma não têm relações entre êles, não se reunem com a membrana celular e, aparentemente, não têm conexões com o espaço perinuclear. O retículo endoplasmático forma um sistema contínuo sòmente no interior de cada coluneta muscular, no sarcoplasma interfibrilar.

A periodicidade de organização do retículo endoplasmático, em fase com a estriação das miofibrilas e suas conexões com o sarcolema e o espaço perinuclear, deram ocasião a uma hipótese a respeito das suas funções prováveis na fibra muscular. Na primeira sugestão (21), o retículo funcionaria como um sistema de canais para o transporte dos metabolitos desde os espaços extracelulares até as diversas partes da célula e formaria, assim, uma espécie de aparelho circulatório intracelular. No caso das fibras musculares estudadas aqui, as largas zonas de sarcoplasma subsarcolêmicas e intercolunares são provàvelmente um lugar de acumulação importante de metabolitos, como o fazem supor as aglomerações densas de glicogênio que aí se encontram. Segundo esta hipótese, o retículo deve ter uma função dupla de transporte, de uma parte para os metabolitos destinados diretamente às miofibrilas, de outra parte, para os metabolitos acumulados no sarcoplasma. Isto implica em que, no sarcoplasma periférico, o retículo tem um desenvolvimento em volume pelo menos igual ao volume do retículo localizado entre as miofibrilas. A raridade das endomembranas neste sarcoplasma marca, ao contrário, uma muito grande desproporção em relação ao retículo tão abundantemente representado no sarcoplasma interfibrilar. Por outro lado, a ausência de continuidade do retículo entre o sarcolema e as colunetas musculares torna igualmente esta hipótese bem improvável aqui. Um

transporte de metabolitos pelo retículo endoplasmático seria todavia possível segundo a concepção de BENNET e PORTER (15), na qual o retículo não teria uma estrutura fixa, mas poderia variar de forma no curso da contração e efetuar migrações no sarcoplasma e nas miofibrilas. As poucas vesículas encontradas no sarcoplasma periférico poderiam então conceber-se como sendo móveis e contendo substâncias em trânsito. Como camada contínua de mitocôndrias subsarcolêmicas impõe um limite ao seu trajeto, o transporte sòmente se faria desde estas mitocôndrias até as mitocôndrias das colunetas musculares, o que se traduziria nas micrografias pelas imagens de junções de endomembranas com as membranas mitocôndrias. Todavia, subsiste sempre a objeção de uma enorme desproporção de volume entre o retículo interfibrilar e os raros perfis periféricos de membranas; é difícil conceber uma compensação para esta desproporção pela grande rapidez dos fenômenos de migração das vesículas periféricas no curso da contração.

A segunda hipótese sugere-se que há uma condução intracelular da excitação da fibra muscular desde o sarcolema até as miofibrilas, pelo intermédio do retículo endoplasmático. Esta idéia, expressa pela primeira vez no século 19 por RETZIUS (17), foi renovada, graças às observações em microscopia eletrônica, primeiramente por BENNETT (16), depois por outros autores (20, 21, 42, 43). No caso das fibras musculares dos caranguejos, a ausência de conexões entre o retículo intrafibrilar e a membrana celular como entre o retículo de duas colunetas musculares vizinhas, torna inconcebível a condução de uma onda de despolarização no interior da fibra. Se a condução intracelular da excitação pelas membranas do retículo endoplasmático pode ser considerada como provável no caso de músculos de Vertebrados (42), é preciso admitir aqui outro processo para efetuar a sua transmissão desde o sarcolema até as miofibrilas, através das camadas espêssas de sarcoplasma que circundam as colunetas musculares. Na realidade, existem importantes diferenças nas modalidades da inervação e nos mecanismos mioneurais entre as fibras musculares estriadas dos Vertebrados e as dos Crustáceos. Nos Vertebrados há em geral uma só terminação nervosa (placa motora) por fibra muscular e a contração nasce, com forma de um repuxo (twitch) do tipo tudo ou nada concomitante à propagação de

um potencial de ação. Em Crustáceos, há grande número de terminações nervosas distribuídas sôbre tôda a superfície da fibra muscular (44) e os músculos que podem responder aos potenciais de junção de valor baixo, produzem contrações muito potentes sem propagação do potencial de ação (45, 46, 47, 48). E' possível que estas diferenças de funcionamento estejam em relação com as diferenças de organização do retículo, verificadas entre as fibras musculares dos Vertebrados e as dos Crustáceos. Nestes últimos, a condução de uma onda de despolarização no interior da célula muscular não seria, pois, mais efetiva do que a passagem de um potencial de ação ao longo da membrana celular.

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RÉSUMÉ

Des coupes ultrafines de muscles provenant de pièces buccales de deux espèces de Crabes, ont été examinées au microscope élec-

tronique. Les principaux caractères de la structure de fibres musculaires sont rapportés avec une étude particulière du reticulum endoplasmatique. Ce dernier est constitué par un réseau de membranes localisé à l'intérieur des colonnettes musculaires, dans le sarcoplasme interfibrillaire. Dans chaque sarcomère, le reticulum se subdivise en trois régions de morphologie différente: 1) en face de la zone I et de la zone A distale, le reticulum est formé d'une double membrane continue formant comme un manchon autour de chaque myofibrille; 2) en face de la zone A proximale, le reticulum, comprenant des tubules et des vésicules en orientation longitudinale, est caractérisé par une discontinuité transversale; 3) en face de la bande H, formé par trois doubles membranes contigues, le reticulum semble affecté d'une discontinuité à la fois transversale et longitudinale. Le reticulum présente encore, au niveau des zones I, des cavités terminales limitées par une membrane double et contenant du sarcoplasme, et situées de part et d'autre de la bande H, des cisternes proximales. L'absence de connexion entre le reticulum des colonnettes musculaires et la membrane plasmique de la cellule musculaire est discutée en fonction des hypothèses actuelles sur le rôle probable du reticulum endoplasmique dans les fibres musculaires striées.

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EXPLICAÇÃO DAS PRANCHAS

Fig. 1 — Micrografia eletrônica com aumento pequeno de um corte transversal passando por duas fibras musculares do exopodito em Carcinus maenas. x 5.500.

Fig. 2 — Micrografia eletrônica de corte transversal da periferia de duas fibras musculares contíguas em Callinectes danae. x 15.000.

Fig. 3 — Corte longitudinal de uma coluneta muscular em Carcinus maenas. x 27.000.

Fig. 4 — Corte longitudinal na parte periférica de uma fibra muscular de Callinectes danae. x 22.000.

Fig. 5 — Micrografia eletrônica dando uma vista geral, em corte transversal, do retículo endoplasmático entre os limites de um sarcômero. x 45.000.

Figs. 6 a 9 — Micrografias eletrônicas de cortes longitudinais, correspondendo mais ou menos ao comprimento de um sarcômero.

Fig. 10 — Corte transversal de miofibrilas passando na maioria pela zona isotrópica em Callinectes danae. x 36.000.

Fig. 11 — Mesmo material que na fig. 10, mas o corte transversal passa na maioria pela zona anisotrópica. x 42.000.

Fig. 12 — Corte transversal ao nível de uma cavidade terminal do retículo endoplasmático. x 35.000.

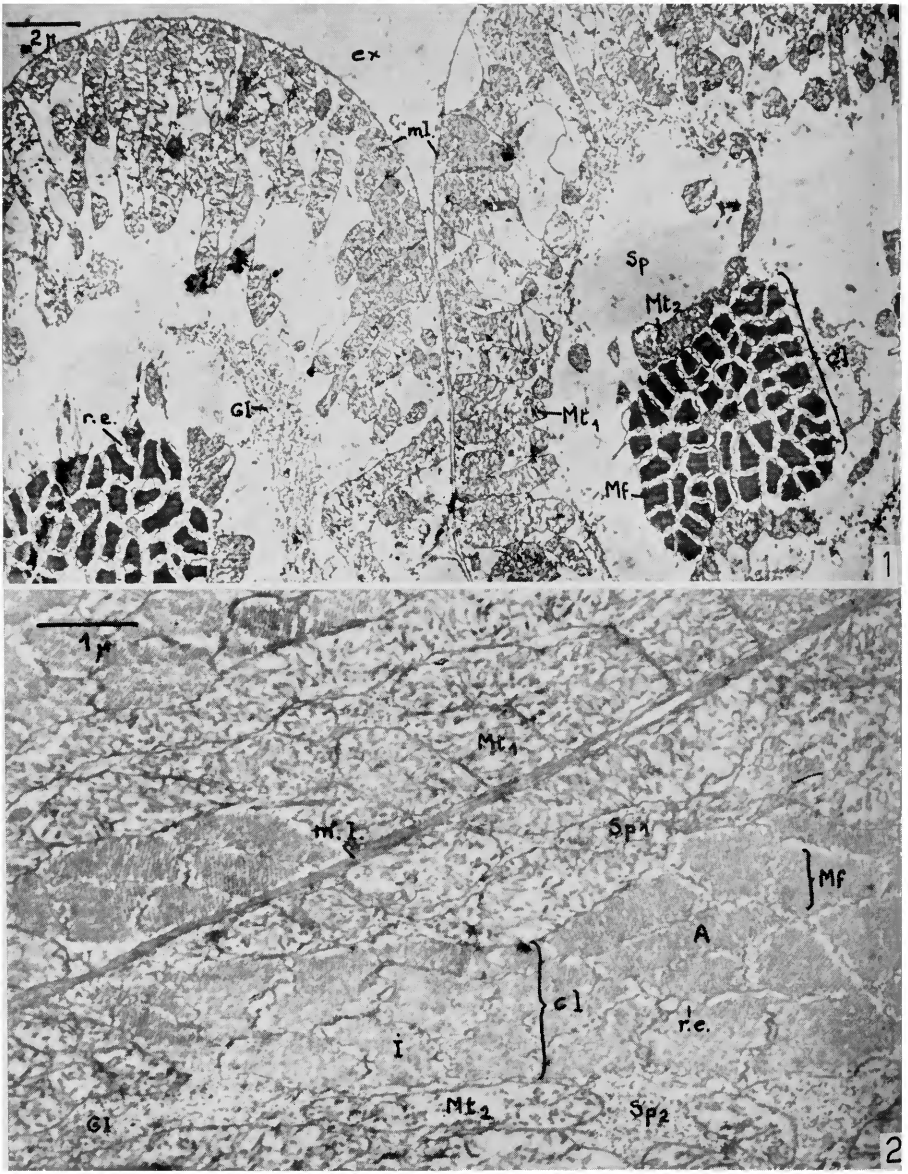
Fig. 13 — Corte transversal ao nível de cisternae proximais do retículo. x 39.000.

Figs. 14 e 14' — Cortes transversais passando pelas três membranas duplas contíguas localizadas em frente do disco H. x 61.000.

Fig. 15 — Micrografia eletrônica da zona subsarcolêmica em corte transversal de uma fibra muscular de Callinectes danae. x 41.000.

A: zona anisotrópica; cl: coluneta muscular; c. p.: cisterna proximal; c. t.: cavidade terminal; ex: espaço extracelular; Gl: acumulação de glicogênio; gr: pequenos grânulos densos; H: disco de Hansen; I: zona isotrópica; M: estria M; m. b.: membrana basal da fibra muscular; m. l.: complexo de membranas do sarcolema; m. p.: membrana plásmica da fibra muscular; Mf: miofibrilas; mf: miofilamentos; Mt: mitocôndrias; Mt_1: mitocôndrias subsarcolêmicas; Mt_2: mitocôndrias pericolunares; r. e.: retículo endoplasmático; r. $e._1$: retículo endoplasmático interfibrilar; r. $e._2$: perfil de membranas no sarcoplasma subsarcolêmico; Sp: sarcoplasma; Sp_1: sarcoplasma subsarcolêmico; Sp_2: sarcoplasma intercolunar; Sp_3: sarcoplasma interfibrilar; t: túbulos; y: forma particular do retículo em frente do disco H, com três duplas membranas contíguas; Z: linha Z.

Explicações mais detalhadas encontram-se no texto.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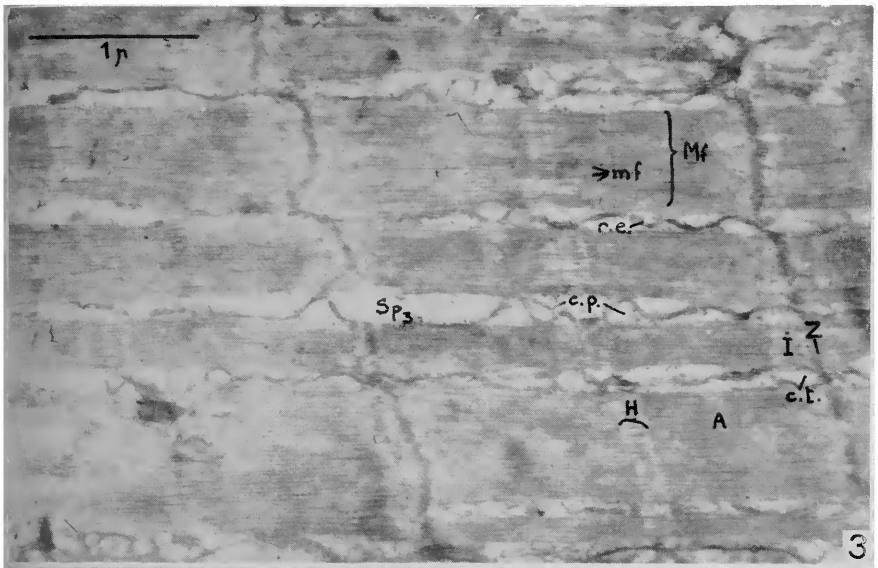
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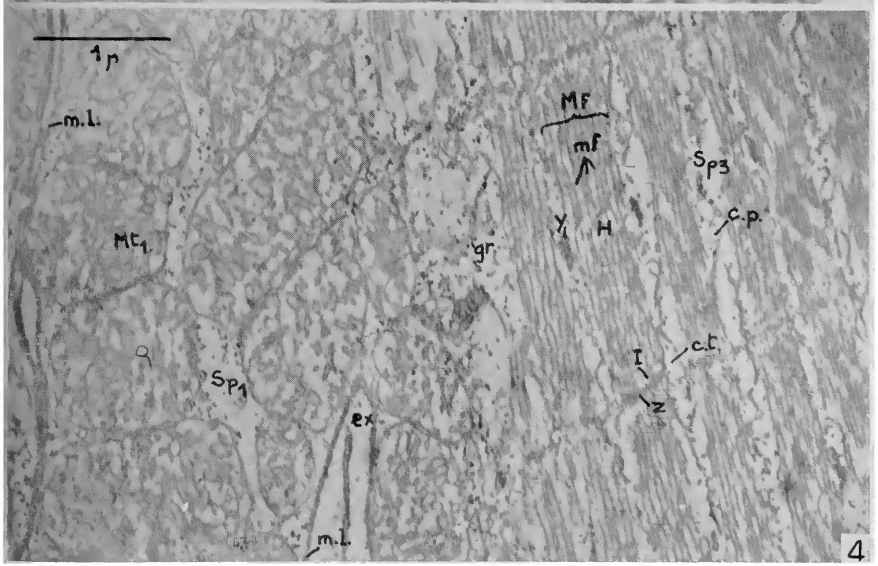
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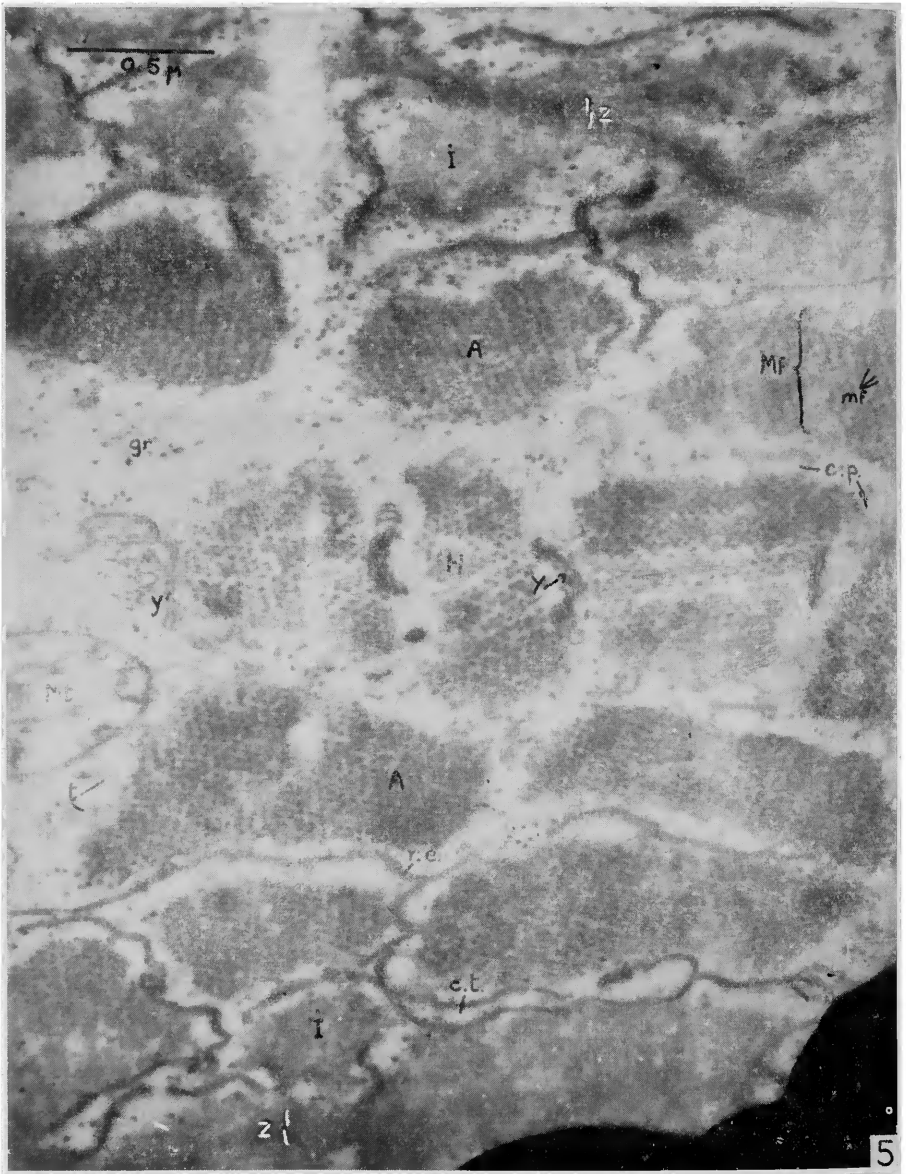
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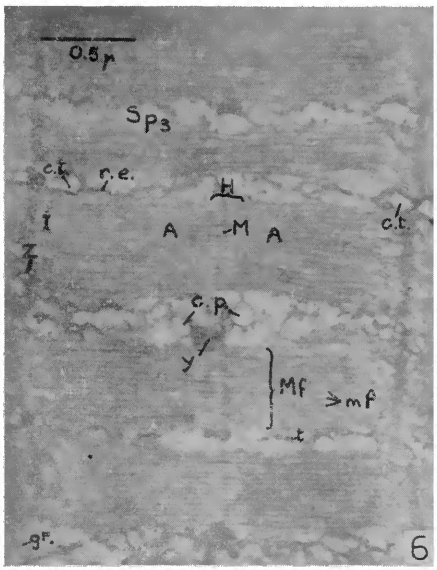
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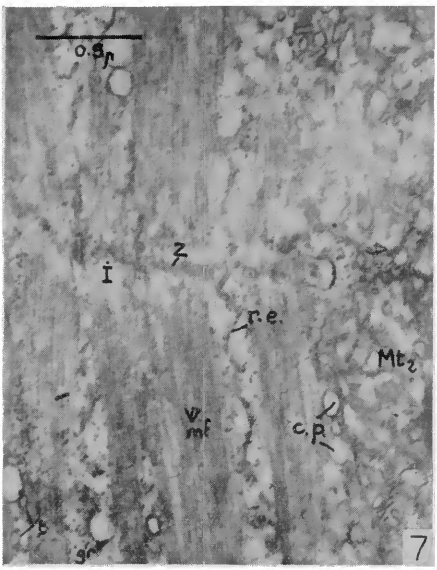
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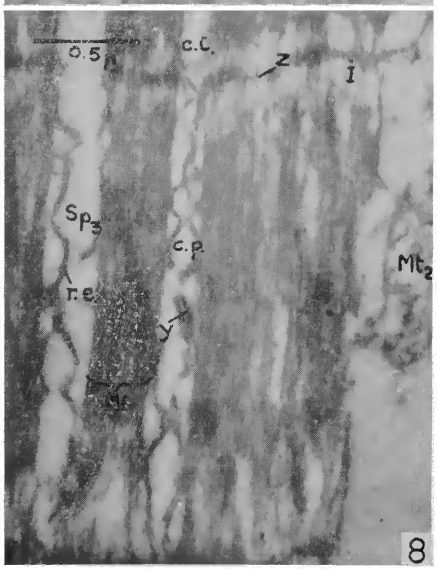
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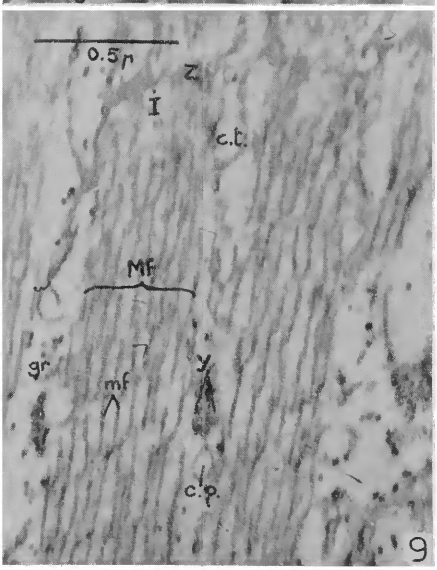
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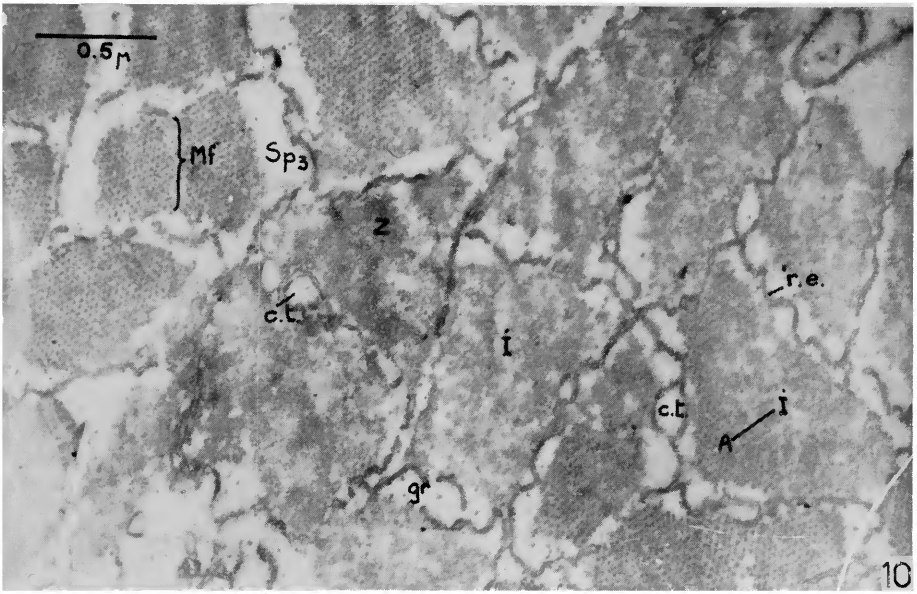

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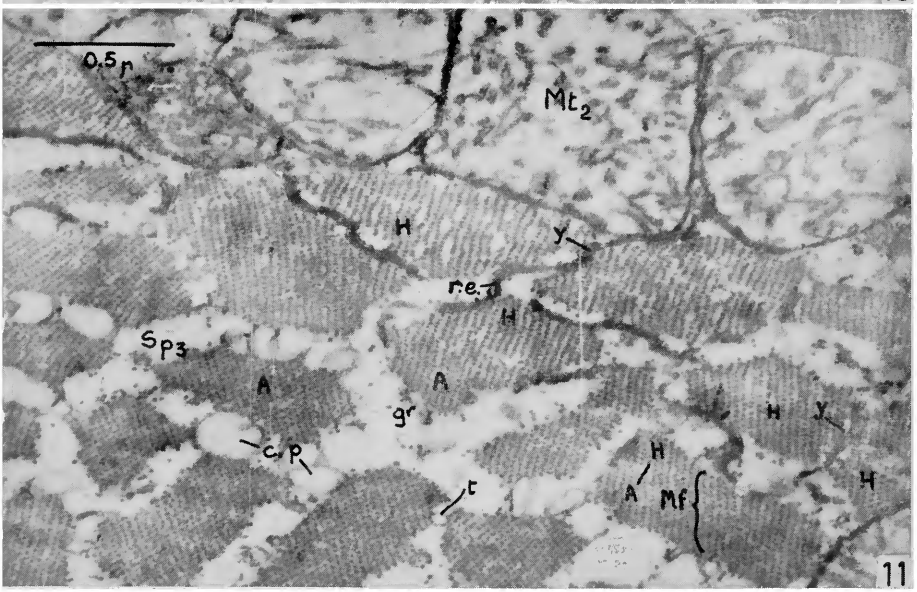

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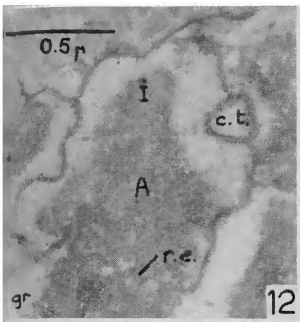
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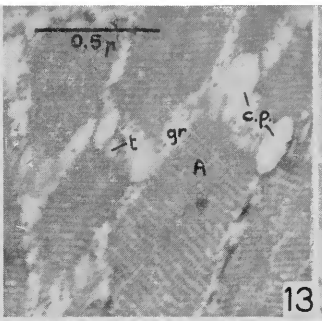
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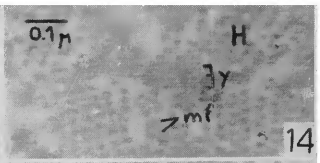
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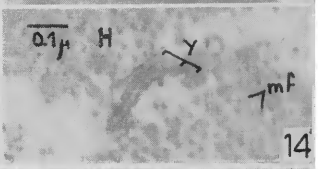
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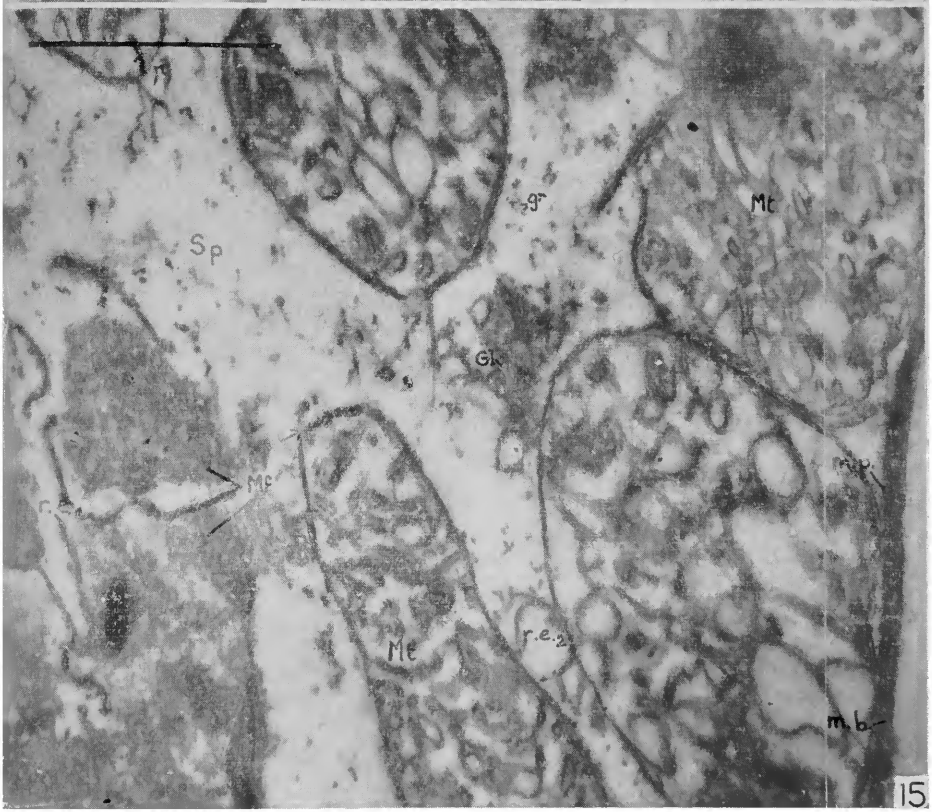
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ON TRICOLIA AFFINIS CRUENTA

by EVELINE and ERNST MARCUS

(with 6 plates)

Thanks to the Director of the Oceanographic Institute of São Paulo, Dr. Ingvar Emilsson, we could continue our studies on marine gastropods at the Base of Research, Bay of the Flamengo near Ubatuba (23° 27' S. 45° 6' W.), whose Head, our friend Dr. Edmundo Nonato, received us with habitual hospitality.

While collecting columbellids we found a trochacean in great numbers together with them. As the small Trochacea are anatomically little known, and this Super-Family is phylogenetically central, we decided to study these snails.

The species belongs to the Phasianellidae. According to Robertson's monograph of the Western Atlantic phasianellids (1958) it is the subspecies *cruenta* Robertson, 1958, of *Tricolia* (*Tricolia*) *affinis* (C. B. Adams, 1850). *T. concinna* (C. B. Adams, 1850) published two pages behind *affinis* is the same species. The references to *concinna* from the coast of São Paulo and Paraná (Lange de Morretes 1949, p. 62; Gofferjé 1950, p. 232) evidently belong to *affinis cruenta* (Robertson 1958, p. 268). The distribution of the subspecies comprises the Caribbean coast of northern South and Central America, ranging as far north as the Grenadines in the Lesser Antilles and south along the coast of Brazil to the State of Sta. Catharina. Sporadically it occurs on the shores of the western Gulf of Mexico (Robertson). The holotype was collected at the same place where we found the snails.

These live a little below the mean low water-line principally on *Sargassum cymosum stenophyllum,* but also on *Galaxaura stupicaulon, Acanthophora spicifera* and others. On *Padina* they are very rare. They inhabit mainly the lower regions of the *Sargassum* where the growth of Hydrozoa, Bryozoa, Tunicata and others is especially thick.

In July 1960 we found 40 two to seven millimeters long snails on a tuft of about 40 stems growing from one holdfast and up to 40 cm high.

SHELL AND OPERCULUM

The following description refers exclusively to the present material of several 1.000 snails of all sizes that we have seen.

Shell solid, ovoid, up to 8 mm in length, 4,5 mm in width. Colour pattern (Fig. 1) consisting in spiral rows of variously sized red spots. If these are small, 40-80 μ in diameter, there occur 32 rows or more on the body whorl; if they are big, 0,15 to 0,3 mm, there are about 20 rows. Between the red spots sometimes opaque white dashes lie over light ground colour. In worn shells the red dots appear as pits; severely worn shells are uniformly pink or quite white. The ground colour is olive or green to cream, or even dark purple. In front of the suture and at the periphery there may be whitish or darker, reddish, greenish or even dark brown axial blotches. Only over the whitish of these blotches occur the above-mentioned white dashes. The presutural and peripheral blotches may fuse, or zig-zag lines or variously shaped ones may occur. Among all our snails we found about ten melanistic shells with black dots on dark grey ground colour. Also the red dots on coloured ground may be substituted by black ones.

Whorls 7 1/2, two of which belong to the white depressed protoconch. The first whorl in front of the protoconch is dark with fine axial pink pattern. Whorls inflated, evenly rounded and smooth. Spire produced at an angle of from 45° to 90° (Fig. 3). Aperture oval, outer lip prosocline. Columellar callus moderately thin, white. Umbilicus of adult shells reduced to a chink. Suture impressed. Operculum (Fig. 4) white, dark olive-green or brown at the margin, paucispiral (oligogyrous), nucleus nearly marginal.

Robertson stressed "regularly spaced large red spots" and "usually low spire (65°-75°)" as characters of the subspecies, but these are not constant. The low spire was already restrained by Robertson who found an elongate spire (about 50°) in two specimens from Pernambuco. In big shells of our material the spire is higher than in smaller ones. In spite of these restrictions we maintain *cruenta* as a subspecies, because its combined characters allow for a separation

from Robertson's other subspecies of *affinis*. Among these only *T. a. beaui* overlaps the range of *cruenta* in the southernmost Lesser Antilles and differs by colour pattern and spiral striae on all postnuclear whorls.

The smallest preserved shell (Fig. 2) is 0,3 mm long and 0,5 mm in greater diameter. It has 2 white nuclear whorls and pale red dots on the following half whorl. The latter, and in older shells the entire first postnuclear whorl, bears convex spiral lines separated by furrows. The apex of young shells is quite, flat, nearly insunk. The angle of the spire is 130° and more. The umbilicus is a deep hole. The operculum is imperforate unlike that of *T. bella* (1958, p. 250, 274), and circular, 0,18 mm in diameter. As it has 4 whorls, it corresponds to the centre of the 0,45 mm long operculum of Fig. 4. The youngest operculum is not calcified yet; its outer surface is slightly depressed so that in this special case the centre is thinnest, and the periphery with its spirals thicker.

Juvenile shells agree with a not yet analyzed species of *Gabrielona* from the Island of São Sebastião (*Eulithidium brevissimum* in Lange's catalogue, p. 62) in several characters (Robertson, p. 259). Therefore we examined radulae (Fig. 8) and opercula of our young snails (Fig. 5) and found dentate (in *Gabrielona,* smooth) lateral teeth and opercula with generally smooth outside (in *Gabrielona,* spirally ridged externally) and thickest in the centre (in *Gabrielona,* thinnest).

JAWS AND RADULA

The radula (ra) works against a pair of jaws (j) which are colourless and united dorsally by cuticle. The mandibular plates are entire, not made up of two portions (Randles 1904, p. 49; Frank 1914, p. 433) as in some trochids. As the jaws are composed of rather flat lying rods, their surface appears scaly. These scales are most conspicuous and jutting in front and in the middle, smaller and flatter on the sides and behind.

The adult radula (Fig. 6) has 43 rows of 58.4.1.4.58 teeth. The rhachidian tooth is a nearly rectangular or more or less rounded plate with smooth borders and without any cusp. The lateral teeth are strongly dentate. They have a principal cusp and inner as well as outer

denticles on the 2 inner, only outer denticles on the 2 outer teeth. The numbers of these denticles are: on the innermost lateral tooth 4 inner and 4 outer ones, on the second 4-5 inner and 4 outer ones, on the third 4-6, and on the fourth 4 outer denticles.

The innermost tooth is broad and has an external wing. Robertson (p. 247) considered this dilatation as a character of Eastern Pacific species of *Tricolia*. Its appearance in our subspecies of *T. affinis* which occurs farthest towards the West, from Colombia (Cartagena) and Panama to Honduras, Mexico and Texas, may point at the Tertiary sea-connection in Central America. The base of the second lateral tooth is roundish. The cusp of the third is specially broad. The base of the fourth is long and expanded on the inner side.

We cannot confirm the existence of a "lateromarginal" plate (Robertson, p. 247) without cusp between the outermost lateral and the innermost marginal tooth and think it must be eliminated from the diagnosis of the subgenus (p. 261). This plate is actually the base of the innermost marginal tooth (Fig. 7). Powell (1951, p. 88, 105) stressed the difficulty to interpret the rhipidoglossid radula owing to the large number and the intricate overlapping of the teeth. The cusp of the first marginal tooth bears 5 outer denticles; that of the three following teeth is broader. From the fourth marginal tooth outwards the cusps decrease. The number of denticles augments to 12 and they are longer and thinner. The outermost marginal teeth are thin and curved plates.

The radula (Fig. 8) of a quite young snail with 0,6 mm long shell has about 17 rows of 11.2.1.2.11 teeth. The median tooth of the oldest rows has a complete cusp with up to 5 denticles on either side. In the younger rows the denticles disappear gradually, and the cusp shortens. The difference between lateral and marginal teeth is less pronounced than in the adult radula. The inner marginal teeth have pointed cusps with 8 outer denticles.

COLOUR, HEAD, EPIPODIUM AND FOOT

The colour of the flesh is green, especially intense on the roof of the mantle cavity. The epidermis bears spots which may be red, brown or black. Some snails have a uniform dark skin. Lighter or darker skin is not correlated with the tone of the shell. The upper

side of the foot is striped or spotted, the sole always light. The green colour is dissolved in alcohol, not the red spots.

The head is distinctly separated from the foot (f) and has a pattern of symmetrical black spots in some specimens (Fig. 10). The cylindrical tentacles (t) are pointed at the end and beset with two rows of sensory papillae which resemble those of *Incisura lytteltonensis* (Bourne 1910, p. 29, pl. 5, f. 27, 28). Equal papillae are rare on the stalks of the eyes (ee) and much more numerous on the epipodial appendages (ei). The cilia of all these papillae are immobile as Robertson (1958, p. 249) indicated correctly.

The eyes are highly developed for an archaeogastropod. They are closed as in the mentioned *Incisura* (l. c., p. 4), fissurellids and turbinids, not open as in the trochids. The shape of the eyes is globular, and they contain a refractive vitreous body. They lie immediately under the epidermis, without a separating precorneal blood sinus, so that inner and outer pellucid (corneal) layer are contiguous in the middle over the convex vitreous body. As in other archaeogastropods there is no lens differentiated within the vitreous humour. The retina agrees with that of *Astraea rugosa* (Hesse 1902, p. 580), but the roundish nuclei of the sensory cells are basal, the longish ones of the pigmented cells are more apical as in the trochid *Gibbula cineraria* (Frank 1914, p. 471-472, text-fig. 55), and contrary to the turbinid studied by Hesse (pl. 35, f. 10).

In the feeding snail mandibles and radula can be seen in ventral view. As in *Tricolia pullus* (Pelseneer 1899, p. 46) the left (ce) and right (rc) cervical (suborbicular) epipodial lobes are different. The left of *T. affinis cruenta* is pectinate, like the "siphon d'entrée" (l. c.) of the branchial side in trochids, the right one is entire, like the "siphon de sortie" of the anal side. In *Tricolia bella* only the pedunculate and digitate left cervical appendage is developed (Robertson 1958, p. 249). The left side comb of *T. affinis cruenta* has up to 7 projections. It does not alter its position, even if sediment is added to the water that is to be inhaled, nor did two nematodes moving about the inhalant opening provoke any noticeable reaction of the comb. This organ is innervated (Fig. 13, ce) but less muscular than the posterior epipodial tentacles (ei).

The latter are symmetrical structures, one in front of and one behind a fold (oe) beset with sensorial papillae. This lateral fringe is continued into a dorsal fold (oe) which hems in the anterior half of the operculum (oc), i. e. anterior in the creeping snail (Fig. 11). The epithelium that lies on the operculum is much higher than that of the back of the foot under the conchinous opercular base. Therefore we suppose that the high epithelium of the pouch secretes the lime of the operculum.

The sole of *T. affinis cruenta* (Fig. 9) does not have the longitudinal furrow that exists in *T. pullus* (Fretter 1955, p. 159) and other species of *Tricolia* and *Phasianella* (Robertson 1958, p. 25). The entire sole and the lateral borders of the foot bear mucus glands. The anterior pedal border is grooved transversely. From the middle of this anterior furrow a 0,18 mm long canal (wz) pierces the foot. Blue-staining glands open into the transverse groove as well as into the canal in its middle. Several trochids have similar structures (Randles 1904, p. 38; Frank 1914, p. 388-389). Mucus glands are accumulated near the posterior border of the foot, where their thick clusters (Fig. 11, oa) open into a shallow median groove.

LOCOMOTION

Corresponding to the undivided sole the muscular locomotion is only sometimes allusively ditaxic. We compared the available trochid *Tegula viridula* (Gm.); it protrudes the two halves of the sole alternately as *Trochus* (Weber 1924, p. 110). In *Tricolia* the position of the anterior border of the foot in relation to the shell is constant when the snail moves forward. It does not move by jerks as we know it of diggers (Olividae, *Hastula*) and climbers (*Anachis*). Our *Tricolia* did not move much about at the temperature of 20°C.

One can watch the snails gliding on the under side of a slide or on the surface film. Here they move by ciliary action on a ribbon of mucus (Kaiser 1959, p. 379) which begins at the anterior border of the foot whose glands were described. The glands of the posterior groove contribute to the ribbon of mucus securing the snails. When the snails glide on the surface film, parts of the sole shift their posi-

tion against others by muscular action. According to Kaiser (p. 375) this secures the contact of the foot with the surface.

If removed from the substratum our snails fall onto their aperture or onto their back. In the glass dish they cannot recover in the latter case nor if they are tilted a little to the right side. They try to attain the substratum, but the short foot is not sufficiently distensible. This insufficience has probably no biological significance, as in the upper littoral the waves will soon roll the snails into a more favourable position with the inner lip nearer to the ground. Laid into the dish in this position the snails feel for a substratum with the anterior border of the foot. According to Weber (1926, p. 393) this is the simplest recovering action of prosobranchs. *Tegula viridula* behaves like *Tricolia affinis; Trochus* searches a hold and fixes itself preferably with the hind tip of the foot (l. c., p. 425-432). If the cephalic tentacles or the anterior pedal border of *Tricolia* come into contact with an object, e. g. the point of a needle, the border grasps it and draws the entire snail after. An equal stimulus touching the epipodial tentacles does not effect any visible reaction. In *Trochus* the epipodial tentacles are tactile (l. c., p. 427).

PALLIAL CAVITY

To the right of the middle the suprapallial border is somewhat recessed (mn) in front of the anus (ar), perhaps a vestige of the slit of *Incisura* (Bourne 1910, pl. 1, f. 2-4, m. s.) which disappeared with the loss of the right gill. An infrapallial narrow glandular fold (xu) runs between shell and operculum in the moving snail as in trochids (Randles 1904, pl. 6, f. 40, ma). The entire border of the mantle bears glands. In front and on the right side, that is under the growing edge of the body whorl, the glands (g) are especially numerous. This increase is brought about by 0,3 mm long ciliated tubular invaginations of the epithelium that lines the mantle furrow. The functioning glandular cells lie near the entrance of the tubules. These are parallel (Fig. 11, g) to the roof of the mantle, perpendicular to its edge (Fig. 12, g) and contiguous with one another. Similar structures occur in the trochid *Monodonta turbinata* (Frank 1914, p. 395-396) and with Frank we suppose that these glands se-

crete the shell or at least its periostracal conchiolin (Graham 1957, p. 136).

A big vessel (ve) accompanies the glands of the mantle furrow and is connected with the system of the efferent branchial vessel (ev). It comes from the lacunae in the foot, runs around the periphery of the mantle where its blood evidently becomes oxygenated. The vessel passes the osphradium (os) and enters the efferent branchial vessel. There are also vessels which come from the anterior margin of the mantle and enter the anterior pallial vein or the transverse pallial vein and therewith the afferent branchial vessel (ac). While these are frequently mentioned for the circulation of trochids (Robert 1900, p. 407; Randles 1904, p. 56-57; Fleure and Gettings 1907, p. 468; Frank 1914, p. 411), the vessel that comes from the roof of the pallial cavity and goes back to the left auricle through the efferent branchial vessel was only recorded by Fleure and Gettings (l. c., p. 468-469). It evidences a respiratory function of the mantle roof.

At the passage of the upper into the under mantle border a band of red staining glands runs into the mantle cavity and accompanies the suture on both sides to an extension of 1,2 mm. A vessel beneath the right glandular band can be followed nearly to the right auricle; it represents a vestige of the right efferent branchial vessel discovered by Thiele (1897, p. 641) in *Gibbula cineraria* and found in several trochids by Robert (1900, p. 405), Randles (1904, p. 57), Fleure and Gettings (1907, p. 467-468) and Frank (1914, p. 410). The last author found and described (p. 470) as glands several bulges in the right angle of the mantle cavity of *Gibbula cineraria,* but identified them with Thiele's sensory organs (1897, pl. 31, f. 8, so). Of this type occurs only one organ (Fig. 13, no) on the left side of the pallial cavity of *Tricolia affinis cruenta,* beside the button-like osphradium (os).

The size of the osphradium in relation to the ctenidium is not bigger than in *Tegula viridula* (Gm.), a trochacean common on rocks in the same region. According to Hulbert and Yonge's view (1937) that the osphradium is a tactile organ concerned with estimating the amount of sediment carried into the mantle cavity one would have expected a bigger osphradium in *Tricolia* than in *Tegula*. Mr. Edmund H. Smith, M. A. of Los Angeles who is at present working in

this Department on *Drupa nodulosa* (C. B. Ad.) and *Thais haemastoma* (L.) kindly informed that the first of these purpurines goes farther into turbid waters than the second and has a proportionally bigger osphradium.

The hypobranchial gland (y) of the present species is weakly developed. It lies to the right of the rectum over a pallial vein. In *Tricolia pullus* there is glandular tissue also to the left of the rectum (Fretter 1955, p. 161). Perhaps a strand of gland cells between anterior mantle border and osphradium can be considered as the left hypobranchial gland of *T. affinis cruenta,* though this gland lies much farther behind in *T. pullus*. Clark's table (1958, p. 61) shows the variation in the topography of the hypobranchial gland in Trochidae and Turbinidae as well as a correlation between an arcuate or straight course of the rectum and the development or absence respectively of a right hypobranchial gland. Also in the present species the right hypobranchial gland is associated with a more or less curved rectum.

The tip of the ctenidium (b) projects from the mantle cavity in some of the preserved snails. On the lower side there are about 50 lamellae hanging free into the cavity, whilst on the upper side they are a little less numerous. They are not placed symmetrically (Fig. 11, b). The hind part of the upper row is enclosed in a chamber formed by the roof of the pallial cavity and the afferent (am) and efferent membranes. These hold the gill for the greater part of its length as in *T. pullus* (Fretter 1955, p. 161). As in this species and contrary to three turbinids (Yonge 1947, p. 475; Clark 1958, p. 62) there is a weak supporting rod (or) in the free extremity of the ctenidium. Also the skeletal rod which bounds the branch of the efferent vessel within the leaflet occurs in our species as in the other Trochacea. Near the attachment to the rhachis every branchial leaflet has a small nick (ni), and these nicks "collectively form a groove which runs the length of the gill" (Clark 1958, p. 59). This feature noted by Clark in *Haliotis, Scutus* and numerous New Zealand trochids and turbinids has been object of a special study of Hatt (1927) who found it in many rhipidoglossan Diotocardia. Bourne (1910, p. 7) seems to be the first to have described it in *Incisura lyttelto-nensis;* he considered (p. 8) the cells of the ridge as secretory. Hatt

observed that they absorb Indian ink and dyes. Clark verified that they are not concerned with sediment disposal.

The branchial filaments on the lower side of the ctenidium are much more efficient to produce the inhalant current than those on the upper side. As in *Turbo* (Yonge 1947, f. 34 B on p. 498) there is also a forwardly directed axial current, carrying sediments from the left filament to the tip, besides the principal current from left to right. The axial current is intermittent. On the floor of the mantle cavity from the hind end forwards runs a streak of cilia (Fig. 10, ci). These beat upwards and forwards leading sediments dropped onto the floor into the exhalant current. Frequently these cilia discontinue beating. Functionally they correspond to those that produce the rejection current of many pectinibranchs and carry sediments along the floor of the mantle cavity (Yonge 1937, p. 693-694; 1938, p. 454-455; 1942, p. 200-201; 1947, p. 479). In sections of a small, 1,6 mm long snail the streak is 1 mm long and 0,1 mm broad. Generally the Trochacea live in clean water on rocks and reefs (Yonge 1947, p. 455). To the exceptions mentioned by Clark (1958, p. 59), trochids found on mud banks and fine sand flats, belong also the species of *Tricolia,* inhabitants of algae and grasses (Fretter 1955, p. 159; Robertson 1958, p. 252). In the bay where our abundant material occurs, sedimentation is intense in the rainy season. So a streak of special cilia under the gill seems to be functionally important.

NERVOUS SYSTEM

In its fundamental traces the nervous system of *Tricolia affinis cruenta* (Fig. 13) agrees with that of the Trochidae (Robert 1900; Randles 1904; Franck 1914), Turbinidae (Bouvier 1887) and, as far as studied, *Phasianella variegata* Lm. (Risbec 1939) whose name to-day is *Ph. rubens* Lm. (Robertson 1958, p. 249). It differs from that of trochids and turbinids by two ganglionar thickenings, one on each side of the abdominal ganglion (ao). Risbec has drawn a single right side ganglion in the supra-intestinal branch of the visceral loop (pl. 6, f. 64) and lettered it sb, suggesting a subintestinal ganglion. In the text (p. 286) however he called it visceral, i. e. abdominal ganglion. His topographic indication "à côté de l'orifice rénal droit"

shows that it corresponds to the abdominal ganglion of the present species.

The cerebral ganglia (ze) of *T. affinis cruenta* are voluminous; the tentacular and optic nerves have separate origins. The long and thin labial commissure (cm) goes out from a large ventrally directed labial lobe of each cerebral ganglion. The cerebral commissure (cz) is broad and posterior to the labial one. Independent from the latter the cerebro-buccal connectives come out from the cerebral ganglia as in *Phasianella rubens* (Risbec 1939, p. 286). The connectives are directed ventrally, they as well as the rather long buccal commissure have no nerve cells. The buccal ganglia (uc) are longish and angled, similar to those of *Turbo setosus* (Bouvier 1887, pl. 2, f. 5, B). They lie dorsal to the radula, ventral to the oesophagus, between these organs. Odontophore, salivary glands and crop are innervated by nerves from the buccal ganglia and the cerebro-buccal connectives.

The cerebro-pedal connectives (co) are shorter than the cerebro-pleural ones (cu). The latter are more lateral and include the static nerve. The pleural ganglia (ur) are fused to the dorsal surface of the pedal ganglia (en) and flank the big statocysts (sz) which contain numerous otoconia. The pedal ganglia are contiguous in the mid line. Each of them gives off a pair of anterior nerves which branch farther in front. Posteriorly each ganglion is continued into a broad cord (wo) coated with nerve cells. From the beginning of each cord spring two lateral epipodial nerves and farther behind several nerves principally to the ventro-lateral parts of the foot. The cords as well as the pedal ganglia are undivided, without a horizontal furrow. Three thin commissures connect the right and left cord. The purely pedal character of the cords first vindicated by Spengel (1881, p. 345) and Pelseneer (1899, p. 49) has been settled by Randles (1904, p. 61-64).

The pleural ganglia (ur) emit the branches of the visceral loop (ei, sv) and the mantle nerves (ne). The latter contain fibres which supply the two columellar muscles (mr, rr), run to the roof of the pallial cavity and unite on the anterior mantle border. A short nerve (ea) connects the nearly coalesced supra-intestinal (iu) and branchial (wa) ganglion with the left pallial nerve. This left zygosis is developed in most Trochacea, but is absent in the calliostomine *Photinula,*

now *Photinastoma, taeniatum* (Frank 1914, p. 461). The occurrence of one, exceptionally two (l. c., p. 459) right zygoses between subintestinal branch of the visceral loop and right pallial nerve is irregular in the Trochacea. Bouvier (1887, p. 32, pl. 1, f. 2, z^1) and Robert (1900, f. 500) found the right zygosis in Turbinidae and Trochidae respectively. Frank (1914, p. 459) verified it in two of his trochids and stated its absence in the third, *Photinastoma taeniatum* (Wood, 1828). Randles (1904) had none in his numerous trochids, and also *Tricolia affinis cruenta* has no right zygosis.

Between the branchial ganglion (wa) and the osphradium (os) lies the above-mentioned sense organ of Thiele (no). The abdominal ganglion is located in the fundus of the pallial cavity between right kidney and right columellar muscle. One of its nerves (on) goes to the gonopore, a second (ma) to the gut where it could be followed to the entrance of the oesophagus into the stomach.

Both branches of the visceral loop contain numerous nerve cells, as was observed by Frank (1914, p. 461). An accumulation of such evidently produced the sometimes developed ganglion in the supra-intestinal branch of *Phasianella rubens* (Risbec 1939, p. 286, pl. 6, f. 64, gn). In *Tricolia affinis cruenta* there are two such accumulations in the subintestinal branch, a right one in the right columellar muscle, and a left under the pallial floor. The nerve that connects this ganglion with the supra-intestinal loop runs under the above-mentioned streak of cilia (Fig. 10, ci). The occurrence of three visceral ganglia resembles the disposition in *Incisura lytteltonensis* (Bourne 1910, p. 27, pl. 1, f. 5), but the ganglia are not homologous.

SHELL MUSCLES

The present species has two columellar muscles (Fig. 14, mr, rr) as Fretter (1955, p. 159-160) discovered in *Tricolia pullus*. The right muscle is longer, thinner and attached to the spindle at the level of the hind end of the mantle cavity. The left muscle is shorter and thicker; its insertion lies farther in front, short behind the level of the osphradium. Also in *Incisura lytteltonensis* the right muscle extends farther back (Bourne 1910, p. 5). In the present state of knowledge (Crofts 1955, p. 740) this right muscle is the post-torsional typical columellar muscle. The presence of a left muscle is not

a novelty but a maintenance of the velum retractor muscle. It is generally considered as a functional adaptation to flattened shells, e. g., Hipponicidae and Cypraeidae. Though the body whorl is enlarged in *Tricolia,* its shell has attained full dextral coiling, it is not flattened, but has a conspicuous spire, and the animal can withdraw completely into the shell. Therefore its two shell muscles appear as a historical reminiscence which can be expected to be found also in other Trochacea.

Among the monobranchiate Aspidobranchia two columellar or shell muscles were hitherto only observed in *Tricolia* and the neritids. According to Bourne's opinion on the origin of the Neritacea quoted and shared by Yonge (1947, p. 492) the two muscles may perhaps be understood as characters which *Tricolia* and the Neritidae have retained independently from zeugobranchiate ancestors. To Robertson (1958, p. 249) it appears unlikely that these muscles indicate any close relationship between the two groups. Bourne's view concerning the origin of the Neritacea from the Zeugobranchia was not generally accepted. Thiele (1935, p. 1083) derives the Neritacea from the Trochacea, and in this sense the two muscles are certainly significative, as Fretter (1955, p. 160) suggested.

ALIMENTARY TRACT

The mouth (Fig. 11, m) is followed by a short oral tube which leads into the buccal cavity (mo). At the entrance of this cavity the mandibles (j) lie on the dorsal and dorso-lateral sides. The floor is occupied by the buccal mass whose anterior ventral and lateral parts are surrounded by diverticula of the cavity. The outer walls of the lateral diverticula are glandular. The buccal mass consists of a muscular sac containing the odontophoral cartilages. The muscles are transversely striated as described by Bourne (1910, p. 18, pl. 3, f. 15), Frank (1914, p. 399) and others. The cartilages are in two pairs whose dorsal mid line is occupied by the radula (ra). The long anterior cartilages (ca) are slightly concave on their inner side, the short posterior ones (sc) concave in front and firmly apposed to the posterior end of the anterior cartilages. These features are similar to those described by Amaudrut (1898, p. 57, pl. 4, f. 33) and Risbec (1939, p. 283, pl. 6, f. 70, not 71) for *Turbo setosus* and *Phasianella*

rubens respectively. The anterior end of the radula and its cushion lies under the jaws. The posterior end of the radula evidences the effect of torsion (Pelseneer 1899, p. 51; Randles 1904, p. 50) twisting around the cartilages and the oesophageal pouches towards the dorsal side (Fig. 15). Its slightly bifid blind end appears apposed to the topographically right wall of the oesophagus as in *Phasianella rubens* (Risbec, l. c.).

The oesophagus (o) begins rather far in front in the dorsal part of the buccal cavity at the level of the cerebral commissure (Fig. 14, cz). A little farther behind the tubular and simple salivary glands (sa) open into the oesophagus. In *Turbo porphyrites* (Risbec 1939, p. 271) and according to Risbec (1955, p. 47) generally in the Turbinidae the salivary glands are also inconspicuous and open immediately behind the cerebral commissure. Behind the entrance of the salivary glands the roof of the oesophagus is dilated transversely in *T. affinis cruenta* (Fig. 15), forming what Frank (1914, p. 431, 444) called "Dachfurche" in trochids. On both sides of the "Dachfurche" dorso-lateral diverticula (au) are developed whose fundi contain glandular epithelium. A glandular fold (zu) arises from the floor of the oesophagus and projects into the lumen as a principally backwards directed languet (Risbec 1939, p. 284: *Phasianella rubens*). Glandular are also the walls of the entrances of the posterior lateral oesophageal pouches (ou). These thin-walled diverticula communicate with the central lumen over a long stretch. Their walls form glandular papillae on their whole inner surface (Fig. 15). Farther behind the pouches unite over the oesophagus forming a crop. Transverse sections reveal the effect of torsion on this part of the anterior gut (Fig. 15) as Amaudrut (1898, p. 189-190, 265) and others described it for *Turbo* and other genera of the Trochacea. The oesophagus is displaced to the left and ventral side, thence also the left part of the crop attains a ventral position. The right part is turned dorsally and lies over the buccal mass. Unlike to the trochids (Robert 1900, p. 392, f. 499) the right pouch of *T. affinis cruenta* is not much bigger than the left.

The thin oesophagus leaves the hind end of the left pouch of the crop, courses under the floor of the mantle cavity and passes into the posterior visceral cavity. Its course is rather straight; its epithe-

lium contains numerous blue staining, high, cup-shaped gland cells. The oesophagus comes from the left and enters the stomach (s) from the ventral side near its middle. One of the ducts (l) of the brown intestinal gland opens into the oesophagus short before its entrance into the stomach, the other into the stomach itself, a little farther behind.

As Fretter (1955, p. 161) observed, the stomach of *Tricolia* is similar to that of the trochid *Monodonta lineata* (Graham 1949, p. 747, f. 17). Different from *Phasianella rubens* (Risbec 1939, p. 285, pl. 7, f. 75, cs) the caecum of *T. affinis cruenta* is wide and not spiral. It contains the major typhlosole (ua) on the topographically right border of the intestinal groove (io). Where this groove is most distinct in transverse sections, it is formed by low cells which lie between the high, apically striated ones of the general gastric epithelium (Randles 1904, p. 54; Bourne 1910, p. 21). To the left of the groove come the ridges of the posterior sorting area (za) alongside of which runs the longitudinal fold (xo) that corresponds to the structure F in Graham's figures (1949). Farther in front the groove is bordered to the left by the minor typhlosole (ui). The two typhlosoles with the groove between them enter the topographically anterior tubular part of the stomach, the style sac, and continue along the whole intestine. For its most part the gastric wall is lined with a cuticle which is raised into a long and thick gastric shield (si) to the right of the major typhlosole. Evidently the cuticle of the shield is renewed from time to time, as it is quite loose in some snails (Fig. 16) and missing in several others. Also Risbec (1939, p. 285) found the cuticle loose in *Phasianella rubens*. The topography of the mucus-producing pouch (mu) and the gastric shield is somewhat different from that in *Monodonta lineata*. We compared the stomach of *Tegula viridula;* it has a long spirally coiled caecum as that of *Calliostoma conuloide* (Graham 1949, f. 18) and is also in further details similar with the latter.

The caecal end of the stomach of *Tricolia affinis cruenta* is apposed to the outer side of the visceral mass; the style sac to the floor of the mantle cavity. The snails browse on the surface of algae covered with detritus. The contents of the stomach are grey, evidently digested masses, in some cases mixed with mucus and including diatoms and animal material as eggs and Foraminifera, and sometimes stones.

At its egress from the style sac the intestine (Fig. 16, i) is directed to the topographically right side. Shortly after it curves to the left and runs backwards on this side. Then it bends to the right and passes through the ventricle (Fig. 12, v) near to the hindmost level attained by the gut (Fig. 14). The forward course on the right side of the mantle cavity is somewhat sinuate but in a variable degree according to contraction. From the level of the renal pores outward the intestine runs detached from the roof of the mantle cavity, on the right side.

Within the intestine the typhlosoles produce a longitudinal groove on the surface of the faecal pellets (ro), but as the folds stand quite near to one another, their two projections into the intestinal lumen bring forth only one incisure together, whilst there are two in the much bigger trochids (Moore 1932). Like *Tricolia pullus* and *Margarites helicinus* (Fretter 1955, p. 161) the present species has an anal gland (Fig. 17, an). It originates by fusion of the typhlosoles in the rectum and thickening of their epithelium. In *T. pullus* and the mentioned trochid (l. c., f. 1C, G) the anal gland is a groove communicating with the rectal lumen, while it is a closed canal in *T. affinis cruenta*. It runs on the side facing the floor of the pallial cavity; on the opposite side there is a short bulge (xi) of the outer side of the cylindrical anal papilla. This yellowish bulge bears a ciliated groove. Topographically the bulge corresponds to the anal gland of *Gibbula cineraria* (Pelseneer 1899, p. 51, f. 153, I; Frank 1914, p. 453), though this lies inside the rectum. Whether the anal glands of *Margarites* and *Tricolia* formed by the typhlosoles are homologous to the long siphons of fissurellids (Graham 1949, p. 754) is not certain; the function of the latter as well as that of the ventral and dorsal rectal structures is unknown.

UROGENITAL SYSTEM

The left kidney or papillary sac (Fig. 12, k) in its usual position, between intestine (i) and pericardium (p), is thin-walled, unlike that of the trochids examined by Randles (1904, p. 45). The number of the papillae increases with age; their structure is similar to that described by Frank (1914, p. 414) and Cuénot (1914, p. 272). An axial blood-space is surrounded by a thick layer of vesicular cells.

Their boundaries are distinct only at the scarcely ciliated surface where the nuclei are located. Some nuclei are farther inwards between the vesicular cells; these nuclei belong to phagocytic amoebocytes (lymphocytes). The nephridial gland (na) lies between kidney and pericardium on the auricular side; the ciliated reno-pericardial communication on the opposite, the right side; the renal pore in front (vo), to the left of the intestine. The outer walls of the left, bigger (ae) and the right, smaller (wr) auricle show slight knobby dilatations which constitute a pericardial gland.

The right kidney (zr) begins behind the pallial cavity where it is situated in front of and beside the stomach. This glandular or posterior lobe communicates with the tubular urinary chamber or ureter (rn) which runs forward in the roof of the pallial cavity to the right of the intestine. Its pore (x) lies level with the left nephropore.

In the females the slit-like urogenital pore opens in the centre of a large and smooth, colourless or orange button. This consists mainly of blue staining gland cells which secrete into the lumen. The bulge of glands around the female opening drawn in Figs. 12 and 18 (x) represents the maximum development of accessory glands in the present species. This contrasts with the sometimes enormous pallial appendage, the nidamental or albumen gland of the trochids that liberate their eggs in clumps or ribbons (Frank 1914, p. 420-21; Fretter 1946, p. 335; 1955, p. 162). Also *Monodonta turbinata* (Frank, text-fig. 25) has a bigger gland than *Tricolia affinis cruenta,* though it is said (Robert 1902, p. 294) to shed its eggs singly into the sea like *Monodonta lineata* and *Tricolia pullus* (Lebour 1937, p. 123, 124).

The male urogenital pore has no glands. In both sexes a narrow diverticulum communicates with the ureter, and the gonadial duct enters this communication short before it opens into the ureter.

Since Perrier (1889, p. 269) the communication of the right kidney with the pericardium was questioned. Ankel (1936, p. 115) indicated absence of the left reno-pericardial communication for the Trochidae, quoting Spillmann (1905). But this author had not found (p. 539) the right communication in *Gibbula cineraria,* as little as Robert (1900, p. 409) in *Monodonta turbinata*. Pelseneer (1899, p. 53) and Frank (1914, p. 421) verified its presence in the mentioned

species. Randles (1904, p. 47-48) observed the right communication in *Gibbula magus* and *Monodonta lineata,* so that its general occurrence in the Trochidae can be considered as settled as in all other Archaeogastropoda (Fretter 1946, p. 334). The left communication first described by Spengel (1881, p. 348) in *Haliotis* was found by all observers of trochids except Gersch (1936, p. 144-145).

The gonad lies in the spire, more on the outside of the whorls than the liver, and also farther apically. The liver does not extend beyond the outer half of the whorl that precedes the body whorl. In mature snails the gonad is much bigger than the digestive gland, unlike that of higher gastropods whose internal fertilization and accessory genital glands are correlated with a proportionally smaller gonad. To the right of the stomach the gonad is in front bordered by the right kidney which it enters with the gonadial duct. This duct is extremely thin-walled, especially in wholly mature snails. Our sectioned material of April 1960 contains spawning specimens with eggs or sperms in the ureter, as well as middle-sized and quite small, recently metamorphosized ones. Our embryological observations were all gathered in the first fortnight of July 1960 during a period of uncommonly cold nights. Evidently *Tricolia affinis cruenta* has no pronounced reproductive periodicity on the coast of Southern Brazil.

The glands around the urogenital aperture are already developed in young females. As far as known, the coverings of the egg of the Archaeogastropoda are produced in the ovary (Fretter 1946, p. 334). Hence the secretion of the accessory glands either hardens the coats or involves the eggs and flows them out of the pallial cavity (Fretter, l. c.). In *T. affinis cruenta* with a weakly developed hypobranchial gland and a gonopore rather distant from the mantle border the second function appears actually needed. Possibly the secretion of the glands around the female pore produces a substance attractive for the males. According to our observations of spawning snails this is however not probable.

The flat epithelium of the outer wall of the olive green ovary underlain by connective tissue and muscle fibres is thrown into high inner folds. There the epithelium is higher and quite distinct; the connective tissue contains blood-spaces. The growing ovocytes project

from the germinal epithelium into the cavity of the ovary. With the fold the germ cell is connected by an alimentary stalk through which pink yolk granules were seen to flow into the ovocyte. The nucleus of the latter lies opposite to the fixed pole. Unlike to *Tricolia pullus* (Lebour 1937, p. 110, f. 1 M) the freed eggs of *T. affinis cruenta* have only one thin membrane whose irregular gibbosities correspond to discoid pads which develop in the ovary. Already around ovocytes of 40 μ diameter these discoid pads appear. They are 5-8 μ thick in the middle, thinner at the margins, and about 15 μ across. Frequently, not always, they have a light spot in the centre. Ovocytes with half the definitive diameter have a complete egg membrane which is interrupted by the stalk, according to Frank (1914, p. 427-428) the future micropyle. Eggs in the ureter and freed ones are 0,12 mm across; free ones of *T. pullus* measure 0,14 mm (Lebour 1937, p. 124). The free eggs of *Gibbula cineraria* and *G. magus* are 0,115 mm in diameter according to Robert (1902, p. 295), whilst Gersch (1936, p. 136) indicated 0,144 mm without investment for those of *G. cineraria*.

In the bright green testis the germinal epithelium on the folds or trabeculae is coated with a thick layer of developing sperms whose definitive minute heads are spherical. The number of male and even of female germ cells is extremely great.

During the first fortnight of July 1960 the snails emitted sperms and eggs every afternoon between 2 and 4 o' clock. This regular diurnal periodicity known of many gastropods (Pelseneer 1935, p. 472-473) was independent of the hour in which the snails were gathered and transferred from moved to still water, and of renewal of the water. Males and females cannot be distinguished externally, and several times all snails in one vial revealed to be only males or only females. Nevertheless they shed their spawn, both males and females, independently of the presence of the other sex. Also in the trochids studied by Gersch (1936, p. 140) the sexes do not exert a reciprocal influence for emission of their germ cells.

In the dish these sink to the bottom, in moved water this process is slower, and on the *Sargassum* part of them may adhere to the sticky film on the branched thallus. The great frequency of the snails proves that the absence of sexual attraction is compensated. The great

number of germ cells and their longevity counterweigh the loss of eggs that are not fertilized. A female sheds about 100 greyish green eggs at a time, as if it emptied a sac of marbles. After some minutes it gives off the same number again, and sometimes a third or fourth lot during the afternoon. The number of sperms in the "cloud of steam" (Robert 1902, p. 288) amounts to many thousands. At about 20°C. the germ cells are alive and fertilizable for 2-3 hours.

The above-mentioned egg-envelope of *Tricolia affinis cruenta* is a primary or "vitelline" membrane formed by the egg itself. It is separated from the cell already in the shed primary ovocyte by a peri-vitelline space which contains a liquid (Fig. 19). A micropyle is not visible. The polocytes are extruded when the ovocytes are discharged from the female body before fertilization. Several sperms pierce the egg membrane (Fig. 21), but only one spermatozoon enters the female cell. The spiral cleavage corresponds, as far as we accompanied it, to that of *Trochus* (Robert 1902), especially the size-relation between macromeres and micromeres is the same. The first six divisions are accomplished very rapidly so that 64-cell stages were reached one hour after fertilization.

The further development was much slower in our dishes where free-swimming trochophores were only found 16 hours after fertilization: probably the lack of suitable facilities had delayed the development. By deficient conditions our early veligers were even arrested (Crofts 1937, p. 226), they did not develop beyond the pre-torsional stage. Most of them had 16 ciliated cells of the prototroch (Fig. 22) and so corresponded to Crofts' stage of figure 41, c (1937). In *Tricolia affinis cruenta* these cells and the apical area are green, the shell is granular. Our veligers with an operculum died on the third day so that the torsion could not be observed. Young metamorphosized snails were found among algae; that whose shell is shown in Fig. 2 corresponds to Robert's figure 82 (1902, pl. 18), of course with different epipodial appendages.

GENERAL REMARKS

By suppression of the right ctenidium, foreshadowed in Haliotidae and Scissurellidae, a left-right respiratory circulation is already established among the Archaeogastropoda (Yonge 1947, p. 491).

Therewith special arrangements for the exhalant current are dispensable, and accessory genital organs for internal fertilization (Neritacea, Meso- and Neogastropoda) could be developed. When the filaments of this left branchia are reduced to a single row an organisation is attained, whose extense ecological range and corresponding morphological diversification evidences its success.

Also a bipectinate single gill does not preclude its bearers from life in turbid water. Certainly most of them avoid water with much mud and silt, but they are not at all restricted to rocks and reefs. Many of them live, as so many other feeders on vegetable substance, in great numbers on seaweeds, e. g. kelp, and eel-grasse. Some are also inhabitants of sand flats, mud banks, mangrove swamps, or rather turbid brackish and fresh water, e. g., *Gibbula umida* (Ankel 1936, p. 74) and occasionally other species of *Gibbula* (van Benthem Jutting 1947, p. 58), *Cantharidus huttoni* (Clark 1958, p. 59), *C. comtessei* (Dakin 1953, p. 243), *Monodonta subrostrata* (Clark, l. c.), *M. obtusata porcata* (Allan 1950, p. 65), sometimes *Tricolia pullus* (van Benthem Jutting 1947, p. 58), *Pictoneritina oualensis* (Allan 1950, p. 74), *Neritina virginea* (on mud flats on the coast of São Paulo) and other Neritinae, several species of the Valvatidae and Adeorbidae. The juvenile *Cyclostrema* from a locality rich in sediments (Emerson and Puffer 1957, p. 32) may be a turbinid, but this is not sure (see Fretter 1956, p. 379). Odd as it may seem, Thiele (1935, p. 1088) considered the bipectinate gill of the Adeorbidae as an adaptation to burrowing habits. Though few will accept this idea, also the opposite, an incompatibility of bipectinate gill and life in turbid water, cannot be stated. A muscular aspidobranch ctenidium can wipe itself against the floor of the pallial cavity and therewith remove sediment from its filaments (Clark 1958, p. 63). In *Valvata* the cilia of the pallial tentacle on the right side create a powerful outgoing current (Yonge 1947, p. 479).

Within the limits traced by their primitive reproductive organs the Trochacea with single long ctenidium and rasping rhipidoglossan radula have been successful. Fundamental uniformity and a great number of species characterize such groups, e. g. the Kalyptorhynchia of the Turbellaria or the Ascophora of the Bryozoa. In these cases natural subdivisions can be recognized only by detailed studies. Thus

Trochidae and Turbinidae differ in few characters, the conchinous and calcareous operculum, the open and closed eyes, and the presence and absence of the supporting rod in the free extremity of the ctenidium (Yonge 1947, p. 475; Clark 1958, p. 62). The difference concerning the branchial ciliation (Yonge, f. 34 B and C) cannot be used for the classification of preserved animals.

Of the 3 last subfamilies of the Trochidae in Thiele's system (1931, p. 58-63) the Stomatiinae were re-established as family (Risbec 1955, p. 65) and *Teinostoma* removed from the Skeneinae (Abbott 1955, p. 139), though not by all (Emerson and Puffer 1957, p. 34). Among the Turbinidae several authors consider the Liotiinae (Thiele 1931, p. 64; Abbott 1955, p. 121) as family (Strong 1934, p. 432; Powell 1951, p. 103; A. Myra Keen 1958, p. 264).

The lack of nacre in the shell of Thiele's Phasianellinae was after 1931 generally considered as sufficient to dissociate them from the Turbinidae. The presence of the skeletal rod in the gill is another character which parts at least *Tricolia* from the turbinids. Operculum and eyes separate *Tricolia and Phasianella* from the Trochidae. By far the most important feature is the occurrence of two shell muscles but this, too, was verified only in *Tricolia*. Risbec (1939) would have noted the two columellar muscles if they were developed in *Phasianella*. The presence of a post-torsional left muscle is not even probable in snails with a spire produced at an angle from 45° to 55° (Robertson 1958, p. 254). The correspondingly rather narrow mantle cavity (Fretter 1951, p. 583) would be still more restricted by this muscle and the function of the gill reduced.

Robertson (1958, p. 251) considers the Phasianellidae diphyletic in origin and quotes Woodring (1928) who distinguished two families, Tricoliidae and Phasianellidae. Certainly one should always try to subordinate systematic categories to one another, in place of coordinating them. But the presence of two shell muscles in *Tricolia,* or perhaps in *Tricolia* and *Gabrielona,* in our opinion outweighs an entirely porcellanous bulimoid shell, so that after the necessary examination Woodring's systematization may become necessary.

An evolution of the Trochacea from the Scissurellidae, as enounced by Thiele (1935, p. 1081) in a general manner, is suggested also by several similar details in the organization of *Incisura lytteltonensis*

and *Tricolia affinis cruenta*. This origin of the trochaceans can be conceived only from a common root with the scissurellids, not from the recent members of this family whose pedal nervous system is the most concentrated among all Rhipidoglossa (Bourne 1910, p. 34).

RESUMO

Tricolia affinis cruenta Robertson, 1958, é freqüente nas algas do litoral superior, principalmente em *Sargassum,* na Base Norte do Instituto Oceanográfico de São Paulo, 14 km. a oeste de Ubatuba.

O ângulo apical da concha (Fig. 2, 3) varia de 130° nos caramujos mais jovens observados, até 45° nos mais crescidos. Também a forma do opérculo (Fig. 2, 4) e o umbigo modificam-se com o crescimento.

Os animais alimentam-se do filme existente na superfície das algas. Deslizam principalmente por meio dos cílios da sola sôbre a fita de muco produzida por glândulas do bordo pedal anterior (Fig. 11, uz). Glândulas da extremidade posterior do pé (oa) alongam a fita, segurando o animal. O bordo anterior e os tentáculos cefálicos (t) são tácteis; os animais não mostram reação quando os apêndices epipodiais (ei) são estimulados mecânicamente. Em oposição a *Trochus,* animais caídos viram-se com a parte anterior do pé, como o faz também *Tegula viridula*. O movimento ditáxico da última espécie ocorre apenas alusivamente em *Tricolia affinis cruenta,* cuja sola é indivisa (Fig. 9). Os olhos são fechados, possuem pelúcida externa e interna contíguas e corpo vítreo sem diferenciação de cristalino. O apêndice epipodial ramificado do lado esquerdo (ce) é pouco móvel, não filtra. Faixa de cílios (Fig. 10, ci) no fundo da cavidade do manto repele sedimentos; êstes cílios são incomuns nos Aspidobranchia, cuja ocorrência é mais freqüente em águas limpas. O influxo de sedimentos é intenso no lugar de achado, no verão.

Vasos do teto da cavidade palial (Fig. 12) evidentemente auxiliam na respiração, pois dêles o sangue passa à aurícula esquerda (ae); há vestígio do vaso branquial eferente direito. No sistema nervoso (Fig. 13) notam-se células nervosas na alça visceral e nos cordões pedais; os últimos não possuem sulco horizontal. Há zigose sòmente no lado esquerdo. O ramo subintestinal da alça visceral contém 3 gânglios, o abdominal (ao) e um de cada lado dêste. Além

do músculo columelar típico, direito depois da torsão (Fig. 14, rr), mantém-se, no lado esquerdo (mr), o retrator do velum larval. O esôfago é rico em glândulas e dilatações (Fig. 15); o ceco do estômago, curto, largo e reto (Fig. 14, ce). A cutícula gástrica, nomeadamente a do escudo (Fig. 16, si), é periòdicamente renovada. Cada rim tem comunicação com o pericárdio. O poro urogenital da fêmea (Fig. 12, 18, x) é provido de glândulas. Entre o ovo e a membrana vitelina (Fig. 19-21) existe espaço peri-vitelino repleto de líquido. Gibosidades irregulares elevam-se na membrana vitelina. Machos e fêmeas emitiram as células germinativas independentemente da presença de indivíduos do outro sexo, na primeira quinzena de julho de 1960, sempre entre 2 e 4 horas da tarde. Os corpúsculos polares formam-se antes da fecundação. A clivagem espiral concorda com a de *Trochus;* a phase de 64 blastômeros alcança-se dentro de 1 hora (20°C.). A retardação ulterior parece ser devida às condições desfavoráveis de manutenção. Os últimos estádios vivos obtidos no aquário foram velígeres jovens, antes da torsão, com 16 células ciliadas do prototróquio (Fig. 22).

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EXPLANATION OF LETTERS OF TRICOLIA AFFINIS CRUENTA

ac — afferent branchial vessel.
ae — left auricle.
am — afferent membrane.
an — anal gland.
ao — abdominal ganglion.
ar — anus.
au — anterior oesophageal pouches.
b — gill.
ca — anterior buccal cartilage.
cc — caecum.
ce — left cervical epipodial lobe.
ci — ciliary streak.
cm — labial commissure.
co — cerebro-pedal connective.
cu — cerebro-pleural connective.
cz — cerebral commissure.
ea — left zygosis.
ee — eye.
ei — epipodial tentacle.
en — pedal ganglion.
eo — lateral epipodial fold.
ev — efferent branchial vessel.
f — foot.
g — glands of mantle border.
i — intestine.
ie — subintestinal branch of visceral loop.
io — intestinal groove.
iu — supra-intestinal ganglion.
j — jaw.
k — left kidney.
l — ducts of digestive gland.
m — mouth.
ma — gastric nerve.
mn — incisure of mantle border.
mo — buccal cavity.
mr — left shell muscle.
mu — gastric mucus pouch.
na — nephridial gland.
ne — pallial nerve.
ni — nick in branchial filaments.

no — Thiele's sensory knob.
nr — branchial nerve.
o — oesophagus.
oa — foot gland.
oc — operculum.
oc — dorsal epipodial fold.
on — genital nerve.
or — supporting rod.
os — osphradium.
ou — posterior oesophageal pouches (crop).
p — pericardium.
ra — radula.
rc — right cervical epipodial lobe.
rn — ureter of right kidney.
ro — faecal mass.
rr — right shell muscle.
s — stomach.
sa — salivary gland.
sc — posterior buccal cartilage.
si — gastric shield.
sv — supra-intestinal branch of visceral loop.
sz — statocyst.
t — tentacle.
ua — major typhlosole.
uc — buccal ganglion and commissure.
ui — minor typhlosole.
ur — pleural ganglion.
uz — canal of pedal gland.
v — ventricle.
va — vessel corresponding to afferent right branchial vessel.
ve — vessel along glands of mantle furrow.
vo — left nephropore.
wa — branchial ganglion.
wo — pedal cord.
wr — right auricle.
x — uro-genital aperture.
xi — ciliated furrow of anal papilla.
xo — longitudinal gastric fold.
xu — glands of infrapallial border.
y — hypobranchial gland.
za — sorting area.
ze — cerebral ganglion.
zr — right kidney.
zu — glandular fold of oesophageal floor.

PLATES

PLATE 1

Tricolia affinis cruenta

Fig. 1 — Shells of living snails from typical locality.
Fig. 2 — Youngest snail in frontal view and from below.
Fig. 3 — Broad shell, 3,5 mm high, and narrow shell, 6,5 mm high.

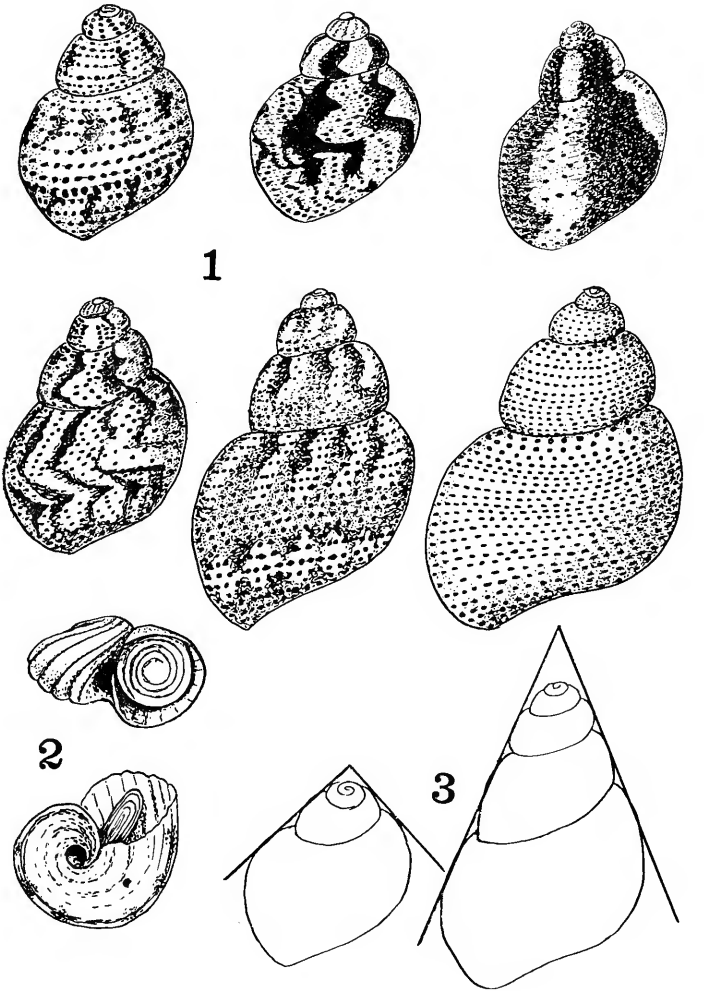
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PLATE 2

Tricolia affinis cruenta

Fig. 4 — Surface view of full-grown operculum, 2,2 mm long.
Fig. 5 — Inside of juvenile operculum, 0,45 mm. long.
Fig. 6 — Part of adult radula.
Fig. 7 — Innermost marginal tooth of adult radula.
Fig. 8 — Part of young radula.
Fig. 9 — Living snail.

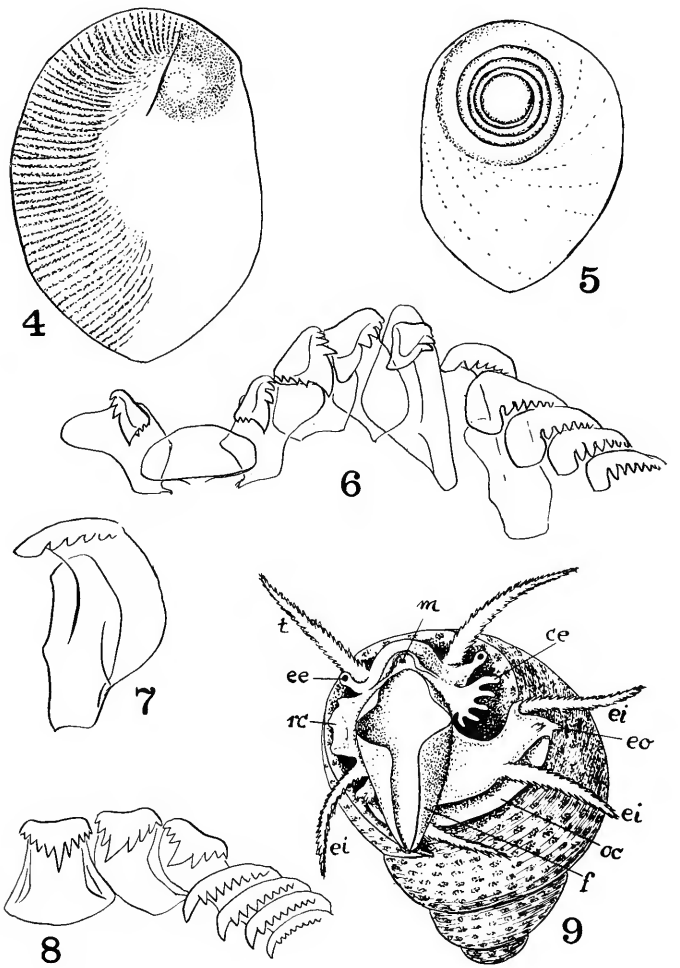
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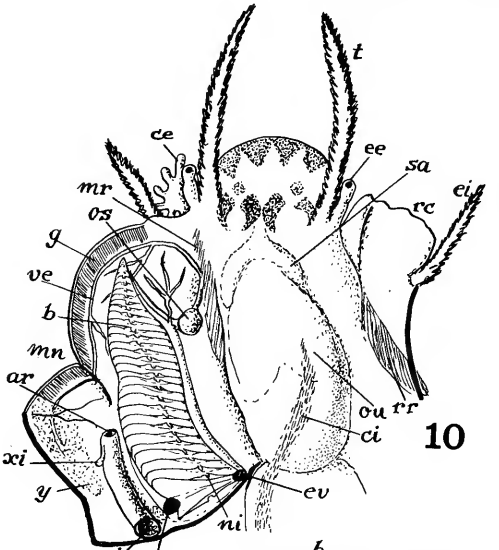
PLATE 3

Tricolia affinis cruenta

Fig. 10 — Anterior part of snail, mantle cavity opened on right side.
Fig. 11 — Combined sagittal section.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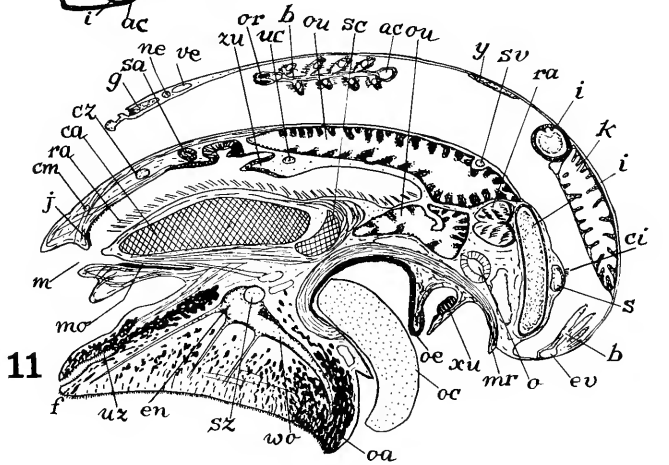
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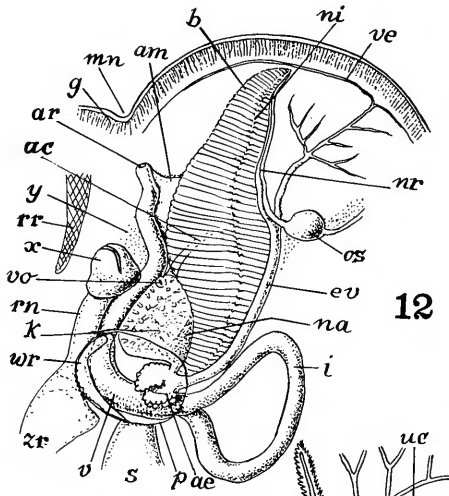
PLATE 4

Tricolia affinis cruenta

Fig. 12 — Inside of pallial roof laid to right side.
Fig. 13 — Nervous system.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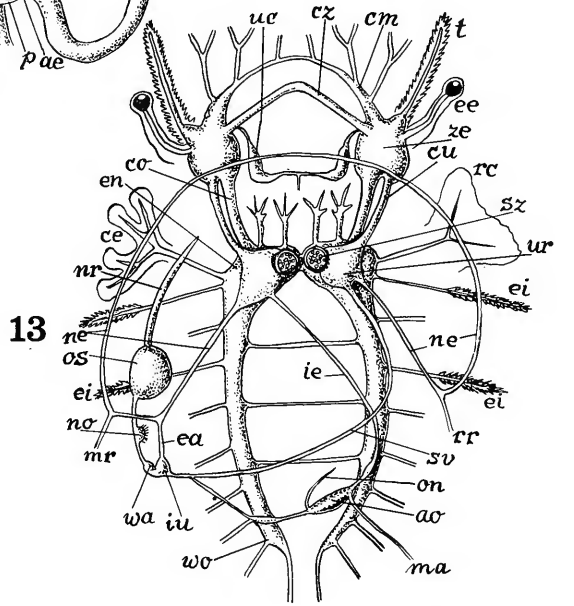
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PLATE 5

Tricolia affinis cruenta

Fig. 14 — Topography of alimentary tract with body whorl drawn unrolled.

Fig. 15 — Transverse sections of anterior gut.

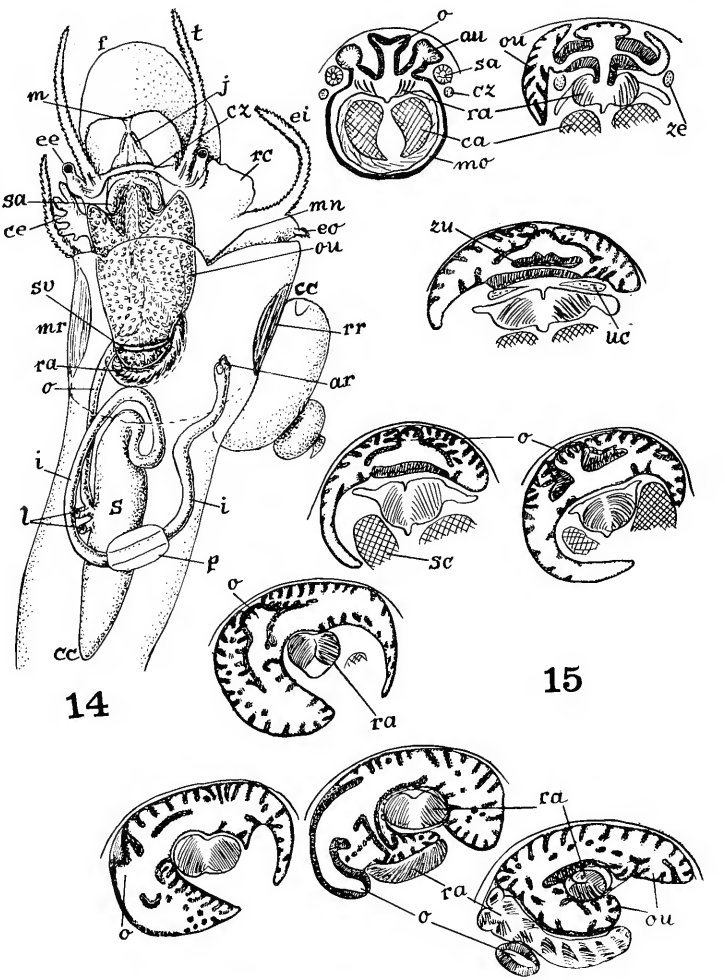
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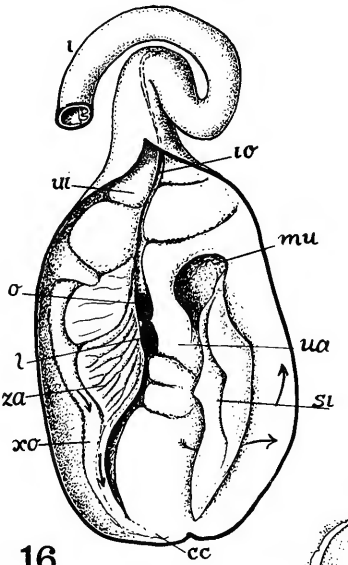
PLATE 6

Tricolia affinis cruenta

Fig. 16 — Stomach.
Fig. 17 — Transverse section of rectum.
Fig. 18 — Section of renal apertures.
Fig. 19 — Unfertilized egg one hour after being shed.
Fig. 20 — Sperm swarming around egg; the tails are so fine that only their movement is visible.
Fig. 21 — Sperms penetrating egg membrane.
Fig. 22 — Young pre-torsional veliger.



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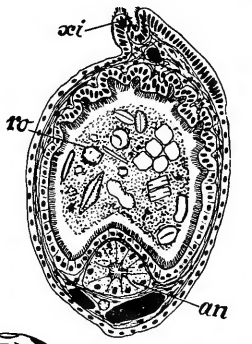

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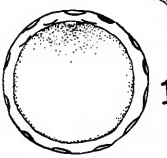

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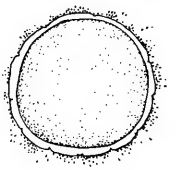

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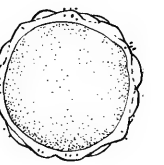

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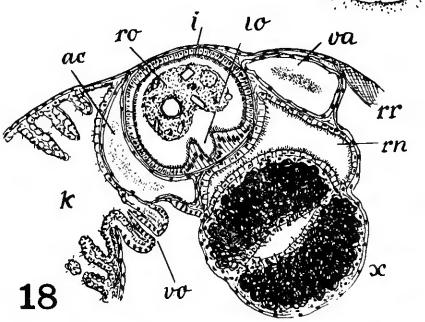

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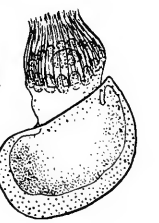

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1961